MONITORING AND ASSESSMENT OF MACROINVERTEBRATE COMMUNITIES IN SUPPORT OF RIVER HEALTH MANAGEMENT IN KWAZULU-NATAL, SOUTH AFRICA

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ABSTRACT

Conservation of freshwater systems is globally weak and generally declining, with rivers and wetlands being the most threatened ecosystems by anthropogenic impacts. Though they are highly import, freshwater ecosystems remain poorly understood and insufficient data often limit conservation efforts on many freshwater ecosystems. KwaZulu-Natal (KZN) Province is an important high water yield area of South Africa, but the sustainability of the rivers is being threatened. Macroinvertebrates are good indicators of water quality and ecosystem degradation, but their biodiversity and ecosystem conservation depend largely on the quality of the available data and the efficiency of the methods used in the data analysis. Each aspect of the research provides results that can be used in current and future conservation planning for rivers and aquatic macroinvertebrates.

The reference condition approach is an effective bioassessment technique closely related to the biological/ecological integrity concept, which is based on the evaluation of the deviation of the ecological quality of a test site's biological community from that of a near-pristine "reference" condition having very similar characteristics. Although the term *reference condition,* is used to describe near-natural or pristine condition, several practitioners believe that only a few pristine ecosystems still exist in the world. Hence, the reference condition (RC) defines the representative of a group of undisturbed or minimally disturbed sites by anthropogenic activities, while biological reference condition is the description of the biological elements that exist under no or very minor anthropogenic activities. This study applied the multivariate method of selecting and validating reference conditions, using ecoregions, river types and seasonal changes as grouping criteria for the reference sites. The ecoregions and river types were more adequate than the seasonal variations in the selection of reference conditions.

Although there is currently no consensus about the most appropriate and informative index, biodiversity indices are essential for environmental monitoring and conservation management decisions. This study compared a series of macroinvertebrate data from the rivers of KZN according to nine diversity indices (total number of species/taxa, total number of individuals, Margalef's, Pielou's, Brillouin's, Hill's, Simpson's, Fisher's and Shannon's indices), one

similarity index (similarity percentage – SIMPER) and three biotic indices (SASS5, ASPT, and MIRAI). There were clear connections between water quality, and abundance of macroinvertebrates with the decrease in the diversity values of macroinvertebrates along pollution gradients. Fisher's index, similarity percentage, SASS5, ASPT and MIRAI were suitable indices for comparing degraded and least degreaded sites in this study. However, small changes in community compositions were better revealed by the Fisher's diversity index, similarity percentage and SASS5. The MIRAI was better than SASS5 as an ecological tool for the rivers of KZN, but it can further be improved by incorporating measures of diversity and taxa richness into the model.

Also, this study examined the effectiveness of macroinvertebrate taxa composition metrics to assess the ecological health of the rivers in KZN. Nine taxa metrics were able to distinguish between reference and impaired sites, through correlation strength with environmental variables and their reliability. The nine metrics were total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, percentage of Odonata taxa, total number of Trichoptera individuals, total number of Gastropoda individuals, total number of Oligochaeta individuals and total number of Coleoptera individuals. This study showed increasing water quality deterioration along the longitudinal gradients of the rivers in KwaZulu-Natal, from the upper reaches towards the lower reaches of the rivers. We found that macroinvertebrate community composition metrics could detect nutrient pollution, organic pollution and physical habitat degradation in KZN rivers. Thus it is recommended that more studies and validation of macroinvertebrate community-based metrics in the assessment of rivers in KZN are conducted. Furthermore, they are relatively cheap and easy to use. Macroinvertebrate community-based indices could be an effective alternative assessment method in the case of the lowland rivers where the lack of quality data often have negative impacts on the use of the biotic indices (SASS5, ASPT and MIRAI).

In addition, this study demonstrated how Bayesian networks can be used to conduct an environmental risk assessment of macroinvertebrate biodiversity and their associated river ecosystem to assess the overall effects of multiple anthropogenic stressors in rivers of KZN. Cause-effect exposure pathways were established between the sources of stressors, habitats and endpoints

(macroinvertebrate biodiversity and river ecosystem wellbeing) using using a conceptual model. The resulting conceptual model was then used to construct the Bayesian network models for each study site (risk regions) to estimate the overall risk from water quality, flow and habitat stressors. The model outputs and sensitivity analysis showed ecosystem threat and river health (represented by MIRAI) as the top factors posing the highest risks to macroinvertebrate biodiversity and the river ecosystem wellbeing respectively. The Bayesian network model was used to estimate the risk across the sites in the current scenario and three other scenarios that could occur if there were inadequate management practices. The current scenario was developed from field data collected during this study, while the other three scenarios were simulated to predict potential risk to the selected endpoints. We further simulated the low and high risks to the endpoints in order to demonstrate that the Bayesian network can be an effective adaptive management tool for decision making. The results of this study demonstrated that Bayesian networks can be used to calculate risk for multiple stressors, and that they are a powerful tool for informing future management strategies for achieving best management practices and policy making in the rivers of KZN.

PREFACE

The data described in this thesis were collected in KaZulu-Natal, Republic of South Africa from September 2014 to April 2016. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Dr. Gordon O'Brien and Professor Colleen T. Downs.

This thesis, submitted for the degree of Doctorate of Philosophy in Zoology in the College of Agriculture, Engineering and Science, University of KwaZulu-Natal, School of Life Sciences, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it was duly acknowledged in the text.

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Olalekan A. Agboola

July, 2017

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.

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Dr. Gordon O'Brien   Professor Colleen T. Downs

Supervisor   Supervisor

July, 2017   July, 2017

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DECLARATION 1 - PLAGIARISM

I, Olalekan A. Agboola, declare that

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DECLARATION 2 - PUBLICATIONS

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis.

Publication 1

OA Agboola, CT Downs & GC O'Brien

Multivariate approach to the selection and validation of reference conditions in KwaZulu-Natal Rivers, South Africa.

Author contributions:

OAA conceived paper with CTD and GOB. OAA collected and analysed data, and wrote the paper. CTD and GOB contributed valuable comments to the manuscript.

Publication 2

OA Agboola, CT Downs & GC O'Brien

Diversity, similarity and biotic indices: insights for ecological applications of macroinvertebrate community structures in the rivers of KwaZulu-Natal, South Africa.

Author contributions:

OAA conceived paper with CTD and GOB. OAA collected and analysed data, and wrote the paper. CTD and GOB contributed valuable comments to the manuscript.

Publication 3

OA Agboola, CT Downs & GC O'Brien

Multimetric assessment of macroinvertebrate community wellbeing and their responses to environmental variable changes in the rivers of KwaZulu-Natal, South Africa.

Author contributions:

OAA conceived paper with CTD and GOB. OAA collected and analysed data, and wrote the paper. CTD and GOB contributed valuable comments to the manuscript.

Publication 4

OA Agboola, CT Downs & GC O'Brien

Risk of water resource use to the wellbeing of macroinvertebrate communities in the rivers of KwaZulu-Natal, South Africa.

Author contributions:

OAA conceived paper with CTD and GOB. OAA collected and analysed data, and wrote the paper. CTD and GOB contributed valuable comments to the manuscript.

Signed…………………………………………

Olalekan A. Agboola

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CHAPTER 1

Introduction

1.1 The river health concept

Freshwater ecosystems conservation is globally generally weak and declining (Dudgeon *et al*., 2006; Butchart *et al*., 2010). Rivers and wetlands are the most threatened freshwater ecosystems, generally caused by various human activities, which alter the ecosystems integrity and functions (Jensen *et al*., 1993, Revenga *et al*., 2000). There is a growing concern for the biodiversity crisis that has engulfed freshwater ecosystems as a result of anthropogenic activities and natural forces (Singh and Singh, (2017). River conservation plans are aimed at identifying the areas or river segments that are impaired or representative of the diversity that require protection within a province or region (Rivers-Moore *et al*., 2007). Insufficient data often limit these conservation efforts (Rivers-Moore *et al*., 2007). Despite their importance and associated threats, freshwater ecosystems remain poorly understood and their representation in biodiversity assessments are still inefficient (Higgins, 2003). These evaluations are resource intensive, thereby creating a scarcity of information on many freshwater ecosystems and the available data for conservation planning are often inadequate for perfect management practices (Abell, 2002; Singh and Singh, 2017).

The health of rivers, their ecological integrity, and methods for their assessment are still a subject of considerable intellectual discourse (Norris and Morris, 1995; Scrimgeour and Wicklum, 1996; Quigley *et al*., 2001; Burnett *et al*., 2006). Although the term "river health" is often related to mean human health to create awareness, river ecosystems have distinct lives without humans (Rapport, 1989; Resh *et al*., 1995). Ecological integrity, according to Angermeier and Karr (1994) may be defined as, "*the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organisation comparable with that of the natural habitat of the region*". A healthy ecosystem is one which: (i) is not in distress; (ii) is resilient (i.e. can recover from stress); and (iii) the risk factors can easily be identified (e.g. pollution sources) (Rapport, 1989).

Furthermore, changes in the environmental conditions of rivers and streams have more recently extended beyond sewage discharge to climatic change effects and anthropogenic impacts causing losses of suitable aquatic habitats and negatively affecting species diversity and the ecosystems' functional diversity (Verdonschot, 2000; Meybeck, 2004; Allan *et al*., 2015). Despite the emergence and continued expansion of the use and knowledge of physico-chemical, biological and ecological assessments, rivers continue to deteriorate (Verdonschot, 2000). Public awareness, political will and inadequate knowledge base are important factors affecting the preservation, restoration and management of freshwater systems. Therefore, sustainable management and conservation of global freshwater resources need to be urgently obtained and these require sufficient and credible scientific information (Balian *et al*., 2008). There is still a need for research on the spatial and temporal dynamics of freshwater biological communities at undisturbed sites (Chaves *et al*., 2005), commonly referred to as reference sites.

1.2 Use of reference conditions in bioassessment

An efficient bioassessment technique receiving renewed interest and closely related to the biological or ecological integrity concept is the reference condition (RC) approach (Reynoldson *et al*., 1997; Chaves *et al*., 2005). The method evaluates the deviation of the ecological integrity of a test site from its near-natural RC, based on its biological community, with similar characteristics (Wright *et al*., 1984; European Commission, 2000; Wallin *et al*., 2003; Bailey *et al*., 2004). Although the RC often depicts a natural or pristine status, researchers observe that no or few truly natural or pristine conditions actually exist globally (Stoddard *et al*., 2006). Therefore, a RC is often defined as a model state of the undisturbed or minimally disturbed site, i.e. minimal exposure to human stressors (Bailey *et al*., 2004).

It is fundamental to measure accurately the existence of the cumulative impacts of the numerous river stressors over time, to protect it. Human activities cause degradation of biological integrity by modifying, destroying and contaminating the features that support and maintain biological communities (Karr *et al*., 1986), thereby causing them to deviate from a natural state. Bioassessment has become a comprehensive and efficient monitoring method because it integrates stressors, water quality variables and morphology (habitat integrity) (Davis and Simon, 1995; Barbour, 1997; Barbour *et al*., 2000; European Commission, 2000; Gerritsen *et al*., 2000). The

need for accuracy has led to the expansion of the assessment of biological integrity (Chaves *et al*., 2005), using the indicators of a good river ecostatus for comparisons between sites of similar characteristics in the absence of degradation (Norris and Thoms, 1999). These indicators are known as RCs. These RCs serve as the control, instead of single sites. A RC represents the best expected condition across similar sites and is represented by many sites (Reynoldson *et al*., 1997).

The knowledge of RCs is fundamental to the development and testing of metrics and indices of biological assessments. The conditions are established from data sets obtained from minimally or least-impacted regional sites (Hughes, 1995; Bailey *et al*., 1998; Stoddard *et al*., 2006; Whittier *et al*., 2007; Herlihy *et al*., 2008) and have legislative backing in several countries (e.g., the Clean Water Act in the USA, 1972; the Water Reform Framework in Australia, 1994 and the National Water Act in South Africa, 1998). Reference sites are the ones without or with minimal anthropogenic stress and satisfying the following criteria according to the European Union Water Framework Directive (EU, 2000): (1) reflecting total or limited undisturbed hydromorphological, physicochemical and biological quality; (2) zero or limited concentrations of peculiar synthetic pollutants when assessed with advanced analytical techniques; and (3) having concentrations of specific non-synthetic pollutants within the normal background limit.

As many factors that can influence biological assemblages need to be considered when establishing RCs in rivers. These may be in the form of large-scale patterns such as ecoregions (Omernik and Griffith, 2014) or small-scale characteristics sush as the watershed area and stream order (Barbour et al. 1999); stream typology (Verdonschot and Nijboer, 2004) and altitude (Bailey et al., 2004). RC models are developed to relate habitat attributes to biological assemblages or diversity, and then used to determine which reference state can be used for comparing a test site. However, reference sites may also be impacted by unknown stressors such as migration barriers, alien species, abnormal physical habitat conditions and the effects of previous impacts (Harding *et al*. 1998; Hughes, 1995; Whittier *et al*., 2007; Zhang *et al*. 2009).

1.3   Ecological indicators

The knowledge of the biological communities as ecological indicators and the abiotic factors of their ecosystems help in the: (i) assessment of impacts of anthropogenic activity on running waters,

(ii) understanding of how climate change modifies freshwater communities, and (iii) determining actual species richness and biodiversity of some particular stream reaches that may be poorly known (Dale and Beyeler, 2001; Chaves *et al*., 2005). The development of dynamic methods for monitoring, evaluating and managing the ecological integrity of a river system is essential to the conservation and preservation of resources, which could be strengthened by incorporating the use of ecological indicators (Dale and Beyeler, 2001). Ecological indicators are used to evaluate the extent of degradation, level of exposure to the stressors and the intensity of ecological responses to the exposure (Hunsaker, 1990; Suter, 1993).

The concept of using ecological indicators is based on the assumption that their presence-absence and community dynamics reflect the fluctuations in the ecological integrity of the system (Ellenberg, 1991; Niemi and McDonald, 2004; Orians and Policansky, 2009). Management goals often influence the choice of ecological indicators; therefore, it is often challenging to determine the best characteristics of the ecosystem which can be useful and efficient for monitoring and modelling of the ecosystem (Dale and Beyeler, 2001). Ecological indicators are categorized as: (i) indicator species (e.g., Weaver, 1995; Mocé-Llivina *et al*., 2003), (ii) keystone species (e.g., Mills *et al*., 1993; Power *et al*., 1996), (iii) ecological engineers (e.g. Zanetell and Peckarsky, 1996; Gibbs *et al*., 2010), (iv) umbrella species (e.g. Launer and Murphy, 1994), (v) link species (e.g. Franklin, 1995), and (vi) special interest or rare species (e.g. Lyons *et al*., 2005).

1.4 Advantages and disadvantages of using macroinvertebrates for biological monitoring in river ecosystems

Amongst the aquatic organisms used for bioassessments, macroinvertebrates have proved to be excellent indicators of good ecological quality of aquatic habitats and their advantages as bioindicators have been described in many studies (e.g. Barbour *et* al., 1999). These advantages include:

a. Macroinvertebrates are a diverse group of organisms and are present in most aquatic habitats.

b. Small order streams do not often support fish, but they do support extensive macroinvertebrate communities.

c. Presence/absence of taxa may provide information about environmental factors (e.g. stream velocity, oxygen content, pH, substrate types and others) and the pollution status of.

d. These taxa are mostly sedentary in lifestyle, they are mostly confined to the part of the river where the conditions (physical and chemical) are suitable (Davies and Day, 1998). This trait makes it possible to easily and efficiently assess the environmental conditions of the site they live.

e. The organisms have abilities to accumulate xenobiotic elements or compounds, thus reflecting the contaminant level in the environment.

f. Different stresses produce different macroinvertebrate communities or taxa compositions.

g. Sampling of macroinvertebrates under a rapid assessment protocol is easy, requires few people and minimal equipment and does not adversely affect other organisms.

h. A negative impact on them may tend to impact the food web and designated uses of the water resource because they are the primary food source for recreationally and commercially important fish.

i. They give an indication of short – long term responses of ecosystems.

However, the use of macroinvertebrates as ecological indicators also has some limitations (Barbour *et* al., 1999). These limitations include:

a. Inadequate identification skills/expertise (which may result in errors especially during the early life stage of macroinvertebrate larvae).

b. The difficulty of obtaining quantitative samples (this is dependent on river gradient and spatial arrangement of habitats).

c. Occurrence and abundance of some species may be different within the same region or river catchment, which makes the assessment of such taxa difficult.

d. Other factors, such as current velocity and substrate types may also determine the occurrence and abundance of macroinvertebrate species (Giller and Malmqvist, 1998;

Rosenberg and Resh, 1993; Linke *et al*., 1999; D'heygere *et al*., 2002; Sandin and Johnson, 2000; De Pauw *et al*., 2006; Hoang, 2009).

e. Some taxa are difficult to identify to species level.

f. There may be seasonal variations in taxa composition.

g. They do not respond to all impacts.

1.5   A brief history of biological monitoring

Globally, biomonitoring protocols were initially developed at small spatial scales (e.g. provinces) or small and medium river basins, suitable to the tradition or for convenience, thereby giving rise to many independent protocols or methods (Carter and Resh, 2001). These different protocols were then refined or modified into national guidelines, so as to standardise them, to produce scientifically valid information for basin or catchment management (Clarke and Hering, 2006; Blocksom *et al*., 2008). Furthermore, conflicts often arise at locations with inter-boundary rivers between states, regions or countries, thereby resulting in different sampling and analytical protocols, various ecological conditions or both (Toset *et al*., 2000; Ward, 2003; Sneddon and Fox, 2006). National biomonitoring programmes represent a variety of legislative or legal mandates, as well as an array of governmental funding initiatives (Dinar *et al*., 2013) (Table 1.1). The unifying aim of biomonitoring programmes is to audit, assess and provide information on freshwater policy and management (Dinar *et al*., 2013).

1.5.1   Australia

The two major river biomonitoring programmes in Australia are: (1) the National River Health Program (NRHP), which was a one-time national assessment of all river catchments conducted between 1997 and 2002, and (2) the Sustainable Rivers Audit (SRA), which existed from 2004 to 2013. It was a large-scale (1 million ha) bi-annual river well-being monitoring programme of 23 catchments across five states. The Australian River Assessment System (AUSRIVAS) was developed through the NRHP (Smith *et al*., 1999; Schiller, 2003). The AUSRIVAS involves a site-based assessment, which can be adapted to regional scales, thereby enabling reporting at the catchment or local levels through the prevailing reference conditions (Davies *et al*., 2010).

1.5.2   United States of America

There are two major national river biomonitoring programmes in the USA, funded by US Environmental Protection Agency (US EPA) and the US Geological Survey (USGS). The USEPA's initial biomonitoring programme was called the Environmental Monitoring and Assessment Program (EMAP) and was a research programme run by EPA's Office of Research and Development (Whittier and Paulsen, 1992; Stoddard *et al*., 2005). Its objective was to develop the necessary tools for monitoring and assessing the status and trends of national ecological resources. The goal of EMAP was to advance the science of ecological monitoring and ecological risk assessment (Messer *et al*., 1991). Also, to provide guidelines for national monitoring by applying advance scientific knowledge of ecosystem drivers and quality, and demonstrate multi-agency monitoring through large regional projects. EMAP developed indicators to monitor the condition of ecological resources (Hunsaker, 1990).

National Aquatic Resources Survey (NARS) has replaced EMAP and routinely monitors the USA's national aquatic resources, through USEPA's Office of Water. The NARS was mandated to execute USEPA's reporting requirements on the status and trends of US waters as stipulated by the Clean Water Act of 1972 (Stern and Mazze, 1974; Taylor and Wayland, 1977). The NARS assessment protocols rely on probabilities because it is demographically challenging and expensive to consolidate all the varying state data reports and previous attempts presented inaccurate reports (Hughes *et al*., 2000). The other agency, USGS' National Water Quality Assessment (NAWQA) monitors the effects of the major land uses (agriculture, urbanisation) on streams and ground water, using sites along different anthropogenic disturbance gradients. Both monitoring programmes, NAWQA and NRSA monitor the physical habitat, water chemistry, algae, macroinvertebrate and fish communities. A national water quality survey of the country's rivers and streams indicated that 55% of the country's flowing waters have degraded biological condition, while 23% are relatively safe (US EPA, 2013).

1.5.3   Europe

The European Commission formulated the Water Framework Directive (WFD) in 2000, as a legal system to promote a common stereotype for the management and protection of freshwater

ecosystems (European Commission, 2000). The objective of the WFD is to monitor and assess the ecological state of EU surface waters and to maintain or restore them to good environmental status by 2015 (European Commission, 2000). The European Union provides funds through multiple research projects in an attempt to centralise the biological assessment and monitoring efforts. All European countries (Austria, Czech Republic, Greece, Italy, Netherlands, Portugal, Germany and Sweden) who are members of the AQEM project (The Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe) make use of Benthic Macroinvertebrates as one of the biomonitoring agents (AQEM, 2002; Hering *et al*., 2006). There are standardised sampling and sample processing guidelines for monitoring benthic macroinvertebrates developed through the AQEM Project.

Also, several European countries (UK, France, Poland Slovakia, Denmark, Latvia and Italy) have adopted the modified version of the AQEM (i.e. the AQEM-STAR methodology; Clarke and Hering, 2006) through the STAR Project Scheme (Standardization of River Classifications: Framework method for calibrating different biological survey results against ecological quality classifications to be developed for the Water Framework Directive). Regardless of the efforts of AQEM and STAR Projects, a "pan-European" sampling, sample processing and data analysis of macroinvertebrate assessment is yet to be developed. However, the European standards (EN) guiding evaluation of benthic macroinvertebrates are: (1) EN-ISO 10870:2012: Water Quality Guidelines for the Selection of Sampling Methods and Devices for Benthic Macroinvertebrates in Freshwaters; and (2) EN 16150:2012: Water Quality Guidance on Pro-rata Multi-Habitat Sampling of Benthic Macroinvertebrates from Wadeable Rivers.

1.5.4   Canada

The Canadian Government through Environment Canada developed the Aquatic Biomonitoring Network (CABIN) to foster interagency collaboration and data sharing, to achieve commensurate and reliable reporting on freshwater ecosystem wellbeing (Reynoldson *et al*., 2003). The programme was developed through research by Environment Canada in the Great Lakes (Reynoldson *et al*., 1995) and the Fraser River Basin in British Columbia (Reynoldson *et al*., 1997). Thus, periodic biological monitoring was applied in these regions until the CABIN national biomonitoring was inaugurated in 1999 (Reynoldson *et al*., 1999). CABIN adopts the Reference

Condition Approach for assessment (Bailey *et al*., 2004), thus establishing and managing an extensive reference database. Environment Canada maintains the CABIN Website, database and training programme for the standard collection, assessment, reporting and distribution of biomonitoring information from different agencies (e.g. provinces municipalities, universities, and industries).

1.5.5   Japan

The first comprehensive taxonomic key to aquatic macroinvertebrates was produced in the late 1950s (Tsuda, 1962). Subsequently, Kolkwitz and Marsson's Saprobic and Beck's Biotic Indices were also developed in 1967 and 1954 respectively (Rosenberg and Resh, 1993). The Japanese aquatic macroinvertebrates identification guides (Kawai, 1985; Kawai and Tanida, 2005) are widely used in Japan and other Asian countries. The pioneer bioassessment protocol, namely the Beck-Tsuda Biotic Index (Tsuda, 1964), was in use for over 20 years in Japan. Other indices, such as the Zelinka–Marvan Saprobic Index (Kyuemon, 1978), the Shannon Diversity Index and the B-IBI (modified from the IBI of Karr (1981)) were also developed to monitor organic pollution and ecosystem wellbeing in Japanese rivers.

Recently, application of bioassessment protocols has been adopted to monitor rivers and streams in Japan. The method has helped to understand the relationships between benthic invertebrate assemblages and ecological characteristics of different rivers and streams in Japan (Kobayashi and Kagaya, 2005; Yoshimura, 2007). The findings of these studies indicated that macroinvertebrate community structures might be impacted by channel characteristics and environmental variables. The long-term restoration of rivers that receive mine effluent was assessed using macroinvertebrate community structure (family richness and abundance of selected taxa) (Watanabe *et al*., 2000).

1.5.6   South Africa

The ecosystem status monitoring of South Africa's inland water resources was developed and is being managed by the Department of Water and Sanitation, through the National Aquatic Ecosystem Health Monitoring Program (NAEHMP) (DWAF, 2008). The National River Health Programme (RHP) component of the NAEHMP is used for monitoring of the ecological status or

wellbeing of rivers (DWAF, 2008). The RHP is used to track the responses of aquatic macroinvertebrates and other biotas such as fish and riparian vegetation to changes in water quality, flow and habitat (quality and integrity), thereby indicating the general wellbeing of each component. There are more than 600 national biomonitoring sites spread across the country (DWAF, 2008). Resource Quality Information Services Directorate (RQIS) of the Department of Water and Sanitation (DWS) manages the RHP database. Survey data are obtained in collaboration with municipal and provincial government departments, as well as water boards, NGOs and academic institutions.

There are established monitoring tools used in the RHP based on the biotic and habitat components utilised in the biomonitoring of South Africa's rivers (DWAF, 2008). These include South African Scoring System (SASS) and Macroinvertebrate Response Assessment Index (MIRAI) for macroinvertebrates (Dickens and Graham, 2002; Thirion, 2007); Fish Response Assessment Index (FRAI) for fish (Kleynhans, 2007); Riparian Vegetation Response Assessment Index (VEGRAI) for vegetation (Kleynhans *et al*., 2008) and Index of Habitat Integrity (IHI) for habitat assessment (Kleynhans *et al*., 2008). The Omnidia software is currently being used for the assessment of diatoms (Lecointe *et al*., 1993). To ensure data quality in the use of macroinvertebrate indices (SASS and MIRAI), practitioners undergo training and accreditation (Dickens and Graham, 2002).

Table 1.1: Summary of global methods of macroinvertebrate biomonitoring protocols.

Area of study	Macoinvertebrate Monitoring Method	Reference
Australia	River Invertebrate Prediction and Classification System (RIVPACS)	Wright, 1995
	Australian Rivers Assessment System (AUSRIVAS)	Smith *et al*., 1999; Schiller, 2003
USA (NARS)	The Hilsenhoff Biotic Index (HBI)	Hilsenhoff, 1988
	Multimetric indices	Kerans and Karr, 1994; Fore *et al.*, 1996; Karr and Chu, 1998
	River Invertebrate Prediction and Classification System (RIVPACS)	Wright, 1995
	BEnthic Assessment of SedimenT (BEAST)	Reynoldson *et al*., 1995; Rosenberg and Resh, 1993
Europe (AQEM-STAR)	Trent biotic index (TBI) - England (1964)	Woodiwiss, 1964
	Chandler's Score – Scotland (1970)	Chandler, 1970
	Extended Biotic Index – UK (1978)	Woodiwiss, 1978
	BMWP Score – UK (1978)	Armitage *et al*., 1983
	Multimetric Macroinvertebrate Index Flanders (MMIF) - Belgium	Gabriels *et al*., 2006
Canada (Canadian Aquatic Biomonitoring Network – CABIN)	The Hilsenhoff Biotic Index (HBI)	Hilsenhoff, 1988
	Modified Biotic Index (BI)	Plafkin *et al*., 1989
Japan	Beck-Tsuda Biotic Index	Tsuda, 1964
	Zelinka–Marvan Saprobic Index	Kyuemon, 1978
	Multimetric analysis	Watanabe *et al*., 2000
	Multivariate analysis	Kobayashi and Kagaya, 2005; Yoshimura, 2007
South Africa (National River Health Monitoring Program)	SASS	Chutter, 1972; 1994; Dickens and Graham, 2002
	MIRAI	Thirion, 2007

1.6   Methods of macroinvertebrate biological monitoring

Organisms visible to the naked eye without the aid of microscopes are called "macro" and invertebrates are animals without backbones (Birmingham, *et al*., 2005; Sharma *et al*., 2006; Heishman and McLusky, 2012). Macroinvertebrates are an indispensable part of aquatic ecosystems and are often used as indicators of water quality and ecological state of these systems (Norris and Thoms, 1999; Whitfield and Elliott, 2002; Borja *et al*., 2009). There is a global acceptability of the valuable roles macroinvertebrates have in the monitoring and assessment of streams and river health because they are regarded one of the best and efficient ways to monitor the state of aquatic ecosystems (Norris and Thoms, 1999; Bonada *et al*., 2006).

Macroinvertebrate community structures and ecology have been an important aspect of research in various river systems. Research on macroinvertebrates has helped improve the assessment of biological resources, their conservation and detection of pollution by providing knowledge of the differences between predicted and actual faunal assemblages (Ormerod and Edwards, 1987). Pristine ecosystems, especially in remote or conserved areas are pertinent for the detection of environmental changes (McCauley *et al*., 2013). Understanding of stream biodiversity patterns has been explored through various local studies, but few studies have been conducted across broad spatial scales (Vinson and Hawkins, 1998). Studies at large and spatial scales are often challenging and expensive because of constraints in sampling across heterogeneous habitats and in the identification of collected specimens (Budy *et al*., 2011).

Classification and ordination tools of assessment of macroinvertebrates based on species and environmental data have been used to detect typical patterns of macroinvertebrate assemblages in river systems and also to predict faunal composition at different sites with the aid of known environmental variables (Malmqvist and Mäki, 1994; Burian, 1997). Macroinvertebrates they play a central role in stream food webs, where they provide integrated information on stream ecosystem structure, function and water quality (Winterbourn, 1999). Also, they provide information on the energy base of the ecosystem, habitat availability and food resources for fish and other aquatic fauna (Whitledge and Rabeni, 1997; Cederholm *et al*., 1999; Covich *et al*., 1999).

Macroinvertebrate species composition and/or diversity is affected by a variety of factors, such as physico-chemistry (Collier, 1995; Jacobsen *et al*., 1997; Winterbourn *et al*., 2000), biogeography (i.e. the distribution of organisms in space and time) (Harding, 1994; Boothroyd and Stark, 2000), and dispersal (Edwards and Sugg, 1993; Boothroyd and Stark, 2000; Winterbourn and Crowe, 2001). Catchment land use is a particularly important factor and is known to greatly influence the physico-chemical conditions and availability of resources (e.g. substrates) in an ecosystem (Thompson and Townsend, 2000).

Biological assessment of rivers has been a valuable alternative to the physical and chemical methods, because it integrates effects of many abiotic or driver variables and better representation of the ecosystem (Hynes and Hynes, 1970; Rosenberg and Resh, 1993; Mattson and Angermeier, 2007), and is being used worldwide. Biological monitoring is especially advantageous in developing countries because it is relatively cheap and easy to perform (Thorne and Williams, 1997). Several biological assessment methods are of international standards and are incorporated into national, regional and local monitoring programs (Barbour *et al*., 1999; Dickens and Graham, 2002; Hering *et al*., 2003; De Pauw *et al*., 2006), serving as a fundamental tool for formulating policy decisions on surface water management (Davis and Simon, 1995).

Extensive research has been conducted on the development and application of biological assessment concepts in both the temperate (Marchant *et al*., 1997; Robinson *et al*., 2000; Lautenschläger and Kiel, 2005) and tropical regions (Capítulo *et al*., 2001; Dickens and Graham, 2002; Mustow, 2002, Moya *et al*., 2007). Although many developing countries still largely depend on physical and chemical methods in the assessment of stream and river water quality, a few, such as South Africa, have made use of the biological monitoring at a national and regional scale (Dickens and Graham, 2002; Fourie *et al*., 2014).

The two primary purposes of biological monitoring of macroinvertebrates are to estimate variables of interest at a site and to make comparisons among sites or time intervals. Many variables, such as the number of taxa, Average Score per Taxon (ASPT: the average score of the water quality sensitivity values of the macroinvertebrates) (Dickens and Graham, 2002) and Saprobic Index (Metcalfe, 1989) are the biological monitoring tools being used to calculate the ecological quality classes resulting from biological assessment systems. These metrics are

calculated from the macroinvertebrate community composition. Various methods have been developed for the collection and processing of macroinvertebrate samples from streams (Barbour and Gerritsen, 1996). The sampled area, mesh size of nets, habitats, taxonomic identification, the intensity of sorting and many other parameters may cause variation in sampling techniques, sample processing and data obtained (Lenat, 1993; Stark *et al*., 2001). Also, methodology influences the accuracy and reliability of bioassessment results, which are expressed as metric values and ecological quality classes (Barbour *et al*., 1996; Haase *et al*., 2004). The sampling method may also be selective for particular species or groups of species depending on their exposure and sensitivity to anthropogenic stress (Barton and Metcalfe-Smith, 1992).

Various methods and tools of biomonitoring have been designed to assess diversity, similarity and biotic indices, as well as multimetric and multivariate parameters (Clarke, 1993; Fenoglio *et* al., 2002; Bonada *et al*., 2006). Diversity indices integrate information on taxonomic richness, dominance and uniformity (Winterbourn, 1999) of the components that are used to illustrate the response of a community to the quality of its environment (Boothroyd and Stark, 2000). Similarity indices are used to compare two or more populations or communities, with the control and reference sites. Similarity indices are mostly used to assess the degree of change caused by a particular impact (Winterbourn, 1999).

1.6.1   Saprobic indices

Saprobes are organisms that live on dead decaying or decaying organic matter (Farlex Partner Medical Dictionary, 2012). Therefore, the saprobic approach to biomonitoring classifies each indicator organism into different "saprobes", according to their level of dependence on decomposing organic nutrients (Metcalfe, 1989). A numerical index, i.e., the saprobic index (SI), which ranges from 1 – 4 is allocated to classify the organisms into oligosaprobic, mesosaprobic and polysaprobic. In addition to this is a consideration for the relative abundance of species, because it is the factor for the derivation of the saprobic index.

The advantage of the saprobic index is that it encompasses a broad range of taxa and communities, making it suitable for all types of rivers. However, its limitations are that it is not consistent in proving particular organic pollution; it is not appropriate for large geographical areas,

and determination of abundance for index calculations are not easy (Bonada *et al*., 2006). Also, it is time-consuming as it involves a high level of identification to species level, hence becoming costly (Carter and Resh, 2001; Bonada *et al*., 2006).

1.6.2 Biotic indices

Implementation of the Clean Water Act in 1972 in the United States prompted the use of various bioassessment methods which were designed to evaluate conditions of aquatic resources (Davies and Jackson, 2006). Biotic indices are coded numerical expressions which are combined into single scores, with a foreknowledge of the tolerance scores of each taxon to pollution (Tolkamp, 1985). Use of biotic indices involves incorporating the desirable features of the saprobic and diversity indices with quantitative measures of species diversity and qualitative information on the ecological sensitivities of individual taxa into a single numerical expression (Hoang, 2009).

A descriptive model, the Biological Condition Gradient, was proposed to describe impacts of stressors on ecological features (Fourie *et al*., 2014). Examples of aquatic biomonitoring methods include the UK's Biological Monitoring Working Party (BMWP) Score System (Hawkes, 1998) and Australia's Stream Invertebrate Grade Number – Average Level (SIGNAL) (Chessman, 2001). South Africa's empirical biotic index, which was developed by Chutter (1972), was a summary of the expected variations in the assemblages of organisms found in organically polluted rivers (Fourie *et al*., 2014). The BMWP was later modified by Chutter (1998) to develop the South African Scoring System (SASS), which is easier to use and more affordable (Fourie *et al*., 2014). The latest version 5 (SASS5) was further modified and upgraded to international standards by Dickens and Graham (2002). The biotic approach is popularly used in Europe (De Pauw *et al*., 1992; Hering *et al*., 2003), North America (Lenat, 1993), Asia (De Zwart *et al*., 1995; Mustow, 2002), South Africa (Chutter, 1994; Thirion *et al*., 1995; Dickens and Graham, 2002) and many tropical countries (Jacobsen, 1998; Fenoglio *et al*., 2002; Astorga *et al.*, 2011).

