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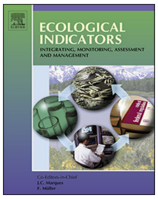

Macroinvertebrates as indicators of ecological conditions in the rivers of KwaZulu-Natal, South Africa

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ABSTRACT

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

1. Introduction

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

Global awareness about the values of bioassessment and biomonitoring is limited (Resh, 2007). It is, therefore, essential to understand the value of the services that high-quality aquatic resources provide to society, to appreciate the importance of bioassessment and biomonitoring (Barbour, 2008). Ecosystem services are the processes by which the environment produces the resources that are often taken for

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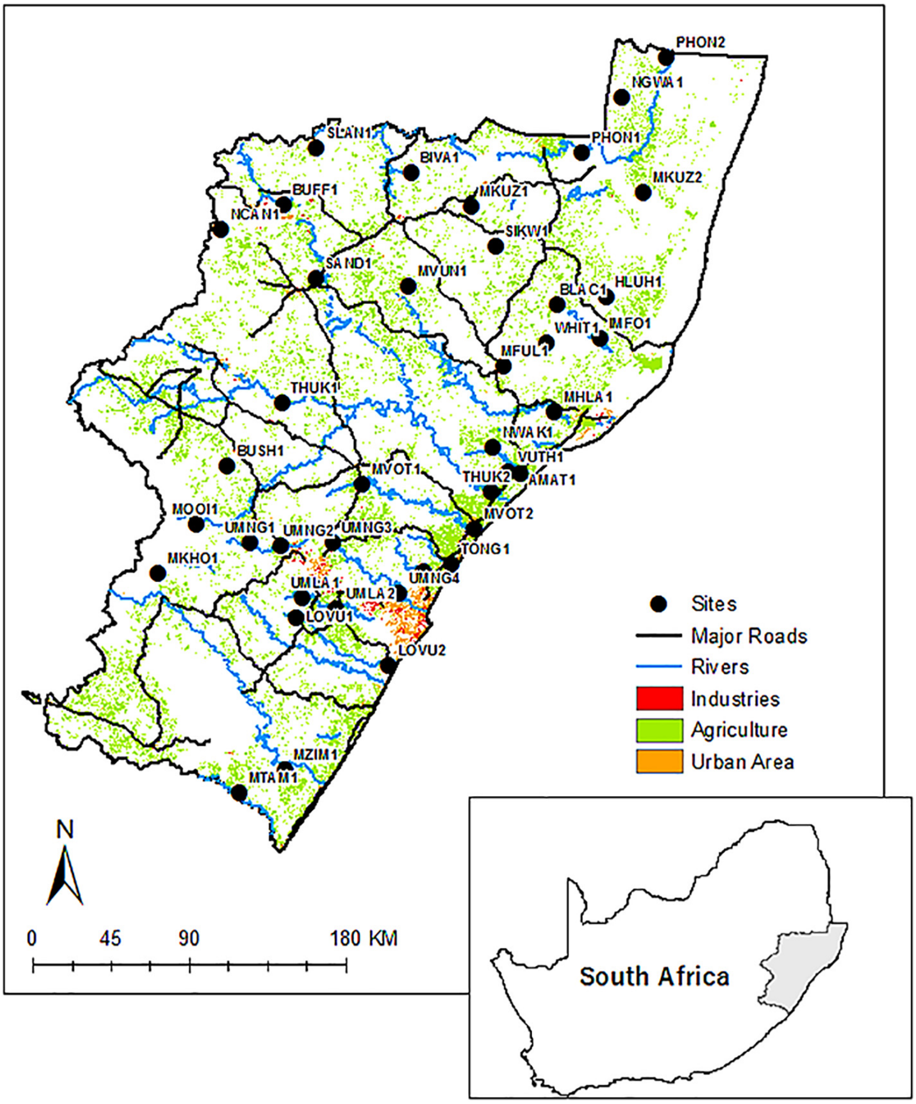

Fig. 1. Map of KwaZulu-Natal rivers studied between 2014 and 2016. (Inset: map of South Africa).

granted, which include clean water, habitat for organisms, nutrients and recreation (Barbour and Paul, 2010). The importance of biota's contribution to the provision of ecosystem services cannot be underestimated (Barbour and Paul, 2010). Maintaining or restoring quality aquatic ecosystem integrity helps to safeguard ecosystem services, and this requires an adequate conservation of all the biological, physical and chemical components (Barbour et al., 2000; Moog and Chovanec, 2000; Barbour and Paul, 2010).

