ADVANCES IN BIOMONITORING FOR THE SUSTAINABILITY OF VULNERABLE AFRICAN RIVERINE ECOSYSTEMS

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ADVANCES IN BIOMONITORING FOR THE SUSTAINABILITY OF VULNERABLE AFRICAN RIVERINE ECOSYSTEMS

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Editorial: Advances in Biomonitoring for the Sustainability of Vulnerable African Riverine Ecosystems

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Editorial on the Research Topic

Advances in Biomonitoring for the Sustainability of Vulnerable African Riverine Ecosystems

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INTRODUCTION

Since recorded time, rivers have facilitated the establishment of human civilizations because of the myriad ecosystem goods and services they offer (Macklin and Lewin, 2015). Rivers provide transportation corridors, supply food in form of fisheries, and are major sources of water for irrigation, domestic use, renewable energy, and industrial development (Ripl, 2003). However, these benefits have come at a great cost to the structural and functional integrity of rivers and linked ecosystems (Dudgeon et al., 2006; Vörösmarty et al., 2010).

The capacity of rivers to sustainably meet human needs for water and ecosystem services is premised on maintaining their ecological integrity, which encompasses the gamut of biological diversity and ecosystem processes that maintain them (Karr, 1993). In river networks, ecological integrity is spatiotemporally dynamic, largely driven by the natural flow regime (Poff et al., 1997), which provides a template for ecological processes and species to thrive. River managers have the challenge of reconciling human needs with the ecological requirements of healthy ecosystems. This requires innovative decision-support tools for assessing and monitoring the ecological status of rivers to guide management and conservation efforts.

This Research Topic presents selected original research articles and reviews on some of the tools used to assess the ecological status of rivers in Africa. The objectives of the special issue are to:

i. contribute to the development of biomonitoring tools (e.g., biotic indices, multimeric indices, models, etc.), that are affordable, rapid and easy to use for enhanced understanding of human impacts on rivers.
ii. give novel insights into the effects of multiple stressors in rivers arising from human activities, such as land-use change, water pollution and excessive water withdrawals,
iii. address methodological challenges related to the use of existing tools used for biomonitoring, and
iv. encourage knowledge sharing and standardization of tools used for biomonitoring rivers in Africa, and promote interdisciplinary collaborations.

ECOLOGICAL STATUS OF AFRICAN RIVERS

Africa is a continent of immense natural heritage, including iconic rivers that fostered the earliest civilizations and continue to be integral to the continent's socio-economic development. Major rivers, such as the Nile, Niger, Orange, Tana, and Zambezi, drain Afromontane headwaters but downstream their flows quench semi-arid and arid lands that grace their journeys to the sea. Although Africa's rivers are amongst the least developed in the world (Grill et al., 2019), there have been ambitious plans to expand water use for irrigation, industry and hydropower (UN-Water Africa, 2003). These developments come with complex social, economic, governance or political challenges that exert multiple demands and stressors on river ecosystems at different spatial and temporal scales (Zeitoun et al., 2013; Fouchy et al., 2019; Birk et al., 2020).

Although data is limited, the status of African freshwater resources is one of a general decline (Darwall et al., 2011; UNEP-WCMC, 2016). Major concerns have been raised on the effects of pollution, urbanization, land-use change, overexploitation of biological resources, agriculture and invasive species on water quality and quantity, aquatic biodiversity, nutrient cycling, and energy sources supporting food webs (e.g., Masese et al., 2017; Fugère et al., 2018; Sayer et al., 2018; Matomela et al., 2021). These impacts are reducing ecosystem services offered by rivers and their floodplains, and undermining human well-being across the continent (IPBES, 2018).

ADVANCES IN BIOMONITORING VULNERABLE AFRICAN RIVERS

To address threats posed by multiple stressors in rivers, a determination of the present ecological condition is needed to guide decisions on management and conservation. For this reason, the development and use of bioassessment and biomonitoring tools is an integral part of integrated water resources management. While efforts have been made to develop indices or models to assess and monitor the status of rivers in Africa (Dallas, 2013; Masese et al., 2013), most countries, except South Africa, lack these tools and expertise on their use.

