DEVELOPMENT OF AN AQUATIC TOXICITY INDEX FOR MACROINVERTEBRATES

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DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in School of Animal Plant and Environmental Sciences, Faculty of Science of the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before for any degree or examination in any other university.

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Date:……18 May 2018…….

ABSTRACT

Rapid biomonitoring protocols employing riverine macroinvertebrates in South Africa utilise the South African Scoring System version 5 (SASS5). The SASS5 was developed as part of the then River Health Programme (RHP) [now River Eco-status Monitoring Programme (REMP)]. The SASS5 index is a cost-effective procedure (utilising limited sampling equipment) that enables speedy evaluation of a riverine ecosystem's health using macroinvertebrates as biological indicators of water quality and ecosystem health. As a result, the SASS5 (including earlier versions) has been widely accepted by water quality practitioners and is increasingly incorporated into Ecological Reserve determinations. However, the SASS is widely criticised for being a 'red flag' indicator of water quality and ecosystem health because it has the ability to show only whether a river is polluted (including the extent of pollution) or not, but cannot differentiate between pollutant types (whether chemical or physical). To trace the pollutants responsible for changes in water quality, practitioners are therefore required to conduct chemical-based water quality assessments.

Chemical analyses can provide accurate measures of the magnitudes of chemical substances present in the river water but they do not readily translate into threshold limits supportive or protective of ecosystems. In South Africa the water quality threshold limits for aquatic ecosystems are provided by the South African water quality guidelines for aquatic ecosystems (volume 7). These guidelines provide threshold limits for the protection of the entire aquatic ecosystem constituting of fish, macroinvertebrates, microinvertebrates, algae and plants. These guidelines are therefore too broad for defining protection thresholds supportive of specific subcomponents (*i.e.* macroinvertebrates) of aquatic ecosystems.

The Aquatic Toxicity Index (ATI) for macroinvertebrates was therefore developed for providing threshold limits for physical and chemical stressors protective of freshwater macroinvertebrates. The ATI is expected to aid water quality practitioners working in the Olifants River and catchments with similar land-uses in at least three ways. Firstly, in interpreting the magnitudes of physico-chemical water quality stressors by providing

varying levels of protection (threshold limits) (*i.e.*PC99, PC95, PC90 and PC80) specific to freshwater macroinvertebrates. Secondly, the ATI is expected to aid in the compression of large volumes of water quality data into manageable quantities (descriptor words and grading symbols). Lastly, conventional water quality reports are replete with technical terminology and symbols emanating from water chemistry and ecotoxicology. While reporting of this kind is accessible to water resource specialists, it may constitute an obstacle for non-technical stakeholders (with no training or experience in water chemistry) like policy makers, political decision makers and the public. These groups generally have neither the time nor the training to study and understand a traditional, technical review of water quality data. Water quality indices are capable of eliminating technical language incurred in water quality reports; hence, they are viewed as necessary tools in reaching multiple audiences by bridging the gaps between the extremes of water quality monitoring and reporting. The ATI is expected to enhance not only accessibility and comprehensibility in all these instances, but utility in general too. Differently expressed, the ATI is expected to aid as a water quality-reporting tool that will help water quality practitioners and managers in communicating technical water quality data to multiple stakeholders even those without training and experience in water chemistry and ecotoxicology.

The development of the ATI for macroinvertebrates was conducted in two phases. First, the derivation of Protection Concentrations (PCs). The PCs were obtained by fitting Species Sensitivity Distribution (SSD) curves on short-term (24-96 hours) median lethal (LC50) data for freshwater macroinvertebrates collected from databases and scholarly publications. Before the estimation of the PCs, the toxicity data had to undergo a preparatory process. This involved the conversion of metal stressors from total metal concentrations to dissolved fractions. Additionally, metal stressors whose toxicity is known to be dependent on water hardness (cadmium, chromium (III), copper, lead, nickel and zinc) were adjusted to reflect their toxicity at six different levels of water hardness using USEPA conversions algorithms. In addition, all ammonia data were converted to reflect the toxicity of ammonia as TAN at pH = 8 and temperature = 25˚C.

The second phase of the index development involved the allocation of index categories. This was to enable the discrimination of stressors' magnitudes into classes. The final product is a five-point scale classification system (A to E) based on four PC levels (PC99, PC95, PC90 and PC80) for freshwater macroinvertebrates obtained by fitting Species Sensitivity Distribution (SSD) functions on the toxicity data. On development, the index was validated using water quality data, riverine macroinvertebrates survey data and flow data collected from the Olifants River catchment between 2015 and 2016. Eight study sites were covered, located in the upper and the lower Olifants system. Four of these were located in the Olifants River main stem and four from four tributaries (Klein Olifants, Blyde, Ga-Selati and Letaba rivers).

The evaluation of the Olifants system based on the assessment of variable-by-variable indicated that Site S5 and S2 (lower Ga-Selati and Klein Olifants) were the most degraded sites in the study, respectively. In addition, the study indicated gross elevation of sulfate, nitrates, pH and copper. The evaluation of the Olifants system using the composite ATI for macroinvertebrates indicated that the system was generally in good condition. However, the identification of the lowest rating score indicated that temperature difference from reference conditions, sulfate, nitrate, zinc and lead were the main variables limiting the water quality of the Olifants system. In addition, the sensitivity analysis of the index conducted as part of the validation process of the index, indicated that temperature difference from reference conditions, sulfate and nitrate were the most important variables in the computation of the index.

Investigations of the relationships between the ATI for macroinvertebrates, SASS5 metrics, MIRAI and measures of flow variability revealed negligible and statistically insignificant associations. These could mainly be attributed to three reasons. Firstly, sampling difficulty, this resulted from high density of filamentous algae and floating aquatic vegetation (posing physical obstructions to sampling) in the river. Such extraneous factors rendered the SASS5 sampling protocol (benthic/kick method), ineffective because of clogging of sampling net and loss of specimens in the sifting of aquatic plants for macroinvertebrates. Secondly, the filamentous algae interfered with the availability and suitability of habitat for aquatic invertebrates. For instance it covered

stones biotopes (stones in current) forming a thick layer of algae on the stones thereby exhibiting the characteristics of vegetation biotopes for most sampling sites, a major impediment for the SASS5, a method that is largely dependent biotope availability. Thirdly, the SASS was developed for organic pollutants while the ATI for macroinvertebrates is largely driven by metal stressors.

Because of toxicity data shortages for regional freshwater macroinvertebrates, a compromise between data availability and quality was considered. For example, toxicity data drawn from global sources were used as the base for the PCs and the index. Despite these limitations, the protection concentrations (numerical sensitivity values) that form the base of the index were comparable with published water quality benchmarks from literature and water quality jurisdictions. In addition, the index has the ability to summarise and discriminate (stressors in terms of concentrations and magnitudes) large quantities of water quality data to facilitate interpretation of the quality of a water's ability to support freshwater macroinvertebrates.

Keywords: Aquatic Toxicity Index, freshwater macroinvertebrates, water quality criteria, species sensitivity distribution, Olifants River

EPIGRAPH

“Water quality indices make it easy for a lay person to judge whether a water source is usable or not and how one source compares to one another, but the development of a water quality index is by no means an easy task. It, in fact, is fraught with several complications and uncertainties” (Abbasi and Abbasi, 2012).

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God bless

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

1.1.1 Current and projected state of water resources and freshwater ecosystems

UNEP (2017) describes freshwater ecosystems as one of the most productive systems on earth and yet the most threatened. In a similar review, Darwall *et al*. (2011) referred to freshwater ecosystems as hotspots for both global biodiversity and biodiversity endangerment. While freshwater is only 0.01% of the world's water and cover only 0.8% of the earth's surface (Dudgeon *et al*., 2006), it is home to 10% of global species (Balian *et al*., 2008). The importance of freshwater ecosystems supporting one tenth of global biodiversity is therefore evident. Yet statistics suggest that as a result of unsustainable human activities within watersheds between 10,000 and 20,000 freshwater species are extinct or critically imperilled (*i.e.* endangered, threatened or vulnerable) (Strayer and Dudgeon, 2010).

As observed by Bunn (2016) the leading threats to freshwater ecosystems biodiversity are population growth, decline in water quantity and habitat transformation. The imperilled state of freshwater species is therefore not surprising particularly in view of observed gross imbalances between waste generation and disposal. For example, estimates suggest that about two million tons of sewerage, industrial and agricultural wastes are discharged into water resources every day (United Nations World Water Assessment Programme, 2003). Yet it was estimated that only 20% of all waste released to the environment was adequately treated to meet stipulated wastewater discharge thresholds (Connor *et al*., 2017). This is even worse in low-income countries, where it was estimated that non-compliant discharges were as high as 92% (Sato *et al*., 2013).

It is therefore expected that the fate of freshwater resources and related ecosystems is gaining prominence in global and national initiatives and programmes. While the United Nations' Millennium Development Goals (MDG) of 2000 to 2015 omitted freshwater ecosystems (United Nations Millennium Development Goals, 2017), the later United Nations' Sustainable Development Goals (SDG) of 2015 to 2030 give more attention to

freshwater ecosystems (United Nations Sustainable Development Goals, 2017). For example, SDG 6.3 aspires for improvements in water quality aimed at 50% reduction in the disposal of untreated waste by 2030. SDG 6.6 aspires for the protection and restoration of water related ecosystems by 2020, while SDG 15.1 puts equal emphasis on the conservation, restoration and sustainable use of inland freshwater ecosystems and related services by 2020.

Similarly, at national level, legal frameworks, regulatory obligations and related programmes afford freshwater attention. In the South African National Water Act (NWA), (Act 36 of 1998) water resources are viewed as ecosystems which should be protected for both current and future human needs. This is done through adherence to Ecological Reserve requirements. The Ecological Reserve is a mondatory allocation of water of specified quality and quantity required to sustain specified river ecosystems (van Wyk *et al*., 2006). The National Environmental Management Act (NEMA) (Act 107 of 1998) makes provision and gives details for the prevention of pollution, minimisation and remediation of pollution. The NEMA also has liability or provisions concerning all environmental resources including water, which the state treats as a subset of the environment in its entirety. Despite these national commitments to protect freshwater resources and related ecosystems, numerous challenges seem to deter or impede attainment of these aspirations.

South Africa is widely described as a semi-arid and water stressed country. It receives about 450 mm mean annual rainfall. This figure is considerably below the world average of 860 mm per year (King and Pienaar; 2011; Kohler, 2016). Water development and use statistics suggest that 98% of South African freshwater has already been allocated (Hedden and Cilliers, 2014). This situation is exacerbated by unsustainable catchment activities. For example, the Rand Water (2017) recognised that in South Africa the scarcity of freshwater is exacerbated by major increase in pollutant fluxes into river systems arising from river catchments caused by urbanisation, deforestation, damming of rivers, destruction of wetlands, industry, mining, agriculture, energy use, and accidental water pollution.

The appalling state of water resources in South Africa is largely compounded by rise in the human population. Statistics South Africa (2017) estimates the country's population to be at 56.5 million people, currently growing at over 1.6% people per year. Consequently, in 2016, the Water Research Commission CEO stated that South Africa could have a freshwater deficit of approximately 1.1 billion cubic metres by 2035. The increased pressures are most likely to impact negatively on both the quality and quantity of the water (DWA, 2011).

1.1.2 The Olifants River catchment

These features are pertinently displayed in the Olifants River catchment. It has been widely classified as one of the most polluted and threatened river systems in South Africa (de Villiers and Mkwelo, 2009; Van Vuuren, 2009; Ashton, 2010). Unsustainable mining activities, industrial and nutrient laden discharges (irrigation return flows) have been repeatedly reported as key sources of the degradation of the Olifants River system (Basson and Rossouw, 2003; de Villiers and Mkwelo, 2009; McCarthy, 2011; Dabrowski, *et al*., 2015; Gerber *et al*., 2015). As a result of these activities there have been numerous reports of crocodile (pansteatitis) and fish deaths (reported since mid-2000s) in the river, eutrophication and microcystis blooms since 2003 (Huchzermeyer, 2012), acid mine drainage (McCarthy, 2011; Dabrowski, *et al*., 2015) and tailing spillages into tributaries *e.g.* the December 2013 Bosveld Phosphate incident. This spillage drained downstream, polluting the Olifants River within the Kruger National Park and further downstream to neighbouring Mozambique (DWA, 2015).

1.1.3 Methods for the classification of water resources

Management decisions pertaining to the protection of water resources in most parts of the world are supported by water quality guidelines or criteria that are recommended by delineated jurisdiction (s). These include the United States Environmental Protection Agency (USEPA), Canadian Council of Ministers of Environment (CCME), Australian and New Zealand Environment and Conservation Council (ANZECC)/Agriculture and Resources Management Council of Australia and New Zealand (ARMCANZ) and the Department of Water and Sanitation (DWS) in South Africa. These water quality

benchmarks are continuously refined to incorporate latest scientific understanding and trends in water resources assessment, monitoring and management.

In the case of South Africa the water quality guidelines for the protection of aquatic ecosystems promulgated in 1996 (DWAF, 1996), served as the primary source of information for determining the suitability of water for aquatic ecosystems (Palmer *et al*., 2004). The DWAF guidelines offered three protection values. These were Target Water Quality range (TWQR), Chronic Effect Value (CEV) and Acute Effect Value (AEV). The TWQR is the most conservative value and the AEV the least restrictive value. All these values were derived from toxicity/tolerance or stressor response relationship data.

The enactment of the NWA created a legal framework that embodies the principles of equity and sustainability. The NWA recognises the importance of the Reserve. The Reserve is a proportion of water intended for ensuring a sustainable balance between the protection of aquatic ecosystems and water demand to support development. For example, it ensures that water of good quality and sufficient quantities is reserved for supporting the functioning of ecosystems, as well as meeting basic human needs. With respect to the Ecological Reserve determination, the NWA provides for classification systems intended for the comprehensive protection of water resources, where Ecological Reserve assessment classes are defined. The eco classification is used in Resource Directed Measures (RDM) studies of rivers: How much water and of what quality does a river need to function?

The determination of an Ecological Reserve requires the use of multiple tools. Some of the tools used in the ecological assessments are those developed through the River Health Programme (RHP). Over the years, one of the most important aspects of the RHP has been the development of biological monitoring and assessment tools (MacKay, 1999). Since 2016, these tools have been reclassified into a new programme referred to as the River Eco-status Monitoring Programme (REMP).

Some of the widely used tools within the REMP are the South African Scoring System Version 5 (SASS5) and the Macroinvertebrate Response Assessment Index (MIRAI) for macroinvertebrates. The SASS5 index is a rapid biomonitoring tool using

macroinvertebrates from lotic systems. These have mostly been identified to family level, to indicate water quality impairment and overall river health (Dickens and Graham, 2002). Employing SASS data, the MIRAI is then used in the provision of a habitat-based cause-and-effect foundation to interpret the deviation of the macroinvertebrate community structure from the reference state (Thirion, 2007; Thirion, 2016). The MIRAI generates Ecological Categories (EC) based on a six-point scale, with ranges from 'A' (pristine) to 'F' (critically modified); all other abiotic drivers and biotic responses used in the determining the Ecostatus of South African waters are rated on this six-point scale. The ECs of the MIRAI are incorporated into the Ecological Reserve determination by integrating the ecological requirements of a macroinvertebrates assemblage and then relating this to flow modifications, in stream habitat structure, water quality modification and connectivity and seasonality. Thirion (2007) recognised that the database of intolerances, substrate preferences and velocity preferences was not comprehensive and further information was required. In addition, the SASS method provides a general indication of the present state of the invertebrate community. It was developed for application in the broad synoptic assessment required for the River Health Programme (RHP) and does not have a particularly strong cause-effect basis.

The SASS5 was developed as part of the then National River Health Programme (RHP) [now River Eco-status Monitoring Programme (REMP)]. The SASS5 index is a cost-effective procedure (utilising limited sampling equipment) that enables speedy evaluation of a riverine ecosystem's health through the use of macroinvertebrates as biological indicators of water quality and ecosystem health (Dickens and Graham, 2002; Fourie *et al*., 2014). As a result, the SASS5 (including earlier versions) has been widely accepted by water quality practitioners and is increasingly incorporated into Ecological Reserve determinations. However, the SASS is widely criticised for being a 'red flag' indicator of water quality and ecosystem health because it has the ability to show only whether a river is polluted (including the extent of pollution) or not, but cannot differentiate between pollutant types (*e.g.* chemical or physical) (Bonada *et al*., 2006; Weerts and Cyrus, 2008). To trace the pollutants responsible for changes in water quality, practitioners are therefore required to conduct chemical-based water quality assessments.

Chemical analyses can provide accurate measures of the magnitudes of chemical substances in the river water but they do not readily translate into threshold limits supportive of ecosystems. In South Africa the water quality threshold limits for aquatic ecosystems are provided by the South African water quality guidelines for aquatic ecosystems (volume 7) (DWAF, 1996). These guidelines provide threshold limits for the protection of the entire aquatic ecosystem constituting of fish, macroinvertebrates, microinvertebrates, algae and plants. These guidelines are therefore too broad for defining protection thresholds supportive of specific subcomponents of the aquatic ecosystems (*i.e*. macroinvertebrates).

The Aquatic Toxicity Index (ATI) for macroinvertebrates was therefore developed for providing threshold limits for physical and chemical stressors protective of freshwater macroinvertebrates. The ATI is expected to aid water quality practitioners working in the Olifants River and similar catchments (river catchments with similar land-uses) in at least three ways. First, in interpreting the magnitudes of physical and chemical water quality stressors by providing varying levels of protection (threshold limits) (*i.e.*PC99, PC95, PC90 and PC80) specific to freshwater macroinvertebrates. Second, the ATI is expected to aid in the compression of large volumes of water quality data into manageable quantities (descriptor words and grading symbols). Lastly, conventional water quality reports are replete with technical terminology and symbols emanating from water chemistry and ecotoxicology. While reporting of this kind is accessible to water resource specialists, it may constitute an obstacle for non-technical stakeholders (with no training or experience in water chemistry) like policy makers, political decision makers and the public (House, 1989; Cude, 2001; Darapu *et al*., 2011; Al-Janabi *et al*., 2015). These groups generally have neither the time nor the training to study and understand a traditional and technical review of water quality data (Al-Janabi *et al*., 2015). Water quality indices are capable of eliminating technical language incurred in water quality reports; hence, they are viewed as necessary tools in reaching multiple audiences by bridging the gaps between the extremes of water quality monitoring and reporting (House, 1989). The ATI is expected to enhance not only accessibility and comprehensibility in all these instances, but utility in general too. Differently expressed, the ATI is expected to aid as a water quality-reporting tool that will help water quality practitioners and managers in

communicating technical water quality data to multiple stakeholders even those without training and experience in water chemistry and ecotoxicology.

1.2 AIMS AND OBJECTIVES OF THE STUDY

The study had four aims; the first aim was to develop a site specific Aquatic Toxicity Index ATI for freshwater macroinvertebrates. The second aim was to characterise the spatio-temporal patterns of the physico-chemical water quality variables and riverine macroinvertebrate community structure of the Olifants River. The third aim of the study was to determine the relationship between the ATI for macroinvertebrates with SASS5 metrics, MIRAI, ATI for fish (Wepener *et al*., 1992) and measures of flow variability. Lastly, to conduct the sensitivity analysis of the ATI for macroinvertebrates.

Aim 1: Develop the ATI for freshwater macroinvertebrates.

Specific objectives:

1. To select appropriate water quality variables for deriving the ATI for macroinvertebrates based on land-use activities within the Olifants River catchment (*e.g.* mining, urban and agriculture) and available ecotoxicity/tolerance data for freshwater invertebrate species from databases and scholarly publications.

2. To estimate population–level benchmark concentrations for protecting freshwater macroinvertebrates from physico-chemical stressors with adequate bioassay data.

Aim 2: To characterise the spatio-temporal patterns of the physico-chemical water quality variables and riverine macroinvertebrate community structure of the Olifants River.

1. To identify physico-chemical water quality variables limiting the water quality of the Olifants River catchment.
2. To identify study sites with gross elevation of pollutants.
3. To compare spatial similarities in riverine macroinvertebrates composition between selected study sites.

Aim 3: To determine the relationships between the ATI for macroinvertebrates with SASS5 metrics, MIRAI, ATI for macroinvertebrates and measures of flow variability.

Specific objectives:

1. To determine the level of correlation between the ATI for macroinvertebrates and the SASS5.
2. To determine the level of correlation between the ATI for macroinvertebrates and measures of streamflow variability.
3. To determine the level of correlation between SASS5 and flow variability.
4. To assess the relationships between the ATI for macroinvertebrates and the ATI for Fish (Wepener *et al*., 1992).
5. To assess the level of agreement (based on inter-rater reliability/agreement) between the ATI for macroinvertebrates, SASS5 metrics and MIRAI version 2 the rating of the state of the Olifants River.

Aim 4: To conduct the sensitivity analysis of the ATI for macroinvertebrates.

Specific objectives:

1. To determine the relative importance of the different water quality variables on the composite index scores.
2. To determine the sensitivity of the index based on the number of input variables.
3. To determine how sensitive the index is to the removal of certain groups of variables *(i.e.* metals, nitrogen compounds, salts and physical variables).

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This chapter has three objectives. First, to review the interrelationships between habitat attributes and the structure and function of riverine organisms. Second, to present a contextual overview of approaches and the theoretical basis of methods used in deriving water quality benchmarks (short-term acute exposures) for the protection of freshwater life adopted by selected water quality jurisdictions globally. Lastly, to give similar attention to reviews of both the theoretical and praxis-based approaches and trends in the development, application and validation of water quality indices.

2.2 FACTORS DETERMINING THE PRODUCTIVITY OF STREAM ORGANISMS

The structure and functioning of stream organisms is determined by numerous environmental factors that are grouped into mainly four components. Those are mainly referred to in literature include water quality, energy inputs, flow regimes and physical habitat structure (Figure 2.1) adapted from Poff *et al*., 1997; Milhous and Bartholow, 2004; Thirion, 2007. Most of the monitoring tools address part of the environmental factors or components. In fact, it does seem practically impossible to derive a tool that will address all possible factors responsible for the structure and functioning of aquatic organisms.

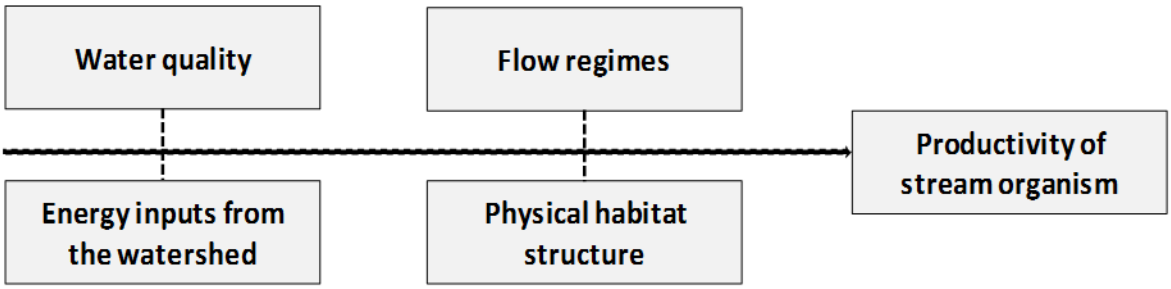

Figure 2.1 Four stream components known to affect the productivity of stream organisms

With respect to riverine invertebrates, several tools have been developed to aid in explaining resultant or observed macroinvertebrate community composition. For example, the Lotic Invertebrate Index for Flow Evaluation (LIFE) has the ability to link invertebrate taxa to flow conditions (Extence *et al*., 1999). However, it explains part of the story because it is biased towards flows, yet the other factors are equally capable at any given point of influencing the structure and functioning of stream organisms. Additionally, the LIFE index has very limited application to most parts of the world, particularly because it has not been calibrated or adapted to most parts of the world besides Britain (Extence *et al*., 1999) and the Mediterranean rivers (Buffagni *et al*., 2009). However, there is no evidence in literature suggesting any attempts to calibrate the LIFE index using South African invertebrate taxa. Similarly, physical habitat indices for invertebrates like the Integrated Habitat Assessment (IHAS) (McMillan, 1998) also address a fraction of the factors and remain silent on the others. On the other hand the Macroinvertebrate Response Assessment Index (MIRAI) (Thirion, 2007; Thirion, 2016) does incorporate most of the components illustrated in Figure 2.1, but just like the South Africa Scoring System (SASS5) index (Dickens and Graham, 2002) it is able to indicate changes in the habitat conditions inferred from observed invertebrates' population dynamics. However, by design these two indices are incapable of tracing the sources of degradation of the riverine ecosystem.

2.1.1 Water quality

Most authors define water quality by relating the suitability of a water body to the requirements of a user or intended uses (*e.g.* Johnson *et al*., 1997; Dallas, 1998). With particular reference to living organisms, this definition acknowledges the differences in tolerances to withstand levels of pollution. In the aquatic environment, there are thousands of physico-chemical variables that can be measured to determine the suitability of a water body to support aquatic organisms (Day, 2000). Nonetheless, the water quality component remains highly prioritised. This is the case because no inquiry into water quality will be complete without yielding evidence on the physico-chemical state of a water body.

2.1.2 Physical habitat structure

The structure and function of aquatic communities is partly determined by habitat in terms of availability and quality. Therefore, it is important that monitoring systems incorporate habitat availability and quality as part of rapid bio-assessments of stream ecosystems (McMillan, 1998; Dickens and Graham, 2002). This is a necessary undertaking because aquatic organisms have varying preferences for biotopes. For example Tubificidae; Oligochaeta are predominantly found in the gravel, sand and mud biotope partly because of their specialised nutritional specialisation (*e.g.* bacteria or diatoms attached to detritus or sand grains) (Giere, 1975). The absence of certain taxa from a site is therefore not always a function of the state of the water column but a mere reflection of the quality and availability of biotopes (Mangold, 2001).

2.1.3 Flow regimes and riverine ecosystems

Flow regime embraces a multifaceted concept comprising flow magnitude, frequency, duration, timing and rate-of-change (Poff *et al*., 1997). Almost all aspects of flow regimes are integral to the functioning of a riverine ecosystem (Poff *et al*., 1997; Richter *et al*., 1997). As a result, the natural flow-regime paradigm suggests that the structure and functioning of riverine ecosystems, and the adaptations of their constituent riparian and aquatic species, are determined and shaped by patterns of temporal variation in river flows (Poff *et al*., 1997). Therefore, natural flow disturbances are considered important for the functioning of an intact ecosystem necessary for controlling population size and spatio-temporal diversity of lotic organisms. Natural flow variability in rivers exist because of natural flow characteristics related to climate, geology and topography (Naiman *et al*., 2008). The natural occurrence of extreme flow events like floods and low flows (because of droughts) is therefore an important natural feature for riverine ecosystems (Lytle and Poff, 2004; Piniewski *et al*., 2017). However, humans have extensively interfered with and modified natural flows of many rivers across the globe through water diversions and damming (Nilsson and Renöfält, 2008). Human interference with natural flows has cascading effects on river ecosystems. As noted by Poff *et al*. (2007) modification of rivers leads to the loss of regionally distinct flow regimes and modification of the timing of

ecologically important flows. These conditions contribute to the proliferation of cosmopolitan and non-indigenous species at the expense of native biota.

Results from past studies have adequately demonstrated the roles of hydrological metrics in shaping the assemblage composition of river ecosystems (Monk *et al*., 2006; Sheldon and Thoms, 2006; Wilding *et al*., 2017). For example, studies relating the biotic composition of riverine macroinvertebrates to extreme high flow events observed considerable reduction in invertebrate abundance (of up to 50%) immediately after floods and hydro-peaks (Bruno *et al*., 2010; McMullen and Lyte, 2012). Extremely high flow events are most likely to modify the physical habitat structure and stream ecosystems through scouring of the riverbed and flushing certain organisms, hence affecting the temporal variation of benthic communities (Brittain and Eikeland, 1988; Jacobsen *et al*., 2013). Consequently, rapid biomonitoring protocols for riverine macroinvertebrates, such as the SASS5, prohibit their use immediately after a flood event (Dickens and Graham, 2002). This is because sampling immediately after a flood most likely reflects the flushing effect of the flood event as opposed to the ecological state of the system under investigation.

Additionally, flow cessation and intermittency have been observed to be on the increase in recent times mainly because of human impacts, including climate change related droughts (Shute *et al*., 2016). This is an area of major concern in hydro-ecology and water management, particularly because the understanding of the impacts of flow cessation on stream organisms is still in an infancy stage and therefore replete with uncertainties. This particular field of study requires further investigation and deeper understanding (Acuna *et al*., 2014; Leigh *et al*., 2016; Stubbington *et al*., 2017), particularly because riverine organisms are largely used as biological indicators of water quality and ecosystem health and integrity (Kenney *et al*., 2009).

Because of limited understanding of the associations between flow intermittency and stream organisms, Dickens and Graham (2002) advised, with precautions, the application of SASS5 in ephemeral streams. This was owing to insufficient understanding and performance of the SASS5 under flow intermittency. Wilding *et al*. (2017) observed that,

as a worst-case scenario, the application of biomonitoring techniques in streams largely characterised by flow intermittency can lead to the misclassification of the ecological status of rivers.

In contrast to flow cessation, urban development and the associated increase in impervious surfaces within watersheds is considered as a key factor affecting variability in stream flow, flashiness (defined by frequent rise and falls of flow levels mainly characteristic of urban streams), baseflow recharge and variability in water quality (O'Driscoll *et al*., 2010; Coles *et al*., 2012). Previous hydro-ecological investigations of the relationships between flow variability, flashiness and baseflow, on the one hand, and macroinvertebrate community structure, on the other, observed strong associations between flow variability and invertebrate assemblage composition (Sheldon and Thoms, 2006; Sprague *et al*., 2006). The frequent rise and fall in stream flow magnitudes have been observed to impact negatively on faunal communities.

As a result, the assessment of the spatio-temporal community structure of riverine organisms should incorporate flow descriptors and/or indicators. For instance, Puckridge *et al*. (1998) held the view that river ecology processes are largely controlled by flow variability. In a similar review, Sheldon and Thoms (2006) stated that habitat connection in rivers is driven by flow variability and it is therefore expected that large-scale connectivity should be reflected in indices of flow variability. Consequently, Sheldon and Thoms (2006) in a study conducted from four Australian rivers observed strong associations between the complex measures of flow variability (coefficient of variation, flash flood magnitude index and hydrological index of variability) and the assemblage composition of riverine macroinvertebrates.

Regardless of attempts to unravel the associations between flow descriptors and river ecosystems, it is difficult to determine which indicators of flow variability directly influence stream biota (Puckridge *et al*., 1998). One of the most commonly used measures of flow variability is the Coefficient of Variation (CV) (Chow, 1964; Jowett and Duncan, 1990). The CV is a value that represents the ratio of standard deviation of stream flow to its mean (Poff, 1996; Pegg and Pierce, 2002). CV can be expressed as a dimensionless

index value [Coefficient of Variation Index (CVI)] or as a percentage (CV %). The higher the CV the greater the relative variability of a river (Arnel, 2014). The Coefficient of Variation Index (CVI) for South African rivers varies from less than one to greater than 10 (Hughes and Hannart, 2003).

The CV describes stream flow variability without regard to the temporal sequence of the flow variations (Baker *et al*., 2004). As a result, the CV is sometimes calculated alongside the Flashiness Index (R-B FI) (Berhanu *et al*., 2015). The Flashiness Index (referred to FI in this study) is an index developed to detect changes in stream/hydrological regimes; *i.e*. it is the ratio of the day-to-day streamflow fluctuations (sums of the path lengths of the flow oscillations) relative to the total flow quantities of the flow duration under consideration (Baker *et al*., 2004). Similar to the CVI, the higher the R-B FI the flasher the river flows.

Total runoff is essentially divided into two components; direct runoff and baseflow. Surface runoff refers to the water that reaches the stream without percolating through the soil while baseflow is mainly the groundwater contribution to the total stream flow (Gibb and O'Hearn, 1981). The chemical water quality of baseflow differs from that of surface runoff (Gibb and O'Hearn, 1981). According to Ku *et al*. (1975) in Hetcher *et al*. (2004) the chemistry of the baseflow component of the total flow differs because, it gets in contact (during percolation) with the subsurface material long enough to leach soluble minerals. This results in higher concentrations of inorganic chemical constituents than in direct runoff.

To determine if there is a relationship between baseflow contributions to the total flow and riverine ecosystems, it is necessary to calculate the Baseflow Index (BFI). BFI is defined as the ratio of the baseflow contribution to the total runoff (Bosch *et al*., 2017). Its determination requires the partitioning of the total flow into two components – the baseflow and the runoff – in a process referred to as the hydrograph analysis (Lim *et al*., 2005). A hydrograph is a graphical representation of river discharge related to time at a flow gauging station (Focazio and Cooper, 1995). Hydrologists use different methods to conduct hydrograph analyses. Broadly, these can be classified as tracer based and non-

tracer based hydrograph analysis techniques (Gonzales *et al*., 2009). Tracer based hydrograph separation techniques use geochemical or isotopic tracers, where it is assumed that the chemical signature of water draining from various sources is constant and unique and that conservation of mass applies and quality including conservative mixing of different components (Jones *et al*. 2006; Gonzales *et al*., 2009). However, these methods have been viewed to be paradoxical largely because they commonly show pre-event waters originating from the subsurface to be a major contributor to the observed rise in stream discharge shortly after a storm event, yet flow is usually considered a relatively slow process (Jones *et al*., 2006).

Non-tracer hydrograph separation methods, on the other hand, are based on the physical characteristics of the stream flow determined from continuous flow data as opposed to the chemical and isotopic tracers. For example, they are focused on the analysis of the recession or depletion curves (Gonzales *et al*., 2009). Similarly, non-tracer baseflow separation methods can be divided into different groups – for example graphical separation methods and filtering separation techniques. Graphical separation methods are commonly used to plot the baseflow component after a flood event (Brodie and Hosteler, 2005). Some typical examples of this method include the constant discharge method, the constant slope method and the concave method.

Filtering separation techniques: these separate the baseflow component of the total flow using streamflow time series processing and filtering procedures (Brodie and Hostler, 2005). Some of the most commonly used techniques include:

- Local Minimum Methods (LMMs): these connect the minimum values of a hydrograph. The discharge under the constructed line represent the baseflow component (Sloto and Crouse, 1996). The advantage of the LMMs is that they are standardised and systematic and, therefore, they can be easily translated into computer programme (Sloto and Crouse, 1996). For example, the LMM is incorporated in the Hydrograph Separation Program (HYSEP) (Sloto and Crouse, 1996) and Web-based Hydrograph Analysis Tool (WHAT) (Lim *et al*., 2005).

- Recursive Digital Filters (RDFs): these are derived from signal analysis (Aksoy *et al*., 2009). RDFs partition the hydrograph into two components, the baseflow and direct runoff. Under this method, it is assumed that high variability (high frequency) in stream flows is caused by direct runoff and low variability (low frequency) is associated with baseflow (Eckhardt, 2005). The RDFs can be easily automated and have been recommended for providing reproducible results (Eckhardt, 2005; Li *et al*., 2013). However, the RDFs have been criticised on the basis that they do not take into consideration the physical processes responsible for baseflow generation as their inputs, but are simply based on streamflow records and filter parameters selected by the user. Additionally, filter parameters are often constrained by the condition that baseflow must not exceed total streamflow or become negative (Furey and Gupta, 2001; Li *et al*., 2013).

2.1.4 Energy and nutrients inputs from watershed and riverine ecosystems

The productivity of aquatic ecosystems is also determined by energy flow (Poff *et al*., 1997; Milhous and Bartholow, 2004). Early studies dating back to the 1920s have emphasised the dependency of ecosystems succession and community survival on energy related controls (Kemp and Boynton, 2004). The supply of energy and related inputs has to be at optimal levels, since excessive supply of nutrients is linked to eutrophication. Previous work by Struijs *et al*. (2011) has indicated the dependency of macroinvertebrates on nutrients stocks (*e.g.* phosphorus) where extremely low nutrients were associated with major reduction in the abundance of aquatic macroinvertebrates.

Hence, the structure and function of stream ecosystems are regulated by a number of interrelated drivers as opposed just one factor (*i.e*. water quality). The potential effects on or contribution of extraneous variables therefore cannot be discounted. Monitoring tools and approaches used in the assessment and evaluation of water for the suitability of aquatic ecosystems must be communicated with full cognizance of the potential contribution of variables not included in the assessments.

2.2 RIVER BIOMONITORING TOOLS EMPLOYING ANIMAL BIOINDICATORS OF WATER QUALITY AND ECOSYSTEM HEALTH IN SOUTH AFRICA

Aquatic biomonitoring refers to the techniques that utilise one or more components of the biota such as fish, macroinvertebrates, diatoms and others, to provide a time and constituent-integrated assessment of an aquatic system (Dallas, 2000). In South Africa biomonitoring has successfully proliferated into the monitoring and assessment of water quality and river health as part of national monitoring programmes (*i.e.* RHP or REMP), environmental consulting and for scientific research purposes. This has resulted in the development and refinement of river biomonitoring tools like SASS5 (Dickens and Graham, 2002), Fish Assemblage Integrity Index (FAII) (Kleynhans, 1999), MIRAI (Thirion, 2007; Thirion; 2016) and the ATI (for fish of the Olifants River) (Wepener *et al*., 1992).

With particular reference to the use of animal bioindicators (*i.e.* fish and macroinvertebrates), macroinvertebrates are recognised as the most valuable organisms for bioassessments (Dickens and Graham, 2002). Day (2000); Dickens and Graham (2002); USEPA (2012) state that macroinvertebrates are valuable bioindicators of water quality and ecosystem health for the following reasons:

- They are affected by the physical, chemical and biological conditions of a river.
- They have limited mobility (relatively sedentary). They cannot escape pollution and therefore show the effects of short-term and long-term pollution events.
- They may show the cumulative impacts of exposure to pollutants.
- They may show the impacts from habitat loss not detected by traditional water quality assessments (*i.e.* measures of the magnitudes of physico-chemical attributes in the river water).
- They are present in large numbers and they are fairly biodiverse.
- They have relative sensitivities to pollution; while some are very tolerant, others are very sensitive to pollution and other aspects of water quality.
- They are relatively easy to sample and identify.

The use of riverine macroinvertebrates for monitoring and assessing river health in South Africa dates back to the Empirical Biological Index (EBI) (Chutter, 1972). This is an index based on species diversity and abundance of aquatic macroinvertebrates. However, water resources managers did not accept the EBI because conducting it was time consuming and it required specialised invertebrate taxonomic knowledge (Barber-James and Pereira-da-Conceicoa, 2016). Consequently, in the 1990s, the SASS (Chutter, 1998) replaced the EBI. The initial SASS underwent several refinements resulting in the current version (SASS5) (Dickens and Graham, 2002). The SASS5 protocol is a standardised procedure for sampling, identification, enumeration and interpretation of the state of a river using riverine macroinvertebrates. The SASS is a rapid macroinvertebrate biomonitoring approach originally developed to monitor organic pollution (Chutter, 1994; Gordon *et al*., 2015).

2.2.1 Riverine macroinvertebrate sampling, identification, enumeration and interpretation of the state of a river using the SASS protocol

Sampling: Riverine macroinvertebrates are sampled using procedures that can be described as multi-biotope (biological habitat types) based and time and area dependent sampling procedure. The collection of the invertebrates is said to be multi–biotope based because invertebrates are sampled from different biotopes covering a wide area. There are three major biotopes considered in SASS: (i) the stone biotope, which constitutes bedrock or any hard surface in or out of current; (ii) The vegetation biotope, which constitute both marginal and aquatic vegetation; and (iii) the gravel, sand and mud biotope. The SASS sampling method can be described as time and space dependent because the time spent sampling from a biotope is specified (*e.g.* the kicking of stone biotopes should be approximated two minutes) and to some extent even the area covered (*e.g.* approximately two metres of vegetation must be sampled). Biotope diversity remains a major focus for the SASS particularly because, it has previously been found to be extremely sensitive to biotope diversity (Chutter, 1995).

Sampling net: invertebrates sampling is conducted using a standardised hand net (size 1000 µm soft mesh net on a 30 cm square frame, with a 135 cm aluminium handle) or

simply a kick-sweep sampling net, utilised through a timed kick sampling method. Everall *et al*. (2017) describes timed kick methods as semi quantitative sampling methods that historically most statutory biomonitoring has relied on.

Identification: The identification of the invertebrates specimen (mostly to family level) is also time dependent; the SASS5 protocol stipulates a 15-minute maximum identification time constraint (Dickens and Graham, 2002).

Enumeration and Interpretation: all identified invertebrate taxa are recorded on a standard SASS5 sheet and awarded quality scores (ranging from 1 to 15). A sensitivity score of 1 is awarded to the most tolerant taxa and 15 to the most sensitive taxa. The different quality scores are awarded on the understanding that the sensitivity/tolerance of invertebrates to pollutants differ from one to the other. This is a common principle underpinning biotic indices *e.g.* Trophic diatom index (Kelly, 1998) Index of trophic completeness for benthic macroinvertebrates (Pavluk *et al*., 2000), diagnostic biotic index for assessing acidity in sensitive streams (Murphy *et al*., 2013), Dragonfly biotic index (Samways and Simaika, 2016). The SASS5 is interpreted from three metrics, SASS score, number of taxa and average score per taxon (ASPT).

- SASS score: sum of the quality/rating scores for the sampled taxa
- Number of taxa : number of macroinvertebrates families sampled and
- ASPT: SASS5 scores divided by the number of taxa.

2.2.2 Macroinvertebrate Response Assessment Index

The Macroinvertebrate Response Assessment Index (MIRAI) was developed as part of a suite of EcoStatus indices (Geomorphological driver assessment index, Physico-chemical driver assessment index, Fish response assessment index, Riparian vegetation response assessment index and Index of habitat integrity) to be used in the Ecological Classification Process (Thirion, 2007; Kleynhans, 2008). The principle underpinning the MIRAI is that biological responses (riverine macroinvertebrates) integrate the effect of the modification of the drivers (hydrology, geomorphology and physico-chemical conditions) (Kleynhans and Louw, 2007; Thirion; 2016).

Employing SASS data, the MIRAI is used in the provision of a habitat-based cause-and-effect foundation to interpret the deviation of the macroinvertebrate community structure from the reference state (Thirion, 2007; Thirion, 2016). Thus, the MIRAI measures the degree of change from natural conditions on a six-point scale, where 0 represents no change and 5 represents maximum change. The MIRAI is calculated from four metrics: flow modification, habitat modification, water quality modification and system connectivity and seasonality. These four metrics measure the degree of change (modification) of observed macroinvertebrate assemblage from natural or reference assemblage. The four metric groups are combined to derive the macroinvertebrate Ecological Category (EC). The MIRAI ECs interpreted on a scale of A-F, where A represents natural conditions and F represents extreme modifications.

In South Africa, most of the biotic indices that use animals (animal component of the aquatic ecostsem) as biological indicators of water quality and ecosystem health are determined from community assemblages by awarding sensitivity scores to sampled taxa and subjecting the sensitivity scores to some mathematical manipulations (Table 2.1). These tools are capable of describing the prevailing conditions of a river (*e.g.* the extent of water pollution) but are unable to identify and distinguish between types of pollutants (*e.g.* metals or nutrients or faecal). As a result, these tools have been described as red flag indicators of water quality and ecosystem health that may inform the need for further investigations of the sources of pollution using other techniques like chemical investigations involving laboratory analysis.

Table 2.1 South African River biomonitoring tools that uses animal biological indicators of water quality and ecosystem health

Tool	Purpose	Index determination basis	Developer
South African Scoring System	Based on the presence of riverine macroinvertebrates families, it reflects changes in water quality with implications for ecosystem health and integrity.	Calculated from riverine macroinvertebrates assemblage	Dickens and Graham, 2002
Macroinvertebrate Response Assessment Index	Measures the modification of macroinvertebrate assemblage from natural or reference assemblage.	Calculated from differences from observed macroinvertebrate assemblage from reference assemblage	Thirion, 2007; Thirion 2016
Fish Assemblage Integrity Index	Developed for assessing the integrity of fish community relative to conditions expected in the absence of human impacts.	Calculated from fish community assemblage by awarding sensitivity scores	Kleynhans, 1999
ATI (fish)	Developed to aid in the routine monitoring of rivers passing through the Kruger National Park.	Calculated from the magnitudes of physical attributes and the concentrations of chemical substances in water in relation with suitability to support fishes of the Olifants River system	Wepener *et al*., 1992

On the contrary, the ATI (for fish) (Wepener *et al*., 1992), is based on a different approach from the other indices (Table 2.1). The ATI for fish describes the suitability of the river water for fishes of the Olifants system by rating physico-chemical (based on magnitudes) constituents of the river water in relation to its suitability to sustain fish (adult *Oreochromis mossambicus* and adult *Clarius gariepinus*).

2.3 DEVELOPMENT OF A WATER QUALITY INDEX

Water quality indices are developed following processes that can best be described as stepwise and continuous. The process can be said to be stepwise because it follows clear and distinct steps; and continuous mainly because to some extent it requires feedback and further calibration after field validation and sensitivity analysis (Mendoza-Salgado *et al*., 2009).

The key steps followed in the development of a water quality index are:

(I) Conception of the water quality index
(II) Selection of water quality variables
(III) Rating and transformation of water quality variables into sub-indices
(IV) Aggregation of sub-indices

2.3.1 Conception of a water quality index

This stage forms the foundation of a water quality index. It is at this stage that a developer responds to the need for an index. Water quality indices by design address specified aspects of the aquatic environment (*i.e.* entire aquatic ecosystems without attention to specified aquatic populations, specific populations (*e.g.* fishes, invertebrates), specific aquatic ecosystem (*e.g*. surface water, groundwater, and estuaries) and specific land-uses or mere targeted pollutants (*e.g.* pesticides because of agricultural activities). This is an important phase of an index development since it defines the scope of the application of the index and partly the subsequent stages of index development (*e.g*. selection of water quality variables (discussed in Section 2.8), rating of water quality variables, and aggregation of sub-indices, index validation and sensitivity analysis).

2.3.1.1 Scope of application: specified and unspecified user

Some indices are general in nature; they respond to no specified water quality requirement of a specified end-user. An example is the United States National Sanitation Foundation Water Quality Index (Ott, 1978). Other indices are somewhat specific. Examples are water quality indices designed for the protection of aquatic life with reference to the water quality requirements for the entire aquatic ecosystem (*e.g.* British Columbia Water Quality Index (Rocchini and Swain, 1995) and the Canadian Council of Ministers of the Environment Water Quality Index (CCME, 2001). Yet further indices are even more specific, for instance those that are designed for the protection of specific population (s) within an ecosystem.

2.3.1.2 Scope of application to specified aquatic ecosystem

Aquatic ecosystems differ from one another; so do their monitoring tools. Some water quality indices are developed for the assessment of specific aquatic ecosystems. Typical examples include the Groundwater Quality Index (Kumari and Rani, 2014), the Estuarine Water Quality Index (Wepener *et al*., 2006), the water quality index for Coastal Zone and Application in the Ha Long Bay (Nguyen *et al*., 2014).

2.3.1.3 Scope of application with reference to specified pollutants

Based on scope, some indices are developed to address a specified group of pollutants. Examples of such indices include the Aquatic Pesticide Toxicity Index for use in Alberta (Anderson, 2008). The water quality variables for this particular index were limited to pesticides known to be generated within the Alberta aquatic systems. The United States Geological Survey's National Water-Quality Assessment (NAWQA) Pesticide Toxicity Index (PTI) assesses the exposure of aquatic organisms to specific pesticides (Munn *et al*., 2015). All water quality evaluations from such indices are skewed towards the specified types of pollutant; because of their narrow focus, they can never give a holistic picture of the state of the water quality of a site.

2.3.3 Rating and transformation of water quality variables

Water quality variables selected for inclusion in a water quality index are measured in varying scales or expressed in different units. For example, physical water quality variables (*e.g.* dissolved oxygen as % saturation or content as mg/L, water temperature in degrees Celsius) differ from chemical variables (*e.g.* metals). To enable aggregation of the different variables into a composite index, the variables have to be transformed into dimensionless sub-indices (Nguyen *et al*., 2013; Sharma and Reddy, 2013). This could be a range of numbers, most commonly between zero and 100, where 100 could represent the best score for a variable (Chaiprasert and Tripetchkul, 2009; Nguyen *et al*., 2013; Shah and Joshi, 2015). Whilst the transformation of variables into dimensionless sub-indices is a common step in the development of water quality indices, some indices are aggregated from actual variable values without any transformations and sub-indexing

(*e.g.* Said *et al*., 2004; Schiff and Benoit, 2007). The transformation step is sometimes followed by the assignment of weights indicating the relative importance of individual index variables (Stoner, 1978; Poonam *et al*., 2015). Some indices, however, do not assign weights to variables *e.g.* the Prati Index (Prati, 1971), and the ATI (Wepener *et al*., 1992). An alternative approach for rating water quality variables is the use of statistical interpolation methods (*i.e.* SSD models) for example Wepener *et al*. (2006).

2.3.4 Aggregation of sub-indices

The last development step is the aggregation of individual sub-indices into a composite or cumulative index score using appropriate mathematical functions. Numerous aggregation functions have been derived and used in developing the previous indices. Some prominent examples include the weighted and un-weighted Solway functions (House and Ellis, 1980; Couillard and Lefebvre, 1985; Wepener *et al*., 1992), geometric functions, weighted and un-weighted arithmetic functions (Landwehr and Deininger, 1976; House and Ellis, 1980; Couillard and Lefebvre, 1985) and harmonic mean function (Cude, 2001). Care is normally advised in selecting an appropriate aggregation function since these are associated with several limitations, like eclipsing, ambiguity and rigidity (Nguyen *et al*., 2001; Abbasi and Abbasi, 2012; Nguyen *et al*., 2014). Eclipsing occurs when the index rating of a site appears to be good, yet is hiding an unacceptable level of a variable. Ambiguity occurs when the index value is too high to the extent of crossing a critical value, yet the individual scores do not (Couillard and Lefebvre, 1985; Singh *et al*., 2008; Abbasi and Abbasi, 2012). Rigidity on the other hand refers to the inability to either add to or remove water quality variables from an index even when need arises (Singh *et al.*, 2008; Abbasi and Abbasi, 2012). A typical example of a rigid aggregation function is the logarithmic proportion used by Said *et al*. (2004) for aggregating five water quality variables for the index they developed for Idaho.

2.4 INDEX VALIDATION

The major concern with the development of water quality-monitoring tools is whether they are able to monitor what they were intended for without being under or over protective. Tools falling on either of the extremes are associated with both financial and

environmental costs. Overprotection provides thresholds that will safeguard the environment while imposing a heavy financial burden on riparian economic activities (Latawiec *et al*., 2011; Whitacre *et al*., 2012). These emanate from costs incurred by overtreatment of wastes to meet stringent wastewater standards. Under-protective criteria, on the other hand, suffer from the inability to identify degraded systems. This is also a general and major concern with tools developed using laboratory based assessments. Thus, caution should be taken with the use of ecotoxicological data generated from laboratory without any field-based assessments since they risk being either under- or over-protective (Merrington *et al*., 2014). It is therefore necessary that set procedures for developing such tools are rigorously adhered to and post-development assessments of the tool conducted.

Based on literature it may be inferred that post-development assessment and evaluation of water quality-monitoring tools is common amongst developers. Developers conduct either index validations, sensitivity analysis or both. Validation of a water quality index refers to a process intended to assess whether the index functions properly and whether it is able to produce reasonable results under specified conditions. This may be conducted through comparing the index's predictions against real water quality data (Rickwood and Carr, 2007).

2.4.1 Index validation process

The validation process of water quality indices differs from one study or from one developer to another. Reviewed literature indicates that most developers validate their tools using data collected from any ecosystem of interest covering reference and impaired sites. Through this approach, the validation could be achieved by comparing index evaluations of less impacted (reference) sites with sites that are known to be degraded. This approach suggests that the major interest of the developer is whether the index is able to discriminate between the levels of degradation or not for example the Multimetric Benthic Macroinvertebrate Index (Jun *et al*., 2012).

Some indices may be evaluated using expert surveys. A practical example of this approach was the validation of the Fuzzy Water Quality Index (Bai *et al*., 2009). The use

of experts is mainly for comparing the extent to which the index results differ from expert surveys based on the evaluations of the field data. For some cases, existing indices could be used for reference evaluation. In such a case, the developers' interest would be in the extent to which the developed index agrees with existing indices (*e.g.* Mohebbi *et al*., 2013; Bai *et al*., 2009).

2.4.2 Flows and dilution effect

Apart from validating the index for the target population(s), it is equally important to assess the possible effect other variables (extraneous variables) may pose on what the index measures and population of concern. Some developers take into consideration the possible effects of seasonality and flows, a set of variables that would possibly interfere with the assumed relationship between water chemistry and ecosystem integrity. As part of the validation of the Water Quality Index for Biodiversity (WQIB) Carr and Rickwood, (2008) investigated the influence of stream flows on the index evaluations. In the case of the validation of the Water Quality Index of the Godavary River in India, particular attention was paid on the dilution effects of flows on the pollutants (Darapu *et al*., 2011). Numerous other factors known to affect water quality could be incorporated in the validation process as a means of gaining deeper understanding of the tool.

2.5 SENSITIVITY ANALYSIS

Index evaluation of water quality of a site for intended uses is largely dependent on the selected variables. Upon development, it is therefore necessary to conduct a sensitivity analysis of the tool. With particular reference to water quality indices, sensitivity analysis is conducted for the assessment of the significance of each water quality variable in the determination of the composite index (Lee, 2006; Zali *et al*., 2011; Derakhshan *et al*., 2015; Sakizadeh, 2015; Scheili *et al*., 2015). It is an important undertaking that enables the developer to receive direct feedback on the appropriateness of each input water quality variable and its relative importance to the composite water quality index (Scheili *et al*., 2015).

Different methods have been developed for conducting sensitivity analyses of indices. For example, Hamby (1994) classified these methods into three groups. These were: (i) those that operate on one variable at a time, (ii) those that rely on the generation of an input matrix and an associated output vector and (iii) those that require a partitioning of a particular input vector based on the resulting outut vector. The procedures required by each method differ. Most publications on sensitivity analysis of water quality indices are those that pay attention to one water quality variable at a time with the relative importance of each vatiable on the composite index informed by statistical analysis (*e.g.* Nasir *et al*., 2011; Zaliu *et al*., 2011; Gazzaz *et al*., 2012; Chang and Liao, 2012; Scheili *et al*., 2015). Most of the recent publications on sensitivity analysis utilise a method known as the '*leave-one-out approach'*. This approach is either conducted alongside correlation analysis (*e.g.* Rickwood and Carr, 2009; Scheili *et al*., 2015) or as part of the Artificial Neural Network (ANN) approach (*e.g*. Zali *et al*., 2011; Gazzaz *et al*., 2012; Azid *et al*., 2016). The Leave-one-out approach is conducted by the removal of each selected variable one by one, recalculation of the index (after the removal of each variable) followed by the statistical comparisons of the index scores resulting from the removal of each variable with the composite index scores (containing all variables).

2.6 WATER QUALITY STRESSORS

2.6.1 Water quality criteria

Water quality criteria are threshold limits for pollutants or other hazard factors in the ambient water environment derived from scientific experiments (*i.e*. bioassays) and extrapolations (*e.g*. assessment factor and SSDs) (Feng *et al*., 2012). Water quality criteria can be classified into generic and site-specific criteria. Generic water quality criteria (also referred to 'one size fits all') are broad scale tools developed to define protection thresholds for aquatic organisms against environmental stressors with no regard for ambient physico-chemical water quality characteristics that may affect or confound the target stressor response relationship (van Dam *et al*., 2014). To counter the limitations of generic water quality criteria water quality jurisdictions have shifted into deriving site-specific water quality criteria (van Dam *et al*., 2014). Site-specific water quality criteria are derived to incorporate differences in water quality characteristics

between sites. As a result, they follow rigorous and complicated processes requiring extensive knowledge of the physical, chemical, and biological characteristics of the water body under consideration (CCME, 1999a). Over the years, water quality jurisdictions have derived water quality criteria mostly to reflect the toxicity modifying effect of water hardness (hardness-based criteria) and through the Biotic Ligand Model (BLM) (Smith *et al*., 2015).

2.6.2 Hardness based water quality criteria

The toxicity of a chemical to an aquatic organism requires the transfer of the chemical from the external environment (*e.g.* water column or sediments) to biochemical receptors on or in the organism (binding surfaces like gills or gill lamellae) at which the toxic effects are elicited (USEPA, 2007). The ability of a chemical to transfer from the external environment into the aquatic organism is dependent on the magnitudes and concentrations of a number of water quality characteristics for example pH, hardness, alkalinity, dissolved organic carbon, humic matter, ionic strength, sulfide, and thiosulfide ions (Davies *et al*., 1994; Rudel *et al*., 2015). Yet hardness remains the main and most well recognised of the modifying factors of metal ionic species (Casares *et al*., 2012). In most cases, water hardness is incorporated to serve as a general surrogate for pH, alkalinity, and ionic strength, because waters of higher hardness usually have higher pH, alkalinity, and ionic strength (USEPA, 2001). Increasing water hardness is understood to ameliorate the toxicity and bioavailability of toxicants to aquatic organisms (Ebrahimpour *et al*., 2010). In this case, hardness (calcium plus magnesium) is viewed as an important inhibiting factor in the transfer of a chemical from the water column to the organism. For instance, hardness reduces the transfer of a chemical from external environment to the receptor through competitive inhibition at binding surfaces (Welch and Lindell, 2002). Where, the non-toxic Ca^{2+} and Mg^{2+} ions compete with the toxic metals for binding sites thereby limiting the permeability of the metals ions (Hunn, 1985; Welch and Lindell, 2002). For example, if Ca^{2+} or Mg^{2+} ion occupy the binding sites, the gill lamellae are protected from being harmed by the toxic ions (Welch and Lindell, 2002). Consequently, most water quality jurisdictions present metal water criteria as hardness-dependent equations and

hardness specific criteria as opposed to single values (generic criteria) (*e.g*. ANZECC and ARMCANZ, 2000; USEPA, 2016).

Water quality jurisdictions incorporate water hardness into their metal criteria using different correction algorithms. The USEPA for example, utilises empirical hardness regressions where LC50 toxicity values at various hardness are normalized to a reference hardness using the regression slopes (USEPA, 2017). The normalized LC50s for each biological species are averaged to derive Species Mean Acute Values (SMAVs) at the reference hardness (USEPA, 2007).

2.6.3 Biotic Ligand Model

Unlike hardness-based water quality criteria (derived from empirical hardness regression models), the BLM explicitly accounts for individual water quality variables (up to ten different physico-chemical variables). Therefore, the BLM approach address ambient water quality variables that were not considered in the hardness algorithms (USEPA, 2003).

A ligand is a complexing chemical (ion, molecule, or molecular group) that interacts with a metal like copper to form a larger complex (USEPA, 2007), and a biotic ligand is defined as a specific receptor within an organism (*e.g.* chemical site on a fish gill) where metal complexation leads to acute or chronic toxicity (Santore *et al*., 2001; USEPA, 2007). The BLM is therefore defined as a predictive tool that allows metal-organism interactions to be taken into account when predicting critical levels of metal to organisms; it does so by incorporating site-specific physical and chemical conditions of the water into the prediction tool (Feng *et al*., 2012). The strength of the BLM concept is that it provides a focus for organising information on how ambient physico-chemical conditions of the water (multiple environmental factors) affect bioavailability and how toxicity results from this accumulation (Erickson, 2013). However, the practical application of BLM is limited by large data requirements, which aimed at improving the prediction of safe environmental concentrations of metals in water as a function of up to ten specific water quality variables (Slaveykova and Wilkinson, 2005). The USEPA BLM based water quality criteria for

copper incorporate alkalinity, dissolved organic carbon, pH, and the major anions (chloride and sulfate) and cations (calcium, magnesium, sodium and potassium) (2007).

2.7 REQUIRED DATA FOR THE DEVELOPMENT OF WATER QUALITY CRITERIA FOR THE PROTECTION OF FRESHWATER ECOSYSTEMS

Water quality criteria, standards and benchmarks for the protection of aquatic life can be developed for the protection of either the entire ecosystem or for specified / target organisms. Derivation of water quality criteria for the protection of the entire aquatic ecosystem requires toxicity data derived from a wide range of biotas consisting of fish, invertebrates, amphibians, algae and plants. Water quality criteria should therefore be diverse and represent a realistic aquatic biological community (Warne *et al*., 2015). In addition, Warne *et al*. (2015) suggest that the criteria should take into account the trophic structure of the ecosystem of interest and should consist of at least invertebrates, phototrophs and organisms associated with nutrient cycling and should contain taxa sensitive to the mode of action of the target stressor. While water quality jurisdictions generally emphasise that a representative sample for target ecosystems can be achieved by selecting from different taxonomic groups constituting a realistic aquatic ecosystem, the United States has additional considerations, where organisms of commercial and recreational importance are given preferential advantage (Table 2.2) (Stephan *et al*., 1985; USEPA, 2013; USEPA, 2016a).

Data requirements for deriving water quality criteria representative of entire aquatic ecosystem vary from one water quality jurisdiction to the other. Water quality criteria for the protection of freshwater life in the United States (Stephan *et al*., 1985; USEPA, 2013; USEPA, 2016a), South Africa (DWAF, 1996) and Canada (CCME, 2007) for instance recommended acceptable toxicity data representing at least one species of freshwater animal obtained from at least eight different families (Table 2.2). The European Union o the other hand is stricter because it requires that the output from an SSD-based quality standards would be considered reliable if the input data points contains preferably more

than 15 input data points obtained from different species covering at least 8 taxonomic groups (EU, 2011).

Meeting the recommended representation (a representative sample) for the development of water quality criteria is a major impediment for most developers and as a result DWAF (1996) states that national water quality criteria are or should only be derived if adequate and appropriate data are available to provide reasonable confidence in the threshold values (DWAF, 1996).

While the focus of most water jurisdictions (including Stephan *et al*., 1985; DWAF, 1996; USEPA, 2013; USEPA, 2016a) is the entire ecosystem, there are very limited hints on how to constitute a representative sample for deriving water quality criteria intended for the protection of specific groups of organisms (*e.g.* macroinvertebrates). Based on the selected water quality jurisdictions (Table 2.2), what appeared to constitute a representative sample for macroinvertebrates are four groups of macroinvertebrates made of crustaceans, insects, annelids and molluscs.