The biotic approach is more convenient to use because it only requires a qualitative sampling without any need to count abundances per taxon and taxa can easily be identified to the family or genus level (De Pauw and Vanhooren, 1983). This advantage has made the biotic approach widely accepted in east Asia (Hoang, 2009), as well as in Africa where taxonomic

knowledge remains a significant constraint in applying bioassessments (Reynoldson and Metcalfe-Smith, 1992; Buss *et al*., 2015). However, another limitation is in determining reference sites or communities, which are used for comparing polluted sites (Economou, 2002; Bailey *et al*., 2004).

The SASS protocol requires an immediate taxa identification in the field after sampling and scored according to the pre-allocated quality values of each taxon, which is indicative of its sensitivity to pollution and disturbance. The ASPT (average score per taxon) is calculated by dividing the SASS score (the total of the sensitivity values) with the total of the number of taxa in the river samples (Dickens and Graham, 2002). SASS has since been reviewed to address the deficiencies identified by researchers and SASS practitioners. The current version 5 (SASS5), is being used as the backbone of the South African River Health Programme to monitor water quality and river health (Dickens and Graham, 2002; Fourie *et al*., 2014).

1.6.3   Diversity indices

Diversity indices for a river describe the response of a community to the quality of its environment in terms of species richness (number of species), abundance (total number of organisms) and evenness (distribution of individuals among the species), based on the hypothesis that impacted river systems or catchments have a lower diversity (Metcalfe, 1989). These diversity indices (species richness, total diversity and evenness) are often applied on an individual basis (Shannon and Weaver, 1963; Hill, 1973). They have previously been used in the United States to assess water quality and as a comparative ecological tool to evaluate tropical streams and rivers (Matagi, 1996; Ometo *et al*., 2000; Buss *et al*., 2002; Moyo and Phiri, 2002).

The advantages of diversity indices are the ease of use and calculation; suitability to all kinds of rivers, irrespective of geographical area. Diversity indices depend on statistical analysis because they are quantitative, which makes them best fit for comparative purposes (Cook, 1976). They are not dependent on the sample size (Pinder *et al*., 1987) and can be used to measure biomass (Mason *et al*., 1985). The limitation is that diversity index values are unable to indicate pollution tolerance or sensitivity of species (Hoang, 2009). Also, data quality may be affected by the sampling method and nature of the study site (Metcalfe, 1989).

1.6.4 Multimetric indices

The first multimetric approach was developed for fish assessment (Karr, 1981; Karr and Chu, 1998). Macroinvertebrate multimetric methods are adaptations of the U.S. Rapid Bioassessment Protocols, which were originally designed to assess the expected faunal community (Plafkin *et al*., 1989). The multimetric approach makes use of a variety of indices (metrics) simultaneously to evaluate site conditions. The multimetric assessment uses the summation of several metrics representing different characteristics of the macroinvertebrate community as one index value or score (Barbour *et al*.,, 2000). Multimetric assessment is based on the assumption that the various aspects of the ecosystem functions and ecological integrity are adequately represented.

Moreover, a combination of several metrics is assumed to be more reliable and robust (Gabriels *et al*., 2010). Classification of metrics is based on different principles of ecological quality assessment (De Pauw *et al*., 2006). The standard multimetric methods being used globally are combinations of species compositions (e.g. % Ephemeroptera, % Trichoptera, % Diptera), species richness (e.g. number of taxa), tolerance (e.g. SASS score and ASPT) and feeding habit (e.g. % predators, % filters). Usage of the multimetric approach is rapidly increasing because of its flexibility (Gabriels *et al*., 2006). However, the multimetric methods require a careful selection of reference sites, which is often overlooked (Winterbourn, 1999).

1.6.5 Multivariate predictive models

The use of multivariate predictive models is increasing for biomonitoring of streams and rivers (Bonada *et al*., 2006). Significant advances have been made in conceptual models and statistical techniques (Leathwick *et al*., 2005; Austin, 2007; Kennen *et al*., 2010; Clapcott *et al*., 2011; Cuffney *et al*., 2011). Multivariate predictive models help practitioners derive response models that better support the needs of bioassessment programmes. Models provide a useful framework for testing hypotheses, determining potential direct and indirect linkages, and directing where further research is required. The expansion and application of multivariate models in stream ecology are helping to address these issues and hopefully, will lead to a broader understanding of ecological and anthropogenic pathways and responses (Oberdorff *et al*., 2001; Cabecinhaa *et al*., 2007; Turak *et al*., 2011).

1.6.6   Ecological quality ratio/index

This assessment approach compares community composition to a stipulated target community, which serves as the reference. The reference community is chosen by one or a combination of the following: field samplings, expert knowledge, historical data and predictive models. This type of assessment is known as the Ecological Quality Ratio (EQR), as defined by the Water Framework Directive (EU, 2000; Wallin *et al*., 2003). The EQR is expressed as a numerical value between zero and one, where zero specifies a severe ecological state and one denotes a good ecological state.

EQR = Index value of observed community / Index value of reference community

Predictive models comprise a central part of the EQR approach. The UK's 'River Invertebrate Prediction and Classification System' (RIVPACS) is an example of EQR (Armitage *et al*., 1983; Wright *et al*., 1993; De Pauw, 2000; Wright *et al*., 2000). The RIVPACS is used to predict the macroinvertebrate taxa that should be present in a river, based on the prevailing physico-chemical conditions. Thus, the target reference community can then be compared with the observed communities. Different metrics or indices, such as the BMWP, ASPT or the number of taxa may be used to calculate the RIVPACS EQI (Sweeting *et al*., 1992). Other similar models have been adapted from the RIVPACS, these include AUSRIVAS (Australian River Assessment Scheme) in Australia (Schofield and Davies, 1996; Parsons and Norris, 1996; Smith *et al*., 1999; Davies *et al*, 2000; Coysh *et al*., 2000; Marchant and Hehir, 2002) and the Benthic Assessment of Sediment (BEAST) in Canada (Reynoldson *et al*., 1995). The application of the EQR approach is currently minimal in most regions because data on reference sites are rare or scarce, but they are commonly used in the temperate regions (Hoang, 2009).

1.7   Importance of river types and ecoregion classifications in biological assessment

Biodiversity is not evenly distributed within any given system or area but arranged into mosaics or patches determined by the climate (environmental conditions), geology (landforms) and evolutionary history (species assemblages and communities) of the area. These patterns are called "ecoregions". The World Wide Fund for Nature (WWF) defined an ecoregion (ecological region) as a "large unit of land or water containing a distinct species diversity, natural communities and

environmental conditions" (Abell *et al*., 2008). Ecoregions do not have fixed boundaries, but rather encompass an area with similar interactive ecological and evolutionary processes. Rivers are generally grouped into ecological units, having limited internal variations in biotic and abiotic components, which are explicitly distinct from those of the neighbouring entities (Hering *et al.,* 2003). River systems classification or typing has enabled the comparison of test sites to appropriate reference conditions (Reynoldson *et al*., 1997; Stoddard *et al*., 2006)). Moreover, river classes ease the planning and development of research, assessment, conservation and management of their ecosystems (Hawkins *et al*., 2000; Chaves *et al.*, 2005).

A suitable river classification is one that gives a reasonable number of practical types for assessment and monitoring programmes; and also offers biologically relevant stream types that incorporate natural biological variability. An example of river typing systems is the European Water Framework Directive which categorises rivers into two systems (Munné and Prat 2004). System A classifies rivers into ecoregions and uses fixed categories for mandatory factors (catchment area, distance from source and geology). In contrast, System B does not give fixed categories for the necessary descriptors and includes two additional obligatory variables namely, latitude and longitude; and a variety of optional physical factors (Munné and Prat 2004; Chaves *et al*., 2005).

In a biological assessment, developing a reference condition for measuring ecosystem changes and accounting for natural variability of the biotic assemblages can be challenging. The concept depicts the capability to differentiate between natural variability and anthropogenic effects. Partitioning a study area into relatively homogeneous ecological regions has been an approach for taking regional variability into account i.e. geographical differences (climatic, hydrological and biogeographic) (Economou, 2002). Partitioning of rivers based on both regional and local characteristics produces classification groups that incorporate natural variability in macroinvertebrate reference conditions.

1.8   Ecological risk assessment

1.8.1   What is an ecological risk?

An ecological risk is defined as the combination of the severity (nature and magnitude) and the probability of effects from a recommended action, though the severity may be dependent on the situation (e.g. the reduction in diversity and mortality rate) (Suter, 2007; Visschers *et al.*, 2009). Risk assessment began in the 17th century in England and the Netherlands with the need for the determination of insurance premiums on merchant ships (Bernstein, 1996; Melnikov, 2003; Hacking, 2006). The broad context of risk assessment includes the use of various techniques (statistical analysis of past events, trend analysis, modelling and professional knowledge) to evaluate how incidents and poorly defined recommendations could affect the future (Slovic *et al.*, 2005). An importance of risk management is the ability to integrate risks from different sources in such a way that minimises risks in the allocation of resources (Cox, 2008).

The capacity to deal with risk and the need for efficient utilisation of resources justify the applicability of risk assessment to environmental management (Ahrens and Rudolph, 2006; Renn, 2006). However, environmental risk assessment started in the 1970s from the United States as a result of the enactment of a series of environmental laws; which included The Clean Air Act of 1970, The Safe Drinking Water Act of 1974 and the Clean Water Act of 1977 (Suter, 2007). Risk assessment has since extended to various fields of study, such as engineering, wildfire management, medicine and environmental regulation (Mulholland and Christian, 1999; Weinstein, 2000; Kolar and Lodge, 2002; Suter, 2007).

1.8.2   Ecological risk assessment

Ecological risk assessment is the systematic evaluation and organization of data, information, assumptions, and uncertainties that help to understand and predict the relationship between stressors and ecological effects in a useful manner for environmental decision making (USEPA, 1998). In the 1990s ecological risk assessment was expanded to accurately reflect the reality of the structure, function and scale of ecological structures (Hunsaker *et al.*, 1990; O'Neill *et al.*, 1997). There have also been other attempts to perform risk assessments from the USEPA paradigm, but each attempt had been affected by the constraints of the risk assessment framework

which was originally designed for single chemicals and receptors (Cook *et al*., 1999; Cormier *et al*., 2000). The prime difficulty in the performance of ecological assessment is the incorporation of the spatial structure of the environment and the coexistence of multiple stressors (Hayes and Landis, 2004).

The traditional three-phase ecological risk assessment method involves problem formulation, risk analysis and risk characterization, while its improved and expanded version is known as the regional or relative ecological risk model (Landis, 2004) (Fig. 1.1). Hence, the relative ecological risk model is suitable for assessment at a larger scale, using the regional factors (e.g. sources of stressors, habitats of the receptors and impacts of the stressors) on the assessment endpoints. Therefore, Landis and Wiegers (1997) defined regional scale ecological risk assessment as the risk assessment at a spatial scale containing a variety of habitats with multiple sources of multiple stressors influencing the multiple endpoints and the risk estimate as a result of the characteristics of the landscape. Moreover, even if only one stressor is being evaluated, all other stressors influencing the assessment endpoints must be considered at the regional scale (Hayes and Landis, 2004).

It is important to have clearly defined endpoints that are socially and biologically relevant in ecological risk assessments (Suter, 1990; Graham et al., 1991). Such endpoints must be accessible to prediction and measurement, as well as being susceptible to the hazard being assessed (Suter, 1990; Gregory et al., 2006). Unfortunately, most ecological assessments do not have such clearly defined endpoints, partly because some of the endpoints of toxicity tests or other effects measurements are used as the assessment endpoints (Suter, 1990; Schlenk, 1999).

Traditional Risk Assessment Components

A

Regional Risk Assessment Components for Invasive Species

B
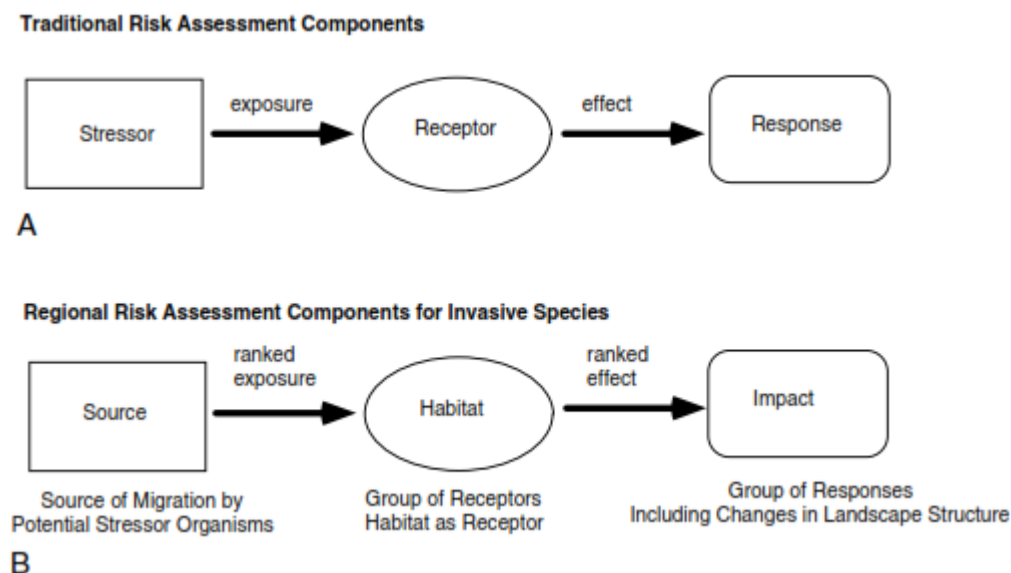
Figure 1.1: Differences between the traditional risk assessment and the regional risk assessment. (Source: Landis, 2004).

1.9 Motivation, aim and objectives

With increasing anthropogenic impacts on rivers in KwaZulu-Natal (KZN), the scarcity of data on the ecology of the aquatic macroinvertebrates of KZN rivers highlights the priority to collect and describe the aquatic macroinvertebrate communities of KZN, at least in the different ecoregions. This paucity of data also emphasises the need for an improvement in river conservation plans and management, through adequate monitoring. In the Western Cape Province for example, streams can be classified by their unique indicators of macroinvertebrate communities (Schael and King, 2005). Moreover, KZN is an important high water-yield area within South Africa; therefore, it is of critical importance to recognise the conservation value of these rivers, by prioritising the conservation of their biological diversity.

This study aimed to assess the ecological wellbeing of macroinvertebrates in KZN rivers, using different approaches and models. A total of 40 sites were selected for this study, but only the sites that are most appropriate for the objectives of each chapter were used accordingly. Details of the study sites are in Appendix 1A. The sites were strategically located within a range of different anthropogenic land uses, from pristine headwater stream conditions to severely impacted systems.

Different macroinvertebrate ecological indices were tested according to their relevance in the assessment of the environmental wellbeing of the rivers. Relationships between macroinvertebrate taxa/indices and river characteristics were analysed using different multivariate statistical and numerical methods.

To achieve the aim, the following objectives were established:

1. Assess the applicability of multivariate statistics in the *a priori* selection of reference conditions for the assessment of river wellbeing in KZN.
2. Compare the performance of the established biological (diversity, similarity and biotic) indices in assessing the wellbeing of macroinvertebrate community structures in the rivers of KZN.
3. Assess the water quality of KZN rivers using macroinvertebrate community composition metrics and their responses to drivers of ecological changes.
4. Conduct an ecological risk assessment of water resource use to the wellbeing and responses of macroinvertebrates and their ecosystems in the rivers of KZN.

1.10   Study outline

The thesis has the following chapters:

Chapter 1: Introduction: describes the river health/wellbeing concept and reviews the development, application and methods of macroinvertebrate based biological assessment of rivers.

Chapter 2: A multivariate approach to the selection and validation of reference sites in Kwazulu-Natal Rivers, South Africa.

 Chapter 3: Diversity, similarity and biotic indices: ecological applications of macroinvertebrate community structures in the rivers of KwaZulu-Natal, South Africa.

Chapter 4: Macroinvertebrates as indicators of ecological conditions in the rivers of KwaZulu-Natal, South Africa.

Chapter 5: Ecological risk of water resource use to the wellbeing of macroinvertebrate communities in the rivers of KwaZulu-Natal, South Africa

Chapter 6: Conclusion: summary of all chapters and management recommendations.

The data chapters were prepared as manuscripts for submission to international peer review journals and some repetition was unavoidable. The particular predictions are presented in each.

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Appendix 1A: Rivers of KwaZulu-Natal used for the study from March 2015 to April 2016.

CODE	RIVER	TRIBUTARY	CATCHMENT	LATITUDE	LONGITUDE	ALTITUDE (m)
AMAT1	Matikulu	Matikulu	Matikulu	-29.072872	31.557742	19
BIVA1	Bivane	Phongolo	Phongolo	-27.529370	30.861440	981
BLAC1	Black Mfolozi	Mfolozi	Mfolozi	-28.191223	31.737514	143
BUFF1	Buffalo	Thukela	Thukela	-27.715620	30.118630	1166
BUSH1	Bushmans	Thukela	Thukela	-29.083510	29.828000	1199
HLUH1	Hluhluwe	Mfolozi	Mfolozi	-28.138560	32.019950	129
IMFO1	Mfolozi	Mfolozi	Mfolozi	-28.359600	31.994340	50
LOVU1	Lovu	Lovu	Lovu	-29.861446	30.261955	868.64
LOVU2	Lovu	Lovu	Lovu	-30.096890	30.822200	8.64
MDLO1	Mdloti	Mdloti	Mdloti	-29.602083	31.009018	105.84
MFUL1	Mfule	Mhlathuze	Mhlathuze	-28.515890	31.436140	710
MHLA1	Mhlathuze	Mhlathuze	Mhlathuze	-28.746950	31.747450	49
MKHO1	Mkhomazana	Mkomazi	Mkomazi	-29.645765	29.431339	2098.68
MKUZ1	Mkuze	Mkuze	Mkuze	-27.692560	31.211290	909
MKUZ2	Mkuze	Mkuze	Mkuze	-27.592270	32.217950	59
MOOI1	Mooi/Mfulankomo	Thukela	Thukela	-29.384345	29.652979	1708
MTAM1	Mtamvuna	Mtamvuna	Mtamvuna	-30.782342	29.950980	285
MVOT1	Umvoti	uMvoti	uMvoti	-29.159860	30.628690	969.03
MVOT2	Umvoti	uMvoti	uMvoti	-29.370004	31.304341	9
MVUN1	Mvunyana	Mfolozi	Mfolozi	-28.118986	30.866828	979
MZIM1	Mzimkhulu	Mzimkhulu	Mzimkhulu	-30.653637	30.220839	156
NCAN1	Ncandu	Thukela	Thukela	-27.851440	29.756630	1450
NGWA1	Ngwavuma	Phongolo	Phongolo	-27.097892	32.068882	120
NWAK1	Nwaku	Matikulu	Matikulu	-28.941420	31.394160	262
PHON1	Pongolo	Phongolo	Phongolo	-27.397500	31.851410	147
PHON2	Pongolo	Phongolo	Phongolo	-26.881417	32.312119	32
SAND1	Mzinyashana	Thukela	Thukela	-28.098820	30.318530	1174
SIKW1	Sikwebezi	Mfolozi	Mfolozi	-27.900330	31.365220	563
SLAN1	Slang	Thukela	Thukela	-27.420670	30.296810	1797
THUK1	Thukela	Thukela	Thukela	-28.743080	30.139480	646
THUK2	Thukela	Thukela	Thukela	-29.172622	31.391921	22
TONG1	Tongati	Tongati	Tongati	-29.559913	31.174085	9.42
UMLA1	uMlazi	uMlazi	uMlazi	-29.756000	30.289000	895
UMLA2	uMlazi	uMlazi	uMlazi	-29.809722	30.500000	635.86
UMNG1	Mgeni	uMngeni	uMngeni	-29.479822	29.969800	1321.22
UMNG2	Mgeni	uMngeni	uMngeni	-29.488134	30.156002	1061.22
UMNG3	Mgeni	uMngeni	uMngeni	-29.464580	30.461970	621
UMNG4	Mgeni	uMngeni	uMngeni	-29.714520	30.868058	281.22
VUTH1	Vutha/Matikulu	Matikulu	Matikulu	-29.067450	31.485960	34
WHIT1	White Mfolozi	Mfolozi	Mfolozi	-28.393483	31.683031	167

Appendix 1B: Map of the study area showing the sites within their respective ecoregions.
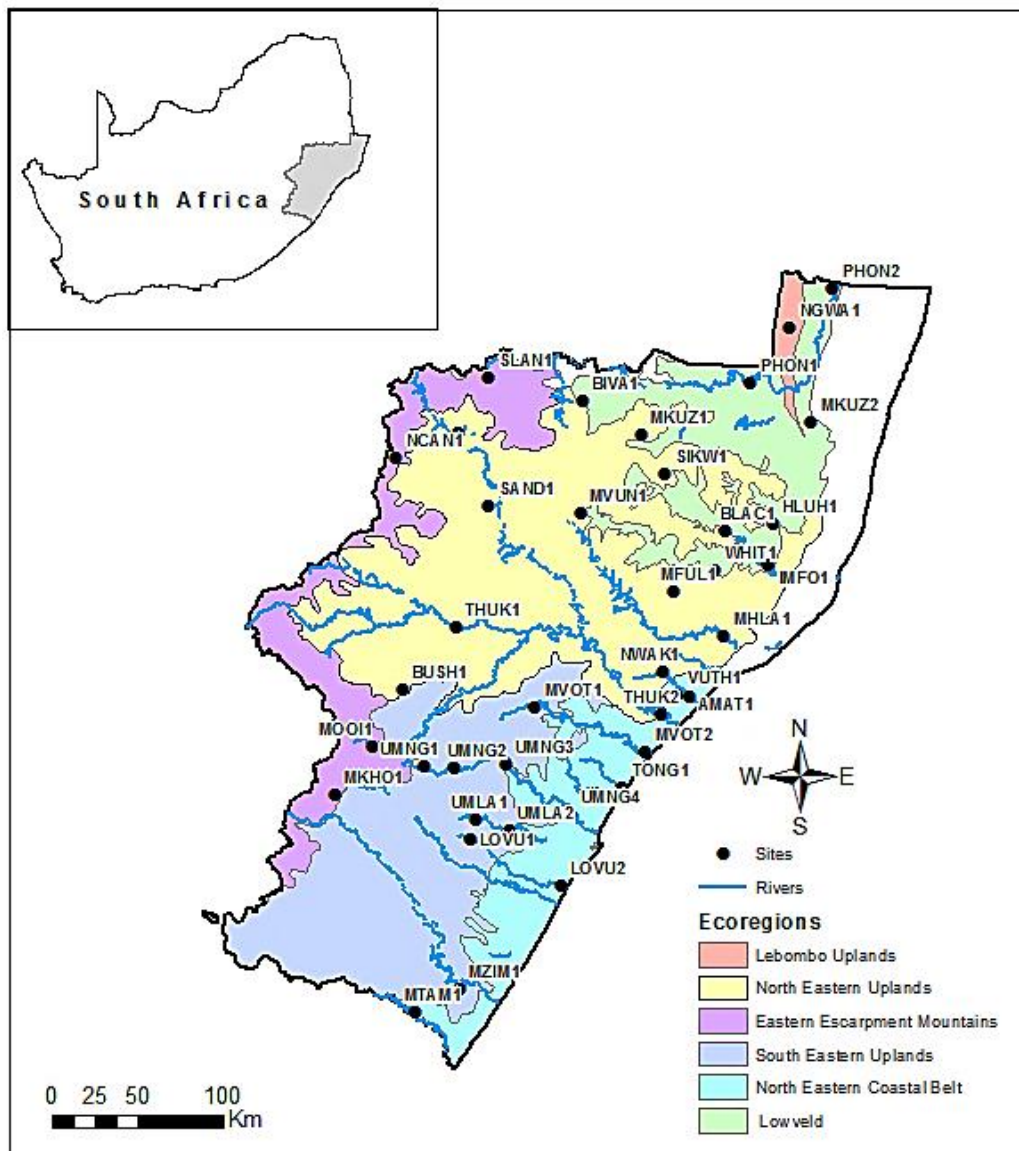
CHAPTER 2

A multivariate approach to the selection and validation of reference conditions in KwaZulu-Natal Rivers, South Africa.

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Summary

1. The use of reference conditions is essential to the monitoring and management of aquatic ecosystems. We examined existing and potential reference sites through historical data, maps and field data collected from river sites in KwaZulu-Natal (KZN), South Africa.

2. In our study, we applied nine criteria that best reflect the characteristics of South African rivers on 24 *a priori* selected reference sites. These nine criteria comprised of catchment conditions (flow modification and natural landscape) and site specific attributes (water quality, human disturbance, river channel, water abstraction, riparian vegetation, riparian zone modification and instream habitat quality).

3. The *a priori* selected reference sites were subjected to validation using multivariate methods such as analysis of similarities (ANOSIM), similarity percentages (SIMPER) and non-parametric multidimensional scaling (MDS) based on the macroinvertebrate fauna by applying a SASS5 threshold considered to be an indicator of undisturbed sites in South African rivers.

4. We identified differences in the macroinvertebrate assemblages of the reference conditions for each river group based on their ecoregions, geomorphology and seasonal variations. Ecoregions and river geomorphology proved more adequate in the grouping of sites with similar reference conditions than the seasons.

5. Our findings indicated that all of the selected sites selection could be considered as valid reference sites; however, caution should be taken in applying this method to the lowland rivers due to their noticeable seasonal variability and habitat instability which tend to alter their reference states. We, therefore, recommend that a type-specific reference condition be developed for the lowland rivers of KZN. Also, statistical validation of reference conditions should be a continuous process.

Keywords: reference conditions, macroinvertebrate, multivariate analysis, geomorphology

Introduction

Using biological methods for the assessment of river water quality and wellbeing is prevalent in most countries, and several of these methods have been standardised. These methods have also been included in national and regional monitoring programs, serving as a basis for policy decisions concerning water quality management (Hering *et al*., 2003; De Pauw *et al*., 2006). Examples of such national and regional biological assessment methods include index of biotic integrity (IBI) (Karr, 1981), riparian, channel environment inventory (RCE) (Petersen, 1992), index of stream condition (ISC) (Ladson *et al*., 1999), river health program (RHP) (Roux, 2001; DWAF, 2008). Recently, the river ecostatus monitoring program (REMP) replaced the earlier RHP of South Africa (DWS, 2016). Ecological reference conditions (RCs) or criteria are the conditions selected through physical, chemical and biological characteristics that are representative of a group of near pristine or "least impacted" sites (Schlacher *et al*., 2014; Bouleau and Pont, 2015). Thus, RCs serve as the foundation for developing biological criteria and enable the determination of the degree of deviation from natural conditions for protecting aquatic ecosystems (Muxika *et al*., 2007; Yurtseven *et al*., 2016).

The first step in the Ecological Classification process is the determination of RCs for each of the biotic components (diatoms, riparian vegetation, invertebrates and fish fauna) of the river ecosystem being surveyed (Kleynhans and Louw, 2007). The RCs provide the fundamentals of measuring anthropogenic impacts, evaluate biological community potential; and spatial and temporal natural fauna distribution (Reynoldson *et al*., 1997; Economou, 2002; Wallin *et al*., 2003; Bailey *et al*., 2004). The RCs do not necessarily represent entirely undisturbed or pristine conditions, they often include minor disturbances (Chaves *et al*., 2006). Low human pressure effects may be allowed in a RC, but a high ecological status must always be achieved (Economou, 2002; Wallin *et al*., 2003; Bailey *et al*., 2004). A RC represents information from numerous similar sites (Reynoldson *et al*., 1997; Wallin *et al*., 2003; Bailey *et al*., 2004). Establishing a RC and specifying ecological class boundaries allows accurate ecological evaluations of each site by comparing data from similar sites with little or no anthropogenic disturbances (Wallin *et al*., 2003; Bailey *et al*., 2004; Chaves *et al*., 2006). Site hydromorphological and physico-chemical attributes

of a RC should meet the criteria of minimal disturbance for reference biological communities to be obtained (Reynoldson *et al*., 1997; European Commission, 2000).

Five different approaches or combinations of the approaches are currently being used in creating RCs for biological indices (Barbour *et al*., 1996; European Commission, 2000; Economou, 2002; Wallin *et al*., 2003). These are: (1) expert judgment, (2) predictive modelling, (3) historical data, (4) extensive spatial surveys, and (5) paleo-reconstruction. An RC established from extensive studies should be a site with minimal exposure to a stressor(s) and must be representative of the river type (Chaves *et al*., 2006). Obtaining survey data is a reliable method for establishing a RC, especially in relatively undisturbed or minimally disturbed sites (Barbour *et al*., 1996; Wallin *et al*., 2003; Bailey *et al*., 2004; Nijboer *et al*., 2004).

Although several studies have assessed the ability of regional classification systems to partition spatial variability, there are differing opinions on the ecological validity of geographic delineators (Dallas, 2002; Dallas, 2004a). For example, water chemistry has been shown to be useful predictors of ecoregions (Ravichandrana *et al*., 1996), while some other studies have shown that ecoregions cannot effectively explain water chemistry patterns (Harding *et al*., 1997). Also, some researchers showed that macroinvertebrate community structures could be used to classify ecoregions (Harding *et al*., 1997; Gerritsen *et al*., 2000), while others have contrasting opinions on the correlation between ecoregions and water chemistry (Hawkins and Vinson, 2000), macroinvertebrate community structures (Marchant *et al*., 2000) and vegetation (Wright *et al*., 1998).

Legislative amendments of the Republic of South Africa have over time modified the functions of the Department of Water and Sanitation (DWS) from merely managing the quality and quantity of water resources to an integrated management of the resources to ensure that the integrity of the ecosystems is not compromised (Thirion, 2016). The REMP involves a significant change in the environmental assessment criteria used for the evaluation of the ecological status of rivers using four dominant biological indicator groups for river research: diatoms, riparian vegetation, invertebrates and fish faunas (Taylor *et al*., 2007; Thirion, 2007; Kleynhans, 2007; Kleynhans *et al*., 2008). Also, REMP requires ecological classification to be based on deviation

from the expected natural condition, which necessitated the characterization of the original status of each water body type, usually designated as the RC.

The widespread human modification of river systems often poses a difficulty in identifying potential reference sites (Chessman and Royal, 2004; Chessman, 2006; Chessman *et al.*, 2008; Dallas, 2013). In South Africa, most possibly minimally impacted sites are those located in the upper reaches of rivers, which may not be useful reference sites for downstream river reaches (Thirion, 2016). Although historical data are often used as supplementary sources of information to characterize reference communities (Ehlert et al., 2002; Nijboer et al., 2004), it is impractical to rely on the historical data for determining RCs for South African rivers, because this information is scanty (Thirion, 2016). We examined the success of the multivariate approach in the selection and validation of reference sites based on macroinvertebrate assemblages in KwaZulu-Natal (KZN) Province, South Africa. We expected that sites within the same classification category (e.g. ecoregions) would have similar RCs in terms of macroinvertebrate assemblages.

Methods

Study area

This study was conducted in the major rivers of KZN. The study sites were spread across KZN covering 17 rivers and five ecoregions (Kleynhans *et al.*, 2005) rivers (Fig. 2.1). The altitudes ranged from 19 to 2098 m a.s.l within a variety of geomorphological zones (Rowntree *et al.*, 2000; Moolman, 2008), ranging from headwater to lowland rivers. Some of the major rivers in this study included the Thukela, uMvoti, uMgeni, Phongolo, uMfolozi, Mooi, Mtamvuna and Buffalo Rivers. The Thukela River is the longest river in the province, while the uMgeni River has five large dams along its course.
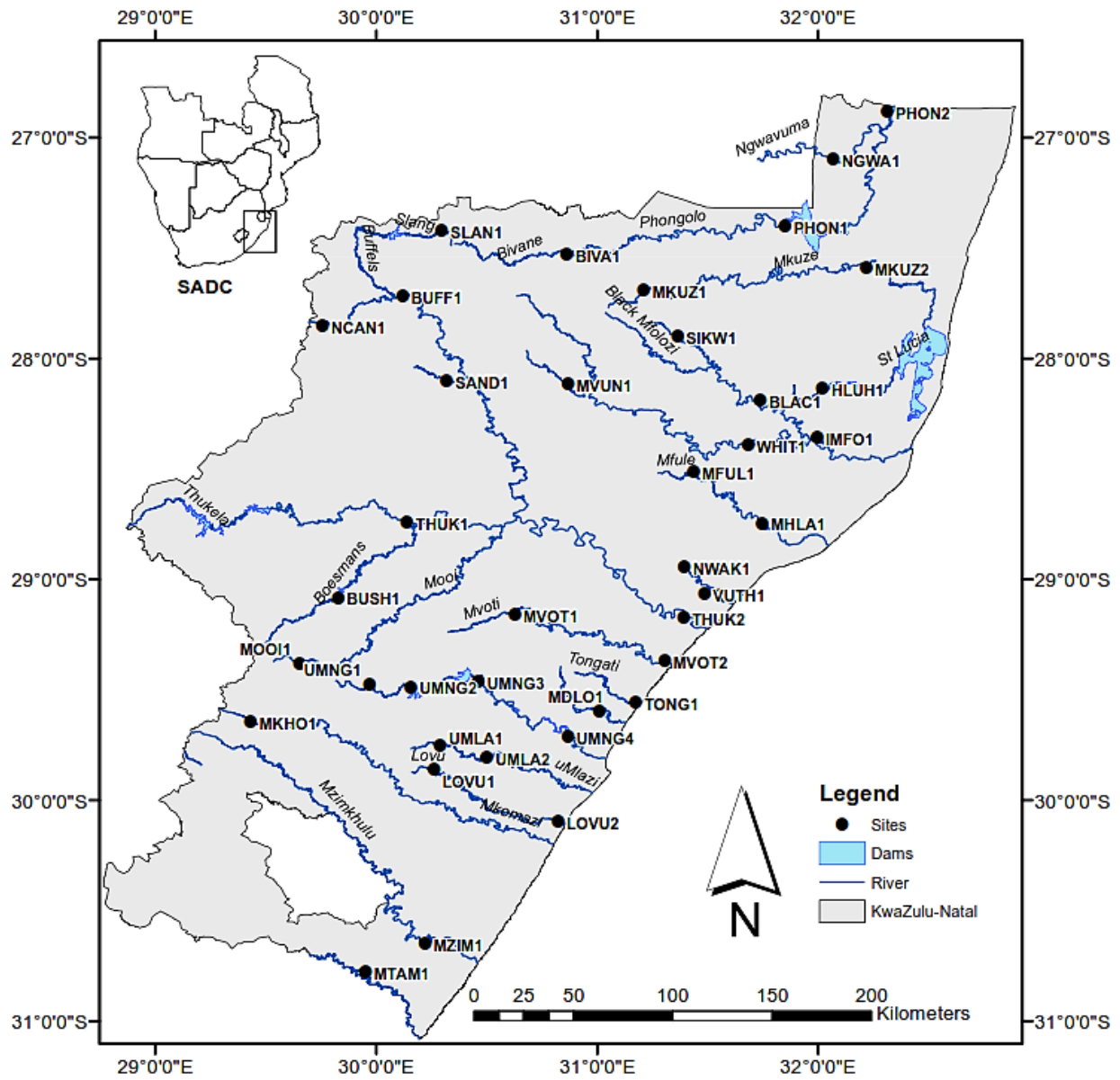
Figure 2.1: River study sites in KwaZulu-Natal, South Africa for the study from March 2015 to March 2016.

