A study of the macroinvertebrate biodiversity and selected physico-chemical parameters of the Marico River

by

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THESIS

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It all starts here ™

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ABSTRACT

The rapid growth in world population, coupled with mega droughts over the past few decades, is progressively responsible for a global decline in water quantity and quality. In South Africa alone, nearly 71% of the main rivers are already regarded as threatened. In order to support the sustainability of freshwater ecosystems, it is of great importance to manage and conserve rivers such as the Marico River, a National Freshwater Ecosystem Priority Area.

Freshwater macroinvertebrates, a diverse group, are often used as indicators for determining the biotic integrity and ecological health of river systems, as well as to monitor environmental change. However, there is limited information on the macroinvertebrate diversity of Southern Africa, especially up to species level.

The aim of this thesis was to determine the aquatic macroinvertebrate biodiversity and to establish whether selected physico-chemical stressors have an adverse effect on the biodiversity of the Marico River.

The first objective of the study was to determine concentrations and values of selected physico-chemical parameters, potentially toxic to aquatic macroinvertebrates, in water and sediment. Water quality variables such as pH, electrical conductivity and temperature were mainly within the tolerance ranges of macroinvertebrates. Some metal concentrations in the water and nearly all metal concentrations in the sediment exceeded quality guideline values. However, results suggested that these metals originated mainly from natural sources such as geological weathering. Metal concentrations in the water were also

considerably lower than in the sediment, which indicated that metals in the sediment were most probably not bioavailable.

The second objective was to conduct comprehensive biodiversity surveys of aquatic macroinvertebrates in the Marico River and selected tributaries. A total of 187 taxa and more than 18 900 specimens were recorded, including both tolerant and highly sensitive species. This supported the general perception that the Marico River is mainly unimpacted by anthropogenic activities. Results showed that biotope and food availability, as well as the measured environmental variables play a significant role in the occurrence, abundance and distribution of species in this river system.

Thirdly, the molluscan diversity in the Marico River was compared with a similar study in the highly impacted Crocodile River. Twenty species were recovered from the Marico River, while only nine species were recovered from the Crocodile River. The relatively high mollusc diversity, as well as the fact that juveniles were present throughout the study, demonstrated that current habitat and environmental conditions were suitable to promote recruitment and the sustainability of diverse mollusc populations in the Marico River and its tributaries.

The fourth objective was to utilize a biomarker approach linking biological response to metal exposure using metallothionein (MT) levels in selected macroinvertebrate families. Results revealed that metals accumulated in the macroinvertebrates in various concentrations, depending on both their proximity to the sediment, as well as their feeding behaviour. Metallothionein levels in the organisms correlated positively with increased metal concentrations in the macroinvertebrates, suggesting that MTs probably act as metal detoxifying proteins and that it may be used as a biomarker for metal exposure.

To conclude, the selected physico-chemical factors measured in the Marico River did not seem to have a detrimental impact on the macroinvertebrates. Both the high total biodiversity and high mollusc diversity compared favourably to similar studies on impacted rivers, supporting the general perception that the Marico River, in view of biodiversity and water quality, is still largely unimpacted by anthropogenic disturbances.

Keywords:

Macroinvertebrate diversity; physico-chemical parameters, water and sediment characteristics, Marico River.

OPSOMMING

Die drastiese toename in wêreldbevolking, tesame met enorme droogtes oor die afgelope paar dekades, is toenemend verantwoordelik vir 'n wêreldwye afname in waterkwantiteit sowel as waterkwaliteit. In Suid-Afrika word byna 71% van die belangrikste riviere reeds as bedreig beskou. Om die volhoubaarheid van varswater ekosisteme te ondersteun, is dit dus baie belangrik om riviere soos die Maricorivier, 'n Nasionale Varswater Ekosisteem Prioriteitsarea, te moniteer en te bewaar.

Varswater makroinvertebrate, 'n diverse groep, word dikwels gebruik as aanwysers vir die bepaling van die biotiese integriteit en ekologiese gesondheid van rivierstelsels, asook om omgewingsveranderinge te moniteer. Daar is egter beperkte inligting oor die makroinvertebraatdiversiteit van Suider-Afrika, veral tot op spesievlak.

Die doel van hierdie proefskrif was om die akwatiese makroinvertebraatdiversiteit te bepaal asook om vas te stel of geselekteerde fisies-chemiese veranderlikes 'n nadelige uitwerking op die biodiversiteit van die Maricorivier het.

Die eerste doel van die studie was om konsentrasies en waardes van geselekteerde fisies-chemiese veranderlikes, potensieel toksies vir akwatiese makroinvertebrate, in die water en sediment te bepaal. Veranderlikes soos pH, elektriese geleiding en temperatuur was hoofsaaklik binne die verdraagsaamheidsgrense van makroinvertebrate. Sommige metaalkonsentrasies in die water en byna al die metaalkonsentrasies in die sediment het óf nasionale, óf internasionale kwaliteitsriglynwaardes oorskry. Die resultate dui egter daarop dat hierdie metale hoofsaaklik vanaf natuurlike bronne soos geologiese verwering afkomstig is. Metaalkonsentrasies in die water was ook aansienlik laer as in die sediment, wat verder daarop dui dat metale in die sediment waarskynlik nie, ten tyde van die opnames, biobeskikbaar was nie.

Die tweede doelwit was om omvattende biodiversiteitopnames van die akwatiese makroinvertebrate in die Maricorivier en geselekteerde sytakke uit te voer. 'n Totaal van 187 taksa, verteenwoordig deur meer as 18 900 individue, insluitend beide verdraagsame en sensitiewe spesies, is versamel. Dit ondersteun die algemene beskouing dat die Maricorivier tot ’n groot mate 'n natuurlike, ongerepte rivier is. Resultate het verder getoon dat biotoop en voedsel beskikbaarheid asook, tot ’n mindere mate, omgewingsveranderlikes, 'n belangrike rol in die voorkoms, getalle en verspreiding van spesies speel.

Derdens is die molluskdiversiteit in die Maricorivier met resultate van 'n soortgelyke studie in die hoogs geïmpakteerde Krokodilrivier vergelyk. Twintig spesies is in die Maricorivier gevind, terwyl slegs nege spesies in die Krokodilrivier gevind is. Die relatief hoë molluskdiversiteit sowel as die feit dat jeugdiges deurentyd teenwoordig was, het getoon dat die huidige habitat en omgewingstoestande geskik is om die aanwas en volhoubaarheid van ’n diverse molluskbevolkings in die Maricorivier te bevorder.

Die vierde doelwit was om 'n biomerker-benadering te gebruik om biologiese respons aan metaalblootstelling te koppel met behulp metallothionienvlakke (MTe) in geselekteerde makroinvertebraatfamilies. Resultate het getoon dat metale in verskillende konsentrasies in die makroinvertebrate geakkumuleer het, afhangende van beide hul nabyheid aan sediment, asook hul voedingsgewoontes. Metallothionienvlakke in die organismes het positief gekorreleer met verhoogde metaalkonsentrasies in die makroinvertebrate, wat daarop dui dat MTe waarskynlik dien as 'n metaal detoksifiserende proteïen en dat dit as 'n biomerker vir blootstelling aan metale gebruik kan word.

Daar kan dus afgelei word dat die geselekteerde fisies-chemiese veranderlikes wat in die Maricorivier gemeet is, nie 'n nadelige uitwerking op die makroinvertebrate gehad het nie. Beide die hoë totale biodiversiteit en hoë molluskdiversiteit vergelyk gunstig met soortgelyke studies op geïmpakteerde riviere. Dit ondersteun die algemene beskouing dat die Maricorivier, in die lig van biodiversiteit en watergehalte, steeds grootliks ongeïmpakteer is deur antropogeniese versteurings.

Sleutelterme:

Makroinvertebraatdiversiteit, fisies-chemiese veranderlikes, water- en sedimenteienskappe, Maricorivier.

CONFERENCE PROCEEDINGS AND PUBLICATIONS

Conference Proceedings

The results of the current investigation were presented at the following conferences:

- Kemp M, Wolmarans CT. *Metal concentrations in the water and sediment of a pristine river system in the North-West Province of South Africa.* 7th International Toxicoloxy Symposium in Africa. Garden Court Hotel, InterContinental O.R. Tambo, South Africa (31 August, 2015).

- Kemp M, de Kock KN, Wepener, V, Wolmarans, CT. *Macroinvertebrate biodiversity, biotope associations and selected environmental variables in a pristine, free-flowing river system, South Africa.* Southern African Society of Aquatic Scientists (SASAqS) 53rd Congress. Skukuza, Kruger National Park, South Africa (26 – 30 June, 2016).

- Kemp M, Wolmarans CT. *Metal concentrations in selected macroinvertebrate families, water and sediment of a pristine river system in the North-West Province of South Africa.* 8th International Toxicoloxy Symposium in Africa. Cairo, Egypt (29-31 August, 2016).

Publications

Previous work done on aquatic macroinvertebrates by the author, but not related to the current investigation, were published as follows:

- de Kock, K.N., Wolmarans, C.T., Kemp, M., and Roets, W. 2013. Korttermynbedreigings vir varswater-Mollusca in die Olifantsrivier en enkele sytakke. *Suid-Afrikaanse Tydskrif vir Natuurwetenskap en Tegnologie*, 32(1), pp.102-107.

- Wolmarans, C.T., Kemp, M., de Kock, K.N., Roets, W., Van Rensburg, L. and Quinn, L., 2014. A semi-quantitative survey of macroinvertebrates at selected sites to evaluate the ecosystem health of the Olifants River. *Water SA*, 40(2), pp.245-254.

- Kemp, M., de Kock, K.N., Wepener, V., Roets, W., Quinn, L. and Wolmarans, C.T., 2014. Influence of selected abiotic factors on aquatic macroinvertebrate assemblages in the Olifants River catchment, Mpumalanga, South Africa. *African Journal of Aquatic Science*, 39(2), pp.141-149.

Peer reviewed publications generated from the current investigation, but which are not part of the thesis, include the following (see ADDENDUM D and E):

- Kemp, M. and Wolmarans, C.T. 2015. Metal concentrations in the water and sediment of a pristine river system in the North-West Province of South Africa. Proceedings of the 7th International Toxicoloxy Symposium in Africa. South Africa.

- Kemp, M. and Wolmarans, C.T. 2016. Metal concentrations in selected macroinvertebrate families, water and sediment of a pristine river system in the North-West Province of South Africa. Proceedings of the 8th International Toxicoloxy Symposium in Africa. Egypt.

STRUCTURE OF THE THESIS

This thesis is presented in article format, as prescribed and approved by the North-West University. Four manuscripts have been generated and submitted to peer reviewed scientific journals. To date, one article **(Manuscript 3)** has been published, while the others are in the process of revision.

- **Chapter 1 – Introduction:** Contains background information with a problem statement, hypotheses, as well as the aims and objectives of the study.

- **Chapter 2 - Study Area:** Contains information on the study area, as well as a detailed description of each study site.

- **Chapter 3 - Manuscript 1:** Selected physico-chemical characteristics potentially toxic to aquatic macroinvertebrates in water and sediment of a relatively unimpacted river, South Africa.

- **Chapter 4 - Manuscript 2:** Influence of biotope associations and selected environmental variables on aquatic macroinvertebrate distribution in a pristine perennial Afro-tropical river system.

- **Chapter 5 - Manuscript 3:** A comparison of the mollusc diversity between the relatively pristine Marico River and the impacted Crocodile River, two major tributaries of the Limpopo River.

- **Chapter 6 - Manuscript 4:** Metallothioneins and metal bioaccumulation in aquatic macroinvertebrates from a relatively unimpacted river system in South Africa.

- **Chapter 7 - Conclusions and Recommendations:** Contains the outcomes of the hypotheses and the findings of Chapters 3 to 6 are integrated in a discussion with concluding remarks and recommendations.

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CHAPTER 1
General Introduction

"We forget that the Water Cycle and the Life Cycle are one"

Jacques Cousteau

"In the beginning God created the heavens and the earth. Now the earth was formless and empty, darkness was over the surface of the deep, and the Spirit of God was hovering over the waters" (Genesis 1:1-2. The Holy Bible NIV, 2011).

Since the beginning of Creation, water has formed an integral part of life. It is the most essential element for all organisms and without water, death would be inevitable.

The rapid growth in world population, coupled with mega droughts over the last few decades are, however, increasingly responsible for a global shortage of water. This situation gives rise to escalating international conflicts regarding the use and distribution of water sources and, as Ismail Serageldin said: "The wars of the 21st century will be fought over water".

The ever-increasing population is not only responsible for a decline in water quantity but also in water quality, a phenomenon which affects humans, as well as all biota that share water with the human race. Impaired water quality is the result of numerous detrimental impacts on our water sources and ecosystems, which ultimately leads to a decline in freshwater biodiversity, also a global phenomenon (Strayer, 2006; Vaughn and Taylor, 1999). Freshwater sources on earth support at least 100 000 species and groups such as fish, amphibians, aquatic reptiles, mammals and invertebrates, are declining at an even more alarming rate than in the most impacted terrestrial environments (Dudgeon et al., 2006). Of these groups, macroinvertebrates, per se, consists of nearly 90 000 species, representing 17 phyla and with approximately 570 described families. According to Strayer (2006), approximately 10 000 freshwater invertebrate species around the world may already be extinct or be at risk of extinction. However, information of the total macroinvertebrate diversity of freshwaters is sadly incomplete and researchers estimate that 20 000 to 200 000 of macroinvertebrate species are still to be discovered (Strayer, 2006).

The question comes to mind: Why is it so important to conserve high species diversity and ensure sustainability?

Loss of species is associated with the loss of ecosystem function. Each species has certain traits to offer to the functioning of an ecosystem and thus, high species diversity equals better ecological functioning and, ultimately, a healthier ecosystem (Purvis and Hector, 2000; Heatherly et al., 2007). In rivers, aquatic macroinvertebrates play a vital role by aiding purification, processing transported organic matter and providing a food source for other fauna such as fish (Weber et al., 2004; Eady et al., 2014). Macroinvertebrates are also widely used as indicators for determining the biotic integrity and ecological health of river systems, as well as to monitor environmental change (Kiffney and Clements, 1994). This is mainly because of their abundance, limited mobility, visibility with the naked eye, known pollution tolerances, wide range of feeding habits and various lengths in their lifespans (Kiffney and Clements, 1994).

The diversity and community structure of these organisms can be influenced by a variety of factors including physico-chemical variables i.e. substrate particle size, turbidity, pH, temperature, electrical conductivity, total dissolved solids, dissolved oxygen, surface flow, nutrients and potentially toxic substances such as metals (Hawkins and Sedell, 1981; Kiffney and Clements, 1994; Dallas, 2004; 2007; Heatherly et al., 2007; Duan et al., 2008; Eady et al., 2014), as well as the availability of suitable habitats or biotopes (Kemp et al., 2014). These influences or stressors may be due to either anthropogenic impacts or from natural processes, i.e. drought causing habitat loss or geological weathering resulting in the deposition of metals in the water and sediment.

Fortunately, macroinvertebrates have certain traits and response mechanisms in order to survive the above mentioned stressors. Some of these morphological and physiological traits include, i.e., specific feeding behaviour or the ability to disperse by flight as adults when water quality has deteriorated. Some species have anal gills, breathing siphons or haemoglobin blood pigment which enables them to live in stagnant, eutrophic and low oxygenated water (Aguilera et al., 1999; Day et al., 2003; Picker et al., 2003), while other species have operculated gills which prevent clogging in silt rich sediments. Macroinvertebrates are also able to respond to stressors such as increased metal concentrations through the induction of metal detoxifying metallothioneins (MTs) (Amiard et al., 2006).

The macroinvertebrate community structure present at a specific site may thus provide an indication of their capability to adapt or respond to environmental stressors. These specific traits or response mechanisms can, unfortunately, only aid in survival until water and habitat quality has deteriorated to such an extent that even the response mechanisms can no longer support survival. In short, this is why it is crucial to manage and conserve our freshwater habitats around the world.

South Africa is a semi-arid country with a mean annual rainfall of 497 mm (Thomas, 1996). With this being said, nearly 71% of the country's main rivers are already regarded as endangered or critically endangered (Nel et al., 2004). For this study, the Marico River was selected because of the fact that its catchment is classified as a National Freshwater Ecosystem Priority Area (Nel, 2011). Currently, the general state of the upper, free-flowing part of the river is still considered as natural to good, characterized by a pronounced biodiversity and overall good water quality (RHP, 2005). However, the Crocodile West and Marico Water Management Area (WMA) is the second most densely populated WMA in South Africa and both these rivers are under increased pressure due to water demands for social and economic development (DWAF, 2004). It is thus of great importance to manage and conserve, amongst others, the Marico River, in order to support the sustainability of freshwater ecosystems in South Africa.

Currently, limited information regarding the macroinvertebrate biodiversity, as well as of the water and sediment quality of this river system is available. This renders it challenging to detect, monitor and remediate possible adverse environmental changes. It is therefore necessary to obtain data regarding the current physico-chemical parameters of the water and sediment, as well as the community structures in order to detect detrimental changes, indicating where and when to implement remediation in the future.

Aim:

The aim of this thesis is to determine the aquatic macroinvertebrate biodiversity and to establish whether selected physico-chemical stressors have an adverse effect on the biodiversity of the Marico River.

During this study, four hypotheses will be tested:

1. The selected physico-chemical parameters in the water and sediment will largely be within the target water quality ranges acceptable for aquatic ecosystems.

2. The macroinvertebrate diversity and abundance will support the general perception that the Marico River is largely unimpacted.

3. The Marico River will exhibit a higher mollusc diversity than the highly impacted Crocodile River, situated in the same catchment area.

4. Metallothioneins as biomarker response will reflect metal exposure to macroinvertebrates in the Marico River.

The primary and secondary objectives will be to:

1. Determine concentrations and values of selected physico-chemical parameters potentially toxic to aquatic macroinvertebrates in water and sediment **(Manuscript 1)**.

- Compare and evaluate these according to specific water quality guidelines for aquatic ecosystems.

2. Conduct comprehensive biodiversity surveys of aquatic macroinvertebrates in the Marico River and selected tributaries **(Manuscript 2)**.

- Assess the influence of biotopes and environmental variables on the distribution of species.

3. Compare molluscan diversity in the Marico River with a similar study in the highly impacted Crocodile River **(Manuscript 3)**.

- Compare current results also with historical data from the National Freshwater Snail Collection Database, North-West University, Potchefstroom Campus.

4. Utilize a biomarker approach linking biological response to metal exposure using metallothionein levels in selected macroinvertebrate families **(Manuscript 4)**.

- Determine whether there are relationships between metal concentrations in the water, sediment and macroinvertebrates.

- Determine whether these relationships are reflected by concomitant MT levels in macroinvertebrates

- Establish whether MTs can be used as a biomarker of metal exposure in a relatively unimpacted system such as the Marico River.

5. Evaluate the river health status in terms of all of the above factors.

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CHAPTER 2
Study Area

As mentioned in the introduction, the Crocodile West and Marico WMA is the second most densely populated WMA in South Africa. Its two major rivers, the Crocodile and Marico, are under increased pressure due to water demands for development (RHP, 2005).

Currently, the Marico River is of great conservation importance as its general state is still considered as natural to good, free from significant organic pollution, with a pronounced biodiversity and overall good water quality (RHP, 2005). The river originates at the dolomitic Marico Eye, near Swartruggens and flows northwards to Derdepoort for about 120 km where it turns north-east to form the border between South Africa and Botswana, for approximately 70 km. The total length of the river is more or less 250 km.

According to DWAF (2004) the lower Marico has slightly reduced water quality and often has no visible surface water flows in the segments downstream of the Marico Bosveld Dam, mainly due to abstraction for irrigation purposes. Substantial volumes of water are also abstracted from the large dolomitic aquifer compartments, which act as the main source of water for this river system (DWAF 2004; Hubert et al., 2006; Smith-Adao et al., 2006). Land uses in the Marico catchment that could have localized impacts on the lower parts of the river include agricultural practices, urban water use and rural domestic water use. The water quality of the tributaries, such as the Klein Marico River and the Sterkstroom, is defined as fair to poor and these rivers are further impacted by slate mining activities, industrialization, urbanization, as well as agriculture (RHP, 2005). The above mentioned stressors may have a detrimental impact by polluting the aquatic environment, while reducing the water and sediment quality.

Mean annual temperatures in the Marico catchment range between 18 and 20°C with mean annual rainfall in the range of 400 to 800 mm (RHP, 2005). The primary lithology surrounding the study area includes quartzite, ferruginous shale, hornfels, ferruginous quartzite, andesite, basaltic lawa, agglomerate, tuff, shale, rhyolite and dacite. Weathering of geological formations in general, combined with human disturbances and basin characteristics such as slope and land cover (Kaufmann et al., 2009) act as the main sources for the formation of sediment.

For the purpose of this study, nine sites in the Marico River, Sterkstroom and Klein Marico River were selected.

Site H1, in the Marico Eye (Spring), is characterized by a deep, clear pool with marginal and aquatic vegetation. Site H2, in the Marico River about 20 km downstream of the Eye, is a fast-flowing cobble stream nestled between the hills, just before the confluence with the Sterkstroom. Both Sites H1 and H2 are situated in largely unimpacted areas. Site H3, in the Sterkstroom, has a rocky substratum with shallow, fast-flowing water, an overhanging tree canopy and little to no aquatic vegetation. Sites H4 (near the town of Groot Marico) and H7 are in the Marico River, situated above and below the Marico Bosveld Dam, respectively. These sites are both characterized by rock and clay substratum with marginal and aquatic vegetation. Site H4 has a fast flowing cobble stream while Site H7 has the impact of a weir with backwaters and bedrock. Sites H5 (near the town of Zeerust) and H6 are in the Klein Marico River, situated above and below of the Klein Maricopoort Dam, respectively. Both these sites have shallow streams with a muddy substratum and little to no aquatic vegetation. The Klein Marico River is further described as being contaminated by effluent from the town of Zeerust (RHP, 2005). Sites H9 and H8 are situated in the Marico River below the Molatedi Dam and near the Derdepoort Botswana border post, respectively. Site H9 is a shallow, rocky stream in which flow is dependent on water being released sporadically from the Molatedi Dam, while Site H8 has deep, slow-flowing water with ample marginal vegetation and a muddy substratum.

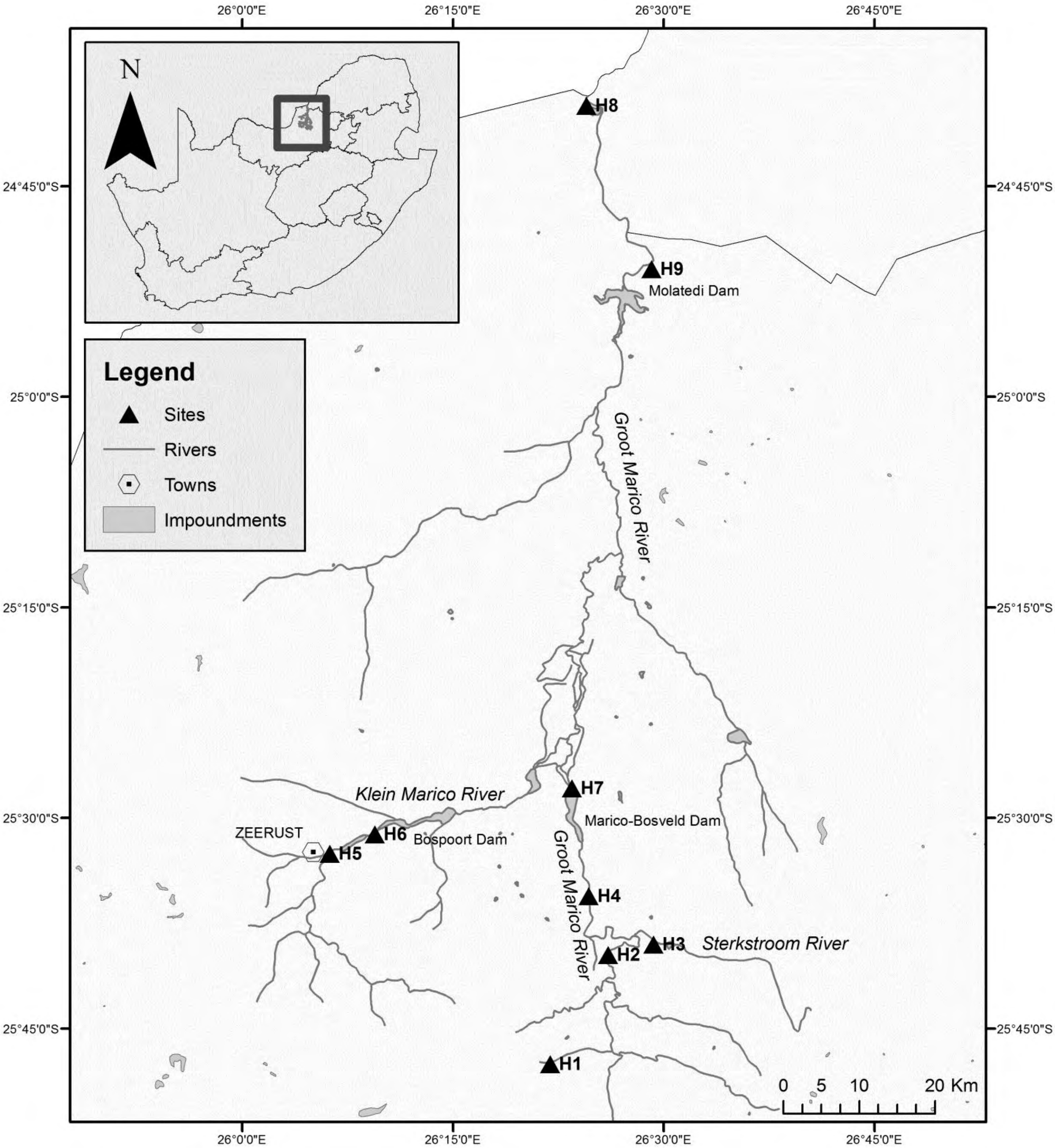

Figure 1: Locations of sampling sites (triangles) within the Marico River catchment, North-West Province, South Africa.

Table 1: Site descriptions, coordinates, altitude, biotopes, land use, as well as the dominant vegetation.

	H1	H2	H3	H4
River	Marico Eye, source of Marico River.	Marico River before its confluence with Sterkstroom, 20 km downstream of the Eye.	Sterkstroom 5 km before its confluence with Marico River.	Marico River 10 km above Marico-Bosveld Dam, after its confluence with Sterkstroom.
Coordinates	S 25'47'32.1	S 25'39'45.1	S 25'39'00.6	S 25'35'33.4
	E 26'21'54'1	E 26'26'01.9	E 26'29'16.3	E 26'24'39.4
Altitude	1480 m	1 197 m	1170 m	1 077 m
Biotopes				
Marginal Vegetation	X	X	X	X
Aquatic Vegetation	X			X
Sand				X
Mud	X			X
Stones in Current		X	X	X
Stones out of Current		X		X
Riffle		X	X	X
Run		X	X	X
Pool	X			
Backwater	X			
Land Use	Recreational diving and water extraction for commercial purposes.	Cultivated land.	Cultivated land.	Cultivated land.
Dominant Vegetation	*Arundo donax, Carex burchelliana, Cyperus sexangularis, Cyperus dives, Phragmites australis, P. mauritianus, Typha capensis, Rumex conglomeratus.*	*Cyperus sexangularis, Nasturtium officinale, Phragmites mauritianus.*	*Cyperus sexangularis.*	*Cyperus sexangularis, Juncus lomatophyllus, Persicaria decipiens, Phragmites australis, Spirogyra, Typha capensis.*

Table 1 continues: Site descriptions, coordinates, altitude, biotopes, land use, as well as the dominant vegetation.

	H5	H6	H7	H8	H9
River	Klein Marico River 5 km above Klein-Maricopoort (Bospoort) Dam	Klein Marico River 1km below Klein-Maricopoort (Bospoort) Dam.	Marico River directly below Marico-Bosveld Dam.	Marico River at Derdepoort.	Marico River 2 km below Molatedi Dam.
Coordinates	S 25'23'44.4	S 25'31'09.2	S 25'27'52.6	S 24'39'15.9	S 24'50'54.2
	E 26'06'08.0	E26'09'25.1	E 26'23'26.9	E 26'24'28.7	E 26'29'07.1
Altitude	1 150 m	1135 m	1 037 m	914 m	926 m
Biotopes					
Marginal Veg	X	X	X	X	
Aquatic Veg				X	
Sand					
Mud	X	X	X	X	X
Stones in Current					
Stones out of Current					
Riffle					
Run	X	X	X		X
Pool		X	X	X	X
Backwater				X	
Land Use	Urbanization and industrialization.	Cultivated land.	Cultivated land.	Game farms.	Inficormal settelemts and cattle grazing.
Dominant Vegetation	*Berula erecta, Hydrocotyle verticillata, Nasturtium officinale, Phragmites mauritianus, Rumex conglomeratus.*	*Cyperus sexangularis, Persicaria decipiens, Typha capensis, Rumex conglomeratus.*	*Cyperus sexangularis, Juncus lomatophyllus, Phragmites mauritianus, Spirogyra.*	*Potamogeton schweinfurthii, Phragmites australis, Juncus lomatophyllus, Cyperus sexangularis, Persicaria decipiens, Typha capensis, Hydrocotyle verticillata.*	*Cyperus eragrostis, Hydrocotyle verticillata, Juncus lomatophyllus, Potamogeton schweinfurthii, Typha capensis.*

Figures 2 and 3: Site H1 – Marico Eye (Spring), source of the Marico River.

Figures 4 and 5: Site H2 – Marico River, 20 km downstream of the Eye.

Figures 6 and 7: Site H3 – Sterkstroom.

Figures 8 and 9: Site H4 – Marico River 10 km above Marico-Bosveld Dam.

Figures 10 and 11: Site H5 – Klein Marico River 5 km above Klein-Marico Bospoort Dam.

Figures 12 and 13: Site H6 – Klein Marico River 1km below Klein-Marico Bospoort Dam.

Figures 14 and 15: Site H7 – Marico River directly below Marico-Bosveld Dam.

Figures 16 and 17: Site H8 – Marico River at Derdepoort.

Figures 18 and 19: Site H9 – Marico River 2 km below Molatedi Dam.

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CHAPTER 3

Selected physico-chemical characteristics potentially toxic to aquatic macroinvertebrates in water and sediment of a relatively unimpacted river, South Africa

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Abstract

This study was conducted to determine concentrations of selected metals in water and sediment, as well as mean values of selected physico-chemical parameters. Five surveys were done during low-flow seasons at nine sites in the Marico River and selected tributaries. Water and sediment samples were collected from all the sites, while selected physico-chemical parameters (turbidity, temperature, EC, pH and velocity) were measured in situ at each site. Water was filtered, sediment was dried and digested and both were analyzed in an inductively coupled plasma mass spectrometry (ICP-MS). The Endecott dry sieving method was used to determine particle size distribution and mineral identification was done by X-ray characterization of the crystalline materials present in fractions <50 µm. In the water, only six metals, including Al, Cr, Mn, Cu, Zn and Pb, exceeded chronic effects values. However, all metal concentrations except Mn at one site, were within the mean natural ranges stated in literature. Most metals including Cr, Fe, Co, Ni, Cu, Zn, As, Cd and Pb, exceeded the sediment quality guideline values. The majority of water quality variables were within the tolerance ranges of macroinvertebrates and may pose limited threat to the organisms. Because of limited anthropogenic impacts in the catchment, the origin of most metals detected is probably from geological weathering of the surrounding primary lithology, resulting in the deposition of secondary minerals. Metal concentrations in the water were considerably lower than in the sediment, which may indicate that metals in the sediment were most probably not bioavailable for aquatic macroinvertebrates at the time of sampling.

Keywords

Metal concentrations, minerals, water quality parameters, sediment particle size, minerals.

Introduction

The water and sediment of rivers naturally contain trace amounts of metals but an increase in these concentrations can, due to accumulation, become potentially toxic to aquatic organisms such as macroinvertebrates (Smith, 1999; Greenfield et al., 2012). Elevated metal concentrations in rivers could be due to natural weathering of the surrounding primary lithology, which then results in the release of secondary minerals. Other natural sources of metals include the decomposition of biotic matter, windblown dust, veld-fires and vegetation (Smith, 1999; Malik et al., 2010). Anthropogenic activities such as mining, urbanization, industrial processing of ores and metals, fertilizer run-off and atmospheric precipitation also contribute to the metal composition in rivers and dams (Greenfield et al., 2007; Koulousaris et al., 2009).

According to Papafilippaki (2008), the behaviour of metals in water is a function of the substrate and suspended sediment composition, as well as water chemistry. When these metals enter aquatic systems, it may get immobilized within the sediments through processes such as adsorption, flocculation and co-precipitation (Barakat et al., 2012; Butu and Iguisi, 2013). Sediments in rivers are thus often regarded as sinks for metals. These metals can be remobilized into the overlying water column, resulting in an increase in bioavailability and concentrations reaching toxic levels, should selected environmental conditions change (Wen and Allen, 1999; Eggelton and Thomas, 2004; Barakat et al., 2012). Aquatic macroinvertebrate populations that are chronically exposed to selected metals may display enhanced tolerance, while acute concentrations of potentially toxic metals (Al, Zn, Cr, Co, Cu, Fe, Mn and Ni), may eliminate sensitive taxa (Kapustka et al., 2004; Chiba et al., 2011). Metal contamination may also reduce species richness, density, growth and reproduction, as well as alter community composition (Maret et al., 2003; Gray and Delaney, 2010). Contaminated rivers may show reduced abundance of metal-sensitive taxa, such as Plecoptera, Epemeroptera and Trichoptera and be dominated by metal-tolerant taxa such as the chironomids (Hickey and Clements, 1998). Depending on the accumulation pattern of metals, invertebrates are, however, able to react to, metabolize and regulate metals within their internal systems through mechanisms such as metallothioneins (Amiard et al., 2006).

Water quality variables including pH, electrical conductivity (EC), turbidity, temperature and velocity may also have an impact on aquatic ecosystems and the survival of biota. Large fluctuations in pH cause stress to the organisms while low pH values can mobilise otherwise bound metals, leading to potentially toxic concentrations (Alabaster and Lloyd, 1980; Dhillon et al., 2013). It is also known that a sudden increase in conductivity or unnatural fluctuations in pH is often used to indicate early stages of water pollution as it is generally due to anthropogenic impacts such as acid mine drainage or agricultural runoff (Dhillon et al., 2013). Increased EC can also lower oxygen concentrations and may create eutrophic conditions, which are harmful to organisms. With regard to turbidity, several authors (Lloyd et al., 1987; Henley et al., 2000; Bilotta and Brazier, 2008) found that it can have a detrimental impact on organisms such as macroinvertebrates by reducing light penetration, decreasing photosynthesis and lowering dissolved oxygen concentrations. Turbidity also affects the survival, growth and feeding of aquatic organisms by reducing visibility of both predators and prey and clogging the gills of some organisms (Lloyd et al., 1987; Bilotta and Brazier, 2008). Fluctuating water temperatures affects the solubility of oxygen, the rates of chemical reactions, as well as metabolic rates and thus control lifecycles of macroinvertebrates (Holmes, 1996), while water velocity influences several of the above factors such as water temperature, dissolved oxygen concentrations, as well as turbidity.