Table 2.2 Minimum data set requirements for the derivation freshwater water quality criteria from selected water quality jurisdictions

Taxonomic representation	United States (Stephan *et al*., 1985; USEPA, 2013; USEPA, 2016a)	South Africa (DWAF, 1996)	Canada (CCME, 2007)	European Union (EU, 2011)
Number of taxonomic groups	• At least one species of freshwater animal in at least eight different families.	• At least one species of freshwater animal in at least eight different families.	• At least eight species selected from to represent fish, amphibians, invertebrates and plants.	• At least ten species belonging to at least eight taxonomic groups
Vertebrates (fish and amphibian)	• The family Salmonidae in the class Osteichthyes, a family in the class Osteichthyes, preferably a commercially recreationally important warm water species (*e.g*., bluegill, channel catfish). • A third family in the phylum Chordata (may be in the class Osteichthyes or an amphibian).	• A representative of the cold-water fishes, *e.g*. from the family Salmonidae in the class Osteichthyes and any family of freshwater fishes in the class Osteichthyes to represent the warm water fishes (*e.g.* Cichlidae, Cyprinidae, Clariidae.	• Three species of fish including at least one salmonoid and one non-salmonoid. • Amphibian.	• Fish (*e.g.* species salmonids, minnows, bluegill, sunfish, channel catfish, *etc*.) • A second family in the phylum Chordata (*e.g*. fish, amphibian, *etc*.).
Invertebrates	• A planktonic crustacean (*e.g*., cladoceran, copepod, *etc*.) • A benthic crustacean (*e.g.*, ostracod, isopod, amphipod, crayfish, *etc*.). • An insect (*e.g*., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, *etc*.) • A family in a phylum other than Arthropoda or Chordata (*e.g*., Rotifera, Annelida, Mollusca*, etc*.)	• Planktonic crustaceans (*e.g*. cladoceran, copepod *etc*.). • Benthic crustaceans (*e.g*. ostracod, isopod, amphipod, crayfish). • A family in a phylum other than Arthropoda or Chordata (*e.g*. Rotifera, Annelida, Mollusca). • Insects (*e.g*. mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito and midge).	• Three aquatic or semi-aquatic invertebrates at least one of which must be a planktonic crustacean (desirably one must be a mayfly, caddisfly, or stonefly).	• A crustacean (*e.g*. cladoceran, copepod, ostracod, isopod amphipod, crayfish *etc*.); • An insect (*e.g*. mayfly, dragonfly, damselfly, stonefly, caddisfly, midge, *etc*.) • A family in a phylum other than Arthropoda or Chordata (*e.g*. Rotifera, Annelida, Mollusca, *etc*.)
Plants	• Limited focus on plants because their toxicity procedures are not well developed.	• Freshwater algae or vascular plants.	• At least one freshwater vascular plant or freshwater algae	• Algae and higher plants (*i.e*.vascular plants).

2.8 Selection of water quality variables

Surface water bodies contain hundreds (Abbasi and Abbasi, 2012) or even thousands (Day, 2000) of water quality constituents arising within watersheds. It is practically impossible and unnecessary to include all or most constituents associated with a catchment in a water quality index. This is more so because a water quality index becomes unwieldy if it contains many water quality variables (Sarkar and Abbasi, 2006; Abbasi and Abbasi, 2012). Preferably, a combination of water quality variables considered adequate to give an overall picture of the prevailing conditions of the water, benchmarked for the protection of target beneficial user(s), should be selected (Terrado *et al*., 2010). Previous reviews of 36-water quality indices by Fernandez *et al*. (2004) indicated that water quality indices can be computed based on as few as one water quality variable to as many as 47.

The selection of water quality variables for inclusion in water quality indices varies from one developer to the other. The selection of variables for the development of a water quality index is mainly dependent on four major approaches:

1. Previously, variables have been selected according to those that are most indicative of pollutants arising from catchment (s) for which the index is developed and those for which data is readily available (Dojlido and Best, 1993; Cude, 2001).
2. Water quality variables that have similar properties need not be considered for selection as input variables for a water quality index, to avoid redundancy (Dunnette, 1979; Sutadian *et al*., 2016).
3. Developers may select water quality variables based on commonly measured variables; for example, the water quality index developed by Horton (1965) is calculated from 10 commonly measured variables in the United States (Tyagi *et al*., 2013).
4. Delphi technique (Dalkey, 1963): the Delphi technique is a widely used and accepted method for gathering data from respondents within their domain of expertise (Hsu and Sandford, 2007). It does so by generating results from the convergence of experts (Shah and Joshi, 2017). The selection of water quality variables using this technique is premised on the understanding that a consensus

of experts provides a more accurate response to a question than a single expert' considerations (Crance, 1987).

5. The use of statistical methods like multivariate statistical techniques (*e.g.* principal component analysis) (Kocer and Sevegili, 2014) and a method widely referred as sensitivity analysis (Azid *et al*., 2016; Zali *et al*., 2011). This approach is premised on the understanding that the development of a water quality index is a continuous process: it must therefore be responsive and flexible to refinements. For example, Zali *et al*. (2011) had to exclude two initially justified and selected water quality variables (biological oxygen demand and chemical oxygen demand) after conducting a sensitivity analysis for the water quality index for use in the Kinta River, Malaysia. In this instance, the two variables were excluded because they were too costly and time consuming yet had minimum correlation for water quality index forecasting (Zali *et al*., 2011).

2.8.1 Cadmium

Cadmium largely occurs in the earth's crust at an estimated concentration of 0.1 mg/Kg (Tchounwou *et al*., 2012). In aquatic environments, cadmium occurs in two oxidative states: (i) the metallic state, a state that is insoluble and rarely occurs in natural waters; and (ii) several salts of divalent salts that are soluble in water (USEPA, 2016a). The ability of cadmium to bioaccumulate in aquatic ecosystems is dependent on various ambient water quality conditions and variables *e.g.* water hardness, temperature, pH and the presence of complexing agents (Reichenbach, 1993). Of the water quality variables that determine the bioaccumulation of cadmium in aquatic organisms, hardness is the only toxicity modifier that most water jurisdictions account for (Table 2.3).

Table 2.3 Acute water quality criteria and guidelines for the protection of freshwater ecosystems for cadmium in South Africa, Canada, Australia and New Zealand and the United States

Locality	WQC Specifications	Numeric criteria	Bioassay toxicants used	Reference
South Africa	Water Quality criteria for total cadmium adjusted to reflect toxicity at different hardness levels	<3 µg/L at H <60 <6 µg/L at H 60-119 <10 µg/L at H 120-180 <13 µg/L at H >180	Not reported	DWAF 1996
Canada	Hardness	<0.11 µg/L at H<60 <1.2 µg/L at H≤60 <2.5 µg/L at H≤120 < 3.8 µg/L at H≤180 <7.7 µg/L at H≥360	Cadmium chloride, cadmium nitrate and cadmium sulfate	CCME 2014
Australia and New Zealand	Hardness-adjusted criteria for dissolved fraction of cadmium, indicating four Hazardous Concentrations	PC99: <0.06 µg/L PC95: <0.2 µg/L PC90: <0.4 µg/L PC80: <0.8 µg/L All levels of protection (PCs) adjusted to H = 30	Not reported	ANZECC and ARMCANZ 2000
United States	Water quality criteria for dissolved cadmium, with cadmium correction to H = 100	<1.8 µg/L adjusted to H= 100	Cadmium chloride, cadmium nitrate and cadmium sulfate	USEPA 2016

ANZECC and ARMANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term exposures (chronic)

H: Water hardness mg/L (as $CaCO_3$)

The water quality criteria for cadmium in most jurisdictions are presented as dissolved concentrations. With a few exceptions – for example the South African water quality guidelines of 1996 – cadmium is presented as total cadmium concentrations (sum of the dissolved and particulate). The South African Department of Water Affairs and Sanitation (DWAF, 1996) preferred the total concentrations as opposed to the dissolved fraction, owing to the relative toxicity of cadmium to freshwater life. This approach was influenced by a principle referred to as the precautionary approach, where the most toxic metals are presented as totals rather than dissolved fractions. From the selected national and international water quality criteria, the aquatic toxicity of cadmium is presented to reflect site-specific water quality conditions. The site-specific conditions in all the considered cases are represented by water hardness as a surrogate variable for the other water quality variables that could potentially affect the toxicity of cadmium to freshwater life.

2.8.2 Chromium

Chromium occurs naturally in the earth's crust (Tchounwou *et al*., 2012). It is the seventeenth most abundant element found in the mantle (Mandina and Magandza, 2013) and among the ten most abundant compounds in the earth's crust (Jacobs and Testa, 2005). In the environment, chromium predominantly occurs in two forms: as trivalent chromium [Cr (III)]; and hexavalent chromium [Cr (VI)] (Byrne *et al*., 2016). The relative quantities of each form of chromium in water exist in an equilibrium, which is predominantly determined by pH and redox potential. For instance at high pH (pH>7) and high redox potential values Cr (VI) remains dominant, whilst Cr (III) occurs predominantly in (pH ≤ 7) (DWAF, 1996; Swietlik, 1998; Henrie *et al.,* 2004). Chromium (III) naturally occurs in the environment, whilst the presence of chromium (VI) in the aquatic environment is mainly attributed to human activities for example the discharge of contaminated effluents (Agency for Toxic Substances and Disease Registry, 1998; Oze *et al*., 2007; Zhitkovich, 2011).

Trivalent chromium is an essential nutrient required by animals for metabolism processes, though only in minute quantities (Richard and Bourg, 1991; Swietlik, 1998). Hexavalent chromium on the other hand is considered highly toxic. The differences in toxicity of the two forms of chromium have been observed to be approximately between 10-100 folds in most cases (Hawley *et al*., 2005). Because of the huge differences in the relative toxicity of these two forms, most water quality jurisdictions do not lump them in total dissolved chromium concentrations, but rather treat them independently by recommending different water quality criteria for each chromium species (Table 2.4).

The toxicity of the two forms of chromium has been reported to be dependent on the ambient water chemistry for example water hardness and pH. The effects of the two toxicity modifiers is limited by empirical evidence and data (DWAF, 1996). Nonetheless, the existing water quality criteria for Cr (III) are hardness-adjusted, whilst the criteria for Cr (VI) exist as default without adjustments for any toxicity modifier (USEPA, 1984; USEPA, 1995; DWAF, 1996; CCME, 1999).

Table 2.4 Acute Water quality criteria and guidelines for the protection of freshwater ecosystems for Chromium (III) and chromium (VI) in South Africa, Canada, Australia and New Zealand and the United States

Locality	Trivalent Chromium [Cr(III)] numeric criteria	Toxicants: Cr (III)	Hexavalent [Cr(VI)] numeric criteria	Toxicants: Cr (VI)	Reference
South Africa	Water quality guideline for Cr (III) without correction for hardness dependency. DWAF (1996) recommended a maximum acute value of <340 µg/L for the protection of freshwater ecosystems against acute effects.	-	Water quality criteria for Cr (VI) presented without any adjustments for hardness dependency. For the protection of freshwater ecosystems against acute effects, a maximum value of <200 µg/L is recommended.	Not reported	DWAF, 1996
Canada	Freshwater quality guideline for Cr (III) presented without correction for water hardness dependency. CCME recommends a maximum of <8.9 µg/L for the protection of ecosystems against acute effects	Not reported	Freshwater quality guideline for Cr (VI) presented without correction for water hardness dependency. CCME recommends a maximum of <1 µg/L for the protection of ecosystems against acute effects	Not reported	CCME, 1999
Australia and New Zealand	No recommended criteria for Cr(III)	-	Water quality criteria recommended for four levels of protection for freshwater life.PC99:<0.01 µg/L PC95:1µg/L, PC90: <6 µg/L and PC80: <40 µg/L	Not reported	ANZECC and ARMCANZ, 2000
United States	Water quality criteria for dissolved Cr (III) corrected to specified water hardness. USEPA recommended <570 µg/L at H = 100 for the protection of freshwater ecosystems against acute effects.	chromium chloride, chromic nitrate, Chromic potassium sulfate	The USEPA water quality criteria for dissolved Cr (VI), is presented without any adjustments for hardness dependency. A default value of <16 µg/L is recommended for the protection of freshwater life against acute effects.	Potassium dichromate, sodium dichromate	USEPA,1984; USEPA ,1995

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures H: stands for hardness mg/L (as $CaCO_3$)

2.8.3 Copper

Copper (Cu) is one of the most abundant trace elements found in the earth's crust and surface waters. Beside natural occurrence of copper in in rocks, mining and discharges of copper-rich effluents by industries (*e.g.* electrical equipment, fabricated metal products, leather and leather producing) have been documented as the major sources of copper in aquatic environments (Patterson *et al*., 1998). Because of its abundance and toxicity, copper is treated as a priority pollutant in South Africa and the United States, for instance (DWAF, 1996; Suedel *et al*., 1996). Most water jurisdictions (national and international) have site-specific criteria for copper, derived to reflect the empirical relationships between copper toxicity and water hardness in the site (s) of interest (Table 2.5). The sole dependency on water hardness to explain the influence of the toxicity of copper on freshwater ecosystems is gradually diminishing, given the latest developments on BLM (*e.g*. USEPA, 2007).

The USEPA criteria for copper are more progressive, since over the past ten years they transitioned from being hardness-dependent into BLM derived. The BLM approach inputs up to ten ambient water variables *i.e.* water temperature, pH, dissolved organic carbon, calcium, magnesium, sodium, potassium, sulfate, alkalinity and sulphide alkalinity, and sulphide. Based on the 'best available science', the BLM is regarded as the best attempt to account for modifying factors in developing site-specific water criteria. The latest published water quality guidelines for copper by ANZECC replaced water hardness as a modifier for copper toxicity with DOC (ANZECC, 2017). The substitution of water hardness by alternative modifiers actually discredits previous studies (Meyer *et al*., 1999), which, based on laboratory experiments, affirmed the ameliorating effects of water hardness on the bioavalability of copper to aquatic animals.

Table 2.5 Acute water quality criteria for freshwater ecosystems for copper in South Africa, Canada, Australia and New Zealand and the United States

Locality	WQC specifications	Numeric criteria	Bioassay toxicants used	Reference
South Africa	Water quality criteria for dissolved copper, adjusted to different water hardness levels	<1.6 µg/L at H: <60 <4.6 µg/L at H: 60-119 <7.5 µg/L at H: 120-180 <12 µg/L at H: >180	Not reported	DWAF, 1996
Canada	Hardness-adjusted criteria for copper, representing three water hardness levels	<2 µg/L at H: 0-120 <3 µg/L at H: 120-180 <4 µg/L at H >180	Not reported	CCME, 2007
Australia and New Zealand	Hardness-adjusted criteria for dissolved fraction of copper, indicating four Hazardous Concentrations (PC99, PC95, PC90 and PC80) at specified water hardness level.	PC99:<1 µg/L PC95:<1.4 µg/L PC90:<1.8 µg/L PC80:<80 µg/L All levels of protection (PCs) adjusted to H = 30	Not reported	ANZECC and ARMCANZ, , 2000
Australia and New Zealand	Water quality criteria dependent on DOC	<1.2µg/L at a DOC of 0.5 mg/L		ANZECC, 2017
United States	Site-specific water quality for copper expressed based on the Biological Ligand Model (current guidelines). Previous guidelines were also site-specific derived to reflect the toxicity of copper with dependency on water hardness	<2.337 µg/L based on normalising chemistry (associated with the BLM variable inputs) The USEPA 1995/1996 guidelines recommended <7.285 µg/L at H = 50	Copper sulfate, copper nitrate and copper chloride	USEPA, 2007; USEPA 1996

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures
H: stands for hardness mg/L (as $CaCO_3$)

2.8.4 Lead

Lead is a non-essential metal; it is stable and persistent in the environment (Mager *et al*., 2010; Esbaugh *et al*., 2011; Richmond and Hu, 2013). Lead is toxic to aquatic organisms even in minute concentrations (Nys *et al*., 2014). Sources of lead in the aquatic environments are both natural sources and numerous human activities, including lead contaminated industrial discharges, point or non-point lead influxes from lead acid battery manufacturing plants (Tchounwou *et al*., 2012). The toxicity of lead to aquatic organisms is determined by several water chemistry attributes, such as calcium, carbonate, alkalinity, pH and DOC (Esbaugh *et al*., 2011). Yet most criteria account only for the

dependency of lead toxicity on the modifying effects of water hardness (Table 2.6). Evidence gathered by Esbaugh *et al*. (2012) from various studies suggests that the calcium component of water hardness is the one that is responsible for ameliorating the toxicity of lead on aquatic organisms and not the magnesium component. Based on these observations therefore, water hardness is a partial modifier.

Table 2.6 Acute water quality criteria and guidelines for the protection of freshwater ecosystems for lead in South Africa, Canada, United States and Australia and New Zealand and British Columbia

Locality	Criteria details	Numeric criteria	Bioassay toxicants used	Reference
South Africa	Water quality criteria for dissolved lead, adjusted to different water hardness levels	<4 µg/L, H:<60 <7 µg/L, H: 60-119 <13 µg/L, H: 120-180 <16µg/L, H: >180	Not reported	DWAF, 1996
Canada (national)	Water criteria corrected to reflect different water hardness ranges.	<1 µg/L, H: 0-60 <2 µg/L, H: 60-120 <4 µg/L, H: 120-180 <7 µg/L, H: >180	-	CCME, 2007
Australia and New Zealand	Hardness-adjusted criteria for dissolved fraction of lead, indicating four Hazardous Concentrations (PC99, PC95, PC90 and PC80).	PC 99:<1 µg/L PC95:<3.4 µg/L PC90:<5.6 µg/L PC80:<9.4 µg/L All levels of protection (PCs) adjusted to hardness 30	Not reported	ANZECC and ARMCANZ, 2000
United States	Water hardness-adjusted water quality criteria for dissolved form of lead	<65 µg/L at H = 100 <67.54 µg/L at H = 50	Lead chloride, lead nitrate and lead acetate	USEPA, 1980; USEPA 1984
British Columbia (Canada-provincial)	Site-specific criteria derived to reflect the effects of water hardness on the toxicity of dissolved and total lead (Pb) on freshwater organisms.	<18 µg/L (dissolved) at H <30 <82 µg/L (dissolved) H = 100 <197 µg/L (dissolved) at H = 200, <330 µg/L (dissolved) at H= 100 <3 µg/L (total Pb) at H ≤ 8	Not reported	British Columbia Ministry of Environment, 1987.

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures H: stands for hardness mg/L (as $CaCO_3$)

Additional evidence from other studies supports the conclusion that the toxicity of lead is dependent on multiple modifiers as opposed to just water hardness. Previous studies conducted for aquatic organisms at different trophic levels all acknowledge the modifying effects of site-specific pH, water hardness and DOC on the bioavailability of lead (Schlekat *et al*., 2010; Nys *et al*., 2014). In light of these observations, it is necessary to

base water quality criteria for lead on BLM as opposed to the sole incorporation of the toxicity modifying effects of water hardness.

2.8.5 Mercury

Mercury rarely occurs in the earth's crust (Hazen *et al*., 2012). Its occurrence in natural waters therefore is largely attributed to human activities as opposed to geological sources (DWAF 1996; Driscoll *et al*., 2013). Some of the well-documented sources of mercury in freshwater systems are discharge of mercury contaminated industrial effluents, mercury contaminated tailing discharges and erosion from tailing dumps (Domagalski, 2001; Beltrans-Pedros *et al*., 2011; Williams *et al*., 2011) and deforestation of mercury contaminated soils. Exposure of organisms to mercury affects the exposed organisms; but, in addition, through bioaccumulation and bio-magnification, mercury gets transferred to higher trophic level organisms (Beltrans-Pedros *et al*., 2011). Freshwater quality criteria for mercury amongst water quality jurisdictions (Table 2.7) are presented to reflect either total mercury, inorganic mercury, methyl mercury, or all forms. Between the two forms of mercury, methyl mercury is considered more toxic (Williams *et al*., 2011) and it is the form of mercury that mostly accumulates in animal tissues (Domagalski, 2001).

Water quality criteria and guidelines from selected national and international water quality jurisdictions (Table 2.7), all recommend default criteria for both forms of mercury. There are no toxicity adjustment algorithms for ambient water quality not even water hardness, a commonly used surrogate. Water quality criteria for mercury could be recommended only as a default, partly because mercury assays with aquatic organisms show very small effect or dependency on water hardness (Rathore and Khangarot, 2002).

Table 2.7 Acute water quality criteria and guidelines for the protection of freshwater ecosystems for mercury in South Africa, Canada, United States and Australia and New Zealand

Locality	Inorganic mercury	Bioassay toxicants used for Mercury (II)	Methyl mercury	Bioassay toxicants used for Methyl mercury	Reference
South Africa	Water quality criteria for total mercury (assuming at least 10% methyl mercury) without any adjustments for ambient water quality for targeted rivers, reaches or ecoregions. A maximum of 1.7 µg/L is recommended for the protection of freshwater ecosystems against acute toxic effects.		Water quality criteria for total mercury (assuming at least 10% methyl mercury) without any adjustments for ambient water quality for targeted rivers, reaches or ecoregions. A maximum of 1.7 µg/L is recommended for the protection of freshwater ecosystems against acute toxic effects.	Not reported	DWAF, 1996
Canada	CCME recommends an interim guideline of 0.026 µg/L (applicable to long-term / chronic exposures)		Interim water quality guideline of <0.004 µg/L.		CCME 2003; CCME 2016)
Australia and New Zealand	Water quality criteria for dissolved fraction of mercury (inorganic) PC99:0.06 µg/L, PC95:0.6 µg/L, PC90:1.9 µg/L, PC80:5.4 µg/L		No recommended criteria		ANZECC and ARMCANZ, 2000
United States	No recommended criteria	Mercuric chloride and mercuric nitrate	Water quality criteria for expressed as default, not taking into consideration the potential influence of ambient water quality conditions of sites/ or region for which they could be applied. The USEPA recommends a maximum of 1.4 µg/L for the protection of freshwater ecosystems.	Methyl mercuric chloride	USEPA, 1984a; USEPA, 2017

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures

2.8.6 Nickel

Nickel is abundantly found in the earth's crust. Nickel is ranked the twenty-fourth most abundant element in the earth's crust (Chowdhury *et al*., 2008). Sources of nickel in aquatic systems are traced from both natural and human activities (Chowdhury *et al*., 2008; Poonkothai and Vijayavathi, 2012). At minute quantities in the environment, nickel is essential as a micronutrient; yet it is a toxicant at elevated concentrations (Poonkothai and Vijayavathi, 2012).

Table 2.8 Acute water quality criteria for the protection of freshwater ecosystems for nickel in Canada, Australia and New Zealand and United States

Locality	Criteria details	Numeric Criteria	Bioassay toxicants	Reference
Canada	Water criteria corrected to reflect different water hardness ranges.	<25 µg/L at H = 0-60 <65 µg/L at H = 60-120 <110 µg/L at H = 120-180 <150 µg/L at H>180	Not reported	CCREM 1987; CCME 2016
Australia and New Zealand	Hardness-adjusted criteria for dissolved fraction of nickel, indicating four PCs	PC99:<8 µg/L PC95:<11 µg/L PC90:<13 µg/L PC80:<17 µg/L PCs adjusted to H = 30	Not reported	ANZECC and ARMCANZ, 2000
United States	Water hardness-adjusted water quality criteria for dissolved form of Nickel.	<470 µg/L adjusted to H = 100	Nickel chloride, nickel sulfate and nickel nitrate	USEPA, 1995

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures

H: stands for hardness mg/L (as $CaCO_3$)

The toxicity of nickel to aquatic organisms has been widely documented to be hardness dependent (*e.g*. Rathore and Khangarot, 2002; Pourkhabbaz *et al*., 2011). In addition, most jurisdictions recommend site-specific water quality adjusted to site-specific water hardness (Table 2.8).

2.8.7 Zinc

Zinc is both an essential micronutrient to aquatic organisms, at low concentrations, and a toxicant, at elevated concentrations (Glover *et al*., 2003). It occurs naturally in rocks and can be released into the aquatic environment through weathering and erosion

processes (DWAF, 1996). The sources of zinc in the aquatic environment could therefore be attributed to both natural and human related sources like industrial discharges and acid mine drainage (Binkman and Johnson, 2011). Most current water quality criteria for zinc are site-specific reflecting the influence of site-specific water hardness on the toxicity/bioavailability of zinc to freshwater organisms; South Africa and Canada are the only exceptional cases (Table 2.9).

Table 2.9 Acute water quality criteria for the protection of freshwater criteria for zinc in South Africa, Canada, British Columbia, Australia and New Zealand and the United States

Locality	Criteria details	Numeric Criteria	Bioassay toxicants used	Reference
South Africa	Criteria for dissolved zinc, a default value without any adjustments for water hardness	<36 µg/L	Not reported	DWAF, 1996
Canada (national)	Criteria value not corrected to any specified water hardness range or value.	<30 µg/L	Not reported	CCREM, 1987; CCME, 2016 (Summary table)
British Columbia (Canada-provincial)	Total maximum allowable concentrations	<33 µg/L when H is less or equal to 90	Not reported	British Columbia, 1999
Australia and New Zealand	Hardness-adjusted criteria for dissolved fraction of zinc, indicating four Hazardous Concentrations (PC99, PC95, PC90 and PC80).	PC99: <2.4 µg/L PC95:<8.0 µg/L PC90:<15 µg/L PC80:<31 µg/L All levels of protection (PCs) adjusted to H = 30	Not reported	ANZECC and ARMCANZ, 2000
Australia and New Zealand	Water hardness and pH dependent criteria based on long-term toxicity data	PC95: 3 µg/L at H = 30 mg/L and pH = 8		ANZECC, 2017
United States	Criteria reflecting dissolved fraction of zinc with toxicity value corrected to specific hardness	66.6 µg/L adjusted to H = 50	zinc sulfate, zinc chloride and zinc nitrate	USEPA,1985; USEPA,1987

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposure H: stands for hardness mg/L (as $CaCO_3$)

In a quest to shift from site-specific water quality criteria based on water hardness into BLM-based zinc water quality criteria in the United States, the International Lead and Zinc Research Organization submitted proposals for the incorporation of BLM into zinc water quality criteria (International Lead and Zinc Research Organization, 2006). More so, because water quality criteria based on BLM would provide better representation of the bioavailability of zinc to aquatic organisms than do hardness-adjusted criteria. In addition to the attempts and proposals by the International Lead and Zinc Research Organization to the USEPA, major advancements have taken place in the European countries and have seen the development of BLMs for both copper and zinc (Peters *et al*., 2009) mainly at research phase.

2.8.8 Chloride

There is a growing concern over increasing concentration of salts in freshwater systems, a condition that is on the rise even in semi-arid regions (Williams, 1987). Salts elevation in freshwater bodies is indicative of both point and non-point sources of pollution (Kincaid and Findlay, 2009). Major sources of chloride in river water range from discharges from sewage treatment plants (Kincaid and Findlay, 2009), agricultural activities (Kefford *et al*., 2005) to geological sources (Mullaney *et al*., 2009).

Unlike other national water quality jurisdictions, the South African water quality guidelines for the protection of freshwater ecosystems (DWAF 1996) do not provide guideline values for chloride (Table 2.10). Suggestions on safe concentrations of chloride are given only by the total dissolved solids guideline. This was despite ever-increasing concerns over the alarming increase of salts in South African freshwater resources and the likely toxicity impacts these salts may have on stream organisms (*e.g.* Kefford *et al*., 2005). This necessitated various investigations directed towards the impacts of these salts on aquatic invertebrates (Palmer *et al*., 2004, Browne, 2005; Kefford *et al*., 2005).

Table 2.10 Acute water quality criteria for the protection of freshwater ecosystems for chloride in Canada, United States, British Columbia and US State Iowa

Locality	Criteria specifications	Numeric Criteria	Bioassay toxicants used	Reference
Canada	Water quality criteria presented as default value-not adjusted to any toxicity modifying variables of any site or region.	<640 mg/L	Sodium chloride, calcium chloride	CCME, 2011
United States (national)	Water quality criteria not adjusted to any toxicity modifying factor (s)	<860 mg/L	Sodium chloride, calcium chloride, potassium chloride, magnesium chloride	USEPA, 1988
British Columbia (Canada-provincial)	Water quality criteria not adjusted to any toxicity modifying factor (s)	<600 mg/L	Not reported	Nagpal *et al*., 2003
US State of Iowa	Water quality criteria not adjusted for hardness and/or sulfate. However, the Iowa Department of Natural Resources does provide options/equations for adjusting the acute criteria for hardness and/or sulfate	<574 mg/L	sodium chloride	Iowa DNR, 2009

The toxicity of chloride has been reported to be dependent on the ambient water quality. Previous work by Elphick *et al*. (2011) points out that the aquatic toxicity of chloride reduces with increasing water hardness. According with this approach is the basis of the water quality criteria for some provincial water quality jurisdictions for chloride *e.g*. Iowa and British Columbia (See Table 2.10). For example, Iowa (USA) and British Columbia (Canada) derived site-specific criteria by incorporating sulfate and water hardness into the chloride criteria for freshwater ecosystems. National jurisdictions are still dependent on default criteria (no adjustments for ambient water quality conditions) for chloride ion *i.e.* Canada and USA without factoring in the toxicity modifying effects of other water quality variables (Table 2.10).

2.8.9 Sulfate

Sulfate occurs naturally in the aquatic environment (Lamare and Singh, 2016). Sulfate stocks and fluxes in surface waters are attributed to both human related and natural sources. Some of the well-documented sources include industrial wastewater, agricultural runoff, streams draining mineralised areas (Elphick *et al*., 2011) and mining (acid mine drainage) (Chen *et al*., 2015).

Table 2.11 Acute water quality criteria for the protection of freshwater ecosystems for sulfate in South Africa, Canada, United States and Australia and New Zealand

Locality	Criteria specifications	Numeric Criteria	Bioassay toxicants used	Reference
British Columbia (Canada-provincial)	Water quality criteria corrected for water hardness	<128 mg/L at H: 0-30 <218 mg/L at H:31-75 <309 mg/L at H: 76-180 <429 mg/L at H: 181-250	Not indicated	Meays and Nordin, 2013
US State of Iowa	Water quality criteria corrected for water hardness and chloride	Lowest hardness and Chloride = 500 mg/L Highest hardness and Chloride = 2000 mg/L	Not indicated	Iowa DNR, 2009

Most national water quality jurisdictions' criteria and guidelines for the protection of freshwater ecosystems reviewed do not have guidelines for sulfate (*e.g.* South Africa, United States, Canada, Australia and New Zealand). Available criteria were for provincial jurisdictions, *i.e*. British Columbia (Canada) and the state of Iowa (USA) (Table 2.11). These two water provincial or state jurisdictions offer site-specific water quality criteria for sulfate. With respect to British Columbia, a hardness-adjusted guideline is available, whilst the state of Iowa has water hardness and chloride adjusted site-specific water quality criteria for sulfate. The hardness dependency of sulfate toxicity is well supported by empirical evidence pointing out that the aquatic toxicity of sulfate to aquatic organisms decreases with an increase in water hardness (Davies and Hall 2007; Elphick *et al*., 2011a).

2.8.10 Ammonia

Ammonia in the aquatic environment is present in two forms: as ionised (NH_4^+); and in unionised form (NH_3). The relative quantities of each of these forms in water exist in an equilibrium that is dependent on the pH, temperature of the water and salinity (Erickson 1985). The toxicity of ammonia is attributed largely to the unionized form as opposed to the ionized form (Smart, 1978). It is primarily for this reason that, in the past, most water jurisdictions expressed water quality benchmarks for unionised ammonia. Most recently, jurisdictions of water quality express ammonia criteria to reflect the contribution of both the ionised and unionised forms. This is referred to as Total Ammonia-Nitrogen (TAN) (Table 2.12). Expressing the toxicity of ammonia as TAN is actually premised on the reasoning that both forms of ammonia (unionized and ammonium ion) are toxic to aquatic organisms at different potencies (Erickson, 1985).

The derivation of water quality criteria for ammonia requires a series of calculations and conversions. These calculations involve the conversion of ammonia to TAN and adjustments to reference pH and water temperature (USEPA, 1999, 2009 and 2013).

Table 2.12 Acute water quality criteria for the protection of freshwater ecosystems in for Ammonia in South Africa, Canada, British Columbia, United States and Australia and New Zealand

Locality	Criteria specifications	Numeric Criteria	Bioassay toxicants used	Reference
South Africa	Water quality criteria for Un-ionized ammonia as NH_3-N.	<100 mg/L (Acute Effect Value) <7 mg/L (Target Water Quality Range)	Not reported	DWAF, 1996
Canada (national)	Flexible with conversions to other forms provided. Reference water temperature and pH specified.	<0.354 mg/L NH_3. Converted to 291.13 µg/L (Total Ammonia-Nitrogen)	Not reported	CCME, 2010
British Columbia (Canada-provincial)	Water quality criteria for total ammonia as NH3-N, water temperature and pH specified.	<19.2 mg TAN/L at Temp = 20 and pH = 7.0	Not reported	Meays, 2009
Australia and New Zealand	Water quality criteria for Total ammonia as NH3-N at pH 8. Indicating for levels of protection.	PC99: <320 µg/L PC95: <900 µg/L PC90: <1430 µg/L PC80: <2300 µg/L All PCs adjusted to pH = 8	Not reported	ANZECC and ARMCANZ, 2000
United States (national)	Short-term exposure to ammonia (one hour average) when salmonids are present.	5.6 mg (TAN)/l: at pH = 8 and temperature = 25°C 24mg (TAN)/L: at pH = 7 and temperature = 20°C	Ammonium chloride, ammonium hydroxide, ammonium sulfate, phosphoric acid diammonium salt, ammonia, ammonium bicarbonate, ammonium phosphate, nitric acid ammonium salt	USEPA, 1999
United States (national)	Water quality criteria TAN with pH and water temperature specified. Criteria reflect conditions when mussels are present and when mussels are absent. Mussels prioritised because they are present in most US rivers and are very sensitive to ammonia.	When mussels are present: 2.9 mg (TAN)/L When mussels are absent: 5.0 mg (TAN)/L Criteria standardised to pH: 8 and temperature: 25°C		USEPA, 2009
United States (national)	Short-term exposure to ammonia (one hour average) when salmonids are present.	2.6mg (TAN)/L: at pH = 8 and temperature = 25°C 17mg (TAN)/L for 1 hour average: at pH = 7 and temperature = 20°C		USEPA, 2013

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures

2.8.11 Nitrate

Sources of nitrate in rivers are numerous, ranging from point to non-point sources. Some of the well-documented sources of nitrate within river catchments include: septic systems, animal waste, fertilizer application, decaying organic matter, atmospheric deposition (Heaton, 1986; Mayer *et al*., 2002; Zeng and Wu, 2015) and sources from the use of nitrogen-containing blasting agents such as ammonium nitrate (Zaitsev *et al*., 2008). In comparison with other nitrogen compounds (*e.g.* ammonia and nitrite), nitrate is considered less toxic (Colt and Armstrong, 1981). Excessive nitrate concentrations in freshwater systems are associated with numerous effects. Some of the widely documented effects include nutrient enrichment and subsequent algal blooms, hypoxia and a general decline in water quality (Zeng and Wu, 2015; USEPA, 2017). Presented in Table 2.13 are the different water quality criteria for nitrate for the protection of freshwater ecosystems.

Table 2.13 Acute water quality criteria for the protection of freshwater ecosystems for nitrate in Canada, British Columbia and Australia and New Zealand

Locality	**Criteria specifications**	**Numeric Criteria**	**Bioassay toxicants used**	**Reference**
Canada (national)	Water quality criteria for nitrate provided for both mg NO_3-N/L and Nitrate as mg NO_3/L	<124 mg NO_3 -N/L <550 mg NO_3/L	sodium nitrate	CCME, 2012
British Columbia (Canada-provincial)	Water quality criteria for nitrate reflecting the maximum allowed nitrate concentration for the protection of aquatic organisms against acute effects	<32.8 mg NO_3-N/L	Not reported	Meays, 2009
Australia and New Zealand	Water quality criteria for nitrate indicating four levels of protection (PC99, PC95, PC90 and PC80)	PC99: <17 µg/L PC95: <700 µg/L PC90:< 3400 µg/L PC80: <17000 µg/L	Not reported	ANZECC and ARMCANZ, 2000

ANZECC and ARMCANZ, 2000 guidelines do not distinguish between short-term (acute) and long-term (chronic) exposures

Unlike ammonia, most jurisdictions do not have water quality criteria for nitrates (Table 2.13). The available water quality benchmarks for nitrate are presented as default, without taking into consideration the ambient water quality of a site. Nonetheless, there is ample evidence supporting the dependency of the toxicity of nitrate on other water quality

variables for example on water hardness (Elphick, 2011 cited in CCME, 2012) and on ionic strength (Baker *et al*., 2017).

2.8.12 Water temperature

Water temperature is an important water quality attribute and a driver for key biochemical and ecological processes (Toffolon and Piccolroaz, 2015). Temperature is known to affect both the physiology and metabolism of aquatic organisms (Dallas and Ross-Gillespie, 2015; Axenov-Gribanov *et al*., 2016). It is therefore a necessary attribute in explaining the spatio-temporal distribution of aquatic organisms (Isaak and Hubert, 2004). Increase in stream water temperature is caused by numerous human activities for example: (i) deforestation of riparian land (Rutherford *et al*., 1997) with resultant loss of stream shading; (ii) heat inputs from power generation plants (Chen *et al*., 2003); and (iii) heated effluents resulting from urban and industrial wastewater discharges (Lezzi and Todisco, 2015).

Tools developed for monitoring the effects of water temperature on aquatic organisms focus principally on two aspects. The protection guidelines can be expressed to reflect permissible maximum water temperature considered safe for aquatic organisms – for example US states of Nebraska and Iowa (cited in Kansas Department of Health and Environment, 2011). Other tools may be based on the water temperature difference from reference conditions. The background temperature may mean three different reference points. These are first, from minimally degraded sites (DWAF, 1996). Second, temperature difference from reference conditions from a site located within the same stream *i.e.* one mile upstream of a study site, a typical background temperature referred to in the National Sanitation Foundation Water quality Index (Said *et al*., 2004; Rajanka *et al*., 2009). Third, permissible water temperature difference from reference conditions within specified period for example within an hour (ANZECC, 1992).

Table 2.14 Acute water quality criteria for the protection of freshwater ecosystems for water temperature in South Africa, Canada and Australia and New Zealand, Nebraska and Iowa

Locality	Criteria specifications	Numeric Criteria [Temperature difference from reference conditions (°C)]	Numeric Criteria [maximum allowable limits (°C)]	Reference
South Africa	Guidelines based on water temperature difference from reference conditions (Degrees Celsius) and percentage change from reference temperature (minimally affected sites).	Should not vary from reference temperature by >2 °C or by >10 %from background temperature	-	DWAF, 1996
Australia and New Zealand	Criteria expresses as temperature difference from reference conditions and also specifies the period beyond which such change can be or cannot be tolerated (for aquaculture). ANZECC provided no trigger value, but they recommend site-specific guideline/trigger value based on 20th and 80th percentiles.	<2°C over 1 hour (guideline value for aquaculture)	-	ANZECC, 1992
US State of Nebraska	Water quality criteria specifying both temperature difference from reference conditions and maximum allowable temperature for both cold and warm water environments	≤3°C	Warm water: 32°C Cold water: 22°C	Kansas Department of Health and Environment, 2011
US State of Iowa	Water quality criteria specifying both temperature difference from reference conditions and maximum allowable temperature for both cold and warm water environments	≤3°C or ≤ 2°C at a rate ≤1°C /hour	Warm water: 32°C Cold water: 20°C (for lakes and most streams)	Kansas Department of Health and Environment, 2011

Most water quality-monitoring tools for temperature (some of which are referred to in Table 2.14) focus on temperature difference from reference conditions as a measure of water quality degradation. The need for comparison with the background temperature make these monitoring tools more site-specific and somewhat data-intensive. The determination and subsequent classification of a site therefore requires adequate ambient and/or reference temperature data to enable sound comparisons. DWAF (1996) for

instance requires good water temperature data from reference sites covering at least a full year. Such data could easily enable the monitoring personnel to determine diurnal and seasonal variability in water temperature (DWAF, 1996; Queensland Department of Environment and Heritage Protection, 2009).

2.8.13 Dissolved oxygen

Dissolved Oxygen (DO) is one of the most important water quality attributes necessary for the survival of aquatic organisms. After water itself, DO is considered the second most important requirement for water inhabiting organisms (Wetzel *et al*., 2001). Aquatic organisms require adequate DO to perform basic and key survival processes like respiration and metabolism (Veesommai *et al*., 2017). Just as with most environmental stressors, aquatic organisms have varying tolerances to dissolved oxygen concentration deficiencies. For example, they differ immensely among species and life stages (juveniles versus adults). Reviewed water quality criteria from different water quality jurisdictions do reflect such differences (Table 2.15). Criteria that are more conservative are recommended for cold-water species and juvenile life stages while relatively less conservative criteria are recommended for their counterparts (warm water organisms and adults).

As shown in Table 2.15, DO criteria are mainly expressed to reflect the minimal concentrations of dissolved oxygen that aquatic organisms can tolerate. More attention is paid to oxygen depletion as opposed to super-saturation, particularly because most impacts of DO are mostly observable at lower concentrations as opposed to higher DO concentrations (Florida Department of Environmental Protection, 2013). Most national and international water quality jurisdictions set the minimum dissolved oxygen criteria between 4 mg/L and 6 mg/L (Elosegi and Sabater, 2013) or a minimum or an instantaneous minimum of >5 mg/L, for example CCME, British Columbia, Alberta, ANZECC (Table 2.15). Alabaster and Lloyd (1982) view this value as representing intentional over-protection or to some extent even unnecessarily high restriction, intended for the satisfactory survival of fish and adequate growth of juveniles.

Table 2.15 Acute water quality criteria for the protection of freshwater ecosystems for dissolved oxygen in South Africa, Canada, British Columbia, Australia and New Zealand, US State of Florida, Alberta and USA

Locality	Criteria specifications	Numerical criteria: DO Concentration (mg/L)	Numerical Criteria (% Saturation)	Reference
South Africa	DO criteria (instantaneous) For the protection of most Southern African aquatic organisms and endemic species adapted to warm water habitats Protection of aquatic organisms against acute (lethality) effects of reduced DO.	-	Target water quality range: 80%-120% saturation 40% sat. (One day min. allowable value)	DWAF, 1996
Canada (national)	The DO criteria specify the organisms group (warm water and cold water) and growth stages, thereby recommending different criteria for warm water organisms and cold-water organisms at different life stages.	Warm water organisms 6 (early stages) 5.5 (other stages) Cold-water organisms 9.5 (Early stages) 6.5(other stages)	-	CCME, 1999
British Columbia (Canada-provincial)	Instantaneous minimum (to be achieved at all times)	5 mg/L(all life stages other than buried forms)	-	Truelson and Bio, 1994
Australia and New Zealand	Criteria derived from non-Australian freshwater organisms. Saturation determined over one diurnal cycle.	-	DO not to drop below 6 mg/L or 80% saturation	ANZECC 1992
Australia and New Zealand	DO guidelines derived from Australian freshwater fishes only. With limited reference to other freshwater organisms	5 mg/L	-	ANZECC and ARMCANZ, 2000
US State of Florida	Recommended minimum criteria, recognising natural fluctuations below the minimum value.	5 mg/L	-	Magley and Joyner, 2008
Alberta (Canada-provincial)	Criteria for the protection of aquatic organisms against acute effects of reduced DO 5 mg/L	5 mg/L (one day minimum)	-	Shaw, 1997
United States (national)	The USEPA DO criteria specify the organisms group (warm water and cold water) and growth stages. Additionally, the maximum period the organisms can tolerate a recommended concentration.	Warm water organisms 5.0-6.0 (early stages) 3.0-5.5 (other stages) Cold-water organisms 5.0-9.5 (Early stages) 4.0-6.5(other stages	-	USEPA, 1986

CHAPTER 3: METHODS

3.1 INTRODUCTION

A holistic view of a water quality index reflect three key areas; (a) details and procedures involved in the development process of the index, (b) assessments of its functionality through literature and field based validations, and (c) details pertaining to the influence of individual input variables on the composite index (sensitivity analysis). However, it should be noted that not all indices address the three development areas at once, most commonly water quality indices will reflect the development steps and procedures and the validation or implementation phase as preferably referred to by some developers. Given that the ATI for macroinvertebrates (this study) was solely developed from desktop-based procedures, post development assessments of the tool were therefore a necessity in order to determine the extent to which its evaluations of water quality were a reflection of field conditions. Addressed in this chapter therefore are the methods used in (i) the development of the Index (ii), validation of the index, and (iii) its sensitivity analysis.

3.2 INDEX DEVELOPMENT

The ATI for macroinvertebrates is underpinned by protection concentrations or trigger values for freshwater macroinvertebrates. The first stages of the index development therefore involved the estimation of the protection concentrations or trigger values. These values were derived using procedures adapted from national and international water quality jurisdictions (*e.g.* ANZECC and ARMCANZ, CCME, DWAF and USEPA). The generic stepwise process followed in deriving the trigger values modified from Stephan *et al*. (1985) and Warne *et al*. (2015) is as illustrated in Figure 3.1.

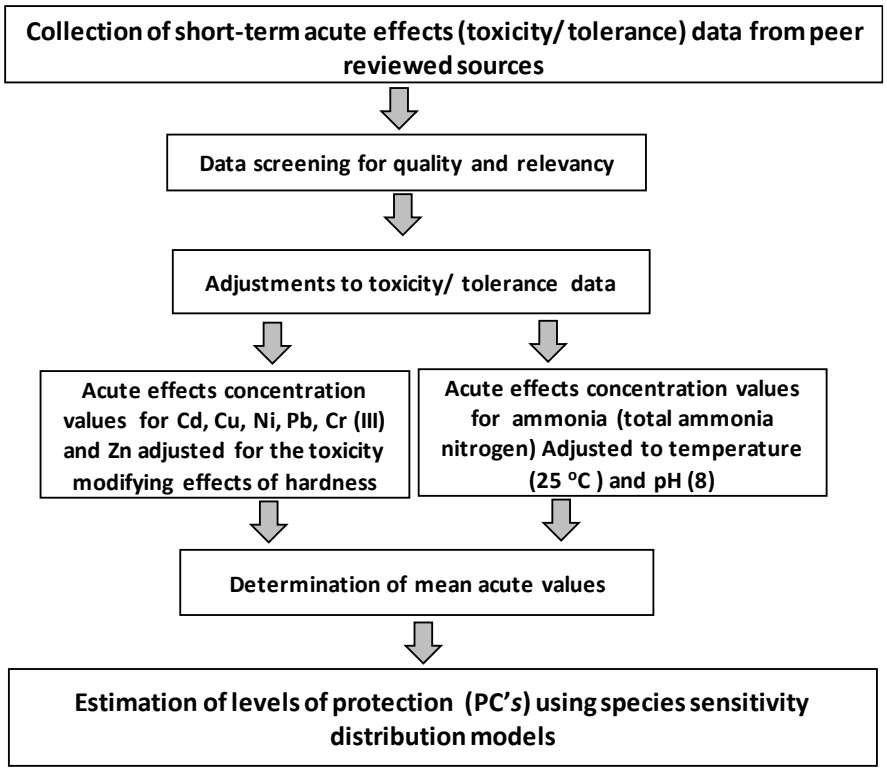

Figure 3.1 Summary of the stepwise process followed in deriving protection concentrations based on SSD models

To account for differences in the sources, processes and tools used in the estimation of the PCs/trigger values from one selected variable to the other, finer details for individual water quality variables are provided in Section 3.3.

3.2.1 Collection and screening of ecotoxicity and ecotolerance data

Trigger values are derived from bioassay data that could either be data generated by the developer or collected from credible ecotoxicity sources. All the data used in the development of the trigger values for the subsequent water quality index in this study were sourced from published ecotoxicity publications and databases.

A large proportion of the ecotoxicity data used in this study were collected from the USEPA ECOTOX Database. The USEPA ECOTOX is an interactive database, which archives and makes available to the public information on the effects of single chemicals on aquatic and terrestrial organisms. Only data meeting the USEPA screening criteria get

stored in the ECOTOX; for instance, the ECOTOX (USEPA, 2017, only accepts toxicity data reported alongside key auxiliary data:

- It documents experimental data reported alongside control group details.
- The control group experiments must have been run concurrently with the treatments in the study.
- Control group survival should not be less than 90%.
- The magnitudes of key water chemistry constituents should be reported and must be within acceptable ranges.
- Mean dissolved oxygen should not be less than 60% saturation.
- If the bioassays used distilled water, the data are only accepted if the water was reconstituted with appropriate salts.

Due to the rigour of the USEPA ECOTOX screening criteria, toxicity data obtained from this database were not screened further for quality but only for relevancy. The interactive nature of the USEPA ECOTOX enables ease of data screening (for relevancy) through the checking and unchecking of appropriate checkboxes. Additional sources were used for stressors where the USEPA ECOTOX data seemed patchy. Eco-tolerance data for physical stressors (*i.e*. water temperature and dissolved oxygen) were collected entirely from original sources, particularly because the USEPA ECOTOX Database only archives chemical data.

Ecotoxicity data collected from other sources had to undergo a screening process, using the predetermined criteria. The filtering of bioassay data prior to use is of major importance particularly because ecotoxicity data vary greatly in quality (Schneider *et al*., 2009). The screening process in this study was based on a predetermined criteria (Table 3.1) adapted from Roux *et al*. (1996), Bejarano *et al*. (2014), Nowell *et al*. (2014) and USEPA (2017).

Table 3.1 Predetermined screening criteria for delimiting ecotoxicological data obtained from scientific publications

Bioassay Information	Criteria	Trade-offs
Target taxa	Freshwater macroinvertebrates occurring in South African rivers or from geographic locations with similar climatic conditions.	Indiscriminate use of data for native and non-native taxa since there were relatively fewer data for native macroinvertebrate taxa.
Test location	Laboratory: Considered data were only from bioassays conducted from a laboratory.	No trade-offs
Endpoint	Median Lethal Concentration (LC50): Only median lethal concentrations were accepted for the study.	No trade-offs
Preferred exposure duration (hours)	Short-term exposure duration: Accepted toxicity data were from bioassays conducted between 24 to 96 hours. In cases where there were multiple test durations for individual genus the longest exposure, duration data were accepted.	No trade-offs
Censored data	Left or right censored or data in a range were rejected	Censored data were not accepted
Measured versus unmeasured concentrations	Preference was given to measured concentrations. Nominal concentrations were only accepted when measured concentrations were limited; less preferred because they tend to overestimate the actual concentrations.	Unmeasured concentrations were accepted because there were relatively fewer measured concentration data for the target contaminants as recommended. [c]
Control mortality	≤10% mortality: Only toxicity data generated from bioassays containing control groups, with at least 90% survival were accepted.	Only data generated from bioassays with a minimum control group survival rate of 90% were accepted
Chemistry of the test medium	Chemical and physical properties of the water must have been reported for the data points to be accepted. Data generated from bioassays conducted using deionized water were all rejected.	Only data meeting the criterion were accepted

Despite these trade-offs, toxicity data for each chemical stressor were selected to represent a wide array of macroinvertebrates covering a minimum of eight families selected from groups considered a representation of the freshwater macroinvertebrates population (Figure 3.2), modified from DWAF (1996), USEPA (2012), Warne *et al*. (2015), and USEPA (2016).

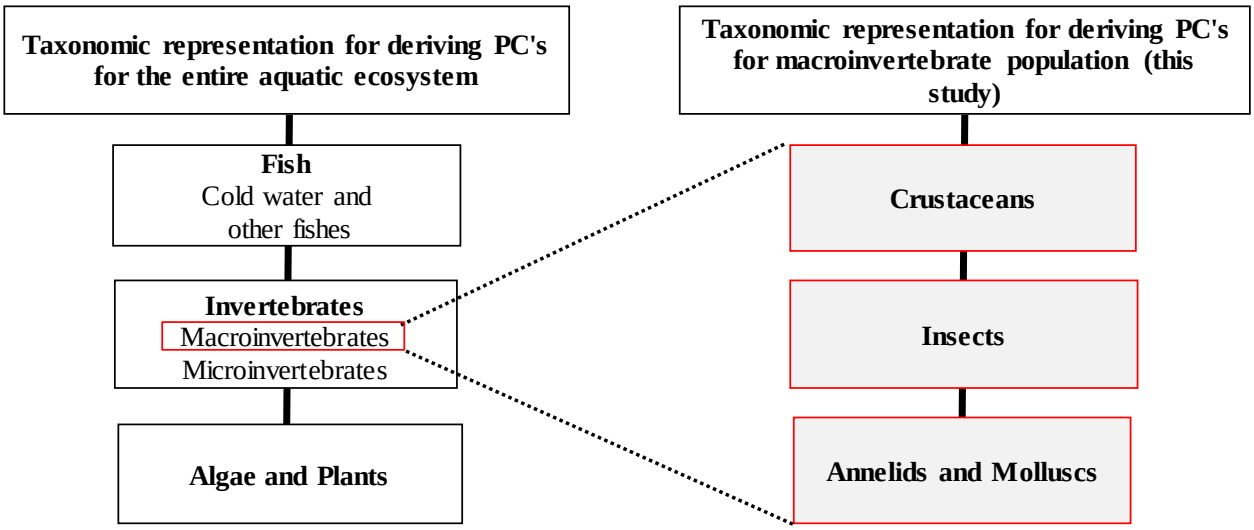

Figure 3.2 Required taxonomic representation for deriving acute freshwater quality criteria for macroinvertebrates

The key assumption in the SSD modelling is that different organisms display varying sensitivities towards the same environmental stressor (Larras *et al*., 2012). In accounting for the variations in sensitivities of different taxa to environmental stressors, it is therefore critical that the input data is representative of the target organisms.

3.2.2 Generation of species sensitivity distribution curves

The last step indicated in Figure 3.1 is the generation of trigger values or the PCs. The PCs form the foundation of the ATI for macroinvertebrates particularly because the index categories are developed from them. The PCs were derived from SSD curve fitting, achieved with the aid of Burrlioz version 2.0 (Barry and Henderson, 2014) for most of the stressors and Inverse Normal Cumulative Distribution Function (ICDF) in Microsoft Excel for dissolved oxygen only. The Burrlioz version 2.0 SSD software is underpinned by statistical processing in the R statistical software programme. The Burrlioz 2.0 generates PCs in accordance with the Australian and New Zealand freshwater and marine water quality guidelines. The software automatically fits one of the Burr Type III family of distributions; either the Burr III, Inverse Pareto, Inverse Weibull or the Log-logistic distributions to collected ecotoxicity data. Generated alongside the distributions are PCs

with their 95% confidence intervals. The Burrlioz 2.0 software derives the 95% confidence intervals using the bootstrap regression technique.

A different approach was adopted for dissolved oxygen because the conventional SSD tools or software could not be utilised for stressors whose effects on aquatic organisms are observable at reduced concentrations as opposed to the more typical elevated magnitudes. An additional challenge with dissolved oxygen was due to limited literature on the estimation of trigger values using SSDs. Alternatively, the Inverse Cumulative Normal Distribution Function (ICDF) was utilised in the estimation of PCs for dissolved oxygen following approaches used by Elshout *et al*. (2013).

3.3 DATA PREPARATION

3.3.1 Metal speciation and laboratory bioassay stock solutions

The index was developed to reflect metal speciation and bioavailability of metals on freshwater macroinvertebrates. With specific reference to metal species, most jurisdictions present their water criteria for chromium and mercury to reflect the toxicity of specific species. For instance, chromium is presented as trivalent chromium [Cr (III)], hexavalent chromium [Cr (VI)] or both and mercury as inorganic mercury [Hg (II)] or methyl mercury. From the sources of data used in this study, in particular the USEPA ECOTOX, the laboratory bioassay data for metals were catalogued based on the chemical that the researcher used in the study and rarely by their oxidative states. The metal species were therefore determined using their molecular formulas, which involved the calculation of their oxidative states as advised by Kinziger, B.P. (Scientist, Science and Engineering, USEPA, CSRA LLC) (pers. comm., 12 October, 2016). As an example, studies where potassium dichromate ($K_2Cr_2O_7$) was used as a stock solution were only considered for Cr (VI). This was the case because $K_2Cr_2O_7$ has no charge, potassium has an oxidative state of +1, oxygen has an oxidative state of -2, and therefore the Cr would have an oxidative state of +6. Similar calculations were conducted for all stock solutions to determine the particular metal species for the two metal stressors [*i.e.* Cr (III) and Hg (II)] used in the study.

The laboratory bioassays for data sources were conducted using specific stock solutions sourced from credible suppliers. Researchers also reported the specific stock solution used in the bioassays; *e.g.* zinc sulfate ($ZnSO_4$), cadmium chloride (CdCl2) and sodium chloride (NaCl). It was only on rare occasions that a researcher reported the test toxicant, for example as zinc (Zn) instead of zinc sulfate ($ZnSO_4$), just as cadmium (Cd) instead of cadmium chloride ($CdCl_2$), or simply as chloride (Cl^-) instead of sodium chloride (NaCl). In this study, therefore in cases where the test toxicant was reported as a solution, several considerations were taken in determining the dominant or more toxic ion. Using the above examples, in laboratory bioassay data where $ZnSO_4$ was used as a test toxicant, the test outputs were used for determining trigger values for zinc (Zn). Such data could not be used for deriving sulfate (SO_4) trigger values particularly because Zn is considered more toxic than SO_4. Observable effects on the test organisms would be due to Zn as opposed to SO_4 (*e.g.* as previously justified by Everitt *et al*., 2002). Similarly, Cd trigger values were derived from various salts [cadmium chloride ($CdCl_2$), cadmium nitrate ($Cd(NO_3)_2$), and cadmium sulfate ($CdSO_4$)] based on the same reasoning. The specific toxicants or chemicals (stock solutions) used in the laboratory bioassay data from literature are presented alongside the original LC50 toxicity values in the Appendices.

3.3.2 Bioavailability

Bioavailability in ecotoxicology refers to a complex and dynamic concept describing the mass transfer and uptake of contaminants into organisms that are determined by substance properties, it is also viewed as a set of processes that incorporate site specific parameters including space and time (Anderson and Hillwalker, 2012). Since the bioavailability and the toxicity of metals is dependent on their form and concentration, the dissolved form is regarded to be a better approximation of the bioavailable fraction of a metal toxicant than the particulate proportion. In this study therefore all metal concentration toxicity values reported as total concentrations were converted to dissolved concentrations using the USEPA multiplicative conversion factors (Table 3.2) (USEPA, 2017).

Table 3.2 Multiplicative conversion factors for total metals to dissolved fractions sourced from the USEPA-aquatic life criteria

Metal	Conversion factors (CFs)
Cadmium	1.136672-[(ln hardness)(0.041838)]
Chromium (III)	0.316
Chromium (VI)	0.982
Copper	0.960
Lead	1.46203-[(ln hardness)(0.145712)]
Mercury II	0.85
Nickel	0.998
Zinc	0.978

The conversion factors for cadmium (Cd) and lead (Pb) are hardness dependent (Table 3.2), so prior to use, their CFs need to be determined based on test hardness magnitudes in mg/L (as $CaCO_3$).

3.3.3 Adjustments for water hardness

The trigger values derived in this study and the subsequent index were standardised to reflect the ameliorating effect of water hardness on the toxicity of six metal stressors [Cu, Cd, Pb, Cr (III), Ni and Zn]. It was necessary to standardise the toxicity of these metals on freshwater macroinvertebrates because literature shows that the toxicity of these metals is hardness dependent, which requires adjustment using published algorithms to enable standardisation to reference water hardness (*e.g.* ANZECC and ARMCANZ, 2000 and USEPA, 1996; USEPA, 2017). For consistency reasons in this study the USEPA 1996 and 2017 algorithms (Equation 3.1) were utilised to standardise the laboratory bioassay data obtained from literature to reference water hardness mg/L (as $CaCO_3$) levels as previously applied by Keithly *et al*. (2004) and DeForest and Genderen (2012).

$$TV\ Href\ = EXP\ (LN(TV) - PS * (LN(Htest) - LN(Href))) \qquad \textbf{Equation 3.1}$$

Where $TV\ H_{ref}$ represents the toxicity value at the reference hardness, *TV* is the toxicity value, H_{test} is the test hardness, *PS* is the pooled slope and H_{ref} is the reference hardness.

The USEPA toxicity versus water hardness adjustments requires pooled regression slopes based on established metal toxicity versus water hardness-empirical relationships

(Table 3.3) sourced from the National Recommended Water Quality Criteria - Aquatic Life Criteria (USEPA, 2017).

Table 3.3 Pooled slopes for calculating short-term trigger values for metals that are hardness dependent

Metal	Acute slope
Cadmium	0.9789
Chromium III	0.8190
Copper	0.9422
Lead	1.273
Nickel	0.8460
Zinc	0.8473

Metals whose toxicities are known not to have dependency on water hardness or have no published conversion algorithms [Cr (VI), Hg (II)] were treated using standard/generic stepwise procedures (Figure 3.1) where mean acute values were determined based on geometric means. The geometric means were further utilised as inputs into the SSD model software [CSIRO Burrlioz version 2.0 (Barry and Henderson, 2014)] for the estimation of the PCs.

In the calculation of the index (validation stage), water hardness was incorporated by first establishing the prevailing water hardness conditions for each study site. Water hardness data were obtained from the South African Department of Water and Sanitation, spanning a period of ten years (2006-2016). Descriptive statistics were performed on the data to establish water hardness values and ranges that would better represent the water at each site.

3.3.4 Chloride and sulfate

The two salts considered in this study were chloride and sulfate. Most jurisdictions do not have recommended freshwater quality criteria or guidelines for chloride and sulfates. Reviews of water quality criteria for the two salts suggest that their toxicities to freshwater ecosystems are dependent on water hardness (*e.g.* Iowa DNR, 2009; Meays and Nordin, 2013) and on each other (Iowa DNR, 2009) (Chapter 2, Literature Review). Whilst it may have been proper to standardise the toxicity values for these salts to account for ambient water chemistry, available bioassay data were limiting and did not allow for the

conversions. Therefore, the estimations of the PCs were carried out using standard procedures adapted for this study (Figure 3.1). The estimation process involved the determination of mean acute values (geometric means) for all selected ecotoxicity data, followed by the estimation of protection concentrations using the CSIRO Burrlioz version 2.0 (Barry and Henderson, 2014).