Site selection and validation

We selected a total of 24 river sites (16 upland rivers and eight lowland) situated above major anthropogenic disturbances for this study, using nine pre-defined criteria, comprising of catchment conditions (flow modification and natural landscape) and site specific attributes (water quality, human disturbance, river channel, water abstraction, riparian vegetation, riparian zone modification and instream habitat quality). The identification and selection of undisturbed or minimally disturbed lowland rivers was difficult, and the few included in this study had the best applicable conditions. Sites with incomplete data sets and unstable habitat conditions were excluded from further analysis. Site validation is essential in the determination of RCs, because it provides the quantitative measurements of both biotic and abiotic variables that characterise a river system and helps to confirm or refine the pre-selection criteria (Barbour *et al*., 1996, Chaves *et al*., 2006). Thus, we adapted the method of Chaves *et al.* (2006) for site validation, where the biological indices used for validation were the riparian vegetation and macroinvertebrate compositions (Table 2.1).

In our study, the Vegetation Response Assessment Index (VEGRAI) level 3 was used to assess the riparian vegetation (Kleynhans *et al*., 2007). The VEGRAI is a semi-quantitative technique that utilises several metrics to describe and rate the ecological status of riparian vegetation. Level 3 VEGRAI requires that the riparian habitat be divided into two defined zones: a) marginal and b) non-marginal zone. Each zone was assessed in terms of the intensity and extent of vegetation modification, invasive alien plant (IAP) infestation or other exotic species, including agricultural species; and changes in the vegetation functional groups and distribution through impacts from water quantity and quality. The VEGRAI index scores range from 0 (critically modified) to 100 (natural indigenous).

The South African Scoring System 5 (SASS5) (Dickens and Graham, 2002) was used to assess the macroinvertebrates wellbeing. The validation process involved the qualitative investigation of macroinvertebrates, habitat quality and water quality. The minimum *a priori* validation criteria for SASS5 and VEGRAI were values >100 and >70 respectively. Water quality variables (temperature, pH, conductivity and other related variables) were not considered in the

validation process because natural or seasonal hydrogeological differences may cause variations or fluctuations in their measurement (Chaves *et al*., 2005; 2006; Meinson *et al*., 2015).

Table 2.1: Selection criteria for minimally disturbed KZN river sites (Adapted from Chaves *et al*., 2006).

Criteria	Spatial Scale	Description	Bibliography
1. Water Quality	Site	Visual inspection of the water quality based on colour, clarity, odour and oil film	Hughes, 1995; Barbour *et al*., 1996
2. Human disturbance	Site	Assessment of the presence of garbage, sewage pipes, industrial effluents pipes and livestock grazing	Hughes, 1995; Barbour *et al*., 1996; Hering *et al*., 2003; Nijboer *et al*., 2004; Sanchez-Montoya *et al*., 2005
3. Flow modification	Catchment	Presence of dams higher than 20 m was considered to disturb the natural flows of the sites irrespective of the distance to the sampling site	Hughes, 1995; Barbour *et al*., 1996; Muhar *et al*., 2000, Ehlert *et al*., 2002; Hering *et al*., 2003; Nijboer *et al.,* 2004; Sanchez-Montoya *et al*., 2005
4. Natural Landscape	Catchment	The level of natural use of the site's drainage area; the degree of usage should be as low as possible for the reference site: <10% of urban and industrial use and <30% of agricultural use	Barbour *et al*., 1996; Hering *et al*., 2003; Sanchez-Montoya *et al*., 2005
5. Natural channel	Site	Presence of bank and bed fixation, artificial channels and small transversal ditches	Hughes, 1995; Barbour *et al*., 1996; Ehlert *et al*., 2002; Hering *et al*., 2003; Nijboer *et al*., 2004; Sanchez-Montoya *et al*., 2005
6. Water Abstraction	Site	Presence of hydropeaking, irrigation canals and water withdrawal for reservoirs, domestic water supply, etc.	Hughes, 1995; Muhar *et al*., 2000; Hering *et al*., 2003; Nijboer *et al*., 2004; Sanchez-Montoya *et al*., 2005
7. Riparian Vegetation	Site	Riparian vegetation cover; ideally should be in near-natural condition, most river types should have total cover and presence of trees in the pristine situation; however temporary or very high-altitude streams can have different cover levels.	Ehlert *et al*., 2002; Sanchez-Montoya *et al*., 2005
8. Riparian zone modification	Site	Presence of recreational facilities, industries or other buildings such as warehouses, croplands and tarred roads (spatial disturbances); it should be covered with natural unmanaged vegetation	Hughes, 1995; Muhar *et al*., 2000; Hering *et al*., 2003; Nijboer *et al*., 2004; Sanchez-Montoya *et al*., 2005
9. Instream Habitat Quality	Site	Presence of snags, roots, wood logs and dead overhanging vegetation; substrates: boulders and stones in upper reaches, cobble and pebbles in middle stretches and sand, clay and lime in lower regions; also assess the sediment retention level	Hughes, 1995; Barbour *et al*., 1996; Ehlert *et al*., 2002; Hering *et al*., 2003, Nijboer *et al*., 2004, Sanchez-Montoya *et al*., 2005

Macroinvertebrates sampling

Field data sampling was conducted on four occasions between March 2015 and March 2016 (March 2015, May 2015, November 2015 and March 2016). Basic *in situ* water quality parameters (temperature, dissolved oxygen, pH and electrical conductivity) were measured at each site on every sampling occasion using the YSI model 556 MPS handheld multi-probe water quality meter. Macroinvertebrate sampling was conducted using a kick net according to the SASS5 protocol (Dickens and Graham, 2002). At each sampling event, macroinvertebrates were sampled from three distinct biotopes: stones (stones-in and stones-out of current), vegetation (marginal and aquatic), and sediment (GSM - gravel, sand and mud). The stones-in-current (SIC) are pebbles and cobbles (2 - 25 cm), and boulders (25 cm). Stone-out-of-current (SOOC) included pebbles and cobbles, and boulders in pools. Marginal vegetation includes vegetation growing on fringes and edges of the rivers, while aquatic vegetation was that mostly growing (may or may not be submerged) inside river channel. Gravel was small stones usually less than 2 cm in diameter, while sand and mud were smaller than 2 mm and 0.06 mm respectively. Unless otherwise stated, the described biotopes are herein referred to as stone, vegetation and GSM.

The SASS5 sampling protocol requires collecting only one sample per biotope group, but care was taken to ensure that all the available biotopes were qualitatively sampled. We sampled each biotope was sampled separately, scored in the field according to the SASS5 protocol, and subsequently preserved these in 80% ethanol for better taxonomic resolution and taxa abundance counts in the laboratory. Three samples were collected from each site during every sampling event or season (i.e. one sample per biotope). Three samples (i.e. one sample from each of the three biotopes) were collected per site at every sampling event or season. Field identification of macroinvertebrates was done to family level, using the identification guides produced by the Department of Water and Sanitation (Gerber and Gabriel, 2002). The estimated abundances of the identified macroinvertebrate families were recorded on the SASS5 sheets. The SASS5 data interpretation is based on the calculation of the SASS score (the sum of the sensitivity weightings for taxa present at a site) and average score per taxon (ASPT). The ASPT is the ratio of the SASS score and the number of taxa (Dickens and Graham, 2002; Dallas, 2004b).

Data analyses

All data analyses were based on the macroinvertebrate data collected from SASS5. Similarities between sites were examined using Analysis of Similarities (ANOSIM), cluster analysis and non-metric multidimensional scaling (MDS) based on macroinvertebrate assemblage composition (Clarke, 1993; Clarke and Warwick, 1994; Clarke and Warwick, 2001). Site classification analysis based on more than two seasons is often recommended because it allows for robustness, hence reducing the temporal variation which could be evident in a one-season site classification (Turak *et al*., 1999; Bailey *et al*., 2004; Dallas, 2004a; Chaves *et al*., 2005). The macroinvertebrate data were transformed to their square roots prior to data analysis using PRIMER multivariate statistical software version 6 (Clarke and Gorley, 2006).

We used the Bray-Curtis resemblance matrix to determine the abundance contribution of each taxon to each of the sites. We also used the similarity percentage (SIMPER) to determine the distinguishing taxa that were responsible for the similarity within groups of sites and the dissimilarity between groups of sites (Clarke and Gorley, 2006). The classification groups were ecoregions (eastern escarpment mountain (EEM), north eastern upland (NEU), south eastern uplands (SEU), north eastern coastal belt (NECB), lebombo uplands (LU) and lowveld (LOWV)), river morphology (lowland and upland) and seasons (summer 2015, autumn 2015, spring 2015 and summer 2016). None of the sites in this study was within the LU ecoregion. Differences in macroinvertebrate compositions among the various classifications were tested by One-way Analysis of Similarities (ANOSIM) using Primer v6.

Results

Macroinvertebrate taxa composition and SASS5

The combined results of the four sampling seasons showed that the macroinvertebrate communities clustered primarily by the river type or geomorphology, with upland streams being approximately 75.5% dissimilar from the lowland rivers of KZN while within-group similarity of the upland sites was 27.1% and the similarity within the lowland sites was 24.1% (Table 2.2). The SIMPER analysis showed that Baetidae had the highest similarity percentage contributions for both upland and lowland groupings at approximately 22.2% and 14.2% respectively, while

Tipulidae contributed the lowest similarity percentage (1.1%) in the upland sites and Notonectidae contributed the lowest similarity percentage (1.2%) in the lowland sites. Atyidae contributed the highest dissimilarity percentage (7.1%) between the upland and lowland sites, while the Athericidae and Tipulidae both contributed the lowest dissimilarity percentage (1.0%) (Table 2.2). For the ecoregions, within group smiliarities were 13.8% (LOWV), 27.9% (NEU), 28.7% (SEU), 29% (EEM) and 31.3% (NECB) (Table 2.3). The cut off for low contributing taxa was 90% as calculated from the Bray-Curtis resemblance matrix, which means that taxa with less than 10% contributions were excluded from the SIMPER analysis. Taxa that contributed to within-group similarity were relatively constant for both river types; the upland group had 24 taxa, while the lowland group had 23 taxa (Table 2.4). The SASS indices clearly distinguished between sites, with the upland sites clearly different from the lowland sites (Fig. 2.2). The ecoregions also clearly separated from each other. Although there were clear separations between sites and between ecoregions, there were some similarities in taxa composition. The similarities in taxa composition between the upland and lowland sites could have been the reason for their mixed clusters at 40% similarity (Fig. 2.2).

Table 2.2: Dissimilarities in macroinvertebrate taxa between upland and lowland rivers of KwaZulu-Natal, South Africa from 2015 to 2016. Mean dissimilarity = 75.50%). Diss = dissimilarity; SD = standard deviation.

Species	Upland Group Mean Abundance	Lowland Group Mean Abundance	Mean Diss	Diss/SD	% Contribution
Athericidae	0.50	0.42	0.76	0.57	1.00
Tipulidae	0.57	0.37	0.75	0.68	1.00
Hirudinea	0.61	0.15	0.76	0.43	1.01
Hydrophilidae	0.58	0.17	0.77	0.46	1.03
Tabanidae	0.47	0.63	0.88	0.74	1.16
Ancylidae	0.49	0.57	0.92	0.57	1.21
Belostomatidae	0.61	0.62	0.96	0.81	1.27
Dytiscidae	0.60	0.47	0.98	0.65	1.30
Aeshnidae	0.80	0.44	1.00	0.70	1.32
Physidae	0.56	0.54	1.07	0.45	1.42
Leptoceridae	0.74	0.84	1.15	0.87	1.53
Veliidae	0.89	0.51	1.15	0.75	1.53
Naucoridae	0.86	0.58	1.18	0.78	1.56
Psephenidae	0.58	1.01	1.18	0.77	1.57
Notonectidae	0.78	0.65	1.19	0.67	1.58
Libellulidae	0.82	0.77	1.3	0.73	1.72
Gyrinidae	0.93	0.66	1.36	0.71	1.80
Philopotamidae	0.51	1.19	1.37	0.60	1.81
Corbiculidae	0.97	0.98	1.45	0.69	1.92
Corixidae	1.27	0.30	1.54	0.45	2.04
Planorbidae	0.82	1.18	1.72	0.54	2.28
Potamonautidae	1.42	1.12	1.76	0.62	2.33
Chironomidae	1.59	1.08	1.79	0.92	2.37
Perlidae	0.93	1.48	1.83	0.43	2.42
Gomphidae	1.01	1.77	1.98	0.90	2.62
Heptagenidae	1.31	1.73	2.07	0.96	2.74
Caenidae	1.86	1.30	2.15	0.99	2.85
Coenagrionidae	1.53	1.88	2.21	0.93	2.92
Elmidae	1.49	1.91	2.20	0.93	2.92
Tricorythidae	2.00	1.07	2.37	0.73	3.14
Simuliidae	2.03	1.23	2.47	0.81	3.27
Oligochaeta	2.11	1.37	2.49	0.71	3.30
Leptophlebiidae	2.27	2.37	2.98	1.00	3.94
Hydropsychidae	2.89	2.12	3.27	1.02	4.33
Thiaridae	0.94	4.10	4.96	0.56	6.57
Baetidae	5.36	4.11	5.15	0.91	6.83
Atyidae	3.09	4.60	5.37	0.93	7.12

Table 2.3: Macroinvertebrate taxa contributing within-group similarities of different river ecoregions of KwaZulu-Natal, South Africa between 2015 and 2016. South eastern uplands (SEU), north eastern coastal belt (NECB), eastern escarpment mountain (EEM), north eastern uplands (NEU) and lowveld (LOWV), x = taxa occurence

Ecoregion	SEU	NECB	EEM	NEU	LOWV
Within Group Similarity (%)	28.69	31.28	28.96	27.85	13.76
Number of Distinguishing Taxa	22	21	22	20	13
Aeshnidae			x		
Ancylidae	x				
Athericidae			x		
Atyidae	x	x	x	x	x
Baetidae	x	x	x	x	x
Belostomatidae	x				x
Caenidae	x	x	x	x	
Chironomidae	x	x	x	x	
Coenagrionidae	x	x	x	x	x
Corbiculidae		x			x
Corixidae			x		
Dytiscidae			x		
Elmidae	x	x	x	x	x
Gomphidae	x	x	x	x	
Gyrinidae	x		x		
Heptageniidae	x	x	x	x	x
Hydropsychidae	x	x	x	x	x
Leptoceridae	x	x			
Leptophlebiidae	x	x	x	x	
Libellulidae		x		x	x
Naucoridae	x		x	x	
Notonectidae		x	x	x	x
Oligochaeta	x	x	x	x	x
Perlidae	x	x		x	
Physidae					x
Planorbidae	x				
Potamonatidae	x	x	x	x	
Psephenidae	x	x			
Simuliidae	x	x	x	x	
Tabanidae		x			
Thiarida		x		x	
Tipulidae			x		
Tricorythidae			x	x	
Veliidae	x			x	x

Table 2.4: Macroinvertebrate taxa contributing to within-group similarity in the upland (27.13%) and lowland (24.05%) rivers of KwaZulu-Natal, South Africa between 2015 and 2016. x = taxa occurrence.

Species	Upland	Lowland
Aeshnidae	x	
Atyidae	x	x
Baetidae	x	x
Belostomatidae		x
Caenidae	x	x
Chironomidae	x	x
Coenagrionidae	x	x
Corixidae	x	
Elmidae	x	x
Gomphidae	x	x
Heptagenidae	x	x
Hydropsychidae	x	x
Leptoceridae	x	x
Leptophlebiidae	x	x
Libellulidae	x	x
Naucoridae	x	
Notonectidae	x	x
Oligochaeta	x	x
Perlidae	x	x
Philopotamidae		x
Planorbidae		x
Potamonautidae	x	x
Psephenidae		x
Simuliidae	x	
Thiaridae		x
Tipulidae	x	
Tricorythidae	x	x
Veliidae	x	
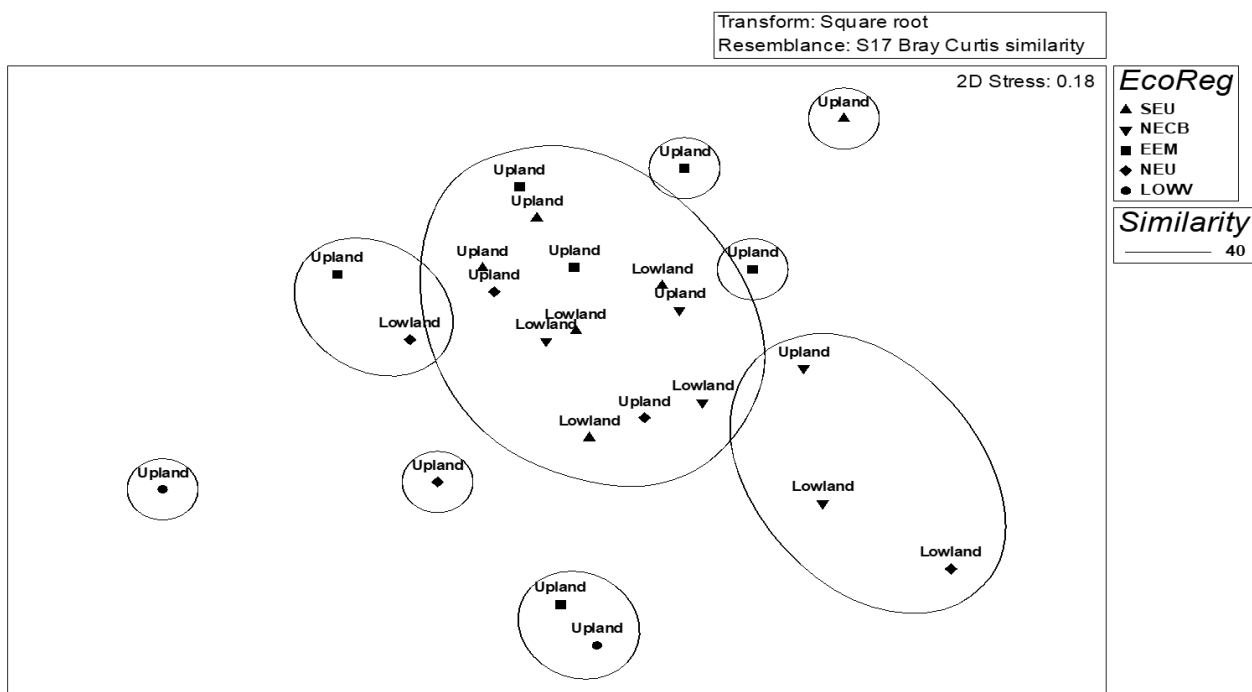
Figure 2.2: Non-metric multidimensional scaling (MDS) ordination showing the classification of sites based on macroinvertebrate taxa collected in summer 2016 in rivers of KwaZulu-Natal, South Africa. Sites were coded by group geomorphology (upland and lowland) and the shapes represent the ecoregions. SEU = south eastern uplands, NECB = north eastern coastal belt, EEM = eastern escarpment mountains, NEU = north eastern uplands, LOWV = lowveld.

Longitudinal gradients

Longitudinal gradients influenced the macroinvertebrate taxa clusters, although in a mixed selection of both upland and lowland KZN river groups (Fig. 2.2, MDS: 2D-stress = 0.18). At 40% similarity, five distinct clusters were formed (Fig. 2.2), MDS 2-D Stress = 0.18). Upland and lowland rivers were 75.5% dissimilar, with several taxa differentiating the groups (Table 2.3). Several sensitive taxa that are typical of headwater streams (e.g. Baetidae, Perlidae, Heptageniidae, Psephenidae and Athericidae) were among the distinguishing taxa. The best predictor variables were SASS score and longitude according to the results of the MDS and distance based redundancy analysis (dbRDA) plot (Fig. 2.3), although the influence of other factors was significant among the classification groups. The result of the dbRDA plot showed that SASS scores influenced 59.1%

of fitted and 15.1% of total variation in macroinvertebrate taxa composition, while longitudes influenced 40.9% of fitted and 10.4% of total variation (Fig. 2.3). SASS score, number of taxa, ASPT, latitude, longitude and altitude were good predictors, while biotopes were not.
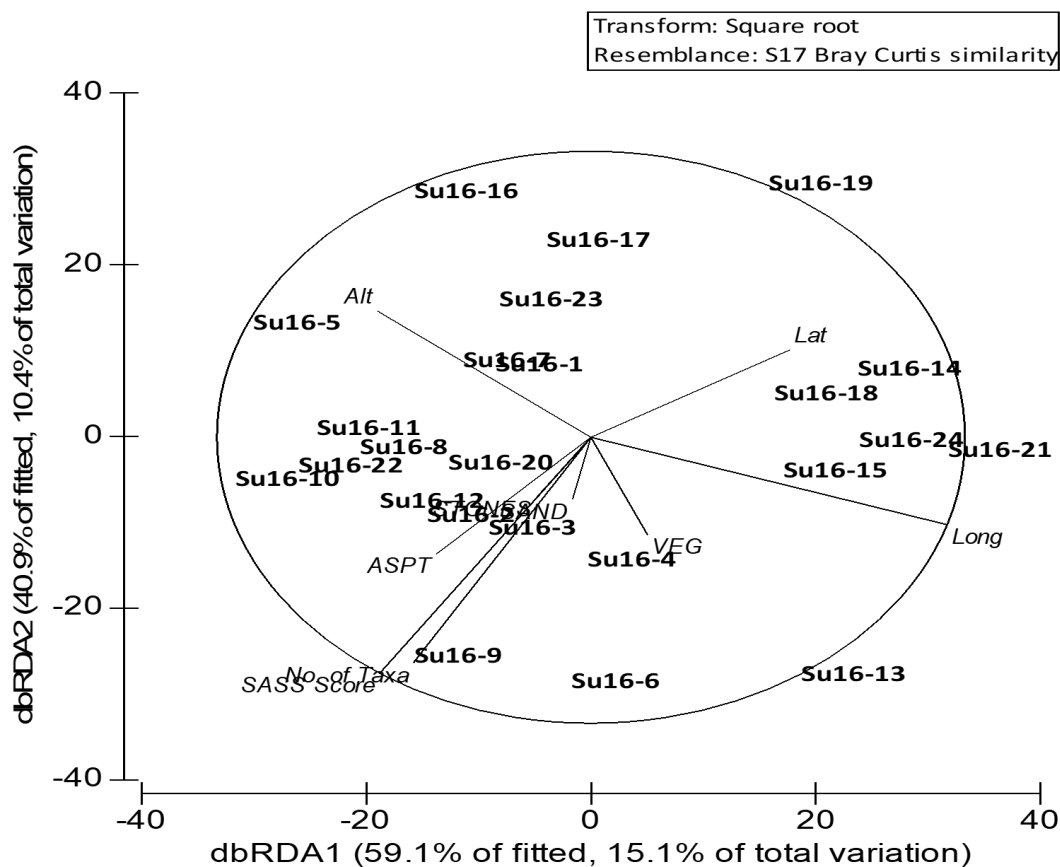
Figure 2.3: Redundancy Analysis (RDA) of SASS indices, biotope availability and geographic location, using macroinvertebrate abundance in KwaZulu-Natal rivers in summer 2016. Alt = altitude, Lat = latitude, Long = longitude, VEG = vegetation, ASPT = average score per taxon, SASS Score = South African Scoring System score, Su16 = summer 2016.

Classification strength

Macroinvertebrate taxa composition within all classification groups of KZN rivers were not significantly different, as indicated by the Global R-values (Table 2.5). Hence their reference conditions can be used interchangeably in assessing the rivers between the groups (Table 2.5). All the groups having significant differences ($p < 0.05$) could not be used as reference sites in assessing the sites between the groups. This showed that all the classification groups had higher within-class similarity than between-class similarity. The ecoregion classification had the largest Global R-value. The pair-wise results suggested that seven pairs of ecoregions were significantly similar, while the three pairs were different (Table 2.5). Macroinvertebrate taxa compositions were considered homogenous within classification groups, but not between groups (Fig. 2.3). The rating, based on the Global R-values showed that ecoregions had the highest classification strength, although they were relatively too weak for between group comparisons. The closer the Global R is to 1, the more positive the result (Clarke, 1993; Clarke and Warwick 1994; 2001).

Table 2.5: Pairwise tests of the analysis of similarity (ANOSIM), indicating the Global R and Statistic R values. All tests with P < 0.05 are significantly similar. The number of sites (n) in each classification group was given wherever possible.

Classification	Group	n	Global R	Statistic R	Significance level
Geomorphology	Upland, Lowland	24	0.061	-	0.115
Seasons	Su15, Au15	-	0.052	0.020	0.102
	Su15, Sp15	-	-	0.034	0.034
	Su15, Su16	-	-	0.059	0.010
	Au15, Sp15	-	-	0.065	0.002
	Au15, Su16	-	-	0.075	0.004
	Sp15, Su16	-	-	0.066	0.005
Ecoregion	SEU, NECB	11	0.087	0.050	0.054
	SEU, EEM	12	-	0.031	0.056
	SEU, NEU	11	-	0.050	0.053
	SEU, LOWV	8	-	0.268	0.038
	NECB, EEM	11	-	0.115	0.003
	NECB, NEU	10	-	0.069	0.012
	NECB, LOWV	7	-	0.236	0.036
	EEM, NEU	11	-	0.034	0.102
	EEM, LOWV	7	-	0.321	0.014
	NEU, LOWV	7	-	0.101	0.167

Discussion

The expectation that there is no ecological class boundary between sites of different ecoregions was rejected, because of high dissimilarities obtained in the pairwise test results. There is close interconnectivity in the establishment of reference conditions and the establishment of ecological quality class boundaries (Wallin *et al*., 2003). Identification of least impacted or reference conditions is important in establishing the ecological status of a river system. However, establishing ecological status or class boundaries can only be possible with the existence of reliable a RC (Economou, 2002; Chaves *et al*., 2006). The inception phase of reference conditions selection is crucial to ecological evaluations (Swetnam *et ai*., 1999). Hence, there is need for careful selection because the reference sites will form the evaluation standards for evaluating other sites (Barbour *et al*., 1996).

Site selection and validation

Many of the lowland rivers of KZN failed the selection criteria, especially in the northern part as there were limited macroinvertebrate biotopes, severe river channel modifications and prevailing drought conditions. The established criteria for the selection and validation of reference conditions for this study involved the inclusion of a certain level of human disturbance or exposure to anthropogenic disturbances (Barbour *et al*., 1996; Economou, 2002; Bailey *et al*., 2004). This is because biomonitoring professionals believe that only a few pristine reference conditions still exist in the world (Stoddard, 2004). It was suggested that the absence of a criterion can be as problematic as selecting the wrong one (Chaves *et al*., 2006).

The River Health Programme of South Africa, which recently metamorphosed into the River Ecostatus Monitoring Program (REMP) (DWS, 2016) and the Water Framework Directive (WFD) (European Commission, 2000) recognise the importance of biological criteria in the validation of aquatic ecosystem status or quality (Chaves *et al*., 2006). This is because biological components of an aquatic ecosystem are good indicators of (1) water quality changes, which may be caused by organic pollution, hazardous substances or nutrient enrichment (eutrophication); (2) habitat modifications by physical disturbance, such as dam construction, canalization, dredging or

other forms of construction activities; and (3) biological pressures on populations, such as the introduction of alien species (Nixon *et al*., 2003; Chaves *et al*., 2006). For example, a decrease in macroinvertebrate diversity and an increase in tolerant taxa are expected in the presence of stressors, which may be indicated by the use of the SASS5 in South African rivers (Dickens and Graham, 2002).

Analysis of similarity

At the ecoregional scale examined in this study, macroinvertebrate assemblages showed distinct separation, as the percentage dissimilarities were high between ecoregions. The lowest dissimilarity percentage occurred between the eastern escarpment mountain (EEM) and north eastern upland (NEU) (Dissimilarity = 69.6%, 36 macroinvertebrate taxa), with EEM comprising six upland sites and NEU comprising of three upland and two lowland sites. The highest dissimilarity percentage occurred between south eastern uplands (SEU) and lowveld (LOWV) ecoregions (Dissimilarity = 81.8%, 35 macroinvertebrate taxa), with SEU comprising of three upland and three lowland sites, and LOWV comprising of two upland sites. Taxa richness between the ecoregions was similar, although taxa compositions were slightly different. While the five ecoregions had a high within-group similarities and taxa richness, the low similarity percentage (13.8%) and taxa richness (13) in the lowveld ecoregion could be a consequence of the low number of sites (2) within the region.

While ecoregional classifications based on macroinvertebrate assemblages are capable of partitioning variability in macroinvertebrate assemblages, an amount of variation in the spatial factors often remain within the classification classes (Dallas, 2004a; Stoddard *et al*., 2006). These factors may be at the level of river type or other aspects such as width, depth, substratum, biotope availability, hydrological-type and canopy cover (Dallas, 2004a; Hawkins *et al*., 2010). This study revealed that some upland and lowland sites were similar within the same ecoregion, though were partitioned by the longitudinal gradients. Hence, supporting the suggestion of Dallas (2004b) that, longitudinal partitioning may be incorporated into bioassessment in South Africa by separating upland sites from the lowland ones. Many studies have reported distinct differentiations in biotic assemblages between montane and non-montane regions (Tate and Heiny, 1995; Dallas, 2004a),

also that topography and climate are good partitions of biotic variation (Hawkins and Vinson, 2000). Our results showed that river types or geomorphology (uplan and lowland river types) have distinct macroinvertebrate assemblages (75% variation), which showed that the RCs of each river type are different in terms of taxa composition. Our study showed macroinvertebrate taxa composition within all classification groups of KZN rivers were not significantly different, as indicated by the Global R-values, this means that sites that fall within the same groups can be used in the comparing of impaired sites in bioassessment. All groups having significant between-group differences ($p < 0.05$) cannot be used as reference sites in assessing each other.

Classification is a major step in bioassessment because it partitions naturally occurring variation among sites and thus allows to specify an ecologically meaningful standard against which potentially impaired sites can be compared (Van Sickle and Hughes, 2000). The ability to detect impairment is a direct function of how well classifications partition natural variation among sites (Hawkins *et al*., 2000a; 2000b). Good classifications are believed to be accurate and thus unbiased in bioassessment (Ostermiller and Hawkins, 2004). Mean similarity dendrograms convey classification strengths through conceptually simple comparisons of within-class and between-class similarities, which make it an attractive non-technical tool for evaluating environmentally-oriented land classifications (Van Sickle, 1997).

Conclusions

River biomonitoring practitioners have often identified potential reference sites using various methods, although the protocols for selecting these sites vary (Davies and Jackson, 2006; Stoddard *et al*., 2006; Dallas, 2013). The advantage of the multivariate approach for selecting reference sites is that, it does not make any prior assumption of the faunal compositions, but it uses a weighting method to predict taxa assemblages or composition, thus making it a useful method for selecting RCs (Reynoldson *et al*., 1997; Legendre and Gauthier, 2014). Cluster and ordination analysis, together with analysis of classification strength of the different ecoregional and faunal classifications suggested that macroinvertebrate assemblages correlate to regional classifications, hence within-class similarity exceeded between-class similarity (Dallas, 2004a).

Regional classification of sites, particularly of reference sites, has a potential for the management of aquatic resources by providing a framework for bioassessment (Omernik and Griffith, 1991; Dallas, 2004a). However, this only holds true if the regional classification reflects actual spatial differences in the ecosystem component or components being managed (Dallas, 2004a; Dallas, 2004b). Choice of classification system may sometimes depend on the ease of assigning new sites to classes (Gerritsen *et al*., 2000). Recently, site classification is often done by predictive models that provide a link between environmental variables and faunal assemblages (Wright, 1995; Smith *et al*., 1999; Kleynhans and Louw, 2007; Thirion, 2007). Homogeneous regions delineated along spatial lines provide for an easier and more logical classification system than non-spatial ones since the grouping of sites is determined by similarity or homogeneity of the region within which the assessment is conducted (Omernik and Griffith, 2014). Fauna classification of sites requires large sets of internally consistent data, obtained from carefully planned and spatially distributed sampling efforts (Van Sickle and Hughes, 2000). SASS score effectively differentiated the upland sites from the lowland sites. The results obtained from the analysis of the SASS score further showed that macroinvertebrate quality values (sensitivity scores) are important in the assessment and classification of RCs when using macroinvertebrates as indicators of the ecosystem. Hence, a high SASS score represents a good RC.

Our results revealed high levels of inconsistent macroinvertebrate data in the lowland rivers of KZN, which was largely due to natural disturbances (e.g. drought) and not pollution or water quality degradation. Majority of the lowland rivers within KZN failed the selection and validation process, especially in the widely used national macroinvertebrate biotic index (SASS5), riparian vegetation cover and biotope or substrate availability. The implication of this is that these sites, especially the small tributary streams may not have effective RCs which could be used in their assessment. Also, there is scarce or paucity of data which could suffice for setting the RCs for these lowland rivers, hence it is recommended that a type-specific RC should be developed for them. This could be achieved by using multivariate analysis and other appropriate statistical tools. However, the selection and validation of RCs should be a continuous process incorporating generation of hypotheses, rigorous data analysis and modification of hypotheses (Gerritsen *et al*., 2000; Dallas, 2004a; Hawkins *et al*., 2010).

Acknowledgements

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CHAPTER 3

Diversity, similarity and biotic indices: insights for ecological applications of macroinvertebrate community structures in the rivers of KwaZulu-Natal, South Africa.

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Abstract

Biodiversity is a complex attribute of natural systems and it is hard to quantify. All biodiversity indices are designed to describe the variation in community dynamics while allowing for comparison between different regions, taxa and trophic levels. Although biodiversity indices are essential for environmental monitoring and conservation management, there is currently little consensus about the most appropriate and informative index. We compared a series of macroinvertebrate data from the rivers of KwaZulu-Natal (KZN), South Africa according to nine diversity indices (total number of species/taxa, total number of individuals, Margalef's, Pielou's, Brillouin's, Hill's, Simpson's, Fisher's and Shannon's indices), one similarity index (similarity percentage – SIMPER) and three biotic indices (South African Scoring System (SASS5), average score per taxon (ASPT) and Macroinvertebrate Response Assessment Index (MIRAI). There were clear connections between various water quality degradations and decrease in the macoinvertebrate indices along pollution gradients. Our study revealed that diversity and biotic indices were useful in comparing polluted and non-polluted rivers of KZN, however small changes in community compositions were better revealed by the diversity indices and the SASS5. Taxa abundance distribution is a simple and powerful tool for describing and comparing diversity from different sites. A comparison of the biological bands and MIRAI revealed that MIRAI is a better tool for ecological classification than the biological bands, especially in the lowland river sites which often have inconsistent data. We found that the MIRAI model was weaker than SASS5 at measuring the biodiversity of macroinvertebrates, hence we suggested that the MIRAI can be improved by incorporating biodiversity and taxa richness measures into its metrics. This is because a good knowledge of biodiversity helps in the improvement of conservation management policies.