With regard to sediments, it is well known that fine sediments (<2000 μm) result in an increase in the adsorption of metals (Salomons and de Groot,1978; Ackermann et al., 1983). It may also be detrimental to benthic organisms as it affects, amongst others, respiration and feeding behaviour (Harrison et al., 2007).

As changes in a single variable also impact on other variables, it is important to understand the combined effect of their interactions, as well as their effect on the distribution of species.

To ensure the survival of species, several countries have developed specific quality guidelines, with chronic and acute values for metal concentrations in the water and sediment (Holmes, 1996, ANZECC, 2000; EPA, 2009), however, no South African guidelines for sediment are available yet.

The Marico River, one of the main rivers in the North-West Province and a tributary of the Limpopo River, is classified as a National Freshwater Ecosystem Priority Area (Nel, 2011). Its general state is considered as natural to good, free from significant organic pollution, with a pronounced biodiversity and overall good water quality (RHP, 2005).

However, little to no information regarding the physico-chemical characteristics of the water, as well as the sediment of this river is currently available. This study therefore aims to determine the concentrations of selected metals, as well as mean values of the physico-chemical parameters. This will then be compared and evaluated according to specific water and sediment quality guidelines for aquatic ecosystems.

Material and Methods

Study area

The Marico River originates at the Marico Eye near the town of Swartruggens in the North-West Province. At its confluence with the Crocodile River, on the Botswana border, these rivers give rise to the Limpopo River. The limited anthropogenic disturbances in the catchment area of the Marico River include two dams in the Marico and one dam in the Klein Marico River, urban development in the town of Zeerust, as well as cultivated land and informal settlements located along the course of the river (RHP, 2005).

Annual temperature and rainfall in the Marico catchment range between 18 and 20°C and 400 to 800 mm, respectively (RHP, 2005). Most of the catchment is situated on the Bushveld Igneous Complex and the primary lithology surrounding the study area includes quartzite, ferruginous shale, hornfels, ferruginous quartzite, andesite, basaltic lawa, agglomerate, tuff, shale, rhyolite and dacite. Weathering of geological formations in general, combined with human disturbances and basin characteristics such as slope and land cover (Kaufmann et al., 2009) act as the main sources for the formation of sediment.

Surveys were conducted at nine samplings sites in the Marico River, Klein Marico River and Sterkstroom (Figure 1). Site H1, the dolomitic Marico Eye, is the source of the Marico River. Sites H1 and H2 are both situated in fairly unimpacted areas. Site H3 is in the Sterkstroom, which is regarded as moderately impacted (RHP, 2005). Sites H4 and H7, in the Marico River are situated upstream and downstream of the Marico Bosveld Dam, respectively. Sites H2, H3, H4 and H7 may all be subjected to agricultural runoff from cultivated lands. Sites H5 and H6, in the Klein Marico River, are situated upstream and downstream of the Klein Maricopoort Dam, respectively. This tributary is described as being in a fair to poor state and is contaminated by effluent from the town of Zeerust (RHP, 2005). Site H8 is located in the Marico River at Derdepoort near the Botswana border and Site H9, also in the Marico River, is just below the Molatedi Dam.

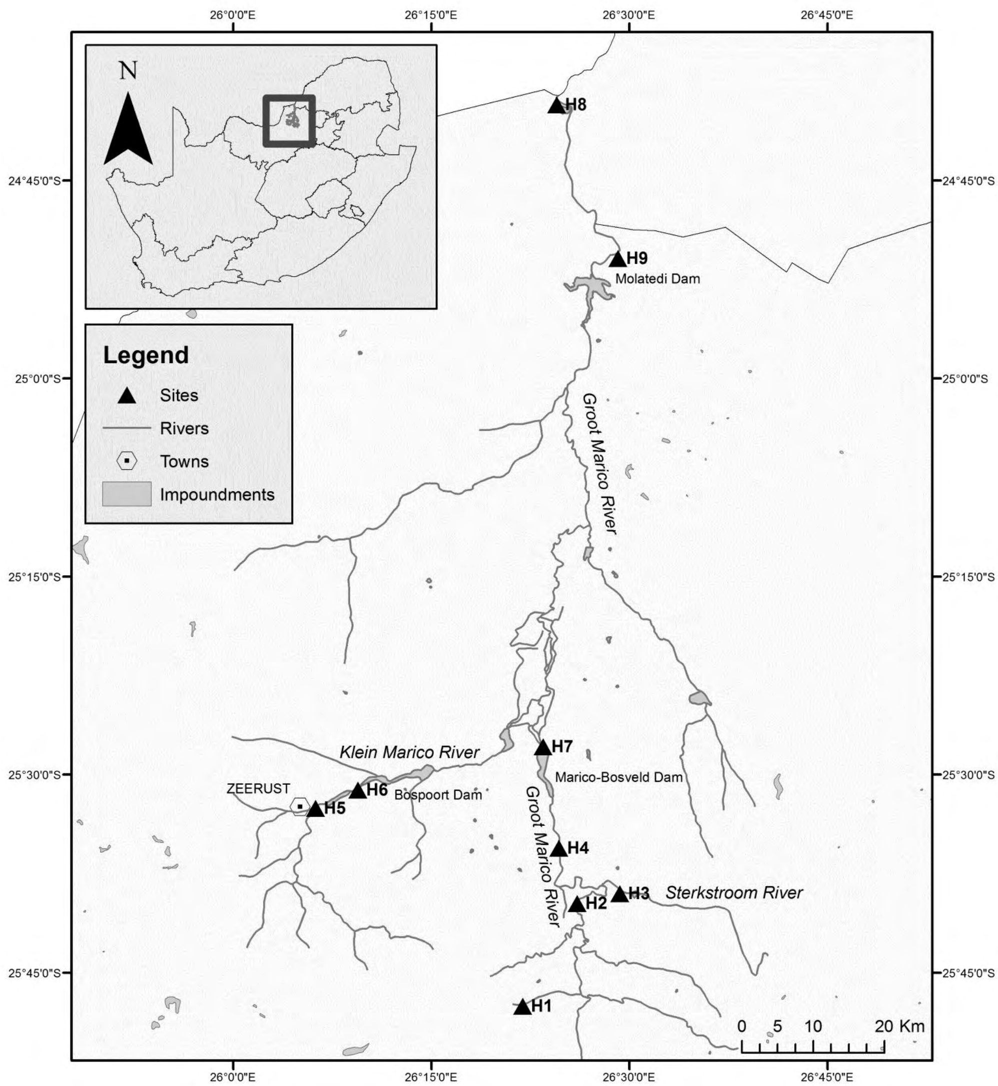

Figure 1: Sampling sites within the Marico River catchment, North-West, South Africa.

Five surveys were conducted during the low-flow periods (May to November), for three consecutive years (2013, 2014 and 2015). The low-flow period was selected based on the fact that the habitat is, to a large extent, stable during this time (Kowalkowski et al., 2007). The fact that the topography of the catchment is generally very flat and that the area is regarded as semi-arid with low rainfall (DWAF, 2004), may limit the migration of metals from the surrounding environment to the river.

Sampling and preparation

Water and sediment samples were collected from all the sites to analyze metals and selected physico-chemical parameters (turbidity, temperature, EC, pH and velocity) were measured in situ at each site. The physico-chemical parameters were measured by making use of a clarity tube and calibrated portable digital instruments including: conductivity meter (DIST 3, HI 98303, Hanna Instruments), pH probe (HI 98128, Hanna Instruments), thermometer (Checktemp, Hanna Instruments) and flow-probe (FP 111, Global Water). The coordinates and altitude of each sampling site were determined with a Garmin Nuvi 500 GPS and from Google Earth. Water temperature is expressed as Degrees Celsius (°C), EC as µS/cm, turbidity as NTU's and velocity or flow as meter/second.

Water and sediment samples (approximately 300ml each) were collected separately in polyethylene bottles which were washed with detergent, rinsed with distilled water followed by 5% nitric acid solution and rinsed again with double distilled water. Samples were then transported to the laboratory and stored at -20°C until it was analyzed. Water samples were filtered through Whatman filter paper (0.45 µm) and sediment samples dried at 60°C. A sub-sample of 30g from the sediment sample was sieved using an Endecott dry sieving system to collect fractions >2000 µm, <2000 µm and <50 µm.

Water metal analysis

The filtrate was used directly for analysis in an inductively coupled plasma mass spectrometry (ICP-MS) using standard recognized techniques. Ultraspec Certified Element Standards (De Bruyn Spectropscopic Solutions, Bryonston, Jhb, SA) were used to set up calibration curves. The percentage recoveries were within an acceptable range (<10%

deviation) (see supplementary material Table S1). All water samples were analysed in triplicate.

Sediment metal analysis

Analyses of metals in the total sediment samples were also performed by making use of an ICP-MS. Samples of 0.5g sediment were digested using aqua regia (HCL: HNO_3 = 3:1) and a microwave digester. For each batch of samples, quality control and quality assurance protocols were applied. Certified reference materials (Resource Technology Corporation and CN Schmidt BV: Trace Elements on Fresh Water Sediments (CNS392-050)) were used and standard calibration runs were performed. The percentage recoveries for the standards and certified reference materials were within an acceptable range (<10% deviation) (see supplementary material Table S1). All sediment samples were analysed in triplicate.

Mineral analyses

Mineral identification was done by characterization of the crystalline materials present in fractions <50 μm. Sample preparation was done using a back loading technique. Samples were scanned using X-rays generated by a Cu X-Ray tube which generates a unique diffractogram for each sample. The diffractogram represented the different phases and phase concentrations of all the crystalline materials present in each sample. The different phases were identified using X'Pert Highscore plus and International Centre for Diffraction Data. Mineral identification was done by making use of ICDD database PDF 4+, 2014 and the ICSD-PANalytical program. The phases were used to do a Rietveld quantification to determine the weight percentage of each mineral in the sample.

Data and statistical analyses

The data obtained from the ICP-MS analyses for both water and sediment samples were recorded in Microsoft Excel 2010 spreadsheets and tables were compiled for each set of data. Metal concentrations were expressed as mg/kg for the sediment and as µg/l for water. The metal concentrations in the water were, where possible, compared to existing water quality guidelines as established for South Africa (SA) (Holmes, 1996), Australia and New Zealand (ANZECC, 2000) and America (EPA, 2009) while the values for the sediment were compared to the guidelines of the EPA (2009) and ANZECC (2000) only. For SA (Holmes, 1996) and the EPA (2009), the "chronic effect values" (CEV) were used, while the values for ANZECC (2000) were based on the "95% level of protection for freshwater species".

The Kolmogorov-Smirnov test (with Dallal-Wilkinson-Lilliefor P values) was used to test all data for normality. One-way analyses of variance (ANOVAs) followed by Tukeys test for post hoc analysis were done to test for significant differences ($p<0.05$) between the mean metal concentrations at the various sites. Particle size and metal concentrations of sediment were assessed through exploratory statistical analysis by making use of a redundancy analysis (RDA). This provides a graphic display, enabling assessments of large datasets and explaining the combination of variables that describe the largest amount of data within a dataset (Gao et al., 2009). All statistics were performed using GraphPad Prism 5 and Canoco (version 5).

Results and Discussion

A total number of 35 metals were measured in each of the water and sediment samples (results not shown). Only selected metals considered as potentially toxic to macroinvertebrates will be discussed.

Water

Table 1 shows the mean, minimum and maximum values recorded for the physico-chemical parameters (temperature, EC, pH, turbidity and velocity) and relevant metal concentrations (µg/l) in the water samples, as well as the natural concentration range and chronic effect values (CEV) for aquatic ecosystems. According to Holmes (1996), the CEV is defined as "the concentration or level of a constituent at which there is expected to be a significant probability of measurable chronic effects to up to 5 % of the species in the aquatic community". These effects can include, amongst others, lethality, interference with breeding, behavioural, feeding or migratory patterns (Holmes, 1996). Figure 2a-l represents the ANOVAs of the metal concentrations in the water and Figure 3a shows the mean metal concentration at the various sites.

From Table 1 it is clear that only six metals, including Al, Cr, Mn, Cu, Zn and Pb, exceeded the CEV. Furthermore, all metal concentrations except Mn at Site H5, were within the mean natural ranges stated in literature (Hem, 1985; Shiller and Boyle, 1985; WHO, 2004).

Taking the different sites into account, it is evident that limited variation occurred in the concentrations of most metals (Table 1 and Figure 3a). This is in accordance with previous studies (de Klerk et al., 2012; Greenfield et al., 2012) and is also reflected by the ANOVAs representing the mean metal concentrations in the water (Figure 2a-l). Aluminium, Ti, Cr, Ni, Ci, Zn, As, Cd and Pb did not show significant variation ($p>0.05$) between the different sites, however, with regard to Mn, Fe and Co concentrations, Site H5 differed significantly ($p<0.05$) from most of the remaining sites. A peak in Ti, Mn, Fe, Co and As concentrations were measured at Site H5 (Table 1), which is situated in the town of Zeerust and which could have been influenced by local anthropogenic activities (RHP, 2005). The drastic increase observed for Fe and Mn can most probably be ascribed to the decomposition of organic matter, leading to a decrease in dissolved oxygen and an increase in the dissolution of these metals. The concentration of Fe at Sites H1 to H4 and H6 to H9 were substantially lower (Table 1), which can be ascribed to well oxygenated water due to water atmospheric interactions.

The elevated As concentration at Site H5 may be due to anaerobic conditions, which could lead to the reduction of Fe (Korte and Fernando, 1991). Korte (1991) speculated that desorption of As from ion oxides occurs in reducing alluvial sediments which could lead to high As in water. Site H5 is further characterised by significantly higher EC (865.3±94.5 µS/cm) and mostly stagnant, turbid water (Table 1). This EC value exceeds the laboratory-derived acute salinity tolerance ($LC(_{50})$ values) of freshwater macroinvertebrates (55-760 µS/cm) (Kefford at al., 2004). The elevated conductivity indicates a high amount of dissolved salts, possibly due to point source pollutants such as wastewater from the town of Zeerust (Michaud, 1991; RHP, 2005) and, as mentioned in the introduction, it can have a detrimental effect on aquatic ecosystems and macroinvertebrates (Bilotta and Brazier, 2008).

As mentioned in the introduction, numerous factors can either anthropogenically or naturally influence the concentration, variation, speciation and bioavailability of metals in a river system. The metals that exceeded the CEV (Al, Cr, Mn, Cu, Zn and Pb), can all originate from anthropogenic sources (Eisenreich, 1980; Driscoll and Postek, 1995; Holmes, 1996). In this study, however, concentrations were most probably from natural origin such as the weathering of minerals identified in the sediment (Table 3). Aluminium, Mn and Fe are of the most abundant elements in the earth's crust (Holmes, 1996) and elevated concentrations in general are visible in natural waters. In contrast to this, Cr, Cu and Pb are rather scarce metals and, while it exceeded the CEV, concentrations measured in the Marico River, were still relatively low.

Although selected metal concentrations exceeded the CEV, various interactions including adsorption, desorption and precipitation may reduce the metals' toxicity and bioavailability to macroinvertebrates (Holmes, 1996; Lin and Chen, 1998; Li et al., 2001a; Fu and Wang, 2011). Microorganisms can also decrease the toxicity of metals via detoxification pathways, such as lowering the metals' redox state, impacting its mobility (Violante et al., 2010).

Except for EC at Site H5, physico-chemical parameters measured were all within the ranges deemed acceptable for aquatic organisms. It will most probably not have any negative impacts, due to the tolerance that macroinvertebrates have for a wide variation for these parameters (Holmes, 1996; Hassell et al., 2006; Tixier et al., 2009; Hussain and Pandit, 2012). The relatively constant pH values might furthermore be explained by the dolomitic geology of the area (RHP, 2005) creating a buffer against variations in pH (Kemp and Wolmarans, 2015).

Table 1: Summarised metal concentrations (µg/l) and selected physico-chemical parameters (mean ±standard error, with minimum and maximum values in parentheses) in the water recorded at nine sites during five surveys, with the corresponding mean natural ranges[+] and guideline values[#].

µg/l	H1	H2	H3	H4	H5	H6	H7	H8	H9	MNR[+]	EPA	SA	ANZECC
Al	22.75±17.54	24±23.23	22.83±14.95	9.64±7.9	13.26±13.14	12.27±7.93	23.55±13.98	16.68±8.59	35.99±20.44	0-50*		20	55
	(0 - 90.73)	(0 - 117.8)	(0 - 80.77)	(0 - 40.8)	(0 - 52.68)	(0 - 39.4)	(0 – 66)	(0 - 44.52)	(0 - 88.26)				
Ti	1.48±0.39	1.53±0.49	1.56±0.43	1.65±0.4	4.27±2	1.24±0.4	2.28±0.86	1.56±0.35	1.98±0.69	0-8.6**			
	(0.47 - 2.901)	(0.6 - 3.31)	(0.47 – 3)	(0.84 - 2.85)	(1.24 - 10.11)	(0.45 - 2.69)	(0.76 - 5.04)	(0.65 - 2.42)	(0.034 - 4.15)				
Cr	3.82±1.22	2.4±1.21	2.5±1.27	2.66±1.39	3.6±1.5	2.63±1.3	2.35±1.23	2.6±1.36	2.94±1.57	0-10*	11	14	1
	(0 - 6.56)	(0 - 5.4)	(0 - 5.72)	(0 - 6.33)	(0.02 - 6.53)	(0 - 5.99)	(0 - 5.33)	(0 - 5.92)	(0 - 7.105)				
Mn	2.28±1.55	2.4±0.97	19.19±16.98	1.21±0.59	280.63±209.27	1.36±0.63	6.57±3.5	1.93±0.61	1.38±0.45	0-200*	100	370	1900
	(0 - 8.1)	(0 - 5.09)	(0 - 87.040)	(0.08 - 2.9)	(0.44 - 903.7)	(0 - 2.952)	(0.49 - 19.33)	(0.05 - 3.69)	(0.04 - 2.53)				
Fe	153.81±30.21	187.01±41.33	132.08±40.01	173.79±23.24	500.08±140.23	324.54±32.84	222.64±20.52	257.84±57.92	253.08±49.69	0-700*	1000		
	(76.28 - 248.6)	(74.75 - 300.1)	(38.47 - 251.5)	(83.15 – 214)	(272.1 – 893)	(222.9 - 414.5)	(164.7 - 278.6)	(153.4 - 472)	(114.6 - 377.3)				
Co	0.30±0.11	0.32±0.1	0.3±0.11	0.3±0.12	1.03±0.32	0.43±0.12	0.3±0.09	0.41±0.14	0.391±0.14	0-1.9*			
	(0.006 - 0.656)	(0.008 - 0.6)	(0.008 - 0.699)	(0.006 - 0.735)	(0.44 - 1.94)	(0.008 - 0.771)	(0.006 - 0.543)	(0.005 - 0.89)	(0.009 - 0.84)				
Ni	2.8±0.88	3.8±1.14	3.56±1.07	2.78±1.2	5.13±1.76	3.85±1.38	2.93±1.30	5.02±1.73	5.78±1.9	0-16.6*	52		11
	(0.09 - 4.72)	(0.02 - 6.57)	(0.08 - 5.79)	(0.07 - 5.73)	(0.05 - 7.62)	(0.054 - 7.809)	(0.09 - 6.21)	(0.12 - 10.22)	(0.1 - 10.11)				
Cu	2.16±1.23	2.66±1.42	3.65±2.38	7.88±6.3	2.77±1.57	2.92±2.27	2.77±1.41	2.55±1.33	3.43±1.95	0-10*		1.5	1.4
	(0 - 6.07)	(0.2 - 7.08)	(0.08 - 12.16)	(0 - 32.82)	(0.017 - 7.16)	(0 - 11.89)	(0.05 - 6.79)	(0 - 5.82)	(0.2 - 11.1)				
Zn	29.9±17.14	36.93±2.26	43.63±26.74	20.02±10.64	14.1±12.35	36.5±26.19	16.62±10.8	21.95±11.49	33.4±21.58	0-29.4***	120	3.6	8
	90 - 81.57)	(0.22 - 102.4)	(0 - 117.8)	(0.16 - 58.46)	(0.1 - 50.98)	(0.228 - 136.1)	(0 - 54.9)	(0.09 - 61.57)	(0.31 - 116.2)				
As	0.188±0.128	0.54±0.19	0.509±0.187	0.64±0.195	0.957±0.492	1.113±0.379	0.45±0.129	0.757±0.196	0.64±0.172	2*		20	13
	(0 - 0.66)	(0.03 - 1.16)	(0.0 - 1.18)	(0.03 - 1.16)	(0.01 - 2.25)	(0.02 - 2.21)	(0.03 - 0.83)	(0.03 - 1.14)	(0.02 - 0.98)				
Cd	0.023±0.021	0.023±0.021	0.027±0.025	0.016±0.013	0.023±0.02	0.018±0.016	0.015±0.013	0.019±0.016	0.023±0.014	1*	0.25	0.5	0.2
	(0 - 0.11)	(0 - 0.11)	(0 - 0.13)	(0 - 0.07)	(0 - 0.084)	(0 - 0.08)	(0 - 0.07)	(0 - 0.08)	(0 - 0.07)				
Pb	3.44±2.49	3.8±2.91	7.78±6.22	3.86±2.88	1.3±1.21	5.84±4.91	5.08±3.97	4.17±.87	6.19±5.11	30*	2.5	1	3.4
	(0 - 12.85)	(0 - 15.04)	(0 - 32.21)	(0 - 14.92)	(0 - 4.91)	(0 - 25.29)	(0 - 20.53)	(0 - 14.72)	(0 - 26.41)				
Temp	19.48±0.76	16.88±1.91	16.02±1.92	18.5±1.62	16.43±2.77	16.64±2.21	19.34±2.17	21.54±1.62	20.12±2.03			5-30	
	(18 - 21.8)	(13 - 23.2)	(11.9 - 22.1)	(14.5 - 23.7)	(10.5 - 22.7)	(11.3 - 22.4)	(12.9 - 24.1)	(18.1 - 27.2)	(15.4 - 27.4)				
pH	6.93±0.3	7.19±0.3	6.77±0.31	7.26±0.41	7.89±0.10	7.44±0.32	7.48±0.3	7.51±0.2	7.82±0.29			6.5-9	
	(6.07 - 7.89)	(6.28 - 8.02)	(6.04 - 7.84)	(6.02 - 8.19)	(7.7 - 8.16)	(6.38 - 8.23)	(6.47 - 8.34)	(6.8 - 7.91)	(7.09 - 8.52)				
EC	350±70.6	288.4±8	107.6±4.9	287.2±15.7	865.25±94.5	617.8±25.2	264±17.1	407.8±51.3	399.4±54.7				
	(253 – 630)	(263 – 312)	(96 – 120)	(241 – 325)	(667 – 1103)	(560 – 696)	(208 – 302)	(256 – 566)	(228 – 548)				
Turb	5±0	5.4±0.24	5.4±0.24	30.2±1.96	203.75±2.11	6.6±0.24	5.2±0.2	15±0.55	29.8±1.46				
	(5 - 5)	(5 – 6)	(5 – 6)	(27 – 35)	(150 – 240)	(6 – 7)	(5 – 6)	(14 – 17)	(27 – 35)				
Flow	0.06±0.02	0.46±0.05	0.104±0.03	0.82±0.04	0.15±0.03	0.2±0.04	0.24±0.05	0.04±0.02	0.26±0.09				
	(0 - 0.1)	(0.3 - 0.6)	(0.02 - 0.2)	(0.7 - 0.9)	(0.1 - 0.2)	(0.1 - 0.3)	(0.1 - 0.4)	(0 - 0.1)	(0 - 0.5)				

Shaded blocks exceed guideline values[#]: EPA=Environmental Protection Agency (2009); SA=South Africa (Holmes, 1996); ANZECC=Australia and New Zealand (2000) and mean natural ranges[+]: *WHO (2004) **Hem (1985) *** Shiller and Boyle (1985).

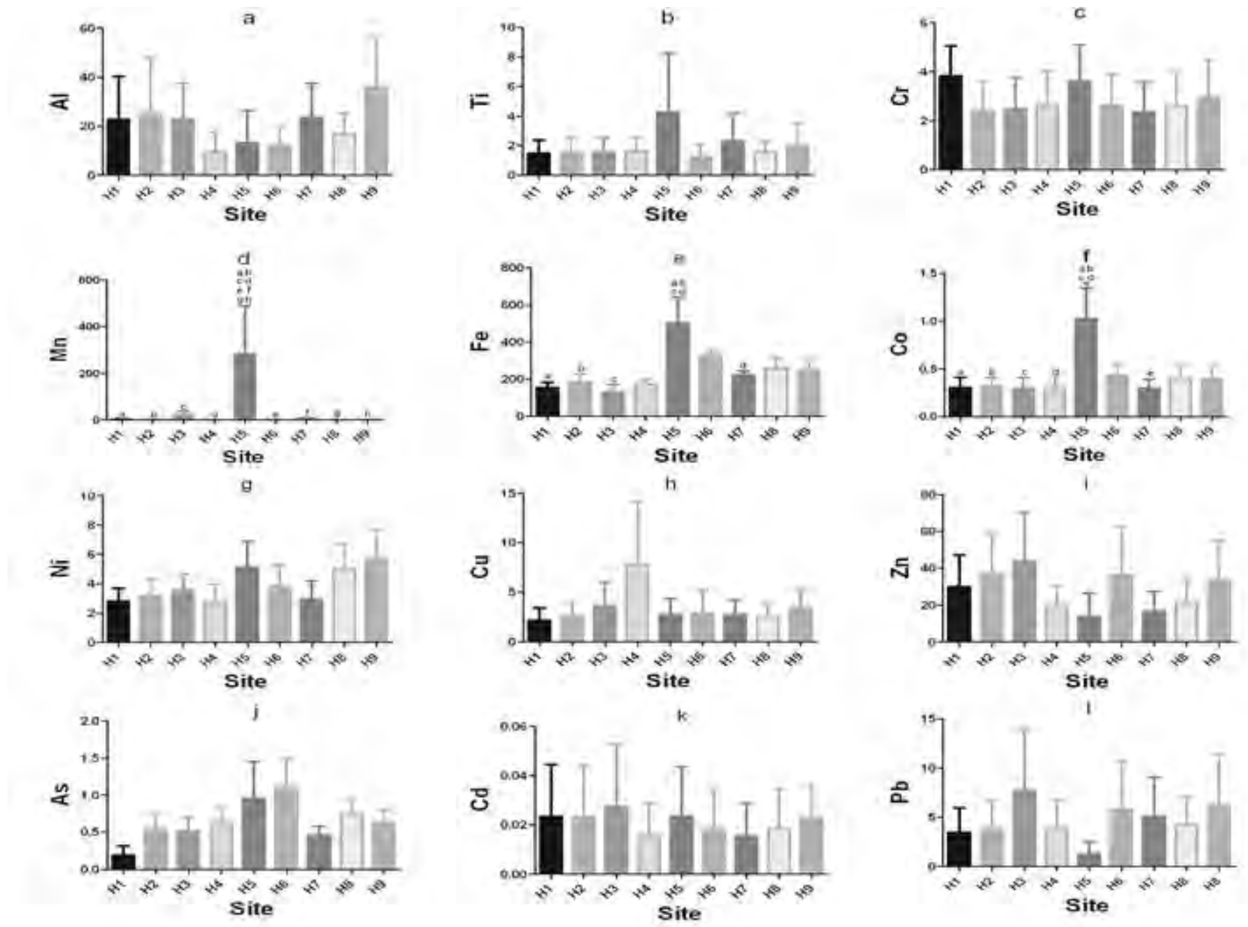

Figure 2a-l: ANOVAs illustrating the mean±standard error of metal concentrations (µg/l) measured in the water. Bars with common superscripts differ significantly (p<0.05).

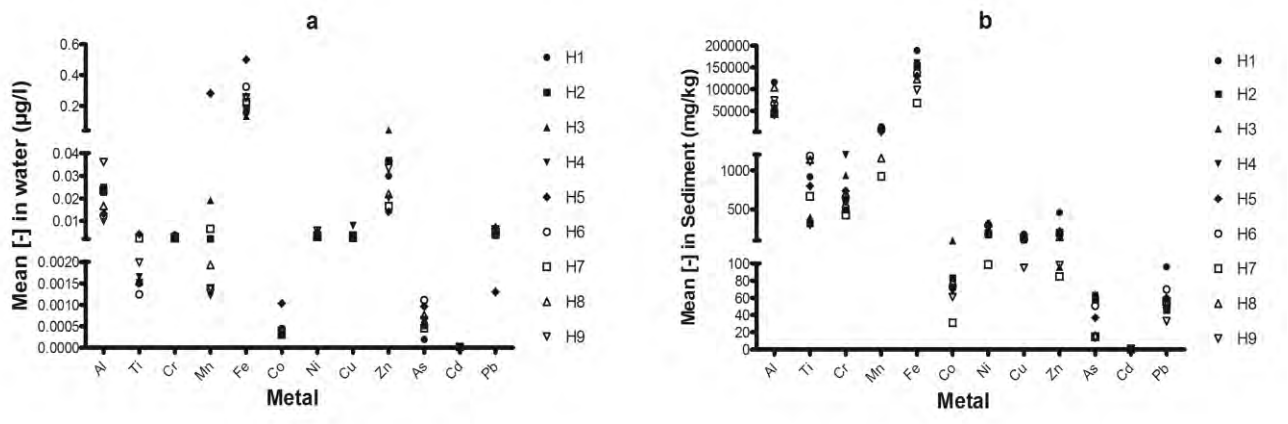

Figure 3a-b: Mean metal concentrations in the water (a) and sediment (b) at the various sites.

Sediment

Table 2 shows the mean, minimum and maximum metal concentrations (mg/kg) in the sediment at each site, the guideline values as well as the percentage of particles <2000 µm. Figure 3b represents the mean metal concentrations at the various sites while Figure 4a-l is the ANOVAs of the metal concentrations in the sediment. The mineral composition of the sediment collected at each site is presented in Table 3. The associations between metal concentrations, sampling sites and mineral composition at each site are depicted in the RDA triplot (Figure 5).

The majority of metals including Cr, Fe, Co, Ni, Cu, Zn, As, Cd and Pb, exceeded the sediment quality guideline values (Table 2). Chromium, Fe, Ni, Cu and As exceeded the guideline values at all the sites while Co and Pb did not exceed the values at Sites H7 and H9, respectively. Zinc did not exceed the guideline values at Sites H3, H7 and H9, while Cd only exceeded the guideline values at Sites H1 and H6.

Even though metal concentrations in the sediment were relatively high, it should be noted that total sediment metal content as such, is not an accurate measure of bioavailability and thus a poor indicator of toxicity (Violante et al., 2010). According to several authors (Wen and Allen, 1999; Eggleton and Thomas, 2004), variation in pH is among the most important chemical factors affecting the mobility and speciation of sediment-bound metals and, as stated already, limited variation in pH was found (Table 1). Other major factors, not measured during this study, that affect the mobility and bioavailability include, amongst others, the redox potential, nature of the sorbents, presence and concentration of organic and inorganic ligands, including humic and fulvic acid, microbial metabolites, root exudates, acid volatile sulphides, microoganisms and nutrients (Allen et al., 1993; Violante et al., 2010).

Metal concentrations in the sediment showed less variation than those in the water (Table 2, Figures 3b and 4a-l) and concentrations measured are in accordance with similar studies done in South Africa (Greenfield et al., 2007). Aluminium, Mn, Cu, As and Cd concentrations did not differ significantly ($p>0.05$) between the different sites (Figure 4a-l). The mean concentrations of Al, Mn, Fe, Cu, Zn, Ni, Cd and Pb were the highest at Site H1, the Marico Eye, while Co and As were the highest at Site H3.
As mentioned before, the Eye is considered as relatively unimpacted (RHP, 2005). The elevated metal concentrations at this site most probably originate from natural sources. The Eye produces approximately 136 000 litres of water per minute, percolating from the dolomitic aquifer, and may be responsible for the continuous erosion of the surrounding geology. This erosion is reflected by the high percentage (74.72%) of fine sediment at Site H1 (Table 2), resulting in increased concentrations of metals (Salomons and de Groot, 1978; Ackermann et al., 1983). According to the literature (NGWA, 2016), the chemical character of groundwater is further subjected to reactions with minerals and gasses as it passes through the rocks and minerals of the earths' crust. Groundwater can thus be highly mineralised depending on the depth of geological origin, as well as the age of the water.

Sites H2, H3, H4 and H7 are subjected to possible agricultural activities that could potentially contaminate the river with metals and fertilizers in runoff surface water. Organic and inorganic fertilizers contain metals such as Cd, Cr, As, Pb, Hg, Ni and V (Mortvedt, 1995), however, most metal concentrations at these sites also did not differ significantly from the other sites (Figure 4a-l). Unlike metal concentrations in the water, concentrations in the sediment at Site H5 did not differ significantly from the rest of the sites (Figure 4a-l).

From Figure 3b, it is clear that Site H7 had lower concentrations of most metals, compared to the other sites. According to Dabrowski and de Klerk (2013), dams may act as sinks for nutrients, dissolved salts and sediment-bound metals. The Marico Bosveld Dam, situated above Site H7, probably acts as a sink as metal concentrations below the dam (Site H7) are considerably lower than measured above the dam (Site H4). This is despite of agricultural activities along the river between these two sites. The relatively increased metal concentrations at Site H9, situated below the Molatedi Dam might be attributed to the confluence of the Klein Marico River with the Marico River, between Sites H7 and H9. The Klein Marico Bospoort Dam, in the Klein Marico River between Sites H5 and H6, did not seem to act as a sink as metal concentrations at these sites did not differ significantly.

Because of limited anthropogenic impacts in the catchment, the origin of most metals detected is probably from geological weathering of the surrounding primary lithology, resulting in the deposition of secondary minerals (Brown et al., 1999), as identified in Table 3. Formations in this Bushveld Igneous Complex are known to be extremely rich in selected minerals (RHP, 2005). Elements in these minerals include, for example, Fe in hematite, goethite, chrysolite and magnetite; Al in kaolinite, pyrophyllite, muscovite and montmorillinite; Na, Ca, and Mg in montmorillinite and Mg, Al and Si in chlinochlore. Quarts, which was the most dominant mineral present at all the sites (Table 3), mostly consist of Si and O (Li et al., 2001b).

The associations between sites and minerals present at these sites, as well as the metals constituting these minerals, are evident in the RDA-triplot (Figure 5). The phenomenon that most metals associated with particles <2000 μm, could be due to the fact that the adsorption of metals is more pronounced with a decrease in particle size (Salomons and de Groot, 1978; Ackermann et al., 1983).