South Africa's freshwater ecosystems are being impacted by anthropogenic development and intensive utilisation of their resources, which is causing a decline in water quality because of several factors (e.g. industrialisation, agriculture and power generation) (Hill, 2003; Oberholster and Ashton, 2008, Ashton et al., 2008). The increase in the demand for water and its associated impacts on the quality of South Africa's freshwater resources started with the large-scale urbanisation, industrialisation and rapid socio-economic changes of South Africa (Roux et al., 1999). The government's responsibility of managing these scarce resources is delegated to the Department of Water and Sanitation (Roux et al., 1999), through the National Water Act of 1998 (RSA, 1998; DWAF, 1998). The current assessment of South African rivers is based on the concept of biological integrity, using fish, invertebrates, riparian vegetation and diatoms as biological indices using established sampling methods for their collection and assessment (Dickens and Graham, 2002; Kleynhans, 2007; Kleynhans et al., 2007). The results of these biological indicators of the freshwater riverine ecosystem are categorised into specific ecological categories representing the river health (DWAF, 2007; Kleynhans and Louw, 2007; Wepener, 2008). Organisms respond to specific stressors in different ways, although these may be obscured in the presence of other stressors (Hering et al., 2006). Their responses to river quality changes are predictable, distinct and taxonomically diverse (Griffith et al., 2005). Apart from differences in the physical and chemical tolerances among taxa, their life histories and biogeography may affect their individual responses to water quality changes (Townsend and Hildrew, 1994).

Ecological assessment of stream conditions requires an evaluation of all the physical and chemical attributes, including the biotic composition and community structures (Karr and Chu, 1998). Earlier water quality monitoring programs focused on the comparison of water chemistry downstream of point-sources, deriving water quality criteria from bioassays (McCarron and Frydenborg, 1997). However, the indices of biotic integrity (IBI) are designed to be sensitive to a wide range of stressors and cumulative disturbances in the ecosystem (Karr, 1993). However, this approach ignored the dynamic responses of *in situ* biological assemblages to chemicals or pollutants (Karr and Chu, 1998). Furthermore, the in-stream conversion of chemicals, the spatial and temporal variation in chemical concentrations and the effects of the interaction of their compounds with other environmental stressors (such as disturbance of the riparian zones and in-stream habitats) were not considered (Karr and Chu, 1998). The taxonomic composition and structures of biological communities incorporate both aspects of exposure and a higher level of responses (Karr et al., 1986; Deshon, 1995; Rosen, 1995). Species traits approach of bioassessment is a promising tool that can provide good interpretations of stressor effects on aquatic systems (Statzner and Beche, 2010; Winemiller et al., 2015). Based on the hypothesis that environmental conditions act as a template for evolutionary combinations of specific organism attributes, we aimed to assess macroinvertebrates' occurrence at different water quality states using taxa-specific indicators. We expected the taxa-specific metrics to give good assessment results in the event of low-quality macroinvertebrate data, especially in the lowland rivers where the widely used biotic indices (SASS, ASPT and MIRAI) for assessing South African rivers are not effective. The information gained in this study is expected to aid stakeholders to better understand the nature of their water resources, as a means of developing appropriate strategies or policies for conserving and managing the river ecosystems. The data can also be used to design measures for mitigating and monitoring environmental changes that can arise from anthropogenic activities within the river catchments.

2. Methods

2.1. Study area

KwaZulu-Natal (KZN) Province is in the southeastern part of South Africa. It has an extensive shoreline along the Indian Ocean and shares borders with the countries of Lesotho, Swaziland and Mozambique. Its climate is classified as subtropical, as it is characterised by hot and humid summers, and mild winters. For our study, we collected water and macroinvertebrate samples from 38 locations within 15 river catchments; Lovu, Matikulu, Mdloti, iMfolozi, Mhlathuze, Mkomazi, Mkuze, Mtamvuna, Mzimkhulu, Phongolo, Thukela, Tongati, uMlazi, uMngeni and uMvoti (Fig. 1). The study sites were selected along the river gradient from the headwaters (upland rivers) to the lowland rivers, with the headwaters being the least impacted sites. The headwaters were used as the reference sites for assessing the impacts of anthropogenic activities along the river continuum. During this study, the major anthropogenic impacts within KwaZulu-Natal Province were commercial sugar-cane production, rapid urbanisation, industrial revolution, large-scale livestock farming, sand mining and indiscriminate domestic waste disposal (Fig. 1).