Keywords: macroinvertebrates, diversity, similarity, biotic indices, MIRAI, SASS5

Introduction

The ecological health of rivers and streams is a vital global water management issue (Arthington *et al*., 2010). Definitions of river health and selection of methods appropriate for its assessment, however, are subject to considerable debate among professionals (Wicklum and Davies, 1995; Norris and Thoms, 1999; Bunn and Davies, 2000). Ecologists often measure biodiversity (Magurran, 2013) and the structure of benthic communities (Wright, 1995), while some promote the distribution and abundance of specific taxonomic groups (Reid *et al*., 1995; Kelly, 1998). Macroinvertebrates are often used as ecological indicators in biological monitoring because of their relative ubiquity, visibility to the naked eyes, ability to live part or their whole lives in the river and also their ability to inhabit a broad range of habitats (Feminella and Flynn, 1999; Bonada *et al*., 2006).

A diversity index is the numerical measure of species diversity in a community that provides information about community compositions rather than the species richness (the number of species) (Mouchet *et al*., 2010; Magurran, 2013). Biological diversity may be evaluated in various ways, but the two most important factors of estimating diversity are richness and evenness. Richness is the total number of different kinds of organisms or taxa in a community, while evenness is the comparison of the similarity of each organism or taxa present within a community (Olszewski, 2004; Leinster and Cobbold, 2012). Therefore all diversity indices are based on two main assumptions: a) stable communities have high diversities, while unstable communities have low diversities, and b) community stability is an index of environmental quality (Washington, 1984; Briones and Raskin, 2003; Lozupone *et al*., 2012); therefore diversity values decrease with environmental degradation and can reveal community differences between sites over time, serving as a valuable indicator of stressors (Kempton, 1979; Lake, 2000; Ravera, 2001; Ives and Carpenter, 2007; Lobera *et al*., 2017; Sundstrom *et al*., 2017).

Although there exist strong relationships between diversity measures, they are not interchangeable, and there has been much debate over which of them is most appropriate in various contexts (Pla, *et al*., 2012; Morris *et al*., 2014). Even after a diversity measure has been chosen, quantifying the biodiversity may still be a problem because a single index cannot adequately summarize the biodiversity (Purvis and Hector, 2000). Magurran and Dornelas (2010) suggested

the use of compound indices wherever the purpose of site ranking by diversity is the primary goal, such as conservation planning for selecting sites to be protected. Conversely, Magurran and Dornelas (2010) also argued against the use of compound indices in the assessment of the effects of external factors on diversity, such as detection of anthropogenic impacts.

Similarity percentage (SIMPER) is a method that allows identification of the taxa that contribute to the similarity and dissimilarity between or within *a priori* defined communities (Clarke and Gorley, 2006). Similarity indices are used to compare the community structures within a site or between two locations at different periods of time; therefore there must be a basis for comparison (Lydy *et al*., 2000). Some of the oldest similarity indices (e.g. Jaccard's and Sorensen's coefficients) are the most widely used in community ecology, but some researchers support the use of percentage similarity (Washington, 1984; Lydy *et al*., 2000).

Several macroinvertebrate biotic indices have been developed for the assessment of aquatic ecosystems, to investigate different kinds of water quality degradation or pollution based on their sensitivity values (Washington, 1984; Ghetti and Ravera, 1994; Şener *et al*., 2017). The basis of biotic indices is the presence or absence of taxa (species, genus, family), which are used as indicators of the pollution level in riverine ecosystems (Junior *et al*., 2015). The commonly used biotic indices in South Africa for assessing water quality or degradation are the South African Scoring System 5 (SASS5), average score per taxon (ASPT) (Dickens and Graham, 2002) and the Macroinvertebrate Response Assessment Index (MIRAI) (Thirion, 2007; 2016).

Although the MIRAI method has not yet been rigorously validated, the method has been successfully tested in perennial rivers and some studies have been able to compare the SASS and MIRAI methods in temporary rivers (Watson and Dallas, 2013; Venter, 2013). Also, there are limited comparative studies between diversity, similarity and biotic indices on South African rivers. Therefore, we aimed to compare the efficiency of diversity, similarity and biotic indices in the assessment of KwaZulu-Natal (KZN) rivers using macroinvertebrate community structures. We expected that the results of this study will add to the awareness of the usefulness of these indices in conservation planning, mitigation management and validation of South Africa's biotic indices (SASS and MIRAI).

Methods

Study area

KwaZulu-Natal Province, known as "the garden province" is located in the southeast of South Africa, and has a relatively long Indian Ocean shoreline. It is home to the iSimangaliso Wetland Park and the uKhahlamba Drakensberg Park, which are UNESCO World Heritage Sites. Tourism is increasingly becoming important to the economy of KZN as a result of the rich biodiversity and efforts at conservation in the province. The study sites were located in a variety of geomorphological zones from headwater to lowland rivers (Rowntree *et al*., 2000; Moolman, 2008) (Fig. 3.1).
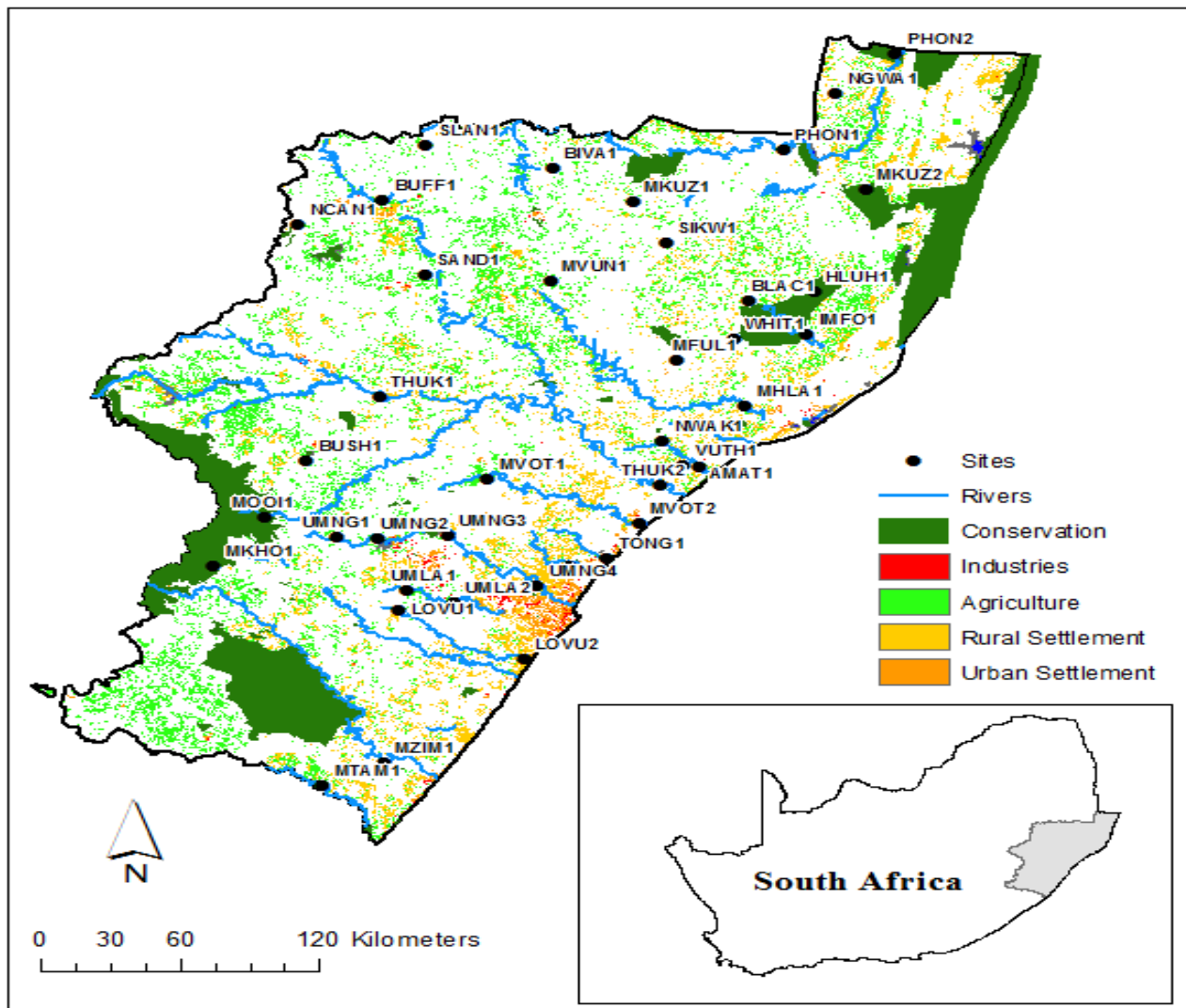
Figure 3.1: Map of KwaZulu-Natal showing the river sites with their associated landuse types from March 2015 to March 2016 (Map of South Africa inset).

Data collection

For this study, field data was collected during four seasons (summer 2015, autumn 2015, spring 2015 and summer 2016) from February 2015 to March 2016 from 38 KZN river sites (Table 3.1). The water quality parameters were grouped into nutrients (phosphate and total inorganic nitrogen); physical variables (pH, clarity/turbidity, temperature, total dissolved solids, dissolved oxygen and electrical conductivity); biological variable (chlorophyll a), microbiological variables (total bacteria, total coliform and *Escherichia coli*); and toxic and complex mixtures (ammonia and fluoride). Water samples for the nutrients, biological variables and microbiological variables were preserved at 4^0C in plastic bottles and sent to the uMgeni Water laboratory for analysis; while the physical variables were measured in the field on every sampling occasion using the YSI model 556 MPS handheld multi-probe water quality meter.

We sampled macroinvertebrates qualitatively according to the protocols of the South African Scoring System version 5 (SASS5) (Dickens and Graham, 2002), using a kick net from three biotope types (stones, vegetation and gravel, sand, mud (GSM)). The net was placed downstream during the sampling events to prevent loss of macroinverterates as the biotopes were kicked. The samples were scored in the field according to the SASS5 protocol to obtain the SASS5 scores and the average score per taxon (ASPT) values. Field identification of macroinvertebrates was done according to the protocols of SASS5 (Dickens and Graham, 2002), using the identification guides produced by the Department of Water and Sanitation (Gerber and Gabriel, 2002) and the samples were subsequently preserved in 80% ethanol for taxonomic resolution and abundance counts in the laboratory. The SASS5 data interpretation is based on the calculation of the SASS score (the sum of the sensitivity weightings for taxa present at a site) and average score per taxon (ASPT), while the ASPT is the ratio of the SASS score and the number of taxa (Dickens and Graham, 2002).

Statistical analyses

We included thirteen indices comprising of nine diversity (Margalef's (Margalef, 1958), total number of species, total number of individuals, Pielou's (Pielou, 1966), Brillouin's (Brillouin, 1956), Hill's (Hill, 1973), Simpson's (Simpson, 1949), Fisher's (Fisher et al., 1943) and Shannon's

indices (Shannon, 1948)), one similarity (similarity percentage – SIMPER (Clarke and Gorley, 2006)) and three biotic indices (SASS5 score and ASPT (Dickens and Graham, 2002); MIRAI (Thirion, 2007; 2016)) in our study. We calculated taxa (family level) abundance distribution for each site because it gives a complete description of the data on diversity (Magurran, 2013). The diversity and similarity indices were calculated from the taxa abundance data using the Primer v6 software based on the Bray-Curtis resemblance matrix (Clarke and Gorley, 2006). We calculated SASS5 scores and ASPT values according to the guidelines of Dickens and Graham (2005), while we used the MIRAI model version 2 to calculate the MIRAI data used (Thirion, 2016).

We normalized the physico-chemical parameters; while we transformed the macroinvertebrate data to their square roots before statistical analyses (Clarke and Gorley, 2006). Pearson's correlation analysis was used to explore the relationships between the physico-chemical variables and the benthic macroinvertebrate indices, using Minitab statistical software 16 (Minitab, 2010). The BEST analysis was used to reduce data complexity through a non-metric multidimensional scaling (MDS) and the redundancy analysis (RDA) to select the best set of indices that best explain the macroinvertebrate assemblages in the rivers (Clarke, 1993; Clarke and Gorley, 2006). Anderson-Darling normality test was further applied to the macroinvertebrates data to test their significance. The results of the MDS ordination was used to generate the clusters of the selected indices using the Bray Curtis similarity matrix (Clarke and Gorley, 2006).

Ecological classification

The two existing South African methods for classifying river sites into their Ecological Categories (EC) or Present Ecological States (PES) are the biological bands (Dallas, 2007) and MIRAI (Thirion, 2007). The biological bands (based on historical SASS5 data) are used to interpret SASS5 data based on the geographical and longitudinal variations (geomorphological zones) of macroinvertebrate assemblages (Dallas, 2007). In generating the biological bands, the ASPT data from each spatial group were plotted as a function of SASS5 score; thus enabling the interpretation to be such that either a higher SASS5 score or higher ASPT score will fall within a band. The biological band method incorporates natural variation in the SASS biotopes (i.e. stones, vegetation, and gravel/sand/mud).

Unlike the biological bands, the MIRAI method has a more holistic approach by incorporating flow, habitat and water quality preferences of macroinvertebrates into the ecological categories. The MIRAI model compares macroinvertebrate occurrence at a site to an expected (reference) assemblage (Thirion, 2007; 2016). Each of these factors or metrics is ranked and weighted according to its importance in determining the ecological category (EC) of the macroinvertebrate assemblage per site. Changes in the abundance and frequency of macroinvertebrate taxa occurrence are measured according to the different metrics on a scale from 0 to 5, with 0 representing no change from the reference condition and 5 being the extreme change from the reference condition. The results from SASS5 and MIRAI are often used to categorize the present ecological states (PES) of South African rivers (Chutter, 1998; Dallas, 2007; Thirion, 2007; 2016). These ecological classes range from Class A to Class F; where A is unmodified natural class, B is largely natural with few modifications, C is moderately modified, D is largely modified, E is seriously modified and F is critically or extremely modified (Kleynhans and Louw, 2007; Kleynhans *et al*., 2008). In this study, we compared the accuracy of the biological bands and MIRAI model in the classification of KZN rivers, using regression and cluster analysis of the total abundance of macroinvertebrates from the laboratory counts data and the Fisher's diversity index.

Results

Physico-chemical variables

Water quality indicators varied between the study sites and most of them, except dissolved oxygen, showed increasing values from the near pristine headwater sites towards the impacted downstream sites (Table 3.1). The patterns of the data showing reduction in quality from headwaters to the downstream sites further emphasized an upstream-downstream water quality degradation gradient between sites located in each river. Mean values of all environmental variables were used for statistical analysis, with temperature ranging between 13.1 to 28.9 ^{0}C, pH ranged between 6.0 to 7.6, total dissolved solids ranged between <1 mg/l and 949.2 mg/l, dissolved oxygen ranged between 2.2 and 31.7 mg/l, clarity ranged between 6 NTU and 240 NTU, electrical conductivity ranged between 82.3 and 1788.7 mS/cm, ammonium ranged from <1 to 8.5 mg/l and Fluoride ranged from <1 mg/l to 0.7mg/l.

Table 3.1: Water physico-chemical parameters of the rivers of KwaZulu-Natal measured in 2015/2016. (TDS = total dissolved solids, Temp = temperature, DO = dissolved oxygen, EC = electrical conductivity).

Site	River	TDS (mg/l)	Temp (^{0}C)	pH	DO (mg/l)	Clarity (NTU)	EC (mS/m)
AMAT1	Matikulu	617.70	27.03	6.71	4.21	6	702.37
BIVA1	Bivane	100.60	21.65	6.78	7.33	13	145.23
BLAC1	Black Mfolozi	240.00	28.87	6.61	5.38	100	419.33
BUFF1	Buffalo	282.90	22.05	6.81	7.87	100	402.90
BUSH1	Bushmans	62.00	21.43	6.74	8.61	6	103.25
IMFO1	Mfolozi	190.00	27.13	6.54	6.41	120	474.67
LOVU1	Lovu	59.00	17.80	6.65	7.44	6	111.75
LOVU2	Lovu	572.80	27.70	6.47	3.79	27	195.57
MDLO1	Mdloti	130.00	23.75	6.82	9.58	8	169.25
MFUL1	Mfule	360.00	20.93	6.18	8.41	<5	273.00
MHLA1	Mhlathuze	230.00	26.27	6.53	8.68	48	316.67
MKHO1	Mkhomazana	44.50	16.20	6.90	9.32	<5	106.50
MKUZ1	Mkuze	465.35	20.90	7.04	5.59	8	611.13
MKUZ2	Mkuze	-	23.30	6.01	9.67	17	1342.00
MOOI1	Mooi	38.50	13.13	6.73	10.33	<5	186.25
MTAM1	Mtamvuna	66.00	19.17	7.24	10.29	10	104.33
MVOT1	uMvoti	85.00	19.73	6.74	6.20	21	163.00
MVUN1	Mvunyana	350.00	25.73	7.19	6.80	48	507.17
MZIM1	Mzimkhulu	87.67	21.77	7.38	9.12	13	204.00
NCAN1	Ncandu	651.84	18.30	6.61	7.39	6	130.00
NGWA1	Ngwavuma	754.00	27.55	6.03	4.33	11	1107.50
NWAK1	Nwaku	176.80	24.33	6.32	7.46	8	229.73
PHON1	Phongolo	767.50	26.37	7.25	11.87	15	355.85
PHON2	Phongolo	475.40	27.05	7.16	5.02	19	1788.70
SAND1	Mzinyashana	546.80	19.00	6.79	5.45	10	483.36
SIKW1	Sikwebezi	230.15	23.85	7.10	31.66	6	268.68
SLAN1	Slang	81.98	15.58	6.59	7.89	6	120.70
THUK1	Thukela	106.00	24.30	7.09	8.52	60	165.35
THUK2	Thukela	178.20	25.95	7.60	7.95	84	213.85
TONG1	Tongati	418.60	22.88	6.54	2.22	10	404.23
UMLA1	uMlazi	64.50	22.93	7.09	7.42	11	116.25
UMLA2	uMlazi	261.35	19.93	6.77	7.21	7	478.63
UMNG1	Mgeni	42.77	15.15	6.36	9.86	6	82.32
UMNG2	Mgeni	54.00	19.70	6.77	8.24	12	221.96
UMNG3	Mgeni	86.38	22.58	6.45	8.27	11	206.00
UMNG4	Mgeni	205.00	22.33	7.54	10.75	<5	311.25
VUTH1	Vutha	949.15	27.27	6.65	6.48	14	1014.93
WHIT1	White Mfolozi	160.00	24.40	6.51	8.38	>240	334.67

Diversity, similarity and biotic indices

The best set of indices from the ordination analysis were Fisher's Alpha, SIMPER, SASS score, ASPT and MIRAI. The selection of the best indices were done using the Bray Curtis resemblance matrix with Akaike information criterion (AICc), with an R value of 0.6 (Fig. 3.2). Results of the diversity indices showed Fisher's alpha index ranged from 4.23 to 18.26. SIMPER mean scores ranged from 2.92 to 60.11 (Table 3.2). Mean SASS scores ranged from 17.7 and 180.3, mean ASPT values ranged from 3.6 to 7.4 and mean MIRAI scores ranged from 22.7 to 88.2 (Table 3.2).
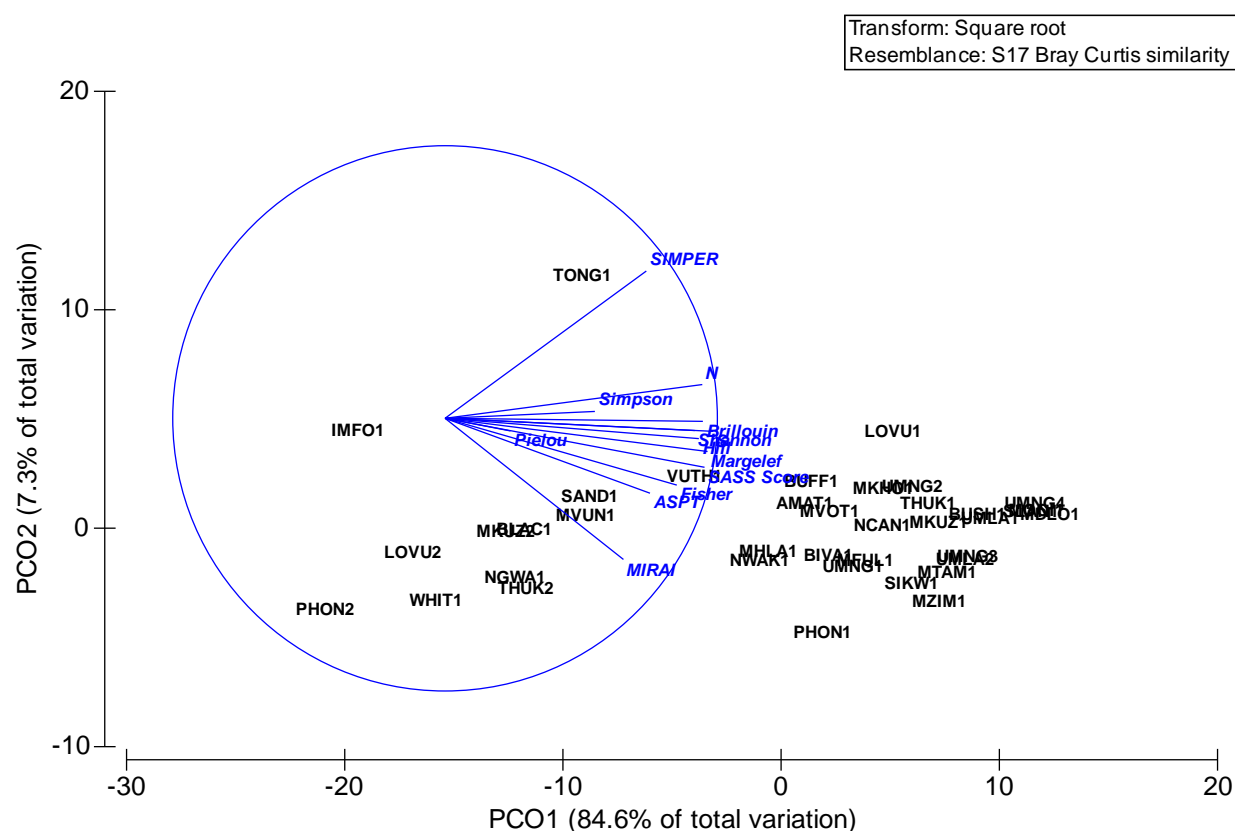
Figure 3.2: Principal coordinate analysis of the diversity, similarity and biotic indices of macroinvertebrates of the rivers of KwaZulu-Natal between 2015-2016. (MIRAI = Macroinvertebrate Response Assessment Index, N = total number of individuals, S = number of taxa, SASS = South African Scoring System, ASPT = average score per taxon, SIMPER = similarity percentage).

Table 3.2: Mean values of diversity, similarity percentage and biotic indices of macroinvertebrates data obtained in KwaZulu-Natal rivers between 2014 and 2016. (S = number species/taxa, N = number of individuals, Fisher = Fisher's index, SIMPER = similarity percentage, SASS Score = South African Scoring System, ASPT = average score per taxon, MIRAI = macroinvertebrate response assessment index, EC = Ecological Category).

Site	River	S	N	Fisher	SIMPER	SASS Score	ASPT	MIRAI	SASS EC	MIRAI EC
AMAT1	Matikulu	27	97	12.36	33.44	93.33	4.79	81.16	C	B
BIVA1	Bivane	30	91	15.56	22.59	94.75	6.25	80.79	F	B
BLAC1	Black Mfolozi	16	41	9.69	14.39	37	4.76	52.72	F	D
BUFF1	Buffalo	26	144	9.26	35.13	70.25	4.37	77.06	F	C
BUSH1	Bushmans	37	143	16.15	41.75	158.5	6.35	81.17	B	B
IMFO1	Mfolozi	9	31	4.23	20.32	17.67	3.77	41.28	F	D
LOVU1	Lovu	33	133	14.05	41.77	119	5.81	41.08	D	D
LOVU2	Lovu	12	22	10.65	6.59	39.5	4.66	38.18	F	E
MDLO1	Mdloti	43	199	16.86	55.76	180.25	6.35	78.83	A	C
MFUL1	Mfule	31	118	13.67	21.35	125	5.87	66.2	D	C
MHLA1	Mhlathuze	23	87	10.21	20.18	113.67	6.53	83.32	A	B
MKHO1	Mkhomazana	28	125	11.23	43.26	125.25	7.42	65.1	A	C
MKUZ1	Mkuze	37	140	16.42	36.23	121.75	5.59	76.98	F	C
MKUZ2	Mkuze	11	27	6.89	25.48	51	4.25	60.97	F	C
MOOI1	Mooi	39	197	14.56	60.11	168.5	6.42	88.19	A	B
MTAM1	Mtamvuna	36	122	17.26	25.38	166	7.11	79.26	A	C
MVOT1	uMvoti	32	92	17.49	25.19	100	6.24	47.88	D	D
MVUN1	Mvunyana	18	38	13.37	18.41	52.37	5.04	42.01	F	D
MZIM1	Mzimkhulu	34	127	15.24	18.65	176.33	6.12	82.21	D	B
NCAN1	Ncandu	36	113	18.26	33.09	99.25	5.83	70.27	E	C
NGWA1	Ngwavuma	15	48	7.46	6.04	41.5	5.19	57.76	F	D
NWAK1	Nwaku	25	74	13.34	18.94	91.67	4.76	74.67	C	C
PHON1	Phongolo	32	114	14.81	8.44	84.67	4.67	78.37	D	C
PHON2	Phongolo	9	23	5.53	2.92	24	3.78	60.97	F	C
SAND1	Mzinyashana	18	76	7.43	8.2	57	3.84	31.91	F	E
SIKW1	Sikwebezi	35	113	17.34	20.91	139.5	5.79	84.16	D	B
SLAN1	Slang	43	206	16.55	50.24	152.75	6.12	82.57	B	B
THUK1	Thukela	32	126	13.8	43.11	131	6.23	79.02	D	C
THUK2	Thukela	15	64	6.19	4.47	59	4.9	55.89	D	D
TONG1	Tongati	16	80	6.01	43.96	26.75	3.6	22.74	F	E
UMLA1	uMlazi	38	173	15.06	40.09	164	6.08	73.87	B	C
UMLA2	uMlazi	42	183	17.07	25.79	149.67	6.82	63.01	A	C
UMNG1	Mgeni	30	109	13.67	19.61	134.5	6.88	66.6	A	C
UMNG2	Mgeni	30	130	12.21	59	128.75	5.72	66.77	D	C
UMNG3	Mgeni	36	138	15.84	29.57	178.5	6.94	78.86	A	C
UMNG4	Mgeni	42	244	14.61	57.22	145	5.82	77.28	B	C
VUTH1	Vutha	22	92	9.14	19.26	74	4.75	38.97	C	D
WHIT1	White Mfolozi	11	22	8.5	9.18	39.33	5.45	55.04	F	D

Correlation and cluster analyses

There were negative correlations between temperature and clarity ($p < 0.01$), total bacterial coliforms and DO ($p < 0.01$), F and clarity ($p < 0.01$); and *E. coli* and clarity ($p < 0.05$). The ordination results showed that physico-chemical variables that mostly affected the macroinvertebrate assemblages were dissolved oxygen, clarity, electrical conductivity, *Escherichia coli* and ammonium. The R value was 0.9.

From the diversity indices category, Fisher's index had the highest discriminant ability by having strong correlations with nine water quality variables out of the 14 tested in this study; while the weakest diversity index (Simpson's) had strong correlations with three water quality variables (Table 3.3). The percentage similarity index (SIMPER) had strong correlations with seven water quality variables. From the biotic indices category, ASPT had the highest discriminant ability by having strong correlations with ten water quality variables, while SASS and MIRAI had strong correlations with nine and six water quality variables respectively (Table 3.3). The five best indices from the MDS ordination showed positive correlations with each other. The highest correlation coefficient was recorded between SASS5 score and ASPT ($r = 0.827$), while MIRAI and SIMPER had the lowest correlation coeeficient ($r = 0.352$) (Table 3.4). Other correlation coefficient values were SIMPER and Fisher's index ($r = 0.418$), Fisher's index and SASS5 score ($r = 0.803$), SIMPER and SASS5 score ($r = 0.595$), ASPT and Fisher's ($r = 0.727$), ASPT and SIMPER ($r = 0.399$), MIRAI and Fisher's ($r = 0.589$), MIRAI and SASS5 score ($r = 0.697$), MIRAI and ASPT ($r = 0.566$) (Table 3.4).

High R^2 values were recorded between SASS5 scores and macroinvertebrate abundance (74.0%); as well as SASS5 and Fisher's diversity index (69.7%). Low R^2 values were recorded between MIRAI and macroinvertebrate abundance (30.3%); and between MIRAI and Fisher's diversity index (34.8%) (Fig. 3.3). The ecological classes obtained from the biological bands were grossly inaccurate in our study, especially for the lowland (ephemeral) rivers, while those obtained from the MIRAI model proved more accurate (Table 3.2). For example, the results from the biological bands showed most of the lowland rivers were in the F (critically modified) category, while the results of the MIRAI showed that they are in better categories of C and D (Table 3.2). The cluster analysis based on macroinvertebrate abundance and Fisher's diversity was able

to partition the impacted KZN sites from the least impacted sites forming two major groups at 80% similarity (Fig. 3.4 and Fig. 3.5). The sub-cluster resolutions of macroinvertebrate abundance (Fig. 3.4A) and Fisher's diversity index (Fig. 3.4B) showed heterogeneous mixtures of the present ecological states for the biological bands (B_PES), although majority of the F category formed separate clusters. The sub-cluster resolutions of the macroinvertebrate abundance (Fig. 3.5A) and Fisher's diversity index (Fig. 3.5B) of the present ecological states for the MIRAI (M_PES) were more orderly arranged than that of the present ecological states for the biological bands.

Table 3.3 Spearman's correlations between macroinvertebrate indices and physico-chemical variables. (S = total number of species/taxa, N = total number of individuals, Margalef = Margalef's index, Brillouin = Brillouin's index, Fisher = Fisher's index, Shannon = Shannon's index, Simpson = Simpson's index, Hill = Hill's index, SIMPER = Similarity percentage, SASS Score = South African Scoring System, ASPT = average score per taxon, MIRAI = macroinvertebrate response assessment index. TDS = total dissolved solids, Temp = temperature, DO = dissolved oxygen, EC = Electical conductivity, PO_4 = Phosphate, TIN = total inorganic nitrogen, Chl-a = chlorophyll a, *E. coli* = *Escherichia coli,* NH_4 = Ammoniun and F = Fluoride).

	S	N	Margelef	Brillouin	Fisher	Shannon	Simpson	Hill	SIMPER	SASS Score	ASPT	MIRAI
TDS (mg/l)	-0.301	-0.300	-0.289	-0.242	-0.253	-0.240	-0.058	-0.313	-0.429**	-0.480**	-0.514**	-0.325*
Temp	-0.587**	-0.577**	-0.563**	-0.591**	-0.475**	-0.578**	-0.400*	-0.574**	-0.543**	-0.571**	-0.559**	-0.235
pH	0.282	0.311	0.263	0.241	0.225	0.204	-0.038	0.274	0.088	0.245	0.103	0.224
DO	0.335*	0.240	0.355*	0.301	0.368*	0.312	0.156	0.344*	0.068	0.382*	0.289	0.441**
Clarity	0.623**	0.622**	0.596**	0.606**	0.494**	0.585**	0.419**	0.560**	0.519**	0.566**	0.434**	0.323*
EC	-0.552**	-0.464**	-0.570**	-0.534**	-0.567**	-0.544**	-0.303	-0.544**	-0.384*	-0.547**	-0.578**	-0.253
PO_4	-0.169	-0.072	-0.203	-0.069	-0.266	-0.086	0.026	-0.141	-0.170	-0.200	-0.348*	-0.311
TIN	-0.162	0.000	-0.223	-0.067	-0.325*	-0.106	0.007	-0.149	0.079	-0.230	-0.399*	-0.359*
Chl-a	-0.331*	-0.279	-0.346*	-0.298	-0.344*	-0.315	-0.129	-0.278	-0.264	-0.293	-0.318*	-0.062
Total Bacteria	-0.501**	-0.456**	-0.494**	-0.496**	-0.437**	-0.498**	-0.331*	-0.531**	-0.402**	-0.583**	-0.470**	-0.473**
Total Coliform	-0.214	-0.180	-0.217	-0.161	-0.215	-0.185	-0.064	-0.224	-0.042	-0.209	-0.284	-0.217
E. coli	-0.416**	-0.418**	-0.377*	-0.374*	-0.245	-0.330*	-0.038	-0.362*	-0.361*	-0.385*	-0.208	-0.243
NH_4	-0.248	-0.111	-0.298	-0.169	-0.369*	-0.202	-0.069	-0.242	0.079	-0.328*	-0.444**	-0.493**
F	-0.597**	-0.519**	-0.598**	-0.584**	-0.543**	-0.573**	-0.290	-0.582**	-0.536**	-0.657**	-0.566**	-0.283

** (p < 0.01); * (p < 0.05)

Table 3.4: Spearman's correlations between macroinvertebrate diversity, similarity and biotic indices. (S = total number of species/taxa, N = total number of individuals, Fisher = Fisher's index, Shannon = Shannon's index, Simpson = Simpson's index, Hill = Hill's index, SIMPER = Similarity percentage, SASS Score = South African Scoring System, ASPT = average score per taxon, MIRAI = macroinvertebrate response assessment index).