Table 2: Summarised metal concentrations (mg/kg; mean±standard error, with minimum and maximum values in parentheses), as well as percentage particle size distribution in the sediment recorded at nine sites during five surveys, with the corresponding guideline values*.

mg/kg	H1	H2	H3	H4	H5	H6	H7	H8	H9	ANZECC	EPA
Al	116186±7720	55977±18655	45804±11826	48359±14140	40969±10899	66245±16358	43495±18138	104384±23037	73644±17331		
	(37298–258060)	(22418 – 126179)	(23707 – 85377)	(14972 – 98379)	(8937 – 57789)	(31636 – 120938)	(6204 - 107224)	(43256 – 165264)	(30690 – 130112)		
Ti	916.79±173.92	318.78±90.06	400.62±73.78	286.9±52.47	799.17±130.32	1184.3±247.59	665.83±193.4	1134.21±308.18	1108.67±235.52		
	(393 - 1402)	(120 - 584)	(181 – 611)	(109 – 398)	(434 – 1027)	(572 – 2013)	(198 – 1173)	(192 – 2053)	(547 – 1884)		
Cr	604.65±69.56	494.89±81	935±157.73	1197.4±367.41	736.12±126.72	523.96±40.45	427.84±59.97	710.2±115.25	604.39±140.15	80	
	(416 - 823)	(270 – 691)	(579 – 1470)	(304 – 2094)	(449 – 1065)	(374 – 611)	(200 – 540)	(459 – 1103)	(285 – 1057)		
Mn	13917±3354	9957.25±7973.32	11290.99±7672.38	2082.12±171.29	4488.44±1597.56	6080.05±2118.21	923.06±292.33	1155.92±261.93	2323.46±318.46		
	(1651 – 20360)	(1186 – 41804)	(1285 – 41804)	(1810 – 2756)	(1342 – 8870)	(3197 – 14467)	(479 – 2074)	(627 – 2058)	(1783 – 3433)		
Fe	188740±29636	154168±20861	151493±19023	160840±39751	130385±17550	137697±18927	67813±15589	123404±19615	98069±21757		20000
	(121255 – 279708)	(87005 – 206316)	(92963- 206316(	(59070 - 278256)	(93957- 177804)	(83820 – 188760)	(30439 – 116740)	(73568 – 168960)	(41910 – 165792)		
Co	82.58±8.69	82.52±25.47	100.61±21.28	66.66±10.59	72.4152±9.82	72.27±9.11	30.5±7.74	78.59±7.74	61.18±14.37		50
	(54.7 - 106.9)	(39.5 – 182)	(64 – 182)	(36.3 - 97.8)	(45.3 – 91)	(41.9 - 94.9)	(10.9 - 52.3)	(60.7 - 104.6)	(25 – 106)		
Ni	310±60.16	178.51±37.93	216.45±37.53	182.9±38.26	225.72±7.79	181.34±32.57	98.95±38.81	326.41±50.3	260.67±49.39	21	22.7
	(183 – 538)	(89 – 274)	(132 – 333)	(81 -303)	(206 – 245)	(108 – 290)	(21.8 - 239.7)	(195 – 441)	(144 – 417)		
Cu	183.26±13.79	110.54±14.14	115.9±19.4	106.4±11.53	127.22±11.4	136.7±20.27	138.98±48.38	154.35±23.52	95.05±25.83	65	31.6
	(133 – 214)	(70.6 – 154)	(70.6 – 180)	(71.6 – 137)	(111 – 159)	(68 – 189)	(23.4 – 300)	(78.6 – 209)	(29.6 – 175)		
Zn	459.81±84.51	208.73±43.15	95.74±11.26	135.65±25.02	217.23±56.91	177.77±35.86	84.6±19.43	145.89±24.56	97.88±21.59	200	121
	(261 – 753)	(93.9 – 354)	(72.1 - 137.9)	(88.2 – 229)	(135 – 378)	(97.7 – 312)	(29.7 – 152)	(75.9 – 221)	(49.2 – 166)		
As	63.16±11.29	57.57±9.41	64.34±7.37	62.66±18.89	37.21±5.09	50.76±7.81	15.34±3.11	16.33±2.07	14.22±3.18	20	9.8
	(42 – 105)	(34.5 - 88.4)	(44.3 - 88.4)	(27.9 – 129)	(25.9 - 50.50	(28.5 - 76.8)	(4.1 - 23.4)	(9.8 - 22.4)	(6.9 - 24.)3		
Cd	1.5±0.61	0.92±0.49	0.86±0.51	0.85±0.49	0.29±0.06	0.99±0.52	0.87±0.5	0.74±0.36	0.2±0.07	1.5	0.99
	(0.26 - 3.57)	(0 - 2.63)	(0 - 2.62)	(0 - 2.560	(0.14 - 0.430	(0 - 2.78)	(0 - 2.63)	(0.003 - 1.94)	(0 - 0.41)		
Pb	96.29±14.93	46.45±4.59	58.9±6.88	46.52±7.24	60.35±0.6	70.39±12.76	55.75±21.39	53.38±9.11	33.15±9.35	50	35.8
	(60.7 – 145)	(37.4 - 64.2)	(37.4 - 75.8)	(30.1 - 70.9)	(58.9 - 61.8)	(26.8 - 99.8)	(5.5 – 127)	(25 – 74)	(11.4 – 63.8)		
<2000μm	74.72%	0	92.43%	0	84.27%	80.17%	55.13%	98.59%	95.33%		
>2000μm	25.28%	100%	7.53%	100%	15.73%	19.83%	44.83%	1.40%	4.67%		

Shaded blocks exceed guideline values*: EPA=Environmental Protection Agency (2009). ANZECC=Australia and New Zealand (2000).

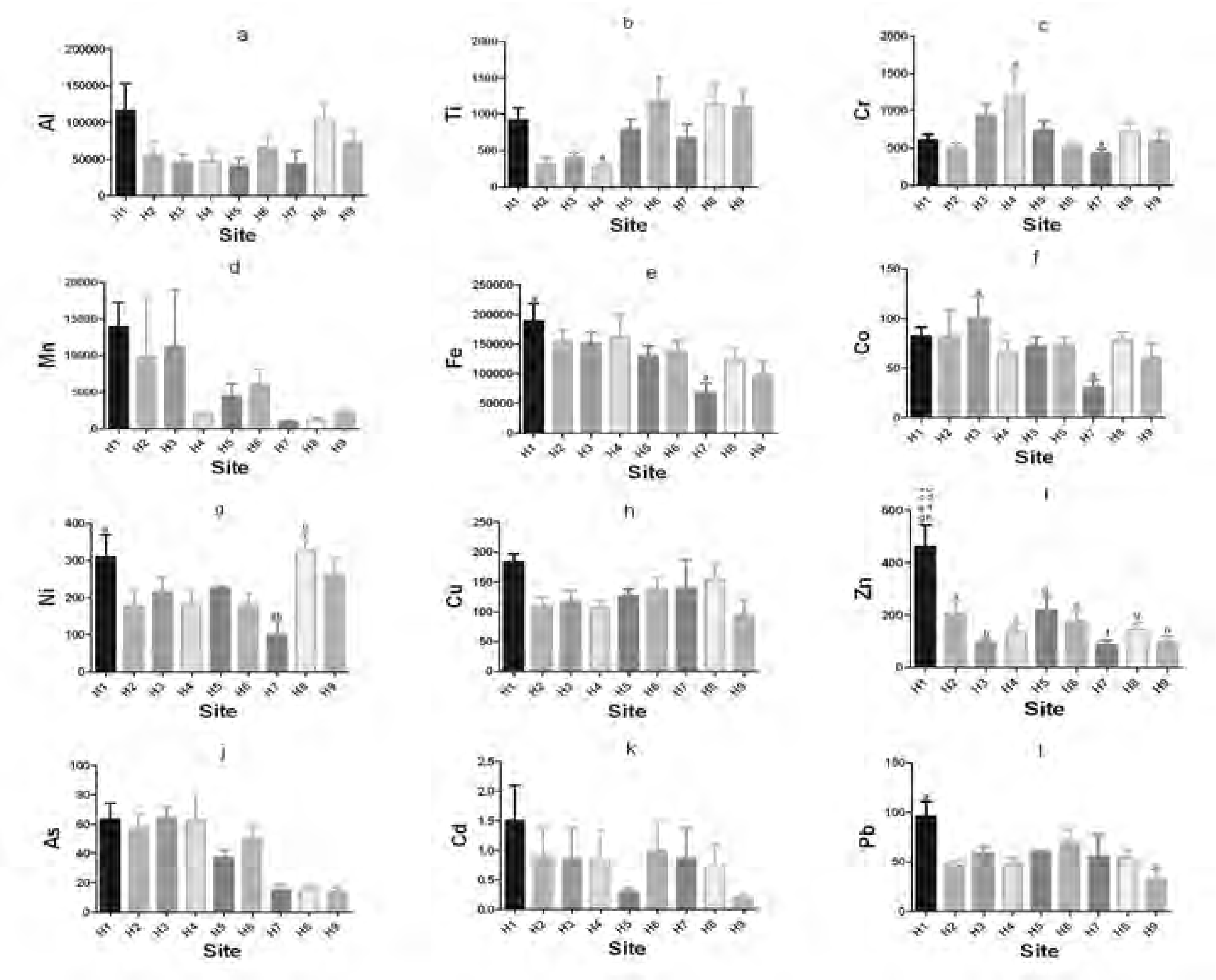

Figure 4a-l: ANOVAs illustrating the mean±standard error of metal concentrations (mg/kg) measured in the sediment. Bars with common superscripts differ significantly (p=<0.05)

Table 3: Minerals identified in the sediment at each of the sites, expressed as a percentage of all the minerals at a specific site.

	Sites and percentage composition								
Minerals	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
Quartz	66.5	73.4	85.5	69.8	81.6	80.3	79.9	99.8	85.2
Hematite	2.2	0	0	0	0	0	0	0	0
Kaolinite	21.1	1.8	3.7	0	3.6	4.7	0	0	2.8
Pyrophyllite	10.2	0	0	0	0	0	0	0	0
Muscovite	0	24.7	10.8	23.3	14.8	15	18.3	0	8
Montmorillonite	0	0	0	0.7	0	0	0	0	0
Clinochlore	0	0	0	6.2	0	0	0	0	0
Goethite	0	0	0	0	0	0	1.7	0	0
Chrysotile	0	0	0	0	0	0	0	0.2	0.1
Magnetite	0	0	0	0	0	0	0	0	1
Calcite	0	0	0	0	0	0	0	0	2.9

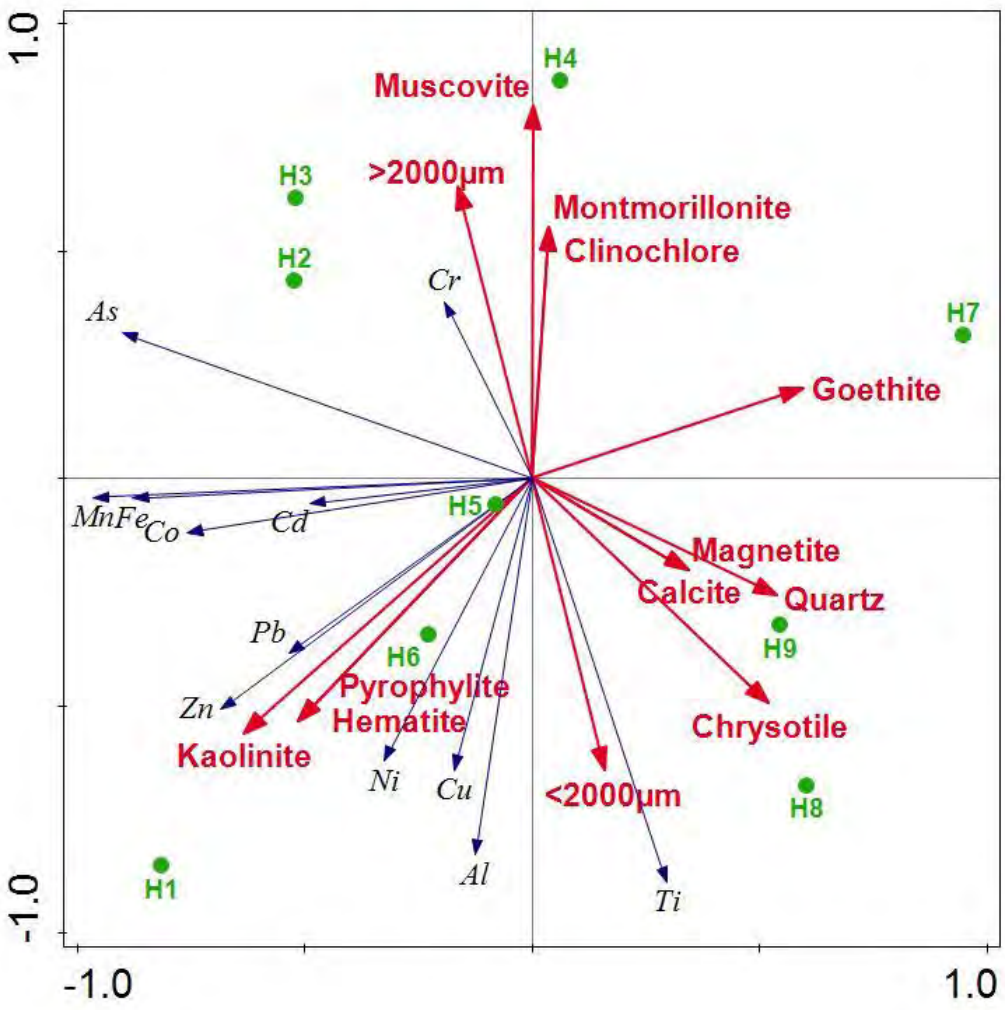

Figure 5: RDA tri-plot of the sites, mineral composition, sediment particle sizes and metal concentrations in the sediment samples. Factor 1 represents 59% of the variance within the data-set while factor 2 represents 20% of the variance within the dataset.

During this investigation the majority of water quality variables were within the tolerance ranges of macroinvertebrates (Holmes, 1996; Hassell et al., 2006; Tixier et al., 2009; Hussain and Pandit, 2012) and, except for the EC at Site H5, may pose limited threat to the organisms. The fact that metal concentrations higher than CEV were present at the Marico Eye, as well as at many sites downstream of the Eye, further suggests that these metals originated most probably, as mentioned before, mostly from natural sources such as geological weathering. Metal concentrations in the water were considerably lower than in the sediment, which may indicate that metals in the sediment were most likely not bioavailable for aquatic macroinvertebrates at the time of sampling. Although metals in water are more representative of the bioavailability to macroinvertebrates, it is important to determine the metal concentration and speciation in the sediment, as it may be remobilised into the water and become bioavailable at some point, should environmental conditions change.

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Supplementary Material

Table S1: Values of certified reference material with concentrations measured for both the sediment and water samples (μg/ml).

	Sediment			Water		
	Reference	**Measured**	**% Recovery**	**Reference**	**Measured**	**% Recovery**
Cr	35	35.1	100.4	0.2	0.22	109.5
Mn	1400	1536.1	109.7	0.1	0.11	107
Co	8.8	9.6	109.5	0.1	0.11	114
Ni	12.8	13.4	104.6	0.2	0.21	105
Cu	1230	1326.6	107.9	0.2	0.21	105.75
Zn	498	525.1	105.4	1	1.06	106.3
As	115	121.9	106.0	0.4	0.42	104.75
Cd	4	4.3	107.8	0.1	0.11	108
Pb	285	309.9	108.7	0.4	0.40	101.2
U	7.8	7.6	97.2			
Ti	1510	1486.4	98.4	0.1	0.11	109
Al				0.4	0.44	108.75
Fe				1	1.06	106

CHAPTER 4

Influence of biotope associations and selected environmental variables on aquatic macroinvertebrate distribution in a pristine perennial Afro-tropical river system

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Abstract

The study was conducted to assess the current macroinvertebrate biodiversity status of the Marico River. Five surveys were done during low-flow seasons of 2013-2015 at nine preselected sites. Vegetation, gravel, sand and stone biotopes were sampled with standard South African Scoring System (SASS) nets for 15 minutes each. Turbidity, flow-rate, pH, water temperature and conductivity were measured in situ at each site. Macroinvertebrates were fixed and preserved in 90% ethanol. At the laboratory, they were sorted, identified, counted and classified as tolerant, moderately sensitive and highly sensitive. A total of 187 taxa were recorded of which 124 taxa were tolerant, 42 moderately sensitive, 12 highly sensitive and for nine, no sensitivity ratings were available. Highly sensitive taxa were collected at almost all of the sites, which indicate that the water quality was good throughout the river system. Mean environmental variables varied between the different sites but did not exceed the target water quality ranges deemed acceptable for aquatic ecosystems. The results show that biotope and food availability, as well as the measured environmental variables play a significant role in the occurrence, abundance and distribution of species in river systems and should be taken into account when assessing the macroinvertebrate diversity of a river.

Keywords

Aquatic macroinvertebrate, biodiversity, biotope associations, water quality, pristine river.

Introduction

The term 'biodiversity' refers to the variability within species, between species and of the ecosystems they live in (Purvis and Hector, 2000; AHSD, 2016). High species diversity leads to better ecological functioning, and ultimately a healthier ecosystem (Purvis and Hector, 2000; Heatherly et al., 2007). It is therefore essential to undertake diversity studies in order to find and describe new species, document and update records of known species, as well as to conserve the diversity of animal and plant species on this planet.

In a river system, aquatic macroinvertebrates play a vital role in ecosystem functionality by aiding purification, processing transported organic matter and providing a food source for other fauna such as fish (Weber et al., 2004; Eady et al., 2014). Because of their abundance, limited mobility, known pollution tolerances, wide range of feeding habits and varied lifespans, these organisms are widely used to determine the biotic integrity and ecological health of river ecosystems, as well as to monitor environmental change (Kiffney and Clements, 1994). The diversity and community structure of macroinvertebrates can be influenced by a variety of factors, including water quality and the availability of suitable habitats or biotopes (Kemp et al., 2014).

Biotopes can be distinguished on the basis of hydraulics (lotic or lentic systems) and substratum (stones, sand and vegetation) (Dallas, 2007). They are subjected to physical and chemical variables such as substrate particle size, surface flow, seasonal variability, perenniality, longitudinal changes, abiotic factors such as water temperature, pH, oxygen, turbidity, as well as the influence of toxic substances like heavy metals (Hawkins and Sedell, 1981; Kiffney and Clements, 1994; Dallas, 2004; 2007; Heatherly et al., 2007; Duan et al., 2008; Eady et al., 2014). Macroinvertebrates have specific biotope preferences and are sensitive to changes of all the above mentioned variables. Thus, either natural or anthropogenic differences or changes in biotope availability and composition, at a specific site, may influence its macroinvertebrate assemblages (Dallas, 2007).

The Marico River is considered as one of the most pristine rivers in South Africa (RHP, 2005; Nel, 2011). The catchment is classified as a National Freshwater Ecosystem Priority Area (NFEPA) while the upper reaches of the river is the last remaining free-flowing river in the north-western region of South Africa (Nel, 2011). The general ecological state of the upper Marico River is still considered as natural to good, free from significant organic pollution, with a pronounced diversity and overall good water quality (RHP, 2005).

There is limited information on the macroinvertebrate diversity of southern Africa, especially up to species level (Smith-Adao et al., 2006). The aim of this study was to undertake a comprehensive study of the macroinvertebrates, in order to determine and document the current diversity and to assess the influence of biotopes and environmental variables on the distribution of species.

Material and Methods

Study Area

The Marico River originates at the Marico Eye near the town of Swartruggens in the North-West Province, South Africa (Figure 1). It meanders in a westerly direction towards the Botswana border and forms an important tributary of the internationally important Limpopo River. Mean annual temperatures in the catchment ranges between 18 and 20°C with a mean annual rainfall of 400 to 800 mm (RHP, 2005). Limited anthropogenic disturbances in the catchment area include three impoundments in the Marico and Klein Marico Rivers, urban development near the town of Zeerust, cultivated land and informal settlements located along the course of the river.

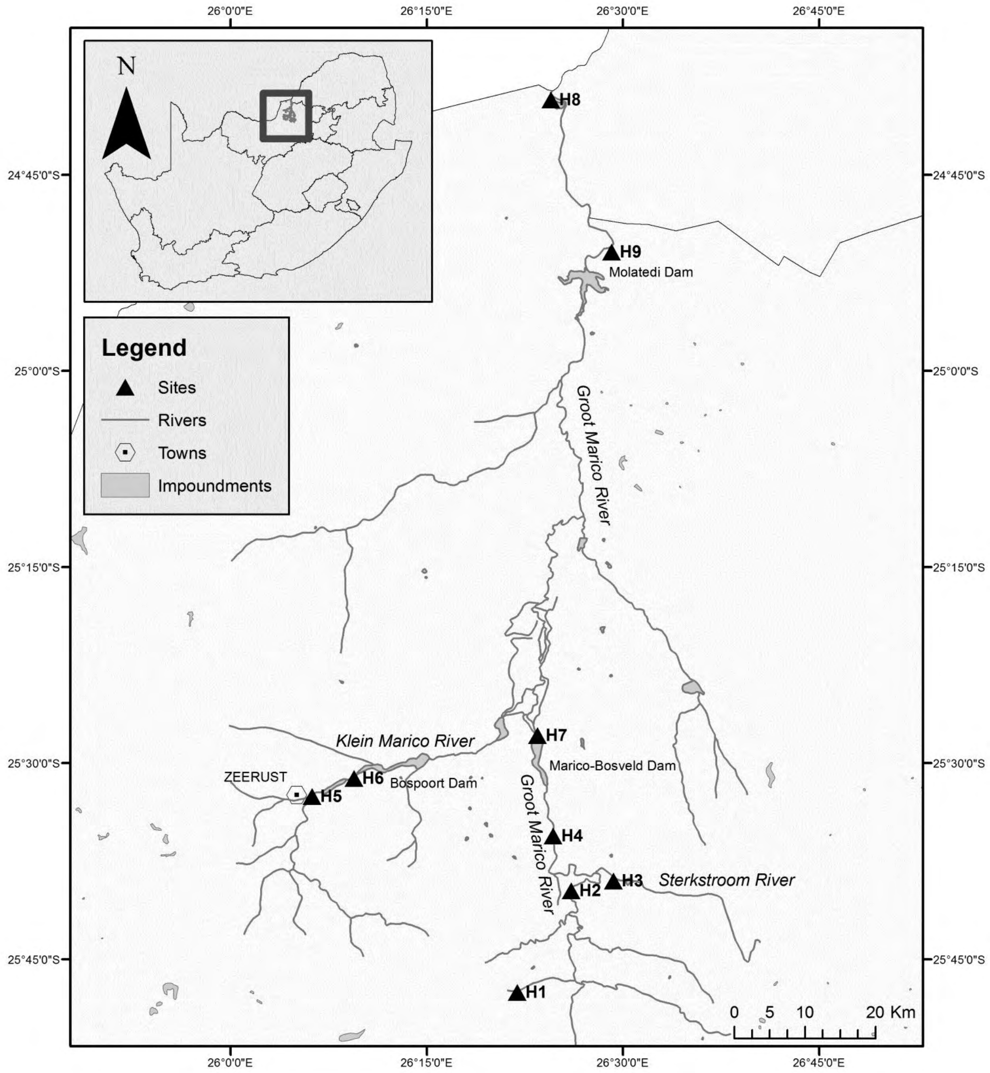

Figure 1: Locations of sampling sites (triangles) within the Marico River catchment, North-West Province, South Africa.

Field Surveys

Nine sites in the Marico River, Sterkstroom and Klein Marico River were selected for this study (Figure 1). To limit natural seasonal variation in biotope availability, surveys were only done during the low-flow seasons, when the highest diversity is usually expected (Dallas, 2004; Khoza et al., 2012; Wolmarans et al., 2014). Five surveys were conducted, three during the early low-flow season (May) and two during the late low-flow season (November) of 2013, 2014 and 2015.

Macroinvertebrates were collected at each site by sampling the vegetation (marginal and aquatic instream), gravel and sand, and stone biotopes with a 30 cm x 30 cm x 30 cm (mesh size 500 µm) sweep net for approximately 15 min each. Marginal and aquatic vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area (Dickens and Graham, 2002; Kemp et al., 2014; Wolmarans et al., 2014). Macroinvertebrates on the mud, sand and gravel biotopes were collected by shuffling the feet whilst sweeping the net over the disturbed area. Stones in and out of current were sampled by carefully lifting the stones by hand and brushing each stone off with a soft brush whilst collecting the macroinvertebrates in a net held downstream from the stone. Contents of the nets were then transferred to a rectangular plastic container (360 x 470 x 80 mm) which was filled with water from the habitat to a level of approximately 40 mm. Most of the debris were then carefully removed by hand. Thereafter the contents of the container were decanted into a cone-shaped net (0.25 mm mesh) suspended on a stand. The contents of the net were then transferred to a plastic container with a tight-fitting lid and 90% ethanol was added to preserve the samples (Kemp et al., 2014).

Samples were transported to the laboratory where each sample was decanted into a rectangular Perspex sorting tray (300 x 200 x 25 mm) with a transparent bottom provided with a 10 mm square grid. All macroinvertebrates were removed by using a stereomicroscope mounted on a sliding stand (Wolmarans et al., 2014). Identification was done with the aid of the 'Guides to the Freshwater Invertebrates of Southern Africa' series published by the Water Research Commission, as well as additional literature (Gerber and Gabriel, 2002a; 2002b). Where possible, organisms were identified up to species level, if not, identification was done up to the genus or family level and enumerated. The South African Scoring System (SASS5) rapid bioassesment index (Dickens and Graham, 2002) was used to classify the macroinvertebrates collected into three groups, namely tolerant (scores 1–5), moderately sensitive (scores 6–9) and highly sensitive (scores 10–15) to organic pollution.

Conductivity, pH, water temperature, flow-rate and turbidity were measured in situ at each site during each survey. This was done by making use of a clarity tube and calibrated portable digital meters including: conductivity meter (DIST 3, HI 98303, Hanna Instruments), pH probe (HI 98128, Hanna Instruments), thermometer (Checktemp, Hanna Instruments) and flow-probe (FP 111, Global Water). The coordinates and altitude of each sampling site were determined with a Garmin Nuvi 500 GPS and from Google Earth. Dominant marginal and aquatic vegetation were identified with the aid of a guide (Gerber et al., 2004) and recorded for each of the sampling sites (results not shown).

Statistical Analyses

A combination of biological indices (species richness, Shannon-Wiener diversity index and Pielou's evenness index) was calculated for each site and survey. Kolmogorov-Smirnov tests (with Dallal-Wilkinson-Lilliefor P values) were used to test all data for normality. One-way analyses of variance (ANOVAs) and Kruskal-Wallis tests (with Dunn's multiple comparison test) were carried out to test for significant differences ($p<0.05$) between the mean biological indices of the various sites. Redundancy analyses (RDAs) were conducted to assess temporal variation for each survey and thereafter, data of the five surveys were grouped to assess spatial variation. Redundancy analysis is a method to extract and summarise the variation in a set of response variables (in this case species distribution) that can be explained by a set of explanatory variables (in this case environmental variables and biotopes). All statistical analyses were done with the aid of GraphPad Prism 5 and Canoco (version 5) software.

Results

Site descriptions and the mean environmental variables with standard errors are depicted in Table 1. Site H4 had the highest variety of biotopes with only pool and backwater being absent (Table 1). Site H9 is the only site from which both aquatic and marginal vegetation was absent, while Sites H1 and H8 are the only sites where both pool and backwater biotopes were present. Regarding the environmental variables, the mean water temperatures ranged from 16±4.3°C at Site H3 to 21.5±3.6°C (Site H8) while mean pH values ranged from 6.8±0.7 at Site H3 to 7.9±0.19 at Site H5. The mean EC ranged from 107.6±11.1 μS/cm at Site H3 to 865.3±189 μS/cm at Site H5. The mean velocity recorded, ranged from 0.04 m/s at Sites H1 and H8 to 0.82±0.08 m/s at Site H4 while the mean turbidity varied between 5±0.1 NTUs (Site H1) and 203.8±44.2 NTUs (Site H5).

Table 1: Site descriptions, coordinates, biotopes, as well as the mean±standard error of selected environmental variables.

	H1	H2	H3	H4	H5	H6	H7	H8	H9
River	Marico Eye, source of Marico River.	Marico River before its confluence with Sterkstroom, 20 km downstream of the Eye.	Sterkstroom 5 km before its confluence with Marico River.	Marico River 10 km above Marico-Bosveld Dam, after its confluence with Sterkstroom.	Klein Marico River 5 km above Klein-Maricopoort (Bospoort) Dam	Klein Marico River 1km below Klein-Maricopoort (Bospoort) Dam.	Marico River directly below Marico-Bosveld Dam.	Marico River at Derdepoort.	Marico River 2 km below Molatedi Dam.
Coordinates	S 25'47'32.1	S 25'39'45.1	S 25'39'00.6	S 25'35'33.4	S 25'23'44.4	S 25'31'09.2	S 25'27'52.6	S 24'39'15.9	S 24'50'54.2
	E 26'21'54'1	E 26'26'01.9	E 26'29'16.3	E 26'24'39.4	E 26'06'08.0	E26'09'25.1	E 26'23'26.9	E 26'24'28.7	E 26'29'07.1
Altitude	1480 m	1 197 m	1170 m	1 077 m	1 150 m	1135 m	1 037 m	914 m	926 m
Biotopes									
Marginal Veg	X	X	X	X	X	X	X	X	
Aquatic Veg	X			X				X	
Sand				X					
Mud	X			X	X	X	X	X	X
Stones in Current		X	X	X					
Stones out of Current		X		X					
Riffle		X	X	X					
Run		X	X	X	X	X	X		X
Pool	X					X	X	X	X
Backwater	X							X	
Temp (°C)	19.48±1.69	16.88±4.27	16.02±4.29	18.5±3.62	16.43±5.54	16.64±4.95	19.34±4.85	21.54±3.63	20.12±4.54
pH	6.93±0.67	7.19±0.67	6.77±0.70	7.262±0.91	7.89±0.19	7.43±0.72	7.47±0.67	7.51±0.45	7.82±0.66
EC (µS/cm)	350±157.79	288.4±17.95	107.6±11.06	287.2±35.20	865.25±188.96	617.8±56.33	264±38.18	407.8±114.77	399.4±122.41
Flowrate (m/s)	0.04±0.05	0.46±0.11	0.104±0.06	0.82±0.08	0.15±0.06	0.2±0.10	0.24±0.11	0.04±0.05	0.26±0.21
Turbidity (NTU)	5±0	5.4±0.55	5.2±0.45	30.2±4.38	203.75±44.23	6.6±0.55	5.2±0.45	15±1.22	29.8±3.27

A total of 187 taxa were identified in the Marico River and its tributaries with a total abundance of more than 18 900 specimens (Table 2). The taxa were classified according to the sensitivity ratings provided for South African aquatic macroinvertebrate taxa (Dickens and Graham, 2002). Of the 187 taxa, 124 taxa are classified as being tolerant to mainly organic enrichment, 42 are moderately sensitive, 12 are highly sensitive and nine of the taxa have no sensitivity rating according to SASS. At least one highly sensitive species was found at all sites, except for Sites H1 and H9. Site H9 had the lowest species richness (SR=45) while Site H4 had the highest species richness (SR=114). Families found at most of the sites and in relatively high numbers include: Sphaeriidae *(Pisidium* spp.*),* Ancylidae *(Burnupia. trapezoidea),* Tubificidae *(T. tubifex* and *B. sowerbyi),* Atyidae *(C. nilotica)*, Baetidae *(A. erepens, Cloeon* and *Procloeon),* Caenidae *(Caenis* sp.*),* Coenagrionidae *(Pseudagrion* spp.*)*, Corixidae (*Micronecta* spp.*),* Ceratopogonidae *(Bezzia* sp.*)*, Chironomidae (*Chironominae* and *Tanypodinae*), Hydropsychidae (*Cheumatopsyche* spp.*)* and Culicidae (*Culicinae* and *Anophilinae)*. Syrphidae was found only once at Site H5, but also in high abundance (Table 2).

Table 2: The taxa, their sensitivity and the number of times they were found at the various sampling sites. Bold numbers indicate where more than 20 specimens of a species were recorded.