2.2. Physico-chemical assessment index

Water and macroinvertebrate samples were collected four times between March 2015 and April 2016 from the rivers of KZN, corresponding to summer 2015, autumn 2015, spring 2015 and summer 2016. We collected water samples for nutrient analyses using 500 ml sterilized, clear, plastic bottles. The water samples were preserved in the field at 4 °C and transported to the uMgeni Water laboratory for nutrient, biological and microbial analyses. Water temperature, electrical conductivity, clarity, pH and dissolved oxygen were measured with the YSI model 556 MPS handheld multi-probe water quality meter (YSI Environmental, USA).

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

2.3. Macroinvertebrate sampling and identification

Macroinvertebrates were qualitatively sampled on four occasions. However, some lowland rivers could not be sampled during the low flows because they were in drought. We used a kick net ($30 \times 30\,cm^2$ frame, 1000 μm mesh) to sample macroinvertebrates from the three biotopes according to the South African Scoring System v5 (SASS5) protocol (Dickens and Graham, 2002). The three biotopes were stones (stones-in and stones-out of current), vegetation (marginal and aquatic) and GSM (gravel, sand and mud). Unless otherwise stated, the described biotopes were herein referred to as stone, vegetation and GSM. Each biotope was sampled separately and preserved in 80% ethanol. The different samples were stained in the field and transported to the laboratory for identification to the lowest possible taxonomic levels and abundance counts. The laboratory identifications were done using a compound microscope and suitable identification keys (Day et al., 2002; Barber-James and Lugo-Ortiz, 2003; De Moor and Scott, 2003; Stals and Moor, 2007).

2.4. Data analyses

Prior to statistical analysis, all macroinvertebrates within each sample were sorted, identified and counted using a compound microscope (Hering et al., 2006; Flinders et al., 2008). We calculated several candidate metrics for macroinvertebrate taxa based on their water quality traits, with particular consideration for the variation of KZN lowland rivers which generally have low macroinvertebrate diversity. The metrics were scrutinised using expert judgement and 19 metrics were eventually selected for statistical analysis (Table 1). The best candidate metrics were identified through a process that included a combination of univariate and nonparametric multivariate methods using Primer v6 statistical software (Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley 2006). Spearman rank correlation was used to identify and eliminate redundant metrics (Rho = 0.65) (Clarke and Warwick, 2001; Clarke and Gorley 2006).

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

Also, a principal coordinate (PCO) analysis was performed on the macroinvertebrate metric scores and study sites in order to explore the relationships of the different indices with each site. This was done using the Bray Curtis Similarity Matrix using Primer v6 statistical software (Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley 2006)

3. Results

3.1. Physico-chemical variables

The RDA model was used to select the best six physico-chemical variables (PAI Score, pH, clarity, EC, *Escherichia coli* and F), at a

Table 1
Definitions and descriptions of selected macroinvertebrate metrics applied to KwaZulu-Natal Rivers in the present study. (Compiled from Barbour et al., 1996; DeShon, 1995; Hering et al., 2004; Baptista et al., 2007).

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

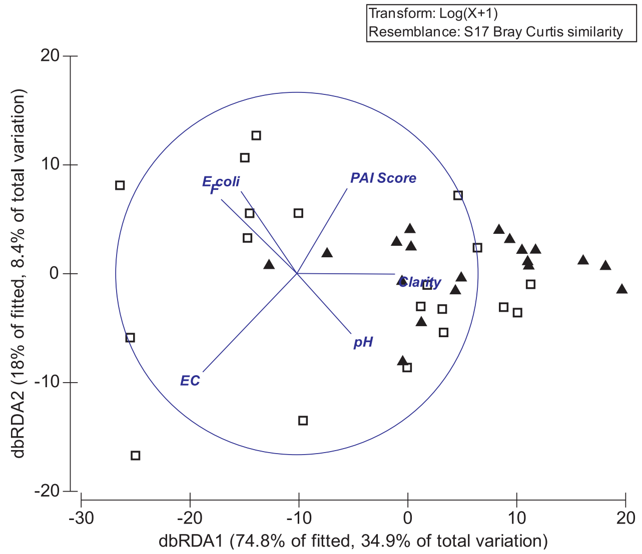

Fig. 2. Redundancy analysis plot of the mean score of the environmental variables measured in the rivers of KwaZulu-Natal, South Africa in 2015–2016 in the present study. (Rho = 0.7. (E. coli = *Escherichia coli*, PAI Score = physico-chemical assessment index, EC = electrical conductivity, F = fluoride). (Triangles are upland rivers, while squares are lowland rivers).