	S	N	Fisher	SIMPER	Shannon	Simpson	SASS Score	ASPT	MIRAI
S	1								
N	0.919**	1							
Fisher	0.892**	0.663**	1						
SIMPER	0.651**	0.768**	0.418*	1					
Shannon	0.932**	0.791**	0.908**	0.573**	1				
Simpson	0.584**	0.447*	0.667**	0.378*	0.811**	1			
SASS Score	0.914**	0.835**	0.803**	0.595**	0.853**	0.495	1		
ASPT	0.722**	0.584**	0.727**	0.400*	0.696**	0.403*	0.827**	1	
MIRAI	0.648**	0.568**	0.590**	0.352*	0.558**	0.255**	0.697**	0.567**	1

Significance = ** ($p < 0.01$); *($p < 0.05$)
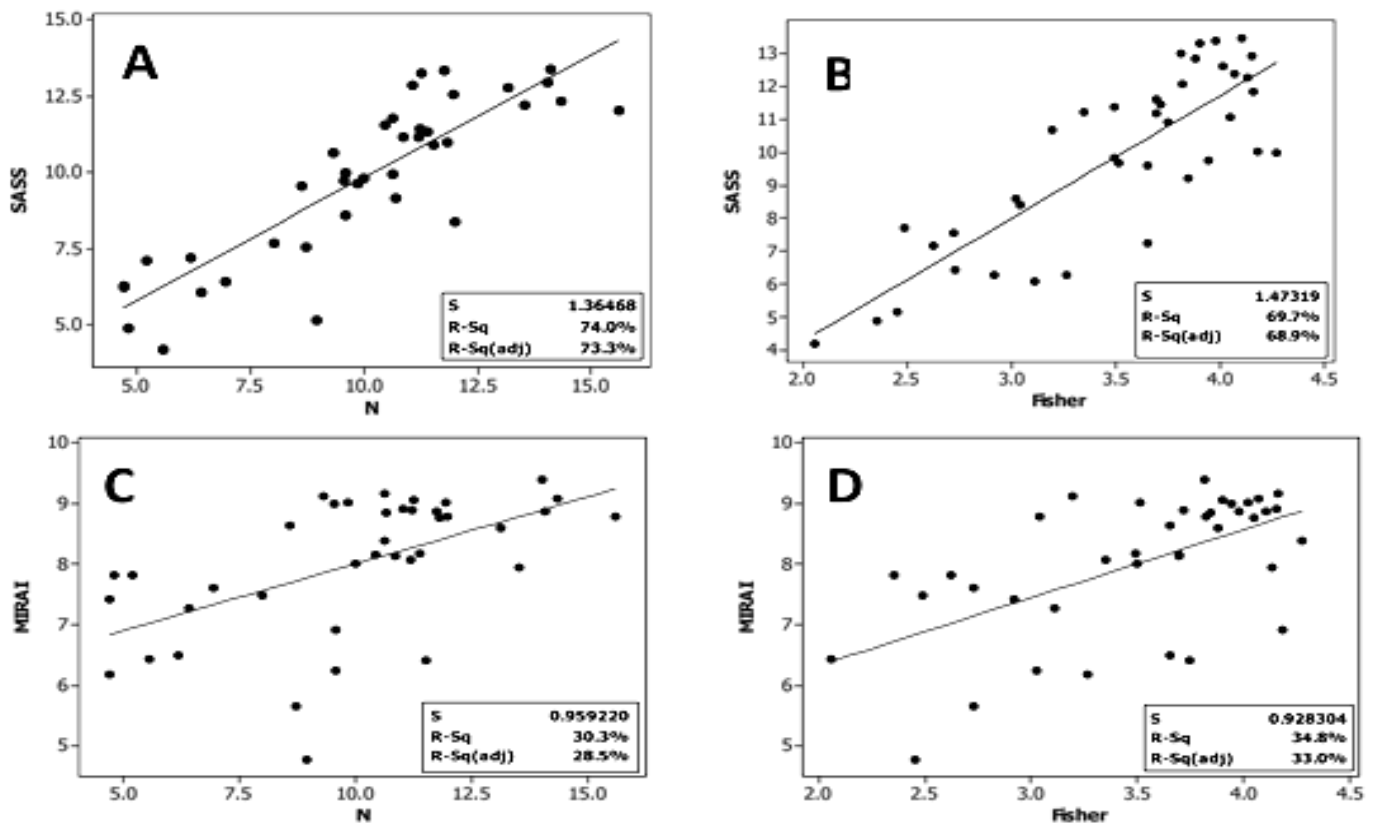
Figure 3.3: Regression between: (A) SASS5 scores and macroinvertebrate abundance (N); p-value = 0.10, (B) SASS5 scores and Fisher's diversity index; p-value = 0.01 (C) MIRAI and macroinvertebrate abundance (N); p-value = 0.01 (D) MIRAI and Fisher's diversity index; p-value = 0.00. S = standard distance data values fall from the regression line; measured in the units of the response variable. The better the equation predicts the response, the lower the S value.
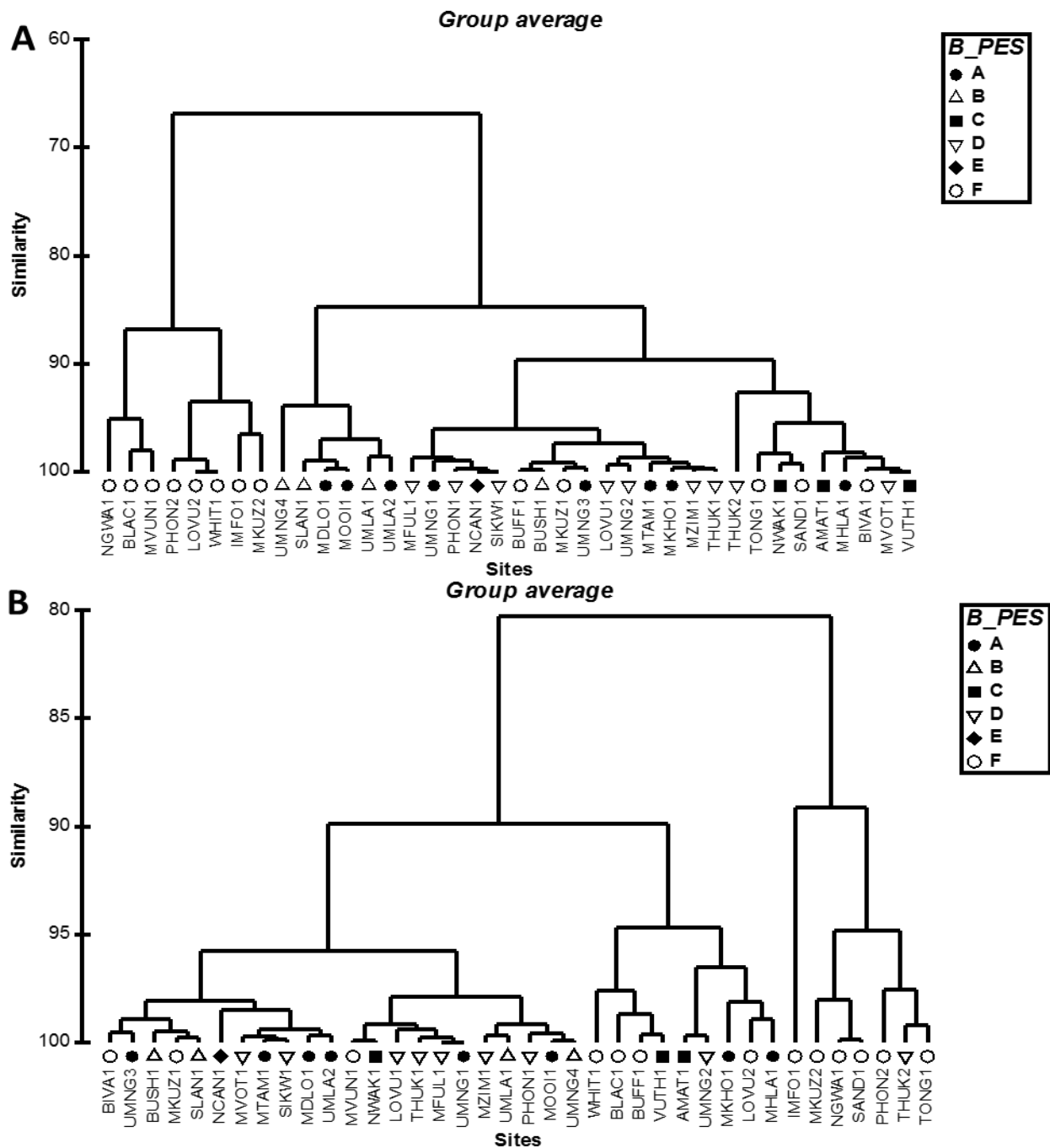
Figure 3.4: Resolution of ecological categories of the rivers of KwaZulu-Natal, South Africa using: (A) macroinvertebrate abundance and B_PES; (B) Fisher's diversity index and B_PES. B_PES = present ecological state from biological bands.
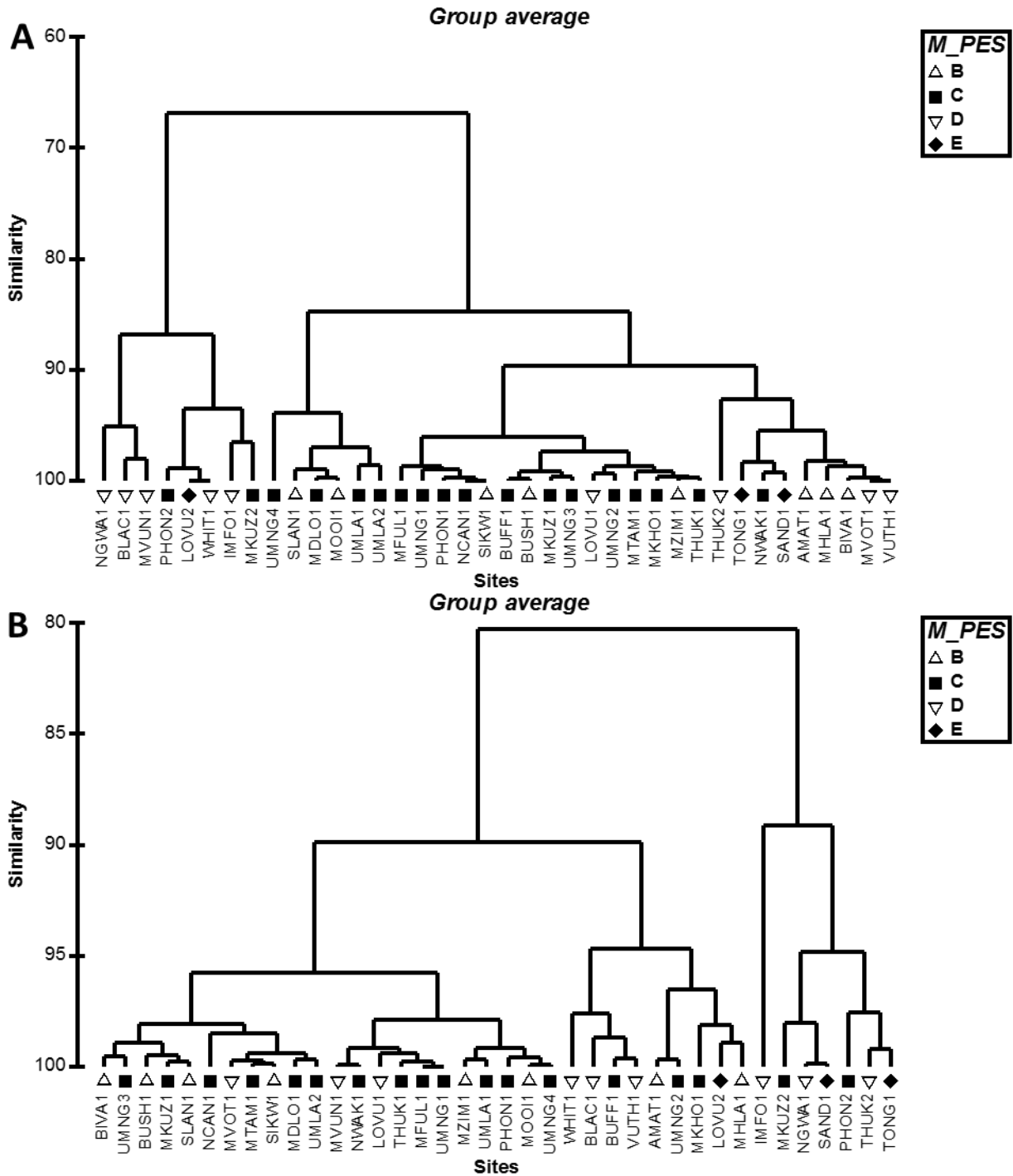
Figure 3.5: (A) macroinvertebrate abundance and M_PES; (B) Fisher's diversity index and M_PES. M_PES = present ecological state from Macroinvertebrate Response Assessment Index (MIRAI).

Discussion

Our findings showed strong correlations between diversity indices, similarity and biotic indices and physico-chemical parameters. The negative correlations from our findings are reflections of changes in macroinvertebrate community compositions or structure in response to elevated levels of such physico-chemical variables in the rivers of KZN. This means that some forms of pollution can be detected using diversity, similarity and biotic indices, although the similarity index in this study was too weak as compared to other forms of indices in the assessment of the rivers of KZN. For example, the pH of measurements in this study showed negative significant correlation with many of the macroinvertebrate indices. This implied that lower pH concentrations had negative impacts on the diversity of the macroinvertebrates in the rivers of KZN. Although some researchers have recorded positive correlations between pH and macroinvertebrate diversity (Anyona *et al.*, 2014), some have also observed significant negative correlations between them (Popoola and Otalekor, 2011).

Another factor which possibly had negative impacts on the low number of individuals or taxa collected at some of the sampled sites in this study was drought, which resulted in limited habitat and could have affected recolonisation and birthrate patterns of macroinvertebrates at such sites by new individuals (Chessman, 2015). In our study, it was evident from our observations and results that the low biodiversity of macroinvertebrates at some of the sites were not necessarily due to pollution, but were as a result of other natural occurences such as limited habitat due to reduced flows at such sites. Our study sites located within industrial and intensive agricultural practices were higly impacted by pollution and this caused a reduction in the macroinvertebrate biodiversity and differences in the indices used in this study. This was shown in the cluster analysis which was able to partition the highly polluted sites from the ecologically viable sites that had lesser levels of impacts. The high diversity indices recorded in most of the upper river reaches inferred that they were relatively stable and minimally impacted by environmental drivers of change.

The high number of macroinvertebrates recorded at some of our study sites can be attributed to relatively less disturbance in or around the river by anthropogenic activities as observed throughout the sampling period compared to other waterbodies that were highly impacted

by anthropogenic activities and inert pollution. Intense urban and agricultural activities greatly influenced the composition and distribution of macroinvertebrates during this study. Industrial, agricultural and urban activities produce pollution that could exert pressure on aquatic ecosystems, resulting in the deterioration of the waterbodies and habitat quality on which the aquatic organisms depend (Wang *et al*., 2012; Morrissey *et al*., 2013; Mabidi *et al*., 2017). Also, a common impact of urbanization and other anthropogenic activities in waterbodies is the reduction in the number of taxa that are less tolerant to modifications in the water quality, but with an increase in the number of pollution-tolerant taxa (Arimoro and Ikomi, 2008; Giorgio *et al*., 2016). We were able to test the diversity, similarity and biotic indices over a wide range of sites because of the variation between numbers of taxa and between numbers of individuals (macroinvertebrate abundance) at the different sampling sites. The taxa abundance distribution (S) is a simple and powerful tool for describing and comparing diversity, however compound indices are often preferred over species richness for the purposes of conservation planning and protection (Ravera, 2001; Magurran and Dornelas, 2010). Hynes (1994) maintains that biotic indices must only be applied to polluted environments, especially those containing readily degradable pollutants. Also, Washington (1984) did not favour the use of biotic indices for the evaluation of changes in community structure, because other forms of stressors different from pollution related ones could also affect community structures. However, these indices are commonly used to evaluate the impact of water quality changes on aquatic communities. Generally, high biodiversity is expected in ecosystems devoid of significant anthropogenic impact (Katsanevakis *et al*., 2014).

Based on our observations and statistical results, the MIRAI model is a better ecological classification tool than the biological bands, especially in the lowland (ephemeral) rivers of KZN where natural occurences (such as drought) make the results of the biological bands unreliable. Generally, the MIRAI results improved the ecological classes of our study sites to one or two categories higher than the classes we obtained from the biological bands, while those that were inflated by the biological bands were corrected by the MIRAI. However, the results of the regression analysis showed that MIRAI is weak at accounting for macroinvertebrate abundance and taxa diversity at the sites. The implication of this is that the MIRAI model is weaker than SASS5 as a biodiversity measuring tool, despite its significant correlation with Simpson's index which is believed to be the strongest diversity index. Fisher's Diversity Index was the strongest

diversity index in this study, although other studies have found Simpson's diversity index to be the best because it is able to account for the number of species and also the relative abundance of each species in a sample (Stirling and Wilsey, 2001; Chalmandrier *et al*., 2015). Also, ASPT is often referred to as a biotic index, but it differs from other biotic indices because it does not consider taxa densities in its computation. However, the ASPT proved to have good pollution detection abilities in this study and its negative correlations with most of the water quality parameters showed its tendency to decrease as water quality degradation increases.

Conclusion

Diversity indices differ in their indications of community changes, but they can all be used to estimate community changes over time or space. Strong correlations between diversity measures may not be surprising because they represent aspects of the same phenomenon (Morris *et al*., 2014). For example, Shannon's diversity and Simpson's diversity indices, differ in their theoretical foundation and interpretation, but they have been found to have strong correlations with each other (Magurran, 2013). The relevance of accurate quantification of the diversity of multiple organism groups is apparent from our analyses, but this may not be possible in real ecosystem models because species interactions are often more complicated in reality (Buckland *et al*., 2005; Beale and Lennon, 2012). Thus, monitoring data may not provide sufficient information to prove these interactions (Buckland *et al*., 2005). Although this may be possible for simple ecosystems, a comprehensive data may be required to identify such complex species interactions (Colwell and Coddington, 1994).

The results of this study affirms that diversity and biotic indices are equally good for the assessment of community changes and water quality degradation, while the similarity index (SIMPER) used in this study was not good enough for the assessment of water quality degradation in the study area. As there are evidences that diversity and biotic indices may be influenced by other sources of stress apart from pollution, therefore there is need for continuous identification of the real nature of the stress before establishing relationships between diversity or biotic indices and the level of pollution (Ravera, 2001; Dauvin; 2016). For example, the low diversity data obtained from some sites in our study were not necessarily as a result of pollution, but rather as a result of other forms of stresses such as droughts and loss of habitat. Therefore, understanding the

major causes of diversity changes will help to improve conservation management and planning (Socolar *et al*., 2016).

Biodiversity monitoring should be robust enough to allow conservation managers and decision-makers to adequately maintain the biodiversity or reduce its loss (Sarkar *et al*., 2006). Thereby giving the opportunities to recognize areas of taxa decline, production or succession (Dale *et al*., 2001). Species abundance distribution emerged as a good assessment tool for comparing polluted and unpolluted sites in this study. However, it may not be reliable in complex situations. Both diversity and biotic indices were reliable in the assessment of the rivers of KZN. Although the MIRAI model proved to be a good tool for the ecological classification of rivers in KZN because of its holistic approach (i.e. incorporating flow, habitat and water quality preferences of macroinvertebrates) to bioassessment, it can further be improved by incorporating biodiversity and taxa richness measures as one of its metrics.

Acknowledgements

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Appendix 3A: Diversity, similarity and biotic indices

Diversity indices

1. Brillouin's index: H = *Log (N! / PROD (Ni!)) / N* where H is the Brillouin's index, *N* is the total number of individuals in the sample and Log is the natural logarithm. (Brillouin, 1956).

2. Fisher's alpha: *S = a*ln (1+n/a)* where *S* is number of taxa, *n* is number of individuals and *a* is the Fisher's alpha (Fisher *et al.*, 1943).

3. Hill's index: *N1 = Exp (H¹)* (Hill, 1973).

4. Margalef's index: *d = (S-1)/*ln*(n)*, where *S* is the number of taxa, and *n* is the number of individuals (Margalef, 1958)

5. Pielou's index: *J¹ = H¹ / Log (S)*; where *H¹* is the Shannon-Weaver index and *S* is the number of species in the community (Pielou, 1966).

6. Shannon's index: *H = -sum ((ni/n) ln (ni/n))* where *ni* is number of individuals of taxon *I* and *ln* is the natural logarithm (Shannon, 1948).

7. Simpson's index: *D = sum ((ni/n)²)* where *ni* is number of individuals of taxon *i* (Simpson, 1949).

8. Total number of species (*S*).

9. Total number of individuals (*N*).

Similarity index

10. SIMPER (Clarke and Gorley, 2006).

Biotic indices

11. SASS score (Dickens and Graham, 2002).

12. ASPT (Dickens and Graham, 2002).

13. MIRAI (Thirion, 2007; 2016).

CHAPTER 4

Macroinvertebrates as indicators of ecological conditions in the rivers of KwaZulu-Natal, South Africa.

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Abstract

This study examined the effectiveness of macroinvertebrate community-based multimetrics to assess the ecological health of 38 rivers in KwaZulu-Natal (KZN) Province, South Africa. The study area comprised of headwater to lowland rivers determined by their hydro-morphology. Of the 40 tested metrics, only 11 core metrics were finally selected because of their ability to distinguish between reference and impaired sites, correlation strength with environmental variables and their reliability. Nine out of the selected metrics had strong correlations with environmental variables and these were total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, percentage of Odonata taxa, total number of Trichoptera individuals, total number of Gastropoda individuals, total number of Oligochaeta individuals and total number of Coleoptera individuals. This study showed increasing chemical deterioration along longitudinal gradients of the rivers in KZN. We found that macroinvertebrate community metrics could detect nutrient pollution, organic pollution and physical habitat degradation in the rivers of KZN. We recommend more studies and validation of macroinvertebrate community-based metrics in the assessment of rivers in KZN, because they are relatively cheap and easy to use. The use of macroinvertebrate community metrics could be an effective alternative assessment method in the case of the lowland rivers where the lack of quality data often has negative impacts on the use of the biotic indices (South African Scoring System (SASS), Average Score Per Taxon (ASPT) and Macroinvertebrate Response Assessment Index (MIRAI)).

Keywords: macroinvertebrates, ecological traits, organic pollution, biomonitoring

Introduction

Globally, river health is of concern with changing land use and anthropogenic effects (Hoekstra and Wiedmann, 2014). River health is the capability of a river system to support and sustain a balanced and robust diversity of organisms that resemble the natural habitat (Norris and Thoms, 1999; Baron and Poff, 2004; Patten, 2016). Pollution causes degradation of water quality; thus, water is often graded into different quality categories according to the pollution levels (Awoke *et al*., 2016). Many countries have established different water quality standards which serve as guides for water quality assessment, although most of these guides are based on chemical concentrations of the pollutants (Keith-Roach *et al*., 2015). Various indicators of environmental degradations may be measured to assess river health deviations from the healthy state or reference conditions (RC) (Palmer *et al*., 2005; Ode *et al*., 2016). The components of a river health assessment may have physical, chemical and ecological linkages or may be a formal monitoring program which may concentrate on a single component or a combination of the components of the river ecosystem (Ladson *et al*., 1999; Kleynhans and Louw, 2007; Clapcott *et al*., 2012). The choice of the components relies heavily on the local ecosystem conditions, the management objectives and the available resources (McDaniels *et al*., 1999; Brody, 2003; Hughes and Rood, 2003; Smith *et al*., 2016). However, a comprehensive monitoring program can generate more information on the river health status, identify the cause of the associated problems and suggest the appropriate management approach that will improve the river health (Tallis and Polasky, 2009; De Fraiture *et al*., 2010; DWA, 2011; Kingsford and Biggs, 2012).

South Africa's freshwater ecosystems are being impacted by development and intensive utilization of their resources, which is causing a decline in water quality as a result of several factors (e.g. industrialization, agriculture and power generation) (Hill, 2003; Oberholster and Ashton, 2008, Ashton *et al*., 2008). Organisms respond to specific stressors, although these may be obscured in the presence of other stressors (Hering *et al*., 2006). The increase in the demand for water and its associated impacts on the quality of South Africa's freshwater resources started with the large-scale urbanization, industrialization and rapid socio-economic changes of South Africa and the management of these resources has considerably improved in recent years (Roux *et al*., 1999). The government's responsibility of managing these scarce resources is delegated to the

Department of Water and Sanitation (Roux *et al*., 1999), through the National Water Act of 1998 (RSA, 1998; DWAF, 1998).

Global awareness about the values of bioassessment and biomonitoring is limited (Resh, 2007). It is, therefore, essential to understand the value of the services that high-quality aquatic resources provide to society, to appreciate the importance of bioassessment and biomonitoring (Barbour, 2008). Ecosystem services are the processes by which the environment produces the resources that are often taken for granted, which include clean water, habitat for organisms, nutrients and recreation (Barbour and Paul, 2010). The importance of biota's contribution to the provision of ecosystem services cannot be underestimated (Barbour and Paul, 2010). Maintaining or restoring quality aquatic ecosystem integrity helps to safeguard ecosystem services, and this requires an adequate conservation of all the biological, physical and chemical components (Barbour et al., 2000; Moog and Chovanec, 2000; Barbour and Paul, 2010).

Current assessment of South African rivers is based on the concept of biological integrity, using fish, invertebrates, riparian vegetation and diatoms as biological indices using established sampling methods for their collection and assessment (Dickens and Graham, 2002; Kleynhans, 2007; Kleynhans *et al*., 2007). The results of these biological indicators of the freshwater riverine ecosystem are categorised into specific ecological categories representing the river health (DWAF, 2007; Kleynhans and Louw, 2007; Wepener, 2008). Their responses to river quality changes are predictable, distinct and taxonomically diverse (Griffith *et al*., 2005). Apart from differences in the physical and chemical tolerances among taxa, their life histories and biogeography may affect their individual responses to water quality changes (Townsend and Hildrew, 1994).

Ecological assessment of stream conditions requires an evaluation of all the physical and chemical attributes, including the biotic composition and community structures (Karr and Chu, 1998). Earlier water quality monitoring programs focused on the comparison of water chemistry downstream of point-sources, deriving water quality criteria from bioassays (McCarron and Frydenborg, 1997). However, the indices of biotic integrity (IBI) are designed to be sensitive to a wide range of stressors and cumulative disturbances in the ecosystem (Karr, 1993). However, this approach ignored the dynamic responses of *in situ* biological assemblages to chemicals or pollutants (Karr and Chu, 1998). Furthermore, the in-stream conversion of chemicals, the spatial

and temporal variation in chemical concentrations and the effects of the interaction of their compounds with other environmental stressors (such as disturbance of the riparian zones and in-stream habitats) were not considered (Karr and Chu, 1998). The taxonomic composition and structures of biological communities incorporate both aspects of exposure and a higher level of responses (Karr *et al*., 1986; Deshon, 1995; Rosen, 1995). Species traits approach of bioassessment is a promising tool that can provide good interpretations of stressor effects on aquatic systems (Statzner and Bêche, 2010; Winemiller *et al*., 2015). Based on the hypothesis that environmental conditions act as a template for evolutionary combinations of specific organism attributes, we aimed to assess macroinvertebrates' occurrence at different water quality states using taxa-specific indicators. We expected that the taxa-specific metrics to give good assessment results in the event of low quality macroinvertebrate data, especially in the lowland rivers where the widely used biotic indices (SASS, ASPT and MIRAI) for assessing South African rivers are not effective. The information gained in this study is expected to aid stakeholders to better understand the nature of their water resources, as a means of developing appropriate strategies or policies for conserving and managing the river ecosystems. The data can also be used to design measures for mitigating and monitoring environmental changes that can arise from anthropogenic activities within the river catchments.

Methods

Study area

KwaZulu-Natal (KZN) is located in the southeastern part of South Africa. It has a long shoreline beside the Indian Ocean and shares borders with three other South African provinces and the countries of Lesotho, Swaziland and Mozambique. Its climate is classified as subtropical, having four seasons; summer, autumn, winter and spring. For our study, we collected water and macroinvertebrate samples from 38 locations within 15 river catchments; Lovu, Matikulu, Mdloti, iMfolozi, Mhlathuze, Mkomazi, Mkuze, Mtamvuna, Mzimkhulu, Phongolo, Thukela, Tongati, uMlazi, uMngeni and uMvoti (Fig. 4.1).
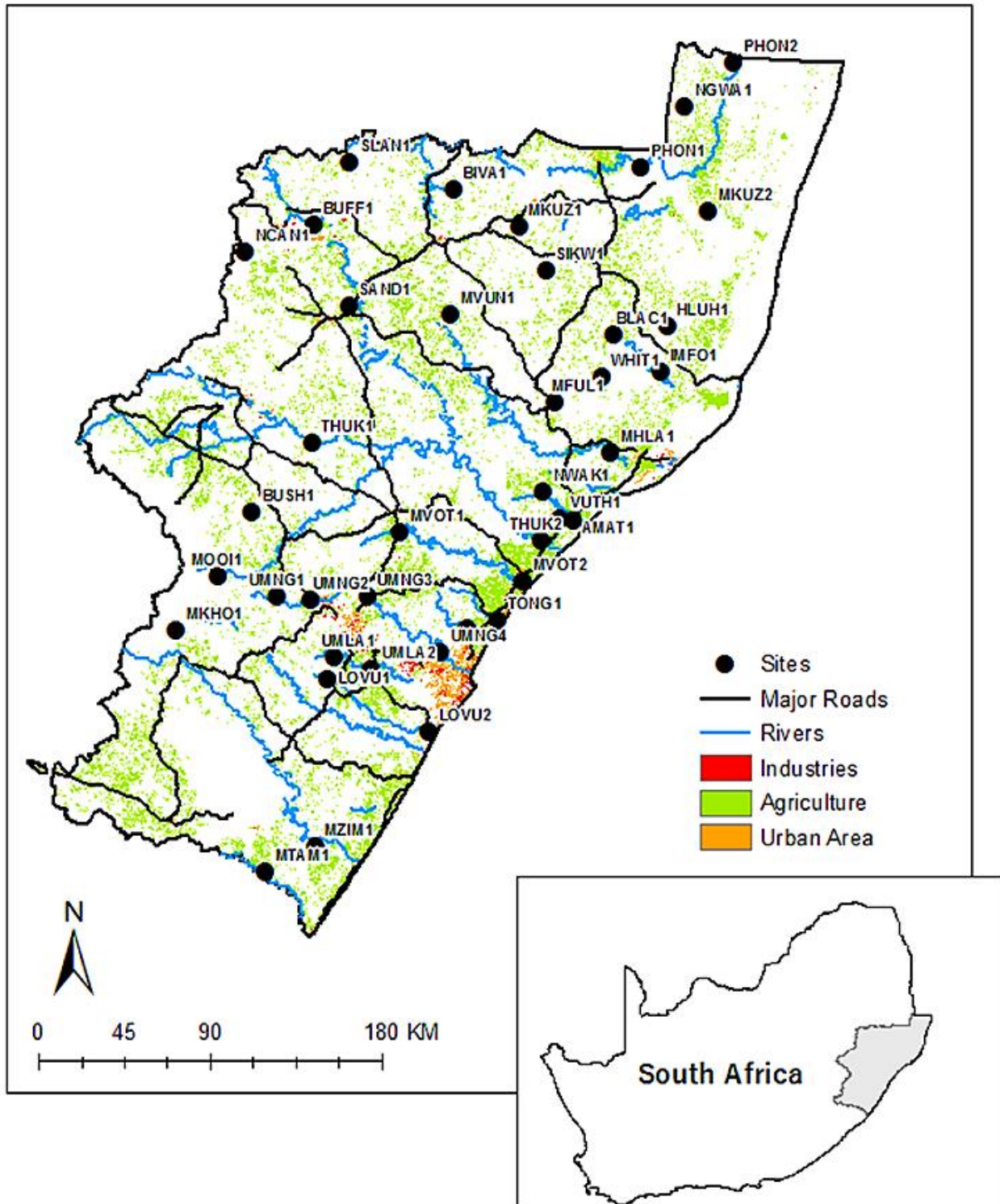
Figure 4.1: Map of KwaZulu-Natal rivers studied between 2014 and 2016. (Inset: map of South Africa).

Physico-chemical assessment index

Water and macroinvertebrate samples were collected for four times between March 2015 and April 2016 from the rivers of KZN. We collected water samples used for nutrient analysis using 500 ml sterilised clear plastic bottles, while two 1L bottles were used to collect water samples for laboratory analyses on each site per sampling occasion. The water samples were preserved in the field at 4^0C and transported to the uMgeni Water laboratory for nutrient, biological and microbial analyses. Temperature, electrical conductivity, clarity, pH and dissolved oxygen were measured with the YSI model 556 MPS handheld multi-probe water quality meter (YSI Environmental, USA).

The results obtained from the measured variables were transformed into the physico-chemical assessment index (PAI) scores for each site according to the Department of Water and Sanitation guidelines (Kleynhans *et al*., 2005; DWAF, 2008). The water quality variables for the calculation of PAI were grouped into nutrients, physical variables, biological variable, microbiological variables, toxics and complex mixtures. The nutrients were phosphate (PO_4) (mg/l) and total inorganic nitrogen (TIN) (mg/l); the physical variables were pH, clarity (cm), temperature (^{0}C), total dissolved solids (TDS) (mg/l), dissolved oxygen (DO) (mg/l) and electrical conductivity (EC) (mS/m); the biological variable was chlorophyll a (Chl-a) (µg/l); the microbiological variables were total bacteria (counts/ml), total coliform (counts/100 ml) and *Escherichia coli* (*E. coli*) (counts/100 ml); toxics and complex mixtures were ammonia (NH_4) (mg/l) and fluoride (F) (mg/l).

Macroinvertebrate sampling and identification

Macroinvertebrates were qualitatively sampled on four occasions between March 2015 and April 2016, corresponding to summer 2015, autumn 2015, spring 2015 and summer 2016. However, some lowland rivers could not be sampled during the low flows because they were either in drought. We used a kick net (30 x 30 cm^2 frame, 1000 µm mesh) to sample macroinvertebrates from the three biotopes according to the South African Scoring System v5 (SASS5) protocol (Dickens and Graham, 2002). The three biotopes were stones (stones-in and stones-out of current), vegetation (marginal and aquatic) and GSM (gravel, sand and mud). Unless otherwise stated, the

described biotopes were herein referred to as stone, vegetation and GSM. Each biotope was sampled separately and preserved in 80% ethanol. The different samples were stained in the field and transported to the laboratory for identification to the lowest possible taxonomic levels and abundance counts. The laboratory identifications were done using a compound microscope and suitable identification keys (Day *et al*., 2002; Barber-James and Lugo-Ortiz, 2003; de Moor and Scott, 2003; Stals and de Moor, 2007).

Data analysis

Macroinvertebrate traits, correlation and redundancy analyses

Prior to statistical analysis, all macroinvertebrates within each sample were sorted, identified to family level and counted using a compound microscope (Hering *et al*., 2006; Flinders *et al*., 2008). We calculated several candidate metrics for macroinvertebrate taxa based on their water quality traits, with particular consideration for the variation of KZN lowland rivers which generally have low macroinvertebrate diversity. The metrics were further scrutinized and 19 metrics were eventually selected for statistical analysis (Table 4.1). The best candidate metrics were identified through a process that included a combination of univariate and nonparametric multivariate methods using Primer v6 statistical software (Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley 2006). Spearman rank correlation was used to identify and eliminate redundant metrics (Rho = 0.65) (Clarke and Warwick, 2001; Clarke and Gorley 2006).

We used redundancy analysis (RDA) to investigate the relationship between the metric scores and environmental variables. The metric scores were initially transformed (log (x + 1)) before the RDA analysis to reduce the effects of extreme parameters that could influence the ordination. A stepwise selection procedure was used in the RDA analysis to obtain the smallest set of statistically significant macroinvertebrate metrics and environmental variables that best contribute to the explained variance in the data. We used Spearman rank correlation to explore the relationships between the macroinvertebrate metrics that were suitable for both lowland and upland river sites using Minitab 16 Statistical Software (Minitab, 2010). Significance was accepted at P < 0.05.

Table 4.1: Definitions and descriptions of selected macroinvertebrate metrics applied to KwaZulu-Natal Rivers. (Compiled from Barbour *et al*, 1994; DeShon, 1995; Hering *et al*., 2004; Baptista *et al*, 2007).

Category	Code	Description	Response to stress
Richness measure	I_Tot_Tax	Total number of macroinvertebrate taxa	Decrease
	Dip_Tax	Number of Diptera taxa	Decrease
	Moll_Tax	Number of Mollusca taxa	Increase
	EPT_Tax	Number of Ephemeroptera, Plecoptera and Trichopetera taxa	Decrease
	Coleop_Tax	Number of Coleoptera taxa	Decrease
	Trich_Tax	Number of Trichoptera taxa	Decrease
	Eph_Tax	Number of Ephemeroptera taxa	Decrease
Composition measure	%EPT	Percentage of the total number of individuals in Ephemeroptera, Plectoptera and Trichoptera taxa	Decrease
	%Chiro	Percentage of the total number of individuals in Chironomidae taxa	Decrease
	%Odon	Percentage of the total number of individuals in Odonata taxa	Decrease
	%Oligo	Percentage of the total number of individuals in Oligochaeta taxa	Increase
	%Coleop	Percentage of the total number of individuals in Coleoptera taxa	Decrease
Abundance measure	Gast_A	Total number of individuals in Gastropoda taxa	Increase
	EPT_A	Total number of individuals in Ephemeroptera, Plecoptera and Trichoptera	Decrease
	Trich_A	Total number of individuals in Trichoptera	Decrease
	Plec_A	Total number of individuals in Plecoptera	Decrease
	Oligo_A	Total number of individuals in Oligochaetae	Increase
	Chiro_A	Total number of individuals in Chironomidae	Increase
	Coleop_A	Total number of individuals in Coleoptera	Decrease

Results

Physico-chemical variables

The RDA model was used to select the best six physico-chemical variables (PAI Score, pH, clarity, EC, *Escherichia coli* and F), at a Spearman Rho value of 0.7 (Fig. 4.2). According to the RDA analysis results, the parameters that best reflect the variability in the environmental data are similar for upland and lowland sites. Physico-chemical assessment index, pH, clarity, total inorganic nitrogen and fluorine were the best water quality variables obtained from the BIOENV analysis, using the Akaike selection criterion (AICc). The RDA ordination of the physico-chemical variables explained 74.8% of fitted and 34.9% of total variation in the data on the first axis, while the second axis explained 18.0% of both fitted and 8.4% of total variation in the data.