3.3.5 Nitrate

Toxicity data for nitrate were obtained and treated using standard procedures adapted for this study. Toxicity data obtained from scientific publications were converted to mg NO_3-N per litre (mg/L) using standard conversion factors sourced from CCME (2012). The toxicity of nitrate to freshwater organisms has been reported to decrease with an increase in water hardness (Elphick, 2011). In this study, however, no water hardness corrections were conducted. Failure to account for modifying effects of water hardness was largely due to limited toxicity data for freshwater macroinvertebrates and uncertainties surrounding conversions. These challenges did not only affect this study as attempts by CCME (2012) to derive hardness-adjusted water quality guidelines for nitrate to freshwater organisms proved futile with the CCME nitrate ion water quality guidelines document citing similar hindrances.

3.3.6 Total ammonia-nitrogen

The derived water quality criteria for this study were derived to reflect the toxicity of TAN to macroinvertebrates at a water temperature of 25°C and pH 8. Prior to utilising the bioassay data (collected from scholarly publications), a series of calculations had to be conducted. These calculations involved the conversion of all ammonia bioassay data expressed in different units to like units and the standardisation of toxicity values to reflect ammonia toxicity at reference conditions (pH = 8, temperature = 25°C). The pH and temperature adjustments of the toxicity were conducted using the algorithms adopted from the USEPA (1999) and USEPA (2009) water quality criteria for ammonia documents. The conversion procedure is as follows:

1. Conversion of all ammonia laboratory bioassay data for ammonia [*i.e.* unionised ammonia to total ammonia (sum of unionised ammonia and ammonium ion]

2. Conversion of total ammonia to TAN, by

$$TAN = Toxicity\ value\ (Total\ Ammonia)/(\frac{14.0067}{17.03}) \qquad \textbf{Equation 3.2}$$

Where, *TAN* is Total Ammonia-Nitrogen, 14.0067 is the average atomic mass of nitrogen (atomic mass unit) and 17.03 is the molecular weight of ammonia (g/mol).

3. Adjustments for pH (from test pH to pH = 8) dependency using Equation 3.3, modified from Equation 11 (USEPA, 1999).

$$AV_{t,8} = \frac{AV_t}{\left(\frac{0.0489}{1+10^{7.204-pH}}+\frac{6.95}{1+10^{pH-7.204}}\right)} \qquad \textbf{Equation 3.3}$$

Where, $AV_{t,8}$ is the acute value at reference temperature and pH = 8, *t* is the temperature, and *0.0489* and *6.95* are synthetic parameters.

4. The last adjustments carried out were for temperature (from test temperature to 25°C) dependency. The conversion algorithm for the adjustments are as shown in Equation 3.4, adopted from USEPA (1999).

$$log(AV_{t,8,25}) = log(AV_{t,8}) - [-0.036(Test_t - 25^oC)] \qquad \textbf{Equation 3.4}$$

Where, *-0.036* is the invertebrate acute slope for water temperature adjustment, $Test_t$ is the test temperature and *25°C* is the temperature for which the conversions are made.

3.3.7 Temperature difference from reference conditions

The water quality criteria and the subsequent water quality index (with categories) for water temperature focused on temperature induced mortalities of macroinvertebrates. The thermally induced mortalities were measured by temperature deviation (increase) above reference water temperature. Steps followed in deriving the water quality criteria for water temperature in this study are as detailed.

- Collection of temperature tolerance data from published bioassay data. The acute thermal endpoints used in this study were tolerance lethal limit or median lethal temperature (LT50) and Incipient Lethal Temperature (ILT). LT50 and ILT were treated as being equivalent (De Vries *et al*., 2008; Dallas and Ketley, 2011) and both expressed as LT50 (denoting the median lethal temperature). Upper incipient lethal temperature is determined relative to the acclimation temperature (Colorado Department of Public Health and Environment, 2011).
- Data screening for quality and relevancy.
- Determination of Temperature Tolerance Interval [(TTI (°C)]. TTI refers to the interval by which temperature may suddenly increase above mean annual water temperature (Urban, 1994) or acclimation temperature (De Vries *et al*., 2008), a change estimate to be protective of 50% of the total population. The TTI was calculated from bioassay data using Equation 3.5 (De Vries *et al*., 2008).

$$TTI = LT50 - Ta \qquad \textbf{Equation 3.5}$$

  Where, *TTI* represents the temperature tolerance interval, *LT50* represents the median lethal temperature and *Ta* is the acclimation temperature (°C).

- Fitting of SSD curves on the TTI data.

3.3.8 Incorporating reference water temperature data into the protection concentrations

Temperature difference from reference conditions for the Olifants River catchment was incorporated into the study by first selecting a stream that would best represent the least impacted conditions of the Olifants. The calculation of index scores for water temperature difference were determined by modifications of the TTI (Equation 3.5) (Urban 1994; De Vries *et al*., 2008). Water temperature deviations (increase) from the 'natural' or reference were determined from water temperature data collected between 2010 and 2011 for the Klaserie River sourced from South African Environmental Observation Network (SAEON-Ndlovu Node, Phalaborwa) as outlined, below (Equation 3.6).

$$Temperature\ difference\ =\ Sample\ Temp\ -\ Ref\ Temp \qquad \textbf{Equation 3.6}$$

Where, *Sample Temp* refers to the water temperature determined *in situ* on the day and time of collection, *Ref Temp* (representing background or near natural conditions) refers to the mean temperature for the specified month and time of the day. The *Ref Temp* was used as a surrogate for acclimation temperature. The water temperature data for the Klaserie River with associated calculations are given in Appendix 20.

3.3.9 Dissolved Oxygen

Previous water quality indices developed for the protection of aquatic life that incorporated Dissolved Oxygen (DO) adopted different approaches. For example, Wepener *et al*. (1992) based DO sub-indices and rankings on statistical (extrapolation) approaches. Whilst the Canadian Council of Ministers of the Environment (CCME) Water Quality Index (CCME, 2001) was formulated through assembling a team of water quality experts who ranked the quality of the selected sites based on available raw data [Mercier, V. (Supervisor, water quality specialist, Water quality monitoring and Surveillance Division, Environment and Climate Change Canada), pers. comm., 08 November 2016]. In this study the DO trigger values for the protection of freshwater macroinvertebrates were estimated using the SSD models adapting an approach and tools previously used by Elshout *et al*. (2013).

The collection and treatment of laboratory bioassay data for DO to freshwater macroinvertebrates followed the generic procedures illustrated in Figure 3.1. For this study, data presented at species level were aggregated to MAVs by calculating arithmetic means for all data points within the same genera and exposure duration. The arithmetic means were preferred over geometric means for the DO mean acute values as suggested by Stephan *et al*. (1985).

The opposing influence of DO on aquatic animals could not allow the estimation of PCs using the CSIRO Burrlioz version 2.0 (Barry and Henderson, 2014) that was used for fitting the SSD curves for all the other stressors. This was the case because SSD software was developed for the determination of PCs for stressors whose effects are observable at elevated concentrations rather than reduced. An alternative approach for estimating

the effects of dissolved oxygen was with inverse cumulative normal distribution function in Microsoft Excel, an approach adapted from Elshout *et al*. (2013). The specific PC's (PCs) were obtained by the command below:

$$[qnorm(p, mu, sigma)]$$

Where, *qnorm* is the inverse cumulative normal distribution function *p*: is the probability of interest (where, 0 < p < 1), *mu*: is the mean of the distribution (LC50s values), *sigma*: is the standard deviation of the distribution (LC50s values). Also obtainable in R-Studio (R-Core Team, 2015) executed by the following syntax:

$$[norminv(p, mu, sigma)]$$

Where, *norminv*: is the inverse of the normal cumulative distribution function, *p*: the probability of interest (where, 0 < p < 1), *mu*: the mean of the distribution and *sigma*: the standard deviation.

3.4 INDEX DEVELOPMENT

3.4.1 Conception of the ATI for macroinvertebrates

The ATI for macroinvertebrates was developed to provide the basis for cause-effect explanations between the magnitudes of physico-chemical water quality variables and observed freshwater macroinvertebrates community structure. The toxicity endpoints that could reasonably account for the presence/absence of macroinvertebrates at a site are the acute (lethal) exposures as opposed to chronic (sub-lethal) exposures. If the index was developed in response to sub-lethal effects (*i.e*. immobilisation, growth and reproduction), long-term exposure associated endpoints could have been appropriate. For practical reasons, through rapid biomonitoring it is not easy to determine or observe sub-lethal effects. For this particular reason, the index is based on short-term (24-96 hours) acute (LC50) exposures, where absence means that the magnitudes of the physico-chemical stressors may have been elevated beyond concentrations that the macroinvertebrates could withstand. The implications of the use of lethality basis on the

index will be strictly binary where absence means mortality and presence means survival (stressors' magnitudes tolerable).

3.4.2 Selection of water quality variables

The ATI for macroinvertebrates is calculated using 14 water quality variables. The selection of these variables was informed by the following considerations:

- Land-uses and pollutants arising from the Olifants River catchment: the Olifants system has been widely classified as one of the most polluted in South Africa because of mining activities plus industrial and nutrient-laden discharges (de Villiers and Mkwelo, 2009; McCarthy, 2011; Dabrowski, *et al*., 2015; Gerber *et al*., 2015). The selection of variables took into consideration the pollutants generated within the catchment. However, not all key pollutants (*e.g.* phosphorus/phosphate/orthophosphates) could be incorporated because of toxicity (stressor-response) data constraints.
- Water quality variables that have similar properties were not considered for selection as input variables for a water quality index, to avoid redundancy (Dunnette, 1979; Sutadian *et al*., 2016).
- Commonly used water quality variables for monitoring and assessing water quality of the Olifants River catchment (Appendix 1).

3.4.3 Rating of water quality variables

Upon selection, water quality variables' concentrations must be linked to water quality. This is achieved through the development of rating curves or attachment of weights to individual variables. The rating or weighting of variables is done either using expert opinion (Delphi method) or purely based on the use of statistical approaches. Through the Delphi method, a team of water quality experts rate or attach weights to selected water quality variables based on their relative importance or their impacts on target users (Tyagi *et al*., 2013; Naubi *et al*., 2016). Most rating methods using expert opinions have been criticised for being subjective (Stoner, 1978; Boyacioglu, 2007; Tyagi *et al*., 2013; Poonam *et al*., 2015). This is because most rating curves are developed based on the developers' judgement or with a few associates (Boyacioglu, 2007).

In this study, the rating of water quality variables adopted the statistical approach, which is an alternative to expert opinion. The rating curves for the selected water quality variables were developed using SSD models. The primary purpose of an SSD is to determine concentrations/magnitudes of physico-chemical stressors that could be tolerated by a specified percentage (PC*p*) of species in an assemblage (Liu *et al*., 2014). In this study, the SSD was utilised to generate four levels of protection (PC99, PC95, PC90 and PC80) of freshwater macroinvertebrates against 14 physico-chemical water quality stressors. Where, PC represents protective concentrations and *p* represents the percentage of species expected to be protected by the concentration. For example, PC99 is an estimated guideline value protective of 99% (or hazardous to 1%) of freshwater macroivertebrates. Similarly, PC95 represents a value protective of 95% (or hazardous to 5%) of the target species.

3.4.4 Transformation of water quality variables

The ultimate goal of a water quality index is to aggregate the magnitudes and concentrations of physico-chemical water quality variables to give an integrated picture of the state of a water body. Since water quality indices are derived from input variables measured on non-commensurate scales, it is therefore necessary to eliminate concentration units by transforming the input variables to common scales or dimensionless scales known as sub-indices (*e.g.* Sadiq and Tesfamariam, 2007). In this study, the sub-indices for each input variable were derived by assigning Hazardous Rank Scores (HRS), ranging between 20 and 100, where 20 corresponds with the worst value and 100 with the best water quality (a procedure modified from Wepener *et al*., 2006). The HRS were derived from the SSD output values, which offered four protection levels for freshwater macroinvertebrates (*i.e.* PC99, PC95, PC90, and PC80).

3.4.5 Aggregation

The sub-indices generated from assigning HRS to the water quality variables were aggregated using the Solway modified un-weighted aggregating function (Equation 3.7) (House and Ellis, 1980).

$$I = \frac{1}{100}\left(\frac{1}{n}\sum_{i=1}^{n} q_{\mathrm{i}}\right)^{2} \qquad \text{Equation 3.7}$$

Where *I* is the final index score *qi* is the equation of the *ith* parameter (as value between 0 and 100) *n* is the number of determinants in the indexing system.

The Solway modified un-weighted aggregating function was selected for aggregating water quality values (sub-indices) obtained from the SSD derived rating curves. This aggregating function has been described as sensitive and unbiased to changes in water quality variables through their ranges (Richardson, 1997). Unlike some aggregation functions, the Solway Modified Un-weighted aggregating function does not suffer from rigidity, making it possible to add or remove variables later during the operation stage of the index. Rigidity with respect to aggregation functions, refers to the inability to either add or remove water quality variable(s) to an index even when need arises (Abbasi and Abbasi, 2012). A limitation that is mainly attributable to the mathematical structure of the aggregation function (Swamee and Tyagi, 2007).

In addition, most water quality indices are interpreted solely based on the composite index score. This particular approach conceals the identity of the specific variable(s) that limit the water's suitability for use (Smith, 1990), a limitation widely referred to as eclipsing. In order to compensate for this limitation Wepener *et al*. (1992) expressed the ATI scores alongside the minimum operator also known as the Smith Index (Smith, 1990) or lowest rating score (Wepener et al., 1999, Gerber et al., 2015). The lowest rating score identifies the input variable limiting the suitability of the water for use and the extent, in terms of severity, to which it occurs (Wepener *et al*., 1999, Gerber *et al*., 2015). As a result, the composite ATI for macroinvertebrates (this study) was expressed alongside the Minimum Operator or the minimally rated variable.

3.4.6 Index categories

The SSD curves/rating produced four levels of protection (PC99, PC95, PC90 and PC80). These four levels of protection were used in deriving the five index' grading categories (A-E). Category 'A' indicates best conditions of the water and Category 'E' indicates worse

conditions of the water along the index scale. The five categories for each chosen stressor were derived using an approach modified from Wepener *et al*. (2006) (Table 3.4).

Table 3.4 Description of the index categories based on the levels of protection derived from SSD models

Dissolved Oxygen (concentration)	Other variables	Grading category
>PC99	<PC99	A
Between PC99-PC95	Between PC99-PC95	B
Between PC95-PC90	Between PC95-PC90	C
Between PC90-PC80	Between PC90-PC80	D
<PC80	>PC80	E

The index categories for dissolved oxygen were slightly different from other variables solely because dissolved oxygen affects aquatic organisms in a manner opposite to that of most variables. For example, aquatic organisms are affected mostly by the depletion of dissolved oxygen as opposed to elevated concentrations.

3.5.1 Calculation and interpretation of the aquatic toxicity index for macroinvertebrates

The calculation of the index involves a series of steps ranging from conversion of units and concentrations to the aggregation of index scores and interpretation of the index outputs as illustrated in Figure 3.3 with illustrative calculations in Appendix 2.

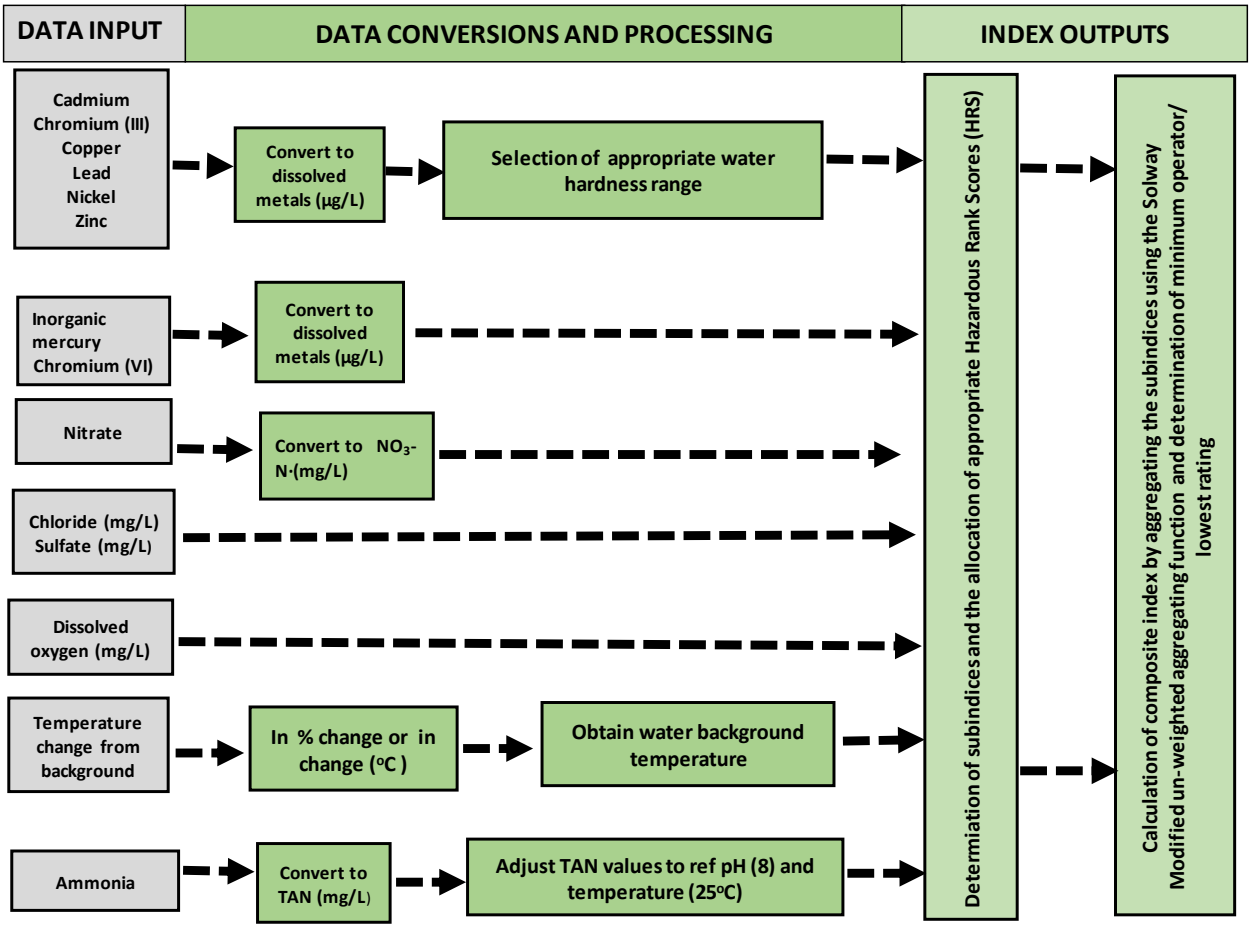

Figure 3.3 Stepwise process for the calculation of the ATI for macroinvertebrates

The determination of the index involved three major steps; data input, processing and output. Since the input of the data differed from variable to variable, some of the input variables included additional steps *e.g*. units' conversion and adjustments for ambient water chemistry. The index outputs phase was divided into two merely for the recognition of sub-indices, which was also necessary for the determination of the minimum operator or lowest rated variable.

3.5.2 Interpretation of the index scores

The composite ATI for macroinvertebrates is interpreted purely on the basis of 14 water quality variables. The index expresses five levels of water quality impairment, which may

subsequently lead to the disappearance of freshwater macroinvertebrates. The index may be interpreted as indicated in Table 3.5.

Table 3.5 Interpretation of the ATI for macroinvertebrates based on the hazard rank scores

Hazard Rank Scores (ranges)	Grading category	Descriptor words	Perceived description and interpretations
81-100	A	High quality	Water perceived to be of natural to near natural conditions, expected to support the survival of a wide variety of freshwater macroinvertebrates including sensitive taxa
61-80	B	Good quality	Water of good quality, indicative of minimal impairments.
41-60	C	Moderate quality	Water with elevated pollutants, less likely to support sensitive and taxa within mid-ranges of tolerance
21-40	D	Poor quality	Water quality conditions that can support only tolerant macroinvertebrates taxa
<20	E	Extremely poor quality	Water indicative of extreme impairment. Conditions that can only support the most tolerant freshwater macroinvertebrates

If invertebrate surveys reveal a picture different from the composite ATI, the index may need to be further explored by tracing variables with relatively lower ratings. In cases where all the sub-indices would be satisfactory external sources of impairment (other stressors not included in the index) would need to be investigated.

3.6 INDEX VALIDATION

3.6.1 Literature-based validation

The literature-based validation was conducted to determine the extent to which the PCs generated in this study compares with those in scientific publications. This involved ranking of toxicants (mainly metals) [using relational operators (</>)] according to their relative toxicities to freshwater organisms as previously done by Otitoloju and Don-Pedro, 2002; Rathore and Khangarot, 2002; Shuhaimi-Othman *et al*., 20120.

3.6.2 Field based validation

Field based validations were conducted using data collected from the Olifants River catchment covering both the upper and the lower Olifants sub-catchments. The field validations were carried out to determine the extent to which the index was able to discriminate between sites with reference to levels of pollution and establish if there was any agreement between the index grading categories and observed macroinvertebrates composition and community structure.

3.6.2.1 Geography of the Olifants River

The Olifants River originates in the highveld, near Bethal in the Mpumalanga Province of South Africa. The river flows eastwards traversing the Kruger National Park into Mozambique where it joins the Limpopo River before discharging into the Indian Ocean. The main tributaries of the Olifants River are the Wilge, Elands, Steelpoort, Blyde, Klaserie and Timbavati rivers. The Olifants River catchment can be divided into three broad sub-catchments, the upper (source to Loskop Dam), Middle (from Loskop Dam to the confluence with the Steelpoort River) and the lower Olifants (covers Steelpoort River sub-catchment and downstream to the South Africa/Mozambique border).

3.6.2.2 State of the Olifants River

The upper Olifants River Sub-catchment, for instance, is dominated by intensive coal mining, coal-fired electricity generation, irrigated agriculture, heavy and light industries, towns and small urban areas (Ashton, 2010). Previous documentation of the most prevalent land-use cover in the upper Olifants showed that grassveld covers approximately 51.6%, agriculture approximately 40.2%, followed by mining with 3.9%, then urban with 1.9%, bushveld with 1.6% and the least being forestry with approximately 0.8% (modified from Dabrowsky and Klerk, 2013). The middle reaches of the Olifants River catchment was characterised by irrigated agriculture, several mines of platinum, chrome and vanadium. Situated within these reaches are ferro-chrome refineries and numerous urban centres (Ashton *et al*., 2010). Studies conducted in the catchment have indicated major pollution for both ground and surface water. Some of the documented

impacts in the catchment include high nutrient and metal concentrations (Dabrowski and de Klerk, 2013). Most of the pollutants in the upper Olifants River catchment could be traced from defunct and existing mines and urban wastewater discharges (DWAF, 2004; Dabrowski and de Klerk, 2013). Similarly, the lower catchment does not present any improvements in water quality either. Several mines such as copper and phosphate mines around Phalaborwa (Mussagy, 2008) mainly dominate the lower Olifants. Recent studies have indicated high nutrient and metal concentrations within the lower Olifants River sub-catchment, which were predominantly attributed to agricultural and mining activities (Gerber *et al*., 2015).

Given the state of Olifants River water quality, numerous concerns have been and can still be raised. Most of the concerns relate to the ecological value of the Olifants River, with particular reference to both pollution and water quantity to supply ecologically sensitive areas dominating the lower Olifants River sub-catchment (*i.e.* game parks including the Kruger National Park) (DWA, 2011). Continued impairments of the Olifants River because of mining and industrial activities will likely jeopardize the viability of the Kruger National Park and other conservation areas, consequently threatening the tourism industry (Couzens and Dent, 2006).

Other concerns could relate to the fact that the Olifants River is a trans-boundary water resource shared with downstream Mozambique. In that regard, South Africa is obliged by both national and international obligations associated with shared water resources/trans-boundary watercourses. The management and benefits sharing of the river is governed by the principles of international law. South Africa as an upstream user has to forego some potential benefits obtainable from the Olifants (including sinking of waste) in recognition of the needs of the downstream Mozambique (Nkomo and van der Zaag, 2004). It is mandatory for South Africa to oblige to these principles particularly because:

- South Africa ratified the SADC Revised Protocol on Shared Watercourses formulated in line with the UN Watercourses Convention; a protocol, which places emphasis on the need for joint efforts in the prevention and preservation of

ecosystem, prevention, reduction and control of pollution and mitigation of harmful conditions.

- The South African Water Services Act (Act 108 of 1997), the National Water Act (Act 36 of 1998) and the National Water Resources Strategy collectively govern the management of South African water resources and provide for international obligations (water resources shared with neighbouring countries through international agreements).

Nonetheless, previous studies and water quality-monitoring reports of the Olifants River catchment suggest that the Blyde and the Klaserie rivers are relatively less impaired tributaries of the Olifants (Ballance *et al*., 2001; Swemmer and Mohlala, 2012). The Blyde River in particular has been reported to have a rejuvenating effect on both the quality and quantity of the state of the Olifants River.

3.6.2.3 Collection and incorporation of ambient physico-chemical data

The ATI for macroinvertebrates is partially site-specific. The index may be considered site-specific because the determination of the index scores took into consideration the ambient water chemistry for various water quality variables. The determination of sub-indices for cadmium, chromium (III), copper, lead, nickel and zinc required the incorporation of water hardness data obtained from the study sites.

3.6.2.4 Water hardness

Six [cadmium, copper, nickel, lead, zinc and chromium (III)] out of 14 of the input variables were corrected for the toxicity ameliorating effect of water hardness. These six metals were adjusted for hardness because (i) their toxicity is known to be water hardness dependant, (ii) conversion algorithms are available from literature, and (iii) there are adequate toxicity data reported with water hardness.

The protection concentrations and the index reflect six water hardness levels (30, 60, 90, 150, 210 and 270 mg/L as $CaCO_3$). Where each specific hardness was selected to represent a range of water hardness (mg/L as $CaCO_3$) in the sequence outlined (Table 3.6).

Table 3.6 Water hardness ranges adopted for incorporating the modifying effects of water hardness on cadmium, chromium (III), copper, lead, nickel and zinc

Water hardness values used for representing ranges in the ATI	Water hardness range
30	<45
60	45-75
90	75-120
150	120-180
210	180-240
270	>240

Water hardness in mg/L (as $CaCO_3$)

The six water hardness ranges were generated to encompass all the water hardness variations in the Olifants River system. This implies that for the six metals stressors (whose toxicity depends on water hardness) an individual concentration could have different interpretations depending on the water hardness of the site from which the water samples were collected.

3.7 SAMPLING LOCALITIES AND FREQUENCY

River water samples were collected and macroinvertebrates surveyed from eight study sites from the Olifants River catchment. Two of the sites were located in the upper Olifants above the escarpment; S1 along the Olifants main stem just south of Witbank and S2 along the Klein Olifants River, a tributary of the Olifants River. The six remaining sites were located in the lower Olifants below the escarpment, with S3 located on the Blyde River. The Blyde River is one of the least impacted sites within the Olifants River catchment (Ballance *et al*., 2001; Swemmer and Mohlala, 2012). In this study, the Blyde was therefore used as a control site upon which the prevailing conditions from the rest of monitoring sites were compared. Sites S4, S6 and S7 were located on the Olifants River (lower Olifants), whilst S5 and S8 were on two tributaries, the Ga-Selati and the Letaba rivers, respectively (Figure 3.4).

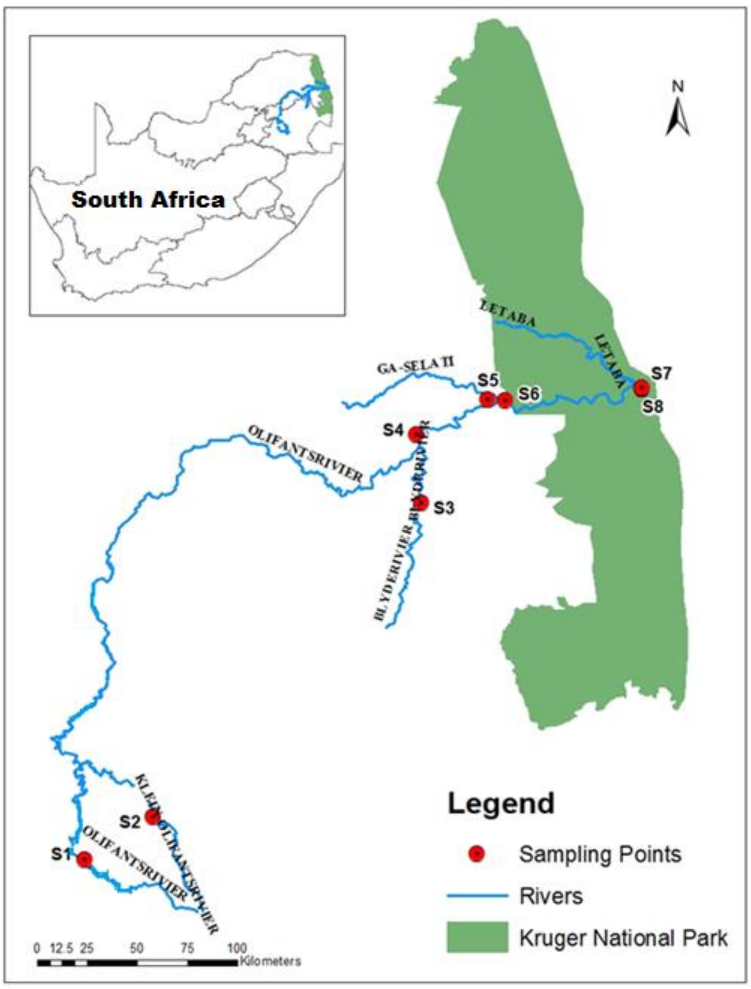

Figure 3.4 Location of study sites within the Olifants River catchment

From these sites, the water samples and ecological data were collected between September 2015 and August 2016. The first samples were collected in September 2015, followed by November 2015 during a severe drought. The November sampling was one of the least successful sampling events since in two study sites (S1 and S8) of the eight there were zero flows. In S1 (Olifants at Witbank) the site was characterised by isolated pools and no continuous flow whilst S8 (Letaba River within the Olifants/Letaba Gorge) was dry. The third sampling round was conducted in March 2016; flow had improved within the Olifants River catchment but S8 was still not flowing. There were great improvements in the water levels in the catchment for the fourth and fifth sampling sessions, in May and August 2016, respectively. However, data for S1 (Olifants at Witbank) could not be collected for both of these sessions due to accessibility issues;

water had dammed up at the site to greater than two metres depth with no measurable flow. The flow intermittency within the catchment (observed for S1, S2 and S8) over the sampling period could be attributed to the 2015/2016 hydrological drought, which was described as the worst recorded drought in Southern Africa in 35 years, driven by the strongest El Nino event in 50 years (FAO, 2016).

3.8 COLLECTION AND ANALYSIS OF PHYSICO-CHEMICAL DATA

3.8.1 *In situ* water quality variables

Water temperature (^{o}C), pH and dissolved oxygen (content and % saturation) were determined in the field, using a calibrated Professional Plus YSI multiparameter meter. River discharge (m^3/s), measured using a Sontek handheld acoustic doppler velocimeter (ADV) flow meter, was determined for study sites located in the upper Olifants catchment (S1 and S2).

3.8.2 Water chemistry

At each study site, sub-surface water samples were collected for the analysis of ammonium, nitrate, sulfate, chloride, cadmium, chromium, copper, lead, mercury, nickel and zinc. After collection all samples were filtered through a 0.45 µm pore size membrane filters and acidified with nitric acid immediately after filtration (pH <2) to keep metals in solution. All metals were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES). Similarly, water samples for the analysis of ammonium, nitrate, sulfate and chloride were filtered through 0.45 µm pore size membrane filters (*in situ*) stored in a mobile freezer at temperature of -5°C and then thawed prior to laboratory analysis (Table 3.7).

Table 3.7 Water quality variables, laboratory methods, and limits of detection

Variables	Instrument	Limit of Detection (LOD)
Dissolved oxygen, pH and temperature	Professional Plus YSI multiparameter meter	-

Nitrate, sulfate and chloride	Ion Chromatography "761 Compact IC" (Metrohm Company, Switzerland) operated with Net 2.3 software	Nitrate (0.0015 mg/L), sulfate (0.0020 mg/L) and chloride (0.0039 mg/L)
Ammonium	Spectrophotometry	0.001 mg/L
Cadmium, chromium, copper, lead, mercury, nickel and zinc	Inductively Coupled Plasma - Optical Emission Spectroscopy (ICP-OES) Spectro Genesis of Germany	Cadmium (0.010 µg/L), Chromium (0.030 µg/L), Copper (0.024 µg/L), Lead (0.08 µg/L), Mercury (0.010 µg/L), Nickel (0.022 µg/L) and Zinc (0.004 µg/L)

3.9 MACROINVERTEBRATE SURVEYS

Water samples from the eight study sites (Figure 3.4) were concurrently collected with macroinvertebrates data. The collection, identification and scoring of the macroinvertebrates followed the SASS5 protocol (Dickens and Graham, 2002). SASS5 is a rapid biomonitoring method for the sampling of riverine macroinvertebrate communities. This method was utilised because it is one of the most widely used and standardised macroinvertebrate sampling procedures in Southern Africa, which has been incorporated into South Africa's River EcoStatus Monitoring Programme (REMP). In addition to macroinvertebrate, data collected using a standardized sampling net, the SASS5 procedures compensate for limitations of the net by including visual observations and hand picking. These supplementary procedures are necessary because from the kicking and netting procedures irrespective of rigor, some organisms may not be dislodged or captured; *e.g*. Gyrinidae (Whirligig beetles) are too fast and cannot be easily captured using the net and Porifera (freshwater sponges) adhere on hard surfaces and cannot be easily dislodged by netting effort.

The SASS5 procedures have limited ability to represent the prevailing macroinvertebrates community in a site. This is particularly the case because sampling of macroinvertebrates biotopes is dependent on a limited time, *i.e*. a specified period for sampling is permitted per biotope. It is therefore imperative that inferences drawn from data collected using this protocol take into consideration its limitations and data should be treated as more of a

sample with full recognition that some taxa may have been missed. This is an important consideration in the context of this study because the macroinvertebrates were collected principally for the validation of the index, where the index evaluations were linked or compared with macroinvertebrate data summarised using the SASS5 metrics (SASS score, number of taxa and average score per taxon) and the Macroinvertebrate Response Assessment Index version 2 (Thirion, 2016).

3.9.1 Sampling difficulty

The sampling of macroinvertebrates during the l study period (2015 to 2016) was largely compromised by flow cessation and dense stands of filamentous algae and other submerged aquatic vegetation for most of the study sites. Because of flow cessation, macroinvertebrate data were collected only on two occasions from Site 1 (S1), located in the upper Olifants main stem and only three times from Site 8 (S8) located along the Letaba River within the Olifants/Letaba gorge. The dense growth of filamentous algae and other submerged aquatic vegetation hampered the use of the net used to sample the various biotopes (Figure 3.5). At any attempt, the net would fill up with algae and other submerged aquatic vegetation that would clog the net openings thereby making it difficult to fully execute the sampling. This made it difficult to separate the macroinvertebrates from the algae prior to macroinvertebrate identification. Nonetheless, prior to identification of the organisms, attempts had to be made to separate the macroinvertebrates from the algae.

All the study sites had large accumulations of filamentous algae and floating aquatic vegetation (Figure 3.5) with the exceptions of S1 (Witbank) and S3 (Blyde River) where algae only occurred in relatively smaller and isolated patches. This could be a result of the near natural state of the Blyde River (S3), which as a result the algal mats become small and patchy due to low organic nutrient accumulation in the water column. Major decreases in the abundances of the algae were observed on the third (March 2016) and fourth (May 2016) sampling sessions. Most likely, the decreases could have been attributed to the flushing and dilution effects of organic material by rainfall events that

were experienced towards the end of February to late April months (see flow hydrographs in Chapter 5).

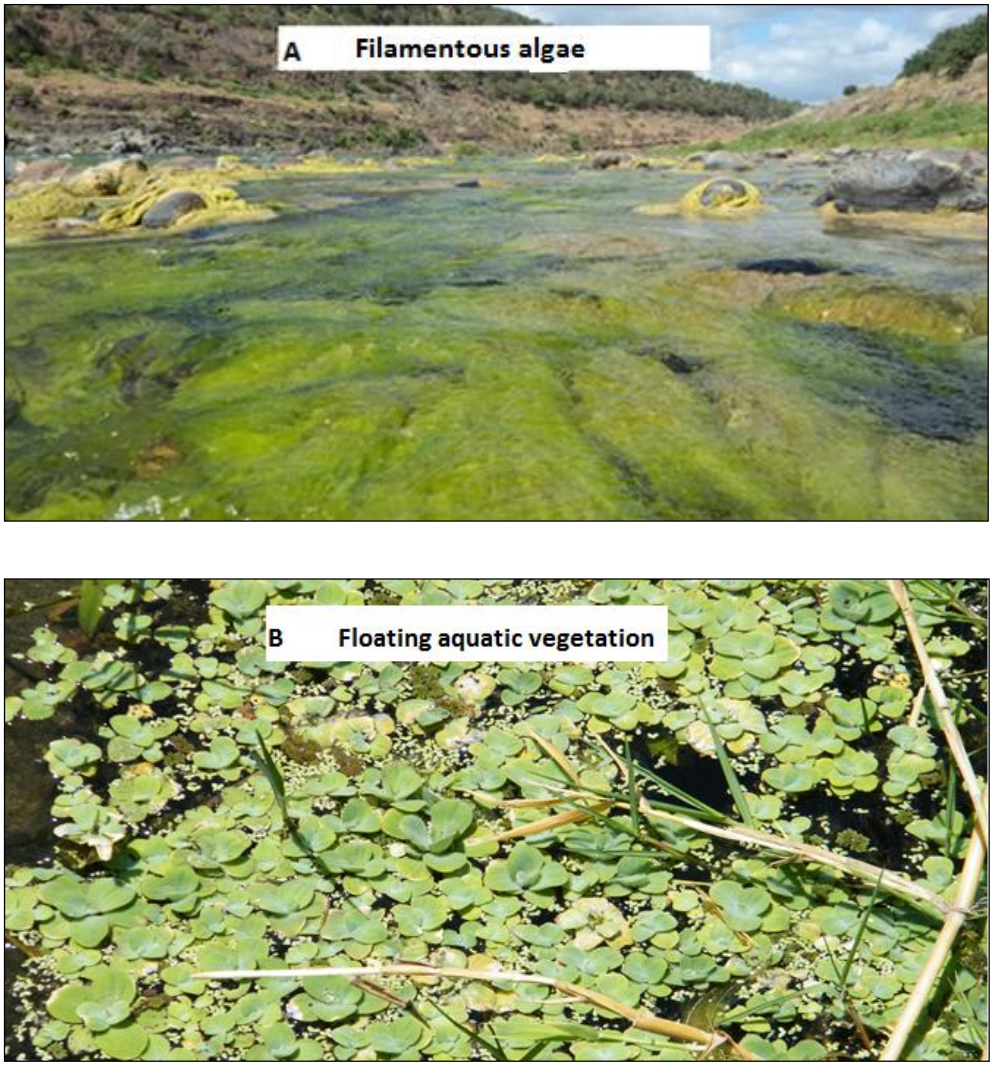

Figure 3.5 Floating aquatic vegetation and filamentous algae of the Olifants River catchment that posed sampling difficulties (physical obstructions) for macroinvertebrate sampling over the study period

3.10 STREAM FLOW CHARACTERISATION

Macroinvertebrate community composition and structure cannot only be explained by water chemistry but by a variety of stream variables and factors. It was therefore necessary to include streamflow variability in the validation phase of the index.

Streamflow variability was assessed in two principal ways; (i) flow variability between sampling sessions and (ii) the average flow rate (discharge m^3/s) at the day of data collection, to account for differences in flows between gauging stations/river reaches the average daily flow rates were standardised by the calculation of exceedance probabilities determined over the sampling period (2015 - 2016).

The effect of stream flow variability on the diversity, community structure and abundance of riverine organisms can be determined from flow characterisation. Various approaches can be utilised to characterise stream flows, the choice of one approach over the other is largely determined by data in terms of availability and quality. For example, ungauged streams and river reaches provide limited scope for the application of detailed flow variability characterisation approaches. In most cases, studies explaining the abundance and diversity of riverine organisms because of flow variability in ungauged stream reaches end up settling for the crudest approaches like dividing flows into wet and dry season flows, where wet seasons assume elevated flows and dry seasons are associated with low flows.

In this study, sampling sites for water samples and macroinvertebrates were partly influenced by the location and availability of flow gauges within the Olifants River catchment. Therefore in this study flow variability determinations for seven (S1, S2, S3, S4, S5, S6, and S7) out of the eight (S8 excl.) study sites were based on flow data obtained from the South African Department of Water and Sanitation (DWS, 2016) stream flow gauging stations (Figure 3.6).

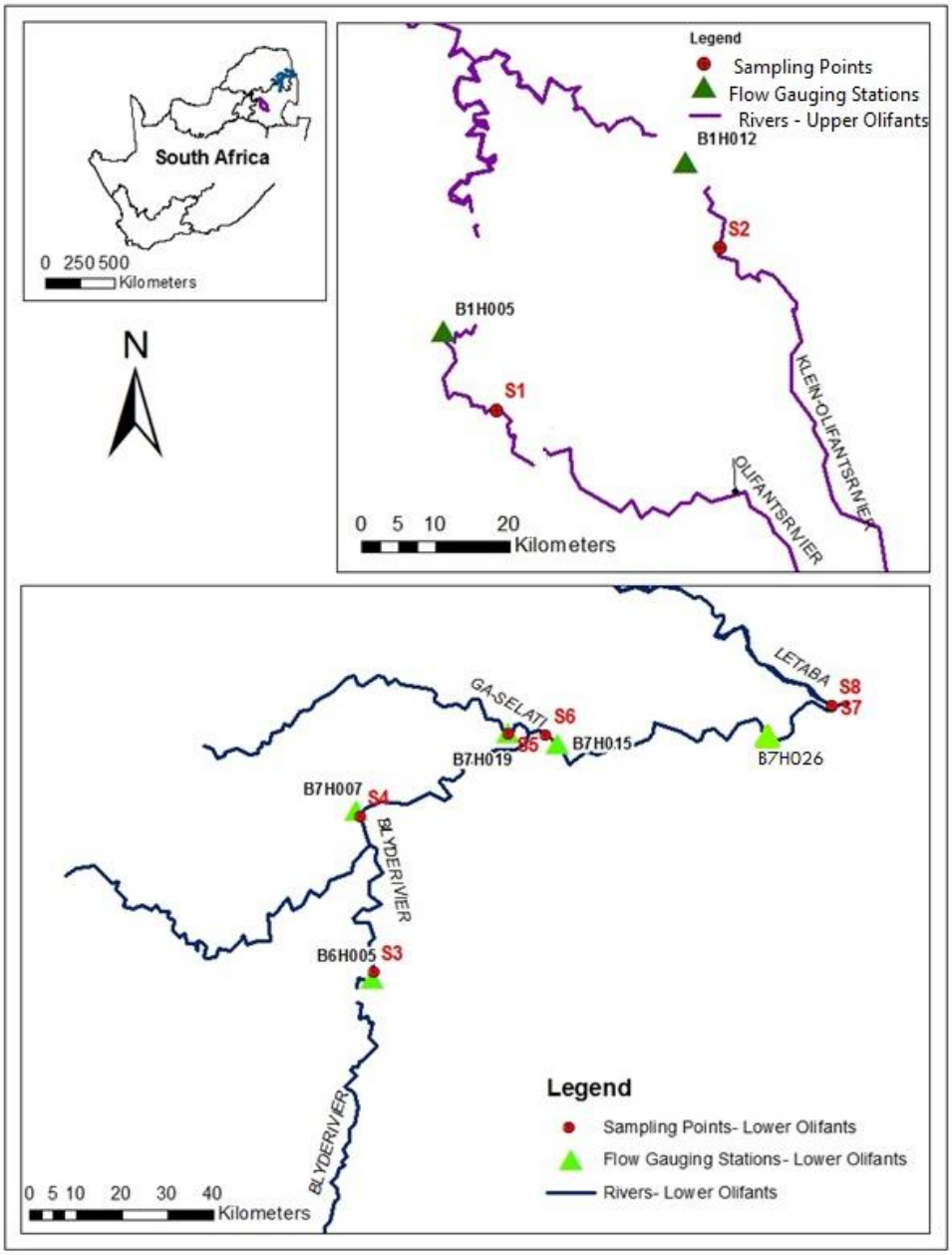

Figure 3.6 Location of sampling points in proximity with associated flow gauging stations

Gauging stations B1H005 and B1H012 (Figure 3.6) were the only flow gauges used for determining flow variability for the upper Olifants River catchment in the study. Flows from B1H005 (Olifants River at Wolwekrans) were used as flow approximates for Site 1 (S1) whilst flows for Site 2 (S2) were approximated from B1H012 (Klein Olifants River at Rondebosch). From the lower Olifants sub-catchment, flow data from five gauging stations were approximated for five corresponding study sites from two tributaries and

along the Olifants River main stem. As shown in Figure 3.6, flow data for Site S3 were obtained from B6H005 (Blyde River at Driehoek). Flows for Site S4 obtained from B7H007 (Olifants River at Oxford). Flow data for Site S5 were based on Gauging Station B7H019 located along the Ga-Selati River at Loole and flow data for Sites 6 and 7 were approximated from B7H015 (Olifants at Mamba Weir in the Kruger National Park) and B7H026 (Olifants River at Balule Weir, Kruger National Park), respectively (Table 3.8).

Table 3.8 Stream flow gauging station information sourced from the South African Department of Water and Sanitation

Study site	Gauging station	River	Location	Area (km^2)	Latitude	Longitude
S1	B1H005	Olifants	Wolwekrans	3256	-26.00655	29.25402
S2	B1H012	Klein Olifants	Rondebosch	1503	-25.80862	29.58640
S3	B6H005	Blyde	Driehoek	2204	-24.51752	30.83174
S4	B7H007	Olifants	Oxford	46583	-24.18473	30.82299
S5	B7H019	Ga-Selati	Loole	2268	-24.03642	31.12821
S6	B7H015	Olifants	Mamba Weir	49826	-24.06628	31.24288
S7	B7H026	Olifants	Balule Weir	53820	-24.05652	31.72091
S8	X	X	X	X	X	X

KNP: Kruger National Park *X: No flow approximations*

The data obtained from these sites were used in the determination of stream flow variability [base-flow Index (BFI), Coefficient of Variation Index - daily flows (CVI daily), Richard Baker Flashiness Index for daily flows (R-B flashiness daily)] were calculated for the period between sampling sessions. Given that the study had five sampling sessions, a total five sets of indices for each gauging station were determined. In addition to the determination of the flow variability (BFI, CVI and FI) based on continuous data, exceedance probabilities for each gauging station on each day of data collection were also calculated and further classified (as a function of the entire period of data collection) using the USEPA, 2007 flow classification scale.

3.10.1 Determination of flow variability in-between sampling events

3.10.1.1 Base-Flow Index

The Base-Flow Index was determined using the United States Geological Survey's (USGS) Web-based Hydrological Analysis Tool (WHAT) (USGS, 2017). The WHAT provides three base-flow separation methods; (i) the local minimum method, (ii) one parameter digital filter and (iii) the two digital filter method. The local minimum method in the WHAT system does not consider 'duration of direct runoff'. Therefore, it could overestimate the baseflow during rainy days (USGS, 2017). The one parameter digital filter method (Lyne and Hollick, 1979; Nathan and McMahon, 1990; Arnold and Allen, 1999 in Lim *et al*., 2005) was used for this study. Through this method, the flows are partitioned into high frequency (direct runoff) and low frequency (base-flow). The separation of the flow components is determined using digital filter Equation 3.8 (Lyne and Hollick, 1979; Nathan and McMahon, 1990; Arnold and Allen, 1999).

$$\mathrm{q}t \;=\; \alpha * qt-1 + \frac{(1-\alpha)}{2} * (Qt - Qt-1) \qquad \textbf{Equation 3.8}$$

Where, q_t is the filtered direct runoff at the t time step (m^3/s); q_t-1 is the filtered direct runoff at the t-1 time step (m^3/s); α is the filter parameter (0.925 selected for this study). Nathan and McMahon (1990) found that the 0.925 filter parameter gave realistic results when compared to manual separation methods. *Qt* is the total stream flow at the t time step (m^3/s); and Q_t-1 is the total stream flow at the t-1 time step (m^3/s).

Based on the hydrograph analyses outputs, the base-flow Index (BFI) for each spatio-temporal point were determined by dividing the mean daily base-flow by the mean daily total flows, as illustrated in Equation 3.9.

$$BFI \;=\; \frac{\Sigma b}{\Sigma d} \qquad \textbf{Equation 3.9}$$

Where, *BFI* is the base-flow index (dimensionless), *b* is the mean daily base-flow (m^3/s) for the entire period of interest and *d* is the mean daily flows (m^3/s) for the entire record of interest.

3.10.1.2 Coefficient of variation of daily flows

Mean hourly and mean daily river flow data for the gauging stations indicated in Figure 3.6 were obtained from the South African Department of Water and Sanitation. These data were used in the determination of the variability of the flows at the selected gauging stations, through the calculation of the Coefficient of Variation Index (CVI) for daily flows. The coefficient of variation of daily flows was calculated by dividing the standard deviation of the flows by the mean flows (Equation 3.10) (Poff, 1996).

$$CVI = \frac{Sd}{\bar{x}} \qquad \textbf{(Equation 3.10)}$$

Where, *CVI* is the coefficient of variation index, *Sd* is the standard deviation of the mean daily flows for the period under consideration and $\bar{x}$ the mean daily flows for the period under consideration.

3.10.1.3 The flashiness index of daily flows

The coefficient of variation describes stream flow variability without regard of the temporal sequence of the variations (Poff, 1996; Baker *et al*., 2004). The CVI was therefore calculated alongside the Flashiness Index (R-B FI) for daily fluctuations (Equation 3.11). The Flashiness Index (FI) is an index developed to detect changes in stream/hydrological regimes; *i.e*. it is the ratio of the day-to-day streamflow fluctuations (sums of the path lengths of the flow oscillations) relative to the total flow quantities of the duration under consideration (Baker *et al*., 2004). The R-BFI (daily) was quantified using Equation 3.11.

$$R - B\ FI_{daily} = \frac{\sum_{i=1}^{n} |q_{i-1} - q_1|}{\sum_{i=1}^{n} q_i} \qquad \textbf{Equation 3.11}$$

Where, $R\text{-}B\ FI_{daily}$ represents the Flashiness Index, q_1 and q_{i-1} are the mean daily flow rates (m^3/s) on day *i* and day *i-1*, respectively (Baker *et al*., 2004).

3.10.1.4 Mean daily flows on the particular day of data collection

To enable statistical (hydrologic) comparisons of the specific flows on the particular days of data collection, exceedance probabilities for all the flow rates on the individual days of

data collection were determined using Equation 3.12. The exceedance probabilities were computed using the Weibull plotting positions formula (Helsel and Hirsch, 1992) using Equation 3.12.

$$P = 100 * \frac{M}{(n+1)} \qquad \textbf{Equation 3.12}$$

Where*, P* is the probability that a given flow of a known magnitude will be equalled or exceeded. *M* is the ranked position of the flow rate on the particular day of data collection (as ranked from highest to lowest flow rate), where *n* is the number of records in the entire sampling period.

3.1.1.5 Zero flows

Study sites located in the upper Olifants River catchment S1 and S2, showed elements of intermittency for the entire period of data collection (393 days). For instance, S1 (gauging station: B1H005) located within the Olifants River main stem had 46% days of zero flow and S2 (gauging station B1H012) located in the Klein Olifants River had 16% days of zero flow. The presence of zero discharges within a stream flow records could be due to two main reasons; (i) the stream literally runs dry (termed true zero flow), or (ii) there is a flow in the river but below the gauging station's detection limit (described as censored zeroes) (Durrans *et al.*, 1999). With an exception of the Letaba River, study sites/gauging stations located in the lower Olifants River had no zeroes in their stream flow records.

3.10.1.6 Treatment of zero flow records in the study

In this study, stream flow data were used for characterising stream flow patterns between sampling sessions (stream flows before sampling events) and for comparing flow magnitudes at points of data collection. For the former case, all zero flows were treated as true zeroes and for the latter they either were treated as true zeroes or censored zeroes. For the point of data collection, physical stream flow determinations conducted out as means of comparing stream gauge readings with the physical stream flow determination using the flow meter. Therefore, on the particular day of data collection, zero flow was only accepted if both the stream flow records and the physical

determination agreed (*e.g*. second sampling session at S1/B1H005; November 2015). In cases where the stream flow records indicated zero flow whilst the flow meter indicated a non-zero flow, the stream record was rejected in favour of the flow meter reading, assuming the zero flow to have occurred because of the flow being below the gauging station's threshold of measurement (*e.g.* second sampling session at S2/B1H012, November 2015).

3.11 SENSITIVITY ANALYSIS

The sensitivity analysis for the ATI for macroinvertebrates was conducted to assess the relative influence/importance/impact of each water quality variable on the composite ATI. This was conducted following the '*leave-one-out approach'*. Through this method, it is possible to determine the relative influence of an individual water quality variable on the composite ATI (calculated from 14 water quality variables). The leave-one-out approach was conducted as follows:

1. Calculation of the composite ATI using water quality data collected from the Olifants River.

2. Removal of each water quality variable (or group of variables) one by one and the recalculation of the ATI resulting from the removal of each variable (reduced indices). This leads to a two-way data split: the composite ATI and the resultant indices from the removal of individual water quality variable or groups of variables.

3. Assessment of the relative influence of each stressor on the composite ATI. This process involved the determination of the strength of the relationships between the indices resulting from the removal of the water quality variables with the composite ATI using the Spearman rank correlation. Low correlation coefficients were interpreted to mean that the composite ATI was less sensitive to the inclusion of the removed variable.

3.12 EXPLORATORY DATA ANALYSIS

3.12.1 Summary statistics

All physico-chemical and riverine macroinvertebrates data were tested for normality using the Shapito-Wilks test, subjected to summary statistical analysis and presented using box-plots in R-studio (R-Core Team, 2015). In this study, box-plots were plotted to provide a graphical presentation of the summary statistics of physico-chemical and invertebrates data measured in the Olifants River between September 2015 and August 2016. The thick lines in the box-plots represent the median values (50^{th} percentile), boxes the 25^{th} (lower quartile) and 75^{th} (upper quartile) percentiles, whiskers represent the maximum and minimum values within 1.5 times the inter-quartile range and small circle symbols represent the outliers beyond this threshold.

3.12.2 Correlation analysis

Correlation analysis is the statistical evaluation of the strength and direction of relationships between variables. In this study correlation analysis was conducted for th assessment of relationships between ATI for macroinvertebrates (this study), SASS5 metrics, measures of flow variability and ATI (Wepener *et al*., 1992). The correlation analysis outputs were presented in a scatter plot matrix containing all pairwise plots (under analysis) shown alongside histograms, kernel density overlays and significance level asterisks. Where, one asterisk (*) denotes $p<0.05$, two asterisks (**) for $p<0.01$ and three asterisks (***) if $p<0.001$.

3.12.3 Principal Component Analysis

Principal Component Analysis (PCA) is a multivariate statistical analysis technique, applied to reduce dimensionality of a data set consisting of a large number of inter-related variables (Vanhatalo and Kulahci, 2016), while retaining as much as possible the variability present in data set (Kebede and Kebedee, 2012). In this study, the PCA bi-plot was plotted using the Multivariate Statistical Package (MVSP) version 3.1 (Kovach, 1998). The bi-plot was interpreted to mean, 90 degrees between vectors indicates that two variables are uncorrelated; zero or 180 degrees between two vectors was interpreted

to mean complete positive or negative correlation as previously interpreted by Buehler *et al*. (2012) and Gerber *et al*. (2015).

3.12.4 Cluster analysis

Cluster Analysis (CA) is a multivariate statistical method, which aims to group a collection of patterns into clusters based on similarity such that objects in the same clusters are similar as possible and those from different clusters are as dissimilar as possible (Irani *et al*., 2016). In this study, the CA was employed for clustering the magnitudes of water quality variables according to sampling months and study sites (based on percentage similarity) and for clustering sampling sites based on riverine macroinvertebrate composition (Sorensen's similarity coefficient). All the CA were performed in the Multivariate Statistical Package (MVSP) version 3.1 (Kovach, 1998).

3.12.5 Inter-rater reliability

Inter-rater reliability (IRR) analysis is a statistical measure for determining how much homogeneity or consensus there is among different raters of the same subject (Wang *et al*., 2015). Traditionally (before 1960), the agreement between raters was measured using percentage agreement (McHugh, 2012). The major limitation of percentage agreement as a measure of IRR is that it lacks the ability to account for chance agreement (McHugh, 2012). In response, Cohen (1960) introduced the Cohen's Kappa, a tool developed to account for the possibility that raters actually guess on at least some variables due to uncertainty and therefore a more robust measure than simple percentage agreement. The IRR of the macroinvertebrates indices used in the study was computed using the Fleiss Kappa, a version/variation of the Cohen Kappa suitable for two or more raters (Fleiss, 1971). Fleiss Kappa is a statistical measure for assessing the reliability of agreement between a fixed number of raters when assigning categorical ratings to a number of items or classifying items (Durivage, 2015; Latha, 2017). The IRR Kappa Fleiss was performed between the ATI for macroinvertebrates and the SASS5 metrics and between the ATI for macroinvertebrates and the MIRAI v2. The Kappa Fleiss was performed in R-studio (R-Core Team, 2015) using the kappa for 'm' raters function [$kappam.fleiss$].

CHAPTER 4: INDEX DEVELOPMENT

4.1 INTRODUCTION

The development of the ATI for macroinvertebrates followed two major development steps. First, the derivation of PCs, upon which the ATI is founded, for each of the 14 environmental stressors to freshwater macroinvertebrates using the SSD models. Second, the generation of sub-indices and index categories using an approach modified from Wepener *et al*., 2006. Given that the derivation of water quality benchmarks/PCs for the protection of aquatic organisms is a process associated with uncertainties, the PCs derived in this study were further compared with those from water quality jurisdictions and scholarly publications.

4.1.1 Cadmium

Ecotoxicity data for cadmium to freshwater macroinvertebrates were collected from literature (see Appendix 3), converted to dissolved-cadmium and adjusted to six water hardness levels using the USEPA conversion algorithms (USEPA, 2017). The toxicity values ranged over four orders of magnitude. The Mean (geometric) Acute Value (MAV) for the freshwater shrimps belonging to the genus *Atyaephy* was the lowest toxicity value (most sensitive taxa to the short-term exposure to cadmium), while the MAVs for the damselfly nymphs belonging to the genus *Ischnura* was the highest MAV (most tolerant taxa to Short-term exposure to cadmium) (Table 4.1).

Table 4.1 Short-term LC50 data (µg/L) for cadmium to freshwater macroinvertebrate genus' collected from scientific publications and adjusted to different water hardness

Genus	Hardness (mg/L) as $CaCO_3$						Genus	Hardness (mg/L) as $CaCO_3$					
	30	60	90	150	210	270		30	60	90	150	210	270
Asellus	83.41	159.6	233.18	375.81	514.48	650.40	*Viviparu*	247.48	473.56	691.91	1115.11	1526.57	1929.86
Chironomus	197.79	378.48	552.99	891.22	1220.07	1542.39	*Varichaeta*	2062.02	3945.64	5764.92	9291.01	12719.27	16079.51
Amnicola	3407.32	6519.84	9526.06	15352.62	21017.54	26570.06	*Biomphalaria*	91.80	175.65	256.64	413.61	566.23	715.82
Niphargus	726.91	1390.92	2032.26	3275.28	4483.81	5668.37	*Lumbriculus*	73.58	140.80	205.72	331.55	453.89	573.80
Macrobrachium	11.21	21.45	31.34	50.51	69.15	87.42	*Orconectes*	6943.81	13286.85	19413.25	31287.26	42831.85	54147.39
Gammarus	7.41	14.18	20.72	33.40	45.72	57.80	*Lirceus*	30.46	58.29	85.17	137.26	187.91	237.56
Atyaephyra	6.14	11.76	17.18	27.68	37.89	47.91	*Branchiura*	5821.46	11139.25	16275.43	26230.20	35908.8	45395.38
Hyalella	6.66	12.74	18.61	29.99	41.06	51.91	*Afrochiltonia*	116.59	223.09	325.96	525.33	719.17	909.17
Ranatra	78.6	150.39	219.74	354.14	484.81	612.89	*Paratya*	302.91	579.61	846.87	1364.85	1868.46	2362.08
Enallagma	16658.28	31875.30	46572.61	75058.48	102754.07	129900.19	*Aedes*	341.74	653.92	955.43	1539.81	2107.98	2664.88
Dugesia	2531.93	4844.80	7078.67	11408.3	15617.82	19743.82	*Atalophlebia*	2448.4	4684.97	6845.15	11031.95	15102.59	19092.47
Nais	43.56	83.34	121.77	196.25	268.67	339.65	*Rhithrogena*	6590.61	12611	18425.78	29695.8	40653.16	51393.13
Trichodrilus	323.88	619.74	905.50	1459.34	1997.81	2525.60	*Ischnura*	67913.97	129952.08	189871.4	306005.15	418917.02	529588.76
Tubifex	311.39	595.84	870.58	1403.07	1920.78	2428.22	*Paraleptophlebia*	203.33	389.07	568.47	916.17	1254.22	1585.57
Lymnaea	73.16	140.00	204.55	329.66	451.3	570.53	*Macromia*	502.81	962.12	1405.74	2265.55	3101.51	3920.88
Radix	241.9	462.87	676.29	1089.94	1492.11	1886.30	*Melanoides*	2350.87	4498.35	6572.49	10592.51	14501.01	18331.96
Dendrocoelum	8142.73	15580.96	22765.15	36689.32	50227.19	63496.48	*Aplexa*	62.73	120.03	175.37	282.64	386.92	489.14
Caecidotea	301.06	576.07	841.69	1356.5	1857.03	2347.63	*Girardia*	520.25	995.49	1454.49	2344.13	3209.08	4056.87
Barytelphusa	498.42	953.72	1393.46	2245.77	3074.43	3886.64	*Pristina*	69.05	132.12	193.04	311.11	425.90	538.42

Procambarus	3423.67	6551.13	9571.78	15426.31	21118.41	26697.58	*Hexagenia*	2386.55	4566.61	6672.22	10753.25	14721.05	18610.14

The PC for freshwater macroinvertebrates to dissolved cadmium were estimated by fitting SSD curves (Burr Type III Distribution) to 40 toxicity values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The six SSD curves were fitted with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.1).