Spearman Rho value of 0.7 (Fig. 2). According to the RDA analysis results, the parameters that best reflected the variability in the environmental data were similar for upland and lowland sites. Physico-chemical assessment index, pH, clarity, total inorganic nitrogen and fluorine were the best water quality variables obtained from the RDA analysis, using the Akaike selection criterion (AICc). The RDA ordination of the physico-chemical variables explained 74.8% of fitted and 34.9% of total variation in the data on the first axis, while the second axis explained 18.0% of both fitted and 8.4% of total variation in the data (Fig. 2). The highest physico-chemical index (PAI) was recorded in the Mzimkhulu River catchment (MZIM1 = 100%) and the lowest score was recorded in the Phongolo River catchment (PHON = 51%) (Table 2). The lowest mean concentration for total dissolved solids was recorded in the Thukela catchment (MOOI1 = 38 mg/l), while the highest was recorded in the Matikulu River catchment (VUTH1 = 949.15 mg/l). The mean water temperature was lowest in the Thukela catchment (MOOI1 = 13.13 °C), while it was highest in the Mfolozi catchment (BLAC1 = 28.87 °C); mean pH was lowest in the Mkuze catchment (MKUZ2 = 6.01), while it was highest in the Thukela catchment (THUK2 = 7.60). For the dissolved oxygen level, the lowest mean measurement was in the Tongati catchment (TONG1 = 2.22 mg/l) and the highest in the Mfolozi catchment (SIKW1 = 31.66 mg/l). The poorest water clarity score was recorded in the Mfolozi catchment (WHIT1 = > 240 NTU), while the best clarity scores were recorded in the Thukela (MOOI1 = < 5 NTU) and uMgeni (UMNG4 = < 5 NTU) catchments. The mean electrical conductivity was lowest in the uMgeni catchment (UMNG1 = 82.32 m*S*/m) and highest in the Phongolo catchment (PHON2 = 1788.70 m*S*/m) (Table 2). All the measurements with zero (0) values were below detection limits (Table 2)

3.2. Macroinvertebrate metrics and water quality

The macroinvertebrate metrics in this study responded to the physico-chemical variables as predicted (Table 1) and these were validated by correlation analysis (Table 3). Percentage of Odonata taxa (%Odon) was strongly correlated with the lowland sites, which showed that Odonata families were abundant in the lowland rivers. Also, Gastropoda was positively correlated with temperature. A high abundance of Chironomidae taxa was recorded at a site below the effluent discharge point of a paper conversion industry.

Eleven macroinvertebrate metrics had general discriminatory abilities in both upland and lowland rivers; and nine of these had strong correlations with physico-chemical variables. These metrics were total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera Plecoptera and Trichoptera taxa, percentage of Odonata taxa, total number of Trichoptera individuals, total number of Gastropoda individuals, total number of Oligochaeta individuals and total number of Coleoptera individuals. (Fig. 3).

The Principal Coordinate Analysis (PCO) ordination explained 52.0% of total variation in the data on the first axis, while the second axis explained 20.2% of total variation in the macroinvertebrate metrics (Fig. 3). The PCO gradients of the macroinvertebrate metrics gave indications of good water quality from the least impacted upper river reaches and increasing impairment towards the downstream sites. The first axis of the PCO ordination plot revealed a correlation with pollution and habitat quality. Most of the sites on the first axis were the sand dominated lowland rivers of which some are affected by periods of

Table 2

Mean scores of environmental data measured in KZN rivers between 2015 and 2016 in the present study, including Standard Deviation values. (PAI Score = physico-chemical assessment index, TIN = total inorganic nitrogen, clarity, Temp = water temperature, TDS = total dissolved solids, DO = dissolved oxygen, EC = electrical conductivity, pH = hydrogen ion concentration and F = fluoride).