The highest physico-chemical index (PAI) was recorded at the Mzimkhulu River catchment (MZIM1 = 100%) and the lowest score was recorded at the Phongolo River catchment (PHON = 51%) (Table 4.2). The lowest score for total dissolved solids was recorded at the Thukela catchment (MOOI1 = 38mg/l), while the highest was recorded at Matikulu River catchment (VUTH1 = 949.15mg/l). Temperature was lowest at Thukela catchment (MOOI1 = 13.13^{0}C), while it was highest at Mfolozi catchment (BLAC1 = 28.87^{0}C); pH was lowest at the Mkuze catchment (MKUZ2 = 6.01), while it was highest at Thukela catchment (THUK2 = 7.60). For the dissolved oxygen level, the lowest measurement was at Tongati catchment (TONG1 = 2. 22mg/l) and was highest at Mfolozi catchment (SIKW1 = 31.66mg/l). Clarity was highest at the Mfolozi catchment (WHIT1 = > 240 NTU), while lowest scores were recorded at Thukela (MOOI1 = < 5 NTU) and uMgeni (UMNG4 = < 5 NTU). Electrical conductivity was lowest at uMgeni catchment (UMNG1 = 82.32mS/m) and highest at Phongolo catchment (PHON2 = 1788.70mS/m) (Table 4.2).
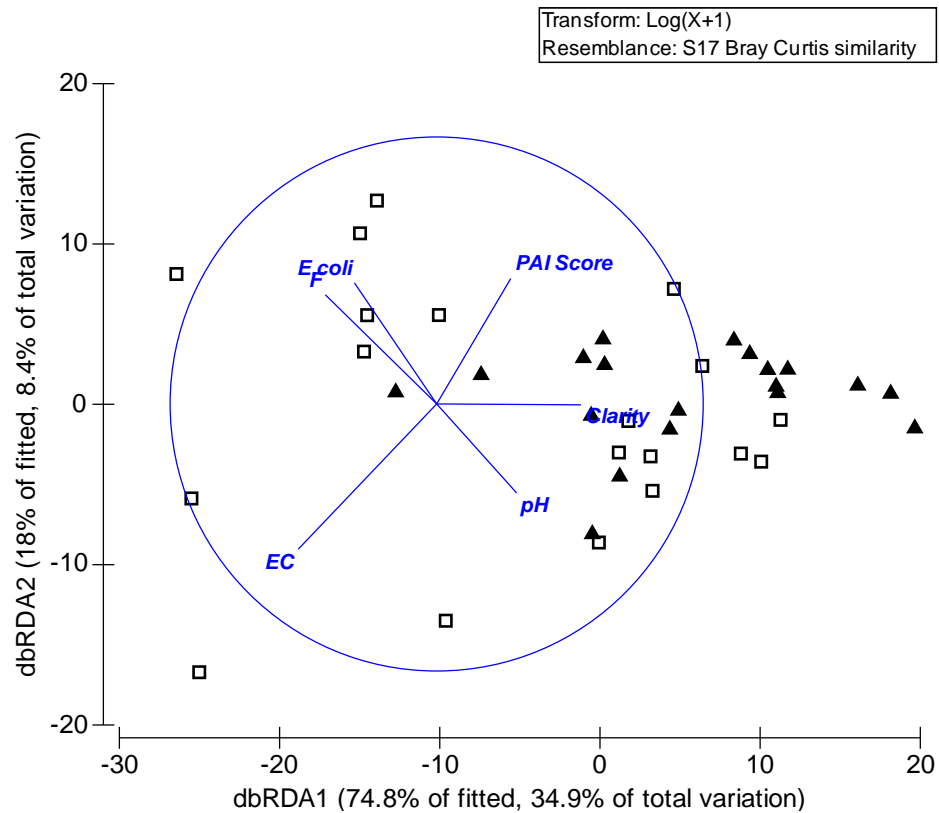
Figure 4.2: Redundancy analysis plot environmental variables measured in the rivers of KwaZulu-Natal, South Africa in 2015 - 2016. (Rho = 0.7. (*E. coli* = *Escherichia coli*, PAI Score = physico-chemical assessment index, EC = electrical conductivity, F = fluoride). (Triangles are upland rivers, while squares are lowland rivers).

Table 4.2: Means of environmental data measured in KZN rivers between 2015 - 2016. (PAI Score = physico-chemical assessment index, TIN = total inorganic nitrogen, clarity, Temp = temperature, TDS = total dissolved solids, DO = dissolved oxygen, EC = electrical conductivity, pH = hydrogen ion concentration and F = fluoride).

Site	PAI Score (%)	TDS (mg/l)	Temp (^{0}C)	pH	DO (mg/l)	Clarity (NTU)	EC (mS/m)	TIN (mg/l)	F (mg/l)
AMAT1	82	617.7	27.03	6.71	4.21	6	702.37	0	0.14
BIVA1	95	100.6	21.65	6.78	7.33	13	145.23	0.33	0
BLAC1	78	240	28.87	6.61	5.38	100	419.33	0	0.41
BUFF1	61	282.9	22.05	6.81	7.87	100	402.9	5.05	0.34
BUSH1	96	62	21.43	6.74	8.61	6	103.25	0	0
IMFO1	80	190	27.13	6.54	6.41	120	474.67	0.14	0.42
LOVU1	93	59	17.8	6.65	7.44	6	111.75	0.18	0
LOVU2	57	572.8	27.7	6.47	3.79	27	195.57	0.19	0.2
MDLO1	97	130	23.75	6.82	9.58	8	169.25	0	0.12
MFUL1	96	360	20.93	6.18	8.41	<5	273	0	0.21
MHLA1	88	230	26.27	6.53	8.68	48	316.67	0.38	0.2
MKHO1	97	44.5	16.2	6.9	9.32	<5	106.5	0	0
MKUZ1	80	465.35	20.9	7.04	5.59	8	611.13	0	0.25
MKUZ2	72	0	23.3	6.01	9.67	17	1342	0	0.37
MOOI1	97	38.5	13.13	6.73	10.33	<5	186.25	0	0
MTAM1	99	66	19.17	7.24	10.29	10	104.33	0.24	0
MVOT1	88	85	19.73	6.74	6.2	21	163	0	0
MVUN1	92	350	25.73	7.19	6.8	48	507.17	0.22	0.36
MZIM1	100	87.67	21.77	7.38	9.12	13	204	0	0
NCAN1	95	651.84	18.3	6.61	7.39	6	130	0	0
NGWA1	64	754	27.55	6.03	4.33	11	1107.5	1.01	0.66
NWAK1	67	176.8	24.33	6.32	7.46	8	229.73	0	0
PHON1	89	767.5	26.37	7.25	11.87	15	355.85	0.43	0.43
PHON2	51	475.4	27.05	7.16	5.02	19	1788.7	0.34	0.34
SAND1	55	546.8	19	6.79	5.45	10	483.36	5.4	0.29
SIKW1	97	230.15	23.85	7.1	31.66	6	268.68	0.12	0.29
SLAN1	95	81.98	15.58	6.59	7.89	6	120.7	0	0
THUK1	93	106	24.3	7.09	8.52	60	165.35	0.38	0
THUK2	97	178.2	25.95	7.6	7.95	84	213.85	0	0.15
TONG1	54	418.6	22.88	6.54	2.22	10	404.23	8.81	0.14
UMLA1	85	64.5	22.93	7.09	7.42	11	116.25	5.29	0
UMLA2	86	261.35	19.93	6.77	7.21	7	478.63	0	0.21
UMNG1	86	42.77	15.15	6.36	9.86	6	82.32	0.23	0
UMNG2	92	54	19.7	6.77	8.24	12	221.96	0.15	0
UMNG3	94	86.38	22.58	6.45	8.27	11	206	0.52	0
UMNG4	96	205	22.33	7.54	10.75	<5	311.25	0	0.16
VUTH1	74	949.15	27.27	6.65	6.48	14	1014.93	0	0
WHIT1	96	160	24.4	6.51	8.38	>240	334.67	0	0.47

Macroinvertebrate traits and water quality

The macroinvertebrate metrics in this study responded to the physico-chemical variables as predicted (Table 4.1) and these were validated by correlation analysis (Table 4.3). Eleven macroinvertebrate metrics had general discriminatory abilities in both upland and lowland rivers; and nine of these had strong correlations with physico-chemical variables. These metrics were total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, percentage of Odonata taxa, total number of Trichoptera individuals, total number of Gastropoda individuals, total number of Oligochaeta individuals and total number of Coleoptera individuals. (Fig. 4.3). The Principal Coordinate Analysis (PCO) ordination explained 52.0% of total variation in the data on the first axis, while the second axis explained 20.2% of total variation in the macroinvertebrate metrics (Fig. 4.3). The PCO gradients of the macroinvertebrate metrics gave indications of good water quality from the least impacted upper river reaches and increasing impairment towards the downstream sites.

The first axis of the PCO ordination plot revealed a correlation with pollution and habitat quality. Most of the sites on the first axis were the sand dominated lowland rivers of which some are affected by periods of droughts and high anthropogenic impacts, especially physical habitat degradation and agricultural practices. Percentage of Odonata taxa (%Odon) was strongly correlated with the lowland sites, which showed that Odonata families were abundant in the lowland rivers. Also, Gastropoda was positively correlated with temperature. A high abundance of Chironomidae taxa was recorded at a site below the effluent discharge point of a paper conversion industry.
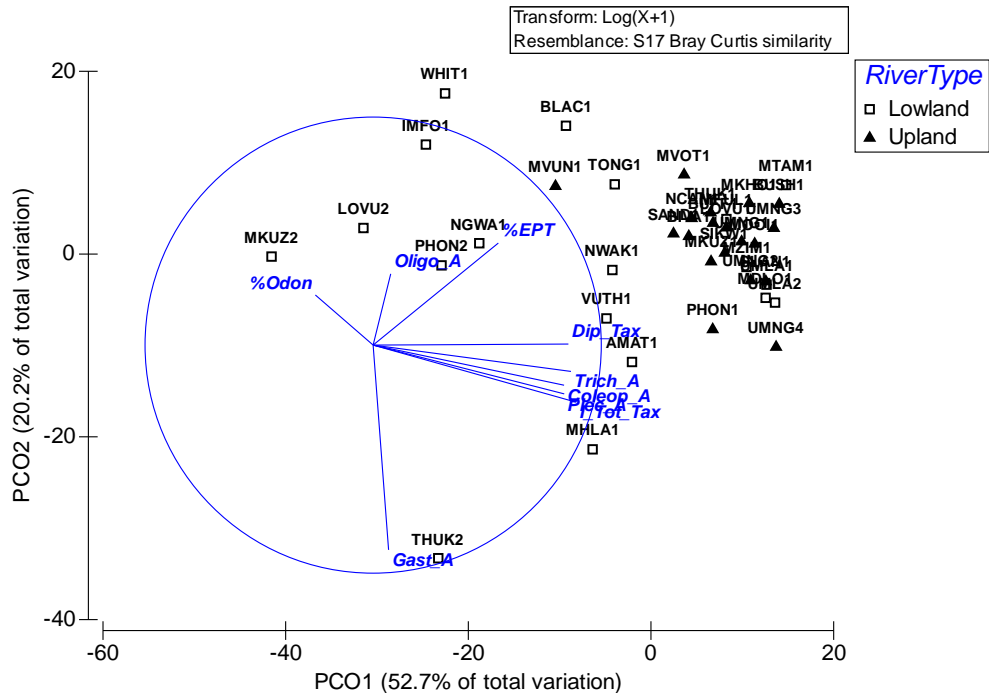
Figure 4.3: Principal coordinate analysis plot of macroinvertebrate metrics sampled in rivers of KwaZulu-Natal in 2015 - 2016. (I_Tot_Tax = total number of taxa, Dip_Tax = total number of Diptera taxa, Plec_A = total number of Plecoptera individuals, %EPT = percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, %Odon = percentage of Odonata taxa, Trich_A = total number of Trichoptera individuals, Gast_A = total number of Gastropoda individuals, Oligo_A = total number of Oligochaeta individuals and Coleop_A = total number of Coleoptera individuals).

Table 4.3: Spearman's correlations between mean water quality data and the mean macroinvertebrate metrics measured from KwaZulu-Natal Rivers in 2015 – 2016. (PAI Score = physico-chemical assessment index, TIN = total inorganic nitrogen, clarity, Temp = temperature, TDS = total dissolved solids, DO = dissolved oxygen, EC = electrical conductivity, pH = hydrogen ion concentration and F = fluoride).

	PAI Score (%)	TDS (mg/l)	Temp (^{0}C)	pH	DO (mg/l)	Clarity (NTU)	EC (mS/m)	TIN (mg/l)	F (mg/l)
I_Tot_Tax	0.617**	-0.301	-0.587**	0.282	0.335**	0.623**	-0.552**	-0.162	-0.597**
Dip_Tax	0.497**	-0.363*	-0.738**	0.229	0.247	0.680**	-0.569**	0.084	-0.507**
Gast_A	0.152	0.161	0.339*	0.315	-0.003	-0.112	0.007	-0.132	0.012
Plec_A	0.410*	-0.378*	-0.207	0.159	0.166	0.161	-0.324	-0.072	-0.378*
%EPT	0.509**	-0.543**	-0.525**	0.062	0.224	0.289	-0.551**	-0.163	-0.374*
%Odon	-0.114	-0.146	0.29	-0.305	0.231	-0.286	0.360*	-0.216	0.403*
Trich_A	0.296	-0.306	-0.508**	0.234	0.189	0.514**	-0.267	0.04	-0.269
Oligo_A	-0.248	0.292	-0.131	-0.106	-0.184	-0.029	-0.02	0.451**	-0.097
Coleop_A	0.368*	-0.398*	-0.514**	0.236	0.101	0.435**	-0.385*	-0.021	-0.517

* $p < 0.05$, **$p < 0.01$

Discussion

The physico-chemical parameters indicated loss of ecological quality or integrity of downstream sites. The impacts on water quality include natural (flood and drought) and anthropogenic impacts (sand mining, agricultural practices, etc.), with the highest impacts occurring downstream, especially those located within agricultural land uses. Five (total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, and total number of Coleoptera individuals) out of the nine final metrics in our study showed significant positive correlations with high PAI scores, and the high PAI scores were reflections of good water quality and that the five metrics increased with improvement in overall water quality. The high scores were obtained from the least impacted or reference sites of the study, while low PAI scores were observed at the impaired sites. The additive or synergistic effects of the PAI components may cause unfavourable conditions for the survival and abundance of sensitive macroinvertebrate taxa at the impacted or polluted sites (Chen and Lu, 2002; Laskowski *et al*., 2010). According to our initial classification of the environmental variables, the

macroinvertebrate metrics were able to detect physical variables (total dissolve solids, temperature, dissolved oxygen, clarity and electrical conductivity), nutrient pollution (total inorganic nitrogen) and toxic pollutant (fluorine). Humans and organisms are often exposed to isolated micropollutants and complex chemicals in their environments or ecosystems (Richardson, 2009; Pal *et al*., 2010). The individual components of these micropollutants and their complex compounds may be harmless at low concentrations (Schwarzenbach *et al*., 2006; Eggen, 2014; Luo *et al*., 2014), however, they may have additive or synergistic effects that can increase their toxic potentials (Heberer, 2002; Schwarzenbach *et al*., 2006).

Our results indicated elevated levels of total inorganic nitrogen at the sites in close proximity of agricultural lands (e.g. TONG1). Elevated total inorganic nitrogen loads are reported to cause nutrient enrichment (eutrophication) and acidification when combined with other chemicals such as phosphorous or ammonia (Schindler *et al*., 1985). Inorganic nitrogen can form compounds with phosphorus to cause eutrophication independently or with acidification (Schindler *et al*., 1985), resulting in loss of biota diversity (Schindler, 1994). Nutrient enrichment from anthropogenic activities has observable impacts on the health of aquatic ecosystems (Wang *et al*., 2007). Organisms that have physiological adaptations to low dissolved oxygen levels can increase in abundance by making use of excess nutrients (Camargo and Alonso, 2006; Bayene *et al*., 2009). High nutrient enrichment may increase primary productivity, oxygen depletion and production of toxic algal blooms (Shiklomanov, 1997). Some of the agricultural practices around the study sites include livestock production, which may increase nutrient runoffs to streams directly (through faecal matter) or indirectly (habitat alteration) (Justus *et al*., 2010).

Fluorine is a very reactive element that does not exist in its natural elemental state, and it may exist in the form of inorganic fluorides or as organic fluoride compounds (e.g., fluorocarbons) (Camargo, 2003). Inorganic fluorides often remain in solution as fluoride ions under low pH conditions inside water (CEPA, 1994). Fluoride ions have enzymatic abilities, which makes them toxic to aquatic and terrestrial biota, for example, the effects of fluoride on algae depends on the concentration, duration of exposure and the algal species (Joy and Balakrishnan, 1990; Rai *et al*., 1998; Camargo, 2003). The level of fluoride toxicity to aquatic invertebrates depends on the concentration, exposure duration and water temperature (Camargo and Tarazona, 1990; Camargo,

2003); thus they can act as inhibiting enzymes by interrupting their metabolic processes (e.g. glycolysis and protein synthesis) (Aguirre-Sierra *et al*., 2013; Ghosh *et al*., 2013; Rani and Naik, 2014).

Water and food contamination with faecal bacteria is a common and persistent problem affecting public health, as well as local and national economies (Stewart *et al*., 2007). The detection of high *E. coli* bacteria in some of our river sites indicated fecal pollution in KZN rivers. Bacterial coliform counts are indicative of faecal contamination, implying poor sanitary conditions (Banwart, 2004). The presence of bacterial coliforms indicated pollution from sewage sources (Edema *et al*., 2001). In this study, the high levels of *E. coli* coliforms detected in the lowland rivers may have been an effect of elevated levels of organic pollution through the faeces of grazing animals in the riparian zone. Majority of the lowland rivers of KZN are located within water stressed or drought ridden northern areas, hence lots of livestock were observed to graze within the riparian zones. Faecal depositions in riparian zones by grazing livestock have been observed to be higher than in pastures that are farther away from rivers (James *et al*., 2007; Bagshaw *et al*., 2008). The trampling of the riparian zone by livestock also impacts on habitat variables, which indirectly influence the biotic integrity of the system (Miltner, 1998; Maret *et al*., 2010). Overgrazing and trampling of the riparian zone can increase nutrient runoff (Zaimes *et al*., 2008). The pollution through organic source may have been the cause for the observed low pH values (Udom *et al*., 2002).

Turbidity (measured as clarity in this study) indicated the amount of particles suspended in water and its high concentrations reduce the habitat quality for aquatic organisms (Said *et al*., 2004). Agricultural wastes, urban runoffs, industrial effluents and domestic wastes contribute to organic pollution of rivers (Singh *et al*., 2005). Increased turbidity in the downstream river site reduced light availability for photosynthetic organisms. Low water clarity affects light penetration, productivity and habitat quality, increased sedimentation and siltation (Wagner *et al*., 2006). Sedimentation and siltation can cause harm to habitat areas for macroinvertebrates and other aquatic life (Ryan, 1991; Novotny *et al*., 2005). Sediment particles also provide attachment for other pollutants (mostly metals and bacteria) (Jiang *et al*., 2009; Wang and Chen,

2009; Mohanty *et al*., 2013). For this reason, turbidity readings are good indicators of potential pollution in a water body (Wagner *et al*., 2006).

Taxa-specific indicators refer to the abilities of specific macroinvertebrate taxa to adapt to certain water quality level, but may not be able to survive in other water quality levels ((Xu *et al*., 2014; Parr *et al*., 2016). For example, species of Oligochaeta and Gastropoda taxa are indicators of organic pollution (Masese *et al*., 2009); Chironomidae are tolerant and can survive in highly polluted water conditions (Al-Shami *et al*., 2010); Annelida is affected by high metal concentrations (Pauwels *et al*., 2013). Elevated levels of pollutants are harmful to aquatic biota, thereby reducing their biodiversity to only the tolerant species (Jackson *et al*., 2016). In our study, hydrology, substrate/habitat availability, seasonal variations (aggravated by periodic flood and drought) and human impacts (e.g. sand mining) limited the macroinvertebrate metrics in KZN lowland rivers.

Oligochaetes and Diptera dominate in polluted water with high concentrations of organic materials and nutrients, but other species cannot survive (Arimoro and Ikomi, 2008; Ikomi and Arimoro, 2014). In our study, the positive correlation between the abundance of Oligochaeta taxa and nutrient enrichment suggested that Oligochaeta taxa increased with an increase in nutrient enrichment. The implication of high inorganic nitrogen in our study indicated that KZN rivers are susceptible to increased productivity from eutrophication, especially at the sites close to agricultural production, which increases oxygen consumption in them and can subsequently lead to low-oxygen (hypoxic) or oxygen-free (anoxic) water bodies (Wang and Widdows, 1991; Welker *et al*., 2013). Both hypoxic and anoxic conditions can lead to fish kills and alteration of ecological structures and function, including low biotic diversity and reduced fish productivity (Camargo and Alonso, 2006; Adams *et al*., 2016).

Members of the Ephemeroptera are sensitive to environmental stress and their presence signifies relatively good conditions of the ecosystem (Fialkowski, 2003). Ephemeroptera larvae are generally microhabitat specialists and they can survive on specific substrates with a certain amount of wave action (Bustos-Baez and Frid, 2003). They are known to burrow into soft areas with shallow flows or in areas of of high sediment depositions (Azrina *et al*., 2006). Therefore, the shallow nature of the lowland rivers in this study could be the factor

contributing to their relative abundance of the Ephemeroptera taxa. The low combined abundance of sensitive macroinvertebrate taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT) in the lowland rivers was not only caused by pollution but was also as a result of the reduced habitat heterogeneity.

Although the families of Odonata taxa were relatively more widespread than other taxa in the sand dominated lowland rivers of KZN during this study, their species richness is being threatened by anthropogenic impacts (Steward and Samways, 1998). Odonata members are sensitive to habitat disturbances and pollution (Adu *et al*., 2015). They have been widely used as indicator of wetland ecosystem quality and for biodiversity studies (Villalobos-Jiménez *et al*., 2016). The abundance of the Odonata larvae in this study at the least impacted sites may be attributed to their relative insensitivity to pH, as evident in our correlation analysis which showed a negative non significant correlation of these taxa with pH (Rychła *et al*., 2011). Our study further revealed a positive significant correlation of the Odonata taxa with electrical conductivity, although some researchers have reported their non significant sensitivity to electrical conductivity (Al Jawaheri and Sahlén, 2017). This observation agrees with the findings of Canning and Cannings (1994) which inferred that Odonata species seems to respond more to habitat form and structure than to its acidity and or general nutrient level.

Although Coleopterans are known to be sensitive to pollution in the aquatic ecosystem, they are also known to possess physiological and behavioural mechanisms that enable them to survive harsh environmental conditions (Nilsson, 2003). These traits may allow them to avoid the deep water habitats that commonly support relatively large and strong predators (Kang and King, 2013). As such they are generally abundant in freshwater bodies. Their ability to survive diverse environmental conditions might explain why they had negative correlations with temperature, electrical conductivity, total dissolved solids and fluorine in this study, which could have favoured their abundance in the rivers of KZN.

Gastropoda have been found to be temperature tolerant (Johnson *et al*., 2015). The significant positive correlation of Gastropoda with temperature in this study confirms their tolerance of thermal pollution, which could have resulted in their high abundance in some of our study sites. Also, Chironomidae was highest at a site below the effluent discharge point of a paper

conversion industry and this is indicative of severe pollution at the site, but no significant correlation was detected between their occurrence and water quality in this study.

Conclusions

The sensitivities of different macroinvertebrate taxa to pollution are often dependent on their life history attributes and feeding behaviours (Luiza-Andrade *et al*., 2017) and consequently different species have considerably different water quality tolerances (Arimoro and Ikomi, 2008; Ikomi and Arimoro, 2014). In this study, we found that patterns of species distribution only give a little understanding of ecosystem functions, but probing the ecosystem processes (e.g. nutrient dynamics) may prove more useful (Harris, 1994). The application of macroinvertebrate ecological trait indices is cheap and provides accurate information about many stressor types and their effects on the river ecosystem. In our study, the use of macroinvertebrate traits approach (majorly at family level of identification) proved to be a useful tool for aquatic ecosystem assessment in KZN rivers. We, therefore, recommend that seasonal variations and factors driving the macroinvertebrate communities to be studied in more detail, as this could help in the development of reference conditions for the application of macroinvertebrate community-based metrics in the region. Also, establishing riparian buffer zones can contribute to erosion control and reduce nutrient runoff from agricultural lands (Novara *et al*., 2013; Bouraoui and Grizzetti, 2014). A suitable buffer serves as a natural filter, which reduces nutrient pollution, sedimentation and chemicals that enter a river and protect the river banks from erosion (Barling and Moore, 1994; Walter *et al*., 2009).

Although it may be difficult to distinguish natural variations in diversity and community composition from the effects of anthropogenic activities, the consistent pattern of taxa composition by a single or only a few taxa at downstream sites indicated impacts from agriculture, nutrient enrichment and drought (Göthe *et al*., 2015). The differences detected when comparing upstream and downstream sites imply that monitoring of macroinvertebrate community composition is useful for assessing management practices and gives an insight into development of a more efficient monitoring of the lowland rivers (Helson and Williams, 2013). Due to the high ecological relevance of macroinvertebrate community composition in biomonitoring, we recommend that

more research is needed to explore the specific tolerance of macroinvertebrates to different chemicals or toxicants impacting their wellbeing in aquatic systems.

Acknowledgements

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CHAPTER 5

Ecological risk of water resource use to the wellbeing of macroinvertebrate communities in the rivers of KwaZulu-Natal, South Africa

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Abstract

The rivers of KwaZulu-Natal, South Africa, are being impacted by various anthropogenic activities that threaten their sustainability. Our study demonstrated how Bayesian networks could be used to conduct an environmental risk assessment of macroinvertebrate biodiversity and their associated ecosystem to assess the overall effects of these anthropogenic stressors in the rivers. We examined the exposure pathways through various habitats in the study area using a conceptual model that linked the sources of stressors through cause-effect pathways. A Bayesian network was constructed to represent the observed complex interactions and overall risk from water quality, flow and habitat stressors. The model outputs and sensitivity analysis showed ecosystem threat and river health (represented by macroinvertebrate assessment index – MIRAI) as the top factors determining risk to macroinvertebrate biodiversity and the ecosystem respectively. The results of this study demonstrated that Bayesian networks can be used to calculate risk for multiple stressors and that they are a powerful tool for informing future management strategies for achieving best management practices and policy making. Apart from the current scenarios which were developed from field data, we also developed three other scenarios to predict potential risks to our selected endpoints. We further simulated the low and high risks to the endpoints to demonstrate that Bayesian network can be an effective adaptive management tool for decision making.

Keywords: Bayesian networks, ecological risk, macroinvertebrates, multiple stressors

Introduction

Water as a natural resource is essential to life, the environment, industrial growth, development, food production, hygiene, sanitation and power generation (Rast, 2009; DWA, 2010). River systems also provide many goods and services upon which society depends, such as maintaining the habitat and integrity of aquatic organisms, transportation of sediment, recreational and eco-tourism centres, disposal sites for effluent and solid wastes (DWA, 2010). Global use of freshwater and its vast resources increased by 10% from 2000 to 2010 due to increase in population growth and economic development (Vörösmarty *et al.,* 2010). These anthropogenic demands on freshwater ecosystems have enormous threats to biodiversity around the world (Dudgeon *et al*., 2006; Richardson *et al*., 2007), through various contaminants which may be chemical, physical, radioactive or pathogenic in nature and may be from multiple sources, including industrial effluents, agricultural run-off, domestic sewage, construction and mining activities (Richardson *et al*., 2007).

Risk assessment is a method used to calculate the probability of the impacts of an unwanted effect on a set of predefined assessment endpoints over a period (Suter, 1993; Walker *et al*., 2001; Landis and Wiegers, 2007; Hines and Landis 2014). Ecological Risk Assessment (EcoRA) is a systematic method of describing and explaining scientific facts, laws and relationships to provide a sound basis for developing adequate protection measures for the environment (US EPA, 2008). A relative risk model (RRM) is a cause and effect model used in the calculation of risks to assessment endpoints due to multiple stressors having impacts on the endpoints of a system or habitat (Landis and Wiegers, 2005). The RRM methodology is an improved and expanded version of the traditional three-phase risk assessment method which involves problem formulation, risk analysis and risk characterization. Landis and Wiegers (1997) developed a framework called the regional-scale ecological risk model for ranking and comparing the risks associated with multiple stressors and this is a useful tool for describing and comparing risks to valued resources (endpoints) within a catchment or region. Risk assessment at a regional scale involves the assessment of multiple habitats with multiple sources of multiple stressors affecting multiple endpoints at a large spatial coverage (Hunsaker *et al*., 1989; Landis and Weigers, 1997). While the traditional risk assessment often has only one endpoint, the regional risk methodology usually has multiple

endpoints (Walker *et al*., 2001). Various stressors impinge on the quality of the environment within any region, and the assessment of these stressors may be bias if there is no objective framework for the evaluation of the risks associated with the stressors (Linkov *et al*., 2006). At the regional scale, considerations of multiple sources of multiple stressors affecting multiple endpoints are allowed (Landis, 2005), because there are often sources for a single stressor (Liu *et al.*, 2010). Also, a regional scale risk assessment allows for landscape characteristics which may affect the risk estimates of the region (Landis, 2005). However, it is difficult to measure, test, model or assess all the components of the environment at a regional scale and the difficulty arises from the high degree of spatial and temporal variability of the regional components (Suter, 1993). The typical impacts considered in risk assessment are mortality, chronic physiological impacts and reproductive effects (Walker *et al*., 2001).

Although the RRM method was initially applied to assess the risk of chemical stressors, it has been successively used in the assessment of non-chemical stressors; such as biological (invasive species) stressors, physical (habitat loss, stream alteration and blockage, land use change) stressors and natural events (climate change) (Moraes *et al*., 2002; Colnar and Landis, 2007; Landis and Wiegers, 2007; O'Brien and Wepener, 2012). Also, the RRM has been adapted to suit a variety of habitats (e.g. freshwater, marine and terrestrial) (Chen and Landis, 2005) and different regions of the world such as South America (Moraes et al., 2002), North America (Colnar and Landis, 2007), South Africa (O'Brien and Wepener 2012), China (Li *et al*., 2015) and Australia (Heenkenda and Bartolo, 2016). A Bayesian network (Bayes Net or BN) is a graphical model that encodes the probabilistic relationships among sources of stressors, habitats and endpoints to estimate the likely risk outcomes through a web of nodes (McCann *et al*., 2006). Bayesian network relative risk model (BN-RRM) is a relative risk model where the linkages between the conceptual models are described by using a Bayesian network (Ayre and Landis, 2012).

Our study aimed to conduct a regional ecological risk assessment of stressors in the rivers of KwaZulu-Natal (KZN) Province, South Africa, to macroinvertebrate biodiversity and ecosystem protection (endpoints) using the BN-RRM approach. We established three objectives in this study. The first objective was to develop a RRM to estimate the relative contribution of risk from stressors to the selected ecological endpoints. The second objective was to determine which

regions and endpoints were at high risk from anthropogenic activities. The third objective was to incorporate one hundred percent (100%) low risk to the endpoints (representing pristine condition or before urbanization and industrial development) into the model to evaluate the relative risk impacts of the sources and habitats to the selected endpoints. We expected this study to give an insight into the threats from the land use types of KZN, reveal their probable risk and lay the foundation for regional ecological risk assessments of the freshwater resources of KZN.

Study area

KZN Province of South Africa was selected for this study and is located within the eastern escarpment drainage region of South Africa, containing four of the 22 primary drainage regions of South Africa, either wholly or partially (Midgley *et al*., 1994). The mean annual rainfall (MAR) is approximately 28.5% (14 800 x 106 m^3a^{-1}) of the national MAR (about 52 000 x $106m^3a^{-1}$) (Rivers-Moore *et al*., 2007) and is drained by the major river systems in the province. Each of the major rivers flows through distinct longitudinal patterns, although they typically exhibit a distinct escarpment zone, with flatter mid-slopes and steep eastern coastal regions (Rivers-Moore *et al*., 2007). In this study, we chose a total of 39 KZN river sites and each site represented a risk region (RR) (Fig. 5.1), based on their sub-quaternary catchments, proximity to risk sources, habitat characteristics and ecological endpoints. The highest (5th) river order in KZN is the Thukela; other long river systems (4th order streams) are the Phongolo, Buffels and Mzimkhulu Rivers (Rivers-Moore *et al*., 2007). The uMvoti and Mhlatuze catchments have the highest drainage densities, the southern KZN regions (Mzimkhulu, Mkomazi and uMgeni catchments) also have relatively high drainage densities, while the northern coastal Zululand regions (Mkuze River and Phongola catchment) have the lowest drainage densities (Rivers-Moore *et al*., 2007). The uMgeni River catchment, spanning 4418 km^2 is reputed to be one of the most reliable large rivers of South Africa (Van der Zel, 1975) and it has five large dams located on its course for domestic water supplies.
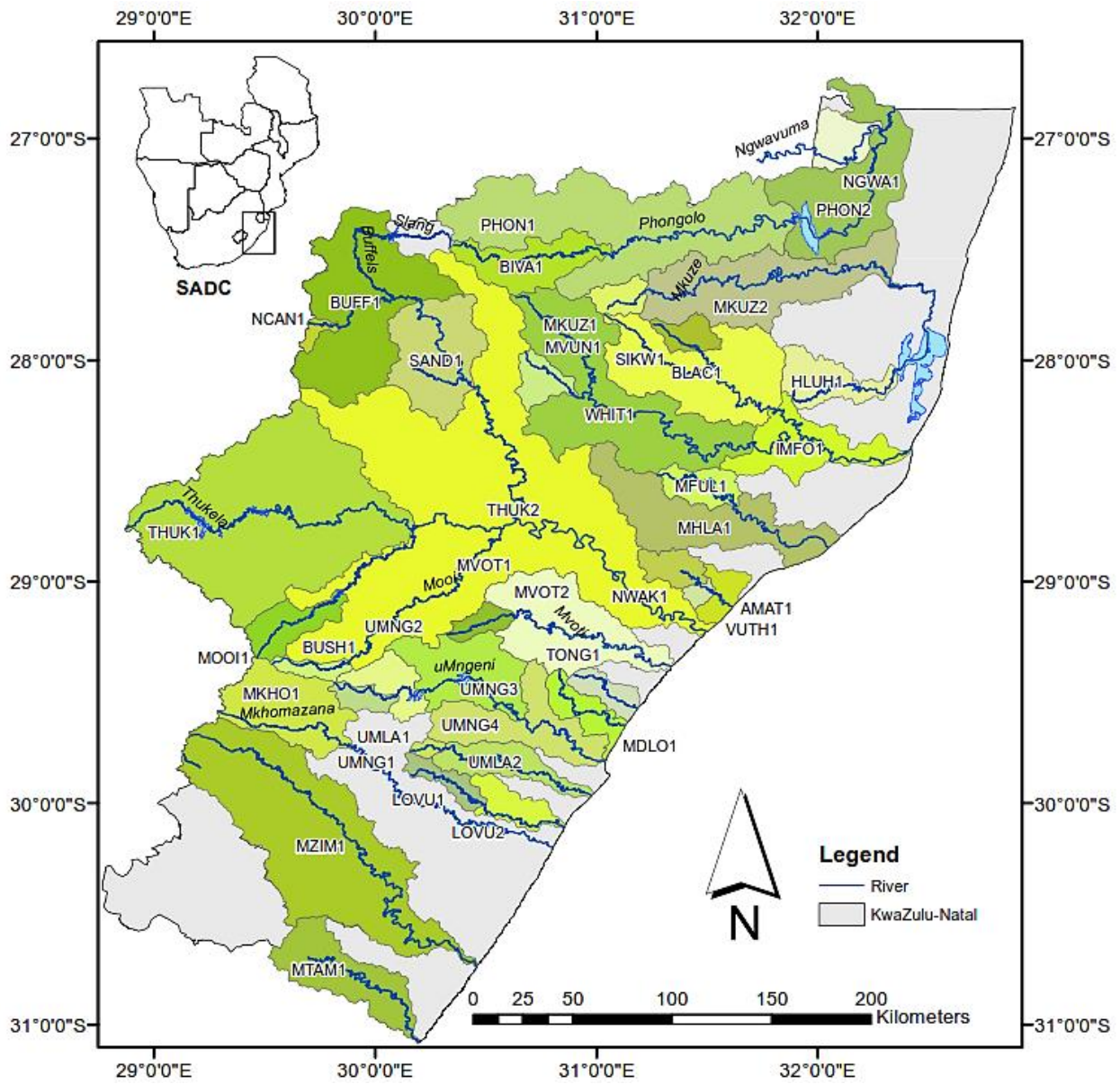
Figure 5.1: Risk assessment regions of KwaZulu-Natal, South Africa, from 2015 to 2016 (Inset: map of Southern Africa Development Countries (SADC)).