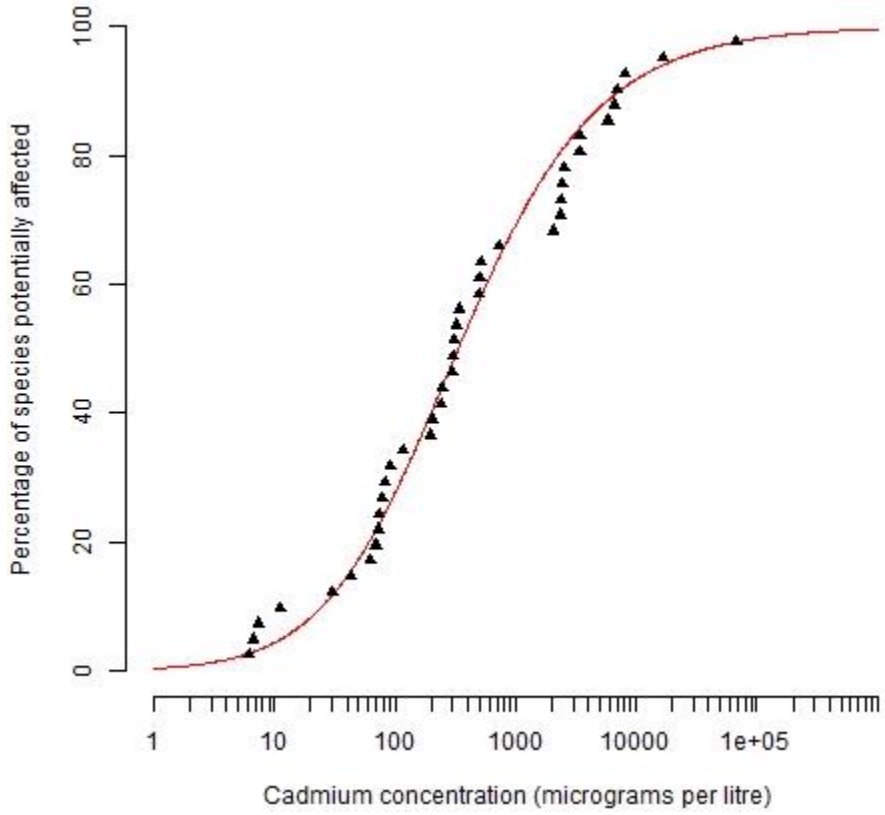

Figure 4.1 SSD (Burr Type III Distribution) for dissolved cadmium fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The fit of the SSD curves on the toxicity values adjusted for the six different water hardness levels were identical. Therefore, only one graph instead of six was presented in this report as a means of illustrating how the SSD model fit into the toxicity data. The four PCs with 95% confidence intervals (in parentheses) are presented in Table 4.2.

Table 4.2 Estimated PCs with 95% confidence intervals (in parenthesis) for dissolved cadmium to freshwater macroinvertebrates indicating four levels of water hardness

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	2.3 (0.038, 14)	4.4 (0.095,23)	6.4 (0.14, 33)	10 (0.23, 55)	14 (0.31, 75)	18 (0.39,95)
PC95	11 (2.5, 37)	22 (5.1,63)	32 (7.5,92)	51 (12,148)	70 (17,202)	89 (21,256)
PC90	25 (11, 64)	47 (22, 117)	69 (32,170)	111 (51,274)	153 (70,376)	193 (89,471)
PC80	61 (27, 138)	116 (54,268)	169 (79,392)	273 (127,632)	374 (173,866)	473 (216,1082)

As expected, the estimated PC increased with increasing water hardness, conforming with the underlying reasoning behind the ameliorating effects of water hardness on the toxicity of cadmium to freshwater organisms.

4.1.2 Chromium (III)

Throughout the literature, the toxicity of Cr (III) to aquatic ecosystems has been shown to be dependent on the ambient water chemistry (ANZECC and ARMCANZ, 2000; USEPA, 1984; USEPA, 1995). Given that, the conversion algorithms for the toxicity dependency of Cr (III) on other water quality constituents have been published by some water quality jurisdictions (*e.g.* USEPA and ANZECC and ARMCANZ). Similarly, in this study, the bioassay data for individual macroinvertebrates were adjusted to reflect the modifying effects of water hardness on the toxicity of Cr (III) to freshwater macroinvertebrates (Table 4.3). The input data into the SSD ranged over two orders of magnitude. The MAV for mayfly nymphs belonging to the genus *Ephemerela* was the lowest toxicity value (most sensitive taxa to the short-term exposure to chromium III), while the MAVs for isopods belonging to the genus *Asellus* was the highest MAV (most tolerant taxa to Short-term exposure to chromium (III) (Table 4.3).

Table 4.3 Short-term LC50 ecotoxicity data (µg/L) for dissolved chromium (III) to freshwater macroinvertebrates collected from scientific publications and adjusted to six water hardness levels

Genus	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
Gammarus	665.49	1174.05	1636.46	2486.56	3275.50	4024.09
Zygoptera	8963.35	15812.98	22041.06	33490.89	44116.95	54199.45
Chironomus	2287.63	4035.80	5625.33	8547.56	11259.55	13832.81
Amnicola	2122.48	3744.44	5219.22	7930.49	10446.69	12834.18
Nais	1934.09	3412.08	4755.96	7226.57	9519.44	11695.01
Ephemerella	461.84	814.77	1135.67	1725.63	2273.14	2792.65
Hydropsyche	14778.88	26072.65	36341.59	55220.21	72740.61	89364.75
Crangonyx	60518.19	106765.15	148815.53	226121.77	297866.21	365940.57
Asellus	91921.10	162165.62	226035.96	343456.44	452429.09	555827.26

Boldface: *denotes higher taxonomic rank*

The PCs for freshwater macroinvertebrates to dissolved Cr (III) were estimated by fitting SSD curves (Inverse Weibull Distribution) to nine toxicity values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The six SSD curves were fitted with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.2).

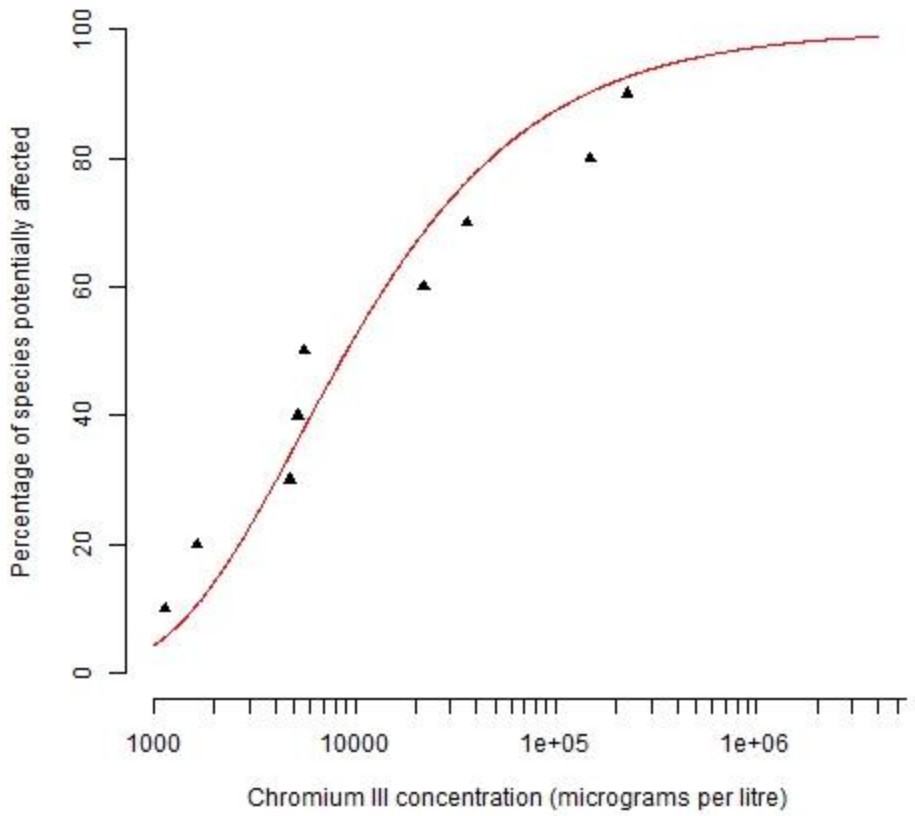

Figure 4.2 SSD (Inverse Weibull Distribution) for dissolved chromium (III) fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The fit of the SSD curves on the toxicity values adjusted for the six different water hardness levels (30, 60, 90, 150, 210 and 270) were identical. Therefore, only one graph instead of six graphs was presented in this report as means of illustrating how the SSD model fit into the toxicity data. The four PCs with 95% confidence intervals are presented in Table 4.4.

Table 4.4 Estimated PCs with 95% confidence intervals (in parenthesis) for dissolved chromium (III) to freshwater macroinvertebrates

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	23 (124, 1049)	411 (219,1851)	573 (305, 2580)	871 (485,3992)	1147 (611,5164)	1409 (750,6344)
PC95	436 (244,1539)	770 (430,2715)	1073 (600, 3784)	1630 (970,621)	2147 (1201, 7573)	2638 (1475,9304)
PC90	640 (360,2112)	1130 (636,3726)	1575 (886,5194)	2393 (1378, 8085)	3152 (1773,10396)	3872 (2179,12772)
PC80	1080 (564,3280)	1905 (995,5786)	2655 (1387, 8065)	4034 (2235,14180)	5314 (2775, 16143)	6528 (3410,19833)

4.1.3 Chromium (VI)

The toxicity of dissolved chromium Cr (VI) is dependent on pH and water hardness (DWAF, 1996). Due to the absence of pH and hardness conversion algorithms to account for the effect of the two toxicity-modifying factors, the levels of protection for Cr (VI) in this study could not be adjusted to reflect the effect of any water quality constituents. Consequently, the Cr (VI) PCs and the subsequent index are not based on unadjusted threshold values (Table 4.5). The MAV for amphipod belonging to the genus *Gammarus* was the lowest toxicity value (most sensitive taxa to the short-term exposure to chromium III), while the MAVs for the stonefly nymphs belonging to the genus *Agnetina* was the highest MAV (most tolerant taxa to Short-term exposure to chromium (VI) (Table 4.5).

Table 4.5 Short-term LC50 ecotoxicity data (µg/L) for dissolved chromium (VI) to freshwater macroinvertebrates collected from scientific publications and aggregated to mean acute values

Taxa	MAVs (µg/L)	Taxa	MAVs (µg/L)
Tanytarsus	56268.60	*Viviparus*	2160.40
Echinogammarus	451.72	*Anculosa*	2252.96
Hyalella	325.69	*Biomphalaria*	64545.56
Macrobrachium	628.48	*Lymnaea*	5862.54
Asellus	14730.00	*Planorbella*	31424.00
Gammarus	124.15	*Elimia*	2356.80
Orconectes	172832.00	*Burnupia*	3700.00
Hydropsyche	274960.00	*Physa*	28160.88
Agnetina	1836340.00	*Dugesia*	16780.42
Stenonema	3437.00	*Aeolosoma*	7958.59
Enallagma	137480.00	*Lumbriculus*	20258.73
Chironomus	39949.31	*Tubifex*	6988.09
Radix	3810.16		

The PCs for Cr (VI) to freshwater macroinvertebrates were estimated by fitting an SSD curve (Burr Type III Distribution) on the MAVs LC50 data. The SSD curve was fitted to over 25 MAVs representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD curve was fitted with an aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.3).

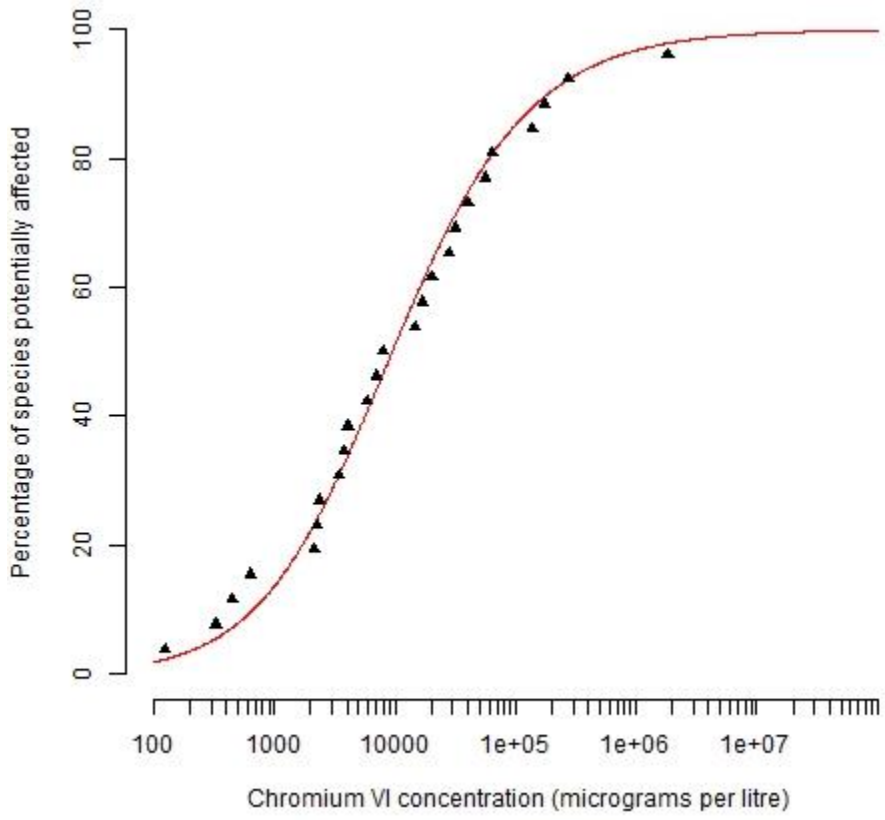

Figure 4.3 SSD (Burr Type III Distribution) for dissolved chromium (VI) fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The resultant SSD outputs were four levels of protection (PC99, PC95, PC90 and PC80). For each level of protection, the SSD software generated three values, Guideline Value (GV), lower 95% confidence Interval (LCI) and the Upper 95% confidence Interval (UCI). These values for each level of protection are presented in the format GV (LCI, UCI), where PC99 (µg/L) = 51 (0.83, 604), PC95 (µg/L) = 292 (50, 1296), PC90 (µg/L) = 667 (222, 2165) and PC80 (µg/L) = 1699 (667, 4583).

4.1.4 Copper

In the past, the toxicity of copper to freshwater organisms has widely been expressed by various water quality jurisdictions (*e.g*. South Africa, Australia, New Zealand, Canada, and the USA) to reflect the toxicity ameliorating effects of water hardness on copper (USEPA, 2007). Most recently published water quality guidelines and criteria have shifted

from water hardness as a sole toxicity modifier into incorporating other potentially influential modifiers. The USEPA water quality criteria for copper for instance, are based on the BLM (USEPA, 2017). The BLM is a mechanism understood to better account for the bioavailability of copper to freshwater organisms than water hardness. Similarly, the ANZECC (2017) chronic water quality guidelines for freshwater life are expressed to reflect the modifying effects of Dissolved Organic Carbon (DOC) instead of water hardness. Incorporation of the BLM was not feasible in this study owing to data unavailability to allow for the full execution of the BLM, while the modifying effects of DOC on copper could not be incorporated owing to uncertainties related to the relationships between DOC and acute effects (Short-term exposures) of copper on freshwater life. It is for these reasons that this study could only incorporate the modifying effects of water hardness to copper rather than any other published toxicity modifiers (Table 4.6).

Table 4.6 Short-term LC50 ecotoxicity data (µg/L) for dissolved copper to freshwater macroinvertebrates collected from publications, adjusted to six water hardness levels (mg/L as $CaCO_3$) and aggregated to mean acute values

Taxa	Hardness (mg/L) as $CaCO_3$						Taxa	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**		**30**	**60**	**90**	**150**	**210**	**270**
Macrobrachium	29.35	56.40	82.64	133.73	183.62	232.68	*Gyraulus*	131.22	252.14	369.44	597.82	820.83	1040.14
Chironomus	134.01	257.49	377.29	610.52	838.26	1062.22	*Pomacea*	12.45	23.93	35.07	56.74	77.91	98.72
Biomphalaria	12.35	23.73	34.77	56.27	77.25	97.9	*Viviparus*	16.58	31.85	46.67	75.52	103.69	131.39
Lumbriculus	37.44	71.95	105.42	170.59	234.22	296.8	*Anculosa*	260.7	500.92	733.98	1187.7	1630.76	2066.45
Amnicola	1716.36	3297.91	4832.28	7819.48	10736.42	13604.9	*Physa*	13.32	25.60	37.51	60.69	83.33	105.59
Gammarus	20.96	40.27	59.01	95.48	131.1	166.13	*Basommatophora*	68.51	131.64	192.88	312.12	428.55	543.04
Hyalella	20.50	39.38	57.7	93.38	128.21	162.46	*Radix*	8.95	17.19	25.19	40.76	55.97	70.92
Penaeus	261.60	502.65	736.51	1191.8	1636.39	2073.59	*Lymnaea*	3.73	7.16	10.5	16.99	23.32	29.56
Asellus	1642.58	3156.15	4624.56	7483.36	10274.92	13020.1	*Corbicula*	4173.35	8018.91	11749.75	19013.18	26105.77	33080.53
Aedes	1672.99	3214.58	4710.18	7621.9	10465.14	13261.15	*Aeolosoma*	1189.6	2285.76	3349.23	5419.64	7441.36	9429.49
Dugesia	511.37	982.58	1439.73	2329.73	3198.81	4053.44	*Planorbella*	77.16	148.26	217.25	351.54	482.68	611.64
Limnodrilus	123.5	237.3	347.71	562.66	772.55	978.95	*Stenonema*	133.18	255.9	374.96	606.75	833.09	1055.67
Nais	18.29	35.15	51.5	83.33	114.42	144.99	*Isonychia*	65.56	125.97	184.58	298.69	410.11	519.68
Tubifex	19.78	38	55.69	90.11	123.72	156.78	*Juga*	20.15	38.72	56.74	91.81	126.06	159.73
Caridina	829.47	1593.79	2335.31	3778.95	5188.63	6574.89	*Fluminicola*	10.75	20.65	30.26	48.96	67.23	85.19
Hydropsyche	53.71	103.21	151.23	244.71	336	425.77	*Orconectes*	828.97	1592.83	2333.91	3776.68	5185.51	6570.94
Hexagenia	17.6	33.82	49.56	80.2	110.11	139.53	*Melanoides*	209.59	402.72	590.09	954.87	1311.08	1661.36
Ephoron	11.97	23	33.7	54.53	74.87	94.88	*Paratya*	103.72	199.3	292.02	472.54	648.81	822.16
Drunella	167.52	321.88	471.64	763.2	1047.9	1327.88	*Girardia*	254.42	488.85	716.3	1159.1	1591.48	2016.67

Rhithrogena	91.68	176.16	258.12	417.68	573.49	726.71	*Elimia*	90.45	173.79	254.64	412.05	565.77	716.92

The PCs for copper were derived from toxicity values ranging over three orders of magnitude, where the MAV for pond snails belonging to the genus *Basommatophora* was the lowest (most sensitive taxa to copper) while the MAV for freshwater clams belonging to the genus *Corbicula* was the least sensitive genera to the short-term exposue to copper. The SSD curves (Inverse Weibull Distribution) were fitted to 40 toxicity values for freshwater macroinvertebrates, representing four groups of macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD curves were fitted with the aid of Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.4).

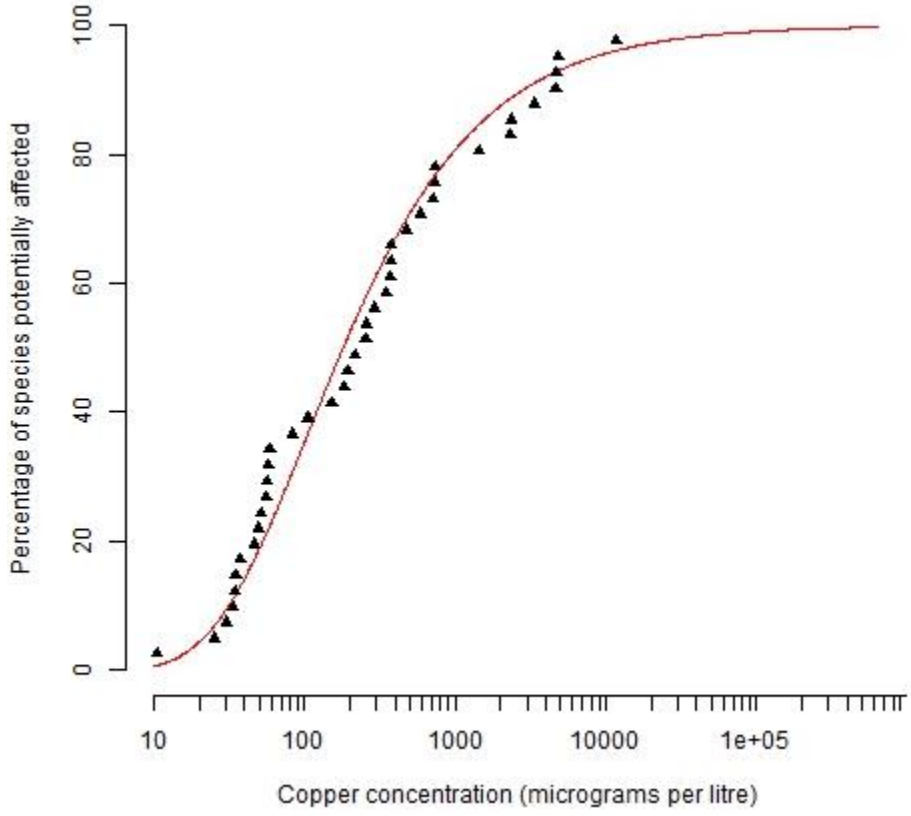

Figure 4.4 SSD (Inverse Weibull Distribution) for dissolved copper fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The outputs from the SSD curves were four levels of protection (PC99, PC95, PC90 and PC80). These levels were estimated to reflect the dependency of copper on water

hardness. Presented in Table 4.7 are the four levels of protection for each of the six water hardness-adjusted thresholds.

Table 4.7 Estimated PCs with 95% confidence intervals (in parenthesis) for dissolved copper to freshwater macroinvertebrates

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	4.1 (2.6,7.5)	7.8 (4.9,14)	11 (7.2,21)	19 (12,34)	25 (16,47)	32 (20,58)
PC95	7.6 (5.2,13)	15 (9.9,25)	21 (15,37)	35 (24,59)	48 (32,81)	60 (40,101)
PC90	11(7.8,19)	21(15,36)	31 (22,53)	51 (35,86)	70 (49,118)	88 (60,148)
PC80	19 (13,32)	36 (25,61)	53 (37,89)	85 (59,144)	117 (81,197)	149 (101,243)

4.1.5 Lead

The toxicity of lead to freshwater organisms is widely expressed to reflect the modifying effects of water hardness (USEPA, 1985; ANZECC and ARMCANZ, 2000). In this study therefore, all toxicity data collected for deriving the PCs of lead to freshwater macroinvertebrates were adjusted to reflect the modifying effects of water hardness on the toxicity of lead. The toxicity data input for the SSD ranged over two orders of magnitude. The most sensitive invertebrate taxa to the short-term to lead were the giant freshwater prawns belonging to the genus *Macrobrachium* while the freshwater crayfish belonging to the genus *Procamburus* were the least sensitive taxa to lead exposure (Table 4.8).

Table 4.8 Short-term LC50 ecotoxicity data for lead to freshwater macroinvertebrates collected from scientific publications adjusted to six water hardness levels and aggregated to mean acute values

Taxa	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
Lymnaea	1686.41	3649.51	7065.86	9909.28	14188.99	18490.82
Elimia	8552.49	18508.25	35833.99	50254.21	71958.45	93774.85
Corbicula	102511.99	221844.02	429514.19	602358.12	862510.03	1124006
Dugesia	80701.03	174643.37	338128.58	474197.37	678998.05	884857
Paratelphusa	2357.33	5101.44	9876.94	13851.59	19833.94	25847.21
Macrobrachium	73.96	160.05	309.87	434.57	622.26	810.91
Lumbriculus	610.4	1320.96	2557.51	3586.7	5135.76	6692.82
Asellus	2480.48	5367.96	10392.96	14575.26	20870.16	27197.58
Procambarus	716598.76	1550776.09	3002471.43	4210717.84	6029281.01	7857241
Chironomus	1595.77	3453.37	6686.11	9376.71	13426.42	17497.04
Enallagma	136642.86	295705.9	572518.83	802910.3	1149678.52	1498239
Viviparus	280.24	606.45	1174.16	1646.66	2357.84	3072.69
Aplexa	525.82	1137.91	2203.12	3089.69	4424.09	5765.39
Nais	1082.44	2342.48	4535.3	6360.38	9107.36	11868.53
Tubifex	33643	72806.09	140960.52	197685.47	283063.68	368883.1

The standardised LC50 values for the MAVs show an inverse relationship between water hardness and toxicity of lead to freshwater macroinvertebrates, where an increase in water hardness is associated with a decrease in the toxicity of lead. The PCs for the invertebrates were estimated by fitting SSD curves (Inverse Weibull Distribution) to 15 toxicity values (Figure 4.5) representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs).

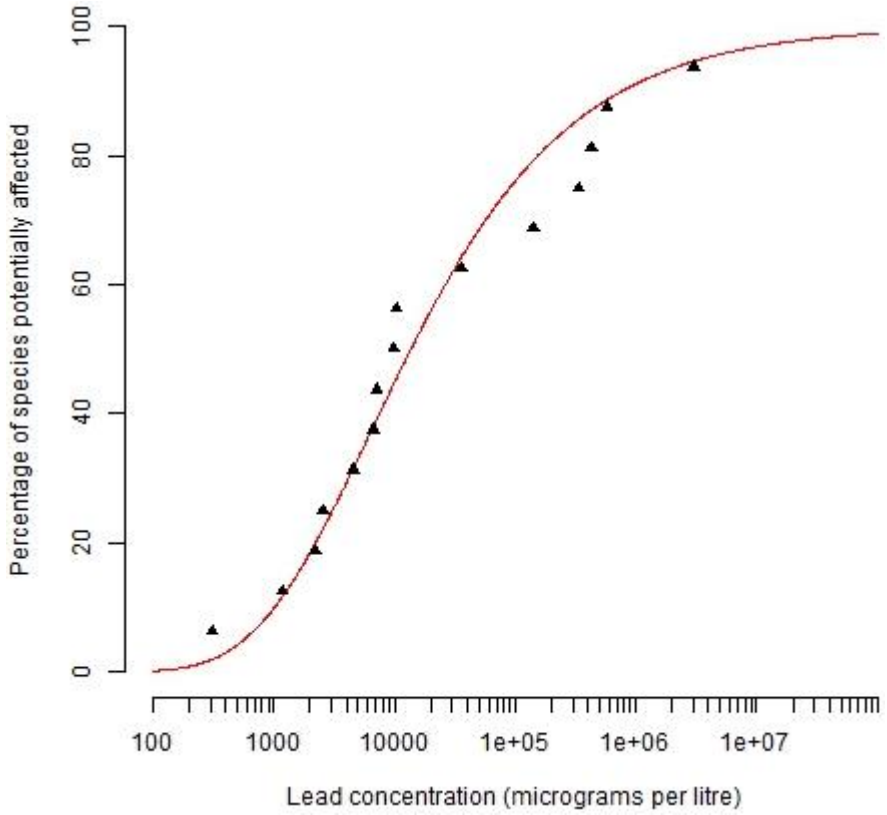

Figure 4.5 SSD (Burr Type III Distribution) for dissolved lead fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

SSD curves were fitted on data normalised for each water hardness level. This resulted in six SSDs, each with four levels of protection (Table 4.9). Given that the SSD graphs were identical, only one graph was presented in this report, for displaying how the SSD curve fits into the LC50 MAVs data.

Table 4.9 Estimated PCs with 95% confidence intervals (in parenthesis) for dissolved lead to freshwater macroinvertebrates

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	54 (17,302)	111 (7.7,653)	182 (57,1022)	304 (56,1777)	454 (143,2545)	573 (148,3317)
PC95	137 (49,611)	293 (90,1292)	461 (164,2063)	797 (260,3588)	1149 (410,5138)	1487 (493,6696)
PC90	241 (88,970)	524 (187,2094)	814 (296,3276)	1423 (510,5697)	2028 (737,8158)	2647 (935,10631)
PC80	522 (190,1950)	1147 (412,4575)	1764 (642,6589)	3110 (1116,12198)	4392 (1598,16410)	5770 (2082,22094)

4.1.6 Inorganic mercury

The toxicity of mercury to aquatic organisms is not expressed to reflect dependency on ambient water quality. Therefore, the PCs for inorganic mercury were not adjusted to reflect the dependency on ambient water chemistry. The SSD input data for inorganic mercury ranged over four orders of magnitude. The black clams belonging to the genus *Villorita* had the lowest MAV, constituting the most sensitive taxa while the stonefly nymphs belonging to the genus *Pteronarcys* had the highest MAV (least sensitive taxa) Presented in Table 4.10 are mean acute values indicating geometric means for median Lethal Concentrations (LC50) for comparable toxicity data.

Table 4.10 Short-term LC50 ecotoxicity data (µg/L) for inorganic mercury to freshwater macroinvertebrates collected from scientific publications and aggregated to mean acute values

Taxa	MAVs (µg/L)	Taxa	MAVs (µg/L)	Taxa	MAVs (µg/L)
Acroneuria	1700	*Ephemerella*	1700.00	*Macrobrachium*	107.92
Aedes	5545.23	*Erpobdella*	201.45	*Nais*	136.00
Aplexa	314.50	*Gammarus*	569.50	*Nemoura*	107.10
Asellus	125.80	*Girardia*	34.00	*Oligochaeta*	195.50
Austropotamobius	17.00	*Gymnometriocnemus*	204.00	*Orconectes*	42.50
Barytelphusa	581.49	*Hyalella*	21.25	*Paramerina*	357.00
Branchiura	430.07	*Hydropsyche*	1700.00	*Paratelphusa*	308.58
Caridina	44.84	*Ilyodrilus*	246.50	*Peloscolex*	280.50
Chironomus	274.32	*Ilyoplax*	25.50	*Poecilobdella*	1194.67
Clunio	238.00	*Ischnura*	6471.05	*Procambarus*	287.54
Culex	1190.00	*Laccotrephes*	671.50	*Pteronarcys*	49300
Culicoides	30.89	*Larsia*	331.50	*Quistadrilus*	212.50
Cypris	110.50	*Limnodrilus*	255.00	*Radix*	16.15
Dugesia	85.00	*Lumbriculus*	85.00	*Rhyacodrilus*	204.00
Echinogammarus	425.00	*Lymnaea*	19.55	*Spirosperma*	425.00
Stylodrilus	119.00	*Varichaeta*	85.00	*Tubifex*	290.91
Tanypus	606.77	*Villorita*	1.33	*Viviparus*	221.00

PCs for freshwater macroinvertebrates against elevated concentrations of inorganic mercury were estimated by fitting a SSD curve (Burr Type III Distribution) using the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.6). The SSD curve was based on 51 toxicity values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs).

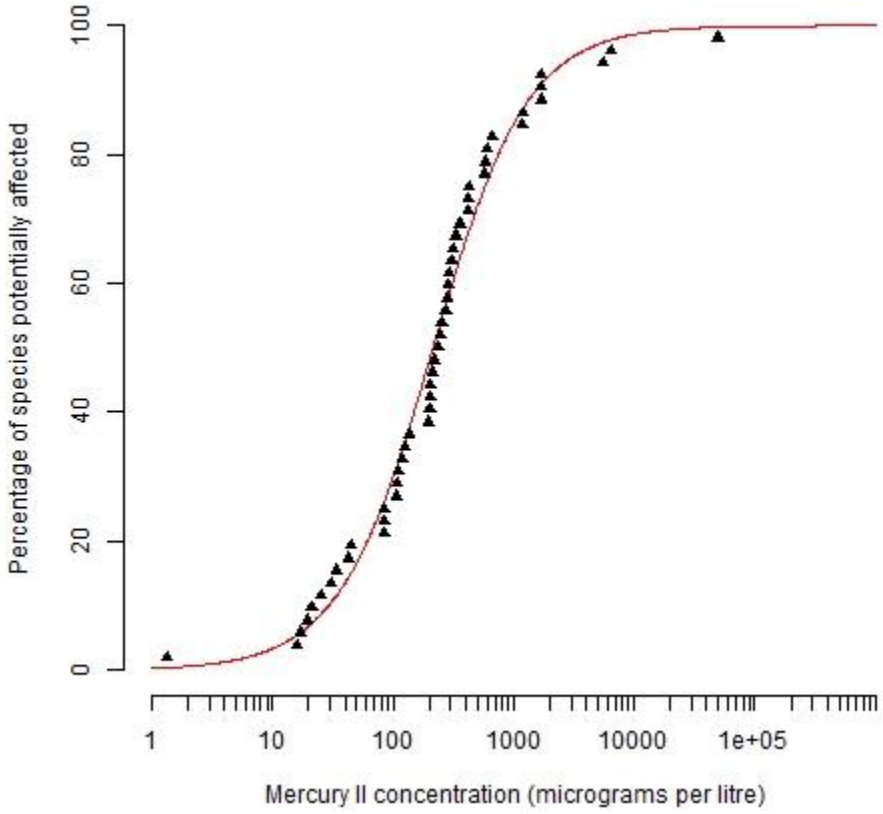

Figure 4.6 SSD (Burr Type III Distribution) for dissolved mercury (II) fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

Each of the four levels of protection is assigned three values; (i) Guideline Value (GV), which is more of the central tendency of the prediction band, (ii) lower 95% confidence interval (LCI), most conservative value among the three, and (iii) the Upper 95% confidence interval (UCI) value, presenting the least conservative guideline value among the three. The specific out values for each level of protection are presented in the format GV (LCI, UCI), where PC99 (µg/L) = 3.2 (0.58, 16), PC95 (µg/L) = 15 (5.5, 38), PC90 (µg/L) = 29 (14, 58) and PC80 (µg/L) = 61 (36,101).

4.1.7 Nickel

The toxicity of nickel to freshwater ecosystems is widely expressed (*i.e.* by water quality jurisdictions) to reflect the ameliorating effects of water hardness. Water hardness adjustments algorithms for Ni are available from various water quality jurisdictions (*e.g.* ANZECC and ARMCANZ (2000) and USEPA (1996). In this study, for consistency, the USEPA (1996) algorithms were utilised for hardness adjustments. Toxicity data for the study were collected from scientific publications. The toxicity data for nickel ranged over two orders of magnitude. The freshwater snails belonging to the genus *Juga* constituted the most sensitive taxa to nickel while the Isopods belonging to the genus *Asellus* constituted the least sensitive taxa to nickel. Presented in Table 4.11 are mean acute values corrected to six water hardness levels.

Table 4.11 Short-term LC50 ecotoxicity data (µg/L) for nickel to freshwater macroinvertebrates collected, adjusted to six water hardness levels (mg/L as $CaCO_3$) and adjusted to mean acute values

Taxa	**Hardness (mg/L) as $CaCO_3$**					
	30	**60**	**90**	**150**	**210**	**270**
Zygoptera	13733.54	24686.17	34787.8	53593.36	71241.87	88119.40
Gammarus	19144.73	34412.85	48494.66	74709.86	99312.12	122839.62
Chironomus	7165.91	12880.79	18151.64	27964.03	37172.7	45979.09
Amnicola	9794.27	17605.3	24809.42	38220.88	50807.17	62843.63
Asellus	24998.25	44934.62	63321.95	97552.48	129676.9	160397.96
Hyalella	330.10	593.36	836.16	1288.18	1712.38	2118.05
Viviparus	2173.24	3906.42	5504.93	8480.78	11273.54	13944.29
Planorbella	923.7	1660.35	2339.77	3604.6	4791.62	5926.77
Dugesia	6560.65	11792.83	16618.48	25602.08	34032.95	42095.51
Lumbriculus	9523.33	17118.28	24123.11	37163.56	49401.66	61105.15
Lymnaea	327.48	588.66	829.53	1277.96	1698.8	2101.25
Nais	3017.49	5423.97	7643.47	11775.37	15653.05	19361.33
Tubifex	8354.35	15017.02	21162.02	32601.77	43337.66	53604.55
Radix	292.92	526.52	741.97	1143.06	1519.48	1879.45
Juga	189.56	340.73	480.16	739.73	983.32	1216.28

The PCs were derived by fitting SSD curves (Burr Type III Distribution) to 15 mean acute values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD curves were fitted using the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.7).

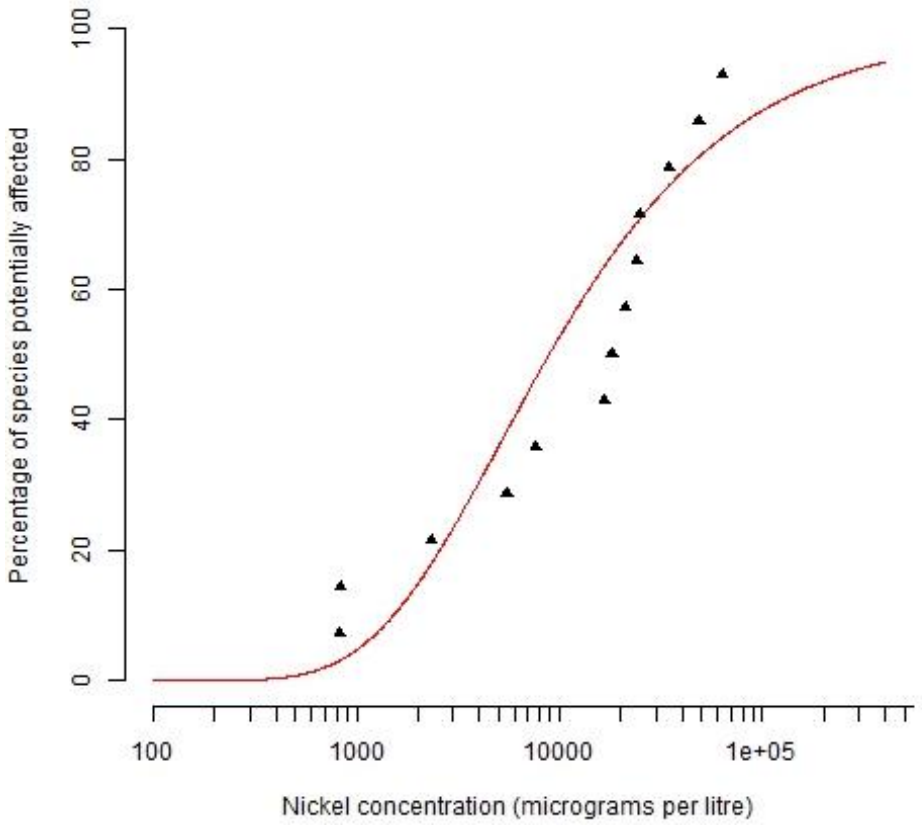

Figure 4.7 SSD (Burr Type III Distribution) for dissolved nickel fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The SSD curves were fitted on each water hardness level, thereby producing six SSD graphs. The graphs were identical throughout the six water hardness levels, therefore only one SSD graph was plotted for showing the graphical fit of the model on the LC50 data. Based on the SSDs four levels of protection (PC99, PC95,PC90 and PC80) for each reference water hardness level were produced, with each having three values ranging from the most conservative (lower 95% confidence interval) to the least conservative value (upper 95% confidence interval) (Table 4.12).

Table 4.12 Estimated PCs with 95% confidence intervals (in parenthesis) for dissolved nickel to freshwater macroinvertebrates

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	17 (4.7,1671)	389 (195,2996)	548 (275,4223)	845 (423,6505)	1123 (563,8647)	1389 (696,10696)
PC95	194 (86,2343)	732 (360,4049)	1031 (507,5706)	1589 (782,8791)	2112 (1039,11685)	2613 (1285,14454)
PC90	559 (278, 2943)	1077 (521, 4757)	1518 (734, 6703)	2339 (1131, 10326)	3110 (1504, 13727)	3846 (1860, 16979)
PC80	1612 (480, 3959)	1824 (863,6522)	2570 (1216, 9191)	3960 (1874, 14160)	5264 (2491,18822)	6511 (3081,23282)

4.1.8 Zinc

The toxicity of zinc to freshwater organisms has been widely reported to be dependent on ambient water chemistry. Most water quality jurisdictions have recommended water quality criteria and guidelines that are hardness dependent (*e.g.* South Africa, Australia, New Zealand, Canada, British Columbia and the United States). However, the latest published water quality guidelines for zinc in freshwaters (chronic) have been derived to account for the modifying effects of both water hardness and pH (ANZECC, 2017). Owing to uncertainties relating to the relationships between pH and the acute effects of zinc to freshwater ecosystems, the levels of protection derived in this study could only account for the ameliorating effects of water hardness on freshwater macroinvertebrates (Table 4.13).

Table 4.13 Short-term LC50 ecotoxicity data (µg/L) for zinc to freshwater macroinvertebrates from scientific publications, adjusted to six water hardness levels and aggregated to mean acute values

Taxa	Hardness (mg/L) as $CaCO_3$					
	30	60	90	150	210	270
Gammarus	13.41	24.12	34.01	52.43	69.72	86.26
Chironomus	11492.01	20675.63	29151.5	44940.04	59765.11	73947.89
Amnicola	10668.57	19194.15	27062.69	41719.91	55482.71	68649.25
Nais	4016.73	7226.63	10189.15	15707.62	20889.33	25846.56
Echinogammarus	2121.9	3817.58	5382.57	8297.79	11035.11	13653.84
Ranatra	529.51	952.66	1343.19	2070.67	2753.76	3407.25
Atyaephyra	1212.06	2180.66	3074.61	4739.83	6303.43	7799.28
Paratya	2728.96	4909.75	6922.47	10671.7	14192.14	17560.06
Penaeus	407.47	733.09	1033.61	1593.42	2119.07	2621.94
Macrobrachium	62.93	113.22	159.64	246.1	327.28	404.95
Caecidotea	3655.53	6576.77	9272.89	14295.11	19010.86	23522.3
Asellus	10651.59	19163.6	27019.63	41653.53	55394.42	68540.01
Lirceus alabamae	2071.07	3726.13	5253.63	8099.02	10770.77	13326.76
Hyalella	167.11	300.65	423.9	653.48	869.06	1075.3
Chloroperlidae	42849.63	77092.09	108695.6	167565.4	222842.8	275725.4
Argia	248206.7	446556.3	629619.7	970623.4	1290818	1597141
Drunella	40740.02	73296.62	103344.2	159315.7	211871.6	262150.6
Baetis	7382.9	13282.8	18728.01	28871.16	38395.33	47506.88
Rhithrogena	35429.7	63742.66	89873.64	138549.4	184254.9	227980.2
Cinygmula	42849.63	77092.09	108695.6	167565.4	222842.8	275725.4
Ephemerella	42849.63	77092.09	108695.6	167565.4	222842.8	275725.4
Planorbella	1001.3	1801.47	2539.98	3915.63	5207.34	6443.09
Radix	792.14	1425.16	2009.4	3097.69	4119.58	5097.19
Physa	568.28	1022.41	1441.54	2222.28	2955.38	3656.71
Elimia	3301.8	5940.38	8375.6	12911.85	17171.28	21246.18
Lymnaea	1206.99	2171.53	3061.73	4719.98	6277.03	7766.62
Viviparus	137.36	247.13	348.44	537.16	714.37	883.89
Anculosa	1988.91	3578.31	5045.22	7777.73	10343.49	12798.1
Corbicula	3129.32	5630.06	7938.08	12237.36	16274.29	20136.32
Nephelopsis	5891.97	10600.42	14946.01	23040.81	30641.64	37913.17
Aeolosoma	11007.4	19803.76	27922.21	43044.95	57244.86	70829.57
Limnodrilus	3526.17	6344.05	8944.76	13789.26	18338.14	22689.94
Girardia	1941.54	3493.08	4925.06	7592.48	10097.13	12493.27
Lumbriculus	1695.36	3050.18	4300.58	6629.79	8816.87	10909.19
Tubifex	7038.35	12662.92	17854.01	27523.79	36603.5	45289.83
Dugesia	7375.17	13268.9	18708.41	28840.93	38355.14	47457.16

The collected toxicity values for zinc ranged over four orders of magnitude. The amphipods belonging to the genus *Gammarus* constituted the most sensitive genera to zinc while the damselfly nymphs belonging to the genus *Argia* constituted the least sensitive invertebrate taxa to the short-term exposure to zinc. The PCs for zinc were estimated by fitting SSD curves (Burr Type III Distribution) to 36 mean acute values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD curves were fitted with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) see Figure 4.8.

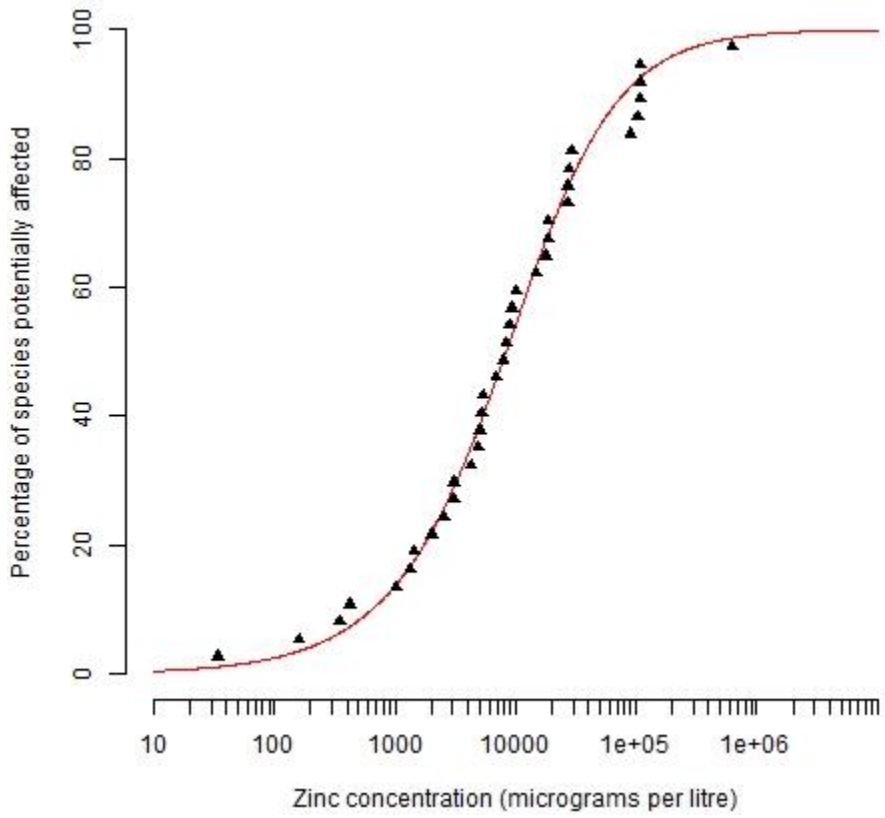

Figure 4.8 SSD (Burr Type III Distribution) for zinc fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

SSD curves were plotted on each of the data sets of toxicity data representing six levels of water hardness. Since the six SSD curves were identical, only one curve was shown in this report. Based on the SSDs, four levels of protection (PC99, PC95,PC90 and PC80) for each reference water hardness level were produced, with each having three values

ranging from the most conservative (lower 95% confidence interval) to the least conservative value (upper 95% confidence interval) (Table 4.14).

Table 4.14 Estimated PCs with 95% confidence intervals (in parentheses) for dissolved zinc to freshwater macroinvertebrates

Levels of Protection (µg/L)	Hardness (mg/L) as $CaCO_3$					
	30	**60**	**90**	**150**	**210**	**270**
PC99	12 (0.59, 178)	21 (1.1, 31)	29 (1.5, 600)	45 (2.4, 943)	59 (3.6, 1253)	74 (4.5,1549)
PC95	99 (19, 430)	178 (34, 798)	250 (48, 1161)	387 (75, 1771)	511 (107, 2305)	634 (128, 2854)
PC90	254 (81,742)	457 (146, 1265)	643 (206,1755)	994 (318, 2684)	1317 (423, 3628)	1632 (517, 4480)
PC80	683 (318, 1438)	1229 (561,2485)	1730 (798, 3460)	2671 (1181, 5258)	3545 (1486, 7159)	4388 (1748, 8871)

4.1.10 Ammonia/ammonium

The toxicity of ammonia to freshwater macroinvertebrates is known to be dependent on water temperature and pH. Empirical relationships between the toxicity of ammonia and water temperature and pH have been established and are widely used among different water quality jurisdictions (DWAF, 1996; CCME, 2010; USEPA, 2013). For this study ammonia data were adjusted to water temperature = 25ºC and pH = 8 reflecting the joint toxicity of ionised and unionised ammonia expressed as nitrogen (TAN). All ammonia adjustments for reference temperature and pH were conducted using the USEPA (1999) and USEPA (2009) conversion procedures.

Table 4.15 Short-term LC50 ecotoxicity for TAN to freshwater macroinvertebrates collected from scientific publications adjusted to pH = 8 and temperature = 25°C and aggregated to mean acute values

Genus	**MAVs TAN (mg /l) at pH = 8, temperature** = 25 **°C**	**Genus**	**MAVs TAN (mg/l) at pH = 8, temperature = 25°C**
Asellus	66.06	*Macrobrachium*	40.91
Baetis	103.43	*Orconectes*	172.96
Callibaetis	46.1	*Pachydiplax*	36.46
Chironomus	159.79	*Paratya*	11.53
Corbicula	4.33	*Philarctus*	84.96
Crangonyx	21.81	*Physa*	22.51
Dendrocoelum	18.37	*Planorbella*	26.75
Drunella	68.09	*Potamopyrgus*	9.36
Gammarus	15.21	*Procambarus*	21.59
Hyalella	3.18	*Scylla*	41
Lumbriculus	58.42	*Turbifex*	33.3

The toxicity data for TAN (after conversions) ranged over one order of magnitude, where freshwater amphipods belonging to the genus *Hyalella* constituted the most sensitive macroinvertebrate taxa to TAN and the freshwater crayfish belonging to the genus *Orconectes* constituted the least sensitive invertebrate genera to TAN. The PCs for TAN were estimated by fitting a SSD curve (Burr Type III Distribution) to 22 mean acute values, representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD curve was fitted using the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.9).

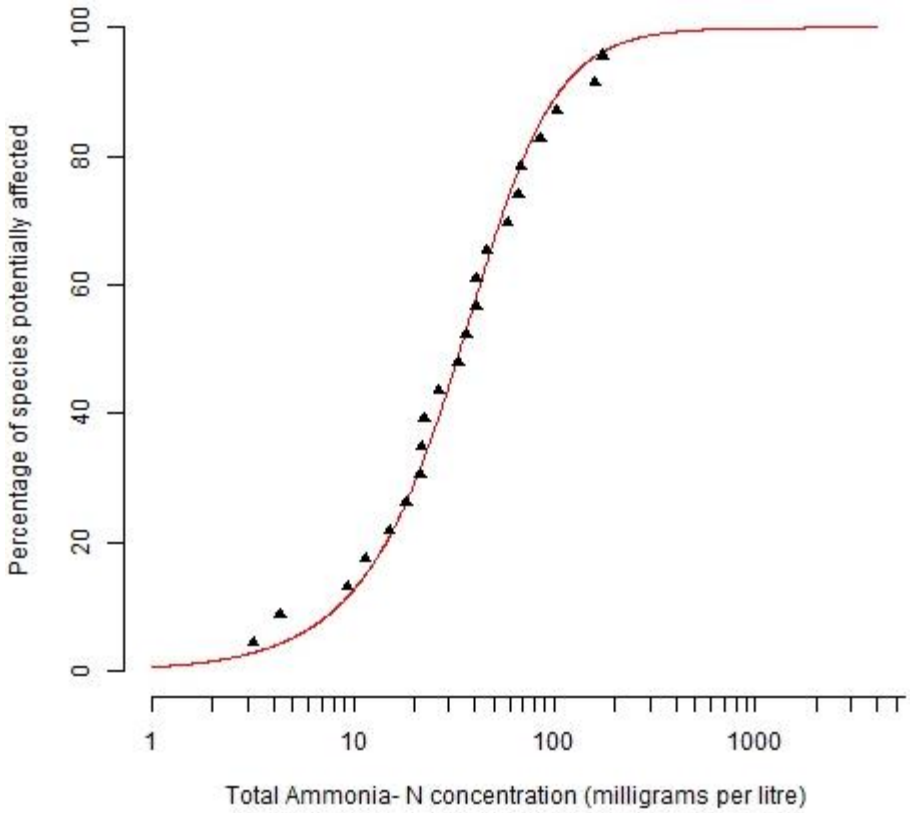

Figure 4.9 SSD (Burr Type III Distribution) for dissolved TAN fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The PCs were estimated for four levels of protection (PC99 to PC80). For each of the PCs the software generated three values; (i) the Guideline Value (GV), (ii) the Lower 95% Confidence (LCI) value, and (iii) the Upper 95% Confidence Interval (UCI). These values were presented in the format GV (LCI, UCI), where, PC99 (mg/L) = 1.4 (0.2, 9.6), PC95 (mg/L) = 4.9 (1.9, 14), PC900 (mg/L) = 8.4 (4.1, 17) and PC80 (mg/L) = 15 (8.1, 24).

4.1.11 Nitrate

The toxicity of nitrate to freshwater organisms has been reported to be strongly influenced by water hardness (Elphick, 2011 cited in CCME, 2012) and on ionic strength (Baker *et al.*, 2017). The PCs for nitrates in this study were not adjusted to account for any toxicity modifier, owing mainly to uncertainties and the absence of conversion algorithms. Toxicity data for nitrate ranged over one order of magnitude. The caddisfly nymphs belonging to the genus *Hydropsyche* constituted the most sensitive genera to nitrate while mud snails

belonging to the genus *Potamorpyrgus* constituted the least sensitive taxa to the short-term exposure to nitrate (Table 4.16).

Table 4.16 Short-term LC50 ecotoxicity for nitrate-nitrogen (mg/L) to freshwater macroinvertebrates collected from scientific publications and aggregated to mean acute values

Taxa	**MAVs (mg/L)**
Hyalella	124.2
Hydropsyche	100.21
Cheumatopsyche	140.47
Lymnaea	733.86
Potamopyrgus	1042
Chironomus	278
Lampsilis	357.11
Sphaerium	371.11
Amphinemura	455.98
Allocapnia	835.89
Megalonaias	937.02

PCs for nitrate on freshwater macroinvertebrates were estimated by fitting a SSD curve (Inverse Pareto Distribution) to 11 toxicity values representing three groups of freshwater macroinvertebrates (crustaceans, insects and molluscs). Annelids were however not represented in the SSD due to data constraints. The SSD curve was fitted using the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.10).

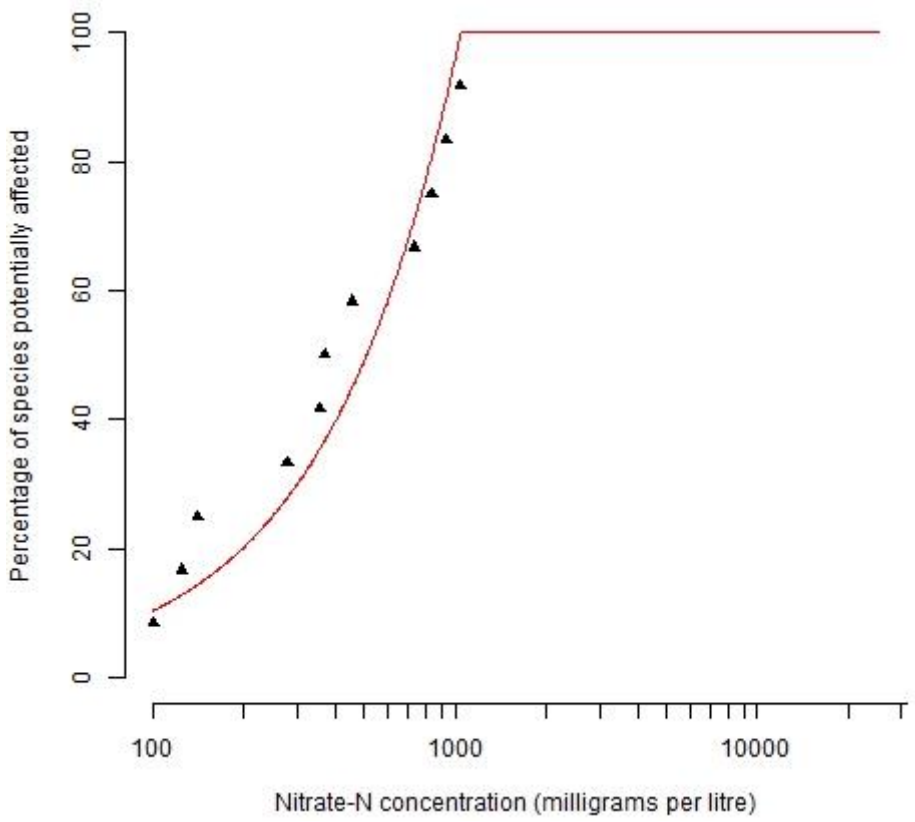

Figure 4.10 SSD (Inverse Pareto Distribution) for dissolved nitrate fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The PCs generated were for four levels of protection (PC99 to PC80). For each of the four levels of protection the software generated three values; (i) Guideline Value (GV), (ii) Lower 95% Confidence Interval (LCI), and (iii) Upper 95% Confidence Interval (UCI). These values were presented in the format GV (LCI, UCI), where, PC99 (mg/L) = 8.8 (0.0036, 84), PC95 (mg/L) = 47 (0.29, 196), PC90 (mg/L) = 96 (1.9, 288) and PC80 (mg/L) = 197(13, 442).

4.1.12 Chloride

The Short-term median lethal data for chloride on freshwater macroinvertebrates were obtained from different sources of ecotoxicity data. The data ranged over one order of magnitude, where the amphipods belonging to the genus *Hyalella* constituted the most

sensitive genera to chloride and the damselfly nymphs belonging to the genus *Argia* constituted the least sensitive taxa to the short-term exposure to chloride (Table 4.17).

Table 4.17 Short-term (24-96 hours) median lethal concentrations (LC50) data ecotoxicity data for chloride to freshwater macroinvertebrate taxa collected from scientific publications and aggregated to mean

Taxa	MAVs (mg/L)	Taxa	MAVs (mg/L)
Lirceus	2970	*Callibaetis*	5000
Lymnaea	3388	*Acroneuria*	10000
Tipula	10000	*Tricorythus*	1503
Agnetina	10000	*Afronurus*	6290
Culex	10200	*Oligoneuriopsis*	4815
Cricotopus	6221	*Baetis*	1569
Planorbella	6150	*Demoreptus*	4370
Gammarus	7700	*Cloeon*	4853
Gyraulus	3700	*Enallagma*	22965
Hyalella	1382	*Caridina*	8568
Chaoborus	5000	*Plea*	6741
Hydropsyche	9000	*Asellus*	6488
Erpobdella	7500	*Isonychia*	2758
Limnodrilus	6800	*Physa*	4234
Stenonema	2500	*Gyraulus*	3095
Chironomus	5867	*Nephelopsis*	4280
Argia	24000		

Chloride toxicity data were not standardised to reflect site-specific water quality conditions or toxicity modifying factors, as it is a procedure with certain metals. While some provincial water quality jurisdictions in Canada (British Columbia) and the US (State of Iowa) (Nagpal *et al.*, 2003 and Iowa, 2009, respectively), have already recommended more site-specific water quality criteria or guidelines for chloride by incorporating the modifying effects of ambient water chemistry. In this study, attempts to derive site-specific trigger values proved futile, because of inadequate availability of key bioassay data to enable the adjustments (*e.g.* water hardness and sulfate concentrations).

PCs for chloride to freshwater macroinvertebrates were estimated by fitting an SSD curve (Inverse Weibull Distribution) to 33 mean acute toxicity values representing four groups of freshwater macroinvertebrates (crustaceans, insects, annelids and molluscs). The SSD was fitted with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.11).

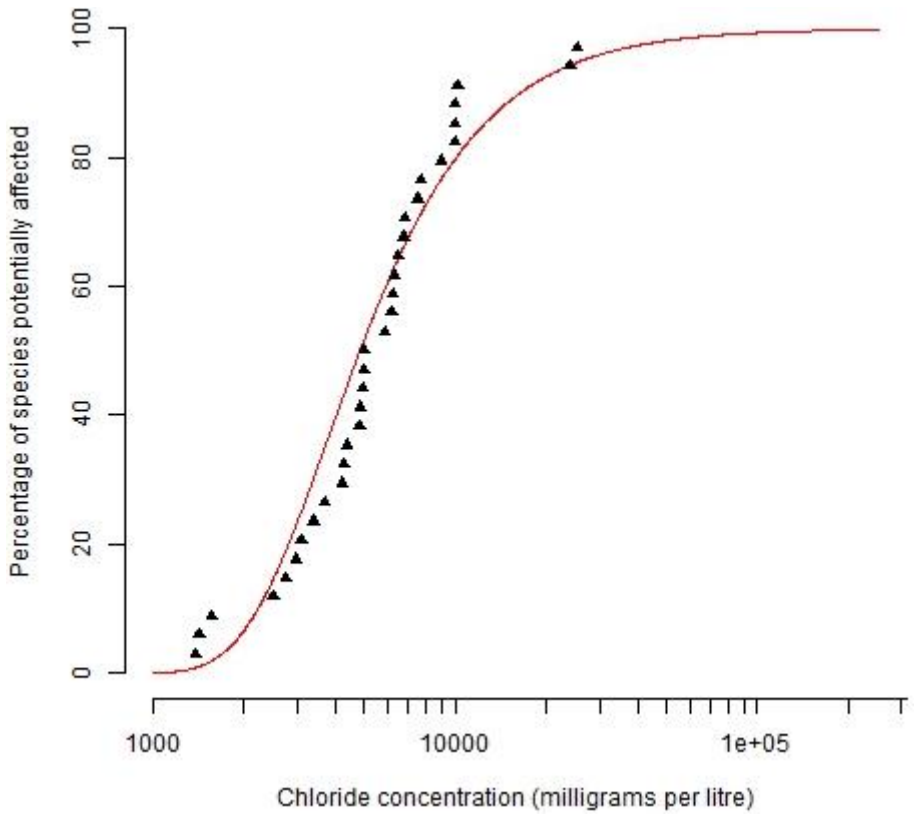

Figure 4.11 SSD (Inverse Weibull Distribution) for chloride fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

The SSD outputs were four levels of PCs ranging from PC99 to PC80. For each level of protection, three values - Guideline Value (GV), Lower 95% Confidence Interval (LCI) and Upper 95% confidence interval (UCI) - produced by the SSD software and are presented in the format GV (LCI,UCI). Where, PC99 (µg/L) = 1420 (1063, 2364), PC95 (µg/L) = 1875 (1433, 2855), PC90 (µg/L) = 2222 (1720, 3198) and PC80 (µg/L) = 2802 (2203, 3782).