Site	PAI Score (%)	TDS (mg/l)	Temp (^{0}C)	pH	DO (mg/l)	Clarity (NTU)	EC (m*S*/m)	TIN (mg/l)	F (mg/l)
AMAT1	82 ± 28.68	617.70 ± 39.17	27.03 ± 2.28	6.71 ± 0.62	4.21 ± 2.60	6 ± 1.95	702.37 ± 119.55	0	0.14 ± 0.05
BIVA1	95 ± 14.27	100.60 ± 22.06	21.65 ± 4.13	6.78 ± 0.56	7.33 ± 1.11	13 ± 3.20	145.23 ± 24.30	0.33 ± 0.29	0
BLAC1	78 ± 14.27	240 ± 90.15	28.87 ± 4.65	6.61 ± 1.02	5.38 ± 3.13	100 ± 1.93	419.33 ± 94.35	0	0.41 ± 0.11
BUFF1	61 ± 10.53	282.90 ± 8.34	22.05 ± 5.50	6.81 ± 0.54	7.87 ± 4.89	100 ± 4.5	402.90 ± 110.62	5.05 ± 0.45	0.34 ± 0.07
BUSH1	96 ± 22.64	62 ± 18.35	21.43 ± 6.22	6.74 ± 0.79	8.61 ± 2.15	6 ± 1.22	103.25 ± 46.85	0	0
IMFO1	80 ± 10.21	190 ± 25.16	27.13 ± 1.43	6.54 ± 0.91	6.41 ± 3.84	120 ± 9.50	474.67 ± 216.52	0.14 ± 0.03	0.42 ± 0.08
LOVU1	93 ± 16.08	59 ± 8.49	17.80 ± 3.51	6.65 ± 0.45	7.44 ± 2,01	6 ± 1.09	111.75 ± 35.53	0.18 ± 0.11	0
LOVU2	57 ± 6.46	572.80 ± 100.58	27.70 ± 0.14	6.47 ± 0.63	3.79 ± 0.71	27 ± 7.78	195.57 ± 274.96	0.19 ± 0.06	0.20 ± 0.03
MDLO1	97 ± 18.87	130 ± 14.14	23.75 ± 4.86	6.82 ± 0.83	9.58 ± 2.17	8 ± 2.99	169.25 ± 103.99	0	0.12 ± 0.06
MFUL1	96 ± 9.18	360 ± 18.57	20.93 ± 4.21	6.18 ± 0.33	8.41 ± 2.38	< 5 ± 0.08	273 ± 193.56	0	0.21 ± 0.05
MHLA1	88 ± 21.21	230 ± 9.87	26.27 ± 7.55	6.53 ± 0.93	8.68 ± 2.01	48 ± 1.50	316.67 ± 6.66	0.38 ± 0.04	0.20 ± 0.08
MKHO1	97 ± 17.74	44.50 ± 10.61	16.20 ± 5.03	6.90 ± 0.64	9.32 ± 2.01	< 5 ± 4.36	106.50 ± 16.84	0	0
MKUZ1	80 ± 17.57	465.35 ± 21.00	20.90 ± 2.86	7.04 ± 0.80	5.59 ± 3.21	8 ± 2.21	611.13 ± 207.72	0	0.25 ± 0.08
MKUZ2	72 ± 15.97	0	23.30 ± 2.95	6.01 ± 0.70	9.67 ± 0.90	17 ± 1.75	1342 ± 510.57	0	0.37 ± 0.05
MOOI1	97 ± 16.11	38.50 ± 4.95	13.13 ± 5.91	6.73 ± 0.65	10.33 ± 2.94	< 5 ± 0.02	186.25 ± 203.39	0	0
MTAM1	99 ± 14.22	66 ± 20.52	19.17 ± 6.21	7.24 ± 0.40	10.29 ± 3.36	10 ± 2.13	104.33 ± 35.92	0.24 ± 0.01	0
MVOT1	88 ± 19.88	85 ± 5.89	19.73 ± 3.32	6.74 ± 0.66	6.20 ± 1.40	21 ± 1.50	163 ± 38.32	0	0
MVUN1	92 ± 14.32	350 ± 10.43	25.73 ± 3.41	7.19 ± 1.34	6.80 ± 1.05	48 ± 1.58	507.17 ± 191.47	0.22 ± 0.03	0.36 ± 0.10
MZIM1	100 ± 17.33	87.67 ± 38.14	21.77 ± 6.12	7.38 ± 0.41	9.12 ± 3.05	13 ± 4.33	204 ± 64.44	0	0