Taxa		H1	H2	H3	H4	H5	H6	H7	H8	H9	Sensitivity
Hydroida											
Hydridae	*Hydra*								1	1	*
Platyhelminthes											
Turbellaria	*Mesostoma*			1	2		1				**
Veneroida											
Corbiculidae	*Corbicula fluminalis africana*				**3**		1	1		3	*
Sphaeriidae	*Pisidium costulosum*		3	3	3						*
	Pisidium langleyanum	3	**3**	4	4	1	**5**	1	1	1	*
	Pisidium viridarium	1	1	4			3				*
Unionidae	*Unio caffer*								1		**
Prosobranchia											
Thiaridae	*Melanoides tuberculata*				**5**				**5**		*
	Melanoides victoriae				1						*
Basommatophora											
Ancylidae	*Ferrissia cawstoni*						1	2			**
	Burnupia trapezoidea	3	**4**	**4**	**4**	2	**5**	2	**5**	1	**
Lymnaeidae	*Lymnaea natalensis*	4	2	1	**4**		1	**5**	**5**	2	*
	Galba truncatula					1					*
	Pseudosuccinea columella				1		1	2			*
Physidae	*Physa acuta*				3						*
Planorbidae	*Ceratophallus natalensis*	1									*
	Bulinus forskalii					1		1	1		*
	Bulinus africanus		1		2			2	**5**		*
	Gyraulus costulatus				**2**			**2**	**2**		*
	Gyraulus connollyi	3	1	2	**2**		1	**3**	3		*
	Biomphalaria pfeifferi	2							3		*
	Bulinus depressus							1	5		*
	Helisoma duryi								1		*
Haplotaxina											
Tubificidae	*Tubifex tubifex*	3	2	2	2	**3**	**5**	**4**	**3**	**4**	*
	Branchiura sowerbyi			1	1		3	**2**	**3**	1	*
Naididae	*Sp.*						1				*
Hirudinea											
Glossiphoniidae	*Marsupiobdella africana*						1				*
	Alboglossiphonia cheili			1							*
	Helobdella stagnalis	3			1			1	2		*
Branchiura											
Dolops	*Dolops ranarum*								1		-
Cladocera											
Daphnidae	*Simocephalus*	3		1			1	1	**1**	1	-
Ostracoda											

Darwinulidae	*Darwinula stevensoni*					1		1			-
Decapoda											
Atyidae	*Caridina nilotica*	4	4	**3**	**5**		**5**	**5**	**5**	**5**	**
Potamonautidae	*Potamonautes*	1	2	3	2	1	2		1	3	*
Anostraca											
Artemiidae	Sp.							1			-
Acarina											
Hydrachnella	Sp.	1						1			**
Ephemeroptera											
Baetidae	*Afroptilum*				**1**						**
	Acanthiops erepens	**4**	**4**	**3**	**3**		**4**	**4**	**4**		**
	Cloeon and Procloeon		**2**	**2**	**2**	**2**	2	**1**	**2**	**5**	**
Caenidae	*Caenis*	**4**	**4**	**2**	**4**	1	**4**	**5**		4	**
Polymitarcyidae	*Povilla adusta*							1			***
Heptageniidae	*Afronurus* morph. 1		**2**	**3**	**3**		1	**1**			***
	Afronurus morph. 2		**1**	1	1						***
Leptophlebiidae	*Euthraulus elegans*		**3**	2	**2**	1	2				**
Oligoneuriidae	*Oligoneuriopsis*						1				***
Tricorythidae	*Tricorythus*		**2**	2	**2**						**
Odonata											
Zygoptera											
Coenagrionidae	*Pseudagrion citricola*	4	**4**	3	**4**	**1**	2	3	**4**	5	*
	Pseudagrion morph. 2	1						1			*
	Pseudagrion morph. 3	1			2	1	1	**3**			*
	Enallagma							2	1		*
	Agriocnemis						1	1			*
Lestidae	*Lestes plagiatus*		1	3	1			2	1		**
Chlorocyphidae	*Platycypha caligata caligata*		**3**	2	4			1	1		***
Anisoptera											
Aeshnidae	*Anax*	4	2	1	**4**	1		**3**	4		**
	Aeshna		1	1	3						**
Corduliidae	*Phyllomacromia picta*				1			1			**
Gomphidae	*Paragomphus genei*		3	3	2		2	2		3	**
	Ceratogomphus pictus		1		2	1	2				**
	Gomphidia quarrei quarrei						**2**				**
	Notogomphus praetorius			1	1						**
Libellulidae	*Notiothemis jonesi jonesi*		1		**2**		4				*
	Trithemis dorsalis		3	3	2		1	**4**	4	1	*
	Orthetrum				1				1		*
	Sympetrum fonscolombii		1								*
	Tetrathemis polleni		1		2	1		1			*
	Diplacodes							1	1		*
	Trithemis werneri	1									*
Plecoptera											

Perlidae	*Neoperla*		4		3						***
Hemiptera											
Belostomatidae	*Appasus*		2	1	3			3	5	2	*
Corixidae	*Micronecta*	2	5	2	**3**	1	**4**	3	3	**3**	*
	Sigara		2	2	4		2	2	3	1	*
	Agraptocorixa	1			1	1			1	1	*
Gerridae	*Neogerris*				1				1		*
	Eurymetra natalensis	1	1	1	2	1	5	3	2	2	*
	Aquarius distanti							2			*
	Limnogonus					1	2	1		1	*
	Gerris swakopensis		2		3	2	2	3	1	1	*
	Tenagogonus							1			*
	Rhagodotarsus hutchinsonii								1		*
Hydrometridae	*Hydrometra*									1	**
Mesoveliidae	*Mesovelia vittigera*		1	2	2		3	3	4	3	*
	Mesovelia morph. 2				2			1			*
Naucoridae	*Laccocoris*				2		1	2	2	2	**
	Neomacrocoris	1									**
Nepidae	*Ranatrinae Ranatra*	1	1		2			1	3		*
	Laccotrephes								2		*
Notonectidae	*Anisops* morph. 1	**2**	2				3	1			*
	Anisops morph. 2					1	1				*
	Enithares	3	3	3	4		3	2	3	3	*
	Nychia limpida		**2**	1	1		1		1	3	*
Pleidae	*Plea pullula*	1	1		1				1		*
	Plea piccanina		2								*
Veliidae	*Rhagovelia*			1	2					1	*
Coleoptera											
Chrysomelidae	Donacinae *Donaciasta*		2	1	2	1					*
Curculionidae	*Neochetina*							1			*
	Pseudobagous longulus	1									*
Dytiscidae	*Nebrioporus*	1			1						*
	Leodytes									1	*
	Hydrovatus	1			1	2					*
	Hydroporinae Yola			1	1			1			*
	Laccophilinae *Laccophilus* morph. 1		**4**	4	1	1	4	5	2		*
	Laccophilinae *Laccophilus* morph. 2								1		*
	Methles	1			1						*
	Cybister							2	1		*
	Hydaticus		1		2	1	1		1		*
	Philodytes	2	1						1		*
	Philaccolus		1								*

	Hydroglyphus		1		**2**	1			2		*
	Derovatellus	2			1		1		1		*
	Clypeodytes				1				1		*
	Hyphydrus	1									*
Elmidae	*Stenelmis*		2	1	1			2			**
	Omotonus			1							**
	Potamodytes				1						**
	Microdinodes				1						**
	Elminae larvae	1	**4**	3	**5**						**
	Lorainae larvae		3	1	2	1	2				**
Gyrinidae	*Orectogyrus*		2	**3**	**4**		1	1			*
	Dineutus			1						1	*
	Gyrinus				1						*
Haliplidae	*Haliplus*				2						*
Hydraenidae	*Hydreana*					1					*
	Parasthetops				1						*
	Ochthebius				1	1					*
Hydraenidae	Hydraenidae larvae	1									**
Hydrochidae	*Hydrochus*								1		-
Hydrophilidae	*Enochrus*				1	1			1		*
	Berosini Berosus								4		*
	Laccobiini Laccobius		2		2		1	2		2	*
	Regimbartia				1		1		1		*
Noteridae	*Neohydrocoptus*				1						*
	Hydrocanthus							1	1		*
Psephenidae	*Afrobrianax ferdyi*		2	2	3		1				***
Scirtidae	*Cyphon*		1				1				*
	Scirtidae larvae		1		1	1					*
Spercheidae	Spercheidae sp.							1			*
Sphaeriusidae	Sphaeriusidae larvae		1				1				*
Trichoptera											
Ecnomidae	*Ecnomus thomasseti*		2	1	3		2	3		1	**
Hydropsychidae	*Cheumatopsyche thomasseti*		**3**	**4**	**3**	1	2	2			**
	Cheumatopsyche morph. 2		2	3	2						**
	Leptonema natalense			1							**
Hydroptilidae	*Thricholeiochiton*		1								**
	Hydroptila cruciata		2		1			**1**			**
Leptoceridae	*Homilia*		2	1	1	1	**1**	1			**
	Leptocerus		1		1						**
	Leptocerina		3		1						**
	Paracetodes		1	1							**
Philopotamidae	*Chimarra*		2	2	**3**	1	1				***

Stenopsychidae	*Stenopsyche*				1						-
Lepidoptera											
Crambidae	*Nymphulinae Nymphula*	1		1	2	1					***
Diptera											
Athericidae	*Athericinae*		2	2	2						***
Blephariceridae	*Eloria*						1				***
Ceratopogonidae	*Bezzia*	4	5	**2**	2	2	**3**	4	4	2	*
	Dasyhelia					**2**	3	1			*
Chaoboridae	*Chaoborus microstictus*	1	1	1	1	1	2	1	2	2	*
Chironomidae	Chironominae *Chironomus*	**2**	2		1				2		*
	Chironominae *Harnishia*	**2**	2	**2**	1	**1**	**5**	**2**		**1**	*
	Chironominae morph. 3	1		**2**			1	1	1	**1**	*
	Tanypodinae morph. 1	**4**	**4**	**4**	**5**	**2**	**5**	**5**	**5**	**2**	*
	Tanypodinae morph. 2				1			1	1		*
	Orthocladinae		1	**1**	1	2		1	1	1	*
Culicidae	Culicinae *Culex*	**4**	1	**2**	**2**	**3**	1	3	**4**	1	*
	Aedes	1				**1**					*
	Anophelinae *Anopheles*	1	**2**	**2**	3	**1**	**3**	5	5	1	*
Dixidae	*Dixa*		1				2				***
Dolichopodidae	Dolichopodidae larvae		1		1					3	*
Ephydridae	*Scatella fusca*				1	**2**					*
Emphididae	*Hemerodromia*		1								**
Leptopodidae	Leptopodidae sp.		1				1				-
Muscidae	*Limnophora*		1				1	1		1	*
Psychodidae	*Clogmia*		1	**1**	1						*
Ptychopteridae	*Ptychoptera*								1		-
Sciomyzidae	Sciomyzidae sp.				1						-
Simuliidae	*Simulium Nevermannia nigritarse*		1	**3**	2	2	**3**	1			*
	Simulium Meilloniellum adersi		2	1	2		**2**	1			*
	Simulium metomphalus hargreavesi		1	1	1		**1**				*
	Simulium paracnephia						1				*
	Simulium Nevermannia loutetense			1							*
	Simulium Lewisellum neavei				1		2				*
Stratiomyidae	Stratiomyidae larvae	1	1		1	1		1	1		*
Syrphidae	*Eristalis*					**1**					*
Tabanidae	Tabanidae larvae		3	2	3	2	2	3	1	1	*
Tipulidae	*Tipula*	1	2		2		1		1		*
	Limnophila	1	2	2	2						*
	Limoniniinae		1		1					1	*
	Antocha		1	1						1	*

Megaloptera											
Corydalidae	Sp.									1	**
Total *n* of taxa: 187	**Species Richness per site**	**49**	**90**	**70**	**114**	**49**	**73**	**78**	**70**	**45**	
Total *n* of specimens: 18 930											

*Tolerant **Moderately sensitive ***Highly sensitive

ANOVAs of the biological indices showed significant differences ($p<0.05$) between various sites. The mean number of specimens collected per site ranged from 127.6±78.7 at Site H9 to 762.3±582.6 at Site H5 (Figure 2a) and Sites H4 and H5 differed significantly from Site H9. The mean species richness ranged from 16±8.6 at Site H5 to 45.8±6.72 at Site H4 (Figure 2b) and there was significant differences between Sites H4, H5, H9 and most of the other sites. Regarding the mean Shannon-Wiener values for diversity, the highest values were calculated for Sites H3, H4, H6, H7 and H8 (*H'*=2.74±0.07, 2.74±0.15, 2.65±0.16, 2.63±0.36 and 2.58±0.20, respectively) and the lowest value for Site H5 (*H'*=1.71±0.5) (Figure 2c). The mean Shannon-Wiener value of Site H5 differed significantly from most of the other sites. The mean Pielou's (*J*) values were the highest at Sites H3, H6, H7, H8 and H9 (J'=0.78±0.03, 0.78±0.02, 0.76±0.06, 0.75±0.06 and 0.75±0.06, respectively) and the lowest at Site H5 (J'=0.64±0.06) (Figure 2d). The evenness only differed significantly between Sites H1 and H6, H3 and H5 and H5 and H6.

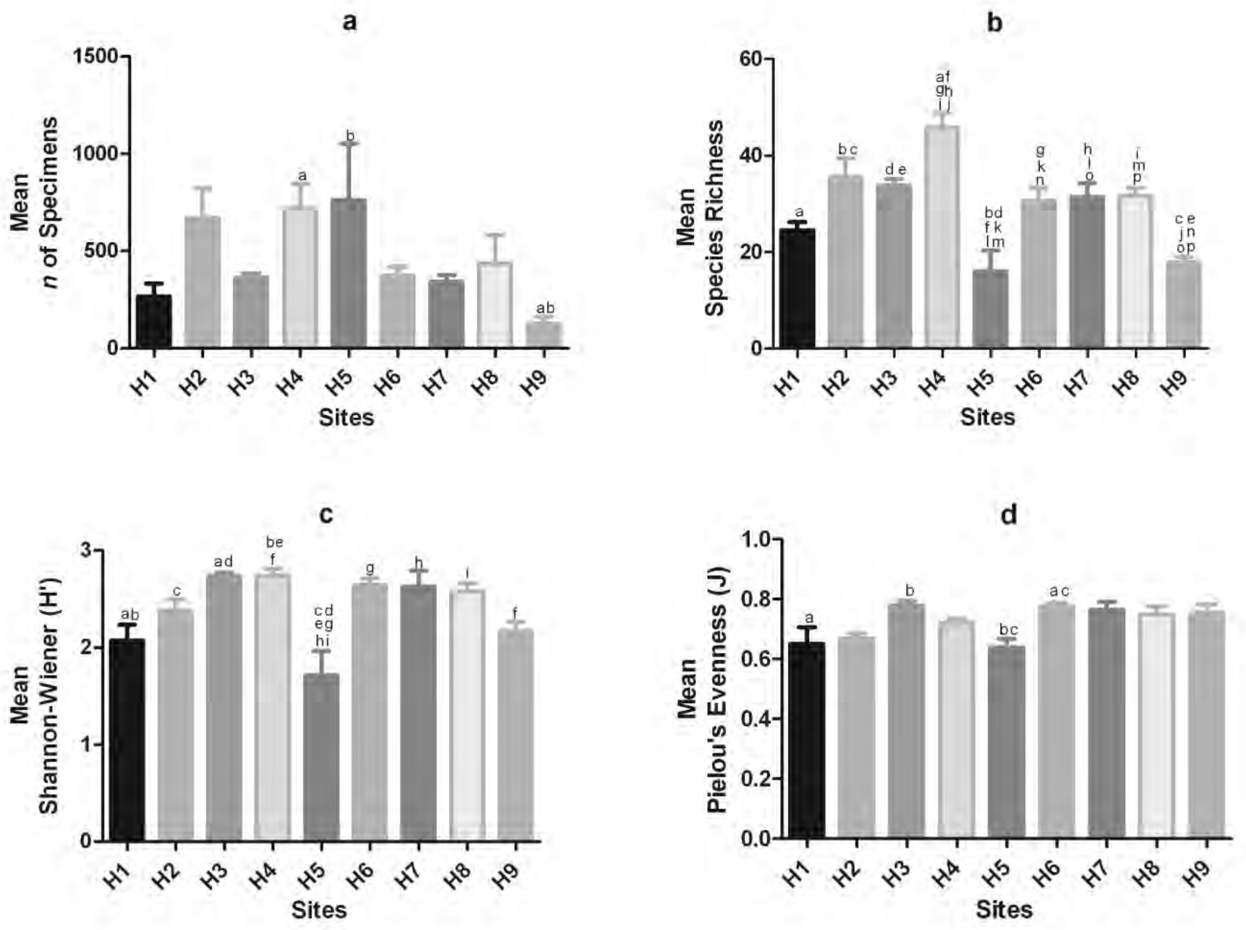

Figure 2a-d: Mean±standard error of the number of specimens (A), species richness (B), Shannon-Wiener (H') (C) and Pielou's Evenness (J) (D) calculated for the various sites. Bars with common superscripts differ significantly ($p<0.05$).

A redundancy analysis (RDA) was conducted for each survey (see supplementary material, Figures S1 – S5). From these figures it is clear that limited temporal variation was evident. The relatively small standard errors on the means of the biological indices calculated per site (Figure 2a-d) also indicate limited temporal variation. These observations motivated the grouping of the five surveys into one RDA (Figure 3). In this RDA tri-plot of the grouped data, the total variation explained was 60.01%, with 37.14% represented on PC axis 1 and 22.87% on PC axis 2. The eigenvalues were 0.371 and 0.229 for axis 1 and 2, respectively. The length of the arrows indicates that temperature, flow, EC and turbidity played a role on the occurrence and distribution of species, while pH had a smaller influence. With regard to the biotopes, only marginal vegetation did not seem to have a significant impact on the occurrence and distribution of species.

In the upper left quadrant, possible associations can be seen between several families, Site H8, temperature, as well as the pool, mud and backwater biotopes. Sites H1 and H9 associated with pH and Site H7 with aquatic vegetation. In the upper right quadrant, the families Corbiculidae, Sciomyzidae, Haliplidae, Stenopsychidae and Physidae associated with sand biotope and Site H4, while Caenidae associated the strongest with stones out of current. Spheariidae and Simuliidae associated with riffle and run biotopes and Sites H2 and H3. In the lower left quadrant, it was evident that Syrphidae, Chironomidae, Ceratopogonidae, Ephydridae and Culicidae associated with EC, turbidity and Sites H5 and H6.

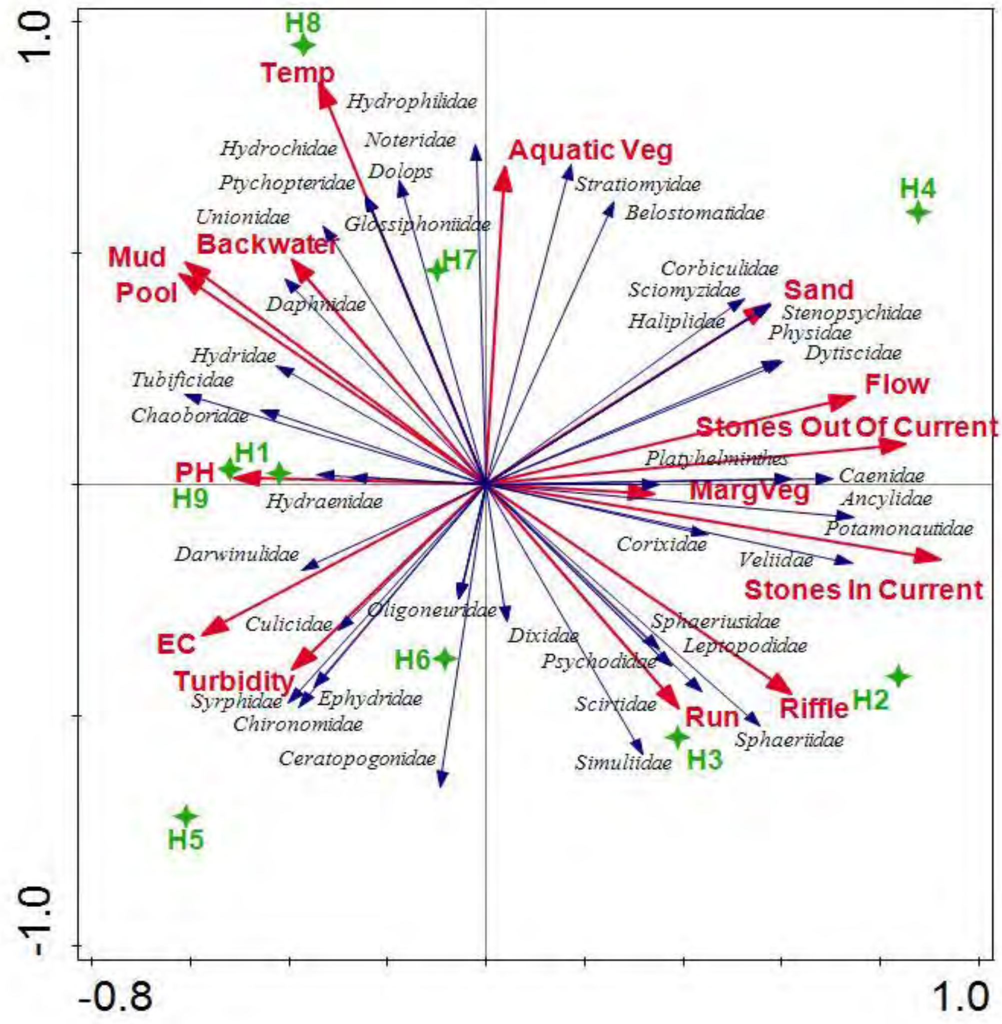

Figure 3: A RDA tri-plot illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 60% of the total variation, with 37.1% being described on the first axis and 22.9% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

Discussion

The fact that the Marico River is considered as a pristine river (RHP, 2005; Kemp et al., 2016), is reflected in the high number of taxa recorded during this study. The 187 taxa identified in the Marico River and tributaries are in contrast to the 94 taxa collected in a similar study in the Olifants River (Kemp et al., 2014). Highly sensitive taxa (Dickens and Graham, 2002) such as Polymitarcyidae, Heptageniidae, Oligoneuriidae, Chlorocyphidae, Perlidae, Psephenidae, Crambidae, Athericidae and Blephariceridae were found at almost all of the sites, which indicate that the water quality was good throughout the river system.

Mean environmental parameters varied between the different sites but, except for EC at Site H5, it did not exceed the target water quality ranges deemed acceptable for aquatic ecosystems (Holmes, 1996). From the RDA (Figure 3) it is clear that pH probably did not have an influence on the distribution of species, while EC and turbidity associated well with Sites H5 and H6 in the Klein Marico River. Although the EC values were considerably higher at these sites, it did not seem to have an influence on the macroinvertebrate distribution in the Marico River downstream of their confluence. Increased turbidity and EC may be caused by various natural and anthropogenic factors but in this case it was most probably due to sewage water discharge and industrial effluent from the town of Zeerust (RHP, 2005). High turbidity levels can adversely affect macroinvertebrate assemblages (Lloyd et al., 1987). It reduces light penetration, which in turn decreases photosynthesis, resulting in lower dissolved oxygen concentrations. It also affects behaviour, feeding and growth of macroinvertebrates as it has a negative impact on visibility of predators and prey and also clogs the gills of organisms (Lloyd et al., 1987; Griffiths et al., 2015).

Numerous factors can have an influence on diversity either at catchment, reach or habitat/site scale (Dallas, 2007). On habitat scale, macroinvertebrates have specific biotope preferences. The importance and effect of biotope availability on diversity, is evident in this study as the different biotopes played an important role in the distribution and abundance of the species (Figure 3). Significant differences ($p<0.05$) in species richness were calculated between several sites (Figure 2b), however, Sites H4, H5 and H9 differed the most from both each other and also from other sites. This is most probably due to the composition of biotopes present at each site. The highest SR was recorded at Site H4 (SR=114, Table 2), where the widest variety of biotopes were present (Table 1), while a considerably lower species richness (SR=45) was recorded at Site H9, where the biotopes appeared to be disturbed and transformed by the sporadic release of large volumes of water from the Molatedi Dam (RHP, 2005). According to Dallas (2007), a change in discharge can directly affect biotope availability, such as reflected by the lack of marginal and aquatic vegetation at Site H9.

The finding that stone biotopes generally support a diverse variety of macroinvertebrates (Wohl et al., 1995; Humphries, 1996; Dallas, 2007) is supported in the current study, where Sites H2 and H4, the only sites at which stones in and out of current were present, yielded the highest mean diversity of species (Figure 2b). Although the tributaries of the Marico River are described as being impacted to some extent (RHP, 2005), Site H3 in the Sterkstroom, had the third highest species richness, the highest diversity (H'=2.74) and most even distribution of species (*J'*=0.78), indicating a healthy ecosystem. The high species richness at this site might be ascribed to the overhanging marginal vegetation that provides organic matter as a food source, as well as the fact that a stones in current biotope was present.

Sites H1 and H5 had the same species richness of 49 but the mean number of specimens and diversity indices differed considerably. Site H1 had a lower mean number of specimens per survey and a higher diversity while Site H5 had a higher mean number of specimens and a lower diversity, which differed significantly ($p<0.05$) from most of the other sites. This is due to the fact that the families Syrphidae and Culicidae were found in very high numbers at Site H5, while Site H1 had a more even distribution of species (*J'*=0.65). These families, together with Chironomidae, Ceratopogonidae, and Ephydridae associated to a large extent with Sites H5, H6, EC and turbidity on the RDA tri-plot (Figure 3). They all have the ability to disperse by flight as adults, while Syrphidae and Culicidae in addition have breathing siphons which enable them to live in stagnant, eutrophic and low oxygenated water

(Aguilera et al., 1999; Day et al., 2003; Picker et al., 2003). These conditions were encountered at Site H5. At Site H1, the Marico Eye, the lower species diversity collected might be ascribed to the lack of both food availability and biotope diversity. According to the River Continuum Concept, headwaters such as Site H1 have less food sources and one might expect to find shredders as the dominant functional feeding group (Hawkins and Sedell, 1981). However, this is based on the assumption that riparian vegetation creates shading and contributes allochthonous detritus as food (Vannote et al., 1980). This was not the case at Site H1, as there was only sparse marginal vegetation which consisted mainly of low-growing plants such as waterparsnip (*Berula erecta*), umbrella sedge (*Cyperus eragrostis*), the common reed (*Phragmites australis*) and bulrush (*Typha capensis*).

Families that were present in high numbers are the ones generally regarded as tolerant or only moderately sensitive to organic enrichment (Dickens and Graham, 2002) and their abundance and occurrence at most of the sites could be attributed to various factors. *Pisidium langleyanum* and *B. trapezoidea*, which associate with fine sediment and stones, respectively (Brown, 1994; de Kock and Wolmarans, 2008), commonly occur in the area and were previously documented at the specific sampling sites (Kemp et al., 2016 – See Chapter 5). The fact that *B. trapezoidea* was recorded at Site H5 during two surveys was unexpected, as this species prefers well oxygenated water (Brown, 1994). Tubificidae and Chironomidae, which were abundant at all of the sampling sites, are both bottom dwellers that have physiological traits such as haemoglobin blood pigment and gills, enabling them to survive extremely low oxygen levels (Day and de Moor, 2002; Day et al., 2003). They also have a preference for and are useful indicators of organic enrichment and low oxygen concentrations (Thirion, 2007; Arimoro at al., 2011; Wolmarans et al., 2014).

The high numbers of *C. nilotica* at Site H8 might possibly be related to water temperature, which associated strongly with Site H8 (Figure 3). Water temperature is an important driver for macroinvertebrate life cycles (Kosnicki and Burian, 2003), for example, at higher temperatures the voltinism of *C. nilotica* is positively affected, eggs hatch faster, sexual maturity is enhanced and abundances may thus be greater (Day et al., 2001), as encountered at this site. Furthermore, other abundant families, including Baetidae, Caenidae, Coenagrionidae, Corixidae and Hydropsychidae have the ability to disperse by flight between favourable waterbodies and all, except Coenagrionidae (Thompson et al., 2003), are multi-voltine, thus reproducing at an increased rate (Sueanna, 2016). Specimens of Ceratopogonidae are generally found in large numbers (Day et al., 2003) while Caenidae, which associated the strongest with stones out of current (Figure 3), are known to prefer slow flowing water in a gravel, sand or mud biotope (Thirion, 2007).

Some families and species were only found at one or two sites and also in low numbers. These include, among others, tolerant species such as *U. caffer, C. natalesnis, B. pfeifferi, H. duryi* and highly sensitive families such as Polymitarcyidae, Heptageniidae, Oligoneuridae and Dixidae. Although Site H1 is regarded as unimpacted and deemed to be the most pristine site encountered during the study, no highly sensitive species were found at this site, while one highly sensitive family (Crambidae) was found at Site H5, where the water quality variables measured, seemed more favourable to tolerant species. This suggests that biotope and food availability probably plays a more decisive role in the occurrence of species than some of the environmental variables that was measured during this study.

Conclusion

The high overall species richness recorded during this study, supports the general perception that the Marico River is an unimpacted, pristine river. As mentioned above, numerous factors can have an influence on biodiversity. The results from this study show that biotope and food availability, as well as the measured environmental variables play a significant role in the occurrence, abundance and distribution of species in river systems and should be taken into account when assessing the macroinvertebrate diversity of a river.

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Supplementary Material

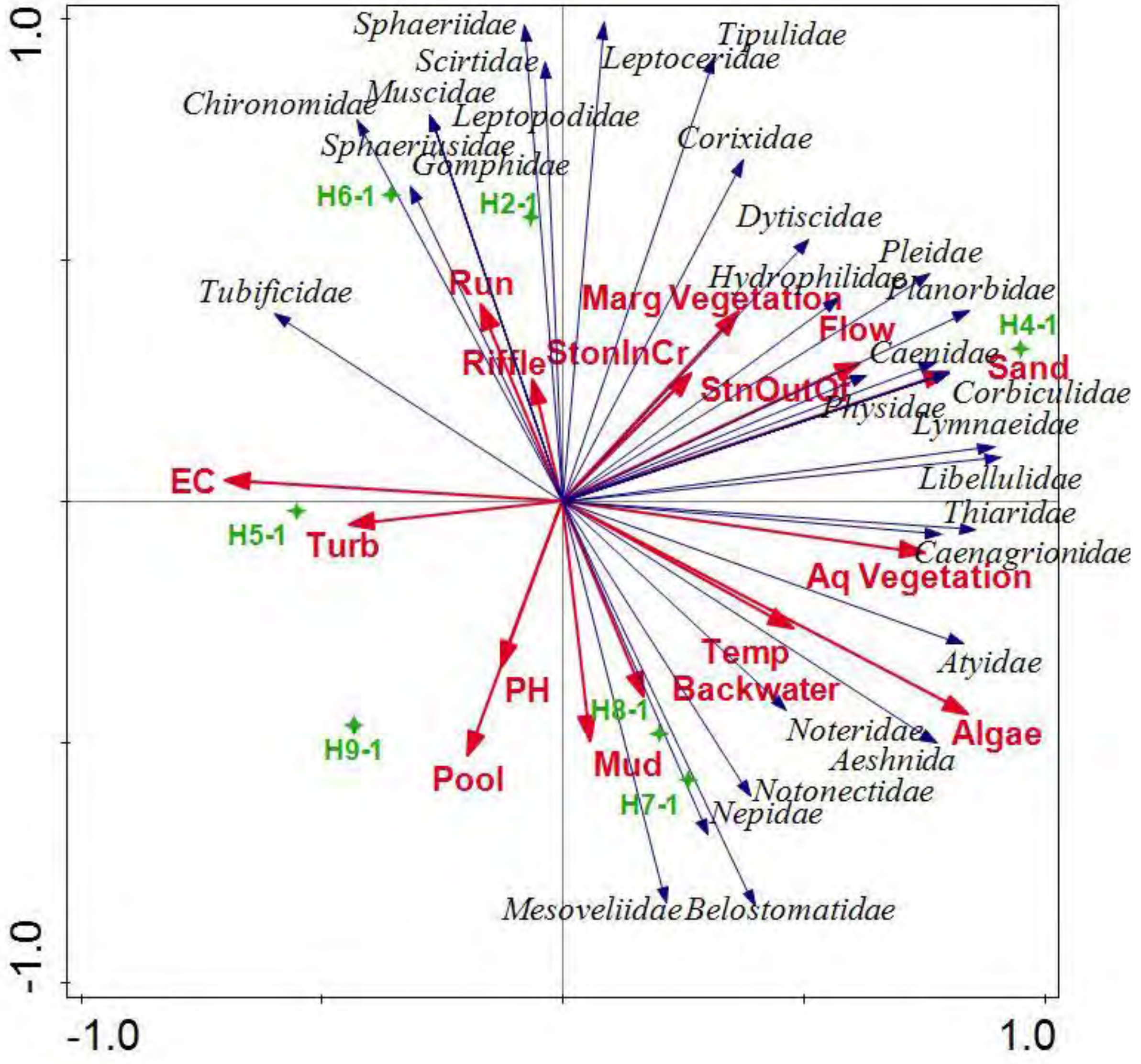

Figure S1: A RDA tri-plot of Survey 1 illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 64.4% of the total variation, with 37.6% being described on the first axis and 26.8% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

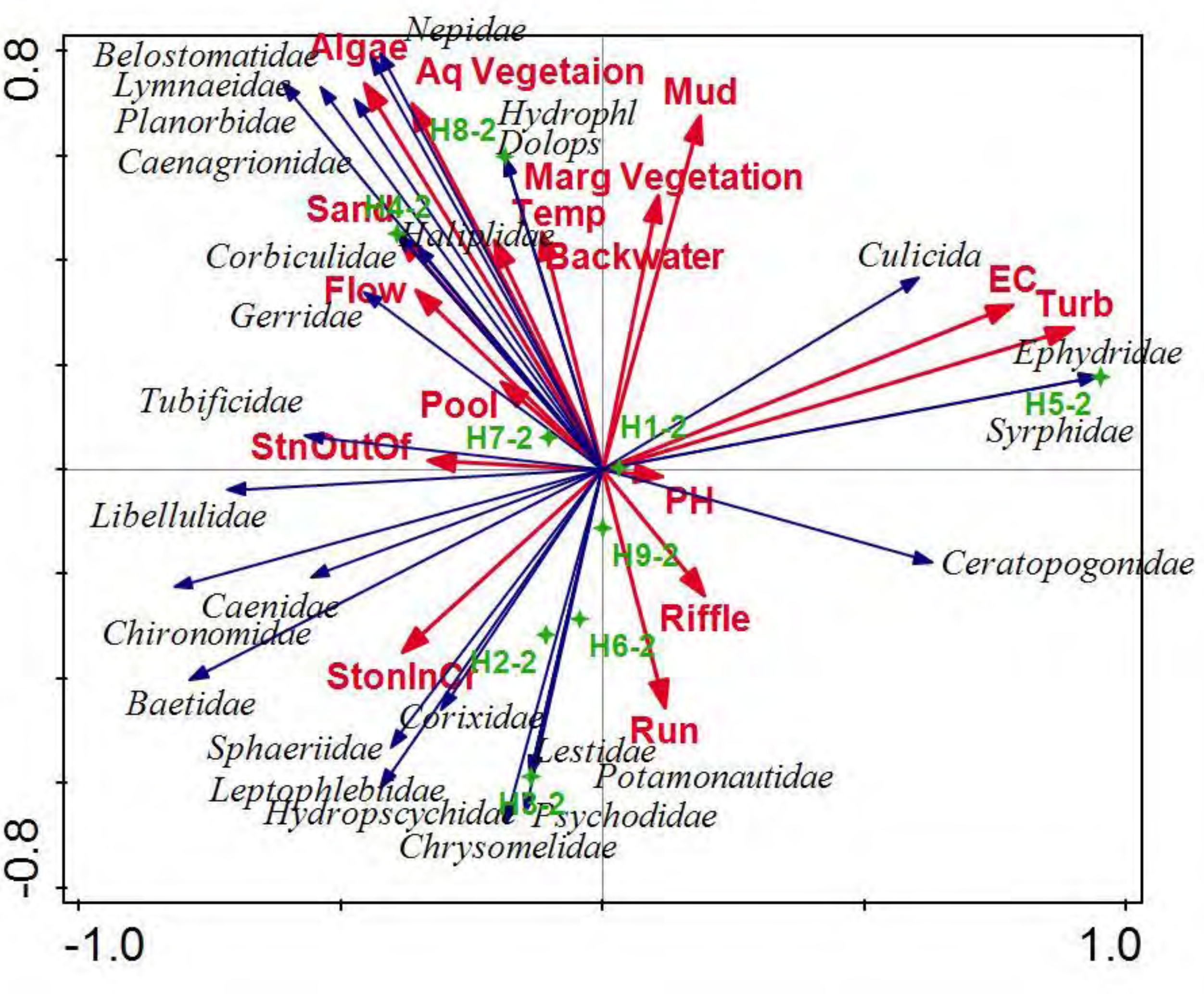

Figure S2: A RDA tri-plot of survey 2 illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 49.3% of the total variation, with 29.6% being described on the first axis and 19.7% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