4.1.13 Sulfate

Relatively few water quality jurisdictions have recommended water quality criteria or guidelines for sulfate to freshwater ecosystems. The available water quality criteria for sulfate are site-specific, derived to reflect the ambient water chemistry (*e.g*. chloride and water hardness). Ecotoxicity data for sulfate from literature were limited and where available relatively few bioassays had additional water chemistry data (chloride and water hardness) to enable necessary corrections. Therefore, the guideline values and subsequent index derived in this study are more default, relating to no specified ambient water chemistry. Presented in Table 4.18 are the mean acute values aggregated (geometric mean) from comparable short-term median lethal concentrations (LC50). The toxicity data for sulfate ranged over one order of magnitude, where water bugs belonging to the genus *Plea* constituted the most sensitive taxa to sulfate and damselfly nymphs belonging to the genus *Enalagma* constituted the least sensitive genera.

Table 4.18 Short-term LC50 ecotoxicity for sulfate to freshwater macroinvertebrates collected from scientific publications and aggregated to mean acute values

Taxa	MAVs (mg/L)
Adenophlebia	8351
Afroptilum	2848
Caddisflies	10546
Caridina	6820
Chironomus	14134
Cloeon	3369
Culex	13350
Enallagma	29760
Hyalella	1998.51
Lampsilis	2362
Lymnaea	5400
Plea	306.58
Tricorythus	660

The PCs for sulfate were estimated by fitting a SSD distribution curve (Inverse Weibull Distribution) on 13 mean acute values, representing three groups of freshwater

macroinvertebrates (crustaceans, insects and molluscs). The SSD curve was fitted with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.12).

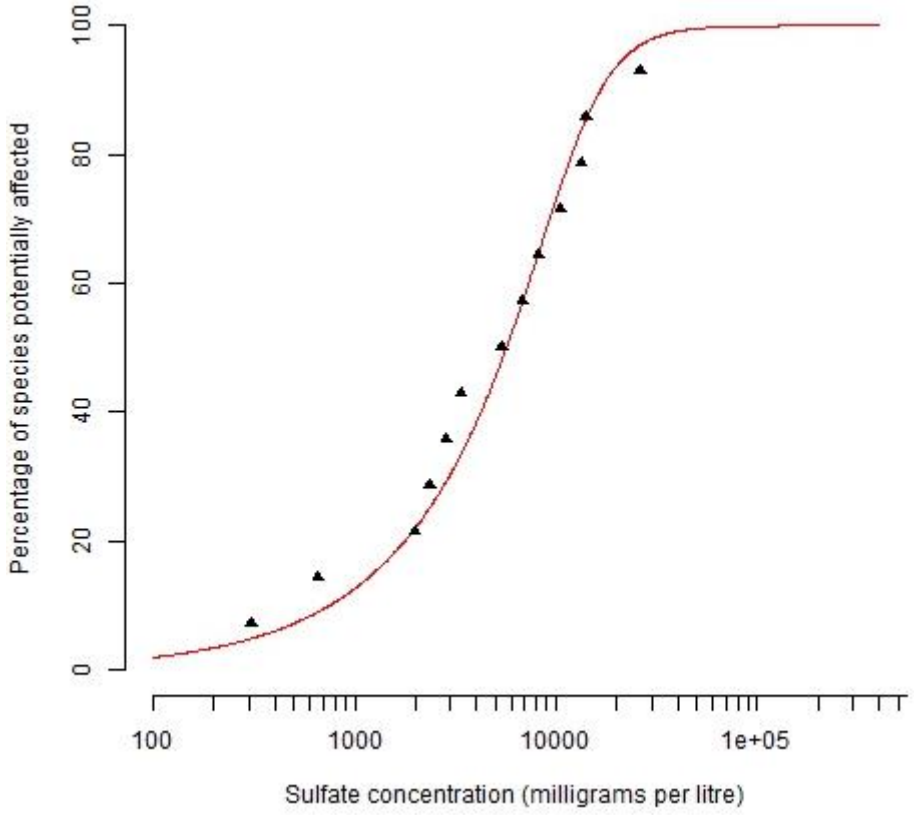

Figure 4.12 SSD (Inverse Weibull Distribution) for sulfate fitted over LC50 toxicity data for freshwater macroinvertebrates obtained from scientific publications

Based on the SSDs four levels of protection (PC99, PC95,PC90 and PC80) for each level were produced, with each having three values ranging from the most conservative, Lower 95% Confidence Interval (LCI), the Guideline Value (GV), which represents the central tendency of the prediction band and the least conservative value, Upper 95% Confidence Interval (UCI). These values are presented in the format GV (LCI, UCI), where, PC99 (mg/L) = 43 (5.9, 1894), PC95 (mg/L) = 316 (108, 2491), PC90 (mg/L) = 747 (322, 2914) and PC80 (mg/L) = 1770 (637, 3778).

4.1.14 Dissolved oxygen

Short-term acute (median lethal concentrations) dissolved oxygen exposures of freshwater macroinvertebrates (mg/L) were collected from literature (Sprangue, 1963;

Nebeker, 1972; Gaufin, 1973; Maltby, 1995) and aggregated (arithmetic means) to produce the MAVs. Presented in Table 40 are the freshwater macroinvertebrate genera with corresponding arithmetic mean acute values aggregated from appropriate LC50 data points. The mayfly nymphs belonging to the genus *Calibeatis* constituted the most sensitive taxa, while the isopods belonging to the genus *Asellus* constituted the least sensitive taxa to reduced dissolved oxygen in freshwater.

Table 4.19 Short-term LC50 data for dissolved oxygen to freshwater macroinvertebrate taxa collected from scientific publications and aggregated to mean acute values

Taxa	MAVs (mg/L)	Taxa	MAVs (mg/L)
Callibaetis	4.40	*Acroneuria*	2.60
Ephemera	4.00	*Pteronarcella*	2.40
Neophylax	3.80	*Pteronarcys*	2.20
Diura	3.60	*Leptophlebia*	2.20
Baetisca	3.50	Hydropsyche	2.00
Limnephilus	3.40	*Drusinus*	1.80
Nemoura	3.30	*Neothremma*	1.70
Arcynopteryx	3.30	*Hexagenia*	1.60
Rhithrogena	3.30	*Hyalella*	0.70
Simulium	3.20	*Asellus*	0.32
Gammarus	3.00		

The dissolved oxygen PCs for freshwater macroinvertebrates were estimated using the inverse cumulative normal distribution function. Preceding the inverse cumulative normal distribution function, the data were tested for normality. The species SSDs derived in this study followed a reverse S-shape (Figure 4.13), which was in agreement with previous work (Elshout *et al.*, 2013). The SSD curve for dissolved oxygen differed from regular SSDs (mainly described as S-shaped), particularly because with most environmental stressors mortality or concentration related effects occurs at higher stressor magnitudes whilst with dissolved oxygen mortality occurs at lower concentrations (Elshout *et al.*, 2013; Florida Department of Environmental Protection, 2013).

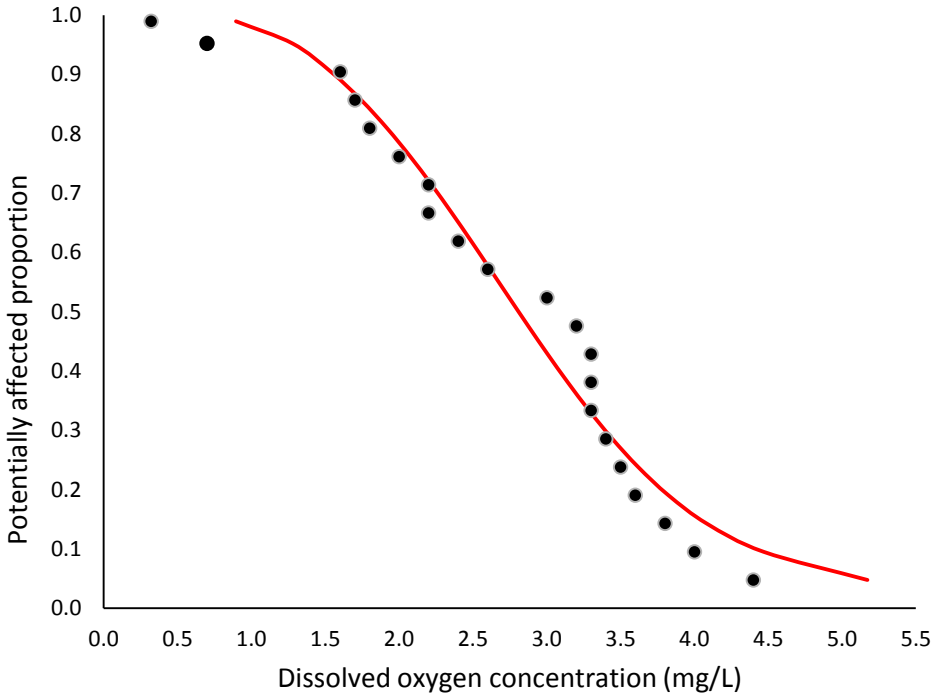

Figure 4.13 SSD for dissolved oxygen fitted through median lethal concentration (LC50) data for freshwater macroinvertebrates (solid black circles) using the inverse cumulative normal distribution function in Microsoft Excel (mean = 2.68; standard deviation = 1.07)

PCs for dissolved oxygen were estimated by fitting an inverse cumulative normal distribution function in Microsoft Excel (Figure 4.13). The inverse cumulative normal distribution function was fitted to 21 data points representing two groups of freshwater macroinvertebrates (crustaceans and insects). Molluscs and annelids were however, not represented in the SSD, due to data constraints. As observed by Mouthon (1996) sensitivity data for molluscs are very limited as opposed to other groups of aquatic macroinvertebrates. Additionally, freshwater molluscs constitute mainly of pulmonate air breathers, with the exception of limpets (Ancylidae sp.) and pelecypoda/bivalves (*e.g.* Corbiculidae, Sphaeriidae and Unionidae spp.). Air breathers are known to withstand low dissolved oxygen concentrations because of their ability to access atmospheric oxygen in addition to the oxygen dissolved in water (Pusey *et al.*, 2004; Brown and Lydeard, 2010). Annelids on the other hand, are known to withstand low to even complete lack of dissolved oxygen (Brinkhurst and Jamison, 1971 cited in Martins, 2008). Their ability to

withstand anoxic conditions could partly explain why their sensitivity data have not been well-documented. Given that both groups (molluscs and annelids) are relatively less sensitive to anoxic conditions, it will be assumed (in this study) that through the protection of the most sensitive groups, the least sensitive will benefit as well.

The estimation of the PCs for dissolved oxygen were PC99 = 5.17 mg/L, PC95 = 4.44 mg/L, PC90 = 4.05 mg/L and PC80 = 3.58 mg/L. these values were obtained using either R-Studio or Microsoft Excel. As expected with dissolved oxygen guideline values, the PCs decreased with a decrease in the PC (mg/L); a trend considered different from other stressors, whose effects are observable mainly at elevated concentrations than reduced.

4.1.15 Temperature difference from reference conditions

In this study water temperature criteria were derived to reflect temperature difference (*i.e.* increase in particular) from background temperature. Water temperature data collected from literature (See Table 4.20), were the median lethal temperature (LT50) together with the temperature acclimation (Ta) for each freshwater macroinvertebrates species. Using the two pieces of information from the bioassays, the temperature difference was determined, which is represented by the Temperature Tolerance Interval (TTI) (Table 4.20). The effects of temperature difference in this study were determined to reflect both differences in degrees Celsius and as percentage change (Δ %). In both cases, the stonefly nymph (*Aphanicerca* sp.) was the most sensitive taxa and the cased caddisfly nymph (*Brachycentus* sp.) was the least sensitive taxa to temperature difference from reference conditions (Table 4.20).

Table 4.20 Short-term LT50 data converted to TTI (˚C) and percentage change (% change) from temperature acclimation collected from scientific publications

Genus	LT50 (˚C)	Ta (˚C)	TTI ˚C (LT50-Ta)	% change	Genus	LT50 (˚C)	Ta (˚C)	TTI ˚C (LT50-Ta)	% change
Anax	33.6	17	16.6	49.4	*Brachycentrus*	32.8	10	22.8	69.51
Chloroniella	31.5	17	14.5	46.03	*Deleatidium*	22.6	15	7.6	33.63
Afronurus	26.3	17	9.3	35.36	*Zephlebia*	23.6	15	8.6	36.44
Athripsodes	29.9	17	12.9	43.14	*Aoteapsyche*	25.9	15	10.9	42.08
Castanophlebia	26.5	17	9.5	35.85	*Pycnocentrodes*	32.4	15	17.4	53.70
Aphanicerca	23.4	17	6.4	27.35	*Pyconocentria*	25	15	10	40
Palaemon	31.3	17	14.3	45.69	*Hydora*	32.6	15	17.6	53.99
Paramelita	24.3	17	7.3	30.04	*Potamopyrgus*	32.4	15	17.4	53.71
Chimarra	25.5	17	8.5	33.33	*Sphaerium*	30.5	15	15.5	50.82
Lestagella	29.5	17	12.5	42.37	*Paratya*	25.7	15	10.7	41.63
Ephemerella	22.9	10	12.9	56.33	*Paracalliope*	24.1	15	9.1	37.76
Symphitopsyche	30.4	10	20.4	67.11	*Lumbriculus*	26.7	15	11.7	43.82
Stenomena	31.8	10	21.8	68.55					

To determine the different levels of protection for freshwater macroinvertebrates against temperature difference as (◦C) a SSD curve (Burr Type III Distribution) was fitted over the determined TTI ◦C with the aid of the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 4.14).

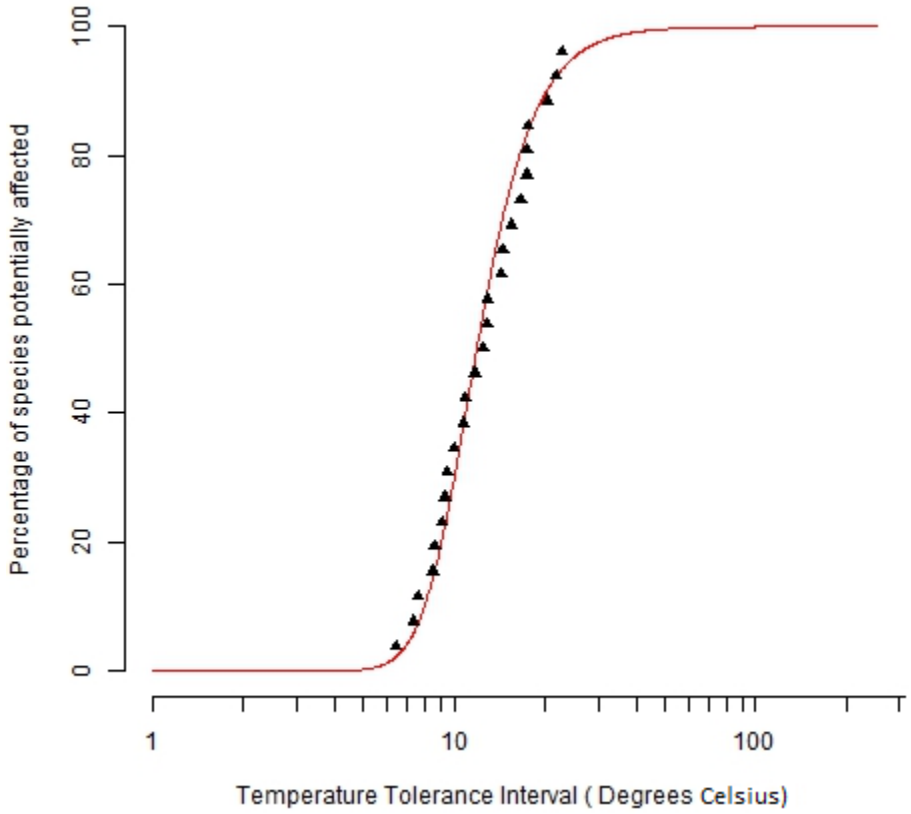

Figure 4.14 SSD (Burr Type III Distribution) for temperature difference (◦C) based on temperature tolerance intervals (TTIs) determined for different acclimation temperatures (Ta)

Similarly, to determine the percentage change that should not be exceeded in order to protect a specified percent of freshwater macroinvertebrates, the SSD curve was fitted on the TTI expressed as % Δ using the Burrlioz version 2.0 (Barry and Henderson, 2014) (Figure 24).

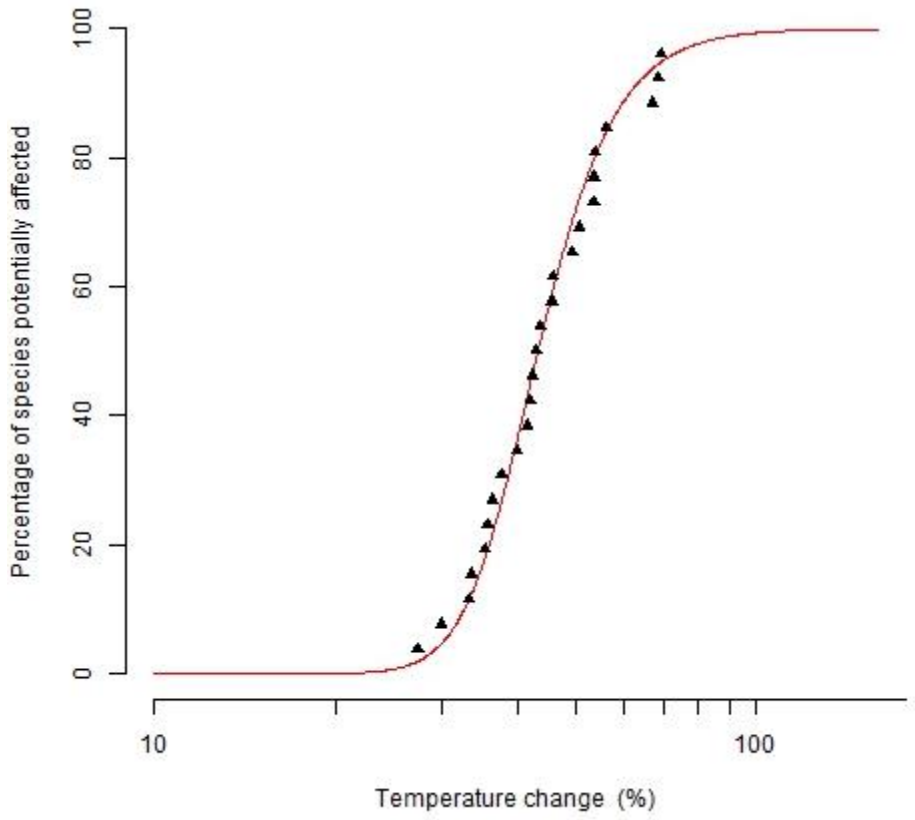

Figure 4.15 SSD (Burr Type III Distribution) for temperature difference (% change) based on temperature tolerance intervals (TTIs) determined for different acclimation temperatures

The SSDs produced using the Burrlioz version 2.0 (Barry and Henderson, 2014) in Figures 4.15 and 4.16, reflected four levels of protection (PC99, PC95, PC90 and PC80). For each of the four levels of protection, the software determined three values; Guideline Value (GV), and the upper and lower 95% confidence intervals (Table 4.21).

Table 4.21 Estimated PCs with 95% confidence intervals (in parenthesis) for water temperature difference (°C and %) to freshwater macroinvertebrates

Levels of protection (µg/L)	Temperature difference (°C)	Temperature difference (%)
PC99	5.8 (2.5, 7.4)	26 (18, 32)
PC95	7.1 (5.1, 8.5)	30 (27, 35)
PC90	8 (6.8, 9.2)	33 (29, 37)
PC80	9.1 (7.9, 11)	36 (32, 40)

The temperature difference - tolerance from temperature acclimation data for freshwater macroinvertebrates were derived to indicate two methods upon which water temperature difference could be based (Table 4.21). As expected, the smaller the deviation from the reference (acclimation temperature) the larger the percentage of freshwater macroinvertebrates likely to be protected, similarly, greater deviations were associated with fewer proportions of organisms likely to be protected.

4.2 DETERMINATION OF THE INDEX CATEGORIES

The ATI for macroinvertebrates was developed to account for the contributory effects of ambient water quality conditions on the toxicity of environmental stressors with known dependency on specific modifying factors (*i.e.* the effects of water hardness on six of the selected metals, and pH and temperature on ammonia). With respect to water hardness, the index was derived to reflect the toxicity ameliorating effects of water hardness on six metal pollutants (Table 4.22 to Table 4.27).

The lowest water hardness level considered in the ATI is 30 mg/L (as $CaCO_3$, a level that was derived for the classification of sites with relatively soft water (hardness <45 as $CaCO_3$) (Table 4.22). At this particular range of water hardness, pollutants are known to be most toxic to aquatic organisms, thereby requiring different numerical protection thresholds (DWAF, 1996; ANZECC and ARMCANZ, 2000). Consequently, the PCs derived for this range are most conservative of the five other hardness ranges.

Table 4.22 Index categories derived for water hardness = 30 mg/L (as $CaCO_3$). Values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC 99**	**PC99—PC95**	**PC95—PC90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.038**	**0.038—2.5**	**2.6—11**	**11.1—27**	**>27**
Chromium III (µg/L)	**<124**	**124—244**	**244.1—360**	**360.1—564**	**>564**
Chromium VI (mg/L)	<0.83	0.83—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<2.6**	**2.6—5.2**	**5.3—7.8**	**7.9—13**	**>13**
Lead (µg/L)	**<17**	**17—49**	**49.1—88**	**88.1—190**	**>190**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—-14	14.1—36	>36
Nickel (µg/L)	**<4.7**	**4.7—86**	**86.1—278**	**278.1—480**	**>480**
Zinc (µg/L)	**<0.59**	**0.59—19**	**19.1—81**	**81.1—318**	**>318**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved oxygen (mg/L)	>5.17	5.17—4.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% Change)	<18	18—27	28—29	30—32	>32

The second water hardness level (Table 4.22) was derived for the classification of sites with relatively harder water. The PCs recommended in this level were derived from toxicity data standardised to reflect the toxicity of selected metals at water hardness of 60 mg/L (as $CaCO_3$), suitable for the evaluations of sites with water hardness falling between 45-75 mg/L (as $CaCO_3$).

Table 4.23 Index categories derived for water hardness = 60 mg/L (as $CaCO_3$). Values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC—99**	**PC99—PC95**	**PC95—PC90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.095**	**0.095—5.1**	**5.2—22**	**22.1—54**	**>54**
Chromium III (µg/L)	**<219**	**219—430**	**430.1—636**	**636.1—995**	**995**
Chromium VI (µg/L)	<0.83	0.83—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<4.9**	**4.9—9.9**	**10—15**	**15.1—25**	**>25**
Lead (µg/L)	**<7.7**	**7.7—90**	**90.1—187**	**187.1—412**	**>412**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—14	14.1—36	>36
Nickel (µg/L)	**<195**	**195—360**	**360.1—521**	**521.1—863**	**>863**
Zinc (µg/L)	**<1.1**	**1.1—34**	**34.1—146**	**146.1—561**	**>561**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved Oxygen (mg/L)	>5.17	5.17—4.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% difference)	<18	18—27	28—29	30—32	>32

The third water hardness level (Table 4.24) was derived for the evaluation of sites with water hardness falling within the 75-120 mg/L (as $CaCO_3$) range. The PCs and index categories in this range were generated from ecotoxicity data standardised to 90 mg/L (as $CaCO_3$) water hardness.

Table 4.24 Index categories derived for water hardness = 90 mg/L (as $CaCO_3$), values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC 99**	**PC99—PC95**	**PC95—PC90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.14**	**0.14—7.5**	**7.6—32**	**32.1—79**	**>79**
Chromium III (µg/L)	**<305**	**305—600**	**600.1—886**	**886.1—1387**	**1387**
Chromium VI (µg/L)	<0.83	0.83—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<7.2**	**7.2—15**	**15.1—22**	**22.1—37**	**37**
Lead (µg/L)	**<57**	**57—164**	**164.1—296**	**296.1—642**	**642**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—14	14.1—36	>36
Nickel (µg/L)	**<275**	**275—507**	**507.1—734**	**734.1—1216**	**1216**
Zinc (µg/L)	**<1.5**	**1.5—48**	**48.1—206**	**206.1—798**	**>798**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved Oxygen (mg/L)	>5.17	5.17—4.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% difference)	<18	18—27	28—29	30—32	>32

The fourth water hardness level of the ATI was derived from ecotoxicity data for freshwater macroinvertebrates standardised to reflect the toxicity of the six metals to water hardness = 150 mg/L (as $CaCO_3$) (Table 4.25). This hardness level is suitable for the evaluation of the water quality of sites with relatively hard water falling between 120-180 mg/L (as $CaCO_3$).

Table 4.25 Index categories derived for water hardness = 150 mg/L ($CaCO_3$). Values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC 99**	**PC99-—PC95**	**PC95—PC90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.23**	**0.23—12**	**12.1—51**	**51.1—127**	**>127**
Chromium III (µg/L)	**<485**	**485—970**	**970.1—1378**	**1378.1—2235**	**2235**
Chromium VI (µg/L)	<0.83	0.85—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<12**	**12—24**	**24.1—35**	**35.1—59**	**>59**
Lead (µg/L)	**<56**	**56—260**	**260.1—510**	**510.1—1116**	**>1116**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—14	14.1—36	>36
Nickel (µg/L)	**<423**	**423—782**	**782.1—1131**	**1131.1—1874**	**1874**
Zinc (µg/L)	**<2.4**	**2.4—75**	**75.1—318**	**318.1—1181**	**>1181**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved Oxygen (mg/L)	>5.17	5.17—.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% difference)	<18	18—27	28—29	30—32	>32

The fifth water hardness level (Table 4.26) was derived from ecotoxicity data for freshwater macroinvertebrates standardised to water hardness = 210 mg/L (as $CaCO_3$). The PCs and subsequent index categories in this hardness level are suitable for the classification of sites with water hardness falling between 180 and 240 mg/L (as $CaCO_3$).

Table 4.26 Index categories derived for water hardness = 210 mg/L (as $CaCO_3$). Values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC 99**	**PC99—PC95**	**PC95—PC90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.31**	**0.31—17**	**17.1—70**	**70.1—173**	**>173**
Chromium III (µg/L)	**<611**	**611—1201**	**1201.1—1773**	**1773.1—2775**	**>2775**
Chromium VI (µg/L)	<0.83	0.83—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<16**	**16—32**	**32.1—49**	**49.1—81**	**>81**
Lead (µg/L)	**<143**	**143—410**	**410.1—737**	**737.1—1598**	**>1598**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—14	14.1—36	>36
Nickel (µg/L)	**<563**	**563—1039**	**1039.1—1504**	**1504.1—2491**	**2491**
Zinc (µg/L)	**<3.6**	**3.6—107**	**107.1—423**	**423.1—1486**	**>1486**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved Oxygen (mg/L)	>5.17	5.17—4.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% difference)	<18	18—27	28—29	30—32	>32

For the classification of sites with extremely hard water [hardness greater than 240 mg/L (as $CaCO_3$)], toxicity data (for the six metals) were adjusted to reflect toxicity at 270 mg/L (as $CaCO_3$) (Table 4.27).

Table 4.27 Index categories derived for water hardness = 270 mg/L (as $CaCO_3$). Values in boldface, indicate water hardness-adjusted stressor toxicity values

Description	High Quality	Good Quality	Moderate Quality	Poor Quality	Extremely Poor Quality
Hazardous Rank Score	**100**	**80**	**60**	**40**	**20**
Index Category	**A**	**B**	**C**	**D**	**E**
Percentiles	**<PC 99**	**PC99—C95**	**PC95—C90**	**PC90—PC80**	**>PC80**
Cadmium (µg/L)	**<0.39**	**0.39—21**	**21.1—89**	**89.1—216**	**>216**
Chromium III (µg/L)	**<750**	**750—1475**	**1475.1—2179**	**2179.1—3410**	**>3410**
Chromium VI (µg/L)	<0.83	0.83—50	50.1—222	222.1—667	>667
Copper (µg/L)	**<20**	**20—40**	**40.1—60**	**60.1—101**	**>101**
Lead (µg/L)	**<148**	**14—493**	**493.1—935**	**935.1—2082**	**>2082**
Mercury II (µg/L)	<0.58	0.58—5.5	5.6—14	14.1—36	>36
Nickel (µg/L)	**<696**	**696—1285**	**1285.1—1860**	**1860.1—3081**	**>3081**
Zinc (µg/L)	**<4.5**	**4.5—128**	**128.1—517**	**517.1—1748**	**>1748**
Ammonia (mg TAN /L)	<0.23	0.23—1.9	1.91—4.1	4.2—8.1	>8.1
Nitrate-N (mg/L)	<0.0036	0.0036—0.29	0.3—1.9	1.91—13	>13
Chloride (mg/L)	<1063	1063—1433	1433.1—1720	1720.1—2203	>2203
Sulfate (mg/L)	<5.9	5.9—108	108.1—322	322.1—637	>637
Dissolved Oxygen (mg/L)	>5.17	5.17—4.44	4.43—4.05	4.04—3.58	<3.58
Temperature (TTI°C)	<2.5	2.5—5.1	5.2—6.8	6.9—7.9	>7.9
Temperature (% difference)	<18	18—27	28—29	30—32	>32

The six water hardness levels for the ATI were determined based on the hardness-standardised stressors. It was only for convenient reasons that in each water hardness level both the hardness-adjusted stressors and those unadjusted were presented. The tables for each hardness level are self-contained for ease in comparison and reference purposes.

4.3 LITERATURE BASED VALIDATION

The production of SSD derived PCs is a lengthy and stepwise process involving collection and screening of large quantities of data and the calculation of geometric means (from data within comparable/acceptable orders of magnitude. It is of note that the quality of the

SSD outputs are largely a reflection of the quality of the input data as opposed to the computational model. More so, computational models operate by logical process and will unquestioningly process erroneous data (garbage in) and produce undesired outputs (garbage out) (Wang, 2017). Based on the Godel's incompleteness theorem (which state that a system cannot define itself) Wang (2017) states that data is irrelevant without context. In this study, therefore, the PCs obtained from this study were compared with those in scholarly publications and national and international water quality jurisdiction. Such comparisons were conducted first to check if there is any consistency in the ranking order of toxicity of metals between previous studies and the current (Table 4.28).

The toxicity of pollutants to freshwater organisms vary, while some pollutants are highly toxic (they induce toxicity to organisms at relatively lower levels of exposure) others induce toxicity at relatively higher concentrations (Jan *et al*., 2015). Numerous ecotoxicity studies have been conducted to rank the relative toxicity of pollutants to freshwater organisms using relational operators (*e.g.* Otitoloju and Don-Pedro, 2002; Rathore and Khangarot, 2002; Shuhaimi-Othman *et al*., 2012).

Based on previous studies and water quality criteria for freshwater ecosystems, mercury, copper and cadmium (in no particular order) appear to be the most toxic metals to freshwater ecosystems, whilst, zinc, lead, chromium and nickel (in no particular order) appear to be relatively less toxic metal stressors (Table 4.28). As observed in this study (freshwater macroinvertebrates), the ranking orders of the metal stressors were consistent with those from published water quality criteria/guidelines or trigger values from water quality jurisdictions and scientific publications.

Table 4.28 Comparison of the ranking order of toxicity of metal stressors (from most to least toxic) for PC values obtained in this study with those obtained from water quality jurisdictions and scientific publications

Hardness	Level of protection/ endpoint	Reference organism/s	Ranking order of toxicity (from most to the least toxic)	Reference
30	HC5/PC95	Macroinvertebrates	Cd>Cu>**Hg (II)** >Zn>Pb>**Cr VI** >Ni >Cr(III)	This study
60	HC5/PC95	Macroinvertebrates	Cd>**Hg (II)** >Cu >Zn >**Cr VI** >Pb>Ni>Cr)III)	This study
90	HC5/PC95	Macroinvertebrates	**Hg(II)** >Cd>Cu >Zn >**Cr VI** >Pb >Ni>Cr III	This study
150	HC5/PC95	Macroinvertebrates	**Hg(II)** >Cd>Cu >**Cr(VI)**>Zn>Pb>Ni>Cr(III)	This study
210	HC5/PC95	Macroinvertebrates	**Hg(II)** >Cd>Cu >**Cr VI** >Zn>Pb >Ni>Cr (III)	This study
270	HC5/PC95	Macroinvertebrates	**Hg(II)** >Cd>Cu >**Cr (VI**)>Zn>Pb>Nil>Cr(III)	This study
12	EC50	Macroinvertebrate (*T. tubifex*)	Hg (II)>Cu>Cd>Cr>Pb>Zn>Ni	Rathore and Khangarot, 2002
45	EC50	Macroinvertebrate (*T. tubifex*)	Hg>Cu>Cd>Cr>Zn>Ni>Pb	Rathore and Khangarot, 2002
170	EC50	Macroinvertebrate (*T. tubifex*)	Hg>Cu>Cr>Zn>Cd>Ni>Pb	Rathore and Khangarot, 2002
300	EC50	Macroinvertebrate (*T. tubifex*)	Hg>Cu>Cd>Cr>Zn>Pb>Ni	Rathore and Khangarot, 2002
18.72 ±1.72	LC50	(Macroinvertebrate) *N. elinguis*	Cu>Cd>Pb>Ni>Zn	Shuhaimi-Othman *et al.*, 2012
-	Lc50	T. *fuscatus, S. huzardi and C. africanus*	Hg>Cu>Cd>Zn>Pb>Ni	Otitoloju and Don-Pedro, 2002

***Boldface**: No hardness adjustments, HC5: Hazardous concentration to 5% PC95: Protective Concentration to 95% LC50: Concentration lethal to 50% of the organisms EC50: Concentration causing sub-lethal effects to 50% of the organisms*

Second, the literature based validation was conducted to determine the extent to which the index' levels of protection agreed or disagreed with published PCs. These investigations were conducted at levels considered comparable *i.e.* at PC95 (concentrations estimated to be protective to 95% of the organisms if not exceeded) or

HC5 (which refers to the concentrations estimated to be hazardous to 5% of the organisms if not exceeded) at comparable water hardness ranges (Appendix 17). For the development of the index, the lower 95% confidence interval value of the PC95 was preferred over the Guideline Value (GV) of the PC 95 and the upper 95% confidence interval values of the PC95. The lower 95% confidence interval values (most cosertvative) were preferred over the other median and the upper 95% confidence interval values because they were comparible with water quality criteria from other studies and water quality jurisdictions.

CHAPTER 5: OLIFANTS CASE STUDY

5.1 INTRODUCTION

This chapter presents the physico-chemical water quality data, riverine macroinvertebrates survey data (collected using SASS5 protocol) and flow data for the Olifants, collected between 2015 and 2016. In addition, inferential and multivariate statistics were used for exploring associations and spatio-temporal variations for each data set.

5.2 PHYSICO-CHEMICAL WATER QUALITY VARIABLES

5.2.1 Chromium

From the 35 water samples collected in this study, total dissolved chromium was detected in only three samples in water collected from S1, S4 and S5. Dissolved chromium concentrations ranged between 4 µg/L and 5 µg/L. For both S4 and S5, chromium was detected from samples collected during elevated stream flows *i.e*. mid-range flows for S4 and moist conditions for S5. The South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) does not provide quality guidelines for total dissolved chromium, but for specific chromium species [*i.e*. Cr (III) and Cr (VI). Nonetheless, the recorded chromium concentrations in the study were within the DWAF target water quality range [Cr (III) = 12 µg/L and Cr (VI) = 7 µg/L].

5.1.2 Nickel

Dissolved nickel concentration values were only recorded from five samples out of the 35. The recorded concentration values ranged between below detection to a maximum of 1504 µg/L. The maximum concentration was recorded from S5 from a stream flow classified as moist conditions for the entire flow duration. In comparison with the mean concentrations for the entire sampling period, sites S5 and S7 had average concentrations above the mean dissolved nickel.

5.1.3 Copper

The concentrations of dissolved copper in this study ranged from below detection to a maximum of 225 µg/L, a concentration recorded from S3 (during a flow event classified as ‘dry conditions’). All recorded concentration values in this study were above those recommended by the South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) at the DWAF water hardness levels. In comparison with the mean concentrations for all sites, Site S3 had average concentration above the mean dissolved copper. The effects of the elevated concentrations of copper for S3 in particular could be a real cause of concern particularly because S3 has relatively lower water hardness thereby offering relatively lower buffering against the toxicity or bioavailability of copper to aquatic organisms. However, that line of reasoning is limited given that latest water quality criteria and guidelines have shifted from the dependency of copper toxicity on water hardness into incorporating other ambient water conditions *i.e*. biotic ligand model and or DOC *e.g*. USEPA (2007) and ANZECC (2017), respectively.

5.1.4 Zinc

The study indicated high concentrations of zinc. For all study sites, the mean concentrations were above the Target Water Quality Range (TWQR) of the South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996). Sites S1 to S4 had the highest zinc concentrations. The sampling month that was associated with the maximum zinc concentrations was November, a month generally characterised by reduced stream flows.

5.1.5 Cadmium

Cadmium is a non-essential element and is highly toxic to freshwater life. Dissolved cadmium concentrations for water samples collected in the study ranged from below detection to the highest value of 10 µg/L. The highest cadmium concentrations were recorded from sites S4 and S5 and were both collected during elevated stream flows, mid-range flows and moist conditions, respectively. The average concentrations for sites S1 to S5 were above the mean cadmium concentration values for the entire study. Due to the extreme toxicity of cadmium to aquatic ecosystems, the South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) express the cadmium guideline as total concentrations as opposed to the dissolved fraction. Nonetheless, all mean dissolved concentrations were within the DWAF, 1996 guidelines.

5.1.6 Lead

Lead is both a toxic and non-essential metal having no nutritional value to living organisms (Edokpayi *et al*., 2016). Dissolved lead concentrations for the study ranged from below detection to a maximum of 1700 µg/L. The maximum value was recorded from S4, a concentration that was detected from a water sample collected during stream flows that were classified as mid-range flows. Out of the 35, dissolved lead was recorded from four occasions, two in S4 and sites S5 and S6. All the four counts were associated with elevated stream flows, one mid-range and three moist conditions. In addition, all the four records were high above the South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996), target water quality range irrespective of the corresponding water hardness levels.

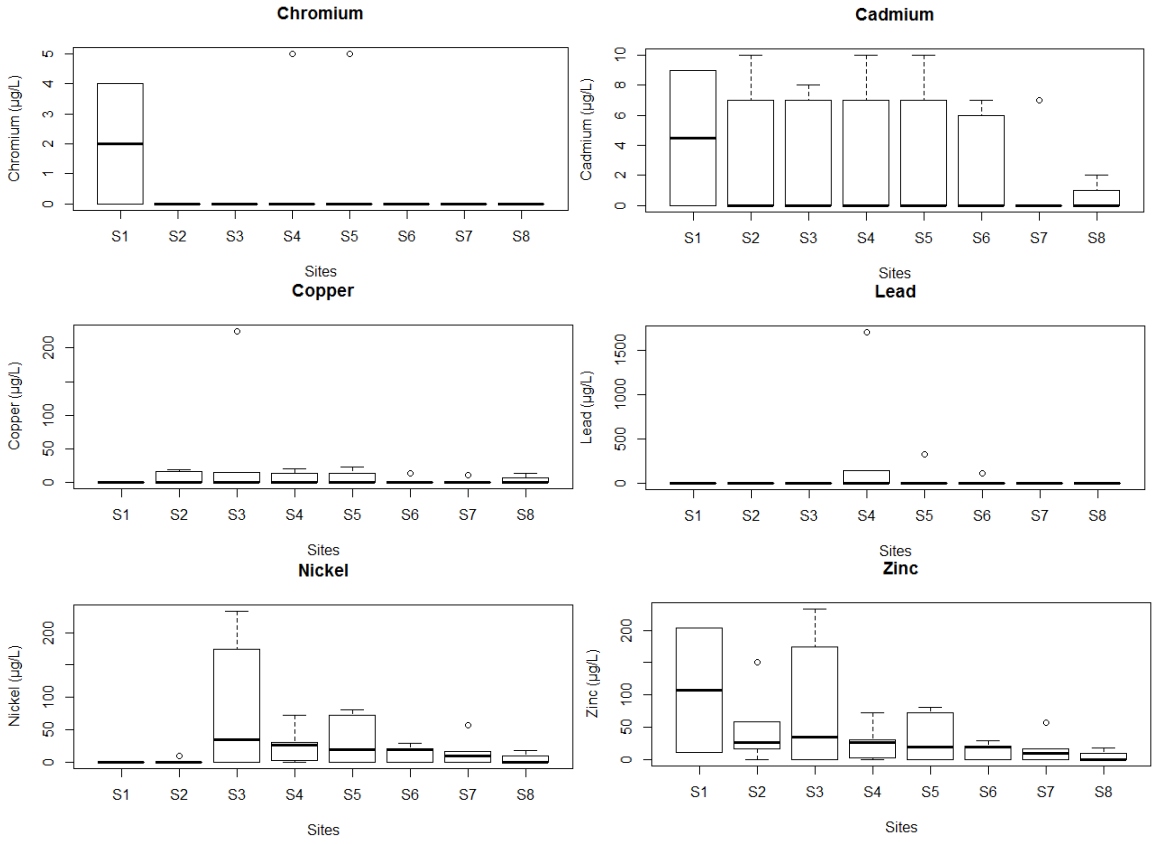

Figure 5.1 Box-plots summarising the concentrations of chromium, cadmium, copper, lead, nickel and zinc for water samples collected from eight sampling stations located within the Olifants Rivers catchment collected between September 2015 and August 2016

The box-plots (Figure 5.1) are graphical representations of the summary statistics of the concentrations of metal pollutants measured from the Olifants River. Thick lines show the median values (50th percentile), boxes the 25th and 75th percentiles, whiskers represent the maximum and minimum values within 1.5 times the inter-quartile range and small circle symbols represent the outliers beyond this threshold.

5.1.7 Ammonium-Nitrogen

Elevated concentrations of ammonium in surface water can be due to both human (*e.g*. industrial emissions, leakage of manure and fertilizer from agricultural activities) and natural activities. Natural sources, however, have been poorly documented (Du *et al*., 2017). Ammonium (NH_4-N) concentrations in the study ranged between below detection

to a maximum concentration value of 0.13 mg/L (measured in S4) under reduced river flows (classified as dry conditions).

5.1.8 Dissolved oxygen

The South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) do not recommend a definitive guideline value for DO as concentration (mg/L) but rather as percent saturation (% sat.). DWAF (1996) recommends a target water quality range 80-120% DO % sat. Study site S1 was the only site that had a DO measurement below the lower bound (80 % sat.) of the recommended range. Sites S2, S5, S6 and S7 had DO measurements for various sampling occasions above 120 % sat. While most studies pay attention to and emphases on dissolved oxygen depletion, it is without doubt that DO super-saturation is associated with some negative effects on aquatic animals as well. Some of the documented effects of DO super-saturation on fish include the bubble disease and ultimate mortality for some species (Li *et al*., 2010; Machova *et al*., 2017). In this study, it was expected that sites S2, S5, S6 and S7, with the highest algal biomass for most parts of the sampling period recorded DO values above the 120 % sat. As stated by Kunlasak *et al*. (2013) in water bodies when the amount of phytoplankton increases, the amount of DO increases as well due to algal photosynthesis during daylight.

5.1.9 PH

According to Chapman and Kimstach (1996), natural waters have pH ranges between 6.0 and 8.5. The pH values in this study ranged between 8.2 and 9.2. The measured pH values were consistent with previous studies of the Olifants River (*e.g.* Mahlatji, 2014; Gerber *et al*., 2015). Water with high pH values is normally an indication of discharge of alkaline compounds either from domestic or industrial discharges (UNEP, 2016). High pH values are of concern since this may affect fish in different ways for example through reduced ability to excrete ammonia or the regulation of ion balance (Carpenter *et al*., 2012 Cited in UNEP, 2016).

5.1.10 Temperature

Water temperature values for the Olifants River ranged from a minimum value of 13.5°c to 30.4°c. The minimum temperature value was recorded in S2 (a site located in the upper Olifants /Highveld) and the maximum value was located in S7 (a site located in the lowveld/Lebombo). As expected the average water temperature values increased with decrease in elevation (from the Highveld to the lowveld/Lebombo), these observations are widely supported by literature (*e.g*. Jackson *et al*., 2017; Xie *et al*., 2017).

5.1.11 Nitrate-Nitrogen

Nitrate is one of the most widespread surface and groundwater contaminants worldwide (Li *et al*., 2012). Nitrate reach surface water via numerous human activities, some of the widely documented include, sewage effluents, commercial fertilizers, animal waste and decaying organic material (Durka *et al*., 1994; Mayer *et al*., 2002). Elevated concentrations of nitrate in surface water can result to numerous ecological problems such as eutrophication and algal blooms (Li *et al*., 2012). Nitrate-nitrogen (NO_3-N) concentrations in the study ranged between below detection (recorded from S7 and S8) to a maximum value of 273 mg/L (recorded from S2). Based on mean nitrate concentrations, sites S2 and S5 had the mean nitrate concentrations above the mean nitrate value aggregated from the 35 observations for the study. The South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) does not recommend a numerical water quality guideline for nitrate instead, nitrate is incorporated in the inorganic nitrogen (NH_4^++NO_2^-+NO_3^-) guidelines.

5.1.12 Chloride

Chloride concentrations in the study ranged from a minimum of 2.9 mg/L to a maximum value of 304.4 mg/L recorded from S3 and S5, respectively. The maximum chloride concentrations were recorded during a flow event that could be classified as dry conditions in this study for the stream flow records ranging from August 2015 to August 2016. Sites S2, S5, and S8 had average chloride values above the mean value

aggregated from the 35 chloride records generated from the eight study sites from the duration of the study.

5.1.13 Sulfate

Sulfate concentrations for the duration of the study ranged from a minimum of 4.7 mg/L to a maximum value of 1114.7 mg/L, recorded from S8 and S2, respectively. Based on site average concentrations, sites S1, S2 and S5 were all above the mean SO_4^{2+} concentration value aggregated from the 35 observations from the eight study sites. The South African water quality guidelines for the protection of aquatic ecosystems (DWAF, 1996) does not provide quality guidelines for sulfate. Elevated sulfate concentrations in surface water is normally associated with acid mine drainage (Gray, 1996; Dabrowski and de Klerk, 2013). The dissolved sulfate derives from the oxidation of metal sulphides such as pyrite, abundant in coal-rich lithologies and precious metal-rich deposits (de Villiers and Mkwelo, 2009). An expected observation in the Olifants River particularly because the Olifants catchment is characterised by extensive coal mining that has been linked to deterioration in the water quality of the Olifants River (Hobbs *et al*., 2008; de Villiers and Mkwelo, 2009).

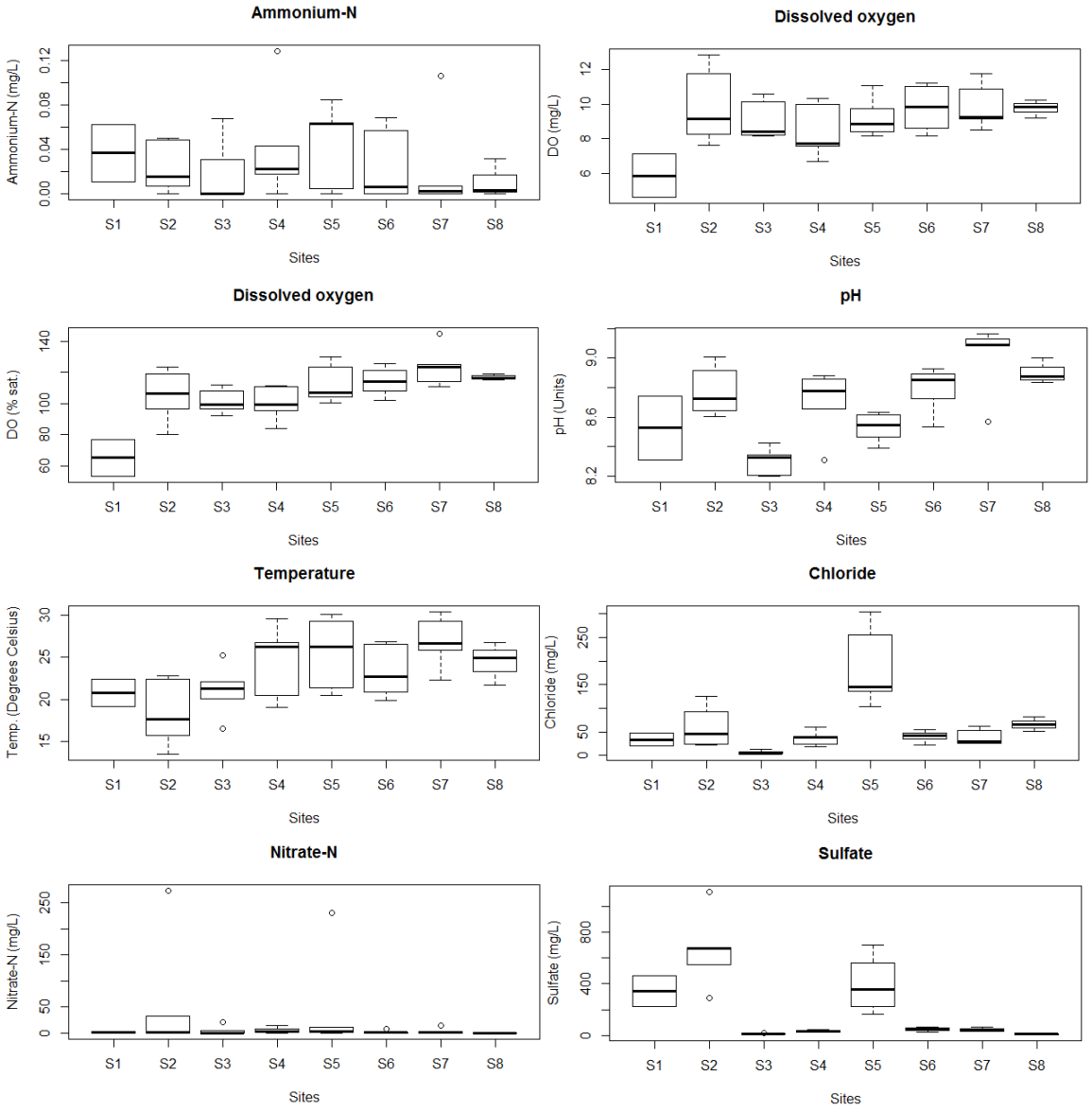

Figure 5.2 Box-plots summarising the magnitudes of ammonium, nitrate, sulfate, chloride, dissolved oxygen, pH and temperature for water samples collected from eight sampling stations located within the Olifants Rivers catchment collected between September 2015 and August 2016

The box-plots (Figure 5.2) are graphical representations of the summary statistics of the concentrations of magnitudes of ammonium, nitrate, sulfate, chloride, dissolved oxygen (concentration and % sat.), pH and temperature measured from the Olifants River. Thick lines show the median values (50th percentile), boxes the 25th and 75th percentiles,

whiskers represent the maximum and minimum values within 1.5 times the inter-quartile range and small circle symbols represent the outliers beyond this threshold.

5.2 MULTIVARIATE STATISTICAL ANALYSIS FOR PHYSICO-CHEMICAL WATER QUALITY VARIABLES

In addition to the interpretation of the magnitudes of the physico-chemical variables, the data were further explored for spatio-temporal variation using the Principal Component Analysis (PCA) and Cluster Analysis (CA). As indicated in the PCA bi-plot (Figure 5.3), the first two axes accounted for 60.0% of the total variation, where 31.1% of the variation is displayed on the first axis and 28.9% is displayed on the second axis. The patterns of correlations among the water quality variables used in the study formed three groups, where metals grouped together (with an exception of nickel), physical variables (dissolved oxygen, temperature and pH) also grouped together and nutrients also showed similar patterns.

In addition to the PCA, a CA was conducted for the determination similarities and clustering of the study sites based on the measured water quality variables. The cluster analysis dendrogram (Figure 5.4) indicates that sites S6 and S4 were the most similar (91.2%) based on the magnitudes of the physico-chemical variables while sites S1 and S8 presented the least percentage similarity (61%). It is of note that the S2 and S5 clustered together, as shown in Figure 5.1 and Figure 5.2 the two sites presented the highest level of impairments in water quality.

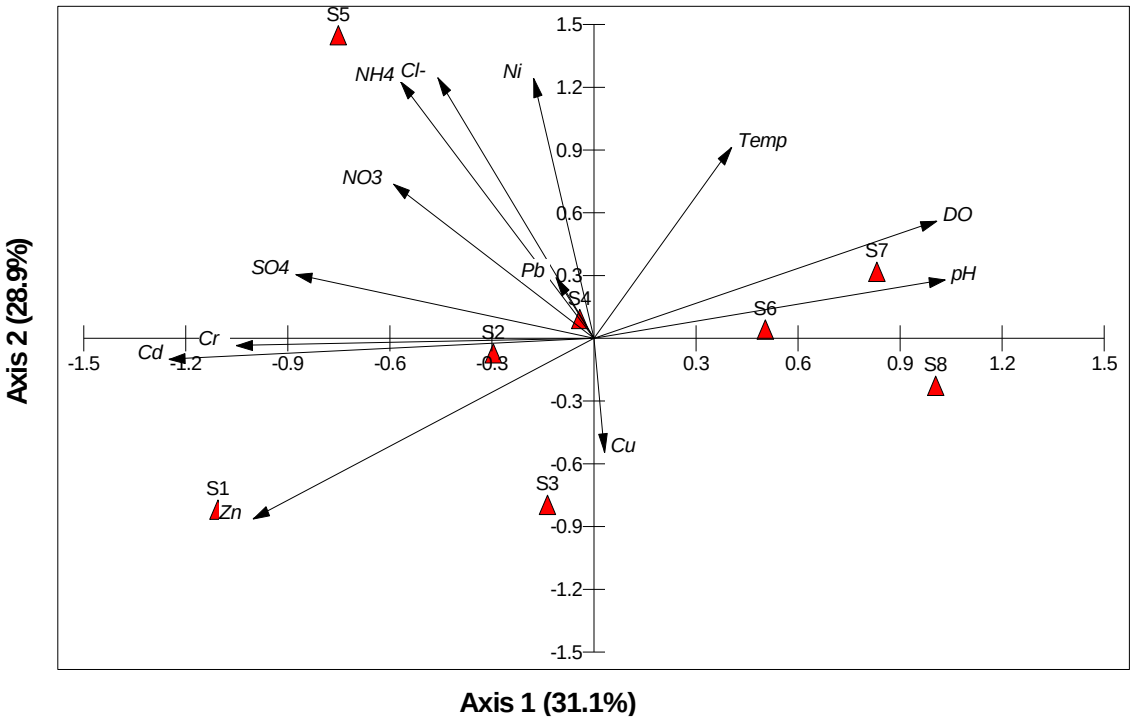

Figure 5.3 Principal component analysis bi-plot for water quality variables measured from the Olifants River Catchment between September 2015 and August 2016

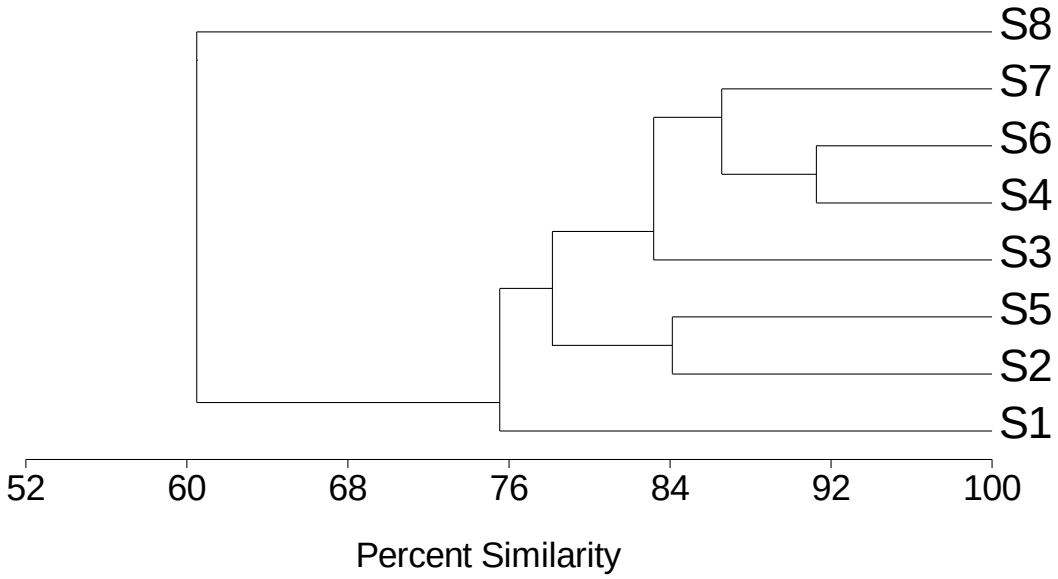

Figure 5.4 Classification of eight study sites (un-weighted pair-group average clustering algorithm) based on the magnitudes of physico-chemical water quality variables (log-transformed) for water quality data collected between September 2015 and August 2016

Temporal variation between sampling months were determined through the calculation of percent similarity. The cluster dendrogram (Figure 5.5) indicated most similarity between the months of March and September (76.3%), followed by the similarity between May and August (74.9%).

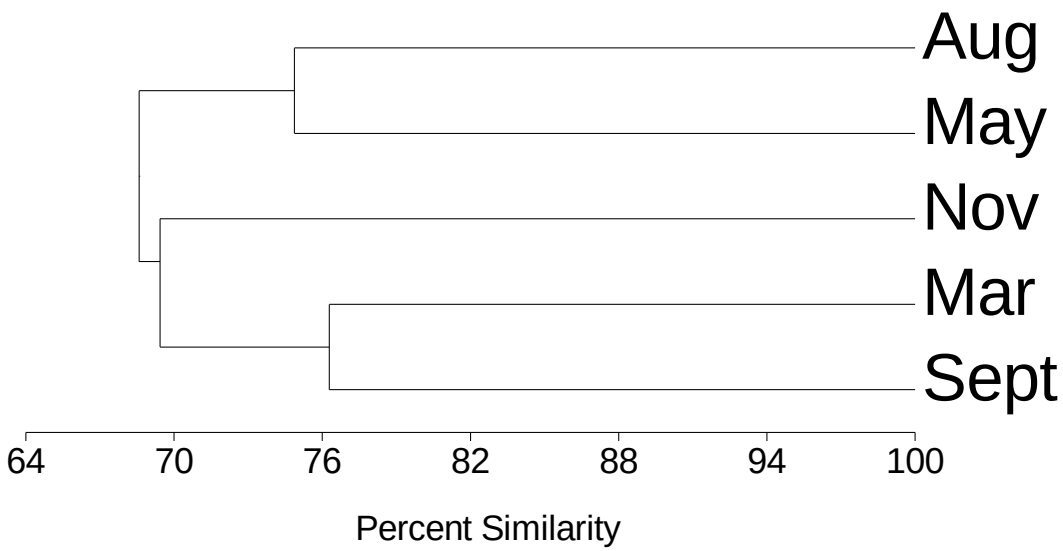

Figure 5.5 Classification of five data collection months (un-weighted pair-group average clustering algorithm determined based on the magnitudes of physico-chemical water quality variables (log-transformed) for water quality data collected between September 2015 and August 2016

Water quality samples collected during the months of September (2015) and March (2016) presented the highest impairments in water quality. It is during these two months that the highest number of maximum concentrations of chemical variables were recorded (Figure 5.1 and Figure 5.2). In agreement with the cluster dendrogram (Figure 5.5), the May and August samples had the least records of the maximum concentrations of chemical variables.

5.3 RIVERINE MACROINVERTEBRATES SURVEY DATA

Riverine macroinvertebrate surveys were conducted between September 2015 and August 2016 from eight study sites located along the Olifants River catchment. Four of the study sites were located along the Olifants River main stem and the other four sites

were located along four tributaries of the Olifants River (Klein-Olifants, Blyde, Ga-Selati and Letaba rivers). Riverine macroinvertebrates were collected, identified and enumerated using the South African Scoring Systems version 5 (SASS5) protocol (Dickens and Graham, 2002). The enumeration of the invertebrates involved the calculation of the three SASS5 metrics (SASS5 score, Number of Taxa and Average Score per Taxon) (Figure 5.6). The SASS5 Index awards Quality Scores (QS) (1 to 15) to macroinvertebrates based on their tolerance/sensitivity to riverine pollutants, where QS of one is attached to the most tolerant taxa and a score of 15 to the most sensitive taxa. In this study, 62 macroinvertebrate taxa/families were identified, 29 of which were tolerant taxa with SASS5 QS between 1 and 5, 25 of the taxa were moderately sensitive, within SASS5 QS range between 6-10 and only five taxa belonged to the most sensitive QS range of 11-15. The classification excludes Beatids and Hydropsychids (see Appendix 18) because their SASS5 sensitivity QSs are determined based on the number of species in the sample as opposed to family level (a common procedure with the other macroinvertebrates).