NCAN1	95 ± 14.02	651.84 ± 33.21	18.30 ± 3.85	6.61 ± 0.47	7.39 ± 0.93	6 ± 0.57	1307.87 ± 7.87	0	0
NGWA1	64 ± 16.50	754 ± 36.87	27.55 ± 3.32	6.03 ± 0.01	4.33 ± 5.18	11 ± 2.05	1107.50 ± 566.39	1.01 ± 0.07	0.66 ± 0.08
NWAK1	67 ± 22,43	176.80 ± 52.04	24.33 ± 3.30	6.32 ± 0.28	7.46 ± 0.76	8 ± 1.53	229.73 ± 41.61	0	0
PHON1	89 ± 19.50	767.50 ± 50.67	26.37 ± 6.19	7.25 ± 1.44	11.87 ± 2.66	15 ± 0.06	355.85 ± 320.05	0.43 ± 0.04	0.43 0.11
PHON2	51 ± 13.45	475.40 ± 100.25	27.05 ± 5.16	7.16 ± 1.63	5.02 ± 2.31	19 ± 0.41	1788.70 ± 857.29	0.34 ± 0.03	0.34 ± 0.09
SAND1	55 ± 18.63	546.80 ± 80.98	19 ± 7.41	6.79 ± 0.74	5.45 ± 1.38	10 ± 2.53	483.36 ± 422.92	5.40 ± 0.03	0.29 ± 0.02
SIKW1	97 ± 27.74	230.15 ± 103.87	23.85 ± 3.21	7.10 ± 0.85	31.66 ± 47.06	6 ± 0.84	268.68 ± 226.88	0.12 ± 0.06	0.29 ± 0.04
SLAN1	95 ± 13.40	81.98 ± 36.10	15.58 ± 5.40	6.59 ± 0.54	7.89 ± 1.87	6 ± 0.67	120.70 ± 16.30	0	0
THUK1	93 ± 20.31	106 ± 45.96	24.30 ± 5.46	7.09 ± 0.93	8.52 ± 3.01	60 ± 1.00	165.35 ± 75.57	0.38 ± 0.02	0
THUK2	97 ± 21.23	178.20 ± 11.60	25.95 ± 1.63	7.60 ± 0.55	7.95 ± 2.12	84 ± 1.39	213.85 ± 22.84	0	0.15 ± 0.04
TONG1	54 ± 9.71	418.60 ± 196.01	22.88 ± 5.09	6.54 ± 0.46	2.22 ± 0.99	10 ± 2.10	404.23 ± 252.05	8.81 ± 1.0	0.14 ± 0.02
UMLA1	85 ± 13.60	64.50 ± 7.78	22.93 ± 10.27	7.09 ± 0.85	7.42 ± 0.77	11 ± 2.76	116.25 ± 28.15	5.29 ± 0.03	0
UMLA2	86 ± 18.72	261.35 ± 92.42	19.93 ± 4.94	6.77 ± 0.71	7.21 ± 1.83	7 ± 0.35	478.63 ± 155.30	0	0.21 ± 0.03
UMNG1	86 ± 22.02	42.77 ± 8.15	15.15 ± 5.02	6.36 ± 0.85	9.86 ± 3.37	6 ± 0.72	82.32 ± 36.03	0.23 ± 0.02	0
UMNG2	92 ± 11.79	54 ± 4.56	19.70 ± 5.07	6.77 ± 0.58	8.24 ± 3.12	12 ± 0.93	221.96 ± 242.65	0.15 ± 0.01	0
UMNG3	94 ± 8.98	86.38 ± 11.84	22.58 ± 3.03	6.45 ± 0.68	8.27 ± 2.37	11 ± 0.34	206 ± 121.90	0.52 ± 0.02	0
UMNG4	96 ± 19.96	205 ± 7.07	22.33 ± 3.71	7.54 ± 1.31	10.75 ± 1.04	< 5 ± 0.03	311.25 ± 26.27	0	0.16 ± 0.02
VUTH1	74 ± 14.18	949.15 ± 71.91	27.27 ± 4.11	6.65 ± 0.65	6.48 ± 1.32	14 ± 0.95	1014.93 ± 416.72	0	0
WHIT1	96 ± 15.48	160 ± 21.89	24.40 ± 4.51	6.51 ± 0.87	8.38 ± 2.52	> 240 ± 4.51	334.67 ± 101.11	0	0.47 ± 0.02