Methods

Our study was conducted using the relative risk model (RRM), which is made up of three main phases: problem formulation, risk analysis and risk characterization (Landis and Wiegers, 1997; 2005).

Problem formulation

This is the information gathering phase of a risk assessment to determine what is at risk (e.g. plants, animals, humans, etc.) and what resources need to be protected (e.g. species of interest, habitat, etc.) (Norton *et al*., 1992). This is also the phase that the chemical, physical and biological characteristics of the study area are outlined, the stressors are identified, the endpoints derived from the region's ecological values, the risk areas are defined and the conceptual model is formulated (O'Brien and Wepener 2012).

Risk sources and stressors

A source is an entity that releases a single or multiple stressors to the environment (e.g. industrial waste of effluent) or the action that produces stressors (US EPA, 2008), while stressors are the physical, chemical or biological substances that can cause an adverse effect (US EPA 2008). Our study focused on the stressors that are influenced or generated by anthropogenic activities and natural events within the study area (Hua *et al*., 2017). The major land use types within these risk regions include conservation/protected lands, forestry, agriculture, urbanization and industrialization. Ecological risk sources relating to the rivers of KZN were grouped into six major categories in this study to describe the effects of their water resource utilization on the selected risk regions. The categories were industrialization (manufacturing, mining and forestry), agriculture (sugarcane, commercial and subsistence farming), natural vegetation, settlements (rural and urban) and construction (roads, rails and dams). All these sources of threats to our endpoints have varying degrees of stress being exerted on the risk regions (Liu *et al*., 2010; Bednarek *et al*., 2014; Lu *et al*., 2015; Mekonnen *et al*., 2016). The stressors evaluated in this study were water quality alteration/abstraction, habitat alteration and flow alteration.

Assessment endpoints and habitats

Assessment endpoints can be made up of a receptor and an attribute (e.g. macroinvertebrate biodiversity as in this study) (US EPA, 2008). The receptor is the biological or ecological component that is exposed to the stressor, while the attribute is the important characteristic of the ecological component to be protected (Hua *et al*., 2017). The assessment endpoints should not only be the characteristics of the receptors and aims of the assessment, but they should also be quantitative measurements of the possible degrees of the impacts to the receptors (Hua *et al*., 2017). For this study, we chose biodiversity and general risk to ecosystem wellbeing or ecological integrity as endpoints. The risk endpoints were chosen to represent the exposure of sources or stressors to the endpoints represented as ecosystem threats and the potential for the endpoints within each region represented as ecoregions and ecological integrity. Ecoregions represent the potential for habitat, which determines the increase or decrease in the diversity of macroinvertebrates. River health provides the indications of existing responses of macroinvertrebrates to the drivers of the ecosystem. Ecosystem threat represents the potential for instream and riparian habitat wellbeing. The instream habitat was selected to represent water quality, flow and habitat stressor states of the risk regions, while riparian habitat was selected to represent the physical habitat structure and the vegetation response assessment index (VEGRAI) of the risk regions.

Risk calculation and simulation

The evidence used in our assessment was obtained from field assessments between September 2014 and March 2016. The RRM was used to develop a conceptual model, which was used to represent the hypothetic relationships between the sources of stressors, stressors, the ecological components (habitats and receptors) and their associated endpoints (Landis and Weigers, 2005; US EPA, 2008) (Fig. 5.2A). The conceptual model was used as the template for developing the BN-RRM using Netica software (Norsys Software Corp., 2014) (Fig. 5.2B). Our RRM was based on a ranking of the stressors and the habitats to generate possible outcomes of their impacts on the ecological receptors and the assessment endpoints (Landis and Weigers, 2005). The ranking was based on the relative magnitude or impact of each stressor and habitat using the quantitative and qualitative data obtained during the study period. The ranks were zero, low, moderate and high,

having 0 – 25%, 26 – 50%, 51 – 75% and 75 – 100% scores respectively; where zero represented no risk, low is the very minimal or negligible risk, moderate was the moderate risk and high was the highest risk of the stressors to the endpoint (S1 Table 4.1). After calculating the risks for the present scenario in the thirty-nine risk regions, three alternative scenarios were proposed and the risks were calculated for each scenario, endpoint and risk region. Scenario 1 represented a low flow situation, scenario 2 represented impacts of limited or degraded habitat, while scenario 3 represented a situation of high water quality degradation. Furthermore, a 100% low risk to the endpoints was simulated in order to characterise the impact of each of the assessment inputs on the risk to the endpoints in each region.
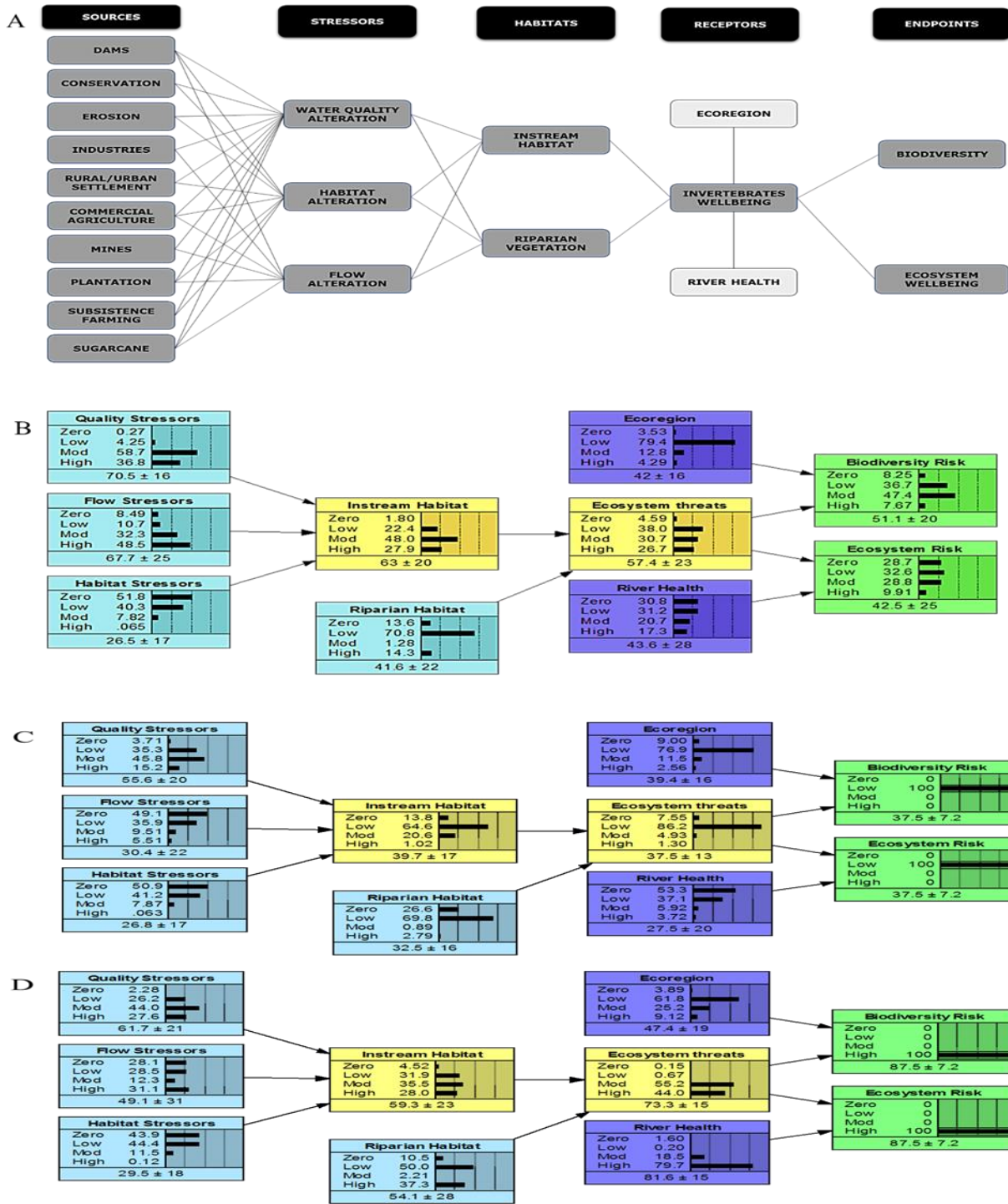
Figure 5.2: A = conceptual model showing linkages between sources, stressors, habitats, receptors and assessment endpoints, while B = Bayesian Network Relative Risk Model, using AMAT1 risk region as an example, C = 100% low risk to endpoints simulation, and D = 100% high risk to endpoints simulation

Uncertainty analysis

From a management perspective, uncertainty is defined as the lack of exact knowledge or assessment confidence, regardless of the cause of the deficiency (Refsgaard *et al*., 2007). Uncertainty is an inevitable factor in ecological risk analysis and this can be analyzed using various tools, such as conceptual models, interval and sensitivity analysis, Monte Carlo simulation, Bayesian networks and decision trees (O'Brien and Wepener 2012; Chen and Liu, 2014). Monte Carlo Simulation tests and Bayesian Networks are the most used of the tools in analysing uncertainty and variability in risk parameters selection and data for stressor–response and exposure models (Hua *et al*., 2017). We linked our causal (sources) probabilistic nodes or networks using conditional probability tables (CPTs), through continuous probability density functions (PDFs) to simulate uncertainties using Monte Carlo tests (Janssen, 2013; Farrance and Frenkel, 2014). To reduce uncertainties in our input data, we used Crystal ball® software in Microsoft Excel® 2013, to run Monte Carlo tests on the risk sources (water quality, flow and habitat stressors) data. Then an entropy reduction was calculated in BN to further reduce the uncertainties by using the "Sensitivity to Findings" tool in Netica (Norsys Software Corp.) (Ayre and Landis, 2012). Entropy reduction is the level of influence an input variable has on a response variable, which means that the greater the entropy value, the greater the degree of influence (Marcot, 2006). We used the sensitivity analysis information for the endpoint variables to determine the input parameters that had the greatest influence on risk estimates and the associated uncertainty (Ayre and Landis, 2012; Landis *et al*., 2017).

Results

Risk calculation and distribution patterns

Our BN approach allowed us to combine empirical data with our expert opinion and scientific literature to construct the CPTs; thus the structure of our BN model revealed our hypothesized understanding of underlying causal relationships, which are not always clear in traditional risk assessments or complex ecological models (Ayre and Landis, 2012).

Our preliminary analysis of the risk sources data showed three regions had high risks of water quality stressors (AMAT1, BUSH1 and SIKW1), with BUSH1 having the lowest score

(26%) and SIKW1 had the highest score (50%). For the flow stressor, ten regions had high risks (BIVA1, BLAC1, BUFF1, HLUH1, IMFO1, LOVU1, MDLO1, MKHO1, MVOT12 and TONG1), with BUFF1 having the lowest score (42%) and TONG1 had the highest score (66%). For the habitat stressor, 29 regions (LOVU2, MFUL1, MHLA1, MKUZ1, MKUZ2, MOOI1, MTAM1, MVOT1, MVUN1, MZIM1, NCAN1, NGWA1, NWAK1, PHON1, PHON2, SAND1, THUK1, THUK2, UMLA1, UMLA2, UMNG1, UMNG2, UMNG3, UMNG4, VUTH1, WHIT1) had high risks; PHON2 had the lowest risk (35%), while PHON1 and WHIT1 had the highest score (56%) (Fig. 5.3).

The risk distributions for each endpoint in the 39 risk regions were generated from the BN output using Netica software (Fig. 5.4 and Fig. 5.5). Often, various distributions may have similar mean values; therefore, it is more important to compare the distributions rather than focus on the mean scores because distributions reflect the actual frequencies from the model calculations (Landis *et al*., 2017). Risk scores suggest general trends, while risk distributions give specific information about the patterns of relative risk and help to compare differences in risk by region (Landis *et al*., 2017). The biodiversity endpoint generally displayed low-moderate risk distribution in our current scenario, except AMAT1, BUSH1 and PHON2 which displayed a zero-low risk distribution and a few other sites showing a high risk. Alternative scenario 1 skewed towards moderate risk at all the study sites for the biodiversity endpoint, the scenario 2 showed a generally high risk at most sites, with a few lowland sites being in a moderate risk. The alternative scenario 3, which represented a high deterioration of water quality due to poor mitigation or management displayed high risk patterns (Fig. 5.4). The ecosystem risk distribution patterns displayed a zero-low risk distribution in the majority of the regions, while some regions (e.g. HLUH1, MVOT2 and TONG1) displayed a medium-high risk pattern. Scenarios 1 generally displayed low-moderate-high risk patterns, while scenario 2 and scenario 3 had a fairly even distribution of medium to high risk (Fig. 5.5).
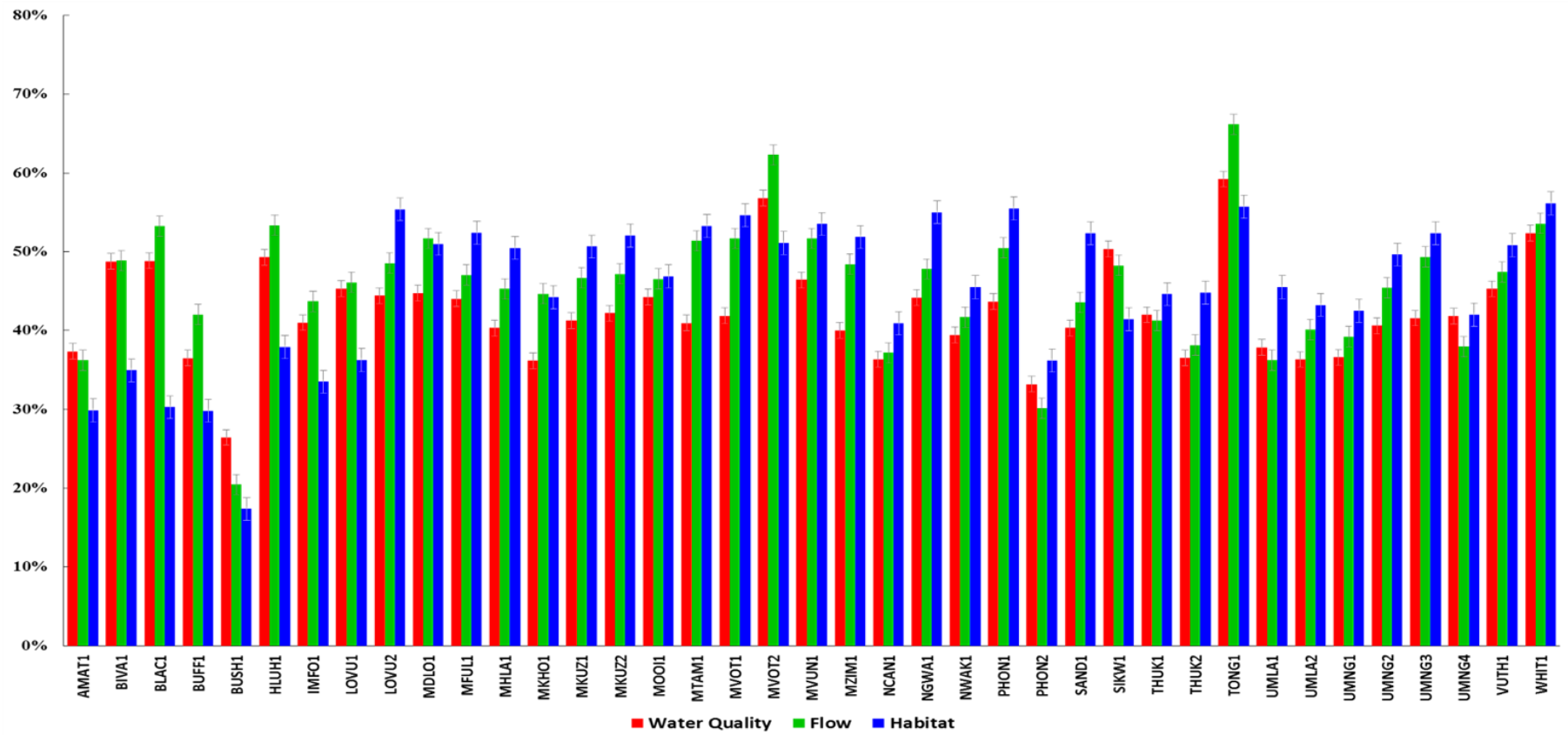
Figure 5.3: Preliminary analysis of the risk sources for rivers of KwaZulu-Natal, South Africa from 2015 to 2016.
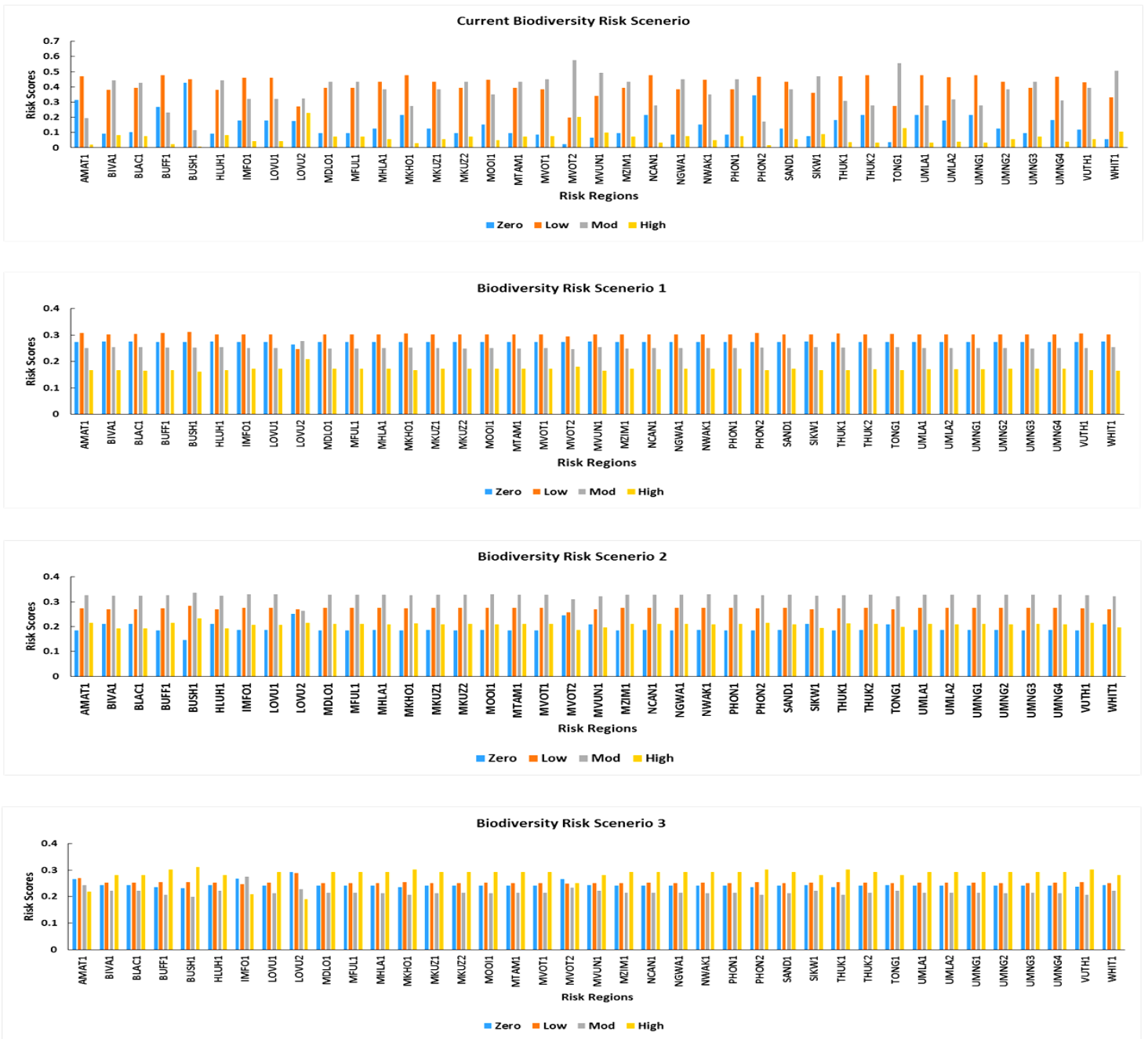
Figure 5.4: Bayesian network risk distributions across the risk regions and in all scenarios of the biodiversity endpoint.
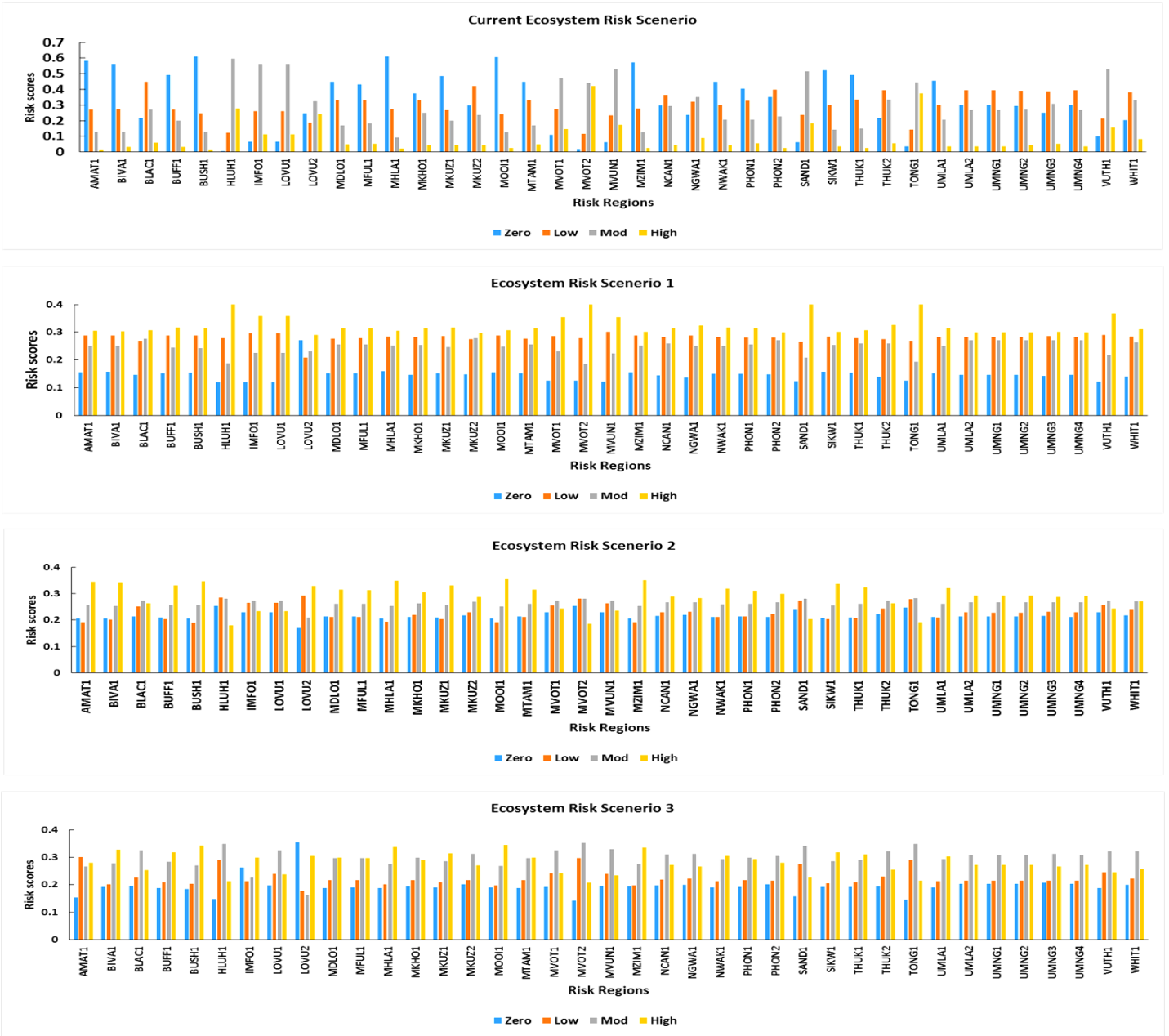
Figure 5.5: Bayesian network risk distributions across the risk regions and in all scenarios of the ecosystem endpoint.

Risk to the endpoints

For the biodiversity endpoint, the lowest and highest risk scores were obtained in the BUSH1 and MVOT2 respectively in the current risk scenario. In scenario 1, the lowest (45.1%) and highest (48.3%) risk scores were obtained from BUSH1 and LOVU2 respectively. In scenario 2 had the lowest risk score (48.5%) and highest risk score (53.9%) from MVOT2 and BUSH1 respectively. For ecosystem endpoint, the BN estimates showed lowest risks at MHLA1 (25.7%), LOVU2 (51%), HLUH1 (47.2%) and LOVU2 (48%) for current scenario, scenario 1, scenario 2 and scenario 3 respectively. The highest risk scores obtained from the BN estimates were from MVOT2 (69.2%), HLUH1 (60%), MOOI1 (56.3%) and BUSH1 (56.8%) for current scenario, scenario 1, scenario 2 and scenario 3 respectively. Final risk to biodiversity was shown in Fig. 5.6, while the final risk to ecosystem was shown in Fig. 5.7. Sites within the industrial and urban areas were mostly at moderate risk in the current scenario for the two endpoints, while the sites within conserved areas had zero to low risks (Fig. 5.6A and Fig. 5.7A). At the alternative scenario 1 (low flow risk), the biodiversity endpoint had moderate risk at all the sites (Fig. 5.6B), while the ecosystem scenario indicated a generally high risk at all sites (Fig. 5.7B). At the alternative scenario 2 (high flow risk), both endpoints had predominantly high risks, with very few lowland river sites being at moderate risk (Fig. 5.6C and Fig. 5.7C). For the alternative scenario 3 (water quality risk), biodiversity endpoint was predominantly high with only a few sites being sites being at moderate risk (Fig. 5.6D), while all the sites were at a high risk for the ecosystem endpoint (Fig. 5.7D).

Figure 5.6: Final biodiversity risk classifications of KwaZulu-Natal rivers studied from 2015 to 2016 based on the present ecological state (A); risk associated with low flow (B); risk associated with limited or degraded habitat (C) and risk associated with poor water quality (D).

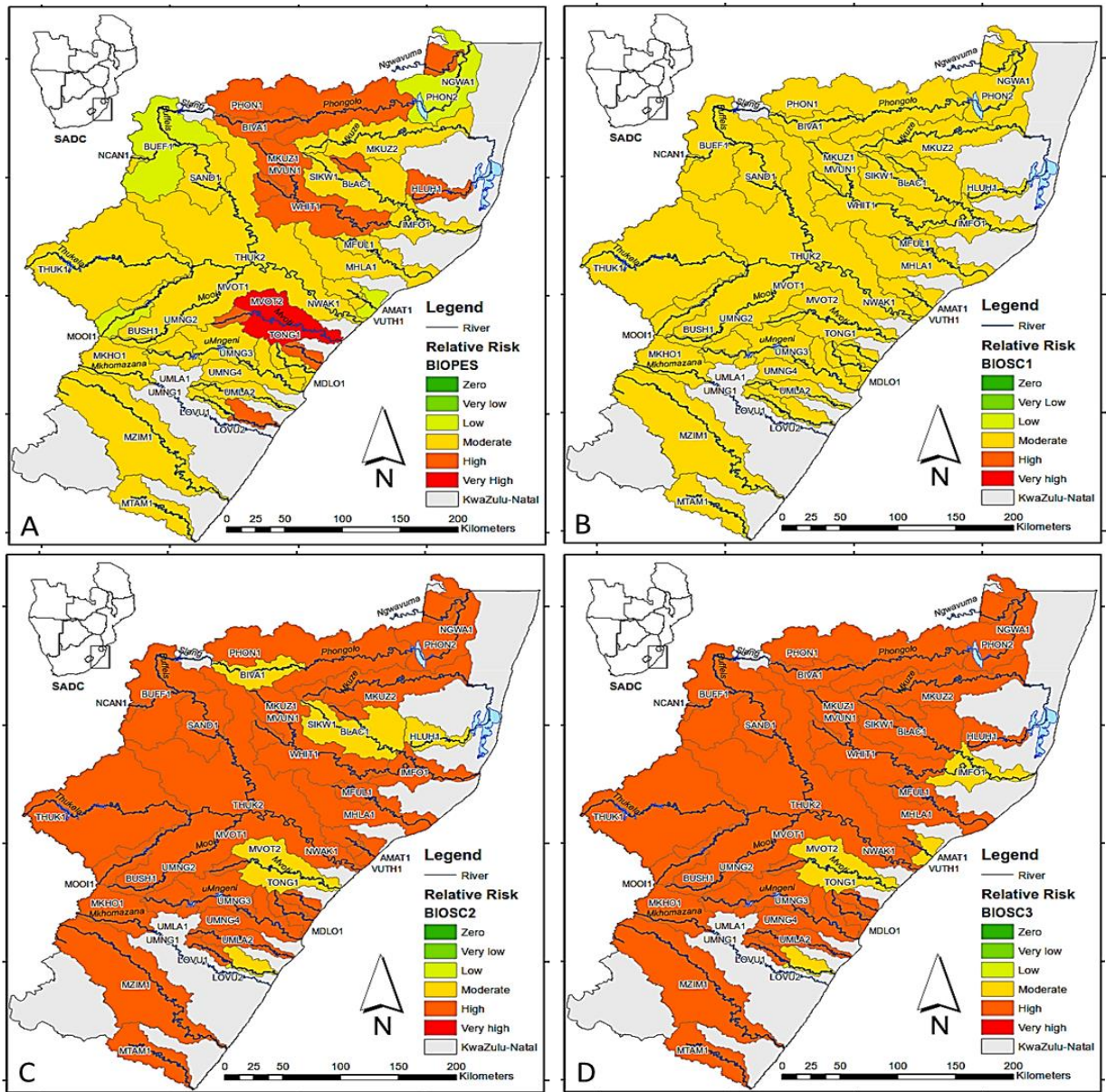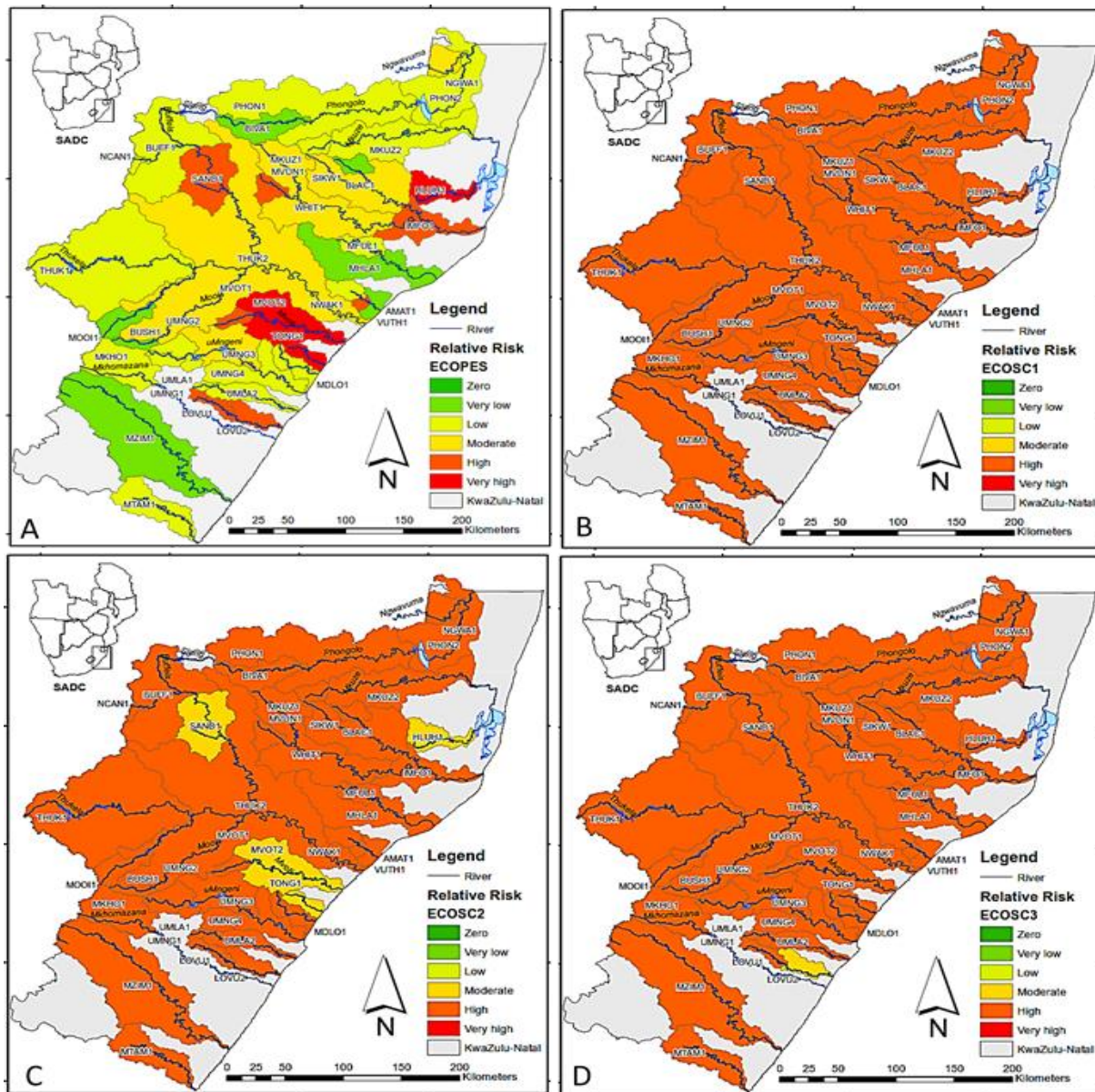Figure 5.7: Final ecosystem risk classifications of KwaZulu-Natal rivers studied from 2015 to 2016 based on the present ecological state (A); risk associated with low flow (B); risk associated with limited or degraded habitat (C) and risk associated with poor water quality (D).