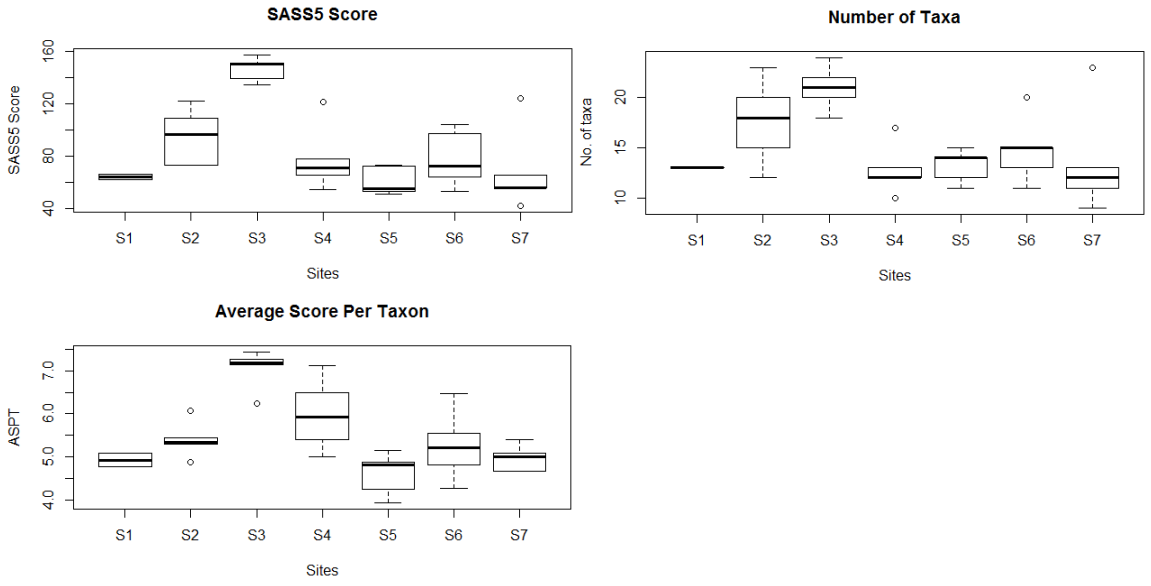

Figure 5.6 Box-plots summarising SASS5 Scores, number of taxa and ASPT for riverine macroinvertebrates collected between 2015 and 2016 from the Olifants River catchment

The box-plots (Figure 5.6) represents the summary statistics of the SASS5 metrics (SASS5 scores, number of taxa and ASPT) sampled from the Olifants River. The thick lines show the median values (50th percentile), boxes the 25th and 75th percentiles, whiskers represent the maximum and minimum values within 1.5 times the inter-quartile range and small circle symbols represent the outliers beyond this threshold.

As indicated in Figure 5.6 the highest SASS5 Scores, number of taxa and ASPT were associated with S3 (located along the lower Blyde River). Theoretically, it was expected that S3 would receive the best SASS5 evaluation scores, particularly because it has previously been identified as one of the least degraded tributaries of the Olifants River (*e.g*. Ballance *et al*., 2001 Swemmer and Mohlala, 2012).

In addition to the determination of the SASS5 metrics for each site, similarities and clusters based on the macroinvertebrates composition were explored through the determination of the Sorensen's Similarity Coefficients using the Un-weighted Pair Group Method with Arithmetic Mean (UPGMA). The Sorensen's similarity coefficients was executed using the Multivariate Statistical Package (MVSP) version 3.1 (Kovach, 1998). The Sorensen's similarity coefficient is the computation of the overlap in taxa between two study sites (Wolda, 1981). It is suitable for binary data (presence/ absence) (Johnston, 1976). It was therefore utilised in this study particularly because the macroinvertebrates data were collected using the SASS5 protocol where taxa abundances are more of estimates or censored values as opposed to specific values, for example abundances are given in ranges (*i.e.* '1' = 1,'A' = 2-10, 'B' = 10-100, 'C' = 100-1000 and 'D'>1000). The classification of the study sites by the riverine macroinvertebrates composition are as indicated in Figure 5.7 and the presence/absence data (Appendix 18).

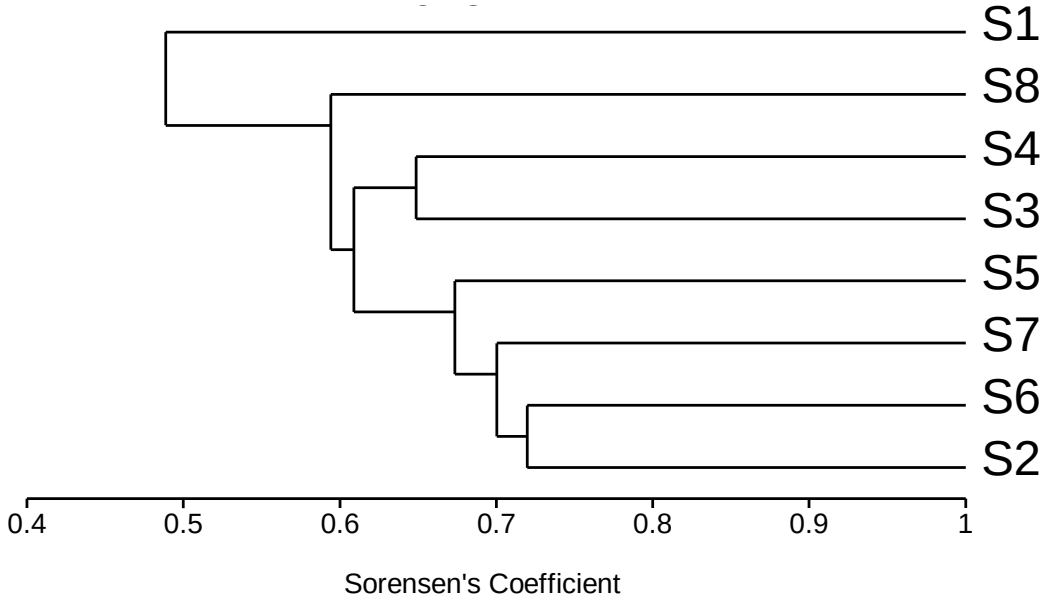

Figure 5.7 Classification of study sites based on similarities (Sorensen's similarity coefficient) of riverine macroinvertebrate composition data collected from the Olifants River system between September 2015 and August 2016

As illustrated by the UPGMA dendrogram the eight study sites split into three groups, where S2, S6, S7 and S5 belong to the first cluster (Cluster I), S3 and S4 belong to the second cluster (Cluster II) and S8 and S1 belong to the third cluster (Cluster III). With respect to similarities S2 and S6 had the highest similarities (72%) and the lowest similarities by macroinvertebrates composition was between S1 and S8 (49%).

5.4 FLOW VARIABILITY

The productivity of aquatic organisms is known to be determined by water quality, flow regimes, energy inputs and physical habitats. Given that the ATI for macroinvertebrates was designed to explain the composition of riverine macroinvertebrates based on physico-chemical water quality variables, it was therefore necessary to investigate the extent to which flows could be used in explaining the resultant macroinvertebrates communities as part of the index validation process. Therefore, the study incorporated three measures of flow variability that are of ecological relevance these were the base-flow Index (BFI), the Coefficient of Variation Index (CVI) (of daily flows index) and Richard

Backer-Flashiness Index (FI). These indices were determined from mean daily flow data obtained from the South African Department of Water and Sanitation (DWS).

5.4.1 Upper Olifants

Study sites located in the upper Olifants River catchment (S1 or Olifants at Wolwekrans and S2 or Klein-Olifants River at Rondebosch) were characterised by low flows and extended periods of flow cessation, low base-flows and high flow variability (figures 5.8 and 5.9) for the extent of the study (2015/ 2016 hydrological year). For instance, study site (S1) was characterised by 46% days with zero flows (for the length of the sampling period) and S2 had 16% zero flow days over the extent of the sampling period (August 2015 to August 2016). The extended periods of flow cessation greatly affected the sampling frequencies for S1 in particular, as a result river water samples and macroinvertebrates surveys could only be conducted two times (September, 2015 and March, 2016).

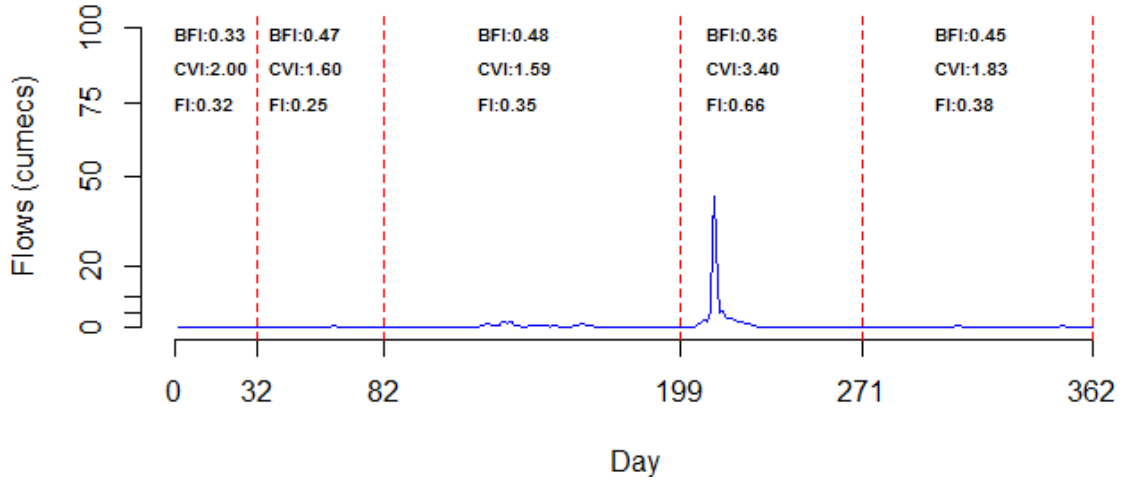

Figure 5.8 Flow hydrograph (flows in linear scale) for Olifants River at B1H005 Gauging Station (Olifants at Wolwekrans) generated from 362 mean daily stream flow records (August 2015-August 2016) presented alongside the BFI, CVI and FI

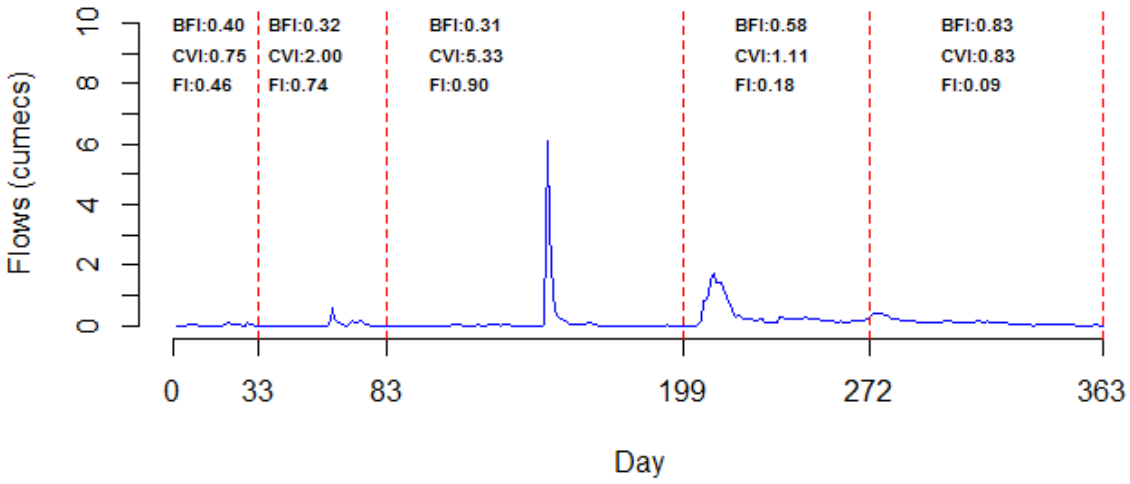

Figure 5.9 Flow hydrograph (flows in linear scale) for Klein-Olifants River at B1H005 Gauging Station (Klein-Olifants at Rondebosch) generated from 363 mean daily stream flow records (August 2015-August 2016) presented alongside the BFI, CVI and FI

While Site S2 had up to 16% days with zero flows based on data obtained from the South African Department of Water and Sanitation (DWS) hydrological records for the sampling period, most of the zero flows could be classified as censored zero flows as opposed to true zeroes based on physical flow determinations using a flow meter. All the zero flows that coincided with the days of data collection were classified as censored zeroes and therefore could not deter us from sampling. It is of note that the intermittent flows of the upper Olifants may have had some effects on the composition and structure of the riverine macroinvertebrates. Flow cessation can greatly modify habitats quality and availability. For example, flow cessation is always associated with loss of specific habitats (*e.g.* riffles), the fragmentation of riverine ecosystem, which could be through the formation of pools that may disappear if the dry season continues, deterioration in water quality, stimulation of algal blooms and accelerated predation (Gasith and Resh, 1999; Lake *et al*., 2003; Boulton, 2003; Acuna *et al*., 2014). Additionally, as noted by Boulton (2003), extremely low flows, intermittent flows and zero flows lead to a substantial loss of aquatic invertebrate taxa in particular atyid shrimps, stoneflies and free-living caddisflies.

5.4.2 Blyde River, Site 3 (Control Site)

As opposed to the upper Olifants sub-catchment, the control site located in the Blyde River (S3) indicated improvements in flow conditions (measures of flow variability). Based on the flow records for the 2015/2016 hydrological year, there were no zero flows (mean daily flows), stream flow variability (CVI and FI) was relatively lower and the base-flow (BFI) contribution was relatively higher than in the upper sub-catchment study sites (Figure 5.10).

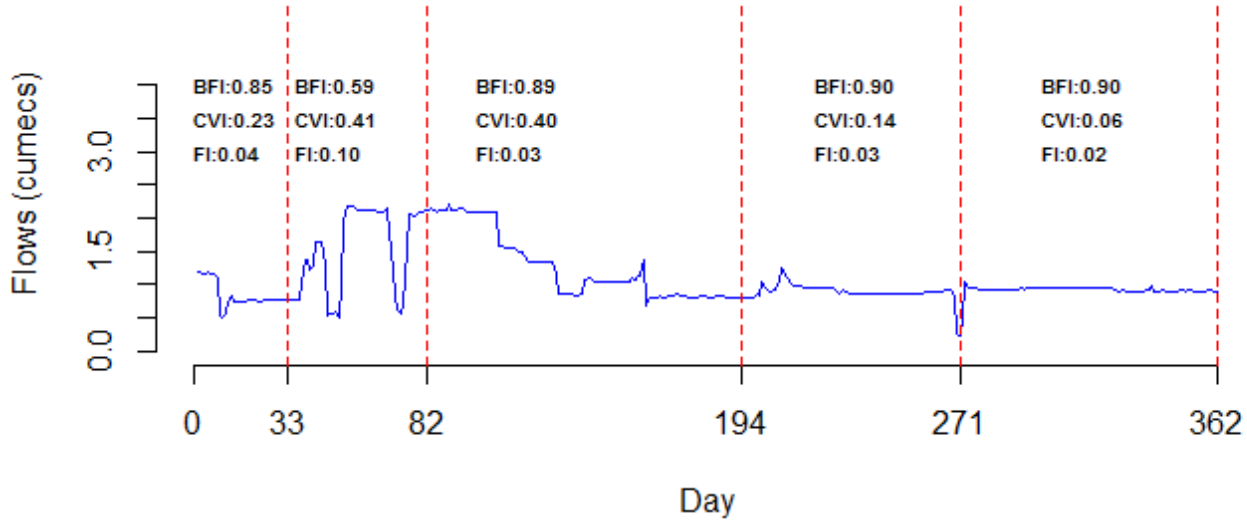

Figure 5.10 Flow hydrograph (flows in linear scale) for Blyde River at B6H005 Gauging Station (Blyde River at Driehoek) generated from 362 mean daily stream flow records (August 2015 - August 2016) presented alongside the BFI, CVI and FI

With no extreme flow events indicated in the S3 flow proxy gauging station, it is less likely that the flow conditions could largely explain the resultant riverine community structure for this site.

5.4.3 Lower Olifants

There were four study sites located in the lower Olifants with proxy gauging stations (S4 to S7) (Figure 5.11). The DWS hydrological records for the four study sites (S4, S5, S6 and S7) indicated improved flow conditions. For instance, flow proxy gauging stations for the four sites indicated no zero flows despite the 2015/2016 hydrological drought. The

flow hydrographs for the lower Olifants sub-catchment were characterised by peaks between the third (March 2016) and fourth (May 2016) sampling occasions.

The overall measures of stream flow variability for the seven study sites based on their proxy gauging stations indicated improved flow conditions for sites located in the lower Olifants than those located in the upper Olifants. The overall flow variability measures indicated that the upper Olifants was highly variable, flashy and the base-flow contributed a smaller proportion of the total stream flows than was the case with the lower Olifants (Table 5.1).

Table 5.1 BFI, CVI and FI for the Olifants River catchment determined for the entire data collection period (August 2015 to August 2016)

Site	BFI	CVI	FI
S1	0.418	2.084	0.392
S2	0.488	2.004	0.474
S3	0.826	0.248	0.044
S4	0.698	0.794	0.218
S5	0.73	0.792	0.16
S6	0.65	0.492	0.286
S7	0.692	0.678	0.236

Sites located in the upper Olifants sites were relatively variable and flashy probably due to the intermittent flow conditions observed over the sampling period. This observation was expected particularly because ephemeral/intermittent streams are known to be more highly variable (high CVI) than perennial streams (Eady *et al*., 2014; Berhanu *et al*., 2015).

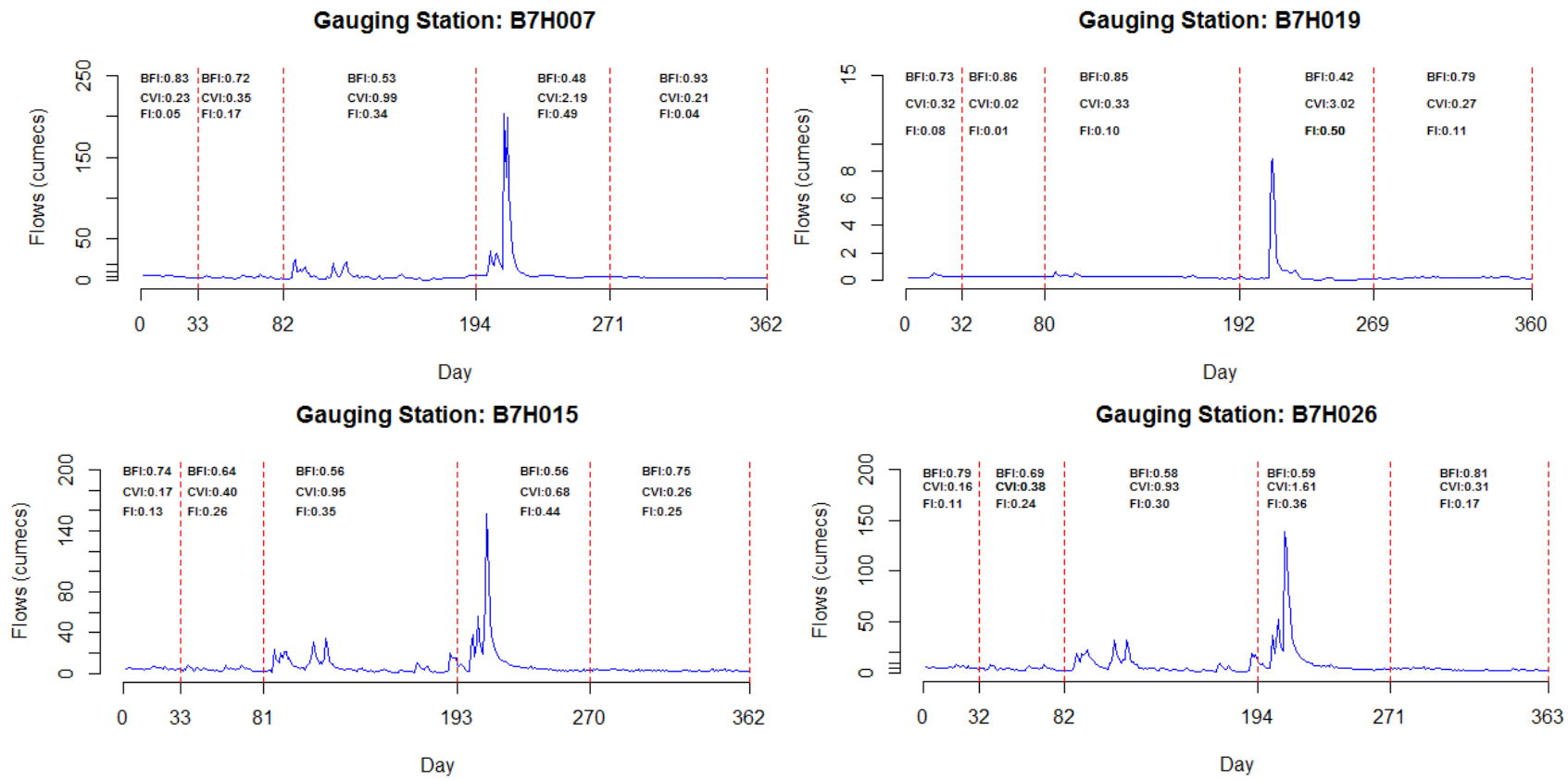

Figure 5.11 Flow hydrographs (flows in linear scale) for B7H007 (Olifants at Oxford) generated from 262 mean daily flow records, B7H019 (Ga-Selati at Loole) generated from 360 mean daily flow records, B7H015 (Olifants at the Mamba Weir) generated from 362 mean daily flows and B7H026 (Balule Weir generated from 362 mean daily flows). Red dotted vertical lines represent day of data collection

5.5 CHARACTERISATION OF FLOWS AT THE DAY OF DATA COLLECTION

In addition to the classification of flows based on flow variability measures, the flows at the dates of data collection were also classified by the determination of exceedance percentiles for the entire data collection period (August 2015 to August 2016). However, exceedance percentiles for S1 and S2 could not be determined because flow data presented in Appendix 19 for the two sites were based on physical determination using a flow meter. The physically determined flow values were preferred in this study because the DWS records indicated zero flows even under conditions when the flow meter detected some trickles. In this study, therefore zero flows from the DWS flow records that coincided with the dates of data collection were treated as censored zeros unless confirmed using a flow meter (true zeros).

River flows were classified by constructing Flow Duration Curves (FDCs) for each gauging station. An FDC is a widely used measure in water resources assessment and management, it is defined as a cumulative frequency curve that shows the percent of time specified discharges were equalled or exceeded during a given period (Masih, 2011). The FDCs were divided into five regions, where flows in the range 0-10% were classified high flows, flow range between 10-40% were classified as moist conditions, 40-60% flow range were classified as mid-range flows, flow range between 60-90%, were classified as dry conditions, and flow range between 90-100% were classified as low flows (USEPA, 2007a). As indicated in Appendix 19, the March 2016 sampling occasion coincided with flow elevation since most of the flows ranged between 10-40% a range indicative of moist conditions. On the other hand, major flow reductions were observed in the last sampling occasion where the flows predominantly ranged between 60-90%, a range indicative of dry conditions. While with the other sampling occasions there are no clear distinctions between the flow magnitudes, where one gauging station may have indicated extreme low the others indicate median flows or even high flows.

The determination of flow exceedance percentiles was necessary in this study because the flows for the 2015/2016 hydrological year did not show obvious lows and highs. Due to the hydrological drought over the data collection period, months that are normally associated with high flows were classified as low to flow cessation in some cases (Appendix 19). It is particularly for that reason that each site/proxy flow gauging station was treated as a single hydrological unit. Even under that condition, the flows widely varied while within each sampling occasion, one gauging station would classify the flow as high the other proxy gauging station records low flow. Principally, because of those differences, the generalization of the flow magnitudes into high flows (rainy season) and low flows (associated with dry season) approach would have been misleading (see differing flow classifications in Appendix 19) in this study consequently that approach was avoided. The effects of flows in the study were therefore based on the measures of flow variability (BFI, CVI and FI)

CHAPTER 6: APPLICATION AND SENSITIVITY ANALYSIS OF THE AQUATIC TOXICITY INDEX FOR MACROINVERTEBRATES

6.1 INTRODUCTION

This chapter focused on the initial application and sensitivity analysis of the ATI for macroinvertebrates. The application and sensitivity analysis processes were conducted using water quality data collected from the Olifants River system.

6.2 GENERALISATION OF WATER HARDNESS FOR THE STUDY SITES

The ATI for macroinvertebrates was derived to reflect the toxicity of the water quality variables (stressors) as modified by the ambient water quality conditions of a site. The determination of the index therefore required the incorporation of water hardness for metal stressors whose toxicity are known to be modified by water hardness *i.e.* Cd, Cr (III), Cu, Pb, Ni and Zn.

To enable the incorporation of water hardness [a measure of dissolved calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions in water] in the index computations, prevailing water hardness for the study sites had to be determined. The water hardness of the eight sites selected for the study were generalised from water hardness data determined from 10 year long water hardness records (from 2006 to 2016) obtained from the South African Department of Water and Sanitation (DWS). The generalised water hardness for each site are shown in Figure 6.1.

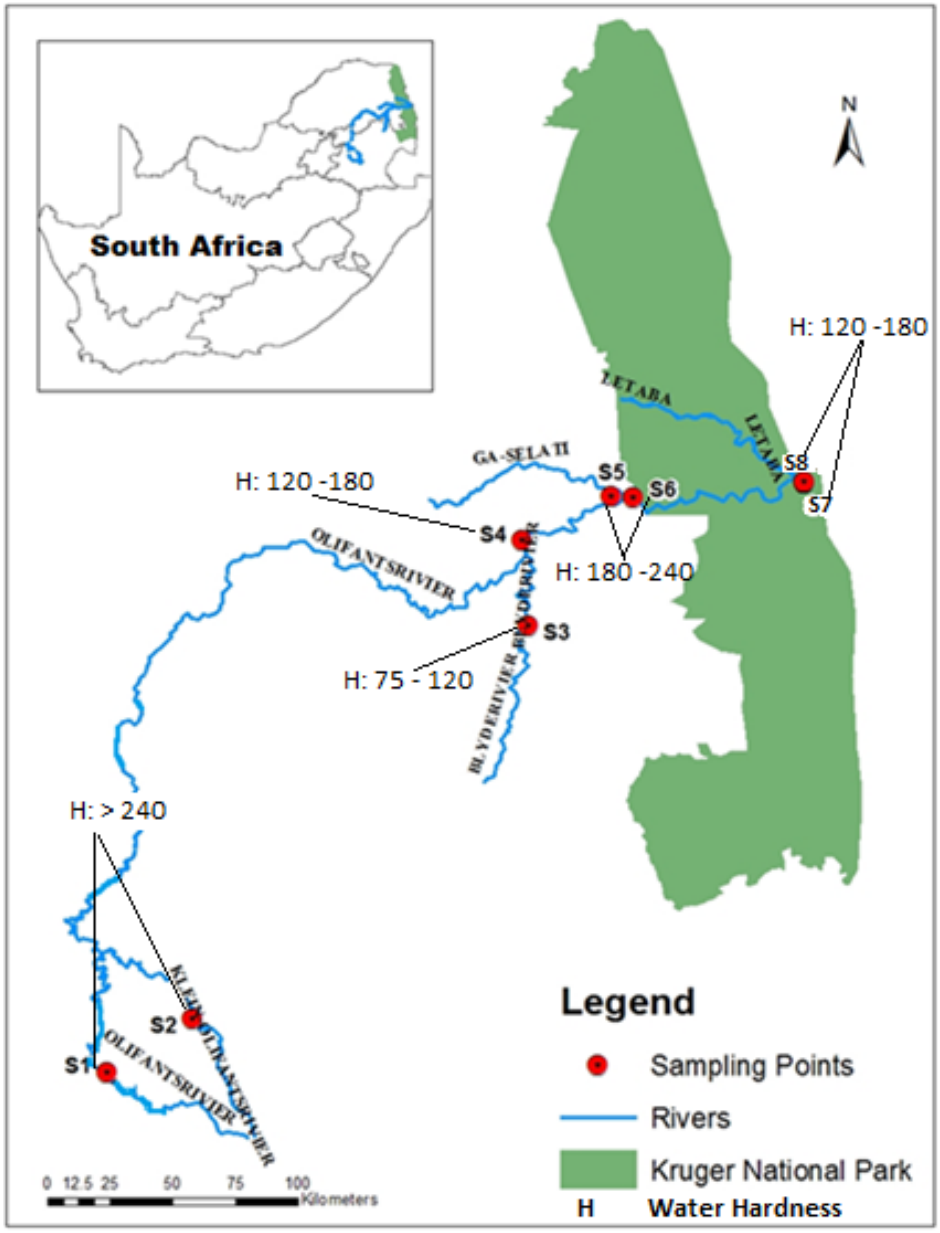

Figure 6.1 Olifants River study sites and corresponding water hardness (mg/L as $CaCO_3$) levels based on water hardness records (2006-2016) sourced from the South African Department of Water and Sanitation.

The water hardness profile for the Olifants River catchment was determined based on the median water hardness values. The median values were preferred as appropriate measures of the central tendency for the water hardness data, particularly because most of the data sets were not normally distributed.

Based on this classification, S3 fell within the 75-120 mg/L (as $CaCO_3$) hardness range, representing the study site with the softest water. S4 and S7/S8 fell within the 120-180 mg/L (as $CaCO_3$) range, S5 and S6 fell within the 180-240 mg/L (as $CaCO_3$) water hardness range and the sites with the hardest water in the study were S1 and S2 (upper Olifants sub-catchment) both falling > 240 mg/L (as $CaCO_3$) water hardness range. The water hardness magnitudes observed from this study (based on the DWS 10 year data set) concurred with previous studies (Wepener *et al*., 1992; Ramollo, 2008; Mahlatji; 2014) where the lower Olifants River catchment was represented by a generalised water hardness value of >120 mg/L (as $CaCO_3$).

6.3 IMPLICATIONS OF THE DIFFERENCES IN WATER HARDNESS BETWEEN THE STUDY SITES

The 10-year long water hardness records revealed that the sites located in the upper Olifants River catchment (S1 and S2) had extremely hard water compared to sites located in the Blyde River (S3) and the lower Olifants River sub-catchment (S4-S8). Implying that the toxicity of Cd, Cr (III), Cu, Pb, Ni and Zn would be relatively high in the Blyde River and the lower Olifants than in the study sites located in the upper Olifants.

6.4 REFERENCE WATER TEMPERATURE

In addition to the baseline data for water hardness, water temperature data from reference conditions (least impacted, surrogate for near natural water temperature conditions) within the Olifants River catchment were also collected. The Blyde and Klaserie rivers are the only tributaries of the Olifants River considered least impacted (Swemmer and Mohlala, 2012). However, the Klaserie River temperature data were preferred because it was data rich; covering monthly and diurnal variations. The South African Environmental Observation Network (SAEON) supplied the Klaserie River temperature data.

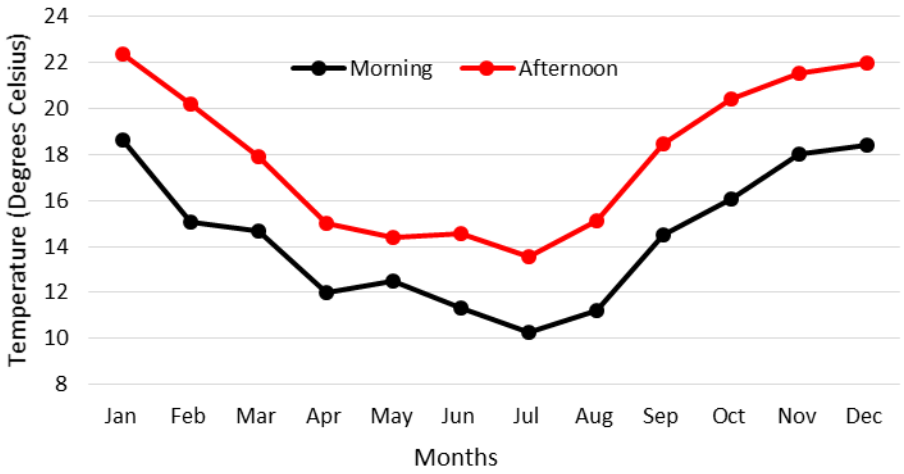

Figure 6.2 Water temperature for Klaserie River (reference site) indicating diurnal and monthly variations generated from data collected between 2010 and 2011

Based on Figure 6.2, minimum water temperatures were observed between June, July and August, a period coinciding with dry season, low flows and low air temperatures in the Olifants River catchment. Maximum water temperatures were observed between the months of November, December and January, which are months that are associated with elevated stream flows and increased air temperatures.

The water temperature difference scores for the ATI were determined by comparing water temperature values measured from this study with those observed from the Klasserie River at comparable times of the day [mornings (06h00 and 12h00) and afternoons (from 12h00 to 18h00)] and monthly variations (Appendix 20).

6.5 COMPUTATION OF THE AQUATIC TOXICITY INDEX FOR MACROINVERTEBRATES

The ATI for macroinvertebrates was determined using 14 physico-chemical water quality variables. From each water sample, the index was computed for the composite index score and the lowest rating score (as illustrated in Figure 3.3). The ATI for macroinvertebrates scores (both composite and lowest rating scores) for the Olifants River catchment are as shown in Figure 6.3.

The lowest index score (composite ATI) was 64 (water of good quality, indicative of minimal impairments in water quality) recorded from S4. The highest score was 88.9 recorded from S8. This reflects water perceived to be of natural to near-natural conditions, expected to support the survival of a wide variety of freshwater macroinvertebrates including sensitive taxa. Based on the composite ATI for macroinvertebrates, therefore, the water was of good quality, with ranges from minimal impairments in water quality to natural conditions. While, temperature difference from the reference conditions was identified as the predominant variable limiting the water quality of the Olifants River system, followed by sulfate and nitrate and to a relatively lesser extent copper, lead and zinc.

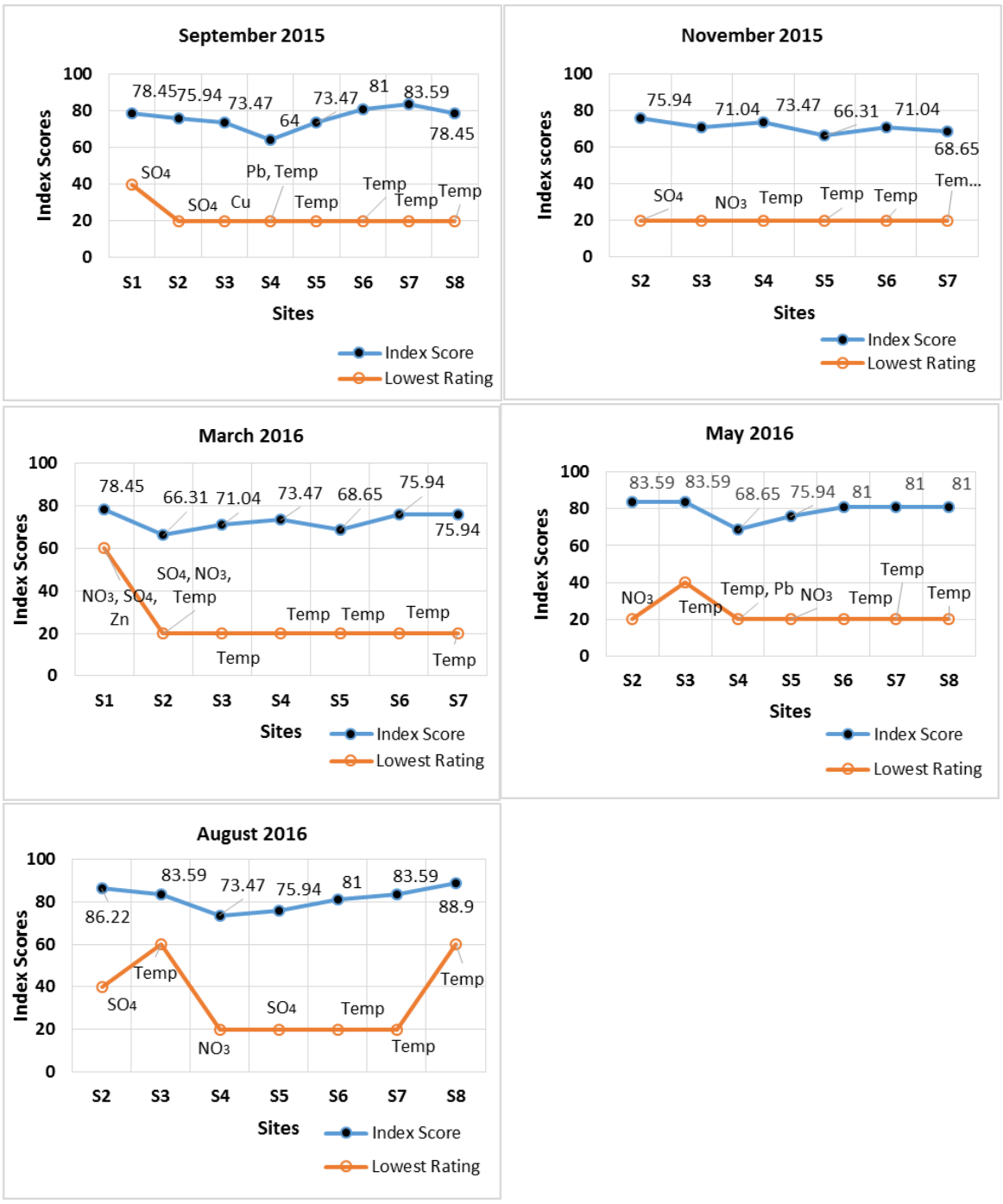

Figure 6.3 ATI for macroinvertebrates (composite index scores and lowest rating) for the Olifants River catchment based on water quality data collected between September 2015 and August 2016

6.6 RELATIONSHIPS BETWEEN ATI FOR MACROINVERTEBRATES, SASS5 METRICS AND FLOW VARIABILITY

The relationships between the ATI for macroinvertebrates, SASS5 metrics (SASS5 score, number of taxa and average score per taxon) and the measures of flow variability (base-flow index, flashiness index and coefficient of variation index) were investigated under two flow conditions. In both, the eighth study site (S8) was excluded from the analysis because there was no flow gauging station that could be used as a surrogate for flows.

Flow Condition 1:

Condition 1 represents the correlation analysis of the associations between the ATI for macroinvertebrates, SASS5 metrics and flow variability based on the seven study sites (S1, S2, S3, S4, S5, S6 and S7). As indicated in Figure 6.4, the relationships between the ATI for macroinvertebrates, SASS5 metrics and flow variability ranged between very weak and weak correlations; all were not significant $p<0.05$.

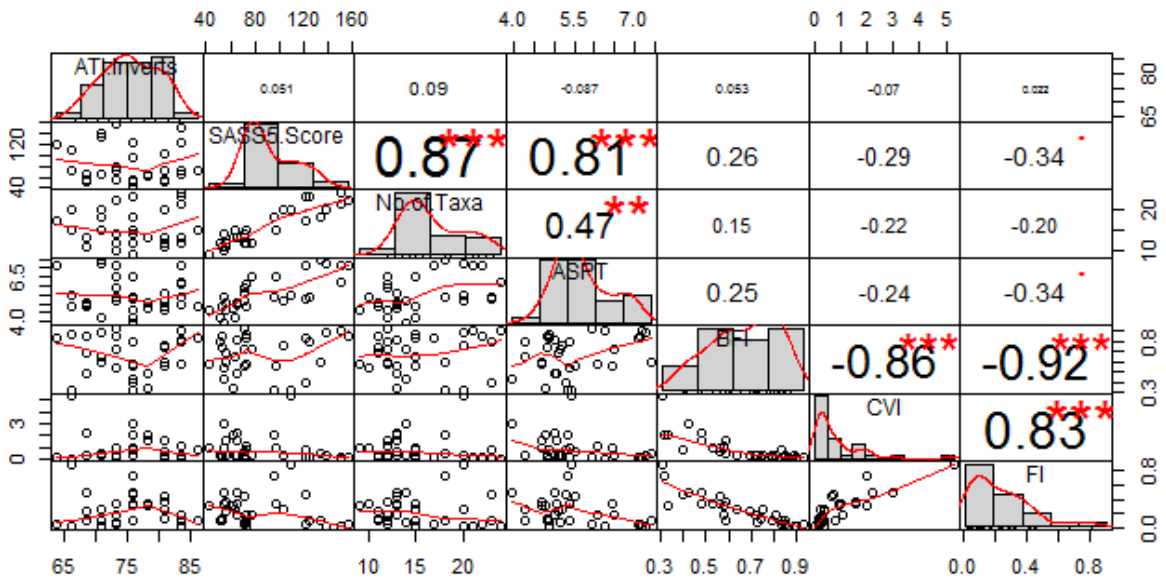

Figure 6.4 Scatter plot matrix of the measures of stream flow variability (BFI, CVI and FI) and SASS5

It is most likely that the relationships between these indices were weak and not statistically significant under flow condition 1 because of possible confounding factors associated with the intermittent/extended period of flow cessation. For the duration of the study period, this accounted for 46% in Site S1 and 16% in Site S2.

Flow Condition 2:

Condition 2 represents the correlation analysis of the associations between the ATI for macroinvertebrates, SASS5 metrics and flow variability based on the five study sites (S3, S4, S5, S6 and S7). On this flow condition, the impact of excluding S1 and S2 (study sites characterised by extended periods of flow cessation) was investigated (Figure 6.5).

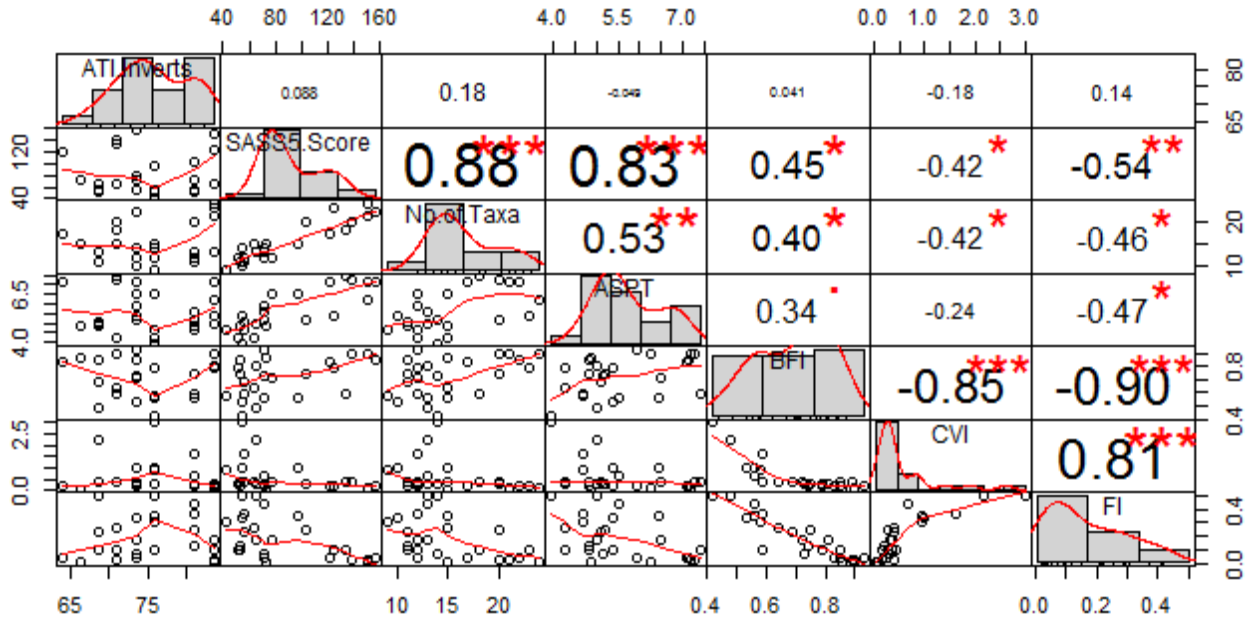

Figure 6.5 Scatter plot matrix of the measures of stream flow variability (BFI, CVI and FI) and SASS5 metrics

As indicated in Figure 6.5, the relationships between the SASS5 metrics and flow variability indicated substantial improvements. With the exclusion of sites S1 and S2 from the analysis, the correlations improved (in strength) from very weak to moderate correlations (without change in the direction of the correlations) that are predominantly statistically significant ($p<0.05$). It is of note, however, that the correlations between the ATI for macroinvertebrates and the SASS5 metrics and flow variability could not indicate any noticeable change because of the exclusion of S1 and S2.

As expected, the relationships between the SASS5 metrics and the BFI were positive and statistically significant ($p<0.05$), implying that macroinvertebrates thrive in flows with higher proportion of base-flow. Similarly, the SASS5 metrics were negatively correlated with the CVI and FI. This implies that high variability in stream flows has negative implications on the community structure of riverine macroinvertebrates.

The strength of the correlations between SASS5 metrics (SASS5 score, number of taxa and ASPT) with the BFI, CVI and FI improved with the exclusion of sites characterised by intermittent flows/flow cessation (flow condition 2). This suggested that the relationships

between riverine macroinvertebrates' community structure and flow patterns are largely dependent on continuous stream flows and suffer with flow intermittency.

6.7 RELATIONSHIPS BETWEEN ATI FOR MACROINVERTEBRATES AND ATI FOR FISH

As part of the validation process of the ATI for macroinvertebrates, it was compared with the ATI for fish (Wepener *et al*., 1992). The object was to determine whether there were any relationships and or concordances between the two indices (See Figure 6.6). The ATI for fish was computed using the WATER 2 software. The output results (composite index and lowest rating variable/minimum operator) are represented in Appendix 21.

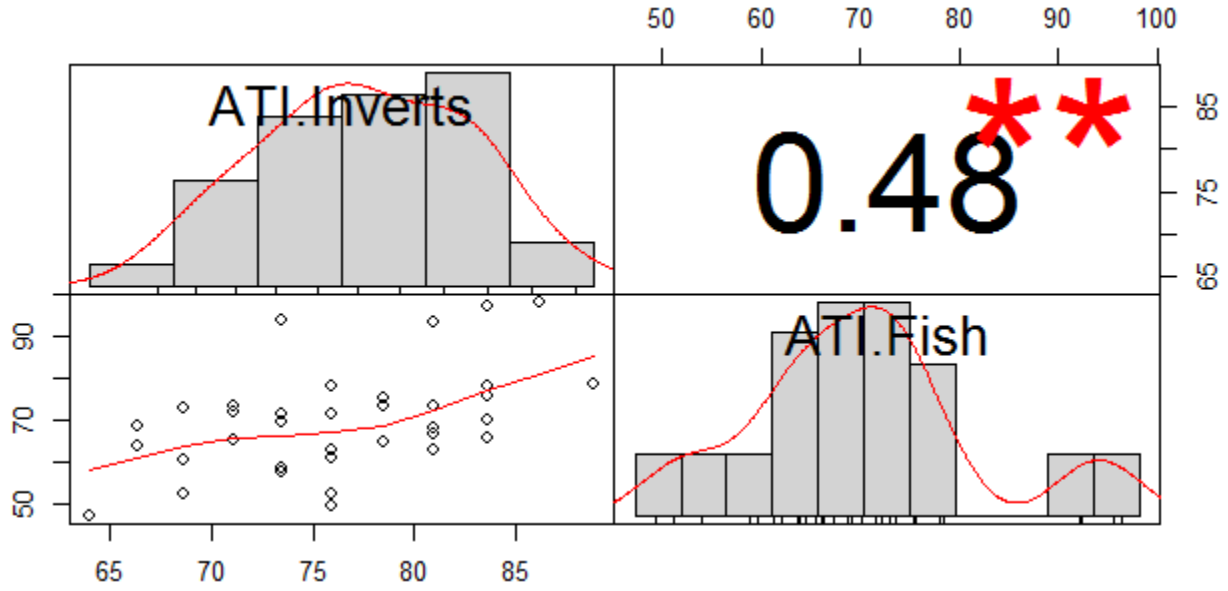

Figure 6.6 Scatter plot matrix of the ATI for macroinvertebrates (ATI Inverts) and ATI for fish (ATI for fish)

The correlation analysis of the relationships between the two indices indicated that the two indices were moderately correlated (r_s = 0.48, $p<0.01$). This implied that an increase in the ATI for macroinvertebrates indicated an increase in the ATI for fish.

6.8 INTER-RATER RELIABILITY OF THE MACROINVERTEBRATES INDICES

In this study, the Fleiss Kappa was used for the determination of the extent to which the different sets of indices (raters) used in the study (ATI for macroinvertebrates, SASS5 metrics and MIRAI version 2.0) rate the water quality/ecosystem health of the eight study sites consistently. Results for the MIRAI V2 are presented in Appendix 22. For the SASS5 raters only the SASS5 score and the number of taxa were used (Table 6.1). The SASS5 ASPT is determined from the SASS5 Score and the number of taxa. It is therefore not an independent rater of water quality and ecosystem health. It is for that particular reason that it was removed from the SASS5 raters, since all raters for the Kappa IRR are assumed to be independent (Cohen, 1960).

Table 6.1 Fleiss Kappa input data (based on rating of the eight study sites, where 1 represented the best rank and 8 the worst rank) for the determination of the inter-rater reliability

Sites	Raters			
	ATI Inverts	SASS5 Score	No of Taxa	MIRAI (Ecological Category)
S1	3	7	6	6
S2	5	2	2	1
S3	6	1	1	2
S4	8	4	7	8
S5	7	8	5	7
S6	4	3	3	5
S7	2	6	4	3
S8	1	5	8	4

The test revealed no consistency (consensus) in the rating of the eight study sites between ATI for macroinvertebrates and the SASS5 metrics (number of taxa and SASS5 score) and the MIRAI. Fleiss Kappa score was 0% ($z = 0$, $p = 1$). In addition, the analysis revealed no consistency between the rating of the sites using the ATI for macroinvertebrates and the MIRAI v2, Fleiss Kappa score was 14.3% ($z = 1.07$, $p = 0.285$). This indicated a slight but insignificant agreement between the two raters

($p<0.05$). This finding was unexpected and it suggests that there is minimal or no consensus between the SASS5 metrics, MIRAI and the ATI for macroinvertebrates.

6.9 SENSITIVITY ANALYSIS OF THE AQUATIC TOXICITY INDEX FOR MACROINVERTEBRATES

The sensitivity analysis of the ATI for macroinvertebrates was conducted using the *leave-one-out* method. This involved removing one water quality variable at a time and recalculating the index. This procedure was carried out for individual variables (for all 14 water quality variables used in the computation of the ATI for macroinvertebrates) and for groups of variables (nitrogen compounds, metals, salts and physical water quality variables). The sensitivity analysis for individual water quality variables indicated that water temperature, sulfate and nitrate were the most important variables-influencing the ATI for macroinvertebrates. This particular observation supports the identification of the lowest rating scores, where temperature, sulfate and nitrate were identified as the top three variables limiting the water quality of the Olifants River in the study.

Table 6.2 Sensitivity of the removal of each variable from the ATI for macroinvertebrates. Decisions on the impact of each variable taken based on the Wilcoxon rank sum test as to whether the exclusion of a variable worsens or improves the reference index. Decisions were based on the differences between the actual median values (median value of the reference index = 75.94)

Reduced Index	Median values for reduced indices	Wilcoxon rank sum test (p-values)	Comment
Temperature	82.4	0.00044	Significantly different at p<0.05. Not considering temperature difference from the reference conditions leads to better reference index scores.
SO_4^{2+}	79.6	0.0058	Significantly different at p<0.05. Not considering SO_4^{2+} leads to better reference index scores.
NO_3-N	82.4	0.00600	Significantly different at p<0.05. Not considering NO_3 leads to better reference index scores.
Pb	74.2	0.69000	Not significantly different at p<0.05. Not considering Pb leads to reduction of the reference index scores.
Cu	74.2	0.65000	Not significantly different at p<0.05. Not considering Cu leads to reduced scores.
Zn	76.9	0.65000	Not significantly different at p<0.05. Not considering Zn leads to better reference scores.
Cd	76.9	0.86000	Not significantly different at p<0.05. Not considering Cu leads to better reference index scores.
Ni	74.2	0.47000	Not significantly different at p<0.05. Not considering Ni leads to reduced reference index scores.
TAN	74.2	0.47000	Not significantly different at p<0.05. Not considering leads to reduced index scores.
Cr (VI)	74.2	0.44000	Not significantly different at p<0.05. Not considering Cr (VI) leads to reduced reference index scores.
Cl^-	74.2	0.84000	Not significantly different at p<0.05. Not considering Cl- leads to reduced reference index scores.
Cr (III)	74.2	0.39000	Not significantly different at p<0.05. Not considering Cr (III) leads to reduced reference index scores.
DO	74.2	0.39000	Not significantly different at p<0.05. Not considering DO leads to reduced reference index scores.
Hg (II)	74.2	0.39000	Not significantly different at p<0.05. Not considering Hg (II) leads to reduced reference index scores.

In addition to the statistical comparisons between the reference index and the reduced indices, the indices were further analysed using Spearman correlations. Correlation analyses were conducted for ranking the reduced indices in relation to their relative

importance in influencing the outputs of the reference index. In this case, weaker correlation between the reference index and the reduced index implied that the reference index was more sensitive to the inclusion of that particular water quality variable (Rickwood and Carr, 2009). Spearman correlations between the reference indices and the reduced indices were all positive ($p<0.0001$). The relative sensitivity of the reference index to the inclusion of the water quality variables varied, where temperature difference (reduced index) had the smallest correlation coefficient ($r_s = 0.81$). This observation suggests that the ATI for macroinvertebrates (reference index) was more sensitive to the inclusion of the water temperature difference than any other variable. On the other hand Cl^-, Cr (III), DO, and Hg (II) had the highest correlation coefficients ($r_s = 1.00$) (Table 6.3). This suggested that the ATI for macroinvertebrates was least sensitive or not sensitive at all to the inclusion of these variables.

Table 6.3 Sensitivity of the ATI for macroinvertebrates to the removal of individual variables from the determination of the composite index. Correlation coefficients for the relationships between the composite ATI for macroinvertebrates and the reduced indices were all positive and significant at $p<0.0001$

Reduced Index	Correlation coefficients (r_s)	Rank
Temperature	0.8113087	1 (Removal had major effect on the index)
SO_4^{2+}	0.8962308	2
NO_3-N	0.8972307	3
Pb	0.9394141	4
Cu	0.9434098	5
Zn	0.9528185	6
Cd	0.968166	7
Ni	0.9941632	8
TAN	0.994595	9
Cr (VI)	0.9956596	10 (Removal had the smallest effect on the composite index values)
Cl^-, Cr (III) DO, Hg (II)	1.00	14 (Removal had no effect on the composite index)

In addition to the statistical analysis for the 14 individual variables, sensitivity analysis was also conducted based on variable groupings *i.e.* metals, nitrogen compounds, salts

and physical. The statistical comparison (Wilcoxon rank sum test) of the reference index with the reduced indices (by group) was significant ($p<0.05$) for nitrogen compounds, metals and physical variables, but not significant for salts. The removal of nitrogen compounds, physical variables and salts led to improved reference index scores (based on the comparison of the median values of reference index with the reduced indices by group) (Table 6.4).

Table 6.4 Sensitivity of the removal of groups of variables from the ATI for macroinvertebrates. Decisions on the impact of each variable were taken based on the Wilcoxon rank sum test. As to whether the exclusion of a variable worsens or improves the reference index, decisions were based on the differences between the actual median values (median value of the reference index = 75.94)

Reduced Index	**Median values for reduced indices**	**Wilcoxon rank sum test**	**Comments**
Nitrogen compounds	81	0.043	Significantly difference at $p<0.05$. Not considering nitrogen compounds in the determination of the reference index would lead to better index scores
Metals	58.8	8.764e-10	Significantly difference at $p<0.05$. Not considering metal stressors in the determination of the reference index would lead to reduced index scores
Salts	78	0.39	Not significantly different at $p<0.05$. The removal of salts from the reference index would lead to improved index scores
Physical	81	0.009351	Significantly different at $p<0.05$. Not considering physical stressors in the determination of the reference index would lead to better index scores

The correlations (r_s) between reference indices and reduced indices by groups were all positive ($p<0.0001$) (Table 6.5). Relationships between the reference index and reduced index for metals were the weakest suggesting that the ATI for macroinvertebrates was more sensitive to inclusion of metal stressors as opposed to the inclusion of physical water quality variables (temperature difference and dissolved oxygen), salts and nitrogen compounds.

Table 6.5 Sensitivity of the ATI for macroinvertebrates to the removal of variables by groups from the composite/reference index, correlation coefficients for the relationships between the composite ATI for macroinvertebrates (reference index) and the reduced indices were all positive and significant at $p<0.0001$

Reduced Index	Correlation coefficient (r_s)	Rank
Metals	0.75	1
Physical	0.82	2
Salts	0.90	3
Nitrogen compounds	0.91	4

The correlation analysis of the relationships between the reference index and the reduced indices (based on groupings of variables) indicates that metal pollutants, with $r_s = 0.75$ (removal had major effect on the index), were the most influential group of variables in the computation of the ATI for macroinvertebrates. Nitrogen compounds with $r_s = 0.91$ (removal had smallest effect on composite index values) were the least influential set of variables in the computation of the ATI (Table 6.5).

CHAPTER 7: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 INDEX DEVELOPMENT

The overriding goal of the research project was the development of the ATI for macroinvertebrates, a quantitative tool for the assessment of river water quality status and trends, developed to aid in the operational management of South African rivers with elevated pollutants as a result of mining, industrial, agriculture and urban related sources of pollution like the Olifants River (East). Efforts to derive a more region specific index were deterred by data constraints for region specific (freshwater macroinvertebrate taxa occurring in South/Southern African rivers or region with similar climatic conditions) toxicity/tolerance data. The major constraints on data availability could be attributed to the fact that toxicity data for freshwater invertebrates available in literature and databases are abundant for standard test species and limited for others across target physico-chemical stressors. More data are available for standard test species because they are easy to culture in a laboratory, have short generation time (Brinkman and Johnston, 2012; Lutnicka *et al*., 2014) and information on their life histories, culturing and bioassay procedures have been widely documented and reviewed (Lohner and Fisher, 1990). The second limitation on data availability could be attributed to the focus of the research on macroinvertebrates, a narrow focus which excludes other invertebrates with abundant data like cladocerans (*e.g*. daphnids) and, lastly, being a general observation acknowledging that South Africa has limited ecotoxicity data for local/native taxa (Jin *et al*., 2015).

The use of non-native taxa and international data in deriving water quality benchmarks protective of native/local taxa is controversial because it is sometimes questionable whether benchmarks derived based on species from one geographical region provide appropriate protection for species in a different region (Sunderam *et al*., 1992; Davies *et al*., 1994; Jin *et al*., 2011). However, such a compromise is not unique to this study because it has been observed from literature that non-native taxa or international toxicity

data are still widely used by water quality jurisdictions (globally) as a basis for the development of their water quality guidelines or criteria. For example, there is evidence of the use of non-native taxa or international toxicity data in water quality criteria for freshwater life in South Africa (DWAF), Canada (CCME) and Australian and New Zealand (Australian and New Zealand Environment Conservation Council) (DWAF, 1996; ANZECC and ARMCANZ, 2000; Bantley *et al*., 2014). In particular, the Australian and New Zealand Environment Conservation Council (ANZECC) decided not to exclude international data in deriving their water quality criteria because excluding these data would have had undesirable consequences for their guideline values. As a result the ANZECC and ARMCANZ (2000) and proposed revisions incorporate all toxicity data irrespective of geographic or climatic differences (Bantley *et al*., 2014).

Nonetheless, studies conducted in China on the appropriateness of such data could not fully invalidate the relevancy and importance of international and or non-native ecotoxicity data as the basis of protecting organisms found in China. Studies by Jin *et al*. (2015) suggested that there is 74% certainty that criteria developed using non-native taxa can protect native Chinese aquatic ecosystems. Wu *et al*. (2015) further, observed no significant difference between native and non-native toxicity data, consequently it was concluded that non-native taxa could be used for ecological risk assessments irrespective of geographic regions where the data was sourced, under data constraints conditions.

7.2 LITERATURE-BASED VALIDATION OF THE ATI FOR MACROINVERTEBRATES

Further assessments of the index involved querying and comparisons of the protection concentration values (which form the foundation of the ATI for macroinvertebrates) with water quality benchmarks suggested by water quality jurisdictions and scholarly publications. The Burrlioz 2.0 SSD software (Barry and Henderson, 2014) used in this study produce three values for each point estimate (Upper and lower 95% confidence interval and median value). The upper 95% confidence interval and the median values were relatively large and not comparable with published water quality benchmarks and guidelines for the protection of freshwater ecosystems. For that particular reason, the

lower 95% confidence interval values were preferred in this study. It is widely suggested that where the median value (50% confidence interval) is inadequate to protect organisms of concern, the lower 95% of the SSD must be used (Newman *et al*., 2000; Tenbrook *et al*., 2010).

The ranking order of the toxicity of metals to freshwater ecosystems produced in this study were comparable with most publications (*e.g*. DWAF, 1996; ANZECC and ARMCANZ, 2000; Rathore and Khangarot, 2002; Shuhaimi-Othman *et al*., 2012). As expected, mercury, copper and cadmium (in no particular order) were the metals most toxic to freshwater organisms, while, zinc, lead, chromium and nickel (in no particular order) appeared to be relatively less toxic metal stressors to freshwater ecosystems.

Comparisons of the protection concentration values, made at PC95, a commonly used level of protection (ANZECC and ARMCANZ, 2000; Liu *et al*., 2014) and comparable water hardness levels, generated in this study were, however, predominantly higher for most metal stressors than those suggested by most water quality jurisdictions (*e.g*. ANZECC and ARMCANZ, CCME, DWAF and USEPA). This observation was, however, contrary to those of Bat *et al*. (2000) where they concluded based on a review of studies from 1971 to 1985 that macroinvertebrates were more sensitive to metals than algae and fish. Similarly, Xin *et al*. (2015) concurred with Bat *et al*. (2000), concluding that vertebrates, fish and cladocerans were more tolerant to heavy metals than macroinvertebrates. The most likely explanation for the observed differences between this study and previous publications for metals could be the differences in the methods used in generating the benchmark values. While some jurisdictions use SSD models, others develop their water quality benchmarks using the Assessment Factor (AF) method or a combination of the two (Nugegoda *et al*., 2013). Another possible explanation for the differences could be the fact that some benchmarks are goal oriented for example deflected towards the protection of imperilled organisms or organisms of socio-economic importance (USEPA, 1999; USEPA, 2013).