droughts and high anthropogenic impacts, especially physical habitat degradation and agricultural practices.

4. Discussion

The physico-chemical parameters indicated loss of ecological quality or integrity of downstream sites. The impacts on water quality included natural (flood and drought) and anthropogenic impacts (e.g. sand mining, agricultural practices), with the highest impacts occurring downstream, especially those located within agricultural land uses. Five out of the nine final metrics in our study showed significant positive correlations with high PAI scores. The five metrics were total number of taxa, total number of Diptera taxa, total number of Plecoptera individuals, percentage of Ephemeroptera Plecoptera and Trichoptera taxa, and total number of Coleoptera individuals. The five metric scores increased with improvement in overall water quality. The high scores were obtained from the least impacted or reference sites of the study, while low PAI scores were observed at the impaired sites. The additive or synergistic effects of the physico-chemical components of a river as indicated by the PAI scores may have caused unfavourable conditions

Table 3

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

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

* $p < 0.05$.

** $p < 0.01$.

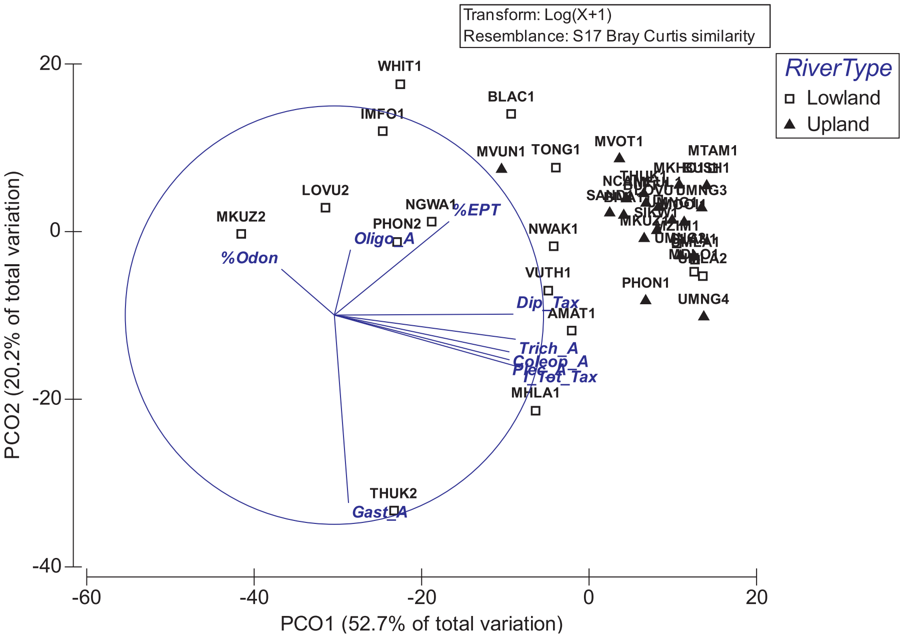

Fig. 3. Principal coordinate analysis plot of macroinvertebrate metrics sampled in rivers of KwaZulu-Natal in 2015–2016 in the present study. (I_Tot_Tax = total number of taxa, Dip_Tax = total number of Diptera taxa, Plec_A = total number of Plecoptera individuals, %EPT = percentage of Ephemeroptera, Plecoptera and Trichoptera taxa, %Odon = percentage of Odonata taxa, Trich_A = total number of Trichoptera individuals, Gast_A = total number of Gastropoda individuals, Oligo_A = total number of Oligochaeta individuals and Coleop_A = total number of Coleoptera individuals).

for the survival and abundance of sensitive macroinvertebrate taxa at the impacted or polluted sites (Chen and Lu, 2002; Laskowski et al., 2010). According to our initial classification of the environmental variables, the macroinvertebrate metrics were able to detect physical variables (total dissolved solids, water temperature, dissolved oxygen, clarity and electrical conductivity), nutrient pollution (total inorganic nitrogen) and a toxic pollutant (fluorine). Humans and organisms are often exposed to isolated micropollutants and complex chemicals in their environments or ecosystems (Richardson, 2009; Pal et al., 2010). The individual components of these micropollutants and their complex compounds may be relatively harmless at low concentrations (Schwarzenbach et al., 2006; Eggen et al., 2014; Luo et al., 2014), however, they may have additive or synergistic effects that can increase their toxic potentials (Heberer, 2002; Schwarzenbach et al., 2006).

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

Fluorine is a very reactive element that does not exist in its natural elemental state, and it may exist in the form of inorganic fluorides or as organic fluoride compounds (e.g., fluorocarbons) (Camargo, 2003). Inorganic fluorides often remain in solution as fluoride ions under low pH conditions inside water (CEPA, 1993). Fluoride ions have enzymatic abilities, which makes them toxic to aquatic and terrestrial biota, for example, the effects of fluoride on algae depends on the concentration, duration of exposure and the algal species (Joy and Balakrishnan, 1990; Rai et al., 1998; Camargo, 2003). The level of fluoride toxicity to aquatic invertebrates depends on the concentration, exposure duration and water temperature (Camargo and Tarazona, 1990; Camargo, 2003); thus, they can act as inhibiting enzymes by interrupting their metabolic processes (e.g. glycolysis and protein synthesis) (Aguirre-Sierra et al., 2013; Ghosh et al., 2013; Rani and Naik, 2014).