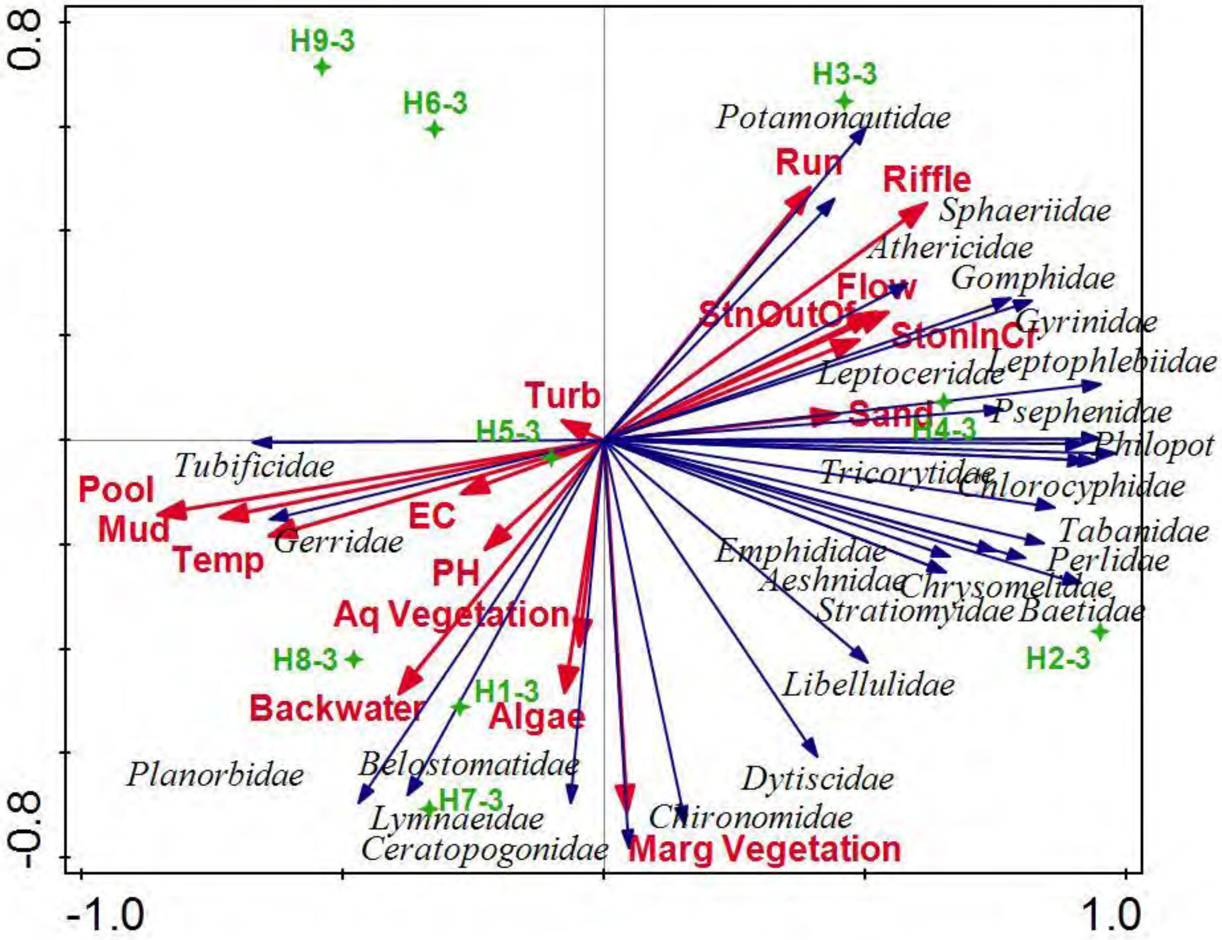

Figure S3: A RDA tri-plot of Survey 3 illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 56.8% of the total variation, with 42.3% being described on the first axis and 14.5% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

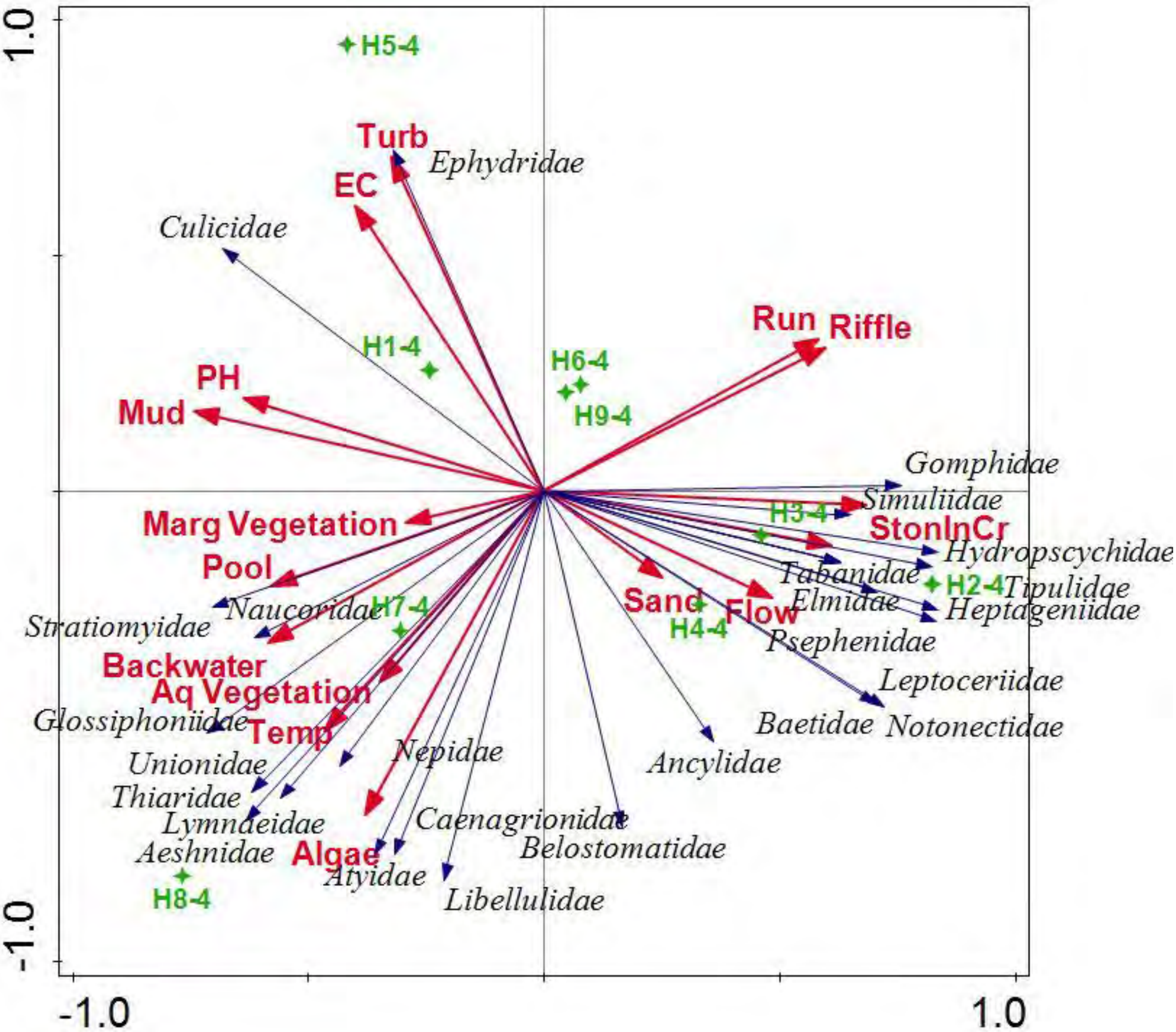

Figure S4: A RDA tri-plot of Survey 4 illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 53.5% of the total variation, with 32.1% being described on the first axis and 21.4% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

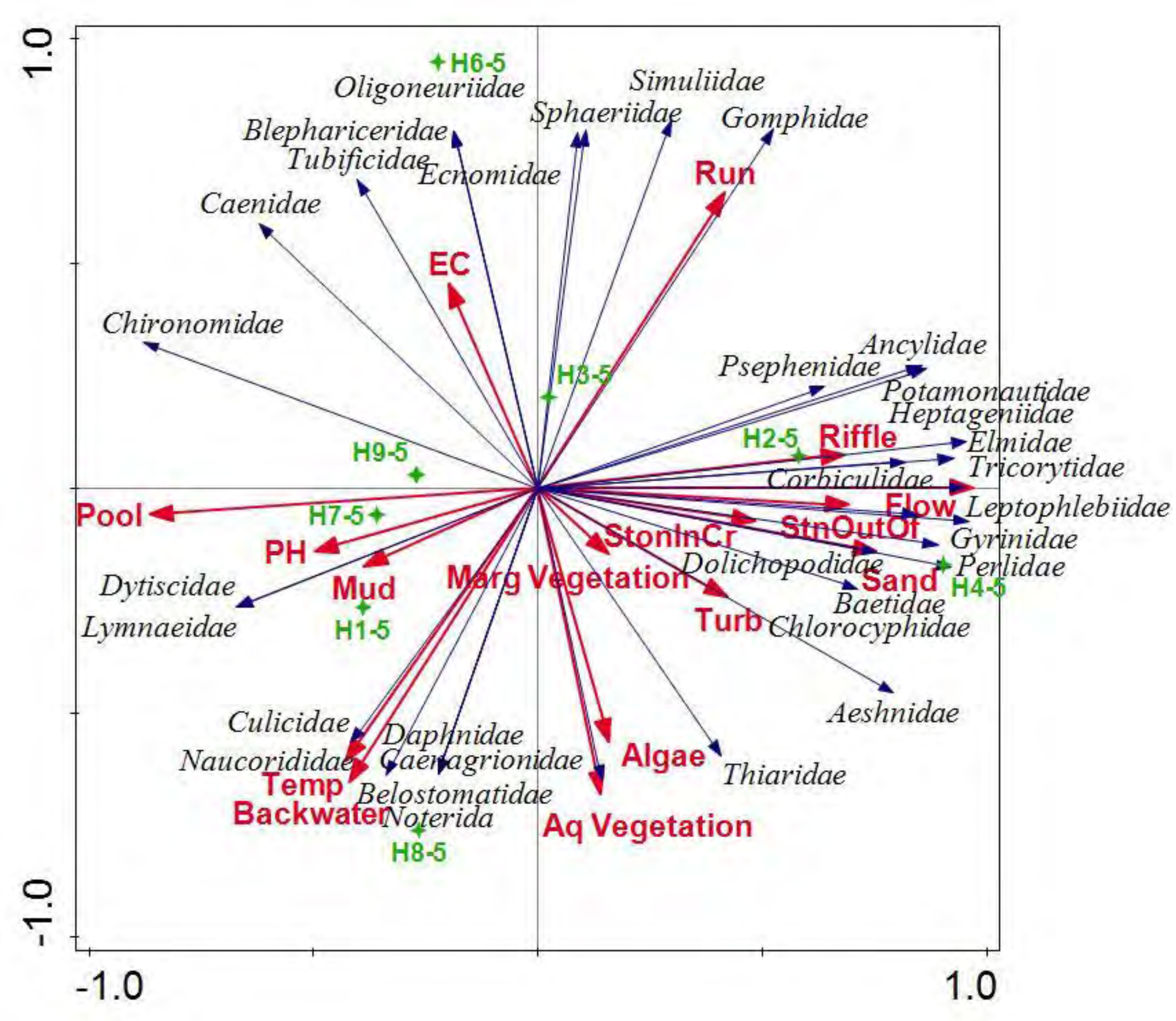

Figure S5: A RDA tri-plot of Survey 5 illustrating associations between sampling sites (green stars), environmental variables and biotopes (red arrows) and families (blue arrows). The tri-plot describes 66.1% of the total variation, with 48.2% being described on the first axis and 17.9% on the second axis. Only taxa of which more than 40% is explained by the model are visualised.

CHAPTER 5

A comparison of the mollusc diversity between the relatively pristine Marico River and the impacted Crocodile River, two major tributaries of the Limpopo River

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Abstract

A study of the freshwater mollusc diversity was conducted at selected sites in the relatively pristine Marico River and the impacted Crocodile River, the main tributaries of the Limpopo River. Four surveys were conducted, two in an early (May 2013 and 2014) and two in a late (November 2013 and 2014) low-flow period. Semi-quantitative surveys were done by sampling the vegetation, as well as the substratum with a standard SASS net for approximately 15 minutes each. Environmental parameters including water temperature, electrical conductivity (EC) and pH were measured at each site. Molluscs were identified up to species level, sorted, counted and the presence of juvenile specimens were recorded. Historical data for the 1/16th degree square grids (loci) in which each of the sampling sites of the current study was located, was extracted from the National Freshwater Snail Collection at the Unit for Environmental Sciences and Management, Potchefstroom Campus of the North-West University. During this study, 20 and nine species were recovered from the Marico and Crocodile Rivers, respectively, as compared to 13 and 12 species on record for these loci, respectively. Juvenile specimens were present during the four surveys at most of the sites. Canonical correspondence analyses were applied which revealed that biotopes, water temperature and EC played the most significant role in the distribution and abundance of species. The relatively high mollusc diversity and the fact that juveniles were present throughout the study, demonstrated that current habitat and environmental conditions were suitable to promote recruitment and the sustainability of diverse mollusc populations in the Marico River and its tributaries. However, in contrast to this, the exploitation of and habitat transformation in the Crocodile River resulted in the decrease of biotopes which eventually could have led to the decrease in diversity and the establishment of *P. acuta*, an exotic invader species.

Keywords:

Freshwater molluscs, biodiversity, Marico River, Crocodile River, environmental variables.

Introduction

Freshwater molluscs are the second most diverse animal phylum on earth (Lydeard et al., 2004). However, they are poorly documented, extremely threatened and have the highest number of recorded extinctions of any major taxonomic group (Vaughn and Taylor, 1999; Lydeard et al., 2004). The decline in freshwater molluscs goes unnoticed due to the poor documentation and monitoring, as only a fragment of molluscan species (<2%) have had their conservation status accurately assessed (Lydeard et al., 2004; Reckendorfer et al., 2006).

Four hundred species of freshwater molluscs were previously identified in Africa (Brown, 1994) and according to de Kock and Wolmarans (1998), the distribution of these molluscs is primarily influenced by the availability of suitable aquatic biotopes. The association of different freshwater molluscs with specific biotopes such as marginal and aquatic vegetation and sediment present at different water velocities and depths, is well known and documented (Brown, 1978; Brown, 1994; Dallas, 2007; Odume et al., 2015). These biotopes are worldwide under constant threat as a consequence of various anthropogenic impacts, geomorphological alterations and the availability of water (Wittmann, 1975; Vaughn and Taylor, 1999; Reckendorfer et al., 2006; Taylor et al., 2007; de Klerk et al., 2012; DWAF, 2012).

With regard to South Africa, nearly 71% of the main rivers are considered as either endangered or critically endangered as the result of exploitation (Nel et al., 2004). The Crocodile River, which is characterised by sprawling urbanization and industrialization of northern Johannesburg and Pretoria and is also under severe threat of pollution due to extensive agricultural activities, the sporadic release of water from impoundments such as the Hartbeespoort Dam and large mining developments north of the Magaliesberg (Wittmann, 1975; Taylor et al., 2007; de Klerk et al., 2012; DWAF, 2012). All of these stressors could have a detrimental effect on the aquatic ecosystems and biotope availability. In contrast, the general state of the Marico River is considered as natural to good, free from significant organic pollution, with a pronounced biodiversity and overall good water quality (RHP, 2005). However, the water quality of two of its main tributaries, namely the Klein Marico River and the Sterkstroom, is defined as fair to poor (RHP, 2005).

Although mollusc surveys were previously done in the Marico and Crocodile River catchment areas, (National Freshwater Snail Collection Database, North-West University, Potchefstroom Campus), limited information regarding the diversity was previously published. Furthermore, in view of the fact that the Marico River is still considered as relatively pristine, while the Crocodile River has been subjected to on-going exploitation and habitat transformation, it is of great importance to survey these areas in order to establish the current status of the mollusc diversity for conservation purposes.

Material and Methods

Study area

The Marico River stretches from its source, the Marico Eye near Swartruggens, for 250 km before it confluences with the Crocodile River to form the Limpopo River. The Crocodile River, originating at Roodepoort, meanders via Hartbeespoort Dam past Thabazimbi to the Botswana border where it joins the Marico River. Nine sites in the Marico River and seven sites in the Crocodile River catchments were selected for sampling. Site selection was influenced by logistical accessibility to the rivers, availability of water and suitable biotopes (Dickens and Graham, 2002; Gerber and Gabriel, 2002; Dallas, 2007). Locations of these sites are depicted in Figure 1 while site descriptions and aquatic biotopes are displayed in Table 1.

Sampling equipment and techniques

Four surveys were conducted, two in an early (May 2013 and 2014) and two in a late (November 2013 and 2014) low-flow period. The coordinates of each sampling site were determined with a Garmin Nuvi 500 GPS.

Semi-quantitative surveys were done by sampling the vegetation, as well as the substratum with a standard SASS net (Dickens and Graham, 2002) for approximately 15 minutes each. Molluscs were collected along the margins of the rivers up to a depth of 70 cm. Aquatic and marginal vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area. Substrata of biotopes consisting mainly of mud, sand, gravel or stones, were sampled by shuffling the feet, whilst continuously sweeping the net over the disturbed area. Contents of the net were then transferred to a rectangular plastic container (360 X 470 X 80 mm) which was filled with water from the habitat to a level of approximately 40 mm. Most of the debris was then carefully removed by hand and scrutinised for specimens that might still be attached. Thereafter the contents of the container was decanted into a cone-shaped Perlon® gauze net (0.25 mm mesh) suspended on a stand. The content of the net was transferred to a plastic container with a tight fitting lid and 90% ethanol was added to preserve the samples. The container was then labelled with relevant site information. The samples were transported to the laboratory where each sample was decanted into a rectangular Perspex® sorting tray (300 X 200 X 25 mm) with a transparent bottom provided with a 10 mm square grid. All molluscs were subsequently removed under a stereomicroscope mounted on a sliding stand and identified up to species level (Walker, 1923; Connolly,1939; Brown,1994; Appleton, 2002). For the indication of recruitment, the presence of juvenile specimens were also recorded.

Historical data

Historical data (1958 to 1984) for the 1/16th degree square grids (loci) in which each of the sampling sites of the current study was located, was extracted from the National Freshwater Snail Collection (NFSC). These data represent species collected in both lotic and lentic waterbodies.

Environmental parameters

Electrical conductivity, pH and water temperature were measured in situ at all sites during each survey using a portable digital thermometer (Checktemp, Hanna Instruments), portable digital conductivity meter (DIST 3, HI 98303, Hanna Instruments) and portable digital pH probe (HI 98128, Hanna Instruments).

Statistical analyses

Canonical correspondence analyses (CCAs) were conducted by making use of CANOCO 5 software, to investigate the distribution and abundance of mollusc species between sites and surveys in relation to the influence of selected environmental variables (water temperature, pH, electrical conductivity (EC) and biotope availability).

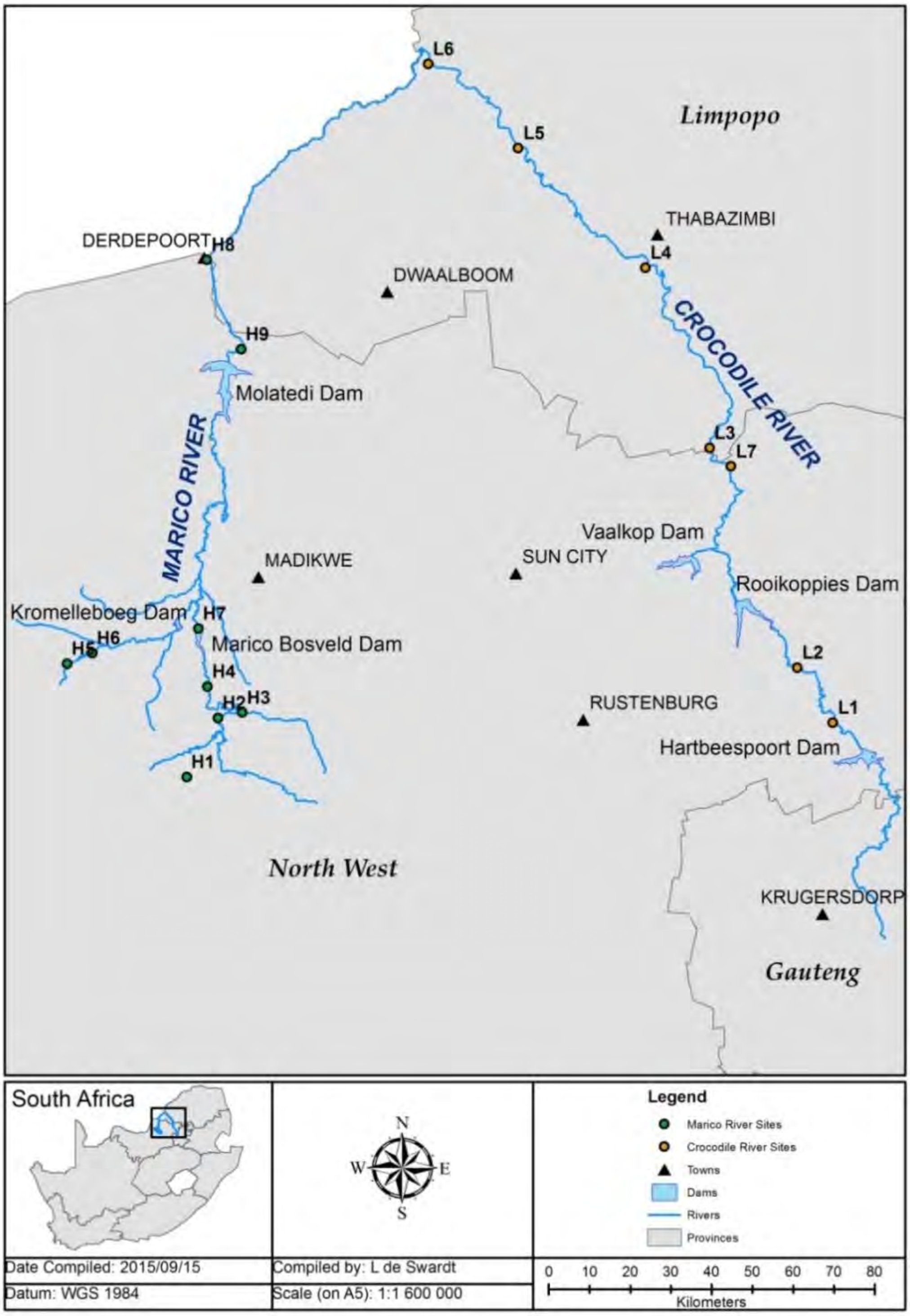

Figure 1: Map of the study area with sampling sites in the Marico and Crocodile Rivers.

Table 1: Site coordinates, river name and aquatic biotopes present at each site.

Site, Coordinates and Altitude	River	Aquatic Biotopes (according to Gerber and Gabriel, 2002)
Marico River		
Site H1 S 25°47'32.1 E 26°21'54'1 1480 m	Marico Eye, source of Marico River.	Abundant marginal and aquatic vegetation, pool and backwaters with stones.
Site H2 S 25°39'45.1 E 26°26'01.9 1197 m	Marico River before its confluence with Sterkstroom, 20 km downstream of the Eye.	Little to no marginal vegetation, stones in current, stones out of current, riffle, run and pool.
Site H3 S 25°39'00.6 E 26°29'16.3 1170 m	Sterkstroom 5 km before its confluence with Marico River.	Little to no vegetation, bedrock, stones in current, riffle, run and pool.
Site H4 S 25'35'33.4 E 26'24'39.4 1 077 m	Marico River 10 km above Marico-Bosveld Dam, after its confluence with Sterkstroom.	Muddy and sandy substratum, filamentous algae, abundant marginal and aquatic vegetation, stones in current, stones out of current, riffle, run and pool.
Site H5 S 25'32'31.4 E 26'06'17.3 1 150 m	Klein Marico River 5 km above Klein-Maricopoort (Bospoort) Dam.	Little to no marginal and aquatic vegetation, filamentous algae, muddy substratum, riffle and run.
Site H6 S 25°31'09.2 E 26°09'25.1 1135 m	Klein Marico River 1km below Klein-Maricopoort (Bospoort) Dam.	Little to no marginal and aquatic vegetation, pool with muddy substratum stones in current, riffle and run.
Site H7 S 25°27'52.6 E 26°23'26.9 1037 m	Marico River directly below Marico-Bosveld Dam.	Marginal and aquatic vegetation, filamentous algae and bedrock.
Site H8 S 24°39'15.9 E 26°24'28.7 914 m	Marico River at Derdepoort.	Abundant marginal and aquatic vegetation, backwater with muddy and sandy substratum, filamentous algae.
Site H9 S 24°50'54.2 E 26°29'07.1 926 m	Marico River 2 km below Molatedi Dam.	Run with muddy substratum, bedrock, stones in current, stones out of current, riffle and pool.
Crocodile River		
Site L1 S 25°40'22.1 E 27°47'25 1104 m	Crocodile River at Brits.	Marginal and aquatic vegetation, filamentous algae, riffle and run with sand and bedrock substratum, stones in current.
Site L2 S 25°33'02.9 E 27°42'40.9 1055 m	Crocodile River downstream of Brits.	Run and pool with sand substratum and filamentous algae.
Site L3 S 25°03'59.6 E 27°31'06.2 936 m	Crocodile River at Koedoeskop Bridge.	Run and pool with muddy substratum and filamentous algae.
Site L4 S 24°40'05.8 E 27°22'35.2 904 m	Crocodile River at Thabazimbi.	Run and pool with sand substratum and filamentous algae.
Site L5 S 24°24'16.8 E 27°05'46.7 905 m	Crocodile River downstream of Thabazimbi.	Run and pool, sand substratum and filamentous algae.
Site L6 S 24°13'07 E 26°53'51.6 836 m	Crocodile River at Rooibokkraal.	Run and pool with sand substratum, sparse marginal and aquatic vegetation, filamentous algae.
Site L7 S 25°06'24.7 E 27°33'55.2 956 m	Pienaars River.	Run and pool with mud and clay substratum, stones in current.

Results

The location of each site is depicted in Figure 1 and site coordinates, river names and biotopes present at each site, are given in Table 1. The mollusc diversity, abundance, species richness (SR) and selected environmental parameters for each of the four surveys, are displayed in Tables 2 and 3. The molluscs on record in the database of the NFSC which were previously collected from the same loci (not necessarily from the same sites) and those species recovered during the present study, are presented in Table 4. Canonical correspondence analyses (CCAs), illustrating associations between species, sampling sites during four surveys, environmental variables and biotope availability, are depicted in Figures 2 and 3.

A total number of 20 species were recovered from the Marico River and tributaries (Table 2). The highest species diversity in the Marico River (SR=12) were recorded for both Sites H4 and H8, while the highest total number of species per survey (9), was also found at these two sites (Table 2). The most common and abundant species were *Burnupia trapezoidea, Lymnaea natalensis* and *Pisidium langleyanum* (Table 2). The lowest species richness was found at Sites H5 and H9, with a total number of four and two species, respectively. Only one specimen of *Galba truncatula, Helisoma duryi* and *Unio caffra* was found during the study. Seven species, not previously on record for this area, were recorded during the present study. However, *Bulinus tropicus*, which was previously recorded in the area (Table 4), was not found. Water temperature and pH did not vary considerably between sites, but seasonal fluctuation in temperature between surveys was observed (Table 2). Electrical conductivity, however, was markedly higher during the third survey at Site H1 and during all the surveys at Site H5.

The length of the arrows in the CCA tri-plot (Figure 2) indicates that algae, pools, stones in current and vegetation biotopes, as well as water temperature played a significant role in the occurrence and distribution of species, while pH, EC and riffle and run biotopes had a minor influence. An association can be seen between *Ferrissia cawstoni, Bulinus forskalii*, Site H7 and algae. *G. truncatula* associated with Site H5, Survey 1, while *H. duryi* and *U. caffra* associated with Site H8, Survey 2 and Site H8, Survey 3, respectively (Figure 2).

Regarding the Crocodile River, a total number of nine species was recovered during the study (Table 2).The highest species diversity (SR=7) was recorded for Site L4, while the highest total number of species per survey (6), was also found at this site. The most common and abundant species were *B. trapezoidea, Corbicula fluminalis africana, Melanoides tuberculata* and *Physa acuta*, while the lowest species richness was found at Site L6 (SR=4) (Table 2). Only one specimen of *Gyraulus costulatus* was found during the study. Five species previously on record for this area were not found (Table 4), however, *Ceratophallus natalensis* and *Gyraulus connollyi* which were not yet on record for this area, were recovered during the present study. Water temperature, pH and EC did not vary considerably between sites, but seasonal temperature fluctuation between surveys was observed (Table 3).

The length of the arrows in the CCA tri-plot (Figure 3) indicates that EC, algae, riffle and stones in current biotopes played a significant role on the occurrence and distribution of species, while pH, run and sand biotopes had a lesser and temperature and vegetation even a smaller influence. An association can be seen between *C. fluminalis africana*, Site L6 and EC. *Melanoides tuberculata* associated with Site L5 and the presence of algae, while *G. connollyi* associated with Site L1 and a riffle biotope.

Table 2*:* Mollusc species collected, species richness and abundances during Surveys 1 to 4 in the Marico River, as well as environmental parameters determined at the time of collection.

Site	H1				H2				H3				H4				H5			
Mollusc species	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
B. pfeifferi		1																		
B. africanus					1									1						
B. forskalii																			1	
B. trapezoidea		1	1	1		2	14	26		2	7	23	6	2		4			1	1
C. fluminalis africana													17	4	3					
G. truncatula																	2			
G. connollyi		4	1	1	6					1		3	84	58						
G. costulatus													73	14						
L. natalensis		5	1	1	5	2				1			172	36	1	1				
M. tuberculata													7	65	4	1				
M. victoriae															1					
**P. acuta*													1	9		1				
P. costulosum					7	4				14	1		2	4						
P. langleyanum		2	1		25	15	1			12	1	1	8	7	5		5			
P. viridarium										7	1	1								
**P. columella*																1				
Total per site/survey	**0**	**5**	**4**	**3**	**5**	**4**	**2**	**1**	**0**	**6**	**4**	**4**	**9**	**9**	**4**	**5**	**2**	**0**	**2**	**1**
Species Richness	**5**				**6**				**6**				**12**				**4**			
Temp (°C)	22	21	18	18	16	23	13	19	12	22	15	19	17	24	15	21	11	22.7	13	19
pH	7.2	7.9	6.8	6.7	7.4	8	7.5	6.8	6	7.8	6.6	7.1	8.1	8.2	7.3	6.8	7.9	8.2	7.7	7.8
EC (µS/cm)	282	253	630	309	295	282	263	290	120	97	96	108	302	308	241	260	773	1103	918	667

*Alien species

Site	H6				H7				H8				H9			
Mollusc species	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	4
B. pfeifferi									4			4				
B. africanus								5	6	16	4	35				
B. depressus								1	1	1	1	1				
B. forskalii							7		1							
B. trapezoidea	12	42	8	1			2		8	5	4	21				
C. fluminalis africana														3	1	1
F. cawstoni	1				4	1										
G. connollyi	9						22	39	3	2	5					
G. costulatus					2			5	4	6		33				
**H. duryi*										1						
L. natalensis	2				3	5	3	26	22	29	7	77	2	2		
M. tuberculata									5	52	3	233				
P. langleyanum	64	86	6	4				1		1						
P. viridarium			1	3												
**P. columella*							2	18								
U. caffra												1				
Total per site/survey	**5**	**2**	**3**	**3**	**3**	**2**	**5**	**7**	**9**	**9**	**6**	**8**	**1**	**2**	**1**	**1**
Species Richness	**6**				**10**				**12**				**2**			
Temp (°C)	11	22	14	21	13	24	16	24	18	27	21	23	18	27	19	21
pH	7.9	8.2	7.5	7.1	7.6	8.3	7.6	7.3	7.9	7.8	7.4	7.7	8.5	8.5	7.4	7.6
EC (µS/cm)	560	696	575	605	265	302	208	249	459	566	256	366	456	548	228	334

*Alien species

Table 3: Mollusc species collected, species richness and abundances during Surveys 1 to 4 in the Crocodile River, as well as environmental parameters determined at the time of collection.

Site	L1				L2				L3				L4				L5				L6				L7			
Mollusc species	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
B. trapezoidea	40	8	79	127	15	9		5					10	6	10	2	1	1	14	3		1			10	7	9	8
C. natalensis			6											6														
C. fluminalis africana					3			1				52	39	28	25	32	4	28	9	25	1	48	25	20			3	5
G. connollyi		1	2	3	2																							
G. costulatus									1																			
L. natalensis	1								20				4	30				9							32	21	4	
M. tuberculata	2		14	15	1			3		1				8	1	1	280	336	138	15	7							
**P. acuta*	3	112	9	862	23	131		145	35	410		3	83	403	12	24	42	42	13		1	3			131	144		6
**P. columella*										1						1												1
Total species per site/survey	**4**	**3**	**5**	**4**	**5**	**2**	**0**	**4**	**3**	**3**	**0**	**2**	**4**	**6**	**4**	**5**	**4**	**5**	**4**	**3**	**3**	**3**	**1**	**1**	**3**	**3**	**3**	**4**
Species Richness	**6**				**5**				**5**				**7**				**5**				**4**				**5**			
Temp (°C)	18.6	21.5	20	22.2	19.4	25.6	17.3	23.2	13.5	23.7	16.1	24.1	18.3	30.6	15.9	24.4	18	28.6	18	28.7	15.6	32.5	20.4	27.2	15.9	26.7	15.7	26.7
EC (µS/cm)	514	501	574	622	613	529	608	732	651	760	621	781	601	652	786	904	612	630	843	888	628	773	753	877	682	819	544	714
pH	8.3	7.12	8.04	8.24	8.64	7.59	7.91	8.41	8.46	9.77	7.91	8.55	9.33	8.95	7.86	8.23	8.54	8.62	8.5	8.81	8.36	9.16	8.48	8.48	8.97	8.94	8.01	8.71

Table 4: Mollusc species on record in the National Freshwater Snail Collection (NFSC) from 1958 to 1984 and species collected during the 2013 and 2014 study.