7.3 FIELD-BASED VALIDATION OF THE ATI FOR MACROINVERTEBRATES

The second focus of the study was on the validation (through application or testing) of the ATI for macroinvertebrates. The index was validated using field data generated from the Olifants River catchment (physico-chemical water quality data, riverine macroinvertebrates survey data and river flow data). It is widely accepted that river ecosystems within the same ecoregion and geomorphological zone would support similar faunal communities (Hering *et al*., 2012; Thirion, 2016; Nautiyal *et al*., 2017). However, in this study, the statistical analysis (Sorensen's similarity coefficient) of the spatial distribution of the composition of the macroinvertebrates did not clearly discriminate between the upper (S1 and S2) and the lower Olifants Sites (S3 to S8). It was unexpected that sites in the upper Olifants would cluster with sites in the lower Olifants River catchment. The interplay between ecoregions and spatial distribution and composition of riverine macroinvertebrates could have been confounded by factors like differences in habitat quality and macroinvertebrates habitat availability (*e.g*. Poff, 1997; De La Rey *et al*., 2008; Menezes *et al*., 2010). Additional explanation may include variations in water quality which in this particular study may have accounted for the differences in the composition and distribution of the riverine macroinvertebrates (*e.g*. Dallas and Day, 1993; Odume and Mgaba, 2016).

With particular reference to the magnitudes of physico-chemical water quality variables sites S2 (located in the Klein Olifants) and S5 (Located in the Ga-Selati) appeared to be the most degraded sites in the study, an observation that is supported by the cluster analysis (dendrogram) where S2 and S5 clustered together (with 85% similarity). It is from these two sites that most of the maximum concentrations of chemical variables were recorded. In agreement with these observations, De Villiers and Mkwelo (2009) rated the Ga-Selati as fair to poor state of quality. The ATI for macroinvertebrates (this study) and the ATI for Fish (Wepener *et al*., 1992) on the other hand both rated S5 as one of the most polluted sites in the study. The high pollutant levels in S5 could be attributed to the various riparian activities leading to increased fluxes of both point and non-point pollution for example the mining activities (Water Research Commission Mine water atlas, 2017).

7.4 COMPUTATION OF THE ATI FOR MACROINVERTEBRATES

The ATI for macroinvertebrates (composite index scores) indicated that the Olifants was in good condition, capable of sustaining a wide variety of freshwater macroinvertebrates. However, site evaluation results for S3, Blyde River, a site expected to be least impaired (control site), was ranked the lowest among the eight sites for the May 2016 and August 2016 sampling events. The most plausible explanation for the low Index scores for S3 could be that the ATI for macroinvertebrates incorporates the modifying effects of water hardness on six metals (Cd, Cr (III), Cu, Ni, Pb and Zn). In these cases, hard water or a high concentration of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (main constituents of total hardness) in the water are associated with increased competition for binding spaces on the gills and tissues of aquatic organisms thereby reducing the uptake of the metals by the organisms. The opposite is expected with relatively soft water (as was the case with the other study sites) (Specht, 2005; Casares *et al*., 2012). In the case of S3 therefore even under conditions where the metal pollutants concentration were relatively lower than most of the other study sites, the ATI would classify them as relatively more toxic due to the increased ameliorating effect of water hardness.

The evaluation of a water body with sole dependency on the composite/overall index score is known to conceal the identity of the variable (s) limiting the water quality (Smith, 1990). To compensate for this limitation some indices incorporate the minimum operator also known as the lowest rating score (variable limiting the water quality) example (Wepener *et al*., 1999; Gerber *et al*., 2015). Similarly, in this study, the ATI classified the water of the Olifants River as of good quality, sole dependency on the composite index value turn to conceal the identity of the water quality variable limiting the water's 'suitability for use (Wepener *et al*., 1999). Consequently, in this study the composite ATI was interpreted alongside the lowest rating score, where, temperature difference from reference conditions was identified as the predominant variable limiting the water quality of the Olifants River system, followed by sulfate and nitrate and to a lesser extent copper lead and zinc.

The second variable identified as a limitation to the water quality in the study was sulfate. Sulfate is normally considered an indicator of acid mine drainage (Gray, 1996; Dabrowski and de Klerk, 2013). The elevated sulfate concentrations could therefore be traced from the numerous mining activities within the catchment and major contributions from tributaries draining intensive mining areas as previously observed by Dabrowski *et al*. (2015). Similarly, nitrate was expected to be identified as one of the lowest rating scores in the catchment. This is the case largely because of the extensive agricultural activities and associated nutrient-laden irrigation return flows within the catchment. For example studies have previously mentioned that irrigation is allocated over 50% of all water abstractions and use from the Olifants River (Basson and Rossouw, 2003; Cullis and Van Koppen, 2007).

7.5 RELATIONSHIPS BETWEEN THE ATI FOR INVERTEBRATES AND THE ATI FOR FISH

As part of the validation of the index, correlation analysis for the relationships between the results of the ATI for macroinvertebrates scores and the Aquatic toxicity ATI the two indices were positive and statistically significant. The correlations between the two aquatic toxicity indices were expected to be positive mainly because both indices were formulated for the protection of freshwater organisms. However, the two indices were moderately correlated (r_s = 0.48) and not strong probably because of several points of departure.

1. While this study focused on macroinvertebrates, the ATI (Wepener *et al*., 1992) focused on the protection of fish.
2. Some of the differences may emanate from the differences in input variables in the computation of the two indices.
3. Incorporation of the toxicity modifiers, where, the ATI for fish was derived for water with hardness >120mg/L ($CaCO_3$), the ATI for macroinvertebrates incorporates a wide range (six) of hardness levels.
4. Differences in the treatment of field data for ammonia. While the ATI for fish purely evaluates ammonia based on magnitudes, the ATI for macroinvertebrates

requires that ammonia field data be converted to TAN with pH = 8 and temperature = 25^{o}C.

7.6 Relationships between the ATI for macroinvertebrates, SASS5 metrics and flow variability

The study indicated weak and negligible relationships between the ATI for macroinvertebrates and the measures of flow variability (BFI, CVI and FI). The correlations between the ATI and the measures of flow variability were very low (r_s<0.03) and insignificant (p<0.05). This observation is contrary to previous findings where for example it has been demonstrated that increase in stream flow may lead to increase in the concentrations of certain pollutants as either a result of weathering (Tiemuer *et al*., 2015) or re-suspension of metals from sediments into the water column (Gerber *et al*., 2015), while others have emphasised a uniform decrease in pollutants' concentrations with increase in flows because of dilution (Tiemuer *et al*., 2015; USEPA, 2017).

Eco-hydrological studies have demonstrated and emphasised the necessity of the incorporation of flow regimes in explaining the community structure of stream organisms (Poff *et al*., 1997; Bunn and Arthington, 2002; Poff *et al*., 2010; Ceola *et al*., 2013). This is particularly the case because stream flows have been widely reported to modify habitat and water chemistry (Brittain and Eikeland, 1988; Lytle and Poff, 2004; Jacobsen *et al*., 2013). As expected, the relationships between the SASS5 metrics and the flow variability measures were predominantly statistically significant. It is of note however that the SASS5 metrics related differently to the stream flow indices, whereby positive relationships were observed between the SASS5 metrics and the CVI and the FI. Literature posit that flashy and highly variable stream flows can have negative effects on stream organisms, since they are normally associated with the scouring of riverbed and the erosion of stream beds (Lu *et al*., 2012). On the other hand strong negative relationships were observed between the CVI and FI with the BFI. These kinds of associations were expected because highly variable streams are normally associated with low base-flows (Smakhtin, 2004; Le Maitre and Colvin, 2008).

It was however unexpected that, the correlations between the ATI for macroinvertebrates and the SASS5 metrics were very weak and negligible. Several confounding factors could have accounted for the lack of association between the ATI for macroinvertebrates and the SASS5 metrics.

1. It is most likely that other physico-chemical water quality variables not included in the index may have affected the relationships between the ATI for macroinvertebrates and the SASS5 metrics. Not only is this explanation limited to the ATI for macroinvertebrates, but a general limitation of water quality indices (McClelland, 1974; Semiromi *et al*., 2011).

2. The riverine macroinvertebrates were sampled during a period-characterised flow reduction and high accumulation of filamentous algae and other submerged aquatic vegetation. The use of benthic/kick sampling methods executed through the SASS5 protocol (Dickens and Graham, 2002), may not have been the best sampling approach for a system with high volumes of submerged/floating aquatic vegetation (posing a physical obstruction to sampling for the invertebrates). This is the case because upon dipping the kick nets into the water the vegetation filled up and clogged the nets thereby making it difficult to sample all biotopes and to separate the invertebrates from the vegetation. Under such compromising situations, it may have been proper to use other complementary samplers and methods, like the surber, drift and hester-dendy samplers. Complementary samplers are necessary because they are capable of capturing divergent invertebrates that benthic and kick methods cannot always capture (Pringle and Ramirez, 1998). For example, surber samplers are capable of capturing rare macroinvertebrates species (Kirk and Perry, 1994).

3. The high densities of filamentous algae on rocks and stone (stone in current) surfaces significantly compromised the availability of SASS5 biotopes. The presence of the filamentous algae in large quantities interfered with the macroinvertebrates physical habitat structure. The filamentous algae physically transformed stone biotopes into vegetation substrate or a hybrid thereof. Physical

habitat structure has been identified as one of the major factors affecting the ecological integrity of riverine ecosystems (Ollis *et al*., 2006) and is therefore recognised as a critical determinant for the composition, diversity and abundance of resident biological communities (Norris and Thoms, 1999; Ollis *et al*., 2006). For instance, the composition, diversity and abundance of riverine macroinvertebrates are determined, partly, by the quantity, quality and diversity of available habitats for invertebrate habitation (Ollis *et al*., 2006). Similarly, the SASS is a multi-biotope method (requiring sampling from all biotopes) and it has been observed to be sensitive to biotope diversity (Chutter, 1995). Additionally, invertebrate variability with reference to the SASS-biotopes (stones, vegetation and gravel, sand and mud biotopes) has been observed to exhibit a degree of biotope specificity, where taxa are known to have specific preference for one biotope over the other (Dallas, 2002). In this study, the accumulation of filamentous algae in particular, might have largely interfered with and transformed biotopes. For example, bedrock and stone biotopes (mostly stones in current) transformed stone biotopes to exhibit the characteristics of vegetation biotopes. As noted by Thirion (2007) the accumulation of filamentous algae on stones/cobbles may affect SASS scores and ultimately lowering the MIRAI invertebrate ecological category than expected.

While the large densities of filamentous algae and floating aquatic plants for most study sites may have affected the general surveys of the macroinvertebrates for a greater proportion of the study, there is no consensus from literature establishing the kind of relationship between aquatic vegetation and macroinvertebrates, (Tonkin *et al*., 2014). Dudley *et al*. (1986) suggested that aquatic vegetation have both positive and negative effects on macroinvertebrates. For example, algae are important sources of food for many aquatic macroinvertebrate herbivores (Shannon *et al*., 1994; Guo *et al*., 2016). Algae provide substrate for attachment by filter feeders, predators and other taxa (Dudley *et al*., 1986), yet they also alter riverine microhabitats by slowing currents, trapping detritus, blocking light, altering oxygen regimes (Dudley *et al*., 1986). Nonetheless, studies by Tonken *et al*. (2014) of the relationships between biomass–macroinvertebrate diversity relationships in 24

streams of Cantabria in Spain uncovered no relationship between benthic periphyton biomass and macroinvertebrate diversity.

4. The SASS method was developed for organic pollution stress on riverine macroinvertebrates (Chutter, 1994; Gordon *et al*., 2015). Previous studies have clearly demonstrated that SASS indices, particularly the SASS scores and ASPT, directly relate to water quality and have clearly been shown to be sensitive to organic pollution (Uys *et al*., 1996). The ATI for macroinvertebrates on the other hand is largely driven by metal pollutants with limited focus on organic pollutants. It is most likely that the correlations between the SASS5 and the ATI for macroinvertebrates were weak and of no statistical significance partly because of this point of departure.

7.7 SENSITIVITY ANALYSIS

In an attempt to get feedback from the individual water quality variables used in the computation of ATI, the sensitivity analysis was conducted for ranking variables based on their relative contribution on the general performance of the index and to assist in prioritise the stressors to monitor. As expected the sensitivity analysis of the ATI for macroinvertebrates, indicated that temperature difference from reference conditions, sulfate and nitrate were the most influential variables on the index. These observations suggest that the computation of the index without the three variables resulted in better index scores. This particular observation supports the identification of the lowest rating scores, where temperature difference from background conditions, sulfate and nitrate were identified as the main three variables limiting the water quality of the Olifants River in the study. On the other hand, the sensitivity analysis process of the ATI for macroinvertebrates rated chloride, chromium (III), dissolved oxygen and mercury (II) as the least important variables in the assessment of the Olifants River. This observation imply that the four variables safely be excluded from the index. However, since the sensitivity analysis was conducted on relatively short-term data, it may therefore be recommended that three of the lowest rated variables (chloride, chromium (III) and dissolved oxygen) be retained and mercury (II) be permanently excluded from the further

computation of the index particularly because it was not detected from all stations throughout all the sampling events. Under similar considerations, Zali *et al.* (2001) excluded two initially justified and selected water quality variables (biological oxygen demand and chemical oxygen demand) after conducting a sensitivity analysis for the water quality index for use in the Kinta River, Malaysia. In that instance, the two variables were excluded because they were too costly and time consuming yet had minimum correlation for water quality index forecasting (Zali *et al*., 2011).

Metal pollutants on the other hand emerged as the most influential group of variables in the ATI evaluations based on the water quality data generated from the Olifants River. It is likely that the reference index appeared to be the more sensitive to the exclusion of metal pollutants primarily because metal pollutants comprised the majority of variables in analysis (57%). Previous work by Rickwood and Carr (2009) suggested that the sensitivity of an index (*e.g*. the water quality index for biodiversity) could be explained based on the number of input variables. In these cases, more significant differences were observed between assessments with fewer variables than with relatively more variables. On the contrary, Lee (2006) observed that the number of input variables in the computation of an index was immaterial, but what seem more relevant are the specifics of which variables are either included or excluded from the computation of the reference index. Omission of these variables would most likely portray a misleading picture about the state of the river.

7.8 CONCLUSIONS

The conclusions for the study were drawn to address each of the four aims of the study. These span from the development of the Aquatic Toxicity for freshwater macroinvertebrates to the sensitivity analysis of the index using physico-chemical water quality data collected from the Olifants River catchment.

Aim 1: Development of an ATI for freshwater macroinvertebrates.

The study set out to develop an ATI for freshwater macroinvertebrates based on short-term (24-96 hours) acute toxicity/tolerance (LC50) exposure endpoints of macroinvertebrates to physico-chemical water quality variables/stressors collected from

databases and scholarly publications. Due to shortage of toxicity data for local freshwater macroinvertebrates, the protection concentrations upon which the index was developed were fitted on data collected from international taxa as opposed to local or region specific data. Irrespective of these limitations, the protection concentrations (numerical sensitivity values) that form the base of the index were comparable with published water quality benchmarks from literature and water quality jurisdictions. In addition, the index has the ability to summarise, discriminate (stressors in terms of concentrations and magnitudes) large quantities of water quality data to facilitate interpretation of a water's suitability to sustain freshwater macroinvertebrates.

Aim 2: Characterise the spatio-temporal patterns of the physico-chemical water quality variables and riverine macroinvertebrates community structure of the Olifants River.

1. The interpretation of the data variable-by-variable indicated gross elevation of pH, sulfate, nitrates and copper. Site S5 (located within the Ga-Selati) and S2 (Klein Olifants site) appeared to be the most impacted sites in the study.

2. The spatial distribution of the composition of the macroinvertebrates did not clearly discriminate between the upper (S1 and S2) and the lower Olifants Sites (S3 to S8), as the highest similarities were observed between upper Olifants and lower Olifants *i.e.* between S2 (Klein Olifants) and S6 (Olifants River at Mamba Weir).

Aim 3: Determine the relationship between the ATI for macroinvertebrates with SASS5 metrics, MIRAI, ATI for invertebrates and measures of flow variability.

1. The study revealed no relationships between the ATI for macroinvertebrates with SASS5 metrics. This was largely due to several confounding factors resulting from the 2015/2016 Southern African drought, where due to inability of the system to flush, there was large accumulation of filamentous algae which largely inhibited the sampling of the macroinvertebrates using the SASS5 protocol (benthic/kick method).

2. There were observed associations between the SASS5 metrics and the measures of flow variability, which were however largely dependent on the inclusion/exclusion of sites with extended periods of flow cessation.

3. Inter-reliability test revealed no consistency of statistical significance in the rating of the eight study sites by ATI for macroinvertebrates, the SASS5 metrics and the MIRAI.

Aim 4: Conduct the sensitivity analysis of the ATI for macroinvertebrates.

1. The sensitivity analysis of the index indicated that temperature difference from reference conditions; sulfate and nitrate were the most influential variables on the evaluations of the ATI for macroinvertebrates. Metal pollutants on the other hand emerged as the most influential group of variables in the ATI evaluations based on the water quality data generated from the Olifants River.

2. There was a general agreement between evaluation of the magnitude of the physico-chemical water quality variables variable-by-variable, the identification of the lowest rating score and the sensitivity analysis. All these measures identified sulfate and nitrate as sources of impairments in the Olifants River.

7.9 RECOMMENDATIONS FOR FUTURE RESEARCH

1. It is recommended that future studies be conducted to generate more toxicity/tolerance data for freshwater macroinvertebrates native to Southern Africa/South Africa. This would enable the further refinement of the index or any other related tool to be founded on region specific toxicity data.

2. It is recommended that further research be conducted to investigate the modifying effects of key ambient physico-chemical conditions of the river water on target environmental stressors other than only water hardness

3. High accumulation of filamentous algae and floating aquatic vegetation inhibit the effective use of benthic/kick macroinvertebrates sampling methods. It is therefore recommended that future validation of the ATI against riverine macroinvertebrates surveys be conducted using complementary/alternative macroinvertebrates sampling methods (*e.g*. a complementary survey approach integrating surber samplers, hester-dendy traps and drift sampling methods).

4. The application and sensitivity analysis of the ATI for macroinvertebrates in a different river system (characteristic of different land-uses) is expected to produce different results as a direct consequence of differences in types of pollutant stocks and fluxes. It is therefore recommended that future studies be carried out using the ATI for macroinvertebrates and that sensitivity analysis be conducted to enhance understanding of the contribution of input variables to the index.

CONFERENCE PRESENTATIONS AND PAPERS EMANATING FROM THE STUDY

1. Mnisi, L. N., Snow G. 2015. Development of an ATI for Macroinvertebrates (Water Quality Criteria). Presented at the Southern African Society of Aquatic Scientists. 28 June to 02 July 2015. Drakensberg, South Africa.

2. Mnisi, L. N., Snow, G. 2016. Towards the development of an ATI for Macroinvertebrates. Presented at the Southern African Society of Aquatic Scientists. 26 to 30 June 2016, Skukuza: Kruger National Park, South Africa.

3. Mnisi, L. N., Snow, G. 2017. Development and application of the ATI for Macroinvertebrates. Presented at the 20th River Symposium and Environmental Flows Conference.18 to 20 September 2017. Brisbane, Australia.

4. Mnisi, L.N., Snow, G. Development of an Aquatic Toxicity Index for Macroinvertebrates (sent for publication, review in progress).

5. Mnisi, L. N., Snow, G. Application and sensitivity analysis of the Aquatic Toxicity Index for macroinvertebrates (sent for publication, review in progress)

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APPENDIX 1: Commonly measured water quality variables in the South Africa with specific reference to the Olifants River catchment (pollutants incorporated in the ATI for macroinvertebrates are in boldface)

Variables	**Wepener *et al*. (1992)**	**DWAF (1996)**	**Bollmohr *et al*. (2008)**	**Ashton and Dabrowski (2011)**	**Gerber *et al*. (2015)**
Aluminum		X	X	X	X
Arsenic		X	X	X	X
Cadmium		X	X		X
Chromium		X			X
Cobalt			X		X
Copper	X	X	X	X	X
Iron		X	X	X	X
Lead	X		X	X	X
Manganese	X	X	X	X	X
Nickel	X		X	X	X
Selenium		X		X	X
Zinc	X	X	X	X	X
Potassium	X			X	X
Sodium			X		X
pH	X				X
Temperature		X			X
Dissolved oxygen	X	X			X
Total Dissolved salts	X	X			X
Ammonia/Ammonium	X	X			X
Chloride					X
Chemical oxygen demand					X
Nitrate					X
Nitrite					X
Phosphorus/phosphate/orthoposphate	X	X		X	X
Sulfate					X
Turbidity	X	X			X
Fluoride	X	X			
Chromium	X	X	X		
Atrazine		X			

Chlorine		X			
Cyanide		X			
Mercury		X			
Endosulfan		X			
Nitrogen		X			
Phenol		X			
Selenium					
Total suspended solids		X			
Barium			X	X	
Beryllium			X	X	
Molybdenum			X		
Strontium			X	X	
Titanium			X		
Vanadium			X	X	
Boron			X	X	
Calcium				X	
Magnesium				X	
Antimony				X	
Bismuth				X	
Silver				X	
Silicon				X	
Tin				X	
Wolfram				X	
Zirconium				X	

APPENDIX 2: Stepwise process for the manual calculation of the ATI for macroinvertebrates: Illustrative calculations

The illustrative calculations of the ATI for macroinvertebrates were based on river water sample data collected from the Blyde River at Blyde River at Driehoek in November 2015.

Step 1: Conversions of units

I. Total Ammonia Nitrogen (TAN): adjusted to pH=8, and temperature to 25^{o}C
II. Nitrates: from NO_3 (mg/L) to NO_3-N (mg/L).

Step 2: Determination of temperature difference from reference sites

Water temperature °C to temperature tolerance interval (as a measure of river water temperature difference from reference temperature) by subtracting reference temperature (*RT*) from measured temperature (*MT*) (see illustration below).

- $TDR = MT - RT$
- $MT = 22.1$
- $RT = 18.1$
- $TDR = 4$

Where *TDR* (^{o}C) represents temperature difference from reference site, *MT* (^{o}C) represents measured temperature and *RT* (^{o}C) represents reference site water temperature (comparable temperature from the Klaserie River at comparable month and time of day).

Step 2: Selecting appropriate hardness for the site

Site 3 water hardness is within 75-120 mg/L as $CaCO_3$, so the stressors were rated using hardness = 90mg/L as $CaCO_3$ (Table 4.24).

Step 3: Sub-indexing (based on hazard rank scores)

Selection of sub-indices by awarding appropriate hazard rank scores obtained from Table 4.24.

A sample calculation of ATI for S3 (Blyde River at Driehoek) in November 2015

N	WQ Variable	Magnitudes	Sub-indices	Grading symbols
1	TAN mg/l (pH=8, Temp=25^{o}C)	0.03	100	A
2	DO (mg/l)	8.40	100	A
3	Temp difference from ref. site (^{o}C)	22.10	80	B
4	Cl^{-} (mg/l)	4.06	100	A
5	NO_3 –N (mg/l)	20.59	20	E
6	SO_4^{2+}-S (mg/l)	18.20	80	B
7	Cr (III) (µg/l)	ND	100	A
8	Cr (VI) (µg/l)	ND	100	A
9	Ni (µg/l)	332.00	80	B
10	Cu (µg/l)	ND	100	A
11	Zn (µg/l)	174.00	60	C
12	Cd (µg/l)	7.60	60	C
13	Pb (µg/l)	ND	100	A
14	Hg (II) (µg/l)	ND	100	A

Step 4: Aggregation of sub-indices

Sub-indices for each variable are aggregated using the Solway modified un-weighted aggregating function (Equation 3.7, main text).

$$I = \frac{1}{100}\left(\frac{1}{n}\sum_{i=1}^{n} q_i\right)^2$$

Where *I* is the final index score *qi* is the equation of the *ith* parameter (as value between 0 and 100) *n* is the number of determinants in the indexing system.

Solway modified un-weighted aggregating function (substitutions for this study)

- $n = 14$
- $(\sum_{i=1}^{n} q_i) = 1180$
- $ATI = \frac{1}{100}\left(\frac{1}{14} * 1180\right)^2$

Composite ATI score

- The composite ATI=71.04 representing water of good quality

Lowest rating score: NO_3-N (with sub-index value of 20)

- NO_3-N is the water quality variable with the lowest sub-index score, implying that it was the determinant limiting the water quality of the Blyde River at Driehoek at the time of sampling.

APPENDIX 3: Acute toxicity cadmium data for freshwater macroinvertebrates sourced from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical name	Species name	Species group	Endpoint	Hardness (mg/L as $CaCO_3$)	Concentration (µg/L)	Reference
Cadmium chloride	*Niphargus aquilex*	Crustaceans	96h-LC50	103.2	2450	Meinel *et al.*, 1989
Cadmium chloride	*Atyaephyra desmarestii*	Crustaceans	96h-LC50	263.43	51.82	Pestana *et al.*, 1988
Cadmium Sulfate	*Lymnaea acuminata*	Molluscs	96h-LC50	375	872	Khangarot and Ray 1988
Cadmium Sulfate	*Radix luteola*	Molluscs	96h-LC50	195	1520	Khangarot and Ray 1988
Cadmium Sulfate	*Varichaeta pacifica*	Worms	96h-LC50	5.3	380	Chapman,1982
Cadmium nitrate	*Biomphalaria glabrata*	Molluscs	96h-LC50	100	300	Bellavere and Gorbi,1981
Cadmium chloride	*Lumbriculus variegatus*	Worms	96h-LC50	30	74	Bailey and Liu,1980
Cadmium chloride	*Lirceus alabamae*	Crustaceans	96h-LC50	152	150	Bosnak and Morgan, 1981
Cadmium chloride	*Branchiura sowerbyi*	Worms	96h-LC50	195	36580	Das and Kaviraj, 1994
Cadmium Sulfate	*Afrochiltonia subtenuis*	Crustaceans	96h-LC50	10	40	Thorp and Lake, 1974
Cadmium Sulfate	*Aedes aegypti*	Insects/Spiders	48h-LC50	44	500	Simonet *et al.*, 1978
Cadmium Sulfate	*Atalophlebia australis*	Insects/Spiders	96h-LC50	10	840	Thorp and Lake,1974
Cadmium Sulfate	*Rhithrogena hageni*	Insects/Spiders	96h-LC50	48	10500	Brinkman and Johnston, 2008
Cadmium Sulfate	*Ischnura heterosticta*	Insects/Spiders	96h-LC50	10	23300	Thorp and Lake,1974
Cadmium chloride	*Paraleptophlebia praepedita*	Insects/Spiders	96h-LC50	67	449	Spehar and Carlson, 1984
Cadmium chloride	*Macromia* sp.	Insects/Spiders	96h-LC50	20	340	Fennikoh *et al.*, 1978
Cadmium chloride	*Melanoides tuberculata*	Molluscs	96h-LC50	18.72	1490	Shuhaimi-Othman *et al.*, 2013
Cadmium chloride	*Girardia tigrina*	Worms	96h-LC50	42.75	740	Safadi, 1998
Cadmium chloride	*Pristina leidyi*	Worms	96h-LC50	95	214.6	Smith *et al.*, 1991
Cadmium	*Dendrocoelum lacteum*	Worms	96h-LC50	87	23220	Ham *et al.*, 1995
Cadmium chloride	*Echinogammarus meridionalis*	Crustaceans	96h-LC50	263.43	44.15	Pestana *et al.*, 2007
Cadmium chloride	*Barytelphusa guerini*	Crustaceans	96h-LC50	112	1820	Venugopal *et al.*, 1997
Cadmium	*Hexagenia rigida*	Insects/Spiders	96h-LC50	79.1	6200	Leonhard *et al.*, 1980
Cadmium chloride	*Procambarus*	Crustaceans	96h-LC50	240	58500	Ramo *et al.*, 1987
Cadmium chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	240	18400	Ramo *et al.*, 1987

Cadmium chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	30.32	1040	Naqvi *et al.,* 1993
Cadmium chloride	*Procambarus* sp.	Crustaceans	96h-LC50	20	5000	Fennikoh *et al.*, 1978
Cadmium chloride	*Aplexa hypnorum*	Molluscs	96h-LC50	44.8	93	Holcombe *et al.*, 1984
Cadmium chloride	*Aplexa hypnorum*	Molluscs	96h-LC50	44.4	93	Phipps and Holcombe, 1985
Cadmium chloride	*Caecidotea* sp.	Crustaceans	96h-LC50	220	2200	Bosnak and Morgan, 1981
Cadmium chloride	*Caecidotea* sp.	Crustaceans	96h-LC50	220	2060	Bosnak and Morgan, 1981
Cadmium Sulfate	*Paratya australiensis*	Crustaceans	96h-LC50	10	60	Thorp and Lake,1974
Cadmium Sulfate	*Paratya australiensis*	Crustaceans	96h-LC50	10	180	Thorp and Lake, 1974
Cadmium chloride	*Orconectes immunis*	Crustaceans	96h-LC50	44.4	10200	Phipps and Holcombe, 1985
Cadmium chloride	*Orconectes virilis*	Crustaceans	96h-LC50	26	6100	Mirenda, 1986
Cadmium Sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	180	1225	Gupta *et al.*, 1981
Cadmium chloride	*Viviparus bengalensis*	Molluscs	96h-LC50	165	1550	Gadkari and Marathe, 1983
Cadmium	*Hyalella azteca*	Crustaceans	96h-LC50	146.9	17.5	Shuhaimi-Othman and Pascoe, 2001
Cadmium nitrate	*Hyalella azteca*	Crustaceans	96h-LC50	290	25	Schubauer-Berigan *et al.*, 1993
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	47.4	23	Call *et al.*, 1981
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	40	7.5	Collyard *et al.*, 1994
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	40	15	Collyard *et al.*, 1994
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	34	8	Nebeker *et al.*, 1986
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	79	6.92	Nebeker *et al.*, 1986
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	40	10	Collyard *et al.*, 1994
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	17	2.8	Suedel *et al.*, 1997
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	259	106	McNulty *et al.*, 1999
Cadmium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	259	121	McNulty *et al.*, 1999
Cadmium chloride	*Ranatra elongata*	Insects/Spiders	96h-LC50	112.4	288	Shukla *et al.*, 1983
Cadmium chloride	*Enallagma* sp.	Insects/Spiders	96h-LC50	15.3	10660	Mackie 1989
Cadmium chloride	*Enallagma* sp.	Insects/Spiders	96h-LC50	15.3	7050	Mackie 1989
Cadmium chloride	*Enallagma* sp.	Insects/Spiders	96h-LC50	15.3	8660	Mackie 1989
Cadmium chloride	*Dugesia tigrina*	Worms	96h-LC50	50	2250	See *et al.*, 1974
Cadmium chloride	*Dugesia* sp.	Worms	96h-LC50	20	4900	Fennikoh *et al.*, 1978
Cadmium chloride	*Dugesia tigrina*	Worms	96h-LC50	40	2200	See *et al.*, 1974

Cadmium chloride	*Nais elinguis*	Worms	96h-LC50	18.72	27	Shuhaimi-Othman *et al.*, 2013
Cadmium chloride	*Nais elinguis*	Worms	96h-LC50	17.89	27	Shuhaimi-Othman *et al.*, 2012
Cadmium chloride	*Trichodrilus tenuis*	Worms	96h-LC50	103.2	1150	Meinel *et al.*, 1989
Cadmium chloride	*Trichodrilus tenuis*	Worms	96h-LC50	103.2	1470	Meinel *et al.*, 1989
Cadmium chloride	*Trichodrilus tenuis*	Worms	96h-LC50	103.2	800	Meinel *et al.*, 1989
Cadmium chloride	*Trichodrilus tenuis*	Worms	96h-LC50	103.2	1050	Meinel *et al.*, 1989
Cadmium chloride	*Tubifex tubifex*	Worms	96h-LC50	128	400	Reynoldson *et al.*, 1996
Cadmium chloride	*Tubifex tubifex*	Worms	96h-LC50	128	3200	Reynoldson *et al.*, 1996
Cadmium chloride	*Tubifex tubifex*	Worms	96h-LC50	128	1700	Reynoldson *et al.*, 1996
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	540	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	170	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	150	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	80	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	600	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	300	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	175	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	87	160	Quinn and Pascoe, 1995
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	170	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	240	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	53	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	450	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	320	Green *et al.*, 1986
Cadmium	*Asellus aquaticus*	Crustaceans	96h-LC50	104.8	230	Green *et al.*, 1986
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	560	Meinel *et al.*, 1989
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	560	Meinel *et al.*, 1989
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	560	Meinel *et al.*, 1989
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	500	Meinel *et al.*, 1989
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	500	Meinel *et al.*, 1989
Cadmium chloride	*Asellus* sp.	Crustaceans	96h-LC50	103.2	560	Meinel *et al.*, 1989
Cadmium chloride	*Chironomus* sp.	Insects/Spiders	96h-LC50	50	1200	Rehwoldt *et al.*, 1973

Cadmium chloride	*Chironomus javanus*	Insects/Spiders	96h-LC50	15.63	60	Shuhaimi-Othman *et al.*, 2011
Cadmium chloride	*Chironomus javanus*	Insects/Spiders	96h-LC50	18.72	60	Shuhaimi-Othman *et al.*, 2013
Cadmium chloride	*Amnicola* sp.	Molluscs	96h-LC50	50	8400	Rehwoldt *et al.*, 1973
Cadmium chloride	*Amnicola* sp.	Molluscs	96h-LC50	50	3800	Rehwoldt *et al.*, 1973
Cadmium chloride	*Macrobrachium lanchesteri*	Crustaceans	96h-LC50	18.72	7	Shuhaimi-Othman *et al.*, 2011
Cadmium chloride	*Macrobrachium lanchesteri*	Crustaceans	96h-LC50	15.63	7	Shuhaimi-Othman *et al.*, 2013
Cadmium chloride	*Macrobrachium rude*	Crustaceans	96h-LC50	116	14	Vijayaraman and Geraldine, 1992
Cadmium chloride	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	41	40.76	Shazili and Ali 1988
Cadmium chloride	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	47.4	22	Call *et al.*, 1981
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	29	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	31	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	94	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	23	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	13	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	94.6	13	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	15	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	60	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	67	54.4	Spehar and Carlson, 1984
Cadmium chloride	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	76.8	49	Call *et al.*, 1981
Cadmium chloride	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	68	62	Call *et al.*, 1981
Cadmium chloride	*Gammarus fossarum*	Crustaceans	96h-LC50	173	6.2	Musko *et al.*,1990
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	117.4	50	McCahon *et al.*, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	113.9	18	McCahon and Pascoe, 1988 a
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	16	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	20	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	145.1	70	McCahon and Pascoe, 1988
Cadmium chloride	*Gammarus pulex*	Crustaceans	96h-LC50	94.6	20	McCahon and Pascoe, 1988 b

APPENDIX 4: Acute toxicity data for chromium (III) to freshwater macroinvertebrates sourced from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical	Species name	Species group	Endpoint	Concentration (µg/L)	Hardness (mg/L as $CaCO_3$)	Reference
Chromium	*Gammarus* sp.	Crustaceans	96h-LC50	3200	50	Rehwoldt *et al.*, 1973
Chromium	**Zygoptera**	Insects/Spiders	96h-LC50	43100	50	Rehwoldt *et al.*, 1973
Chromium	*Chironomus* sp.	Insects/Spiders	96h-LC50	11000	50	Rehwoldt *et al.*, 1973
Chromium	*Amnicola* sp.	Molluscs	96h-LC50	12400	50	Rehwoldt *et al.*, 1973
Chromium	*Amnicola* sp.	Molluscs	96h-LC50	8400	50	Rehwoldt *et al.*, 1973
Chromium	*Nais* sp.	Worms	96h-LC50	9300	50	Rehwoldt *et al.*, 1973
Chromium chloride	*Ephemerella subvaria*	Insects/Spiders	96h-LC50	2000	44	Warnick and Bell, 1969
Chromium chloride	*Hydropsyche betteni*	Insects/Spiders	96h-LC50	64000	44	Warnick and Bell, 1969
Chromium chloride	*Crangonyx pseudogralis*	Crustaceans	96h-LC50	291000	50	Martin and Holdich, 1986
Chromium chloride	*Asellus aquaticus*	Crustaceans	96h-LC50	442000	50	Martin and Holdich, 1986

***Boldface**: denotes higher taxonomic rank (sub-order)*

APPENDIX 5: Acute toxicity data for chromium (VI) to freshwater macroinvertebrates sourced from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical name	Species name	Species group	Endpoint	Concentration (µg/L)	Reference
Potassium dichromate	*Tanytarsus dissimilis*	Insects/Spiders	48h-LC50	57300	Call *et al.*, 1983
Potassium dichromate	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	460	Pantani *et al.*, 1997
Potassium dichromate	*Hyalella curvispina*	Crustaceans	96h-LC50	200	Peluso *et al.*, 2011
Potassium dichromate	*Hyalella curvispina*	Crustaceans	96h-LC50	550	Peluso *et al.*, 2011
Potassium dichromate	*Macrobrachium lamarrei*	Crustaceans	96h-LC50	640	Murti and Shukla 1983
Potassium dichromate	*Asellus intermedius*	Crustaceans	96h-LC50	15000	Ewell, *et al.*, 1986
Potassium dichromate	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	94	Call *et al.*, 1983
Potassium dichromate	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	67	Call *et al.*, 1983
Potassium dichromate	*Gammarus fasciatus*	Crustaceans;	96h-LC50	320	Ewell, *et al.*, 1986
Potassium dichromate	*Orconectes rusticus*	Crustaceans	96h-LC50	176000	White, 1983
Potassium dichromate	*Hydropsyche* sp.	Insects/Spiders	48h-LC50	280000	Roback, 1965
Potassium dichromate	*Agnetina capitata*	Insects/Spiders	96h-LC50	1870000	White, 1983
Potassium dichromate	*Stenonema rubrum*	Insects/Spiders	48h-LC50	3500	Roback, 1965
Potassium dichromate	*Enallagma aspersum*	Insects/Spiders	96h-LC50	140000	White, 1983
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	23000	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	65320	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	23110	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	89900	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	38560	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	90680	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	39160	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	67650	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	69260	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	76500	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	67030	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	17230	Larrain *et al.*, 1997

Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	65890	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	20340	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	74160	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	90630	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	21340	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	20300	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	20940	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	19350	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	65270	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	16640	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	19370	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	17300	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	90920	Larrain *et al.*, 1997
Potassium dichromate	*Chironomus* sp.	Insects/Spiders	48h-LC50	75490	Larrain *et al.*, 1997
Potassium dichromate	*Burnupia* sp.	Molluscs	96h-LC50	3700	Palmer *et al.*, 2004
Potassium dichromate	*Viviparus bengalensis*	Molluscs	96h-LC50	2200	Gupta *et al.*,1981
Potassium dichromate	*Anculosa* sp.	Molluscs	48h-LC50	800	Cairns *et al.*, 1978
Potassium dichromate	*Anculosa* sp.	Molluscs	48h-LC50	1200	Cairns *et al.*, 1978
Potassium dichromate	*Anculosa* sp.	Molluscs	48h-LC50	7800	Cairns *et al.*, 1978
Potassium dichromate	*Anculosa* sp.	Molluscs	48h-LC50	3700	Cairns *et al.*, 1978
Potassium dichromate	*Biomphalaria glabrata*	Molluscs	24h-LC50	115000	Bellavere and Gorbi, 1981
Potassium dichromate	*Biomphalaria glabrata*	Molluscs	48h-LC50	66200	Bellavere and Gorbi, 1981
Potassium dichromate	*Biomphalaria glabrata*	Molluscs	96h-LC50	37300	Bellavere and Gorbi, 1981
Potassium dichromate	*Lymnaea acuminata*	Molluscs	96h-LC50	5970	Khangarot *et al.*, 1982
Potassium dichromate	*Planorbella trivolvis*	Molluscs	96h-LC50	32000	Ewell *et al.*, 1986
Potassium dichromate	*Elimia livescens*	Molluscs	48h-LC50	2400	Cairns *et al.*, 1976
Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	31600	Academy of Natural Sciences, 1960
Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	49000	Academy of Natural Sciences, 1960
Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	17300	Academy of Natural Sciences, 1960
Potassium dichromate	*Physella heterostropha*	Molluscs	96h-LC50	17300	Patrick *et al.*, 1968

Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	40600	Academy of Natural Sciences, 1960
Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	17300	Academy of Natural Sciences, 1960
Potassium dichromate	*Physa heterostropha*	Molluscs	96h-LC50	49000	Academy of Natural Sciences, 1960
Potassium dichromate	*Dugesia tigrina*	Worms	96h-LC50	7300	See, 1976
Potassium dichromate	*Dugesia tigrina*	Worms	96h-LC50	40000	Ewell *et al.*, 1986
Potassium dichromate	*Aeolosoma headleyi*	Worms	48h-LC50	7000	Cairns *et al.*, 1978
Potassium dichromate	*Aeolosoma headleyi*	Worms	48h-LC50	12100	Cairns *et al.*, 1978
Potassium dichromate	*Aeolosoma headleyi*	Worms	48h-LC50	8600	Cairns *et al.*, 1978
Potassium dichromate	*Aeolosoma headleyi*	Worms	48h-LC50	10000	Cairns *et al.*, 1978
Potassium dichromate	*Aeolosoma headleyi*	Worms	48h-LC50	4800	Cairns *et al.*, 1978
Potassium dichromate	*Lumbriculus variegatus*	Worms	96h-LC50	32000	Ewell *et al.*, 1986
Potassium dichromate	*Lumbriculus variegatus*	Worms	96h-LC50	13300	Bailey and Liu, 1980
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	7220	Maestre *et al.*, 2009
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	6290	Maestre *et al.*, 2009
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	4890	Maestre *et al.*, 2009
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	15500	Reynoldson *et al.*, 1996
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	4990	Maestre *et al.*, 2009
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	5490	Maestre *et al.*, 2009
Potassium dichromate	*Tubifex tubifex*	Worms	96h-LC50	9800	Reynoldson *et al.*, 1996

APPENDIX 6: Acute toxicity data for copper to freshwater macroinvertebrates sourced from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical name	Species name	Species group	End-point	Hardness (mg/L as $CaCO_3$)	Conc. (µg/L)	Reference
Copper chloride hydroxide	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	48.07	50	Lombardi *et al.*, 2000
Copper chloride hydroxide	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	46.55	60	Lombardi *et al.*, 2000
Copper chloride	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	41	10.42	Shazili and Ali, 1998
Copper sulfate	*Macrobrachium rude*	Crustaceans	96h-LC50	116	18	Vijayaraman and Geraldine, 1992
Copper sulfate	*Macrobrachium lanchesteri*	Crustaceans	96h-LC50	18.72	32	Shuhaimi-Othman *et al.*, 2013
Copper sulfate	*Macrobrachium dayanum*	Crustaceans	96h-LC50	268	418	Lodhi *et al.*, 2006
Copper sulfate	*Macrobrachium lamarrei*	Crustaceans	96h-LC50	268	304	Lodhi *et al.*, 2006
Copper sulfate	*Macrobrachium lanchesteri*	Crustaceans	96h-LC50	15.63	32.3	Shuhaimi-Othman *et al.*, 2013
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	36	467	Nebeker *et al.*, 1984
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	84	773	Nebeker *et al.*, 1984
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	84	1690	Nebeker *et al.*, 1984
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	71	298	Nebeker *et al.*, 1984
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	84	1446	Nebeker *et al.*, 1984
Copper chloride	*Chironomus tentans*	Insects/Spiders	96h-LC50	71	1200	Nebeker *et al.*, 1984
Copper	*Chironomus riparius*	Insects/Spiders	96h-LC50	151	700	Taylor *et al.*, 1981
Copper	*Chironomus* sp.	Insects/Spiders	96h-LC50	50	30	Rehwoldt *et al.*, 1973
Copper sulfate	*Chironomus plumosus*	Insects/Spiders	96h-LC50	80	200	Fargasova, 2003
Copper sulfate	*Chironomus riparius*	Insects/Spiders	96h-LC50	130	43	Milani *et al.*, 2003
Copper sulfate	*Chironomus javanus*	Insects/Spiders	96h-LC50	15.63	170	Shuhaimi-Othman, *et al.*, 2011
Copper sulfate	*Chironomus tentans*	Insects/Spiders	96h-LC50	64	135	Suedel *et al.*, 1996
Copper sulfate	*Chironomus tentans*	Insects/Spiders	96h-LC50	64	57.1	Suedel *et al.*, 1996
Copper sulfate	*Chironomus javanus*	Insects/Spiders	96h-LC50	18.72	170	Shuhaimi-Othman *et al.*, 2013
Copper nitrate	*Biomphalaria glabrata*	Molluscs	96h-LC50	100	40	Bellavere and Gorbi, 1981
Copper sulfate	*Lumbriculus variegatus*	Worms	96h-LC50	130	320	Ewell *et al.*, 1986
Copper nitrate	*Lumbriculus variegatus*	Worms	96h-LC50	290	500	Schubauer-Berigan *et al.*, 1993
Copper nitrate	*Lumbriculus variegatus*	Worms	96h-LC50	290	270	Schubauer-Berigan *et al.*, 1993

Copper nitrate	*Lumbriculus variegatus*	Worms	96h-LC50	290	130	Schubauer-Berigan *et al.*, 1993
Copper sulfate	*Orconectes rusticus*	Crustaceans	96h-LC50	112.5	3000	Hubschman, 1967
Copper	*Amnicola* sp.	Molluscs	96h-LC50	50	9300	Rehwoldt *et al.*, 1973
Copper	*Amnicola* sp.	Molluscs	96h-LC50	50	900	Rehwoldt *et al.*, 1973
Copper chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	1400	Pantani *et al.*, 1995
Copper chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	3000	Pantani *et al.*, 1995
Copper chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	6100	Pantani *et al.*, 1995
Copper chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	720	Pantani *et al.*, 1995
Copper chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	590	Pantani *et al.*, 1997
Copper chloride	*Gammarus* sp.	Crustaceans	96h-LC50	182	133	Naddy *et al.*, 2002
Copper nitrate	*Gammarus pulex*	Crustaceans	96h-LC50	230	329	Bascombe *et al.*, 1990
Copper chloride	*Gammarus italicus*	Crustaceans	96h-LC50	240	170	Pantani *et al.*, 1997
Copper	*Gammarus pulex*	Crustaceans	96h-LC50	151	37	Taylor *et al.*, 1991
Copper chloride	*Hyalella azteca*	Crustaceans	96h-LC50	130	210	Milani *et al.*, 2003
Copper sulfate	*Hyalella* sp.	Crustaceans	96h-LC50	90	170	Giusto and Ferrari 2008
Copper sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	64	47.2	Suedel *et al.*, 1996
Copper sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	64	674	Suedel *et al.*, 1996
Copper sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	40	40	Collyard *et al.*, 1994
Copper nitrate	*Hyalella azteca*	Crustaceans	96h-LC50	290	87	Schubauer-Berigan *et al.*, 1993
Copper nitrate	*Hyalella azteca*	Crustaceans	96h-LC50	290	17	Schubauer-Berigan *et al.*, 1993
Copper nitrate	*Hyalella azteca*	Crustaceans	96h-LC50	290	24	Schubauer-Berigan *et al.*, 1993
Copper sulfate	*Penaeus chinensis*	Crustaceans	96h-LC50	6.5	80	Zang *et al.*, 1993
Copper sulfate	*Penaeus chinensis*	Crustaceans	96h-LC50	6.5	52	Zang *et al.*, 1993
Copper sulfate	*Asellus meridianus*	Crustaceans	96h-LC50	25	680	Brown 1976
Copper sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	32000	Ewell *et al.*, 1986
Copper sulfate	*Asellus meridianus*	Crustaceans	96h-LC50	25	650	Brown, 1976
Copper sulfate	*Aedes aegypti*	Insects/Spiders	72h-LC50	44	2500	Simonet, 1978
Copper sulfate	*Gyraulus circumstriatus*	Molluscs	96h-LC50	100	425	Wurtz and Bridges, 1961
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	151	27.17	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	65	82.11	Rogevich *et al.*, 2008

Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	64	46.75	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	156	41.29	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	60	20.28	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	327	30.58	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	64	44.55	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	72	35.65	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	60	37.74	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	176	30.29	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	58	20.32	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	68	31.96	Rogevich *et al.*, 2008
Copper sulfate	*Pomacea paludosa*	Molluscs	96h-LC50	68	64.625	Rogevich *et al.*, 2008
Copper sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	240	88	Khangarot *et al.*, 1981
Copper sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	180	88	Gupta *et al.* 1981
Copper sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	190	390	Gupta *et al.* 1981
Copper sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	205	66	Gupta *et al.* 1981
Copper sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	195	60	Gupta *et al.* 1981
Copper sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	210	Cairns *et al.*, 1978
Copper sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	300	Cairns *et al.*, 1978
Copper sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	1000	Cairns *et al.*, 1978
Copper sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	53	Wurtz, 1962
Copper sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	16	Wurtz, 1962
Copper sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	69	Wurtz, 1962
Copper sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	13	Wurtz, 1962
Copper sulfate	*Basommatophora* sp.	Molluscs	96h-LC50	80	83	Howard *et al.*, 1964
Copper sulfate	*Basommatophora* sp.	Molluscs	96h-LC50	6.2	35	Howard *et al.*, 1964
Copper sulfate	*Radix luteola*	Molluscs	96h-LC50	195	27	Khangarot and Ray, 1988
Copper sulfate	*Radix luteola*	Molluscs	96h-LC50	315	172	Mathur *et al.*, 1981
Copper sulfate	*Lymnaea acuminata*	Molluscs	96h-LC50	375	34	Khangarot *et al.*, 1982
Copper sulfate	*Lymnaea acuminata*	Molluscs	96h-LC50	240	34	Khangarot *et al.*, 1981
Copper sulfate	*Corbicula manilensis*	Molluscs	96h-LC50	63.5	40	Cherry *et al.*, 1980

Copper sulfate	*Corbicula manilensis*	Molluscs	96h-LC50	21	670000	Chandler and Marking, 1979
Copper sulfate	*Corbicula manilensis*	Molluscs	96h-LC50	17	2600	Harrison *et al.*, 1984
Copper sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	2300	Cairns *et al.*, 1978
Copper sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	1000	Cairns *et al.*, 1978
Copper sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	2600	Cairns *et al.*, 1978
Copper sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	2000	Cairns *et al.*, 1978
Copper sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	1650	Cairns *et al.*, 1978
Copper sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	3200	Ewell *et al.*, 1986
Copper sulfate	*Dugesia dorotocephala*	Worms	96h-LC50	41.7	190	Rauscher, 1988
Copper sulfate	*Dugesia tigrina*	Worms	96h-LC50	40	1770	See, 1976
Copper sulfate	*Limnodrilus hoffmeisteri*	Worms	96h-LC50	100	400	Wurtz and Bridges, 1961
Copper sulfate	*Nais elinguis*	Worms	96h-LC50	18.72	7	Shuhaimi-Othman *et al.*, 2013
Copper sulfate	*Nais elinguis*	Worms	96h-LC50	17.89	7	Shuhaimi-Othman *et al.*, 2012
Copper sulfate	*Nais* sp.	Worms	96h-LC50	50	90	Rehwoldt *et al.*, 1973
Copper sulfate	*Tubifex tubifex*	Worms	96h-LC50	247.5	80	Khangarot and Rathore, 2004
Copper sulfate	*Tubifex tubifex*	Worms	96h-LC50	128	70	Reynoldson *et al.*, 1996
Copper sulfate	*Tubifex tubifex*	Worms	96h-LC50	128	90	Reynoldson *et al.*, 1996
Copper chloride	*Tubifex tubifex*	Worms	96h-LC50	130	160	Milani *et al.*, 2003
Copper hydroxide	*Caridina laevis*	Crustaceans	96h-LC50	128	3390	Sucahyo *et al.*, 2008
Copper chloride	*Hydropsyche angustipennis*	Insects/Spiders	96h-LC50	210	350	Van der Geest *et al.*, 1999
Copper chloride	*Hexagenia* sp.	Insects/Spiders	96h-LC50	130	73	Milani *et al.*, 2003
Copper chloride	*Ephoron virgo*	Insects/Spiders	96h-LC50	210	77	Van der Geest *et al.*, 2000
Copper chloride	*Ephoron virgo*	Insects/Spiders	96h-LC50	210	79	Van der Geest *et al.*, 2000
Copper sulfate	*Drunella grandis*	Insects/Spiders	96h-LC50	36.4	209.38	Brinkman and Vieira, 2008
Copper sulfate	*Rhithrogena hageni*	Insects/Spiders	96h-LC50	44	137	Brinkman and Vieira 2008
Copper sulfate	*Girardia tigrina*	Worms	96h-LC50	42.75	370	Safadi, 1998
Copper sulfate	*Elimia livescens*	Molluscs	96h-LC50	154	440	Paulson *et al.*, 1983
Copper sulfate	*Planorbella trivolvis*	Molluscs	96h-LC50	130	320	Ewell *et al.*, 1986
Copper	*Stenonema* sp.	Insects/Spiders	48h-LC50	110	471.88	Dobbs *et al.*, 1994
Copper	*Isonychia bicolor*	Insects/Spiders	48h-LC50	110	232.30	Dobbs *et al.*, 1994

Copper chloride	*Juga plicifera*	Molluscs	96h-LC50	21	15	Nebeker *et al.*, 1986
Copper chloride	*Fluminicola virens*	Molluscs	96h-LC50	21	8	Nebeker *et al.*, 1986
Copper Sulfate	*Melanoides tuberculata*	Molluscs	96h-LC50	18.72	140	Shuhaimi-Othman *et al.*, 2013
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	16	21.88	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	17.5	34.38	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	13.2	92.71	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	12.7	83.33	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	11.9	54.17	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	16.4	29.17	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	15.6	56.25	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	13.5	76.04166667	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	14.7	73.96	Daly *et al.*, 1990
Copper	*Paratya australiensis*	Crustaceans	96h-LC50	14.1	77.08	Daly *et al.*, 1990
Copper	Zygoptera sp.	Insects/Spiders	96h-LC50	50	4600	Rehwoldt *et al.*, 1973

APPENDIX 7: Acute toxicity data for lead to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species scientific name	Species Group	Endpoint	Test conc. (µg/L)	Test Hardness (mg/L as $CaCO_3$)	Reference
Lead acetate	*Lymnaea emarginata* ssp. *angulata*	Molluscs	48h-LC50	14000	154	Cairns *et al.*, 1976
Lead acetate	*Elimia livescens*	Molluscs	48h-LC50	71000	154	Cairns *et al.*, 1976
Lead acetate	*Corbicula manilensis*	Molluscs	96h-LC50	1023320	178	Labrot *et al.*, 1999
Lead	*Dugesia tigrina*	Worms	96h-LC50	160000	50	See *et al.*, 1974
Lead chloride	*Paratelphusa hydrodromus*	Crustaceans	96h-LC50	18925	150	Victor, 1994
Lead chloride	*Macrobrachium lanchesteri*	Crustaceans	96h-LC50	35	15.63	Shuhaimi-Othman *et al.*, 2011
Lead nitrate	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	108.5873025	41	Shazili and Ali, 1988
Lead chloride	*Lumbriculus variegatus*	Worms	96h-LC50	8000	290	Schubauer-Berigan *et al.,* 1993
Lead chloride	*Lumbriculus variegatus*	Worms	96h-LC50	8000	290	Schubauer-Berigan *et al.,* 1993
Lead chloride	*Lumbriculus variegatus*	Worms	96h-LC50	8000	290	Schubauer-Berigan *et al.,* 1993
Lead Nitrate	*Lumbriculus variegatus*	Worms	96h-LC50	1800	30	Bailey and Liu, 1980
Lead Nitrate	*Asellus meridianus*	Crustaceans	48h-LC50	2800	25	Brown, 1976
Lead Nitrate	*Asellus meridianus*	Crustaceans	48h-LC50	3500	25	Brown, 1976
Lead Nitrate	*Asellus meridianus*	Crustaceans	48h-LC50	1000	25	Brown, 1976
Lead Nitrate	*Asellus meridianus*	Crustaceans	48h-LC50	1750	25	Brown, 1976
Lead Nitrate	*Procambarus clarkii*	Crustaceans	96h-LC50	751570	30.32	Naqvi and Howell, 1993
Lead Nitrate	*Chironomus javanus*	Insects/Spiders	96h-LC50	720	15.63	Shuhaimi-Othman *et al.*, 2011
Lead Nitrate	*Enallagma* sp.	Insects/Spiders	96h-LC50	60000	15.3	Mackie, 1989
Lead Nitrate	*Viviparus bengalensis*	Molluscs	96h-LC50	2540	165	Gadkari and Marathe, 1983
Lead Nitrate	*Aplexa hypnorum*	Molluscs	96h-LC50	1340	60.9	Call, 1981
Lead Nitrate	*Nais elinguis*	Worms	96h-LC50	580	17.89	Shuhaimi-Othman *et al.*, 2012
Lead Nitrate	*Tubifex tubifex*	Worms	48h-LC50	450000	224	Qureshi *et al.*, 1980

APPENDIX 8: Acute toxicity data for inorganic mercury to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species scientific name	Species group	Endpoint	Conc. (µg/L)	Refernce
Mercuryic chloride	*Acroneuria lycorias*	Insects/Spiders	96h-LC50	2000	Warnick and Bell 1969
Mercuryic chloride	*Aedes aegypti*	Insects/Spiders	48h-LC50	7600	Slooff *et al.*, 1983
Mercuryic chloride	*Aedes aegypti*	Insects/Spiders	48h-LC50	5600	Slooff, 1982
Mercuryic chloride	*Aplexa hypnorum*	Molluscs	96h-LC50	370	Holcombe *et al.*, 1983
Mercuryic chloride	*Asellus aquaticus*	Crustaceans	48h-LC50	148	Slooff, 1983
Mercuryic chloride	*Austropotamobius pallipes* ssp. *pallipes*	Crustaceans	96h-LC50	20	Boutet and Chaisemartin, 1973
Mercuryic chloride	*Barytelphusa cunicularis*	Crustaceans	96h-LC50	450	Chourpagar and Kulkarni, 2011
Mercuryic chloride	*Barytelphusa cunicularis*	Crustaceans	24h-LC50	1040	Chourpagar and Kulkarni, 2011
Mercuryic chloride	*Branchiura sowerbyi*	Worms	96h-LC50	80	Chapman *et al.*, 1982
Mercuryic chloride	*Branchiura sowerbyi*	Worms	96h-LC50	3200	Chapman *et al.*, 1982
Mercuryic chloride	*Caridina rajadhari*	Crustaceans	48h-LC50	52.75	Ghate, 1984
Mercuryic chloride	*Chironomus plumosus*	Insects/Spiders	96h-LC50	400	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Chironomus plumosus*	Insects/Spiders	96h-LC50	400	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Chironomus plumosus*	Insects/Spiders	96h-LC50	400	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Chironomus riparius*	Insects/Spiders	96h-LC50	547	Rossaro *et al.*, 1986
Mercuryic chloride	*Chironomus riparius*	Insects/Spiders	96h-LC50	100	Rossaro *et al.*, 1986
Mercuryic chloride	*Clunio gerlachi*	Insects/Spiders	96h-LC50	280	Vedamanikam *et al.* 2013
Mercuryic chloride	*Culex pipiens*	Insects/Spiders	48h-LC50	1400	Slooff *et al.*, 1983
Mercuryic chloride	*Culicoides furens*	Insects/Spiders	96h-LC50	30	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Culicoides furens*	Insects/Spiders	96h-LC50	40	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Culicoides furens*	Insects/Spiders	96h-LC50	40	Vedamanikam and Shazilli, 2008
Mercuryic chloride	*Cypris* sp.	Crustaceans	48h-LC50	130	Qureshi *et al.*, 1980
Mercuryic chloride	*Dugesia bengalensis*	Worms	96h-LC50	100	Aditya and Bandyopadhyay, 1995
Mercuryic chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	500	Pantani *et al.*, 1997
Mercuryic chloride	*Ephemerella subvaria*	Insects/Spiders	96h-LC50	2000	Warnick and Bell, 1969
Mercuryic chloride	*Erpobdella octoculata*	Worms	48h-LC50	237	Slooff, 1983

Mercuryic chloride	*Gammarus italicus*	Crustaceans	96h-LC50	670	Pantani *et al.*, 1997
Mercuryic chloride	*Girardia tigrina*	Worms	96h-LC50	40	Safadi, 1998
Mercuryic chloride	*Gymnometriocnemus mahensis*	Insects/Spiders	96h-LC50	240	Vedamanikam *et al.,* 2013
Mercuryic chloride	*Hyalella curvispina*	Crustaceans	96h-LC50	25	Peluso *et al.*, 2013
Mercuryic chloride	*Hydropsyche betteni*	Insects/Spiders	96h-LC50	2000	Warnick and Bell, 1968
Mercuryic chloride	*Ilyodrilus frantzi*	Worms	96h-LC50	290	Chapman and Mitchell, 1986
Mercuryic chloride	*Ilyoplax gangetica*	Crustaceans	96h-LC50	30	Amte and Sawant, 1992
Mercuryic chloride	*Ischnura elegans*	Insects/Spiders	48h-LC50	7613	Slooff, 1983
Mercuryic chloride	*Laccotrephes* sp.	Insects/Spiders	48h-LC50	790	Parthasarathi and Ranganathan, 1999
Mercuryic chloride	*Larsia* sp.	Insects/Spiders	96h-LC50	390	Vedamanikam *et al.*,2013
Mercuryic chloride	*Limnodrilus hoffmeisteri*	Worms	96h-LC50	180	Chapman *et al.*, 1982
Mercuryic chloride	*Limnodrilus hoffmeisteri*	Worms	96h-LC50	500	Chapman *et al.*, 1982
Mercuryic chloride	*Lumbriculus variegatus*	Worms	96h-LC50	100	Bailey and Liu, 1980
Mercuryic chloride	*Lymnaea acuminata*	Molluscs	96h-LC50	23	Khangarot *et al.*,1982
Mercuryic chloride	*Macrobrachium hendersodayanus*	Crustaceans	96h-LC50	68	Patil and Kaliwal, 1986
Mercuryic chloride	*Macrobrachium lamarrei*	Crustaceans	96h-LC50	70	Murti and Shukla, 1984
Mercuryic chloride	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	430	Kaoudl *et al.*, 2011
Mercuryic chloride	*Nais communis*	Worms	96h-LC50	160	Chapman and Mitchell, 1986
Mercuryic chloride	*Nemoura cinerea*	Insects/Spiders	48h-LC50	126	Slooff, 1983
Mercuryic chloride	*Oligochaeta*	Worms	96h-LC50	230	Chapman *et al.*, 1982
Mercuryic chloride	*Orconectes limosus*	Crustaceans	96h-LC50	50	Boutet and Chaisemartin, 1973
Mercuryic chloride	*Paramerina minima*	Insects/Spiders	96h-LC50	420	Vedamanikam *et al.*, 2013
Mercuryic chloride	*Paratelphusa hydrodromus*	Crustaceans	96h-LC50	349.3	Ananthalakshmikumari *et al*., 1990
Mercuryic chloride	*Paratelphusa hydrodromus*	Crustaceans	96h-LC50	377.3	Ananthalakshmikumari *et al.*, 1990
Mercuryic chloride	*Peloscolex ferox*	Worms	96h-LC50	330	Chapman *et al.*, 1982
Mercuryic chloride	*Poecilobdella viridis*	Worms	24h-LC50	1411	Shelar and Kanse, 2011
Mercuryic chloride	*Poecilobdella viridis*	Worms	24h-LC50	1400	Shelar and Kanse, 2011
Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	790	Del Ramo *et al.*, 1987
Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	350	Del Ramo *et al.*, 1987

Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	140	Del Ramo *et al.*, 1987
Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	790	Medina *et al.*, 1991
Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	350	Medina *et al.*, 1991
Mercuryic chloride	*Procambarus clarkii*	Crustaceans	96h-LC50	140	Medina *et al.*, 1991
Mercuryic chloride	*Pteronarcys californica*	Insects/Spiders	48h-LC50	58000	Elder and Gaufin, 1974
Mercuryic chloride	*Quistadrilus multisetosus*	Worms	96h-LC50	250	Chapman, 1982
Mercuryic chloride	*Radix luteola*	Molluscs	96h-LC50	19	Khangarot and Ray 1988
Mercuryic chloride	*Rhyacodrilus montanus*	Worms	96h-LC50	240	Chapman *et al.*, 1982
Mercuryic chloride	*Spirosperma nikolskyi*	Worms	96h-LC50	500	Chapman *et al.*, 1982
Mercuryic chloride	*Stylodrilus heringianus*	Worms	96h-LC50	140	Chapman *et al.*, 1982
Mercuryic chloride	*Tanypus complanatus*	Insects/Spiders	48H-LC50	860	Vedamanikam, 2013
Mercuryic chloride	*Tanypus complanatus*	Insects/Spiders	96h-LC50	330	Vedamanikam, 2013
Mercuryic chloride	*Tanypus complanatus*	Insects/Spiders	24h-LC50	1220	Vedamanikam, 2013
Mercuryic chloride	*Tanypus complanatus*	Insects/Spiders	72h-LC50	750	Vedamanikam, 2013
Mercuryic chloride	*Tubifex tubifex*	Worms	96h-LC50	280	Fargasova, 1994
Mercuryic chloride	*Tubifex tubifex*	Worms	96h-LC50	140	Chapman *et al.*, 1982
Mercuryic chloride	*Tubifex tubifex*	Worms	96h-LC50	1250	Chapman *et al.*, 1982
Mercuryic chloride	*Tubifex tubifex*	Worms	96h-LC50	280	Fargasova, 1999
Mercuryic chloride	*Varichaeta pacifica*	Worms	96h-LC50	100	Chapman *et al.*, 1982
Mercuryic chloride	*Villorita cyprinoides* ssp. *cochiensis*	Molluscs	96h-LC50	1.57	Abraham *et al.*, 1986
Mercuryic chloride	*Viviparus bengalensis*	Molluscs	96h-LC50	260	Seth *et al.*, 1990

APPENDIX 9: Acute toxicity data for nickel to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical	Species Scientific Name	Species Group	Endpoint	Conc. (µg/L)	Hardness	Reference
Nickel	Zygoptera	Insects/Spiders	96h-LC50	21200	50	Rehwoldt *et al.*, 1973
Nickel	*Gammarus* sp.	Crustaceans	96h-LC50	13000	50	Rehwoldt *et al.*, 1973
Nickel chloride	*Gammarus fasciatus*	Crustaceans	96h-LC50	100000	130	Ewell *et al.*, 1986
Nickel chloride	*Gammarus fasciatus*	Crustaceans	96h-LC50	100000	130	Ewell *et al.*, 1986
Nickel Sulfate	*Chironomus javanus*	Insects/Spiders	96h-LC50	5320	15.63	Shuhaimi-Othman *et al.*, 2011
Nickel	*Chironomus* sp.	Insects/Spiders	96h-LC50	8600	50	Rehwoldt *et al.*, 1973
Nickel	*Amnicola* sp.	Molluscs	96h-LC50	11400	50	Rehwoldt *et al.*, 1973
Nickel	*Amnicola* sp.	Molluscs	96h-LC50	21200	50	Rehwoldt *et al.*, 1973
Nickel	*Amnicola* sp.	Molluscs	96h-LC50	14300	50	Rehwoldt *et al.*, 1973
Nickel chloride	*Asellus intermedius*	Crustaceans	96h-LC50	75000	130	Ewell *et al.*, 1986
Nickel chloride	*Asellus intermedius*	Crustaceans	96h-LC50	100000	130	Ewell *et al.*, 1986
Nickel chloride	*Hyalella azteca*	Crustaceans	96h-LC50	3051.102	98	Keithly *et al.*, 2004
Nickel chloride	*Hyalella azteca*	Crustaceans	96h-LC50	1900	290	Schubauer-Berigan *et al.*, 1993
Nickel chloride	*Hyalella azteca*	Crustaceans	96h-LC50	890	290	Schubauer-Berigan *et al.*, 1993
Nickel chloride	*Hyalella azteca*	Crustaceans	96h-LC50	2000	290	Schubauer-Berigan *et al.*, 1993
Nickel chloride	*Viviparus bengalensis*	Molluscs	96h-LC50	9915	180	Gupta *et al.*, 1981
Nickel chloride	*Planorbella trivolvis*	Molluscs	96h-LC50	3200	130	Ewell *et al.*, 1986
Nickel chloride	*Dugesia tigrina*	Worms	96h-LC50	32000	130	Ewell *et al.*, 1986
Nickel chloride	*Dugesia tigrina*	Worms	96h-LC50	32000	130	Ewell *et al.*, 1986
Nickel	*Dugesia tigrina*	Worms	96h-LC50	16800	50	See *et al.*, 1974
Nickel Sulfate	*Dugesia tigrina*	Worms	96h-LC50	2550	40	See *et al.*, 1974
Nickel chloride	*Lumbriculus variegatus*	Worms	96h-LC50	32000	130	Ewell *et al.*, 1986
Nickel chloride	*Lumbriculus variegatus*	Worms	96h-LC50	100000	290	Schubauer-Berigan *et al.*, 1993
Nickel chloride	*Lumbriculus variegatus*	Worms	96h-LC50	48000	130	Ewell *et al.*, 1986
Nickel chloride	*Lumbriculus variegatus*	Worms	96h-LC50	26000	290	Schubauer-Berigan *et al.*, 1993
Nickel chloride	*Lumbriculus variegatus*	Worms	96h-LC50	75000	290	Schubauer-Berigan *et al.*, 1993

Nickel Sulfate	*Lymnaea acuminata*	Molluscs	96h-LC50	2780	375	Khangarot *et al.*, 1982
Nickel Sulfate	*Nais elinguis*	Worms	96h-LC50	645	17.89	Shuhaimi-Othman *et al.*, 2012
Nickel	*Nais* sp.	Worms	96h-LC50	14100	50	Rehwoldt *et al.*, 1973
Nickel Sulfate	*Tubifex tubifex*	Worms	48h-LC50	7000	34.2	Brkovic-Popovic and Popovic, 1977
Nickel chloride	*Tubifex tubifex*	Worms	48h-LC50	82	30.1	Brkovic-Popovic and Popovic, 1977
Nickel chloride	*Tubifex tubifex*	Worms	48h-LC50	8700	34.2	Brkovic-Popovic and Popovic, 1977
Nickel chloride	*Tubifex tubifex*	Worms	48h-LC50	61400	261	Brkovic-Popovic and Popovic, 1977
Nickel chloride	*Radix luteola*	Molluscs	96h-LC50	1430	195	Khangarot and Ray, 1988
Nickel chloride	*Juga plicifera*	Molluscs	96h-LC50	239	26	Nebeker *et al.*, 1986
Nickel chloride	*Juga plicifera*	Molluscs	96h-LC50	237	59	Nebeker *et al.*, 1986

APPENDIX 10: Acute toxicity data for zinc to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species scientific name	Species group	Endpoint	Hardness	Conc. (µg/L)	Reference
Zinc nitrite	*Gammarus pulex*	Crustaceans	96h-LC50	230	77	Bascombe *et al.*,1990
Zinc	*Chironomus* sp.	Insects/Spiders	96h-LC50	50	18200	Rehwoldt*et al.*, 1973
Zinc Sulfate	*Chironomus plumosus*	Insects/Spiders	96h-LC50	80	32600	Fargasova, 2003
Zinc Sulfate	*Chironomus javanus*	Insects/Spiders	96h-LC50	15.63	5570	Shuhaimi-Othman, 2011
Zinc	*Amnicola* sp.	Molluscs	96h-LC50	50	20200	Rehwoldt*et al.*, 1973
Zinc	*Amnicola* sp.	Molluscs	96h-LC50	50	14000	Rehwoldt*et al.*, 1973
Zinc Sulfate	*Nais elinguis*	Worms	96h-LC50	17.89	912	Shuhaimi-Othman *et al.*, 2012
Zinc	*Nais* sp.	Worms	96h-LC50	50	18400	Rehwoldt*et al.*, 1973
Zinc chloride	*Echinogammarus tibaldii*	Crustaceans	96h-LC50	240	25900	Pantani *et al.*, 1997
Zinc Sulfate	*Echinogammarus meridionalis*	Crustaceans	96h-LC50	263.43	6670	Pestana *et al.*, 2007
Zinc chloride	*Ranatra elongata*	Insects/Spiders	96h-LC50	112.4	1658	Shukla *et al.*, 1983
Zinc Sulfate	*Atyaephyra desmarestii*	Crustaceans	96h-LC50	263.43	7810	Pestana *et al.*, 2007
Zinc Sulfate	*Paratya australiensis*	Crustaceans	96h-LC50	10	1100	Thorp and Lake, 1974
Zinc Sulfate	*Penaeus chinensis*	Crustaceans	96h-LC50	6.5	100	Zang *et al.*, 1993
Zinc Sulfate	*Penaeus chinensis*	Crustaceans	96h-LC50	6.5	130	Zang *et al.*, 1993
Zinc Sulfate	*Macrobrachium rosenbergii*	Crustaceans	96h-LC50	41	83	Shazili and Ali, 1988
Zinc Sulfate	*Caecidotea* sp.	Crustaceans	96h-LC50	220	20220	Bosnak and Morgan *et al.*, 1981
Zinc Sulfate	*Caecidotea* sp.	Crustaceans	96h-LC50	220	20000	Bosnak and Morgan *et al.*, 1981
Zinc Sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	4700	Ewell *et al.*, 1986
Zinc Sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	100000	Ewell *et al.*, 1986
Zinc Sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	5500	Ewell *et al.*, 1986
Zinc Sulfate	*Asellus intermedius*	Crustaceans	96h-LC50	130	100000	Ewell *et al.*, 1986
Zinc Sulfate	*Asellus communis*	Crustaceans	96h-LC50	20	56000	Wurtz and Bridges, 1961
Zinc Sulfate	*Asellus communis*	Crustaceans	96h-LC50	100	38500	Wurtz and Bridges, 1961
Zinc Sulfate	*Lirceus alabamae*	Crustaceans	96h-LC50	152	8450	Bosnak and Morgan *et al.*, 1981

Zinc Sulfate	*Lirceus alabamae*	Crustaceans	96h-LC50	152	8300	Bosnak and Morgan *et al.*, 1981
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	100	436	Eisenhauer, 1999
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	40	200	Collyard *et al.*, 1994
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	40	200	Collyard *et al.*, 1994
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	40	350	Collyard *et al.*, 1994
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	290	1500	Schubauer-Berigan *et al.*, 1993
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	290	1200	Schubauer-Berigan *et al.*, 1993
Zinc Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	290	290	Schubauer-Berigan *et al.*, 1993
Zinc	*Hyalella azteca*	Crustacean	96h-LC50	146.9	1613	Shuhaimi-Othman and Pascoe, 2001
Zinc Sulfate	*Chloroperlidae*	Insects/Spiders	96h-LC50	51.1	68800	Brinkman and Johnston, 2012
Zinc Sulfate	*Argia* sp	Insects/Spiders	96h-LC50	20	180000	Wurtz and Bridges, 1961
Zinc Sulfate	*Drunella doddsi*	Insects/Spiders	96h-LC50	49.8	64000	Brinkman and Johnston, 2012
Zinc Sulfate	*Baetis tricaudatus*	Insects/Spiders	96h-LC50	42.3	10100	Brinkman and Johnston, 2012
Zinc Sulfate	*Rhithrogena hageni*	Insects/Spiders	96h-LC50	44.4	50500	Brinkman and Johnston, 2008
Zinc Sulfate	*Cinygmula* sp.	Insects/Spiders	96h-LC50	51.1	68800	Brinkman and Johnston, 2012
Zinc Sulfate	*Ephemerella* sp.	Insects/Spiders	96h-LC50	51.1	68800	Brinkman and Johnston, 2012
Zinc Sulfate	*Planorbella campanulata*	Molluscs	96h-LC50	20	1270	Wurtz, 1962
Zinc Sulfate	*Planorbella campanulata*	Molluscs	96h-LC50	100	3030	Wurtz, 1962
Zinc Sulfate	*Planorbella campanulata*	Molluscs	96h-LC50	100	1270	Wurtz, 1962
Zinc Sulfate	*Planorbella campanulata*	Molluscs	96h-LC50	20	870	Wurtz, 1962
Zinc Sulfate	*Radix luteola*	Molluscs	96h-LC50	315	6130	Mathur *et al*., 1981
Zinc Sulfate	*Radix luteola*	Molluscs	96h-LC50	195	11000	Khangarot and Ray, 1987
Zinc Sulfate	*Radix luteola*	Molluscs	96h-LC50	198	8010	Khangarot and Ray, 1987
Zinc Sulfate	*Radix luteola*	Molluscs	96h-LC50	203	1680	Khangarot and Ray, 1987
Zinc Sulfate	*Radix luteola*	Molluscs	96h-LC50	195	1680	Khangarot and Ray, 1987
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	1110	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	1390	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	3160	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	1110	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	350	Wurtz, 1962

Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	1700	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	48h-LC50	100	1390	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	303	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	100	434	Wurtz, 1962
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	434	Wurtz, 1962
Zinc chloride	*Physa gyrina*	Molluscs	96h-LC50	36	1274	Nebeker *et al.*, 1986
Zinc Sulfate	*Physa heterostropha*	Molluscs	96h-LC50	20	434	Wurtz, 1962
Zinc Sulfate	*Elimia livescens*	Molluscs	48h-LC50	154	13500	Cairns *et al.*, 1976
Zinc Sulfate	*Lymnaea acuminata*	Molluscs	96h-LC50	375	10490	Khangarot *et al.*, 1982
Zinc Sulfate	*Viviparus bengalensis*	Molluscs	96h-LC50	180	641	Gupta *et al.*, 1981
Zinc Sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	4600	Cairns *et al.*, 1978
Zinc Sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	4800	Cairns *et al.*, 1978
Zinc Sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	2800	Cairns *et al.*, 1978
Zinc Sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	1650	Cairns *et al.*, 1978
Zinc Sulfate	*Anculosa* sp.	Molluscs	48h-LC50	45	1900	Cairns *et al.*, 1978
Zinc Sulfate	*Corbicula manilensis*	Molluscs	96h-LC50	63.5	6040	Cherry *et al.*, 1980
Zinc	*Dugesia tigrina*	Worms	96h-LC50	50	7400	See *et al.*, 1974
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	130	32000	Ewell *et al.*, 1986
Zinc Sulfate	*Dugesia tigrina*	Worms	96h-LC50	40	5480	See, 1976
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	165	36660	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	63	3180	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	165	53050	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	63	8120	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	63	5850	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	165	41710	Gray, 1995
Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	165	59650	Gray, 1995

Zinc Sulfate	*Nephelopsis obscura*	Worms	96h-LC50	63	9480	Gray, 1995
Zinc Sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	15600	Cairns *et al.*, 1978
Zinc Sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	15000	Cairns *et al.*, 1978
Zinc Sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	18100	Cairns *et al.*, 1978
Zinc Sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	13500	Cairns *et al.*, 1978
Zinc Sulfate	*Aeolosoma headleyi*	Worms	48h-LC50	45	17600	Cairns *et al.*, 1978
Zinc Sulfate	*Limnodrilus hoffmeisteri*	Worms	96h-LC50	100	10000	Wurtz and Bridges 1961
Zinc Sulfate	*Girardia tigrina*	Worms	96h-LC50	42.75	2680	Safadi, 1998
Zinc Sulfate	*Lumbriculus variegatus*	Worms	96h-LC50	290	5000	Schubauer-Berigan *et al.*, 1993
Zinc Sulfate	*Lumbriculus variegatus*	Worms	96h-LC50	130	22000	Ewell *et al.*, 1986
Zinc Sulfate	*Lumbriculus variegatus*	Worms	96h-LC50	290	5000	Schubauer-Berigan *et al.*, 1993
Zinc Sulfate	*Lumbriculus variegatus*	Worms	96h-LC50	290	5000	Schubauer-Berigan *et al.*, 1993
Zinc chloride	*Lumbriculus variegatus*	Worms	96h-LC50	30	6300	Bailey and Liu, 1980
Zinc Sulfate	*Tubifex tubifex*	Worms	48h-LC50	0.1	110	Brkovic-Popovic and Popovic, 1977
Zinc Sulfate	*Tubifex tubifex*	Worms	48h-LC50	261	60200	Brkovic-Popovic and Popovic, 1977
Zinc Sulfate	*Tubifex tubifex*	Worms	48h-LC50	34.2	2980	Brkovic-Popovic and Popovic, 1977
Zinc Sulfate	*Tubifex tubifex*	Worms	48h-LC50	34.2	2570	Brkovic-Popovic and Popovic, 1977
Zinc chloride	*Tubifex tubifex*	Worms	48h-LC50	224	130000	Qureshi *et al.*, 1980

APPENDIX 11: Acute toxicity data for ammonia to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species Scientific Name	Species Group	Temp. (°C)	pH	Endpoint	Form of ammonia	Conc. (µg/L)	Reference
Ammonium chloride	*Asellus racovitzai*	Crustaceans	4	8	96h-LC50	Unionized	4080	Arthur *et al.*, 1987
Ammonium chloride	*Asellus racovitzai*	Crustaceans	22	7.8	96h-LC50	Unionized	4190	Arthur *et al.*, 1987
Ammonium chloride	*Baetis rhodani*	Insects/Spiders	13.1	8.15	24h-LC50	Unionized	8200	Khatami *et al.*, 1998
Ammonium chloride	*Callibaetis skokianus*	Insects/Spiders	13.3	7.9	96h-LC50	Unionized	3970	Arthur *et al.*, 1987
Ammonium chloride	*Callibaetis skokianus*	Insects/Spiders	10.8	7.7	96h-LC50	Unionized	2590	Arthur *et al.*, 1987
Ammonium chloride	*Callibaetis* sp.	Insects/Spiders	11.9	7.81	96h-LC50	Total Ammonia	108000	Thurston *et al*., 1984
Ammonium chloride	*Cherax quadricarinatus*	Crustaceans	28	8.3	96h-LC50	Total Ammonia	29300	Rouse *et al.*, 1995
Ammonium chloride	*Cherax quadricarinatus*	Crustaceans	28	7.5	96h-LC50	Total Ammonia	45900	Meade and Watts, 1995
Ammonium chloride	*Chironomus riparius*	Insects/Spiders	21.7	7.685	96h-LC50	Unionized	9400	Monda *et al.*, 1995
Ammonium chloride	*Chironomus riparius*	Insects/Spiders	21.4	7.675	96h-LC50	Unionized	6600	Monda *et al.*, 1995
Ammonium chloride	*Corophium* sp.	Crustaceans	21	6.25	96h-LC50	Total Ammonia	5500	Hyne and Everett, 1998
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	13.3	8	96h-LC50	Unionized	2710	Arthur *et al.*, 1987
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	13	8.2	96h-LC50	Unionized	2930	Arthur *et al.*, 1987
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	24.9	8	96h-LC50	Unionized	1340	Arthur *et al.*, 1987
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	12	7.5	96h-LC50	Unionized	360	Prenter *et al.*, 2004
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	12.1	8	96h-LC50	Unionized	4640	Arthur *et al.*, 1987
Ammonium chloride	*Crangonyx pseudogracilis*	Crustaceans	4	8	96h-LC50	Unionized	2270	Arthur *et al.*, 1987
Ammonium chloride	*Crangonyx* sp.	Crustaceans	20	7.95	96h-LC50	Unionized	2050	Diamond *et al.*, 1993
Ammonium chloride	*Drunella grandis*	Insects/Spiders	12.8	7.84	96h-LC50	Total Ammonia	259000	Thurston *et al*., 1984

Ammonium chloride	*Drunella grandis*	Insects/Spiders	12	7.85	96h-LC50	Total Ammonia	319000	Thurston *et al*., 1984
Ammonium chloride	*Drunella grandis*	Insects/Spiders	13.2	7.84	96h-LC50	Total Ammonia	196000	Thurston *et al*., 1984
Ammonium chloride	*Gammarus duebeni*	Crustaceans	12	7.5	96h-LC50	Unionized	1155	Prenter *et al.*, 2004
Ammonium chloride	*Gammarus pseudolimnaeus*	Crustaceans	18.1	7.53	96h-LC50	Total Ammonia	22000	Call *et al.*, 1984
Ammonium chloride	*Gammarus pulex*	Crustaceans	12	7.5	96h-LC50	Unionized	381	Prenter *et al.*, 2004
Ammonium chloride	*Gammarus pulex*	Crustaceans	12	7.5	96h-LC50	Unionized	1544	Prenter *et al.*, 2004
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	7.41	96h-LC50	Total Ammonia	140000	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	7.49	96h-LC50	Total Ammonia	17500	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	8.21	96h-LC50	Total Ammonia	24000	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	6.43	96h-LC50	Total Ammonia	105000	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	7.31	96h-LC50	Total Ammonia	64000	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	6.55	96h-LC50	Total Ammonia	204000	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	23	6.28	96h-LC50	Total Ammonia	82000	Whiteman *et al.*, 1996
Ammonium chloride	*Hyalella azteca*	Crustaceans	23	6.91	96h-LC50	Total Ammonia	9200	Whiteman *et al.*, 1996
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	8.45	96h-LC50	Total Ammonia	35200	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	6.5	96h-LC50	Total Ammonia	22800	Ankley *et al.*, 1995
Ammonium chloride	*Hyalella azteca*	Crustaceans	23	6.85	96h-LC50	Total Ammonia	9700	Whiteman *et al.*, 1996
Ammonium chloride	*Hyalella azteca*	Crustaceans	25	8.3	96h-LC50	Total Ammonia	39800	Ankley *et al.*, 1995
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9.56	72h-LC50	Unionized	1000	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	8.5	72h-LC50	Unionized	2180	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	8.76	72h-LC50	Unionized	3000	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9	72h-LC50	Unionized	2020	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9.5	72h-LC50	Unionized	540	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	8.71	72h-LC50	Unionized	2000	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9	72h-LC50	Unionized	1450	Strus *et al.*, 1991

Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9.04	72h-LC50	Unionized	2000	Strus *et al.*, 1991
Ammonium chloride	*Macrobrachium rosenbergii*	Crustaceans	29	9.21	72h-LC50	Unionized	1000	Strus *et al.*, 1991
Ammonium chloride	*Orconectes immunis*	Crustaceans	17.1	7.9	96h-LC50	Unionized	12120	Arthur *et al.*, 1987
Ammonium chloride	*Orconectes immunis*	Crustaceans	4.6	8.2	96h-LC50	Unionized	18810	Arthur *et al.*, 1987
Ammonium chloride	*Orconectes nais*	Crustaceans	26.5	8.3	96h-LC50	Unionized	2590	Evans, 1979
Ammonium chloride	*Pachydiplax longipennis*	Insects/Spiders	12	7.95	96h-LC50	Unionized	1990	Diamond *et al.*, 1993
Ammonium chloride	*Pachydiplax longipennis*	Insects/Spiders	20	7.95	96h-LC50	Unionized	3450	Diamond *et al.*, 1993
Ammonium chloride	*Paratya curvirostris*	Crustaceans	15	8.1	96h-LC50	Unionized	750	Richardson, 1997
Ammonium chloride	*Paratya curvirostris*	Crustaceans	15	7.5	96h-LC50	Unionized	770	Richardson 1997
Ammonium chloride	*Penaeus chinensis*	Crustaceans	29	7.6	96h-LC50	Total Ammonia	5800	Zang *et al.*, 1993
Ammonium chloride	*Physa gyrina*	Molluscs	24.9	8	96h-LC50	Unionized	1410	Arthur *et al.*, 1987
Ammonium chloride	*Physa gyrina*	Molluscs	5.5	8.2	96h-LC50	Unionized	1720	Arthur *et al.*, 1987
Ammonium chloride	*Physa gyrina*	Molluscs	4	8	96h-LC50	Unionized	1310	Arthur *et al.*, 1987
Ammonium chloride	*Physa gyrina*	Molluscs	12.1	8.1	96h-LC50	Unionized	2050	Arthur *et al.*, 1987
Ammonium chloride	*Physa gyrina*	Molluscs	12.8	8	96h-LC50	Unionized	1470	Arthur *et al.*, 1987
Ammonium chloride	*Physa gyrina*	Molluscs	13.3	8	96h-LC50	Unionized	1780	Arthur *et al.*, 1987
Ammonium chloride	*Physa* sp.	Molluscs	22.2	7.55	96h-LC50	Total Ammonia	30600	Call *et al.*, 1984
Ammonium chloride	*Planorbella trivolvis*	Molluscs	22	7.9	96h-LC50	Unionized	1680	Arthur *et al.*, 1987
Ammonium chloride	*Planorbella trivolvis*	Molluscs	12.9	8.2	96h-LC50	Unionized	2270	Arthur *et al.*, 1987
Ammonium chloride	*Potamopyrgus jenkinsi*	Molluscs	15	7.8	96h-LC50	Unionized	315	Watton and Hawkes, 1984
Ammonium chloride	*Potamopyrgus jenkinsi*	Molluscs	15	7.6	96h-LC50	Unionized	490	Watton and Hawkes, 1984
Ammonium chloride	*Potamopyrgus jenkinsi*	Molluscs	15	7.75	96h-LC50	Unionized	850	Watton and Hawkes, 1984
Ammonium chloride	*Procambarus clarkii*	Crustaceans	12	7.95	96h-LC50	Unionized	1990	Diamond *et al.*, 1993
Ammonium chloride	*Procambarus clarkii*	Crustaceans	20	7.95	96h-LC50	Unionized	1210	Diamond *et al.*, 1993
Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	33560	Neil *et al.*, 2005
Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	47130	Neil *et al.*, 2005
Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	37520	Neil *et al.*, 2005
Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	50000	Neil *et al.*, 2005

Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	20630	Neil *et al.*, 2005
Ammonium chloride	*Scylla serrata*	Crustaceans	28.3	8.01	48h-LC50	Total Ammonia	43540	Neil *et al.*, 2005
Ammonium chloride	*Stenelmis sexlineata*	Insects/Spiders	25	8.7	96h-LC50	Unionized	6590.2	Hazel *et al.*, 1979
Ammonium chloride	***Corbicula manilensis***	**Molluscs**	**25**	**8**	**96h-LC50**	**Total Ammonia**	**2250**	**Schelller, 1997 in USEPA, 2009**
Ammonium chloride	***Corbicula manilensis***	**Molluscs**	**29.4**	**8.05**	**96h-LC50**	**Total Ammonia**	**6316**	**Belanger *et al.*, 1991**
Ammonium chloride	***Corbicula manilensis***	**Molluscs**	**30.3**	**8.05**	**96h-LC50**	**Total Ammonia**	**2125**	**Belanger *et al.*, 1991**
Ammonium chloride	***Turbifex turbifex***	**Worms**	**12**	**8.2**	**96h-LC50**	**TAN**	**66670**	**Stammer, 1953 in USEPA, 2009**
Ammonium chloride	***Dendrocoelum lacteum***	**Worms**	**18**	**8.2**	**96h-LC50**	**TAN**	**22370**	**Stammer, 1953 in USEPA, 2009**
Ammonium chloride	***Lumbriculus variegatus***	**Worms**	**23**	**7.56**	**96h-LC50**	**TAN**	**286000**	**Besser *et al.*, 1998 in USEPA, 2009**
Ammonium chloride	***Lumbriculus variegatus***	**Worms**	**23**	**6.69**	**96h-LC50**	**TAN**	**302000**	**Besser *et al.*, 1998 in USEPA, 2009**
Ammonium chloride	***Lumbriculus variegatus***	**Worms**	**25**	**8.1**	**96h-LC50**	**TAN**	**43500**	**Schubauer-Berigan *et al.*, 1995**
Ammonium chloride	***Lumbriculus variegatus***	**Worms**	**25**	**8.1**	**96h-LC50**	**TAN**	**34000**	**Schubauer-Berigan *et al.*, 1995**
Ammonium chloride	*Corbicula manilensis*	Molluscs	20	8.05	48h-LC50	Total	1720	Belanger *et al.*, 1991

Boldface: *Denotes toxicity data collected from original source and not from USEPA ECOTOX (2016)*

APPENDIX 12: Acute toxicity data for nitrate to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species Scientific Name	Species Group	Endpoint	Conc. (µg/L)	Reference
Sodium nitrate	*Hydropsyche occidentalis*	Insects/Spiders	96h-LC50	466036	Camargo and Ward, 1995
Sodium nitrate	*Hydropsyche occidentalis*	Insects/Spiders	96h-LC50	482870	Camargo and Ward, 1992
Sodium nitrate	*Hydropsyche occidentalis*	Insects/Spiders	96h-LC50	400472	Camargo and Ward, 1992
Sodium nitrate	*Hydropsyche occidentalis*	Insects/Spiders	96h-LC50	431039	Camargo and Ward, 1992
Sodium nitrate	*Cheumatopsyche pettiti*	Insects/Spiders	96h-LC50	568369	Camargo and Ward, 1992
Sodium nitrate	*Cheumatopsyche pettiti*	Insects/Spiders	96h-LC50	681334	Camargo and Ward, 1992
Sodium nitrate	*Lymnaea* sp.	Molluscs	96h-LC50	3251000	Dowden and Bennett, 1965
Sodium nitrate	*Potamopyrgus antipodarum*	Molluscs	96h-LC50	4616060	Alonso and Camargo, 2003
Sodium nitrate	*Chironomus dilutus*	Insects/Spiders	48h-LC50	1231540	USEPA, 2010
Sodium nitrate	*Lampsilis siliqoidea*	Molluscs	96h-LC50	1582000	USEPA, 2010
Sodium nitrate	*Sphaerium simile*	Molluscs	96h-LC50	1644000	USEPA, 2010
Sodium nitrate	*Hyalella azteca*	Crustaceans	96h-LC50	550206	Pandey *et al.*, 2011
Sodium nitrate	*Amphinemura delosa*	Insects/Spiders	96h-LC50	2020000	USEPA, 2010
Sodium nitrate	*Allocapnia vivipara*	Insects/Spiders	96h-LC50	3703000	Sourcek and Dickinson, 2011
Sodium nitrate	*Megalonaias nervosa*	Molluscs	96h-LC50	4151000	USEPA, 2010

APPENDIX 13: Acute toxicity data for chloride to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species Scientific Name	Species Group	Endpoint	Conc. (µg/L)	Reference
Sodium chloride	*Lirceus fontinalis*	Crustaceans	96h-LC50	2970000	Birge *et al.*, 1985
Sodium chloride	*Lymnaea* sp.	Molluscs	48h-LC50	3388000	Dowden and Bennett, 1965
Sodium chloride	*Tipula abdominalis*	Insects/Spiders	96h-LC50	10000000	Blasius and Merritt, 2002
Sodium chloride	*Agnetina capitata*	Insects/Spiders	96h-LC50	10000000	Blasius and Merritt, 2002
Sodium chloride	*Culex* sp.	Insects/Spiders	48h-LC50	10200000	Dowden and Bennett, 1965
Sodium chloride	*Cricotopus trifasciatus*	Insects/Spiders	48h-LC50	6221000	Hamilton *et al.*, 1975
Sodium chloride	*Planorbella campanulata*	Molluscs	96h-LC50	6150000	Wurtz and Bridges, 1961
Sodium chloride	*Gammarus pseudolimnaeus*	Crustaceans	96h-LC50	7700000	Blasius and Merritt, 2002
Sodium chloride	*Gyraulus circumstriatus*	Molluscs	72h-LC50	3700000	Wurtz and Bridges, 1961
Sodium chloride	*Hyalella azteca*	Crustaceans	96h-LC50	1382000	Elphick *et al.*, 2011
Sodium chloride	*Chaoborus americanus*	Insects/Spiders	96h-LC50	5000000	Benbow and Merritt, 2004
Sodium chloride	*Hydropsyche* sp.	Insects/Spiders	48h-LC50	9000000	Roback, 1965
Sodium chloride	*Erpobdella punctata*	Worms	72h-LC50	7500000	Wurtz and Bridges, 1961
Sodium chloride	*Limnodrilus hoffmeisteri*	Worms	72h-LC50	6800000	Wurtz and Bridges, 1961
Sodium chloride	*Stenonema rubrum*	Insects/Spiders	48h-LC50	2500000	Roback, 1965
Sodium chloride	*Chironomus dilutus*	Insects/Spiders	96h-LC50	5867000	Elphick *et al.*, 2011
Sodium chloride	*Argia* sp.	Insects/Spiders	96h-LC50	24000000	Wurtz and Bridges, 1961
Sodium chloride	*Callibaetis fluctuans*	Insects/Spiders	96h-LC50	5000000	Benbow and Merritt, 2004
Sodium chloride	*Acroneuria abnormis*	Insects/Spiders	96h-LC50	10000000	Blasius and Merritt, 2002
Sodium chloride	***Tricorythus tinctus***	**Insects/Spiders**	**96h-LC50**	**1689**	**Palmer *et al.*, 2004**
Sodium chloride	***Tricorythus tinctus***	**Insects/Spiders**	**96h-LC50**	**1337**	**Palmer *et al.*, 2004**
Sodium chloride	***Afronurus peringueyi***	**Insects/Spiders**	**96h-LC50**	**6290000**	**Palmer *et al.*, 2004**
Sodium chloride	***Oligoneuriopsis lawrencei***	**Insects/Spiders**	**96h-LC50**	**4815000**	**Palmer *et al.*, 2004**
Sodium chloride	***Baetis harrisoni***	**Insects/Spiders**	**96h-LC50**	**1569000**	**Palmer *et al.*, 2004**
Sodium chloride	***Demoreptus natalensis***	**Insects/Spiders**	**96h-LC50**	**4370000**	**Palmer *et al.*, 2004**
Sodium chloride	***Cloeon virgiliae***	**Insects/Spiders**	**96h-LC50**	**4853000**	**Palmer *et al.*, 2004**

Sodium chloride	***Enallagma* sp.**	**Insects/Spiders**	**96h-LC50**	**24407**	**Palmer *et al.*, 2004**
Sodium chloride	***Enallagma* sp.**	**Insects/Spiders**	**96h-LC50**	**21608**	**Palmer *et al.*, 2004**
Sodium chloride	***Caridina nilotica***	**Crustaceans**	**96h-LC50**	**8568000**	**Palmer *et al.*, 2004**
Sodium chloride	***Plea pullula***	**Insects/Spiders**	**96h-LC50**	**6741000**	**Palmer *et al.*, 2004**
Sodium chloride	*Asellus communis*	Crustaceans	96h-LC50	8250000	Wurtz and Bridges, 1961
Sodium chloride	*Asellus communis*	Crustaceans	96h-LC50	5100000	Wurtz and Bridges, 1961
Sodium chloride	*Isonychia bicolor*	Insects/Spiders	96h-LC50	3380000	Echols *et al.*, 2010
Sodium chloride	*Isonychia bicolor*	Insects/Spiders	96h-LC50	2250000	Echols *et al.*, 2010
Sodium chloride	*Physa heterostropha*	Molluscs	96h-LC50	4100000	Wurtz and Bridges, 1961
Sodium chloride	*Physella integra*	Molluscs	96h-LC50	5000000	Benbow and Merritt, 2004
Sodium chloride	*Physa heterostropha*	Molluscs	96h-LC50	6200000	Wurtz and Bridges, 1961
Sodium chloride	*Physa heterostropha*	Molluscs	96h-LC50	5100000	Wurtz and Bridges, 1961
Sodium chloride	*Physa gyrina*	Molluscs	96h-LC50	2540000	Birge *et al.*, 1985
Sodium chloride	*Physa heterostropha*	Molluscs	96h-LC50	3500000	Wurtz and Bridges, 1961
Sodium chloride	*Gyraulus parvus*	Molluscs	96h-LC50	3009000	Soucek *et al.*, 2011
Sodium chloride	*Gyraulus circumstriatus*	Molluscs	96h-LC50	3200000	Wurtz and Bridges, 1961
Sodium chloride	*Gyraulus parvus*	Molluscs	96h-LC50	3078000	Soucek *et al.*, 2011
Sodium chloride	*Nephelopsis obscura*	Worms	96h-LC50	4269830	ENVIRON International Corp., 2009
Sodium chloride	*Nephelopsis obscura*	Worms	96h-LC50	4269830	ENVIRON International Corp., 2009
Sodium chloride	*Nephelopsis obscura*	Worms	96h-LC50	4270280	ENVIRON International Corp., 2009
Sodium chloride	*Nephelopsis obscura*	Worms	96h-LC50	4310420	ENVIRON International Corp., 2009

Boldface: *Denotes toxicity data collected from original source and not from USEPA ECOTOX (2016)*

APPENDIX 14: Acute toxicity data for Sulfate to freshwater macroinvertebrates collected from USEPA ECOTOX (2016) shown alongside original sources of data

Chemical Name	Species Scientific Name	Species Group	Endpoints	Conc. (µg/L)	Reference
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1879000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1616000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1938000	Illinois National History Survey,2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1684000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1799000	Illinois National History Survey, 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2955000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1562000	Illinois National History Survey, 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	4336000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1226130	Aquatic Toxicology Group 1998
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	5259000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	569000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	512000	Soucek and Kennedy, 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2203000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1901000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1563000	Illinois National History Survey, 2005 i
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1691000	Illinois National History Survey 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1820000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2240000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2002000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2101000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	3144000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1480000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1387000	Illinois National History Survey , 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1448000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	3796000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2000000	Soucek, 2007

Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1438000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1433000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1580000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2725000	Davies and Hall, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2724000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1854000	Illinois National History Survey, 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	4145000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2740000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1470000	Illinois National History Survey, 2005
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	3462000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1679000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1621000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1830000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1779000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1226130	Pickard *et al.*,1999
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2840000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	4046000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2121000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2002000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	4345000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	1767000	Soucek, 2007
Sodium Sulfate	*Hyalella azteca*	Crustaceans	96h-LC50	2855000	Soucek and Kennedy, 2005
Sodium Sulfate	***Tricorythus***	**Insects/Spiders**	**96h-LC50**	**660000**	**Goetsch and Palmer, 1997**
Sodium Sulfate	***Culex* sp.**	**Insects/Spiders**	**96h-LC50**	**13350000**	**Dowden and Bennett, 1965**
Sodium Sulfate	***Chironomus tentans***	**Insects/Spiders**	**96h-LC50**	**14134000**	**Soucek and Kennedy, 2005**
Sodium Sulfate	***Lymnaea* sp.**	**Molluscs**	**96h-LC50**	**5400000**	**Dowden and Bennett, 1965**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**7736000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**10379000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**10320000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**6363000**	**Palmer *et al.*, 2004**

Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**6303000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**7978000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**8598000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Adenophlebia auriculata***	**Insects/Spiders**	**96h-LC50**	**10379000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Enallagma* sp.**	**Insects/Spiders**	**96h-LC50**	**31703000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Enallagma* sp.**	**Insects/Spiders**	**96h-LC50**	**26224000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Enallagma* sp.**	**Insects/Spiders**	**96h-LC50**	**31703**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Cloeon virgiliae***	**Insects/Spiders**	**96h-LC50**	**3369000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Caridina nilotica***	**Crustaceans**	**96h-LC50**	**6820000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Afroptilum sudafricanum***	**Insects/Spiders**	**96h-LC50**	**3096**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Afroptilum sudafricanum***	**Insects/Spiders**	**96h-LC50**	**2755**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Afroptilum sudafricanum***	**Insects/Spiders**	**96h-LC50**	**2708**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Caddisflies* sp.**	**Insects/Spiders**	**96h-LC50**	**9803000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Caddisflies* sp.**	**Insects/Spiders**	**96h-LC50**	**11345000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Plea pullula***	**Insects/Spiders**	**96h-LC50**	**9999000**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Plea pullula***	**Insects/Spiders**	**96h-LC50**	**9400**	**Palmer *et al.*, 2004**
Sodium Sulfate	***Lampsilis***	**Molluscs**	**96h-LC50**	**2362000**	**Wang *et al.*, 2015**

Boldface: *Denotes toxicity data collected from original source and not from USEPA ECOTOX (2016)*

APPENDIX 15: Acute tolerance data for dissolved oxygen to freshwater macroinvertebrates collected from scientific papers shown alongside original sources of data

Species scientific name	Species group	Endpoint	Tolerance value (mg/L)	Reference
Acroneuria lycorias	Insects/Spiders	96h-LC50	3.6	Nebeker, 1972
Acroneuria pacifica	Insects/Spiders	96h-LC50	1.6	Gaufin, 1973
Hexagenia limbata	Insects/Spiders	96h-LC50	1.4	Nebeker, 1972
Hexagenia limbata	Insects/Spiders	96h-LC50	1.8	Gaufin, 1973
Ephemera subvaria	Insects/Spiders	96h-LC50	3.9	Nebeker, 1972
Ephemera doddsi	Insects/Spiders	96h-LC50	5.2	Gaufin, 1973
Ephemera grandis	Insects/Spiders	96h-LC50	3	Gaufin, 1973
Hydropsyche betteri	Insects/Spiders	96h-LC50	2.9	Nebeker, 1972
Hydropsyche betteri	Insects/Spiders	96h-LC50	2.6	Nebeker, 1972
Hydropsyche betteri	Insects/Spiders	96h-LC50	2.3	Nebeker, 1972
Hydropsyche betteri	Insects/Spiders	96h-LC50	1	Nebeker, 1972
Hyalella Azteca	Crustaceans	24h-LC50	0.7	Sprangue *et al.*, 1963
Asellus aquaticus	Crustaceans	24h-LC50	0.32	Maltby, 1995
Diura knowltoni	Insects/Spiders	96h-LC50	3.6	Gaufin, 1973
Nemoura cinctipens	Insects/Spiders	96h-LC50	3.3	Gaufin, 1973
Arcynopteryx sp.	Insects/Spiders	96h-LC50	3.3	Gaufin, 1973
Pteronarcella badia	Insects/Spiders	96h-LC50	2.4	Gaufin,1973
Callibaetis montanus	Insects/Spiders	96h-LC50	4.4	Gaufin, 1973
Rhithrogena robusta	Insects/Spiders	96h-LC50	3.3	Gaufin, 1973
Drusinus sp.	Insects/Spiders	96h-LC50	1.8	Gaufin, 1973
Limnephilus ornatus	Insects/Spiders	96h-LC50	3.4	Gaufin, 1973
Neophylax sp.	Insects/Spiders	96h-LC50	3.8	Gaufin, 1973
Neothremma alicia	Insects/Spiders	96h-LC50	1.7	Gaufin, 1973
Simulium vittatum	Insects/Spiders	96h-LC50	3.2	Gaufin, 1973
Gammarus limnaeus	Crustaceans	96h-LC50	3	Gaufin, 1973
Pteronarcys dorsata	Insects/Spiders	96h-LC50	2.2	Nebeker, 1972
Leptophlebia nebulosa	Insects/Spiders	96h-LC50	2.2	Nebeker, 1972
Baetisca laurentina	Insects/Spiders	96h-LC50	3.5	Nebeker, 1972

APPENDIX 16: Acute tolerance data for temperature [median Lethal Temperature (LT50 and Temperature acclimation (Ta)] to freshwater macroinvertebrates collected from scientific papers shown alongside original sources of data

Species scientific name	Species group	Endpoint	LT50 (˚C)	Ta (˚C)	Reference
Anax imperator/speratus	Insects/Spiders	96h-LT50	33.6	17	Dallas and Ketley, 2011
Chloroniella peringueyi	Insects/Spiders	96h-LT50	31.5	17	Dallas and Ketley, 2011
Afronurus barnardi	Insects/Spiders	96h-LT50	26.3	17	Dallas and Ketley, 2011
Athripsodes sp.	Insects/Spiders	96h-LT50	29.9	17	Dallas and Ketley, 2011
Castanophlebia sp.	Insects/Spiders	96h-LT50	26.5	17	Dallas and Ketley, 2011
Aphanicerca capensis	Insects/Spiders	96h-LT50	23.4	17	Dallas and Ketley, 2011
Palaemon capensis	Crustacean	96h-LT50	31.3	17	Dallas and Ketley, 2011
Paramelita nigroculus	Crustacean	96h-LT50	24.3	17	Dallas and Ketley, 2011
Chimarra ambulans	Insects/Spiders	96h-LT50	25.5	17	Dallas and Ketley, 2011
Lestagella penicillata	Insects/Spiders	96h-LT50	29.5	17	Dallas and Ketley, 2011
Ephemerella invaria	Insects/Spiders	LT50	22.9	10	De Kozlowzki and Bunting, 1981
Symphitopsyche morosa	Insects/Spiders	LT50	30.4	10	De Kozlowzki and Bunting, 1981
Stenonema ithaca	Insects/Spiders	LT50	31.8	10	De Kozlowzki and Bunting, 1981
Brachycentrus lateralis	Insects/Spiders	LT50	32.8	10	De Kozlowzki and Bunting, 1981
Deleatidium spp.	Insects/Spiders	LT50	22.6	15	Quinn et al., 1994
Zephlebia dentata	Insects/Spiders	LT50	23.6	15	Quinn *et al*., 1994
Aoteapsyche colonica	Insects/Spiders	LT50	25.9	15	Quinn *et al*., 1994
Pycnocentrodes aureola	Insects/Spiders	LT50	32.4	15	Quinn *et al*., 1994
Pyconocentria evecta	Insects/Spiders	LT50	25	15	Quinn *et al*., 1994
Hydora sp	Insects/Spiders	LT50	32.6	15	Quinn *et al*., 1994
Potamopyrgus antipodarum	Molluscs	LT50	32.4	15	Quinn *et al*., 1994
Sphaerium novaezelandiae	Molluscs	LT50	30.5	15	Quinn *et al*., 1994
Paratya curvirostris	Crustacean	LT50	25.7	15	Quinn *et al*., 1994
Paracalliope fluviatilis	Crustacean	LT50	24.1	15	Quinn *et al*., 1994
Lumbriculus variegatus	Worms	LT50	26.7	15	Quinn *et al*., 1994

APPENDIX 17: Comparison of protective concentrations generated from this study with those recommended by water quality jurisdictions with particular focus on the minimum and maximum values

Stressor	This Study		Modifying Factor (s)	Published Criteria		Rank order	Comment
	PC95 (LCI 95%)	PC95 (GV)		Min. Value (MinV)	Max. Value (MaxV)		
Ammonia (mg TAN /L)	1.9	4.9	Temp (25°C) and pH (8)	-	29[10]	LCI < GV < MaxV	Derived PC95 was more conservative than published (comparible) TAN guideline at reference pH and Temp.
Cadmium (µg/L)	7.5 at H = 90	32 at H = 90	Hardness	1.8 at H = 100[9]	5.7[1] (Conv. DF) at H = 60-119	MinV < MaxV < LCI<GV	Derived PC95 was less conservative than published Cd guidelines
Chloride (mg/L)	1433	1875	Nil	574[16]	860[12]	MinV < MaxV < LCI < GV	Derived PC95 was less conservative than published Cl^- guidelines
Chromium III (µg/L)	600 at H = 90	1073 at H = 90	Hardness	8.9 H = Unadjusted[8]	570 H = 100[5]	MinV < MaxV < LCI < GV	Derived PC95 was less conservative than published Cr (III) guidelines
Chromium VI (µg/L)	50	292	Nil	1[8]	200[1]	MinV < LCI < MaxV< GV	Derived PC95 lay between published guidelines for Cr (VI).
Copper (µg/L)	9.9 at H = 60	15 at H = 60	Hardness	1.6 at H<60 [1]	7.285 at H = 50[2]	MinV < MaxV < LCI < GV	Derived PC95 was less conservative than published Cu guidelines at comparible water hardness
Dissolved oxygen (mg/L)	4.44		Nil	3.0[12]	9.5[8]	MinV < GV < MaxV	Derived PC95 lay between published guidelines for short-term exposure of freshwater organisms to DO (mg/L saturation).
Lead (µg/L)	164 at H = 90	461 at H = 90	Hardness	2 at H = 60-120[6]	82 at H = 100[7]	MinV < MaxV < LCI < GV	Derived PC95 was less conservative than published Pb guidelines at comparable water hardness
Mercury II (µg/L)	5.5	15	Nil	0.026[6]	**0.6**[4]	MinV < MaxV < LCI < GV	Derived PC95 was less conservative than published Hg (II) guidelines.
Nickel (µg/L)	507 at H = 90	1031 at H = 90	Hardness	65 at H = 60-120[6]	470 at H = 100[5]	MinV < MaxV < LCI <GV	Derived PC95 was less conservative than published Ni guidelines.
Nitrate (mg NO_3-N/L)	0.2	47	Nil	32.8	124[11]	LCI < MinV <	Derived PC95 was more conservative than published guidelines.

						GV < MaxV	
Sulfate (mg/L)	108	316	Nil	128[15] (hardness and chloride adjusted)	2000[16] (hardness and chloride adjusted)	LCI < MinV < GV< MaxV	Derived PC95 for SO_4 was more conservative than published guidelines.
Temperature (Δ°C)	5.1	7.1	Nil	-	2[1]		Derived PC95 was less conservative than published (comparable) guidelines for temperature difference (°C)
Temperature (%Δ)	27	30	Nil	-	10[1]		Derived PC95 was less conservative than published (comparable) guidelines for temperature difference (% difference)
Zinc (µg/L)	19 at H = 30	99	Hardness	8.0 at H = 30[4]	66.6 at H = 50[3]	MinV < LCI < MaxV < GV	Derived PC95 lay between published guidelines for Zn.

1: DWAF, 1996 2: USEPA, 1996 3: USEPA, 1987 4: ANZECC and ARMCANZ, 2000 5: USEPA, 1995 6: CCME, 2007 7: Nagpal et al., 2001 (British Columbia) 8: CCME, 1999 9: USEPA, 2017 10: USEPA, 2009 11: CCME, 2012 12: USEPA, 1986 13: Meays, 2009 (British Columbia) 14: CCME, 2012
15: Meays and Nordin, 2013 16: Iowa DNR, 2009. H: Hardness (mg/L as $CaCO_3$) Conv. DF: After being converted to dissolved fraction

APPENDIX 18: Generalized/overall list of riverine macroinvertebrates taxa collected [present (1) and absent (0)] from each of the eight study sites throughout the sampling period

TAXON	S1	S2	S3	S4	S5	S6	S7	S8
Porifera	1	1	1	0	0	1	1	0
Coelenterata	0	0	0	0	0	1	0	0
Turbellaria	0	1	1	1	0	1	0	1
Oligochaeta	0	1	1	1	1	1	1	1
Hirudinea	0	1	1	1	1	1	1	1
Potamonautidae	1	0	1	1	1	0	0	0
Atyidae	1	1	1	0	0	1	0	0
Hydracarina	1	1	1	0	0	0	0	0
Perlidae	0	0	1	0	0	0	0	0
Baetidae 1 sp	0	0	0	0	1	0	0	0
Baetidae 2 sp	1	1	1	1	1	1	1	1
Baetidae >2 sp	0	0	1	1	0	1	1	1
Caenidae	0	1	0	0	1	0	1	1
Heptageniidae	0	0	1	1	0	0	0	0
Leptophlebiidae	0	0	1	0	0	1	1	1
Oligoneuridae	0	1	0	0	0	1	0	0
Prosopistomatidae	0	0	0	1	0	0	1	0
Tricorythidae	0	0	0	1	0	1	0	1
Chlorocyphidae	0	0	1	1	1	1	0	0
Synlestidae	0	1	0	1	0	0	1	0
Coenagrionidae	1	1	1	1	1	1	1	1
Lestidae	0	0	0	1	1	1	1	0
Platycnemidae	0	0	0	0	0	0	0	1
Protoneuridae	0	0	1	1	0	0	0	0
Aeshnidae	1	1	0	1	1	1	0	0
Corduliidae	0	0	0	1	0	1	0	0
Gomphidae	0	1	1	1	1	1	1	1
Libellulidae	0	1	1	1	1	1	1	1
Crambidae	0	0	1	0	0	0	0	0
Belostomatidae	1	0	0	0	1	0	0	1
Corixidae	1	1	0	0	1	1	0	0
Gerridae	0	0	1	0	1	1	1	1
Naucoridae	1	1	1	1	1	1	1	1
Notonectidae	0	1	0	0	0	1	0	0
Pleidae	1	1	0	0	1	0	0	0
Veliidae	1	1	1	1	1	1	1	0

Ecnomidae	0	1	0	1	0	1	0	0
Hydropsychidae 1 sp	0	1	0	1	1	1	1	0
Hydropsychidae 2 sp	1	1	1	1	1	1	0	1
Hydropsychidae > 2 sp	0	0	1	1	0	0	1	1
Philopotamidae	0	0	1	0	0	0	0	0
Hydroptilidae	0	0	0	0	0	1	0	1
Lepidostomatidae	0	0	0	1	0	0	0	0
Leptoceridae	0	0	1	1	0	0	0	1
Pisuliidae	0	0	0	1	0	0	0	0
Dytiscidae	1	1	1	1	0	1	1	0
Elmidae	0	1	1	0	1	1	1	1
Gyrinidae	1	1	1	0	1	1	1	0
Hydraenidae	0	1	0	0	0	0	1	0
Hydrophilidae	0	1	0	0	0	0	1	0
Psephenidae	0	0	1	0	0	0	0	0
Athericidae	0	0	1	0	0	0	0	0
Ceratopogonidae	1	1	1	1	1	1	1	1
Chironomidae	1	1	1	1	1	1	1	1
Culicidae	1	1	1	0	0	1	1	0
Dixidae	0	1	0	0	0	0	0	0
Psychodidae	0	0	1	0	0	0	0	0
Simuliidae	0	1	1	1	1	1	1	0
Tabanidae	0	0	1	0	0	1	0	1
Tipulidae	0	1	0	0	0	0	0	0
Ancylidae	1	1	1	1	0	0	0	0
Lymnaeidae	0	0	0	0	0	1	1	0
Physidae	1	1	0	0	1	1	1	1
Planorbinae	0	0	1	0	0	0	0	0
Thiaridae	0	0	1	1	1	1	1	1
Corbiculidae	0	1	1	1	1	1	1	1

1: Denotes present in any of the sampling occasions *0: Denotes absent throughout the sampling period*

APPENDIX 19: Flow magnitudes for seven study sites generated from flow proxy gauging stations with associated classes for data collected between August 2015 and August 2016

Site	Date	Flow (m^3/s)	Exceedance %	Exceedance % (Range)	Flow class
S1	September 2015	**0.10**	NC	NC	NC
S2	September 2015	**0.10**	NC	NC	NC
S3	September 2015	0.78	89.81	60-90	Dry conditions
S4	September 2015	3.2	59.5	40-60	Mid-range flow
S5	September 2015	0.24	27.98	10-40	Moist conditions
S6	September 2015	4.45	43.8	40-60	Mid-range flows
S7	September 2015	3.39	64.84	60-90	Dry conditions
S8	FC	FC	FC	FC	FC
S1	FC	FC	FC	FC	FC
S2	November 2015	**0.02**	NC	NC	NC
S3	November 2015	2.1	7.44	0-10	High flows
S4	November 2015	1.98	92.29	90-100	Low flows
S5	November 2015	0.24	27.98	10-40	Moist conditions
S6	November 2015	2.29	87.6	60-90	Dry conditions
S7	November 2015	2.08	89.29	60-90	Dry conditions
S8	FC	FC	FC	FC	FC
S1	March 2016	0.51	NC	NC	NC
S2	March 2016	0.80	NC	NC	NC
S3	March 2016	0.8	87.05	60-90	Dry condition
S4	March 2016	5.85	15.7	10-40	Moist conditions
S5	March 2016	0.23	39.34	10-40	Moist conditions
S6	March 2016	9.72	14.05	10-40	Moist conditions
S7	March 2016	11.53	12.36	10-40	Moist conditions
S8	FC	FC	FC	FC	FC
S1	NC	-	NC	NC	NC
S2	May 2016	0.55	NC	NC	NC
S3	May 2016	0.25	99.72	90-100	Low flows
S4	May 2016	4.45	31.13	10-40	Moist conditions
S5	May 2016	0.1	83.93	60-90	Dry conditions
S6	May 2016	4.04	49.59	40-60	Mid-range flows
S7	May 2016	4.01	54.4	40-60	Mid-range flows

S8	May 2016	NC	NC	NC	NC
S1	August 2016	-	NC	NC	NC
S2	August 2016	0	NC	NC	NC
S3	August 2016	0.8	87.05	60-90	Dry conditions
S4	August 2016	2.60	74.38	60-90	Dry conditions
S5	August 2016	0.12	80.06	60-90	Dry conditions
S6	August 2016	2.75	87.6	60-90	Dry conditions
S7	August 2016	2.21	87.64	60-90	Dry conditions
S8	August 2016	NC	NC	NC	NC

NC: Denotes flows could not be classified FC: Denotes flow cessation Exceedance %: The probability/ percentage that the flow of that magnitude can be equalled or exceeded

APPENDIX 20: Background water temperature for the Olifants River catchment based on data collected (between 2010 and 2011) from the Klaserie River by South African Environmental Observation Network

Month	Time	n	Mean	Median
January	Morning	103	18.17	18.62
	Afternoon	108	22.52	22.34
February	Morning	87	15.16	15.05
	Afternoon	54	20.35	20.19
March	Morning	93	14.01	14.66
	Afternoon	32	17.83	17.9
April	Morning	90	11.71	12.02
	Afternoon	30	14.61	15.01
May	Morning	147	12.69	12.49
	Afternoon	31	14.31	14.37
June	Morning	134	12.08	11.33
	Afternoon	116	17.05	14.56
July	Morning	155	11.69	10.29
	Afternoon	180	15.98	13.58
August	Morning	128	11.41	11.22
	Afternoon	185	15.67	15.14
September	Morning	117	14.49	14.53
	Afternoon	210	18.59	18.47
October	Morning	120	16.26	16.06
	Afternoon	217	20.34	20.44
November	Morning	115	18.16	18.01
	Afternoon	208	21.74	21.54
December	Morning	120	18.33	18.39
	Afternoon	190	21.97	21.99

Morning: indicate data collected between 06h00 and 12h00, Afternoon: data collected between 12h00 and 18h00

APPENDIX 21: ATI for fish scores (Wepener *et al*., 1992) scores (composite) with corresponding lowest rating scores per study site and sampling event for water quality data collected from the Olifants River catchment between September 2015 and August 2016

Site	Month/Sampling event	Composite ATI Fish	Lowest rating score
S1	Sep-15	73.44	Zinc
S1	Mar-16	64.65	Fluoride
S2	Sep-15	62.88	Orthophosphates
S2	Nov-15	78.24	Zinc
S2	Mar-16	68.64	Fluoride
S2	May-16	65.57	Fluoride
S2	Aug-16	98.17	pH
S3	Sep-15	57.53	Zinc
S3	Nov-15	65.38	Orthophosphates
S3	Mar-16	72.02	Fluoride
S3	May-16	69.84	Fluoride
S3	Aug-16	97.2	Copper
S4	Sep-15	47.26	Orthophosphates
S4	Nov-15	69.72	Nickel
S4	Mar-16	71.33	Fluoride
S4	May-16	60.22	Fluoride
S4	Aug-16	93.8	pH
S5	Sep-15	58.47	Orthophosphates
S5	Nov-15	63.73	Nickel
S5	Mar-16	52.39	Fluoride
S5	May-16	52.31	Fluoride
S5	Aug-16	49.28	Orthophosphates
S6	Sep-15	73.45	Zinc
S6	Nov-15	73.45	Nickel
S6	Mar-16	71.25	Fluoride
S6	May-16	68.23	Fluoride
S6	Aug-16	93.45	pH
S7	Sep-15	75.65	Zinc
S7	Nov-15	72.76	Nickel
S7	Mar-16	61.01	Fluoride
S7	May-16	62.68	Fluoride
S7	Aug-16	78.28	Zinc
S8	Sep-15	75.35	Zinc
S8	May-16	66.59	Fluoride
S8	Aug-16	78.74	Zinc

APPENDIX 22: Results for the macroinvertebrate response assessment index

Site	Modification metrics			MIRAI	
	Flow	Habitat	Water Quality	Invertebrate EC	EC Category
S1	18	34	37	69.97	C
S2	13	13	27	81.98	C/B
S3	12	14	30	81.1	C/B
S4	38	34	31	65.6	C
S5	41	25	34	66.8	C
S6	23	31	27	72.7	C
S7	18	18	26	79.5	C/B
S8	12	14	41	77.3	C