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

Turbidity (measured as clarity in this study) indicated the number of particles suspended in water and its high concentrations reduce the habitat quality for aquatic organisms (Said et al., 2004). Agricultural wastes, urban runoffs, industrial effluents and domestic waste contribute to organic pollution of rivers (Singh et al., 2005). Increased turbidity in the downstream river site reduced light availability for photosynthetic organisms. Low water clarity affects light penetration, productivity and habitat quality, increased sedimentation and siltation (Wagner et al., 2006). Sedimentation and siltation can cause harm to habitat areas for macroinvertebrates and other aquatic life (Ryan, 1991; Novotny et al., 2005). Sediment particles also provide attachment for other pollutants (mostly metals and bacteria) (Jiang et al., 2009; Wang and Chen, 2009; Mohanty et al., 2013). For this reason, turbidity readings are good indicators of potential pollution in a water body

(Wagner et al., 2006).

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

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

Members of the Ephemeroptera are sensitive to environmental stress and their presence signifies relatively good conditions of the ecosystem (Fialkowski et al., 2003). Ephemeroptera larvae are generally microhabitat specialists and they can survive on specific substrates with a certain amount of flow (Bustos-Baez and Frid, 2003). They are known to burrow into soft areas with shallow flows or in areas of high sediment depositions (Azrina et al., 2006). Therefore, the shallow nature of the lowland rivers in this study could be the factor contributing to their relative abundance of the Ephemeroptera taxa. The low combined abundance of sensitive macroinvertebrate taxa such as Ephemeroptera, Plecoptera and Trichoptera (EPT) in the lowland rivers was not only caused by pollution but was also because of the reduced habitat heterogeneity.

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

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

Gastropoda have been found to be temperature tolerant (Johnson et al., 2015). The significant positive correlation of Gastropoda with temperature in this study confirms their tolerance of thermal pollution, which could have resulted in their high abundance in some of our study sites. Also, Chironomidae was highest at a site below the effluent discharge point of a paper conversion industry and this is indicative of severe pollution at the site, but no significant correlation was detected between their occurrence and water quality in this study.

5. Conclusions

The sensitivities of different macroinvertebrate taxa to pollution are often dependent on their life history attributes and feeding behaviours (Luiza-Andrade et al., 2017) and consequently different species have considerably different water quality tolerances (Arimoro and Ikomi, 2008; Ikomi and Arimoro, 2014). In this study, we found that patterns of species distribution only give a little understanding of ecosystem functions but probing the ecosystem processes (e.g. nutrient dynamics) may prove more useful (Harris, 1994). The application of macroinvertebrate ecological trait indices was effective and provided accurate information about many stressor types and their effects on the river ecosystems. Although it may be difficult to distinguish natural variations in diversity and community composition from the effects of anthropogenic activities, the consistent pattern of taxa composition by a single or only a few taxa at downstream sites indicated impacts from agriculture, nutrient enrichment and drought (Göthe et al., 2015). The differences detected when comparing upstream and downstream sites imply that monitoring of macroinvertebrate community composition is useful for assessing management practices and gives an insight into development of a more efficient monitoring of the lowland rivers (Helson and Williams, 2013). Due to the high ecological relevance of macroinvertebrate community composition in biomonitoring, we recommend that more research is needed to explore the specific tolerance of macroinvertebrates to different chemicals or toxicants impacting their wellbeing in aquatic systems.

In our study, the use of macroinvertebrate metrics approach (majorly at family level of identification) proved to be a useful tool for aquatic ecosystem assessment in KZN rivers. We, therefore, recommend that seasonal variations and factors driving the macroinvertebrate communities to be studied in more detail, as this could help in the development of reference conditions for the application of macroinvertebrate community-based metrics in the region. Also, establishing riparian buffer zones around the sand dominated lowland rivers of KZN can contribute to erosion control and reduce nutrient runoff from agricultural lands (Novara et al., 2013; Bouraoui and Grizzetti, 2014). A suitable buffer serves as a natural filter, which reduces nutrient pollution, sedimentation and chemicals that enter a river and protect the river banks from erosion (Barling and Moore, 1994; Walter et al., 2009).

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