	Marico River and Catchment		**Crocodile River and Catchment**	
Mollusc species	**1958 – 1984**	**2013 - 2014**	**1958 – 1984**	**2013 - 2014**
B. pfeifferi	X	X	X	
B. africanus	X	X	X	
B. depressus	X	X	X	
B. forskalii	X	X	X	
B. tropicus	X		X	
B. trapezoidea	X	X	X	X
C. natalensis				X
C. fluminalis africana	X	X	X	X
F. cawstoni		X		
G. truncatula		X		
G. costulatus	X	X	X	X
G. connollyi	X	X		X
**H. duryi*		X		
L. natalensis	X	X	X	X
M. tuberculata	X	X	X	X
M. victoriae		X		
**P. acuta*		X	X	X
P. costulosum		X		
P. langleyanum		X		
P. viridarium		X		
**P. columella*	X	X	X	X
U. caffra	X	X		

*Alien species

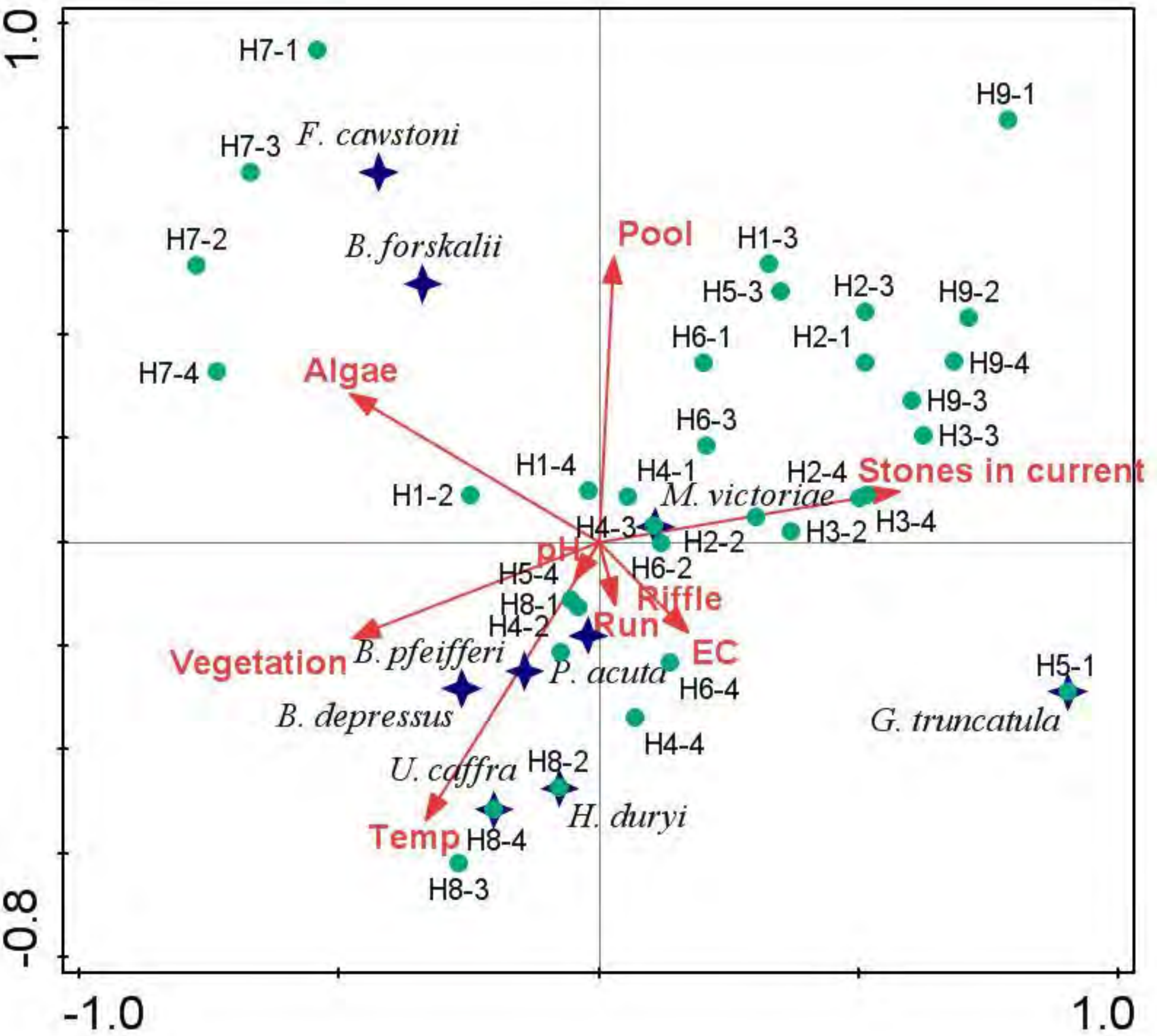

Figure 2: CCA tri-plot illustrating associations between species (blue stars), sampling sites in the Marico River during four surveys (green dots), environmental variables (red arrows) (electrical conductivity [EC], water temperature and pH) and biotope availability (pool, riffle, run, stones in current, vegetation and the presence of algae). Only taxa for which more than 10% is explained by the model are depicted. The tri-plot describes 57.26% of the total variation, with 38.52% being described on the first axis and 18.74% on the second axis.

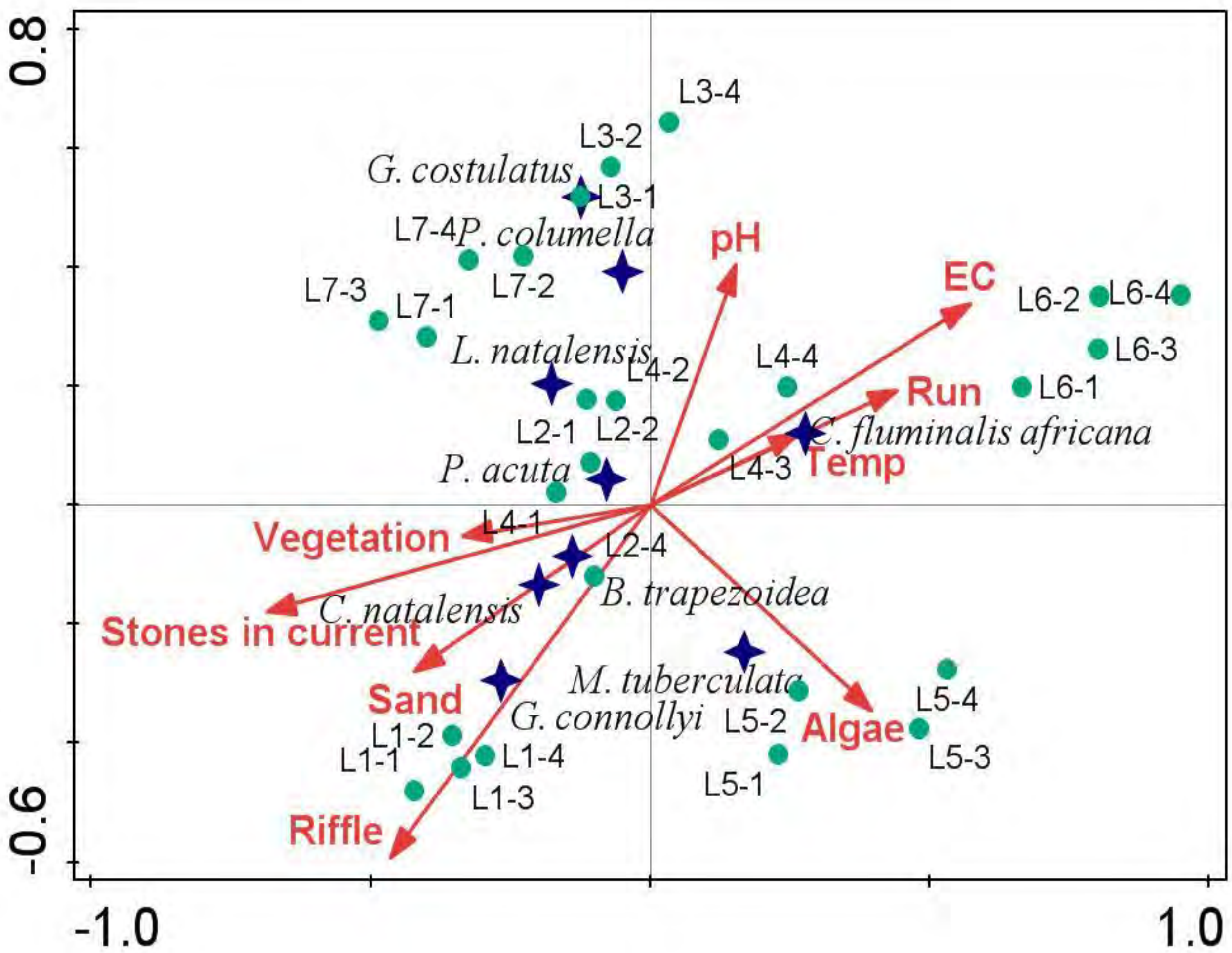

Figure 3: CCA tri-plot illustrating associations between species (blue stars), sampling sites in the Crocodile River during four surveys (green dots), environmental variables (red arrows) (electrical conductivity [EC], water temperature and pH) and biotope availability (riffle, run, stones in current, sand, vegetation and the presence of algae). The tri-plot describes 66.92% of the total variation, with 40.75% being described on the first axis and 26.17% on the second axis.

Discussion

From historical data in the database of the NFSC it is clear that the Marico and Crocodile River areas had almost a similar molluscan diversity, with only one more species recorded from the Marico River (Table 4). The results of the current study, however, show a dramatic change in this similarity. The number of species found in the Marico River area increased from 13 to 20 species, while the number of species in the Crocodile River area decreased from 12 to nine species.

Of the 13 species on record for the Marico River area, only *B. tropicus* was not recovered during the present study, probably due to the fact that this snail is primarily found in lentic waterbodies such as dams and lakes and various ephemeral habitats, which were included in the previous surveys (de Kock et al., 2002). Of the 12 species on record for the Crocodile River area, only seven were collected during this study, while two species, *C. natalensis* and *G. connollyi* were recorded for the first time. Of the 21 species recovered during this study, only seven species were found in both the Marico and Crocodile Rivers. Six of these seven species can be considered as generalists which are able to utilise both sediment and vegetation biotopes, while the seventh species, *C. fluminalis africana*, is a bottom dweller and not dependent on vegetation biotopes (de Kock and Wolmarans, 2007a).

The importance of the effect of biotope availability on biodiversity (Dallas, 2007) is supported by the high species richness (SR=12) found at Sites H4 and H8 in the Marico River, where a large selection of biotopes were present (Table 1). The low species richness (SR=2) found at Site H9 was possibly due to the fact that this site was situated directly downstream from the Molatedi Dam, where the constant release of large volumes of water every four to six weeks (RHP, 2005) possibly resulted in gross disturbance and transformation of the aquatic biotopes, as reflected by the absence of marginal and aquatic vegetation (Table 1). Furthermore, this site was also negatively impacted by the clearing of riparian vegetation and grazing by cattle from a nearby informal settlement as observed during the surveys. With regard to Site H5, located close to Zeerust, the low mollusc diversity (SR=4) could most likely be ascribed to the lack of suitable biotopes and possible negative impacts from the nearby industrial and urban activities (RHP, 2005) as obvious signs of littering and eutrophication were observed. The fact that *B. trapezoidea, L.*

natalensis and P. langleyanum were the most commonly occurring species in the Marico River and tributaries, could be attributed to their wide distribution in the area, as previously recorded (Database of the NFSC; de Kock et al., 2001; de Kock and Wolmarans, 2008). The single specimen of *M. victoriae* collected at Site H4 was unexpected, as this locality is well beyond its distribution range previously reported by de Kock and Wolmarans (2009).

The ordination in the CCA tri-plot (Figure 2) shows that, regarding the environmental variables, pH and EC did not play a significant role in the species occurrence and distribution, but water temperature and the presence or absence of different biotopes, did play a significant role. The fact that water temperature played such a significant role in the distribution of mollusc species, is in accordance with the results of similar studies in Africa (Tchakonté et al., 2014; Abd El-Wakeil et al., 2015). Water temperature is further considered as one of the major factors influencing reproduction of molluscs (de Kock and van Eeden, 1982). However, the lower temperature during the May surveys and the higher temperature during the November surveys did not prevent recruitment, as juvenile specimens of different generations of the majority of species were present at most of the sampling sites.

The association of *G. truncatula* with Site H5 and *H. duryi* and *U. caffra* with Site H8, is most probably due to the fact that they were found only once and in single numbers at these sites.

A comparison between the historical data and the results of the current study is not really feasible as the historic data have been collected by many different people over several decades. However, it is still clear that a higher diversity was currently found in the Marico River, possibly due to the fact that previous surveys did not concentrate on the Marico River per se, as well as to the fact that this river has not been seriously impacted by anthropogenic activities during the past three decades (RHP, 2005).

Of the nine species found in the Crocodile River, the occurrence of several of these, namely *C. fluminalis africana, B. trapezoidea, G. costulatus*, *G. connollyi* and *C. natalensis,* could be expected, as they are widely distributed in South Africa (Database of the NFSC; de Kock and Wolmarans, 2004; 2006; 2007a; 2007b) and predominantly associate with sediment biotopes which were available at almost all of the sites. However, the limited availability of marginal and aquatic vegetation at most of the sites, did not prevent the occurrence of *P. acuta*, *L. natalensis* and *P. columella* which are generalists in respect of their biotope preferences (de Kock et al., 1989; Brown, 1994; de Kock et al., 2001). As in the case of the Marico River, the ordination in the CCA tri-plot (Figure 3) shows that biotope availability played a significant role on species occurrence and distribution. In the Crocodile River, however, EC was also an important factor in this respect and these results are in agreement with the conclusions of Tchakonté et al. (2014) and Abd El-Wakeil et al. (2015). The presence of filamentous algae, an indication of organic enrichment, and the considerably higher recorded EC values at most of the sites throughout the study, could be attributed to the anthropogenic stressors mentioned above.

Three alien species, namely *H. duryi, P. acuta*, and *P. columella* were found during this study. Of these, *P. columella* is on record as the third most widespread freshwater snail species in South Africa (de Kock and Wolmarans, 2008). However, during this study it was found only in a few sites and also in limited numbers in both the Marico River (Sites H4 and H7) and the Crocodile River (Sites L3, L4 and L7). The fact that *P. acuta* was found at all the sites in the Crocodile River, often in high numbers, is not surprising as it has attributes such as a superior fecundity rate as reported by Appleton and Brackenbury (1998) and the ability to migrate upstream (Appleton and Branch, 1989). Furthermore, this species can serve as an indicator of organic enriched water and commonly occurs in areas where sewage pollution is obvious and according to Brown (1994) seems to be closely associated with human activities. *Helisoma duryi*, of which only one specimen was found at Site H8, is widespread in artificial waterbodies but not invasive (Appleton, 2003). Of the 41 records of this species in the database of the NFSC reported by de Kock and Joubert (1991), only 15 were from natural habitats.

The relatively high mollusc diversity and the fact that juveniles were present throughout the study, demonstrated that current habitat and environmental conditions were suitable to promote recruitment and the sustainability of diverse mollusc populations in the Marico River and its tributaries. However, in contrast to this, the exploitation of and habitat transformation in the Crocodile River resulted in the decrease of biotopes which eventually could have led the decrease in diversity and the establishment of *P. acuta*, an exotic invader species.

Acknowledgments

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CHAPTER 6

Metallothioneins and metal bioaccumulation in aquatic macroinvertebrates from a relatively unimpacted river system in South Africa

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In submission to *Bulletin of Environmental Contamination and Toxicology*

Abstract

The Marico River is still, to a large extent, unimpacted by anthropogenic activities. However, metal concentrations in the water and sediment, originating mainly from natural sources, occasionally exceeds target quality guidelines. Macroinvertebrates are capable to react to, metabolize, and regulate these metals within their internal systems through processes such as the induction of metallothioneins (MTs). This study aimed to 1) determine whether there are relationships between metal concentrations in the water, sediment and macroinvertebrates 2), determine whether these relationships are reflected by concomitant MT levels in macroinvertebrates and 3), establish whether MTs can be used as a biomarker of metal exposure in a relatively unimpacted system such as the Marico River. Positive correlations were evident between metal concentrations in all the macroinvertebrate families and in the sediment while no correlations were found between metal concentrations in the water and organisms. Metal concentrations were the highest in Baetidae, Caenidae and Chironomidae, all sediment associated and sediment dependant feeders. Metallothionein levels correlated positively with most metals. The results indicated that MTs are induced in response to an increase in most of metal concentrations. This phenomenon therefore renders MTs as a suitable biomarker for metal exposure, even in a relatively unimpacted river system.

Keywords

Metal exposure; bioaccumulation; aquatic macroinvertebrates; biological response; metallothioneins; Marico River.

Introduction

Aquatic macroinvertebrates are exposed to a variety of physico-chemical factors in water that may be detrimental or advantageous to their survival and reproduction. According to literature (Holmes, 1996; Hassell et al., 2006; Tixier et al., 2009; Hussain and Pandit, 2012) macroinvertebrates in general demonstrate wide tolerance ranges for variations in parameters such as velocity, pH, EC temperature and turbidity. They are also capable of accumulating essential and non-essential metals present in the surrounding water and sediment (Amiard et al., 2006). The concentrations of these metals demonstrate enormous variability across metals and taxa (Rainbow, 2007) and may be influenced by several factors such as, amongst others, species, size, gender, functional feeding groups and temporal variability (Farag et al., 1998).

Metal concentrations can reach chronic or acute levels and may have adverse effects on macroinvertebrates. Populations that were chronically exposed to metals often display enhanced tolerance, while acute concentrations may eliminate sensitive taxa and reduce biodiversity (Kapustka et al., 2004). Hickey and Clements (1998) found that rivers with excessive metal concentrations generally have a low species-richness, reduced abundance of metal-sensitive taxa, such as mayflies, and may be dominated by metal-tolerant taxa such as Chironomidae (Kiffney and Clements, 1994).

Fortunately, depending on the accumulation patterns of metals which entail adsorption, absorption and metals associated with material in the organisms gut (Maret et al., 2003), invertebrates are able to react to, metabolize, and regulate metals within their internal systems (Hyne and Maher, 2001; Amiard et al., 2006). One such response to high metal concentrations is the induction of metallothioneins (MTs). Metallothioneins are non-enzymatic proteins with low molecular weight and high cysteine content. The synthesis of these proteins may be influenced by various factors such as metal contamination or accumulation, as well as by biotic and abiotic factors such as the reproductive cycle of organisms or temperature (Geffard et al., 2007). The function of MTs is still, to a large extent, an uncertainty, however, they are known to play a role in the homeostasis of essential metals and are allegedly involved in the detoxification of surplus amounts of trace metals (Amiard et al., 2006). Additionally, they may also serve as a biomarker for metal exposure (Amiard et al., 2006).

In Chapter 3 it was demonstrated that the metal concentrations in the Marico River system cannot be attributed to direct human impacts. The variable metal concentrations in water and sediment were rather attributed to natural processes such as geological weathering (Chapter 3). The aims of this Chapter were therefore to 1) determine whether there are relationships between metal concentrations in the water, sediment and macroinvertebrates 2), determine whether these relationships are reflected by concomitant MT levels in macroinvertebrates and 3), establish whether MTs can be used as a biomarker of metal exposure in a relatively unimpacted system such as the Marico River.

Material and Methods

Study Area

The study was conducted in the North-West and Limpopo Provinces, at eight samplings sites in the Marico River and selected tributaries (Klein Marico River and Sterkstroom) (Figure 1). The Marico River, a tributary of the Limpopo River, is classified as a National Freshwater Ecosystem Priority Area (NFEPA) (Nel, 2011). Its general state is considered as natural to good, free from significant organic pollution, with a pronounced biodiversity and overall good water quality (RHP, 2005).

Site H1 is the dolomitic Marico Eye, the source of the Marico River. Sites H1 and H2 are both situated in unimpacted areas. Site H3 is in the Sterkstroom, described as moderately impacted (RHP, 2005). Sites H4 and H6 are in the Marico River upstream and downstream of the Marico Bosveld Dam while Site H5 is situated in the Klein Marico River, downstream of the Klein Maricopoort Dam. This tributary is described as being in a fair to poor state and is considered as being contaminated by effluent from the town of Zeerust (RHP, 2005). Site H8 is located in the Marico River at 3de Poort near the Botswana border and Site H7, also in the Marico River, is just below the Molatedi Dam. Macroinvertebrates, water and sediment samples were collected from all the sites during the low-flow period (May) of 2013.

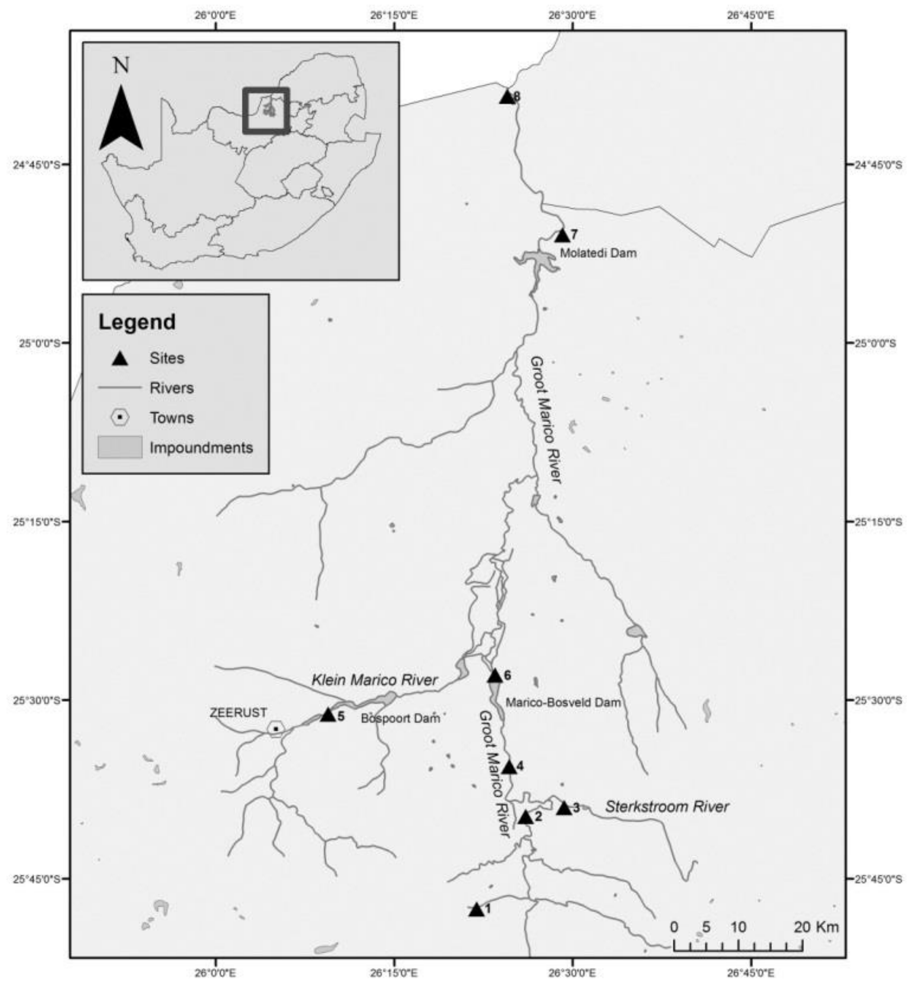


Figure 1: Sampling sites within the Marico River catchment, North-West, South Africa.

Physico-chemical parameters were measured in situ by making use of a clarity tube and calibrated portable digital meters including: conductivity meter (DIST 3, HI 98303, Hanna

Instruments), pH probe (HI 98128, Hanna Instruments), thermometer (Checktemp, Hanna Instruments) and flow-probe (FP 111, Global Water). The coordinates and altitude of each sampling site were determined with a Garmin Nuvi 500 GPS and from Google Earth. Water temperature is expressed as Celsius (°C), EC as µS/cm, turbidity as NTU's and velocity or flow as meter/second.

Macroinvertebrates were collected by sampling the vegetation (marginal and aquatic instream), gravel and sand, and stone biotopes with a 30 cm x 30 cm x 30 cm (mesh size 500 µm) sweep net for approximately 15 min each. Marginal and aquatic vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area (Dickens and Graham, 2002; Kemp et al., 2014; Wolmarans et al., 2014). Macroinvertebrates on the mud, sand and gravel biotopes were collected by shuffling the feet whilst sweeping the net over the disturbed area. Stones in and out of current were sampled by carefully lifting the stones by hand and brushing each stone off with a soft brush whilst collecting the macroinvertebrates in a net held downstream from the stone. The contents of the nets were decanted into a plastic tray and families represented by high numbers and a variety of functional feeding groups were identified and collected for metal and MT analyses. At each site, specimens for MT analyses were put in a sterilised snap cap, filled with standard Hendrikson storage buffer (Wepener at al., 2005) and stored in liquid nitrogen. Specimens for metal analyses were transferred to a plastic container with a tight-fitting lid and 90% ethanol was added to preserve the samples (Kemp et al., 2014).

Water and sediment samples (approximately 300ml each) were collected separately in polyethylene bottles which were washed with detergent, rinsed with distilled water followed by 1% nitric acid solution and rinsed again with double distilled water. Samples were then transported to the laboratory and kept in a refrigerator until analyzed.

Metal analyses

Only selected metals considered as potentially toxic to macroinvertebrates were measured.

Water

Water samples were filtered through Whatman filter paper (0.45 μm) and the filtrate was used directly for analysis in an inductively coupled plasma mass spectrometry (ICP-MS) using standard recognized techniques. Ultraspec Certified Element Standards (De Bruyn Spectropscopic Solutions, Bryonston, Jhb, SA) were used to set up calibration curves. The percentage recoveries were within an acceptable range (<10% deviation) (see supplementary material Table S1). All samples were analysed in triplicate.

Sediment

Analyses of metals in the total sediment samples were also done by making use of an ICP-MS. Samples of 0.5g sediment were digested using aqua regia (HCL: HNO_3 = 3:1) and a microwave digester. For each batch of samples, quality control and quality assurance protocols were applied. Certified reference materials (Resource Technology Corporation and CN Schmidt BV: Trace Elements on Fresh Water Sediments (CNS392-050)) were used and standard calibration runs were performed. The percentage recoveries for the standards and certified reference materials were within an acceptable range (<10% deviation) (see supplementary material Table S1). All samples were analysed in triplicate.

Macroinvertebrates

Seven families (Notonectidae, Coenagrionidae, Atyidae, Libellulidae, Baetidae, Caenidae and Chironomidae), categorised into feeding groups, were selected for metal analysis (see supplementary material Table S2). Feeding groups were based on the type of material ingested, as well as the organism's interaction with sediment during feeding (Smock, 1983). From each of these seven families, three replicate samples (consisting of one or more specimens, depending on their size) were selected. Samples were weighed and transferred to a Teflon multicell container with 1ml 65% chemically pure nitric acid added to each cell. Samples were digested under pressure at 70°C for 24 hours. Digested samples were diluted up to 14ml with double distilled water and analysed with an ICP-MS. Metal concentrations are expressed as µg/l/mg wet weight. For each batch of samples, quality control and quality assurance protocols were applied. Certified reference material for mussel tissue (ER-CE78k) was used and standard calibration runs were performed.

Macroinvertebrate metallothionein analysis

Metallothionein concentrations were analysed in four families (Gerridae, Aeshnidae, Atyidae and Coenagrionidae) represented by the highest abundance, using a modified version of Viarengo et al. (1997) and Wepener et al. (2005). From each of the four families, three replicate samples (consisting of one or more specimens, depending on their size) were selected. Samples were kept on ice during the entire procedure. Samples were weighed and homogenised in 500 µl homogenising buffer. The buffer consisted of Tris–HCl (0.02 M, adjusted to pH 8.6), Sucrose (0.5 M), Leupeptine (0.006 mM), phenylmethylsulphonylflouride (PMSF, 0.0005 M) and b-Mercaptoethanol (0.01%). The homogenate was centrifuged at 30 000 g (4°C) for 20 minutes. The supernatant was transferred to another vial after which absolute ethanol (500 µl at -20°C) and absolute chloroform (40 µl) were added. Samples were centrifuged again at 7 000 rpm (4°C) for 10 minutes. Here after three volumes of absolute ethanol (20°C) were added and samples were incubated at -20°C for 1-2 hours to allow for pellet formation.

The resulting supernatant was decanted while pellets were rinsed two times with 1 ml washing buffer (consisting of 87% absolute ethanol + 1% chloroform + 12% homogenising buffer). After each rinse, samples were centrifuged at 3 000 rpm (4°C) for 20 min and washing buffer was decanted. Remaining washing buffer was removed by exposing the pellets to a nitrogen gas and pellets were resuspended in 150 µl NaCl (0.25 M) and 150 µl

Tris (5 mM)-EDTA (4 mM) (pH 7). A standard GSH (reduced glutathione, 1.627 mM) calibration curve was prepared by adding 210 µl of DTNB (0.43mM in 0.2M Sodium phosphate buffer, pH 8) and 15 µl of the blank, standards and samples to a microtitre plate (in triplicate). The plate was incubated for 15 minutes at room temperature and sample absorbencies were read on an ELx Universal microplate reader at 412 nm. The calibration curve was used to determine MT concentrations expressed as nM/wet weight (Viarengo et al., 1997, Wepener et al., 2005). The content of MT was calculated as follows: 1 nM SH= 1 nM GSH= 0.055 nM MT.

Statistical analyses

Kolmogorov-Smirnov tests (with Dallal-Wilkinson-Lilliefor P values) were used to test for normality of data. Since data were non-parametric, one-way analyses of variance (ANOVAs) followed by Dunns test for post hoc analysis was done to test for significant differences ($p<0.05$) in the metal concentrations in the water and sediment as well as in the remaining physico-chemical parameters in the water between the various sites.

To test for possible relationships between metal concentrations in the macroinvertebrate families and metal concentrations in the water and sediment, Spearmans' nonparametric correlation tests were done for each site.

One-way analyses of variance (ANOVAs) followed by Dunns test for post hoc analysis were done to test for significant differences ($p<0.05$) in the concentrations of each metal between the different families. To test if metal concentrations in invertebrates correlate with MT levels in invertebrates, Spearmans' nonparametric correlation tests and linear regressions were done for each metal.

Results and Discussion

No significant spatial variation in both metal concentrations and other physico-chemical parameters were evident (results of ANOVAs not shown). The limited spatial variation was also evident from the results in Chapter 3. As expected (Bervoets et al., 1994; Williamson et al., 1996), metal concentrations in the water (Figure 2a) were considerably lower than those measured in the sediment (Figure 2b). Only Cu and Zn concentrations in the water exceeded the South African Chronic Effect Values (CEV) for aquatic ecosystems (Holmes, 1996). South Africa has no quality guideline values for sediment, however, most metal concentrations in the sediment exceeded international guideline values (EPA, 2009; ANZECC, 2000). Although the metal concentrations in the water were substantially lower than in the sediment, predictions regarding the organisms' reaction to different metal concentrations are difficult.

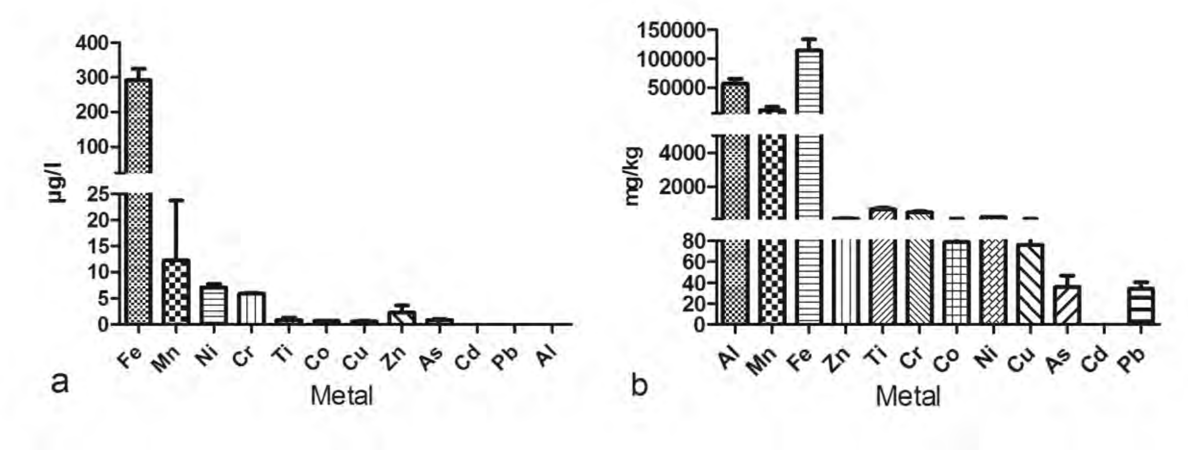


Figure 2a and b: Mean metal concentrations in the water (a) and sediment (b) measured at the various sites.

During this study it was found that, regardless of the organisms' feeding behaviour or interaction with sediment, positive correlations were evident between metal concentrations in all the macroinvertebrate families and in the sediment while no correlations were found between metal concentrations in the water and organisms (results of correlation statistics not shown). This is congruent with other studies (Miller at al., 1992; Santoro et al., 2009; Corbi et al., 2010), where linear correlations between As, Cd, Cr and Zn concentrations in invertebrates and sediment were evident. Numerous factors such as species, size, gender, functional feeding groups and temporal variability (Farag et al., 1998), may influence the metal accumulation in macroinvertebrates. The positive correlation of metals present in organisms and sediment may be due to adsorbed, absorbed, as well as ingested metals (Smock, 1983; Cairns et al., 1984; Miller et al., 1992), phenomena that varies greatly between species (Chapman et al., 1980; Rainbow, 2007).

There were no significant differences in metal bioaccumulation in macroinvertebrates between the different sites and, therefore, all bioaccumulation data were pooled per family. Comparing the mean concentrations of metals measured in the macroinvertebrate families (Figure 3), Al, Mn, Fe and Zn were present in the highest concentrations in all the families. The dominance of Al, Mn and Fe were also visible in the sediment, as reflected by their concentrations of 56500, 1066 and 114237 µg/g (Figure 3), respectively. These elevated concentrations are in accordance with findings of Gerber et al. (2016) and might be ascribed to the fact that these are some of the most abundant metals in the earths' crust (Holmes, 1996).

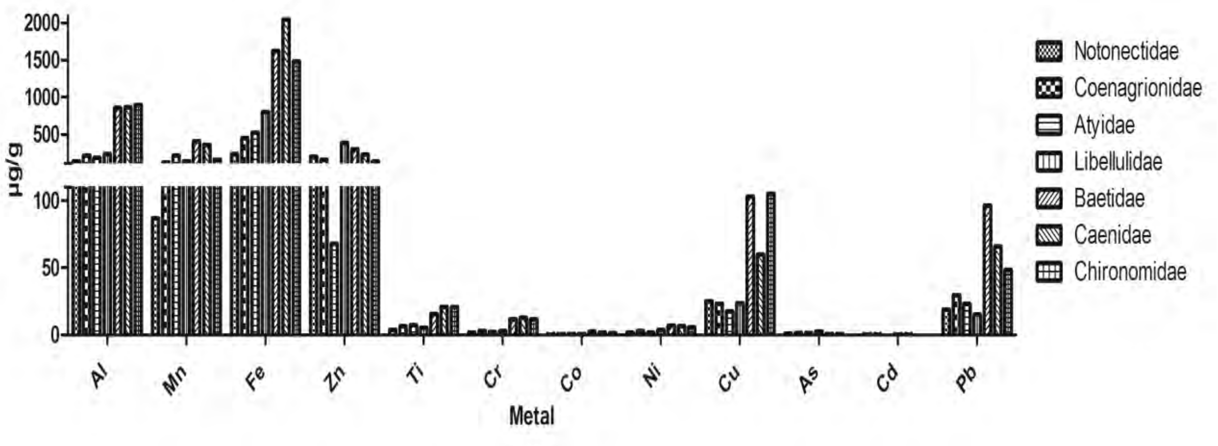


Figure 3: Mean concentrations of metals (µg/g wet weight) in the different families.

Feeding habits are known to play a significant role in the metal concentrations of macroinvertebrates (Smock, 1983) and it was found that the metal bioaccumulation for most metals (i.e. Al, Ti, Cr, Fe, Co, Ni, Cu and Pb) were significantly higher in the mayfly and non-biting midge families (Baetidae, Caenidae and Chironomidae) (Figure 4a-l). This is congruent with results of Smock (1983), who established that these families are both sediment associated and sediment dependant feeders that may ingest sediment and detritus whilst feeding. Notonectidae, Coenagrionidae and Libellulidae are all predators (carnivores) and the fact that they had the lowest metal concentrations is also consistent with literature (Smock, 1983; Corbi et al., 2010).