Low risk simulation

An advantage of the BN model is that it can be directly used as an adaptive management tool by setting the state of an endpoint to the desired level and essentially solving the model "backwards" (Ayre and Landis, 2012). For this study, we set our endpoints to 100% low risk. The 100% low risk simulation represented the resource management goals for South African rivers (DWA, 2012). Using AMAT1 region, our 100% low risk simulation altered the risk distributions in the BN model and also gave insights into the input parameters posing the highest risk to the endpoints (Fig. 5.8A). Water quality stressors posed the highest risk (55.6%) to the biodiversity endpoint, while river health (measured as the macroinvertebrate response assessment index (MIRAI)) posed the highest risk (81.6%) to the ecosystem endpoint. Habitat stressors posed the lowest risk to both biodiversity (27.8%) and ecosystem (29.5%) endpoints (Fig. 5.8B).

All the input parameters skewed towards zero or low risk in the low risk simulation, except water quality stressors that skewed towards moderate risk (Fig. 5.8A). The habitat stressors skewed towards zero risks in the low risk simulation (Fig. 5.8A). The flow stressors, riparian habitat, ecosystem threats and instream habitat had higher scores at the current risk scenarios than at the low risk simulation (Fig. 5.8B). The ecoregion, habitat stressors and water quality stressors were fairly the same for both current scenario and low risk simulation, but river health input had lower scores for the current scenario than at the low risk simulation (Fig. 5.8B). The habitat stressors were fairly stable in both low and current risk (Fig. 5.8B).
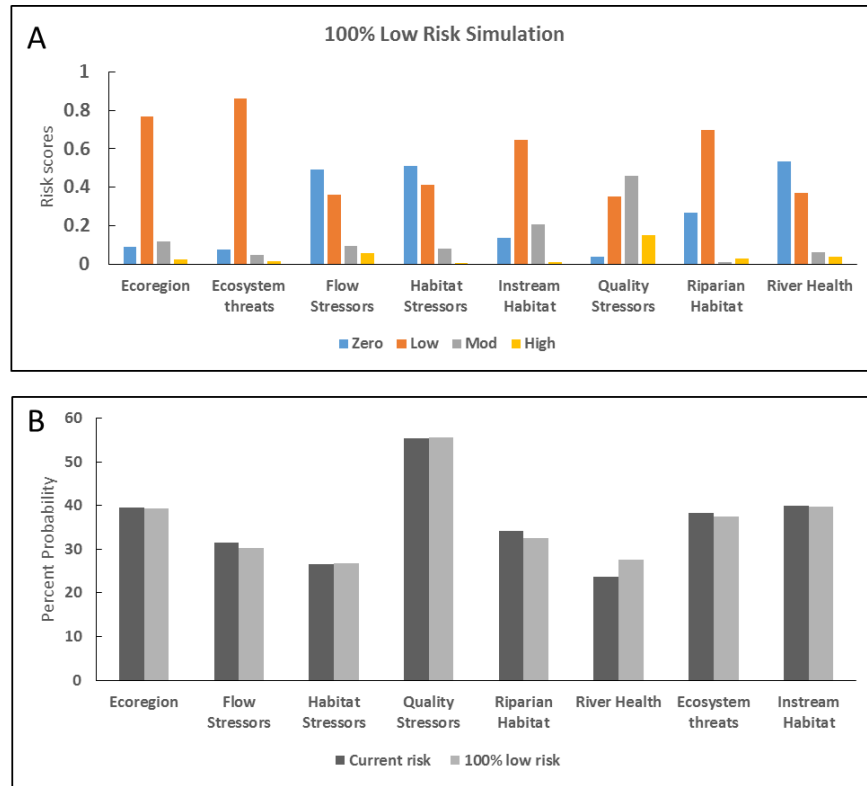
Figure 5.8: Low risk simulation of KwaZulu-Natal rivers; A = risk distribution for the low risk simulation, B = comparison between low risk and current risk scenarios.

Uncertainty

Our sensitivity analysis indicated that ecosystem threats were the highest contributor to the overall risk to biodiversity, while river health was the highest contributor to the overall risk to the ecosystem and the lowest contributor to both endpoints was habitat stressor (Table 5.1). As expected, there was generally a high probability of endpoints to be at high risk during scenario 3 and the high risk simulation, but those risk probabilities were reduced in the low risk simulation.

Table 5.1: Sensitivity analysis for endpoints, showing the percent of calculated entropy for each endpoint attributed to input nodes. Percentage is expressed relative to the calculated entropy for each endpoint.

Paremeters	Risk to Biodiversity	Risk to Ecosystem
Ecosystem Threats	40.6	5.34
Instream Habitat	11.5	2.22
Flow Stressors	4.72	1.02
Riparian Habitat	6.89	0.98
Quality Stressors	1.03	0.19
Ecoregions	0.6	NA
Habitat Stressors	0.53	0.09
River Health	NA	30.6

NA = the parameter was not an input parameter to the endpoint.

Discussion

Surface water abstraction for agricultural irrigation and industrial activities have compromised water demand and quality in the rivers of KZN. Pollution from nutrients and organic compounds are impacting on water quality in the middle and lower reaches of the rivers and this worsens during low flow conditions. Some of the lowland rivers suffer from impacts of drought conditions during low flow periods. The purpose of our study was to apply the BN-RRM in assessing the impacts of multiple stressors on the wellbeing of KZN rivers using macroinvertebrates as our indicator species and incorporating different management alternatives into the models. As demonstrated in this study, BN can be used as an adaptive management tool for ecological risk assessments of multiple stressors, whether they are from chemical or non-chemical sources (Landis *et al*., 2017). Bayesian Network models can be used interactively to visually communicate responses of endpoints to variables, compare risk regions and can be used as a risk communication tool to compare risk under theoretical scenarios (Landis *et al*., 2017). Our BN succeeded in calculating the overall risk to the two endpoints selected for this study and identified ecosystem threats and river health as the most influential contributors to the risk to biodiversity and ecosystem

respectively in the study area; while habitat stressors had the lowest risk contribution to both endpoints. The development of risk models and calculation of current risk within the study area was the initial step in assessing the risk to the macroinvertebrate biodiversity and ecosystem wellbeing. We obtained region-specific data during our extensive sampling program for the model parameters and this data were used in the calculation of risk to the endpoint in our current scenario.

With the BN model, we were able to account for potential synergistic effects of variables and the effects of ecosystem threats through the conditional probability tables (CPTs), which allowed for complex ecological interactions to be incorporated into the model's complexity (Maxwell *et al*,. 2015; Landis *et al*., 2017). For example, the CPT for Instream Habitat was selected in this study to represent the integrated variable for water quality (quality stressors), flow stressors, habitat stressors and determinants of physical habitat (Davies and Day, 1993). The CPTs were established using Netica ratio equations whereby when water quality is observed in a high risk rank state the relative importance of flow and habitat was hypothesised to be at lower risk states. Thereafter when the flow is in a high rank state, the other variables are weighted lower and such was done to habitat when it is in a high rank state. When variables were in a zero to moderate risk state, they were all weighted equally. It is these synergistic effects that may explain why ecosystem threat was the disturbance that most strongly influenced the level of potential risk to biodiversity endpoint (Landis *et al*., 2017). Also, input parameters and CPTs can easily be refined or updated to reflect current knowledge of the river sites, thereby reducing uncertainty in the data which may be caused by incomplete data and sampling errors (Marcot *et al*., 2006; Landis *et al*., 2017). Also, it is possible for new data to be added to BN risk models to reflect new knowledge of the system (Fuster-Parra *et al*., 2016). Thus, access to new data will greatly reduce uncertainty and reflect a more accurate risk evaluation (Landis *et al*., 2017).

Evaluating uncertainties is necessary for policy or management decision making, but care has to be taken as such information may easily be misused (Aven and Krohn, 2014). It is difficult to predict future risk characteristics, therefore, not properly addressing risks and its associated uncertainties may lead to short term solutions, which could be insufficient in the long term (Refsgaard *et al*., 2013; Refsgaard *et al*., 2014). Decision support models help a decision-maker to evaluate the consequences of various management alternatives (Holzkamper *et al*., 2012).

However, awareness of the various sources of uncertainty may help to ascertain justified decisions (Uusitalo *et al*., 2015). Thus a useful model should include information about the uncertainties related to each of the decision options, because the certainty of the desired outcome may be a central criterion for the selection of the management policy (Uusitalo *et al*., 2015). Uncertainty in BN risk model results reflects in the risk distributions for each node; where uncertainty increases as the risk distribution increases (Holt *et al*., 2014).

Not only are BNs networks effective at synthesizing the interactions of multiple stressors and calculating risk, but they may be used to identify parameters for remediation and model the impacts of different management scenarios. By evaluating the BN models in reverse, the overall risk output may be manually altered to identify specific conditions of stressors to achieve management decisions. Another advantage of using BNs in risk assessments is their ability to model risk reduction scenarios for best management practices (Johns *et al*., 2017; Landis *et al*., 2017). The input parameters in the BN may be altered to model the predicted conditions under different management strategies or upon implementation of best management practice (Duggan *et al*., 2015; Herring *et al*., 2015; Johns *et al*., 2017). Using BN, we identified the stressors contributing the highest risks, which were water quality stressors for biodiversity and river health for ecosystem endpoints in this study. The current BN for our endpoints showed the frequency distributions for all input parameters. As the model was changed to simulate a low-risk scenario, the distribution for all the input parameters changed to give indications of the critical inputs in the model that need to be closely monitored to attain a 100% low. The distribution changes not only reflected a change in the risk state for those nodes, but it was also a reflection of the reduction in the model's uncertainty. Many ecological risk assessment (EcoRA) and even some probabilistic models are not capable of such analysis without being entirely changed to a new framework.

Flowing water is the defining characteristic of rivers (Nadeau and Rains, 2007), with important influence on aquatic biota (Bunn and Arthington, 2002). Flow alteration in rivers is often the most severe and continuing threat to their ecological sustainability and associated floodplain wetlands (Pringle, 2001). However, water resource managers often have a difficulty in assessing the flow velocity a river needs to maintain its ecosystem, while still enabling water abstraction for other uses (Vörösmarty *et al*., 2010). Natural flows periodically include low flow periods as a

result of precipitation deficits. Low flows are seasonal, but may also be induced by anthropogenic activities which cause a deviation from the natural flow regime (Al-Faraj and Scholz, 2014). Artificial flow reductions are those created by human activities, such as dam closure, groundwater abstraction and water diversion (Adams *et al.*, 2016). Demand for water gets to the peak during dry periods of the year when streams have naturally low flows, which are worsened by water abstraction (Mishra and Singh, 2010). Flow alteration exerts a direct physical influence on aquatic biota and indirectly influences substrate composition, water chemistry, nutrient availability, organic substances, as well as in-stream habitat availability and suitability (Dewson *et al.*, 2007).

In our study, the current scenario indicated that the lowland river sites had the highest risk to the endpoints. As demonstrated by the current scenario of our study, the impact of low flow was greatest in the lowland rivers where habitat diversity was limited and habitat conditions were severely altered. Also in the current scenario, our study showed that the endpoints were at high risk within the proximity of agricultural lands and industries (e.g. MVOT2, TONG1 and LOVU2), while the regions within minimally impacted upstream areas were at low risk (e.g. MKHO1 and AMAT1). The high risk of the BUSH1 region to the biodiversity in scenarios 2 and 3; and ecosystem in scenario 3 may be due to the impacts of the densely populated villages in its upper catchment, through domestic wastes. Also, the MVOT2 is highly impacted by the industrial activities (paper and sugar mills) along its course and their effluent discharge points form confluences with the lower part of the river, which makes it the highest risk region in the current scenarios of our endpoints. In scenario 3, LOVU2 had the lowest risk, while BUSH1 had the highest risk.

Habitat structure affects biota community composition in freshwater ecosystems, with species diversity and abundance often influenced by structural complexity and heterogeneity (Tews *et al.*, 2004). Previous studies have shown that macroinvertebrates can be influenced by both complexity and heterogeneity (Barnes *et al.*, 2013). Hence, structural features of their habitats have consequently become a central focus in river management (Feld *et al.*, 2011). During low flows, there may be adverse effects of habitat heterogeneity as a result of fragmentation, which disrupts essential biological processes such as dispersal and resource acquisition (Saunders *et al.*, 1991). However, not all species in an ecosystem are equally affected by spatial structures in either

heterogeneous or fragmented state (Steffan-Dewenter and Tscharntke, 2000). The severity of reduced flow has an important influence on invertebrate responses because it determines the magnitudes of changes in the environment, habitat diversity, sedimentation and availability of food resources (e.g. periphyton) (Lake, 2000). During our study, there were limited habitat diversity and connectivity in the lowland streams as a result of drought (low flow), while a diverse range of suitable microhabitats remained available in the upland rivers. As observed in this study, reduced flows in perennial rivers may cause decreases in taxonomic richness (Poff and Zimmerman, 2010). A loss of taxonomic richness in the upland sites may be attributed to the loss of habitat types (e.g. fast flows or rapids) during the low flows, hence resulting in the generally low-moderate risk to the endpoints of this study in the current scenario, and a resultant high risk in the alternative scenarios. Also during the low flow scenario, changes in macroinvertebrate biodiversity (community composition and taxa richness) could probably result in increased habitat suitability for some species and decreased suitability for others (Gore *et al*., 2001), hence this will result in high risks to biodiversity and ecosystem wellbeing as demonstrated in our alternative scenarios. Furthermore, the drift behaviour of macroinvertebrates enables them to leave a stream reach or seek refuge in more favourable patches of the river in events of unsuitable low flow conditions (Verdonschot *et al*., 2014). This drift behaviour enables organisms to escape unfavourable conditions either actively or passively (James *et al*., 2008). Studies have shown that passive drift decreases during low flow conditions, while other studies have shown that active drift increases during periods of low flow (Naman *et al*., 2016). Active drifts during low flow are often caused by insufficient water velocities to meet nutritional, physiological and habitat requirements (Brooks and Haeusler, 2016). Active drift may also be a predator avoidance behaviour and this may increase if predator density increases during the low flow (Naman *et al*., 2016). Active drifts may, therefore, cause a reduction in biodiversity as demonstrated by our alternative risk scenarios.

Conclusion

Our study has demonstrated that subtle changes in environmental management may result in large changes in the risk distribution of sensitive endpoints and that the BN-RRM risk assessment plays a critical role in adaptive management schemes (Carriger *et al*., 2016). Also, the BN-RRM model's intrinsic flexibility makes it a powerful tool for resource management because alternative

management scenarios can easily be evaluated for desired objectives (Landis *et al*., 2017). Moreover, the graphic interface of the model results makes it a valuable tool for collaborative resource management (Carriger *et al*., 2016). This study provides the foundation for assessing the effects of multiple stressors in rivers of KZN using macroinvertebrate biodiversity and ecosystem as assessment endpoints over a regional spatial scale and incorporating site-specific information. This study lays the foundation for future risk assessment for the rivers of KZN. Furthermore, specific chemicals or ecological stressors should be integrated into this risk framework for future studies in KZN; for example, the effects of invasive alien biota or chemicals on biological endpoints can be investigated using this model. The model created in this research also provides a foundation for assessing the impacts of adaptive management strategies, and these models may be adapted to the evaluation of risk changes for best management practices in the rivers of KZN. Rivers of KZN are being impacted by pollution from different anthropogenic land uses across longitudinal gradients. These anthropogenic sources include effluents from domestic wastes, industrial effluents from the paper and sugar mills, agricultural practices and water abstraction. All these anthropogenic impacts pose risks to the endpoints of the rivers if not properly regulated or managed. Hence the river systems will continue to deteriorate. Deteriorated river systems will consequently not be able to meet their ecological functions. Strict adherence to environmental laws on the treatment and discharge of wastewater by industries should be enforced, as this will help to improve the water quality of the high risk regions (e.g. MVOT2 and TONG1).

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S1 Table 5.1: Justification for the risk assessment of the rivers of KwaZulu-Natal, South Africa, 2015 – 2016.

Land use category	Threat description	Occurrence and integrity range	Ranks	References
Dams	Dams alter aquatic ecology and river hydrology upstream and downstream, affecting water quality, quantity and breeding grounds. They create novel and artificial types of aquatic environment for the life span of the dam. Water quality of the impounded river is characterized by impacts along the longitudinal profile of the river also both upstream and downstream of the dam.	Upstream	Zero	Helland-Hansen et al., 1995; Kingsford, R. T. (2000). Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. Austral Ecology, 25(2), 109-127; McCartney, M. P., Sullivan, C., Acreman, M. C., & McAllister, D. E. (2000). Ecosystem impacts of large dams. Thematic review II, 1; Mandal, R. B. & Jha, D. K. (2014). Impacts of Damming on Ichthyo-fauna Diversity of Marshyangdi River in Lamjung district, Nepal. *Our Nature*, 11, 168-176.
		Upstream	Low	
		Upstream	Moderate	
		Downstream	High	
Conservation	Conserved or protected areas are partial solutions to mitigating habitat degradation, though only a few of these are created for for freshwater resources. Conservation of freshwater habitats are often incidentally included within terrestrial reserves.	Absent	Zero	Nel, J. L., Roux, D. J., Maree, G., Kleynhans, C. J., Moolman, J., Reyers, B., Rouget, M. and Cowling, R.M. (2007). Rivers in peril inside and outside protected areas: a systematic approach to conservation assessment of river ecosystems. *Diversity and Distributions*, 13, 341-352; Roux, D.J., Nel, J.L., Ashton, P.J., Deacon, A. R., de Moor, F. C., Hardwick, D., Hill, L., Kleynhans, C. J., Maree, G. A., Moolman, J. and Scholes, R. J. (2008). Designing protected areas to conserve riverine biodiversity: lessons from a hypothetical redesign of the Kruger National Park. *Biological Conservation*, 141, 100-117.
		0 - 10%	Low	
		>10 - 20%	Moderate	
		>20%	High	
Erosion	Erosion is considered in as a stressor source because its spatial and temporal occurence may lead to pollution inputs and habitat loss for the invertebrates (e.g. through the removal of substrates and sedimentation downstream).	Absent	Zero	Dotterweich, M. (2013). The history of human-induced soil erosion: geomorphic legacies, early descriptions and research, and the development of soil conservation—a global synopsis. Geomorphology, 201, 1-34; Reusser, L., Bierman, P., & Rood, D. (2015). Quantifying human impacts on rates of erosion and sediment transport at a landscape scale. *Geology*, 43, 171-174.
		Present, with vegetation >10%	Low	
		Present, with vegetation <10%	Moderate	
		Present, without veg >10%	High	
Light Industry	Industrial activities are the principal human activities that are posing a heavy burden on the environment. Industrial activities are all about the utilisation of resources and energy to produce goods and services. Inevitably during the process, wastes are generated. These wastes have a profound impact on such resources as water, air, land, biodiversity, etc.	Absent	Zero	Shrivastava, P. (1995). The role of corporations in achieving ecological sustainability. Academy of management review, 20(4), 936-960; Liu, J., Chen, Q., & Li, Y. (2010). Ecological risk assessment of water environment for Luanhe River Basin based on relative risk model. Ecotoxicology, 19(8), 1400-1415; Dunlap, R. E., & Jorgenson, A. K. (2012). Environmental problems. The Wiley-Blackwell Encyclopedia of Globalization. Chicago.
		1%	Low	
		<5%	Moderate	
		>5%	High	
Heavy Industry		Absent	Zero	
		<1%	Low	
		>1 - <5%	Moderate	
		>5%	High	
Rural Settlement	Occurrence and intensity of rural settlements; settlements with no formal wastewater treatment works (WWTW)	Absent	Zero	Ceola, S., Laio, F., & Montanari, A. (2015). Human-impacted waters: New perspectives from global high-resolution monitoring. Water Resources Research, 51, 7064-7079.
		<10%	Low	
		11 - 60%	Moderate	
		>60%	High	
Urban Settlement	Occurrence and intensity of urban settlements	Absent	Zero	
		1-10%	Low	
		10 - 20%	Moderate	
		>20%	High	
Commercial Agriculture	Agriculture source includes crop production and livestock productions. Crop production can increase the levels of various chemicals	Absent	Zero	Walker, R., Landis, W., & Brown, P. (2001). Developing a regional ecological risk assessment: A case study of a Tasmanian
		<1%	Low	

Land use category	Threat description	Occurrence and integrity range	Ranks	References
	including nutrients such as nitrogen, phosphorus and pesticides. Nitrogen and phosphorus concentrations can reach high levels from fertilizer and manure applications.	1.1 - 5%	Moderate	agricultural catchment. *Human and Ecological Risk Assessment*, 7(2), 417-439.
		>5%	High	
Mines	The effects of mining activities are far reaching and liable to affect the ecosystem for many years. Mining industries require large amounts of water for their work, through a series of processes. The water comes into contact with heavy metals, harmful chemicals, radioactive waste and even organic sludge.	Absent	Zero	Schwarzenbach, R. P., Egli, T., Hofstetter, T. B., von Gunten, U., & Wehrli, B. (2010). Global water pollution and human health. *Annual Review of Environment and Resources*, 35, 109-136.
		<1%	Low	
		>1 - <5%	Moderate	
		>5%	High	
Plantation	Plantation sources include formal afforestation within the province including alien commencial plantation of trees. Plantations may have deleterious effects on stream biota depending on the management practices. E,g. timber harvesting operations have significant effects on both water quantity and water quality.	Absent	Zero	Campbell, I. C., & Doeg, T. J. (1989). Impact of timber harvesting and production on streams: a review. Marine and Freshwater Research, 40(5), 519-539; Kreutzweiser, D. P., Capell, S. S., & Good, K. P. (2005); Macroinvertebrate community responses to selection logging in riparian and upland areas of headwater catchments in a northern hardwood forest. Journal of the North American Benthological Society, 24(1), 208-222.
		Present, <5%	Low	
		Present, >5.1-50%	Moderate	
		Present, >50%	High	
Susbstistence Farms	Vegtable farming without formal irrigation and limited commercial sale of produce.	Absent	Zero	Kilonzo, F., Masese, F. O., Van Griensven, A., Bauwens, W., Obando, J., & Lens, P. N. (2014). Spatial–temporal variability in water quality and macro-invertebrate assemblages in the Upper Mara River basin, Kenya. Physics and Chemistry of the Earth, Parts A/B/C, 67, 93-104.
		Present, <5%	Low	
		Present, >5 - 40%	Moderate	
		>40%	High	
Sugarcane		Absent	Zero	Nhiwatiwa, T., Dalu, T., & Brendonck, L. (2017). Impact of irrigation based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Science of The Total Environment, 587, 316-325.
		Present, <5%	Low	
		Present, >5.1-40%	Moderate	
		Present, >40%	High	
VEGRAI	Unmodified natural (A)	Unmodified natural	Zero	Kleynhans, C. J., Mackenzie, J., & Louw, M. D. (2008). River EcoClassification: Manual for EcoStatus Determination (Version 2) Module F: Riparian Vegetation Response Assessment Index (VEGRAI). Water Research Commission and Department of Water Affairs and Forestry, Pretoria. WRC Report No. TT333/08.
	Few/moderately modifications (B/C)	Largely natural or moderately modified	Low	
	Largely modified (D)	Largely modified	Moderate	
	Seriously/Extremely modified (E/F)	Seriously or critically modified	High	
MIRAI	Unmodified natural (A)	Unmodified natural	Zero	Thirion, C. (2007). MODULE E: Macroinvertebrate Response Assessment Index (MIRAI). River ecoclassification manual for ecostatus determination (Version 2): Joint Water Research Commission and Department of Water and Sanitation and Forestry report.
	Few/moderately modifications (B/C)	Largely natural or moderately modified	Low	
	Largely modified (D)	Largely modified	Moderate	
	Seriously/Extremely modified (E/F)	Seriously or critically modified	High	
Ecoregions	The geomorhpological location of the ecoregions determine the potential habitat quality of the river system. The ecoregional approach is intended to provide a more pragmatic basis from which ecological similarities between ecosystems can be derived and from which expected conditions can be specified. This level of typing is based on the premise that ecosystems and their components display regional patterns that are reflected in spatially variable combinations of causal factors such as climate, mineral availability (soils and geology), vegetation and physiography (Omernik, 1987).	Poor	Zero	Omernik, J. M., & Bailey, R. G. (1997). Distinguishing between watersheds and ecoregions. *Journal of the American Water Resources Association*, 33, 935-949.
			Low	
		Moderate	Moderate	
		Good	High	

CHAPTER 6

Conclusion: Synthesis of research findings

6.1 Introduction

This chapter is a summary of the main research findings according to the aim and objectives of this thesis. The benefits of this study to freshwater biomonitoring in South Africa are discussed in a comparative analysis of the different approaches applied. The chapter concludes with a proposed framework for improving the environmental water quality of the rivers of KwaZulu-Natal (KZN), a brief conclusion and recommendations.

Deterioration of water quality and biotic integrity by anthropogenic activities are threatening freshwater ecosystem sustainability, human health and socio-economic development (Malaj *et al*., 2014; Mei *et al*., 2016). Industrial waste discharges and agricultural runoffs are the major pollution sources degrading the biotic integrity and water quality of South Africa's freshwater resources (Van Ginkel, 2011). Many environmental factors, including physical, structural and chemical variables determine macroinvertebrate community composition (Murphy and Davy-Bowker, 2005). The use of freshwater macroinvertebrates as indicators of water quality in biomonitoring assessment requires considerable understanding of the factors involved in determining the conditions (Nicacio and Juen, 2015; Colin *et al*., 2016). Research on the response of macroinvertebrate assemblages to habitat conditions can improve the understanding of environmental stress (King *et al*., 2015).

Integrated applications of appropriate statistical and ecological modelling tools can help to extract important information about pollution sources that are vital for selecting and prioritising appropriate restoration measures for rivers. The results of this study indicated that the water quality variables affecting the composition, distribution and abundance of macroinvertebrates in the rivers of KZN are: (1) pH, (2) clarity, (3) electrical conductivity, (4) dissolved oxygen, (5) ammonium and (6) *Escherichia coli*. These water quality variables are associated with the following major pollution sources and physical degradations in the rivers of KZN:

- Industrial wastewater discharge may be the cause of low pH values as recorded at some sites. This may tend to increase metal concentrations in the water column and metals tend to be more toxic at lower temperatures because of their higher solubility at that state.
- Untreated domestic wastewater caused excessive nutrient loads.
- Runoffs from agricultural lands caused excessive nutrient loads.
- Pathogenic microbes exist in the rivers of KZN.
- The drought conditions greatly affect the lowland rivers, causing loss of biodiversity and habitat. The loss of biodiversity and habitat were because there was not enough water in the rivers to maintain the ecosystem services.
- Anthropogenic activities (e.g. sand mining and livestock grazing) had noticeable impacts on the riparian zones.

6.2 Research findings

South Africa makes use of regional reference condition approach in the interpretation of river biomonitoring data and this enables the comparison of data from monitoring sites to established reference conditions or benchmarks from a near pristine or "least-impacted" site or sites (Dallas, 2013). The regional reference condition approach of South Africa also incorporates the verification of spatial framework and potential variability of physical, seasonal and habitat factors (Dallas, 2013). This regional approach is not often adequate in the assessment of some major rivers, especially the shallow lowland rivers of KZN because it is hard to find appropriate reference conditions for comparison. The established criteria for the selection and validation of reference conditions for our study involved the inclusion of certain level of human disturbance or little exposure to anthropogenic disturbances (Bailey *et al*., 2004). It was suggested that the absence of a criterion can be as equally problematic as selecting the wrong one (Chaves *et al*., 2006). In this study, many of the lowland KZN sites failed the selection criteria, especially in the northern part of KZN where natural factors cause a lot of impairment to the stability of the river ecosystems and rendering them unsuitable as good reference sites majorly because of the insufficient data obtained from them (Chapter 2). The established reference sites in this study were able to generate reference conditions for the classification of sites in this study into groups (ecoregions, river geomorphology and seasons) (Chapter 2). The implication of this is that similar ecoregions had similar reference

conditions, while they do not have the same reference conditions when compared with other ecoregions (Dallas, 2013) (Chapter 2). The findings of this study indicated that lowland river type-specific reference conditions are needed for their assessment within KZN (Chapter 2). Potential users may get discouraged by the complexities of multivariate methods of reference condition selection and validation; they are more desirable because they require no prior assumptions in choosing groups of reference sites or in comparing test sites with the reference group (Reynolds *et al*., 1997; Muxica *et al*., 2007).

Diversity, similarity and biotic indices were used to test a wide range of river sites having a considerable variation in the numbers of macroinvertebrate taxa and numbers of individuals within each taxon at the sampling sites (Chapter 3). However, these indices are commonly used in evaluating the impacts of water quality changes on aquatic communities. Research has shown that diversity and biotic indices may be influenced by other sources of stress other than pollution, therefore it is important to investigate the major causes of diversity changes (Ravera, 2001). Biodiversity monitoring should not be limited to maintainance of the historical or current species or taxa abundances, but should rather be robust enough to allow conservation managers and decision-makers to sustainably maintain the biodiversity or reduce its loss (Sarkar *et al*., 2006). Thus giving the opportunities for early recognition of taxa decline, productivity and succession (Dale *et al*., 2001; Mori *et al*., 2017). In this study, species abundance distribution emerged as a good assessment tool for comparing polluted and unpolluted sites, however it may not be reliable in complex situations (Glaser *et al*., 2014) (Chapter 3). Diversity and biotic indices were more reliable in the assessment of KZN rivers than similarity index (SIMPER) in this study (Chapter 3). The macroinvertebrate response assessment index (MIRAI) was a good tool for the ecological assessment of the rivers of KZN, however efforts should be made to improve its quality by incorporating a robust diversity measure such as the species diversity or evenness into the metrics (Chapter 3).

This study explored the responses of macroinvertebrate taxonomic traits in terms of abundance (total number of individuals), composition (relative abundance) and richness (total number of taxa within a family or genus) to assess the pollution levels of the rivers of KZN (Chapter 4). The redundancy analysis (RDA) graph showed a consistent degradation of KZN rivers

along a longitudinal gradient. The responses of the macroinvertebrate assemblages to degradations in the rivers of KZN were consistent with information obtained from literature reviews; for example, abundance of Oligochaeta taxa decreased along pollution gradients as predicted at the sites with degraded water quality (Chapter 4). A total of nine taxa traits had high discriminant abilities in differentiating the minimally impacted sites from the degraded sites. This study revealed that macroinvertebrate trait metrics were able to detect physical (total dissolved solids, temperature, dissolved oxygen, clarity and electrical conductivity), nutrient (total inorganic nitrogen) and toxic (fluorine) drivers of change in the rivers of KZN. Five of the metrics in this study (total number of macroinvertebrate taxa, total number of diptera taxa, plecoptera abundance, percentage of ephemeroptera, plecoptera and trichoptera and coleoptera abundance had significant positive correlations with high physico-chemical assessment index (PAI), which indicated their sensitivity to good water quality (Chapter 4). Elevated nutrient loads were apparent at the sites close to agricultural practices and livestock productions. Presence of *Escherichia coli* bacteria in the rivers of KZN are indications of fecal contamination probably from sewage sources in the urban settlements and poor sanitary conditions at the rural settlements (Suthar, 2009). The drought conditions during this study period may have caused the low pH levels at the lowland rivers, indicating a high acidity that causes a loss or decline in the abundance of sensitive taxa in the rivers.

One of the objectives of this study was to apply the Bayesian Network Relative Risk Model (BN-RRM) in assessing the impacts of multiple stressors on the wellbeing of KZN rivers using macroinvertebrates as the bioindicator species and incorporating different management alternatives into the models. Anthropogenic demands on freshwater ecosystems have enormous threats to biodiversity around the world (Dudgeon *et al*., 2006; Richardson *et al*., 2007), through various contaminants which may be chemical, physical, radioactive or pathogenic. These contaminants may be from single or multiple sources, including industrial effluents, agricultural run-off, domestic sewage, construction and mining activities) (Richardson *et al*., 2007). This study estimated the risks of multiple stressor sources to the wellbeing of macroinvertebrates (biological endpoint) and their associated ecosystem (habitat endpoint) from the rivers (risk regions) in KZN using a Bayesian network model (Chapter 5). The two endpoints had varying risk distributions across all the four scenarios that were simulated in this study. For this study, conditional

probability tables (CPTs) were used to reduce complex ecological interactions and the potential synergistic effects of their input variables and the equations were inserted into the Bayesian network input nodes (Mkrtchyan *et al.,* 2017). Also, simulations of the low and high risks to the study endpoints (macroinvertebrate biodiversity and ecosystem wellbeing) were calculated (Chapter 4) to represent good and bad management options for the rivers of KZN. The graphic interface of the model results made it a valuable tool for collaborative resource management because it was easy to visualize the risk distributions across the risk regions (Carriger *et al*., 2016). In this study, the stressors posing the highest risks in KZN rivers were water quality stressors for biodiversity and river health for ecosystem endpoints. This study was able to demonstrate that subtle changes in environmental management decisions may result in large changes in the risk distribution of sensitive endpoints and that the BN-RRM risk assessment plays a critical role in adaptive management schemes (Carriger *et al*., 2016).

6.3 Conclusions

This study has contributed to the field of biomonitoring in South Africa by demonstrating the importance of an integrated approach to the assessment of environmental water quality and providing insights into selecting the appropriate biomonitoring approach for use. It highlighted the importance of careful selection and validation of reference conditions as benchmarks for comparing data from impacted sites and the need for the development of type-specific reference conditions for the lowland rivers (Chapter 2), then it provided information on the benefits of measuring diversity for adequate management policy decision making (Chapter 3). Furthermore, the study was able to highlight an alternative assessment method for river water quality based on macroinvertebrate community assemblages, especially for the lowland rivers which are often characterised by insufficient data for adequate assessment and comparison (Chapter 4). The Bayesian network for risk assessment in this study was shown to be a good adaptive management tool as the results are easy to interpret and the model can easily be manipulated to suit management goals (Chapter 5).

6.4 Recommendations

1. Sand mining activities within the riparian zones should be regulated as this will reduce its impacts on the habitat loss and fragmentation of the rivers.

2. Creation of buffers within the riparian zones will help to reduce agricultural runoffs from causing unnecessary nutrient enrichment in the rivers.

3. The use of diversity indices should be encouraged and combined with the current biomonitoring tools of macroinvertebrates, as they give more information about biodiversity; thus helping to make accurate management decisions about the conservation and preservation of the macroinvertebrate biodiversity as well as the river ecosystems.

4. The MIRAI model can be improved by incorporating a measure of biodiversity into its metrics.

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