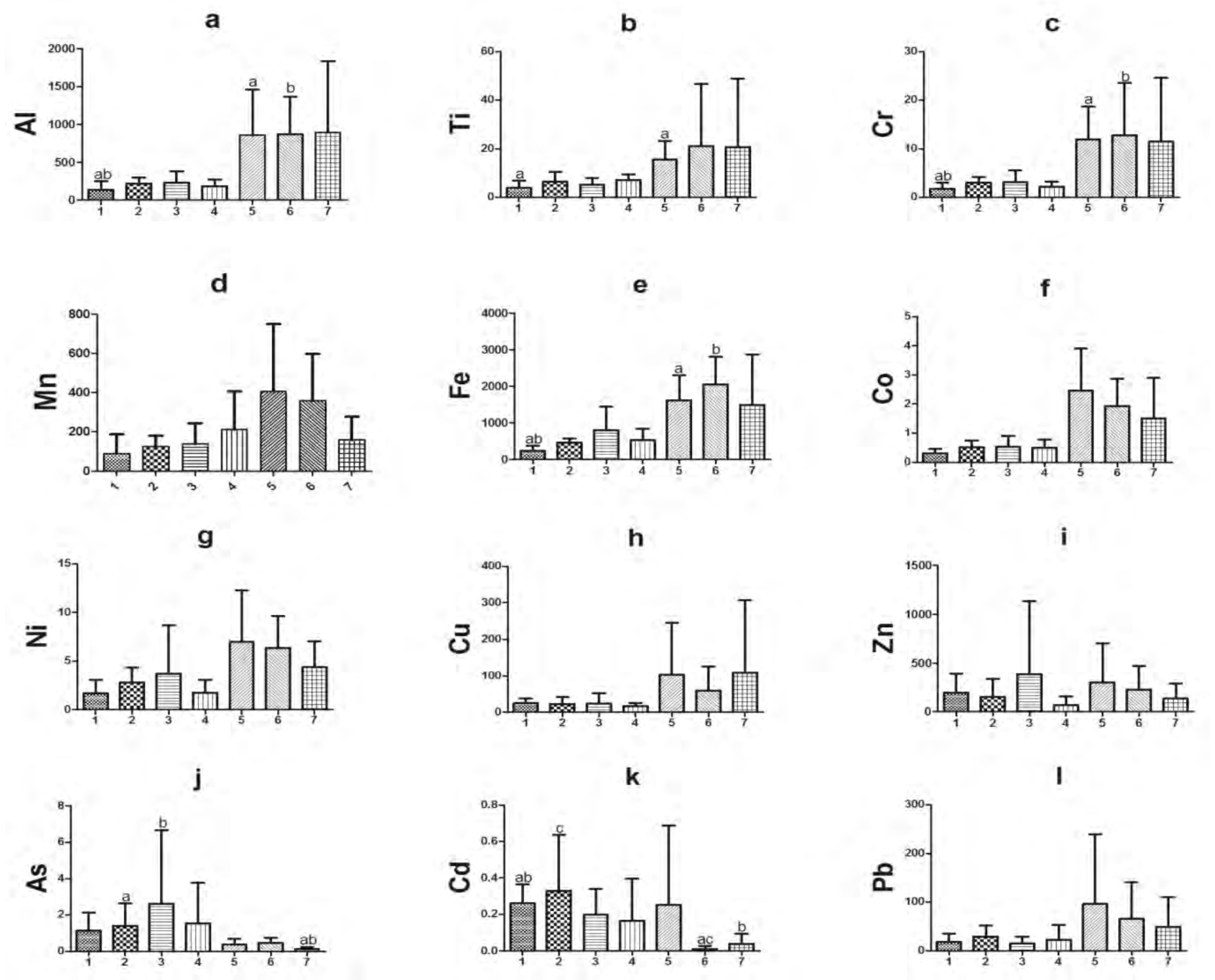


Figure 4a-l: Mean±standard error (µg/g wet weight) of metal concentrations in the different families. Bars with common superscripts differ significantly ($p<0.05$).

Legend: 1 – Notonectidae. 2 – Coenagrionidae. 3 – Libellulidae. 4 – Atyidae. 5 – Baetidae. 6 – Caenidae. 7 – Chironomidae.

Metallothionein levels varied between the four families (Figure 5). Gerridae, a predacious family, had the highest levels of MTs while Atyidae, a filter feeding family, had the lowest levels of MTs. Unfortunately, only two families (Atyidae and Coenagrionidae) had sufficient material to determine both MT levels and metal concentrations. Results of the analyses on these two families will be used to determine whether there is a relationship between metal bioaccumulation and MT induction.

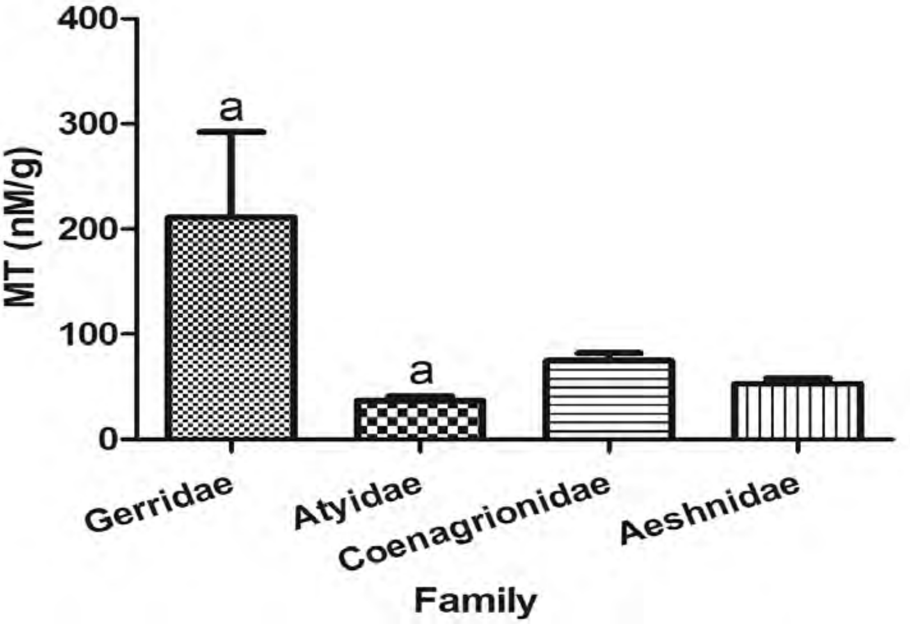
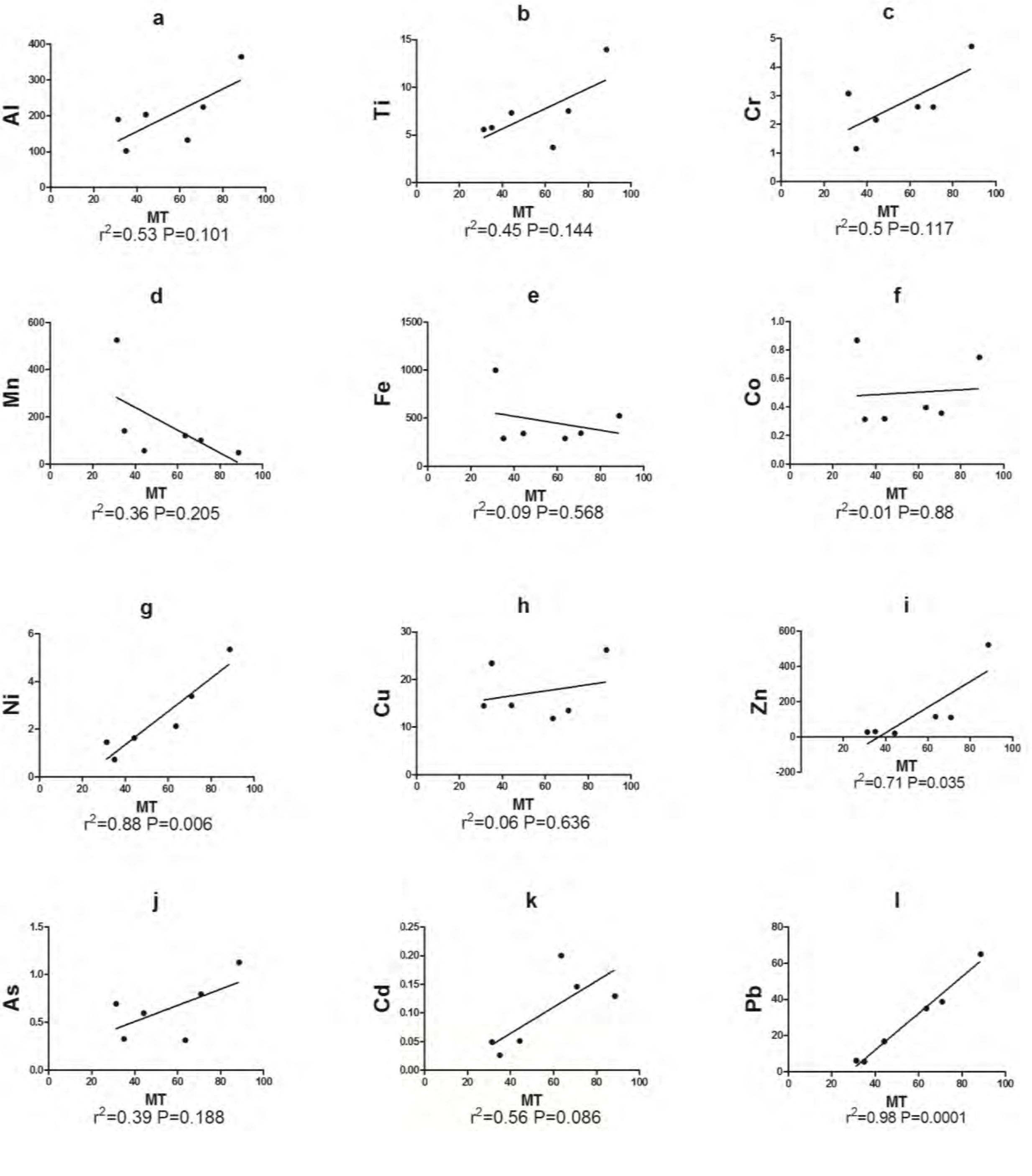


Figure 5: Mean±standard error of MT levels (nM/g wet weight) in four families. Bars with common superscripts differ significantly ($p<0.05$).

Taking the MTs into account, the correlation analyses between MT levels and metal bioaccumulation in the organisms in total, belonging to Atyidae and Coenagrionidae, are depicted in Figure 6a-l. Metallothionein levels correlated positively with most metals, with the exception of Mn, Fe and Co (Figure 6a–l). The only significant correlations ($p<0.05$), however, were between MT levels and Ni, Zn and Pb concentrations in the macroinvertebrates. Although various factors such as the reproductive cycle of organisms or temperature (Geffard et al., 2007), can influence MT synthesis, the results of the current investigation indicate that MTs are induced in response to an increase in most metal concentrations. This phenomenon therefore renders MTs as a suitable biomarker for metal exposure, even in a largely unimpacted river system with relatively low metal concentrations, a conclusion supported by Freitas et al. (2012).



Figures 6a-l: Linear correlations of metal concentrations (µg/g wet weight) and MT levels (nM/g wet weight) in macroinvertebrates.

In a study of the macroinvertebrate biodiversity in the Marico River (see Chapter 4), a total of 187 taxa, including highly sensitive families (Kiffney and Clements, 1994; Dickens and

Graham, 2002) such as, Oligoneuriidae, Heptageniidae, Perlidae, Psephenidae, Chlorocyphidae, Crambidae, Blephariceridae and Athericidae were recorded. This is in contrast to the 94 taxa recorded in the Olifants River, (Kemp et al., 2014) which is frequently described as being highly contaminated by metals (McCarthy, 2011; Netshitungulwana and Yibas, 2012; Gerber et al., 2015). Furthermore, Wolmarans et al. (2016) found no correlation between the metal concentrations in the sediment and the diversity and abundance of benthic macroinvertebrates in the Marico River.

In conclusion, it was found that, although metals in the water and sediment exceeded some quality guideline values and accumulated in macroinvertebrates, they most probably do not affect the community assemblages in a negative way. The fact that MT levels correlated positively with increased metal concentrations in the macroinvertebrates, suggests that MTs may be used as a biomarker for metal exposure. However, it is recommended that additional factors such as gender, size and seasons should also be taken into account, as it can all play a role in MT induction.

Acknowledgments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for financial support and infrastructure.

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Supplementary Material

Table S1: Values of certified reference material with concentrations measured for both the sediment and water samples (µg/ml).

	Sediment			Water		
	Reference	**Measured**	**% Recovery**	**Reference**	**Measured**	**% Recovery**
Cr	35	35.1	100.4	0.2	0.22	109.5
Mn	1400	1536.1	109.7	0.1	0.11	107
Co	8.8	9.6	109.5	0.1	0.11	114
Ni	12.8	13.4	104.6	0.2	0.21	105
Cu	1230	1326.6	107.9	0.2	0.21	105.75
Zn	498	525.1	105.4	1	1.06	106.3
As	115	121.9	106.0	0.4	0.42	104.75
Cd	4	4.3	107.8	0.1	0.11	108
Pb	285	309.9	108.7	0.4	0.40	101.2
U	7.8	7.6	97.2			
Ti	1510	1486.4	98.4	0.1	0.11	109
Al				0.4	0.44	108.75
Fe				1	1.06	106

Table S2: Families and functional feeding groups.

Family	Feeding Group
Notonectidae	Predator/Carnivore
Coenagrionidae	Predator/Carnivore
Libellulidae	Predator/Carnivore
Atyidae	Filter Feeder
Baetidae	Scraper/Sediment Associated
Caenidae	Collector/Sediment Associated
Chironomidae	Herbivore/Sediment Dependant

CHAPTER 7
Conclusions and Recommendations

The aim of this thesis was to determine the aquatic macroinvertebrate biodiversity and to establish the possible influence that selected physico-chemical stressors might have on this in the Marico River. During this study, some shortcomings were identified and a few recommendations can be made for future investigations. However, all of the objectives were achieved and it seemed to be largely effective in answering the research question.

Four hypotheses were generated and the outcomes were as follows:

1. *The selected physico-chemical variables in the water and sediment will be largely within the target quality ranges acceptable for aquatic ecosystems.*

Results revealed that water quality variables such as pH, EC and temperature were mainly within the tolerance ranges of macroinvertebrates. However, some metal concentrations in the water and nearly all metal concentrations in the sediment exceeded quality guideline values. Therefore, this first hypothesis can be rejected.

2. *The macroinvertebrate biodiversity and abundance will support the general perception that the Marico River is largely unimpacted.*

This hypothesis can be accepted as the high overall species richness and presence of sensitive species supported the general perception that the Marico River is relatively unimpacted.

3. *When compared to an impacted river in the same catchment area, the Marico River will exhibit a higher mollusc diversity.*

Twenty species were recovered from the Marico River, while only nine species were recovered from the Crocodile River. This hypothesis can thus be accepted.

4. *Metallothioneins as biomarker response will reflect metal exposure to macroinvertebrates in the Marico River.*

Metallothionein levels correlated positively with increased metal concentrations in the macroinvertebrates, which suggested that MTs may be used as a biomarker for metal exposure. This hypothesis can therefore also be accepted.

During this study, a total diversity of 187 taxa, which were represented by more than 18 900 specimens, was recorded. These included both tolerant and highly sensitive species. Physico-chemical variables such as pH, EC and temperature were mainly within the tolerance ranges of macroinvertebrates and results further suggested that biotope and food availability probably played a more decisive role in the occurrence of species than some of these environmental variables measured during the study. This means that when using macroinvertebrates as indicators, the biotopes and food availability at each sampling site should also be taken into account, and not only the environmental variables normally associated with water quality. The absence of sensitive species does thus not necessarily imply that the water is of low quality, but it could just mean that the specific biotope, preferred by the species, is not present or that there is a lack of food availability.

While some metal concentrations exceeded the target quality values, results suggested that these metals originated mainly from natural sources such as geological weathering. Metal concentrations in the water were also considerably lower than in the sediment, which may indicate that metals in the sediment were most probably not bioavailable. From the results it was clear that metals in the macroinvertebrates accumulated in various concentrations, depending on both their proximity to the sediment, as well as their feeding behaviour. However, the biodiversity compared favourably to similar studies in highly metal-contaminated river systems, suggesting that the metal concentrations most probably did not affect the community assemblages in a negative way. The fact that MT levels correlated positively with increased metal concentrations in the macroinvertebrates, suggests that MTs probably act as metal detoxifying proteins and that it may be used as biomarkers for metal exposure.

To conclude, the selected physico-chemical variables measured in the Marico River did not seem to have a detrimental impact on the macroinvertebrates. Both the high total biodiversity and high mollusc diversity compared favourably to similar studies on impacted rivers. These findings support the general perception that the Marico River, in view of biodiversity and the selected physico-chemical variables, is still largely unimpacted by anthropogenic disturbances.

"The Greatest Threat to Our Planet Is the Belief That Someone Else Will Save It"

Robert Swan

Recommendations

- The current investigation was successful in providing baseline data as reference for future studies. As concluded, the river is still, to a large extent, unimpacted, however, there are rumours of prospecting and mining of nickel in the area. It is thus recommended that surveys should be continued if any kind of development or impacts such as these were to be conducted, in order to monitor and manage possible environmental changes.

- It is also recommended to conduct surveys at other sites in the catchment such as the Molemane and Molopo Springs, to determine the biodiversity and possible impacts on these areas.

- Point sources of pollution in the town of Klein Marico should be investigated, in order to manage the water health of the Klein Marico River, as it may impact on the Marico River after their confluence.

- Regarding the MTs, it is known that various factors may influence their induction and it is recommended that additional factors such as gender, size of the organisms, as well as the effect of seasons should also be taken into account.

ADDENDUM A

Co-authors Letters of Consent

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7 November 2016

PhD THESIS – M KEMP
PERMISSION TO INCLUDE MANUSCRIPTS FOR EXAMINATION PURPOSES

As supervisor and co-author of four manuscripts first authored by Mrs Kemp, I hereby approve that the manuscripts listed below be included as part of the requirements for fulfilment of the PhD degree, and that these manuscripts be submitted for examination of Mrs Kemps' thesis.

The four manuscripts are as follows:

Manuscript 1
Selected physico-chemical characteristics potentially toxic to aquatic macroinvertebrates in water and sediment of a relatively unimpacted river, South Africa.
In submission to *Environmental Toxicology and Chemistry*

Manuscript 2
Influence of biotope associations and selected environmental variables on aquatic macroinvertebrate distribution in a pristine perennial Afro-tropical river system.
In submission to *Limnologica*

Manuscript 3
A comparison of the mollusc diversity between the relatively pristine Marico River and the impacted Crocodile River, two major tributaries of the Limpopo River.
Published in *Water SA*, 2016, *42*(2), pp.253-260

Manuscript 4
Metallothioneins and metal bioaccumulation in aquatic macroinvertebrates from a relatively unimpacted river system in South Africa.
In submission to *Bulletin of Environmental Contamination and Toxicology*

A letter of confirmation of submission/acceptance from the respective journal editorial offices for each of the above manuscripts is included in the thesis.

Sincerely,

K. N. de Kock

Professor K.N. de Kock

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4 November 2016

PhD THESIS – M KEMP
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Sincerely,

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A letter of confirmation of submission/acceptance from the respective journal editorial offices for each of the above manuscripts is included in the thesis.

Sincerely,

Prof C.T. Wolmarans

ADDENDUM B

Confirmation of Article Submissions

Documents of correspondence have been formatted without changing the contents, to fit the style of this thesis.

Manuscript 1 (Chapter 3)

Wednesday - October 19, 2016

Dear Mrs. Kemp:

Your manuscript entitled "Selected physico-chemical characteristics potentially toxic to aquatic macroinvertebrates in water and sediment of a relatively unimpacted river, South Africa." by Kemp, Mathilde; Wolmarans, Cornelius; de Kock, Kenne; Wepener, Victor, has been successfully submitted online and is presently being given full consideration for publication in Environmental Toxicology and Chemistry.

Your **manuscript ID is ETCJ-Oct-16-00737**. Please mention this in all future correspondence to the office for questions.

You can view the status of your manuscript at any time by checking your Author Center after logging in to https://mc.manuscriptcentral.com/etc.

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We appreciate your submission to Environmental Toxicology and Chemistry. Thank you.

Sincerely,

Environmental Toxicology and Chemistry Editorial Office

Manuscript 2 (Chapter 4)

Tuesday - August 23, 2016

Ref: LIMNO_2016_67

Title: Influence of biotope associations and selected environmental variables on aquatic macroinvertebrate distribution in a pristine perennial Afro-tropical river system

Journal: Limnologica

Dear Mrs. Kemp,

Thank you for submitting your manuscript for consideration for publication in Limnologica. Your submission was received in good order.

To track the status of your manuscript, please log into EVISE® at: http://www.evise.com/evise/faces/pages/navigation/NavController.jspx?JRNL_ACR=LIMNO and locate your submission under the header 'My Submissions with Journal' on your 'My Author Tasks' view.

Thank you for submitting your work to this journal.

Kind regards,

Limnologica

Wednesday - September 7, 2016

Reference: LIMNO_2016_67

Title: Influence of biotope associations and selected environmental variables on aquatic macroinvertebrate distribution in a pristine perennial Afro-tropical river system

Journal: Limnologica

Dear Mrs. Kemp,

I am currently identifying and contacting reviewers who are acknowledged experts in the field. Since peer review is a voluntary service it can take time to find reviewers who are both qualified and available. While reviewers are being contacted, the status of your manuscript will appear in EVISE® as 'Reviewer Invited'.

Once a reviewer agrees to review your manuscript, the status will change to 'Under Review'. When I have received the required number of expert reviews, the status will change to 'Ready for Decision' while I evaluate the reviews before making a decision on your manuscript.

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http://www.evise.com/evise/faces/pages/navigation/NavController.jspx?JRNL_ACR=LIMNO

Kind regards,

Limnologica

Manuscript 3 (Chapter 5)

Tuesday - February 17, 2015

Dear Mrs Kemp

Thank you for submitting this paper for possible publication in *Water SA*. The paper has been accepted for review, and has been assigned a **reference number of 3184**. Please make a note of this reference number and quote it in future enquiries. Please ensure that you have complied with the submission requirements below:

SUBMISSION CHECKLIST

- This is the authors' own, original work and has not been, nor will be, submitted for publication elsewhere during the period of review.
- All authors have agreed to the submission and their e-mail addresses are provided in the letter of submission.
- The total length of the manuscript does not exceed 8 000 words in the case of a research paper or rapid communication, or 4 000 words in the case of a short communication or technical note.
- Four reviewers have been recommended and their contact details (e-mail) provided.

Please note: By submitting a manuscript to *Water SA* authors transfer copyright to the journal, for the full term thereof throughout the world, subject to acceptance of the manuscript for publication in *Water SA*.

Kind regards

Tamsyn Sherwill

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Editor: *Water SA*
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Wednesday - December 2, 2015

Dear Ms Kemp

Your above-referenced paper has been reviewed now and I am pleased to inform you that it has been accepted for publication in *Water SA*, subject to minor revision. I herewith attach the review report. Please can you consider all of the comments made and let me have your response*, along with a suitably revised version of the paper. Please also pay attention to the required *Water SA* style and format in your revision of the paper. It is the author's responsibility to check that all references cited are listed in the reference list, and vice versa.

All co-authors must agree to the submission of the revised manuscript. Evidence of their agreement must be included when submitting the revised manuscript and all co-authors must be copied on the e-mail used to submit the revised manuscript to *Water SA*.

*The 'response' must be in the form of a document (e.g. using the review report and inserting comments on this) explaining how you have addressed (or, alternatively, motivating why you have chosen not to address) each of the reviewers' comments (other than those referring to minor typographical or grammar corrections).

I look forward to hearing from you.

Kind regards

Tamsyn Sherwill

Manuscript 4 (Chapter 6)

Thursday - November 3, 2016

Dear Mrs Kemp:

Thank you for submitting your manuscript, "Metallothioneins and metal bioaccumulation in aquatic macroinvertebrates from a relatively unimpacted river system in South Africa.", to Bulletin of Environmental Contamination and Toxicology.

The submission id is: BECT-D-16-01300

Please refer to this number in any future correspondence.

Your manuscript will be sent for review shortly. During the review process, you can track your manuscript by accessing the following web site:

http://bect.edmgr.com/

Your username is: hildekemp1

Your password is: available at this link
http://bect.edmgr.com/Default.aspx?pg=accountFinder.aspx&firstname=Mathilde&lastname=Kemp&email_address=hilde.kemp@nwu.ac.za

With kind regards,

The Editorial Office

Bulletin of Environmental Contamination and Toxicology

ADDENDUM C

Published PDF Version and Guide to Authors of Manuscript 4 (Chapter 5)

A comparison of mollusc diversity between the relatively pristine Marico River and the impacted Crocodile River, two major tributaries of the Limpopo River, South Africa

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ABSTRACT

A study of the freshwater mollusc diversity was conducted at selected sites in the relatively pristine Marico River and the impacted Crocodile River, the major tributaries of the Limpopo River. Four surveys were conducted, two in an early (May 2013 and 2014) and two in a late (November 2013 and 2014) low-flow period. Semi-quantitative surveys were done by sampling the vegetation, as well as the substratum, with a standard SASS net for approximately 15 min each. Environmental parameters including water temperature, electrical conductivity (EC) and pH were measured at each site. Molluscs were identified up to species level, sorted, counted, and the presence of juvenile specimens recorded. Historical data for the 1/16th degree square grids (loci), in which each of the sampling sites of the current study was located, were extracted from the National Freshwater Snail Collection at the Unit for Environmental Sciences and Management, Potchefstroom Campus of the North-West University. During this study, 20 and 9 species were recovered from the Marico and Crocodile Rivers, respectively, as compared to 13 and 12 species on record for these loci, respectively. Juvenile specimens were present during the four surveys at most of the sites. Canonical correspondence analyses were applied which revealed that biotopes, water temperature and EC played the most significant role in the distribution and abundance of species. The relatively high mollusc diversity and the fact that juveniles were present throughout the study, demonstrated that current habitat and environmental conditions were suitable to promote recruitment and the sustainability of diverse mollusc populations in the Marico River and its tributaries. However, in contrast to this, the exploitation of and habitat transformation in the Crocodile River has resulted in the decrease of biotopes which eventually could have led to the decrease in diversity and the establishment of *P. acuta*, an exotic invader species.

Keywords: freshwater molluscs, biodiversity, Marico River, Crocodile River, environmental variables

INTRODUCTION

Freshwater molluscs are the second most diverse animal phylum on earth (Lydeard et al., 2004). However, they are poorly documented, extremely threatened and have the highest number of recorded extinctions of any major taxonomic group (Lydeard et al., 2004; Vaughn and Taylor, 1999). The decline in freshwater molluscs goes unnoticed due to poor documentation and monitoring, as only a fragment of molluscan species (<2%) have had their conservation status accurately assessed (Lydeard et al., 2004; Reckendorfer et al., 2006).

Four hundred species of freshwater molluscs have been previously identified in Africa (Brown, 1994) and, according to De Kock and Wolmarans (1998), the distribution of these molluscs is primarily influenced by the availability of suitable aquatic biotopes. The association of different freshwater molluscs with specific biotopes, such as marginal and aquatic vegetation and sediment present at different water velocities and depths, is well known and documented (Brown, 1978; Brown, 1994; Dallas, 2007; Odume et al., 2015). These biotopes are under constant threat worldwide as a consequence of various anthropogenic impacts, geomorphological alterations and the changing availability of water (Wittmann, 1975; Vaughn and Taylor, 1999; Reckendorfer et al., 2006; Taylor et al. 2007; De Klerk et al., 2012; DWA, 2012).

With regard to South Africa, nearly 71% of the main rivers are considered as either endangered or critically endangered as the result of exploitation (Nel et al., 2004). The Crocodile River, which is characterised by sprawling urbanization and industrialization in the region of northern Johannesburg and Pretoria, is also under severe threat of pollution due to extensive agricultural activities, the sporadic release of water from impoundments such as the Hartbeespoort Dam, and large mining developments north of the Magaliesberg (Wittmann, 1975; Taylor et al., 2007; De Klerk et al., 2012; DWA, 2012). All of these stressors could have a detrimental effect on the aquatic ecosystems and biotope availability. In contrast, the general state of the Marico River is considered to be 'natural' to 'good', free of significant organic pollution, with a pronounced biodiversity and overall good water quality (RHP, 2005). However, the water quality of two of its major tributaries, namely, the Klein Marico River and the Sterkstroom, is defined as 'fair' to 'poor' (RHP, 2005).

Although mollusc surveys have previously been done in the Marico and Crocodile River catchment areas, (National Freshwater Snail Collection Database, North-West University, Potchefstroom Campus), limited information regarding diversity has previously been published. Furthermore, in view of the fact that the Marico River is still considered to be relatively pristine, while the Crocodile River has been subjected to ongoing exploitation and habitat transformation, it is of great importance to survey these areas in order to establish the current status of the mollusc diversity for conservation purposes.

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Study area

The Marico River stretches from its source, the Marico Eye near Swartruggens, for 250 km until its confluence with the Crocodile River to form the Limpopo River. The Crocodile River, originating at Roodepoort, meanders via Hartbeespoort Dam past Thabazimbi to the Botswana border where it joins the Marico River. Nine sites in the Marico River and seven sites in the Crocodile River catchments were selected for sampling. Site selection was influenced by logistical accessibility to the rivers, availability of water and suitable biotopes (Dickens and Graham, 2002; Gerber and Gabriel, 2002; Dallas, 2007). Locations of these sites are depicted in Fig. 1 while site descriptions and aquatic biotopes are displayed in Table 1.

Sampling equipment and techniques

Four surveys were conducted, two in an early (May 2013 and 2014) and two in a late (November 2013 and 2014) low-flow period. The coordinates of each sampling site were determined with a Garmin Nuvi 500 GPS.

Semi-quantitative surveys were done by sampling the vegetation, as well as the substratum, with a standard SASS net (Dickens and Graham, 2002) for approximately 15 min each. Molluscs were collected along the margins of the rivers up to a depth of 70 cm. Aquatic vegetation and marginal vegetation were sampled by pushing the net vigorously into the vegetation and moving it backwards and forwards through the same area. Substrata of biotopes consisting mainly of mud, sand, gravel or stones were sampled by shuffling the feet whilst continuously

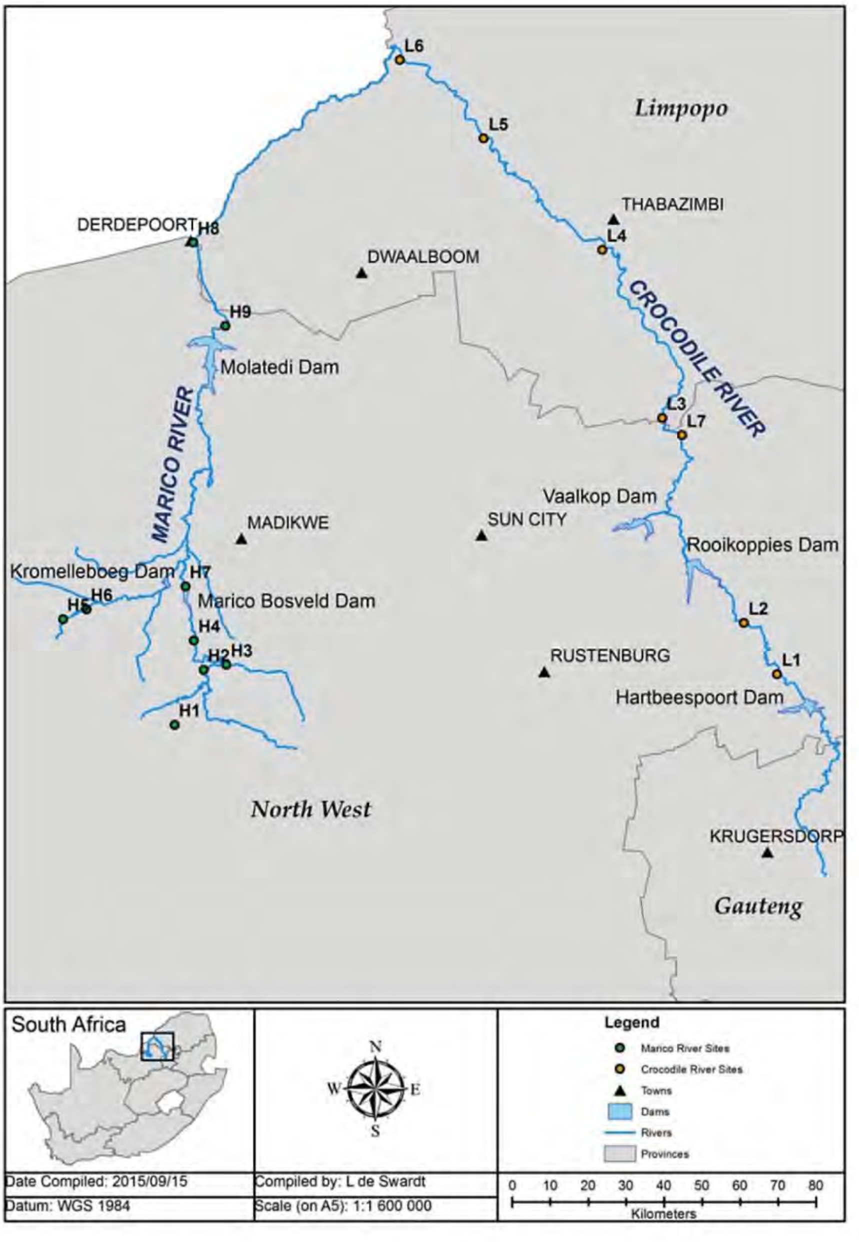

Figure 1
Map of the study area

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TABLE 1 Site location, river name and aquatic biotopes present at each site		
Site, coordinates and altitude	**River**	**Aquatic biotopes (according to Gerber and Gabriel, 2002)**
Marico River		
Site H1 S 25°47′32.1 E 26°21′54.1 1 480 m	Marico Eye, source of Marico River	Abundant marginal and aquatic vegetation, pool and backwaters with stones
Site H2 S 25°39′45.1 E 26°26′01.9 1 197 m	Marico River before its confluence with Sterkstroom, 20 km downstream of the Eye	Little to no marginal vegetation, stones in current, stones out of current, riffle, run and pool.
Site H3 S 25°39′00.6 E 26°29′16.3 1 170 m	Sterkstroom 5 km before its confluence with Marico River	Little to no vegetation, bedrock, stones in current, riffle, run and pool
Site H4 S 25°35′33.4 E 26°24′39.4 1 077 m	Marico River 10 km above Marico-Bosveld Dam, after its confluence with Sterkstroom	Muddy and sandy substratum, filamentous algae, abundant marginal and aquatic vegetation, stones in current, stones out of current, riffle, run and pool
Site H5 S 25°32′31.4 E 26°06′17.3 1 150 m	Klein Marico River 5 km above Klein-Maricopoort (Bospoort) Dam	Little to no marginal and aquatic vegetation, filamentous algae, muddy substratum, riffle and run
Site H6 S 25°31′09.2 E26°09′25.1 1 135 m	Klein Marico River 1 km below Klein-Maricopoort (Bospoort) Dam	Little to no marginal and aquatic vegetation, pool with muddy substratum stones in current, riffle and run
Site H7 S 25°27′52.6 E 26°23′26.9 1 037 m	Marico River directly below Marico-Bosveld Dam	Marginal and aquatic vegetation, filamentous algae and bedrock
Site H8 S 24°39′15.9 E 26°24′28.7 914 m	Marico River at Derdepoort	Abundant marginal and aquatic vegetation, backwater with muddy and sandy substratum, filamentous algae
Site H9 S 24°50′54.2 E 26°29′07.1 926 m	Marico River 2 km below Molatedi Dam	Run with muddy substratum, bedrock, stones in current, stones out of current, riffle and pool
Crocodile River		
Site L1 S 25°40′22.1 E 27°47′25 1 104 m	Crocodile River at Brits	Marginal and aquatic vegetation, filamentous algae, riffle and run with sand and bedrock substratum, stones in current
Site L2 S 25°33′02.9 E 27°42′40.9 1 055 m	Crocodile River downstream of Brits	Run and pool with sand substratum and filamentous algae
Site L3 S 25°03′59.6 E 27°31′06.2 936 m	Crocodile River at Koedoeskop Bridge	Run and pool with muddy substratum and filamentous algae
Site L4 S 24°40′05.8 E 27°22′35.2 904 m	Crocodile River at Thabazimbi	Run and pool with sand substratum and filamentous algae
Site L5 S 24°24′16.8 E 27°05′46.7 905 m	Crocodile River downstream of Thabazimbi	Run and pool, sand substratum and filamentous algae
Site L6 S 24°13′07 E 26°53′51.6 836 m	Crocodile River at Rooibokkraal	Run and pool with sand substratum, sparse marginal and aquatic vegetation, filamentous algae
Site L S 25°06′24.7 E 27°33′55.2 956 m	Pienaars River	Run and pool with mud and clay substratum, stones in current

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sweeping the net over the disturbed area. Contents of the net were then transferred to a rectangular plastic container (360 × 470 × 80 mm) which was filled with water from the habitat to a level of approximately 40 mm. Most of the debris was then carefully removed by hand and scrutinised for specimens that might still be attached. Thereafter the contents of the container were decanted into a cone-shaped Perlon gauze net (0.25 mm mesh) suspended on a stand. The content of the net was transferred to a plastic container with a tight-fitting lid and 90% ethanol was added to preserve the samples. The container was then labelled with relevant site information. The samples were transported to the laboratory where each sample was decanted into a rectangular Perspex sorting tray (300 × 200 × 25 mm) with a transparent bottom provided with a 10 mm square grid. All molluscs were subsequently removed under a stereomicroscope mounted on a sliding stand and identified up to species level (Walker, 1923; Connolly, 1939; Brown, 1994; Appleton, 2002). To obtain an indication of recruitment, the presence of juvenile specimens was also recorded.

Historical data

Historical data (1958 to 1984), for the 1/16th degree square grids (loci) in which each of the sampling sites of the current study was located, were extracted from the National Freshwater Snail Collection (NFSC). These data represent species collected in both lotic and lentic waterbodies.

Environmental parameters

Electrical conductivity, pH and water temperature were measured in situ at all sites during each survey using a portable digital thermometer (Checktemp, Hanna Instruments), portable digital conductivity meter (DIST 3, HI 98303, Hanna Instruments) and portable digital pH probe (HI 98128, Hanna Instruments).

Statistical analyses

Canonical correspondence analyses (CCAs) were conducted using CANOCO 5 software, to investigate the distribution and abundance of mollusc species between sites and surveys in relation to the influence of selected environmental variables (water temperature, pH, electrical conductivity (EC) and biotope availability).

RESULTS

The location of each site is depicted in Fig. 1 and site coordinates, river names and biotopes present at each site are given in Table 1. Mollusc diversity, abundance and species richness (SR), and selected environmental parameters for each of the four surveys, are displayed in Tables 2 and 3. The molluscs on record in the NFSC database which were previously collected from the same loci (not necessarily from the same sites), as well as those species recovered during the present study, are presented in Table 4. Canonical correspondence analyses (CCAs), illustrating associations between species, sampling sites during four surveys, environmental variables and biotope availability, are depicted in Figs 2 and 3.

A total number of 20 species were recovered from the Marico River and tributaries (Table 2). The highest species diversity in the Marico River (SR=12) was recorded for both Sites H4 and H8, while the highest total number of species per survey (9) was also found at these two sites (Table 2). The most common and abundant species were *Burnupia trapezoidea*, *Lymnaea natalensis* and *Pisidium langleyanum* (Table 2). The lowest species richness was found at Sites H5 and H9, with a total number of 4 and 2 species, respectively. Only one specimen of *Galba truncatula*, *Helisoma duryi* and *Unio caffra* was found during the study. Seven species not previously on record for this area were recorded during the present study. However, *Bulinus tropicus*, which was previously recorded in the area (Table 4), was not found. Water temperature and pH did not vary considerably between sites, but seasonal fluctuation in temperature between surveys was observed (Table 2). Electrical conductivity, however, was markedly higher during the third survey at Site H1 and during all the surveys at Site H5.

The length of the arrows in the CCA tri-plot (Fig. 2) indicates that algae, pools, stones in current and vegetation biotopes, as well as water temperature, played a significant role in the occurrence and distribution of species, while pH, EC and riffle-and-run biotopes had a minor influence. An association can be seen between *Ferrissia cawstoni*, *Bulinus forskalii*, Site H7 and algae. *G. truncatula* associated with Site H5, Survey 1, while *H. duryi* and *U. caffra* associated with Site H8, Survey 2 and Site H8, Survey 3, respectively (Fig. 2).

Regarding the Crocodile River, a total number of 9 species was recovered during the study (Table 2). The highest species diversity (SR = 7) was recorded for Site L4, while the highest total number of species per survey (6), was also found at this site. The most common and abundant species were *B. trapezoidea*, *Corbicula fluminalis africana*, *Melanoides tuberculata* and *Physa acuta*, while the lowest species richness was found at Site L6 (SR=4) (Table 2). Only one specimen of *Gyraulus costulatus* was found during the study. Five species previously on record for this area were not found (Table 4); however, *Ceratophallus natalensis* and *Gyraulus connollyi*, which were not yet on record for this area, were recovered during the present study. Water temperature, pH and EC did not vary considerably between sites, but seasonal temperature fluctuation between surveys was observed (Table 3).

The length of the arrows in the CCA tri-plot (Fig. 3) indicates that EC, algae, riffle and stones in current biotopes played a significant role on the occurrence and distribution of species, while pH, run and sand biotopes had a lesser, and temperature and vegetation an even smaller, influence. An association can be seen between *C. fluminalis africana*, Site L6 and EC. *Melanoides tuberculata* associated with Site L5 and the presence of algae, while *G. connollyi* associated with Site L1 and a riffle biotope.

DISCUSSION

From historical data in the database of the NFSC it is clear that the Marico and Crocodile River areas had a similar molluscan diversity, with only one more species recorded from the Marico River than the Crocodile (Table 4). The results of the current study, however, show a dramatic change in this similarity. The number of species found in the Marico River area increased from 13 to 20 species, while the number of species in the Crocodile River area decreased from 12 to 9 species.

Of the 13 species on record for the Marico River area, only *B. tropicus* was not recovered during the present study, probably due to the fact that this snail is primarily found in lentic waterbodies such as dams and lakes and various ephemeral habitats, which were included in the previous surveys (De Kock et al., 2002). Of the 12 species on record for the Crocodile River

Table 2
Mollusc species collected, species richness and abundances during Surveys 1 to 4 in the Marico River, as well as environmental parameters determined at the time of collection

Site	H1				H2				H3				H4				H5				H6				H7				H8				H9			
Mollusc species	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
B. pfeifferi		1																											4			4				
B. africanus					1									1														5	6	16	4	35				
B. forskalii																			1									1	1	1	1	1				
B. trapezoidea		1	1	1		2	14	26		2	7	23	6	2		4			1	1							7		1							
C. fluminalis africana													17	4	3						12	42	8	1			2		8	5	4	21				
G. truncatula																	2																	3	1	1
G. connollyi		4	1	1	6					1		3	84	58							1				4	1										
G. costulatus													73	14							9						22	39	3	2	5					
L. natalensis		5	1	1	5	2				1			172	36	1	1									2			5	4	6		33				
M. tuberculata													7	65	4	1														1						
M. victoriae															1						2				3	5	3	26	22	29	7	77	2	2		
**P. acuta*													1	9		1													5	52	3	233				
P. costulosum					7	4				14	1		2	4							64	86	6	4				1		1						
P. langleyanum		2	1		25	15	1			12	1	1	8	7	5		5						1	3												
P. viridarium										7	1	1															2	18								
**P. columella*																1																1				
Total per site/survey	0	5	4	3	5	4	2	1	0	6	4	4	9	9	4	5	2	0	2	1	5	2	3	3	3	2	5	7	9	9	6	8	1	2	1	1
Species richness	5				6				6				12				4				6				10				12				2			
Temp (°C)	22	21	18	18	16	23	13	19	12	22	15	19	17	24	15	21	11	22.7	13	19	18.6	21.5	20	22.2	19.4	25.6	17.3	23.2	13.5	23.7	16.1	24.1	18.3	30.6	15.9	24.4
pH	7.2	7.9	6.8	6.7	7.4	8	7.5	6.8	6	7.8	6.6	7.1	8.1	8.2	7.3	6.8	7.9	8.2	7.7	7.8	514	501	574	622	613	529	608	732	651	760	621	781	601	652	786	904
EC (µS/cm)	282	253	630	309	295	282	263	290	120	97	96	108	302	308	241	260	773	1103	918	667	8.3	7.12	8.04	8.24	8.64	7.59	7.91	8.41	8.46	9.77	7.91	8.55	9.33	8.95	7.86	8.23

**Alien species*

Table 3
Mollusc species collected, species richness and abundances during Surveys 1 to 4 in the Crocodile River, as well as environmental parameters determined at the time of collection

Site	L1				L2				L3				L4				L5				L6				L7			
Mollusc species	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
B. trapezoidea	40	8	79	127	15	9		5					10	6	10	2	1	1	14	3		1			10	7	9	8
C. fluminalis africana			6											6														
C. natalensis					3			1				52	39	28	25	32	4	28	9	25	1	48	25	20			3	5
G. connollyi		1	2	3	2																							
G. costulatus									1																			
L. natalensis	1								20				4	30				9							32	21	4	
M. tuberculata	2		14	15	1			3		1				8	1	1	280	336	138	15	7							
**P. acuta*	3	112	9	862	23	131		145	35	410		3	83	403	12	24	42	42	13		1	3			131	144		6
**P. columella*										1						1												1
Total spp. per site/survey	4	3	5	4	5	2	0	4	3	3	0	2	4	6	4	5	4	5	4	3	3	3	1	1	3	3	3	4
Species richness	6				5				5				7				5				4							
Temp (°C)	18.6	21.5	20	22.2	19.4	25.6	17.3	23.2	13.5	23.7	16.1	24.1	18.3	30.6	15.9	24.4	18	28.6	18	28.7	15.6	32.5	20.4	27.2	15.9	26.7	15.7	26.7
EC (µS/cm)	514	501	574	622	613	529	608	732	651	760	621	781	601	652	786	904	612	630	843	888	628	773	753	877	682	819	544	714
pH	8.3	7.12	8.04	8.24	8.64	7.59	7.91	8.41	8.46	9.77	7.91	8.55	9.33	8.95	7.86	8.23	8.54	8.62	8.5	8.81	8.36	9.16	8.48	8.48	8.97	8.94	8.01	8.71

**Alien Species*

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Table 4
Mollusc species on record in the National Freshwater Snail Collection (NFSC) from 1958 to 1984 and species collected during the 2013 and 2014 study

Mollusc species	Marico River and Catchment		Crocodile River and Catchment	
	1958–1984	2013–2014	1958–1984	2013–2014
B. pfeifferi	✗	✗	✗	
B. africanus	✗	✗	✗	
B. depressus	✗	✗	✗	
B. forskalii	✗	✗	✗	
B. tropicus	✗		✗	
B. trapezoidea	✗	✗	✗	✗
C. natalensis				✗
C. fluminalis africana	✗	✗	✗	✗
F. cawstoni		✗		
G. truncatula		✗		
G. costulatus	✗	✗	✗	✗
G. connollyi	✗	✗		✗
**H. duryi*		✗		
L. natalensis	✗	✗	✗	✗
M. tuberculata	✗	✗	✗	✗
M. victoriae		✗		
**P. acuta*		✗	✗	✗
P. costulosum		✗		
P. langleyanum		✗		
P. viridarium		✗		
**P. columella*	✗	✗	✗	✗
U. caffra	✗	✗		

**Alien species*

area, only 7 were collected during this study, while 2 species, *C. natalensis* and *G. connollyi* were recorded for the first time. Of the 21 species recovered during this study, only 7 were found in both the Marico and Crocodile Rivers. Of these 7 species 6 can be considered as generalists which are able to utilise both sediment and vegetation biotopes, while the 7th species, *C. fluminalis africana*, is a bottom dweller and not dependent on vegetation biotopes (De Kock and Wolmarans, 2007a).

The importance of the effect of biotope availability on biodiversity (Dallas, 2007) is supported by the high species richness (SR = 12) found at Sites H4 and H8 in the Marico River, where a large selection of biotopes was present (Table 1). The low species richness (SR = 2) found at Site H9 was possibly due to the fact that this site was situated directly downstream from the Molatedi Dam where the constant release of large volumes of water every 4 to 6 weeks (RHP, 2005) possibly resulted in gross disturbance and transformation of the aquatic biotopes, as reflected in the absence of marginal and aquatic vegetation (Table 1). Furthermore, this site was also negatively impacted by the clearing of riparian vegetation and grazing by cattle from a nearby informal settlement, as observed during the surveys. With regard to Site H5, located close to Zeerust, the low mollusc diversity (SR = 4) could be ascribed to the lack of suitable biotopes and possible negative impacts from the nearby industrial and urban activities (RHP, 2005), as obvious signs of littering and eutrophication were observed. The fact that *B. trapezoidea*, *L. natalensis and P. langleyanum* were the most commonly occurring species in the Marico River and tributaries, could be attributed to their wide distribution in the area, as previously recorded (NFSC database; De Kock et al., 2001; De Kock and Wolmarans, 2008). The single specimen of *M. victoriae* collected at Site H4 was unexpected, as this locality is well beyond the distribution range previously reported by De Kock and Wolmarans (2009).

The ordination in the CCA tri-plot (Fig. 2) shows that, regarding the environmental variables, pH and EC did not play a significant role in species occurrence and distribution, but water temperature and the presence or absence of different biotopes did play a significant role. The fact that water temperature played such a significant role in the distribution of mollusc species, is in accordance with the results of similar studies in Africa (Tchakonte et al., 2014; Abd El-Wakeil et al., 2015). Water temperature is further considered as one of the major factors influencing reproduction of molluscs (De Kock and Van Eeden, 1982). However, the lower temperature during the May surveys and the higher temperature during the November surveys did not prevent recruitment, as juvenile specimens of different generations of the majority of species were present at most of the sampling sites.

The association of *G. truncatula* with Site H5 and *H. duryi* and *U. caffra* with Site H8, is most probably due to the fact that these species were found only once and in single numbers at these sites.

A comparison between the historical data and the results of the current study is not really feasible as the historic data have been collected by many different people over several decades. However, it is still clear that a higher diversity is currently found in the Marico River, possibly due to the fact that previous surveys did not concentrate on the Marico River *per se*, as well as the fact that this river has not been seriously impacted by anthropogenic activities over the past three decades (RHP, 2005).

Of the 9 species found in the Crocodile River, the occurrence of several of these, namely *C. fluminalis africana*, *B. trapezoidea*, *G. costulatus*, *G. connollyi* and *C. natalensis*, could be expected, as they are widely distributed in South Africa (Database of the NFSC; De Kock and Wolmarans, 2004; 2006; 2007a; b) and predominantly associate with sediment biotopes that were available at almost all of the sites. However, the limited availability of marginal and aquatic vegetation at most of the sites did not prevent the occurrence of *P. acuta*, *L. natalensis* and *P. columella*, which are generalists in respect of their biotope preferences (De Kock et al., 1989; Brown, 1994; De Kock et al., 2001). As in the case of the Marico River, the ordination in the CCA tri-plot (Fig. 3) shows that biotope availability played a significant role in species occurrence and distribution. In the Crocodile River, however, EC was also an important factor in this respect and these results are in agreement with the conclusions of Tchakonte et al. (2014) and Abd El-Wakeil et al. (2015). The presence of filamentous algae, an indication of organic enrichment, and the considerably higher recorded EC values at most of the sites throughout the study, could be attributed to the anthropogenic stressors mentioned above.

Three alien species, namely *H. duryi*, *P. acuta*, and *P. columella*, were found during this study. Of these, *P. columella* is on record as the third most widespread freshwater snail species in South Africa (De Kock and Wolmarans, 2008). However, during this study it was found only in a few sites and also in limited numbers in both the Marico River (Sites H4 and H7) and the Crocodile River (Sites

L3, L4 and L7). The fact that *P. acuta* was found at all the sites in the Crocodile River, often in high numbers, is not surprising as it has attributes such as a superior fecundity rate, as reported by Appleton and Brackenbury (1998), and the ability to migrate upstream (Appleton and Branch, 1989). Furthermore, this species can serve as an indicator of organic-enriched water, commonly occurs in areas where sewage pollution is obvious and, according to Brown (1994), seems to be closely associated with human activities. *Helisoma duryi*, of which only one specimen was found at Site H8, is widespread in artificial waterbodies but not invasive (Appleton, 2003). Of the 41 records of this species in the NFSC database reported by De Kock and Joubert (1991), only 15 were from natural habitats.

The relatively high mollusc diversity, and the fact that juveniles were present throughout the study, demonstrated that current habitat and environmental conditions were suitable to promote recruitment and the sustainability of diverse mollusc populations in the Marico River and its tributaries. However, in contrast to this, the exploitation of and habitat transformation in the Crocodile River resulted in the decrease of biotopes which eventually could have led to the decrease in diversity and the establishment of *P. acuta*, an exotic invader species.

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ADDENDUM D
Manuscript A

Metal concentrations in the water and sediment of a pristine river system in the North-West Province of South Africa.

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This study was undertaken to determine the metal composition in the Groot Marico catchment. Eight sites were selected in the Marico River, Klein Marico River and Sterkstroom, where water and sediment samples were collected for metal analysis. Selected physico-chemical parameters were measured *in situ*. With the exception of the pH at Site H3, all the physico-chemical parameters recorded, were within the DWAF Target Water Quality Ranges and only a few of the metal concentrations (P, Cu and Pb) exceeded the guideline values for freshwater systems. From the results, it appears that the water of the tributaries was not impacted to such an extent that it had any substantial effect on the water of the Groot Marico River. The elevated levels of metals were possibly due to natural geological weathering and thus poses little to no threat for the ecosystems in this study area.

Keywords: Metal concentration, water, sediment, river

1. Introduction

In South Africa, nearly 71% of the main rivers are considered as either endangered or critically endangered, mainly due to anthropogenic activities such as mining and industrialisation, contaminating these waterbodies. It is thus of great importance to conserve the few remaining so-called pristine rivers such as the Marico River, a tributary of the Limpopo River and one of the main rivers in the North-West Province.

No information is currently available regarding the metal composition of this river system. This study aims to contribute in closing the knowledge gap regarding the metal concentrations in the catchment, as well as the possible influence of tributaries by sharing the data obtained from two seasonal surveys.

2. Materials and Methods

The study was conducted in the North-West and Limpopo Provinces, at eight samplings sites in the Marico River and selected tributaries (Klein Marico River and Sterkstroom) (Figure 1).

Sites were selected based on the importance and possible impacts of the tributaries on the Marico River. Site H1 is the dolomitic Marico Eye, the source of the Marico River. Sites H1 and H2 are both situated in unimpacted areas. Sites H3 is in the Sterkstroom, described as moderately impacted. Sites H4 and H7 are in the Marico River upstream and downstream of the Marico Bosveld Dam while sites H5 and H6 are situated in the Klein Marico River, upstream and downstream of the Klein Maricopoort Dam, respectively. This tributary is described as being in a fair to poor state and is contaminated by effluent from the town of Zeerust. Site H8 is situated in the Marico River at 3de Poort near the Botswana border.

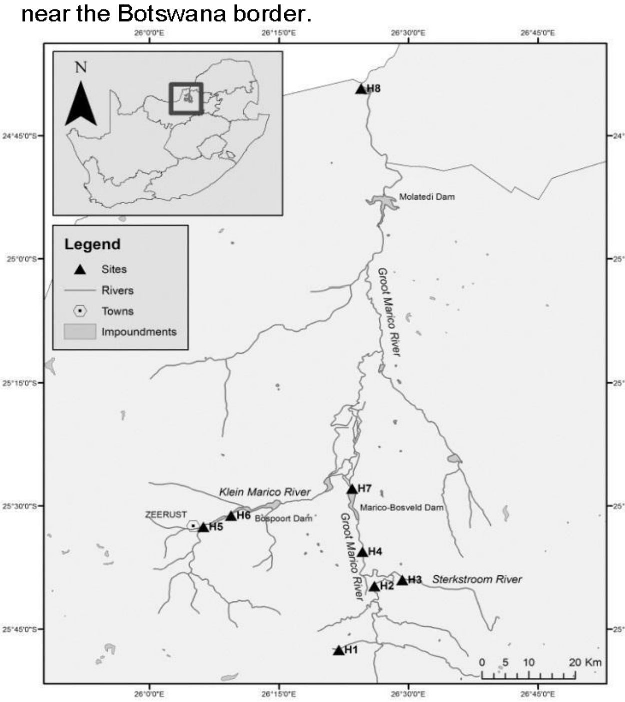

Figure 1: Sampling sites (triangles) within the Marico River catchment, North-West, South Africa.

Two surveys were conducted, one during a high-flow period (April 2013) and one during a low-flow period (June 2013). Water and sediment samples were collected separately in polyethylene bottles, from all the sites. Physico-chemical parameters including water temperature (Temp), electrical conductivity (EC) and pH were measured *in situ* at all the sites during each survey with portable digital instruments. Water samples were filtered and the filtrate was used directly for a baseline analysis in an ICP-MS using standard recognized techniques. Sediment analyses were carried out after air drying the samples at 70°C for 48 hours before the EPA Method (3050B) was used to digest the samples for ICP-MS analyses.

The values were, where possible, compared to existing target water quality guidelines (TWQG) as established for South Africa (Holmes, 1996) and only the metals that exceeded these values will be discussed.

3. Results and discussion

A total number of 35 metals were detected in both the water and sediment samples from all the sampling sites (data not shown). The fact that these metals were all present at both the anthropogenically impacted and unimpacted sites, suggests that it is most probably from a geological origin.

When comparing and evaluating these metal concentrations to the water quality guidelines, only three elements exceeded the guideline values. These include phosphorous (P), copper (Cu) and lead (Pb). As for the sediments, no guideline values have yet been established for South Africa.

The levels of P and Cu exceeded the TWQR at the majority of the sites during the high-flow season, possibly due to runoff surface water. In contrast to this, the increased levels of Pb at all the sites during the low-flow season may indicate a possible concentration effect. Copper, a common environmental metal, was the highest at Site H1, the Marico Eye, and Site H7 in the Marico River. The main sources of Pb in the aquatic environment are generally regarded as originating from anthropogenic impacts however, as in this study; it can also be released into the water through the weathering of sulphide ores. High P concentrations at most sites, possibly originates from phosphorous bearing rocks and the decomposition of organic matter. At Site H5 however, the elevated P concentrations during both the flow periods might be ascribed to the sewage water discharge from the town of Zeerust.

Although the pH values and EC varied between the different sites and the two flow periods, it was, with the exception of pH at Site H3, never above or below the target water quality ranges acceptable for aquatic ecosystems (Holmes, 1996). From the results, it appears that the water of the tributaries was not impacted to such an extent that it had any substantial effect on the water of the Groot Marico River**.** The dolomitic geology of the area is further responsible for a buffer capacity, explaining the relatively constant pH values and ultimately stable metal concentrations throughout the system.

The fact that elevated levels of metals were present at the source of the Marico River (Site H1), where no known impacts are described in literature, and at many sites downstream of the Eye, indicates that these metal concentrations were most likely from natural sources such as geological weathering and not from any specific anthropogenic impacts.

4. Acknowledments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for financial support and infrastructure.

5. References

Holmes, S., (ed) 1996, 'South African Water Quality Guidelines. Volume 7: Aquatic Ecosystems', Department of Water Affairs and Forestry. The Government Printer, Pretoria. 145p.

Table 1: Metal concentrations (µg/ℓ) and selected physico-chemical parameters recorded from each site, as well as the target water quality range* values.

Site	Flow	Temp (°C)	EC (µS/cm)	pH	P	Cu	Pb
H1	High	20.7	256	7.14	76.64	6.4	0.06888
Marico Eye	Low	21.8	282	7.2	1.224	0.1703	4.213
H2	High	22.4	310	7.7	81.15	0.951	0.07446
Marico River	Low	15.8	295	7.38	1.538	0.1968	3.977
H3	High	22.3	137	6.45	78.03	0.8948	0.06687
Sterkstroom	Low	11.9	120	6.04	0.9869	0.07734	6.259
H4	High	25.3	297	6.98	83.97	0.004728	0.07462
Marico River	Low	17.2	302	8.05	0.9115	0.1773	4.277
H5	High	23.4	951	8.29	554.7	0.001177	0.06606
Klein Marico River	Low	10.5	773	7.86	742.3	0.01733	4.912
H6	High	21.5	522	7.61	85.83	1.018	0.07154
Klein Marico River	Low	11.3	560	7.9	0.8468	0.1056	3.782
H7	High	25.5	271	7.17	85.56	4.507	0.07395
Marico River	Low	12.9	265	7.61	0.9116	0.2239	4.779
H8	High	24.7	436	8.7	81.76	0.9921	0.06197
Marico River	Low	17.7	456	8.52	0.6633	0.1661	5.909
TWQR* Values				6.5 - 9	5-250	<0.3	<0.5

ADDENDUM E

Manuscript B

Metal concentrations in selected macroinvertebrate families, water and sediment of a pristine river system in the North-West Province of South Africa.

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This study was undertaken to determine the metal concentrations in selected macroinvertebrate families, as well as in the water and sediment in the Groot Marico catchment. Eight sites were selected in the Marico River and tributaries where macroinvertebrates, water and sediment samples were collected for metal analyses. Seven families including Notonectidae, Coenagrionidae, Atyidae, Libellulidae, Baetidae, Caenidae and Chironomidae were analysed to determine their metal concentrations. A total of 35 metals were detected in the macroinvertebrates, water and sediment. Metal concentrations in the sediment were higher than those in the water and the slightly elevated levels of metals were probably due to natural geological weathering. Metal concentrations were the highest in specimens of Baetidae, Caenidae and Chironomidae, which is most probably due to the fact that these families live in close proximity to the sediments while the other families reside in the water, on the surface or on plants.

Keywords: Metal concentration, water, sediment, river, macroinvertebrates

Introduction

Aquatic macroinvertebrates can take up and accumulate both essential and non-essential trace elements in the water and sediment, and the concentrations of these metals show enormous variability across metals and invertebrate taxa. Excessive metal concentrations can have adverse effects on these organisms, however, invertebrates are able to react to, metabolize, and thus regulate, both essential and non-essential metals within their internal systems.

This study was conducted to determine the baseline metal concentrations in selected macroinvertebrate families, as well as in the water and sediment of a pristine river system.

Material and methods

The study was conducted in the North-West and Limpopo Provinces, at eight samplings sites in the Marico River and selected tributaries (Klein Marico River and Sterkstroom) (Figure 1).

Site H1 is the dolomitic Marico Eye, the source of the Marico River. Sites H1 and H2 are both situated in unimpacted areas. Sites H3 is in the Sterkstroom, described as moderately impacted. Sites H4 and H7 are in the Marico River upstream and downstream of the Marico Bosveld Dam while sites H5 and H6 are situated in the Klein Marico River, upstream and downstream of the Klein Maricopoort Dam, respectively. This tributary is described as being in a fair to poor state and is contaminated by effluent from the town of Zeerust. Site H8 is situated in the Marico River at 3de Poort near the Botswana border.

One survey was conducted during the low-flow period of 2013. Macroinvertebrates, water and sediment samples were collected from all the sites.

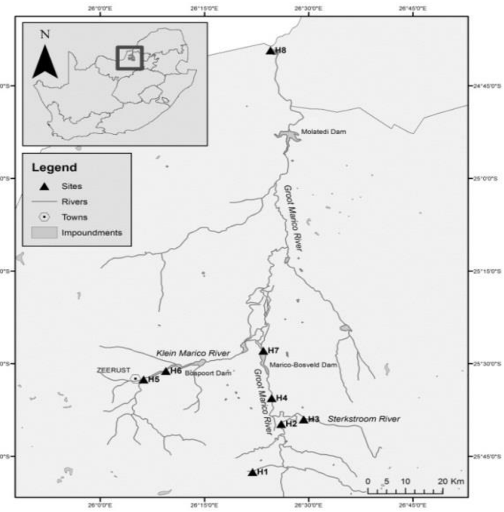

Figure 1: Sampling sites within the Marico River catchment, North-West, South Africa.

Macroinvertebrates were collected by sampling the marginal and aquatic vegetation, as well as the substratum, using a Perlon® gauze net. Samples were preserved with 70% ethanol and transported to the laboratory for identification and analyses.

Macroinvertebrates were identified up to family level. From each sample, three specimens per family were selected, weighed and placed in a multiple cell Teflon container with 1ml 65% nitric acid per cell. Samples were then digested under pressure at 70 °C for 24 hours. Digested samples were diluted with double distilled water and analysed with an ICP-MS.

Water samples were filtered and the filtrate was used directly for analysis in an ICP-MS using standard recognized techniques. Sediment analyses were carried out after air drying the samples at 70°C for 48 hours before the EPA Method (3050B) was used to digest the samples for ICP-MS analyses. Only selected metals will be discussed.

Results and discussion

A total number of 35 metals were detected in both the water and sediment samples from all the sampling sites (data not shown). Only selected metals were used for statistical analyses and discussion. Metal concentrations in the sediment were higher than those in the water, however, the fact that these metals were all present at both the anthropogenically impacted and unimpacted sites, suggests that it is most probably from a geological origin (Kemp and Wolmarans, 2015).

Seven families including Notonectidae, Coenagrionidae, Atyidae, Libellulidae, Baetidae, Caenidae and Chironomidae were analysed to determine their metal concentrations. A total number of 35 metals were also detected in the different macroinvertebrate samples at the various sites. Concentrations varied considerably between the different families as well as between the different sites (data not shown). The mean metal concentrations were the highest in the families Baetidae, Caenidae and Chironomidae. This is most probably due to the fact that these families are known to live in close proximity to the sediments while the other families reside either in the water, on the surface or on plants.

According to the PCA tri-plot (Figure 2), metal concentrations in the macroinvertebrate families are strongly associated with the metal concentrations in both the water and sediment. This is in accordance with literature, where linear correlations between metal concentrations in macroinvertebrates and sediments where found (Santoro et al. 2008). Furthermore, on the PCA tri-plot, Baetidae, Caenidae and Chironomidae also associates slightly more with the metal concentrations in the sediment while Notonectidae, Coenagrionidae, Atyidae and Libellulidae associate slightly more with the metal concentrations in the water.

Concentrations of Al, Mn, Fe and Zn were constantly the highest in all of the families, as well as in the water and sediment, which is not surprising as these are the most common elements in the earths' crust.

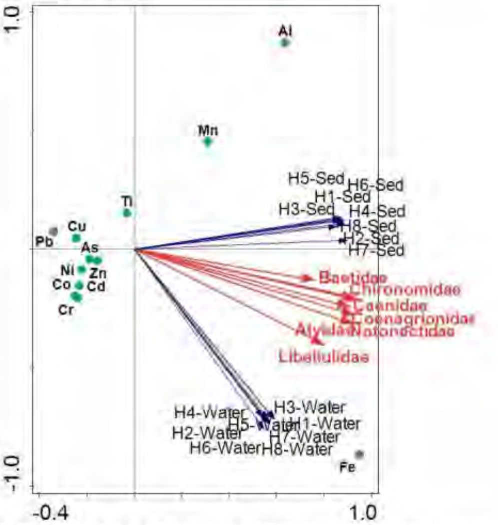

Figure 2: PCA tri-plot of the metal concentrations in the water and sediment at the various sites as well as the mean metal concentrations in the selected families. The tri-plot describes 79.48% of the total variation, with 68.17% being described on the first axis and 11.31% on the second axis

To conclude, positive correlations were evident between the metal concentrations in the selected macroinvertebrate families and the metal concentrations in the water and sediment. As the metal concentrations in the water and sediment were most probably from geological origin, it does not, under the current circumstances, pose a serious threat to the organisms.

Acknowledments

We are indebted to the Unit for Environmental Sciences and Management, North-West University, Potchefstroom, South Africa for financial support and infrastructure.

References

Santoro, A., Blo, G., Mastrolitti, S. and Fagioli, F., 2009. Bioaccumulation of heavy metals by aquatic macroinvertebrates along the Basento River in the South of Italy. *Water, air, and soil pollution*, *201*(1-4), pp.19-31.

Kemp, M. and Wolmarans, C.T. 2015. Metal concentrations in the water and sediment of a pristine river system in the North-West Province of South Africa. 7th International Toxicology Symposium in Africa, Johannesburg, South Africa, pp. 55 – 56.

Table 1: Mean metal concentrations (ppb/mg) in macroinvertebrate families collected at the various sites.

ppb/mg	Notonectidae	Coenagrionidae	Atyidae	Libellulidae	Baetidae	Caenidae	Chironomidae
Al	137.27±114.32	215.74±82.10	184.96±85.33	234.79±149.44	855.09±604.23	865.52±499.70	892.81±945.22
Ti	3.87±2.97	6.52±3.95	7.22±2.33	5.32±2.72	15.60±7.68	21.01±25.61	20.77±28.12
Cr	1.82±1.20	3.02±1.17	2.24±0.98	3.16±2.3967	11.86±6.81	12.74±10.78	11.50±13.09
Mn	86.89±99.73	123.37±56.39	211.56±193.54	137.90±104.99	405.52±344.79	357.38±240.34	157.59±118.90
Fe	232.84±138.32	450.11±122.28	523.13±315.50	799.24±638.77	1619.59±677.16	2046.97±762.19	1479.99±1391.61
Co	0.30±0.15	0.50±0.24	0.50±0.28	0.53±0.37	2.46±1.45	1.93±0.94	1.50±1.39
Ni	1.71±1.37	2.80±1.54	1.76±1.34	3.72±4.96	7.00±5.26	6.37±3.30	5.60±4.01
Cu	25.12±12.88	23.27±18.56	17.60±7.89	23.61±29.37	103.01±142.69	59.83±65.67	105.24±181.45
Zn	197.44±193.69	153.57±183.63	67.91±87.78	385.81±748.78	298.82±406.12	226.37±244.17	134.93±155.49
As	1.13±1	1.41±1.23	1.55±2.23	2.62±4.05	0.40±0.31	0.48±0.27	0.12±0.14
Cd	0.26±0.10	0.33±0.31	0.17±0.23	0.20±0.14	0.25±0.44	0.01±0.02	0.04±0.05
Pb	18.85±17.04	29.62±22.20	23.10±30.23	15.10±14.47	96.46±143.20	65.98±74.51	48.48±61.75