As a contribution to the use of biotic indices in Africa, Dallas, 2013 has presented a review on important methodological considerations for developing new or adapting existing macroinvertebrate-based biotic indices for bioassessment. Similarly, Achieng et al. and Tampo et al. present the application of multimetric indices in rivers based on fishes and macroinvertebrates, respectively. Other interesting biomonitoring approaches covered in this Special Topic include the use of macroinvertebrate traits as indicators of ecological health (Edegbene et al.) and the use of host-parasite dynamics as bioindicators of heavy metal pollution in rivers (Keke et al.). Considering that identification of reference conditions is a prerequisite for biomonitoring programs (Dallas, 2013), Agboola et al. demonstrate the use of the multivariate approach in the selection of reference sites, and in their second paper, they show how to conduct an ecological risk assessment of stressors in rivers in KwaZulu Natal, South Africa.

The use of citizen science as an environmental monitoring approach in many African countries is quite limited (Requier et al., 2020). To address this need, Aura et al. used indigenous knowledge to develop a multimeric index, called the "Citizen Index of Ecological Integrity (CIEI)," for bioassessment of rivers. Further, Ndiritu et al. developed a biomonitoring framework based on the United States Environmental Protection Agency's Biological Condition Gradient (BCG; USEPA, 2016) to support the rehabilitation of the upper Tana River basin in Kenya.

WAY FORWARD

Innovative approaches and management solutions are required to achieve water-related sustainable development of African economies. The governments, research institutions, private sector, and civil society need to be involved in addressing the inherent problems. Below we highlight three areas of action that should be prioritized for improved conservation and management of rivers in Africa.

1. Plans to develop water resources should espouse principles of integrated water resources management (IWRM) (Dirwai et al., 2021). IWRM seeks to develop and manage water in a manner that maximizes economic and social benefits for multiple water users without degrading ecosystems (Meran et al., 2021).
2. While striving to supply water to millions of people in sub-Saharan Africa who lack access to safe drinking water and sanitation (Armah et al., 2018), the effects of water withdrawals on the ecological condition of rivers should be monitored. This calls for water allocation planning, water accounting and environmental flows assessments to apportion available water for river ecosystems and abstractive uses.
3. The complex mix of multiple stressors acting on rivers in Africa calls for multidisciplinary, multi-institutional, and transboundary (where necessary) collaborations and stakeholder participation to develop decision-support tools to guide the conservation and management of river ecosystems at multiple scales.

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All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Biomonitoring of Effects and Accumulations of Heavy Metals Insults Using Some Helminth Parasites of Fish as Bio-Indicators in an Afrotropical Stream

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Studies on biomonitoring the aquatic environment using host-parasite dynamics as bio-indicators of effects and accumulators of heavy metals insults are still scarce, particularly in the tropics. In our study, we aimed at elucidating the possible use of helminth parasites of fish in monitoring and controlling heavy metal pollution. Samples were collected from an anthropogenically polluted river in north central region of Nigeria over a period of 24 months (September 2014 and October 2016). Water, fish muscle, and fish parasites samples of three dominant fish species were collected, processed, and analyzed for copper, lead, manganese, iron, zinc, and chromium. The metal concentrations in parasites of: *Clarias gariepinus* was in the order of Fe > Zn > Cr > Mn > Pb > Cu; *Tilapia zillii* was in the order of Fe > Zn > Mn > Cu > Cr > Pb; and that of *Raiamas nigeriensis* was in the order of Fe > Zn > Cr > Mn > Cu > Pb. The CCA ordination revealed strong relationships between fish parasites and heavy metals pollution. Generally, Fe, Zn, Mn, Cu, Cr, and Pb concentrations in the parasites of all fish species were clearly higher than those in the muscles of the fish hosts. Pb was not detected in the fish muscles of *Raimas nigeriensis* but was detected in the parasites of the fish, thus indicating high bioaccumulation capacity of the parasites. The close linkage between *Eustrongylides* sp. and zinc could mean that *Eustrongylides* sp. was an ideal surrogate for zinc pollution. This study revealed that intestinal helminthic parasites can be ideal surrogates for both effects and accumulation bioindication of heavy metal pollution.

Keywords: aquatic pollution, bioaccumulation, bioindication, biomonitoring, ecotoxicology, heavy metals, parasites

INTRODUCTION

Aquatic ecosystem has various sources of pollution, resulting from human activities such as industrial processes, amplified urbanization, and waste discharge (Aladaileh et al., 2020). Furthermore, processes such as weathering of rocks, human-induced emissions from mining, and other mining-related processes are ultimately likely to elevate heavy metals concentrations in water bodies, leading to increased pollution (Sankhla et al., 2016). Elevated levels of heavy metals (e.g., cadmium, lead, and mercury) are naturally found in rocks and sediments (Waheed et al., 2020). Given that a broad range of these heavy metals are persistent and are not naturally degradable, they aggregate in the sediments of streams (Sures et al., 2017). Heavy metals are biologically classified into two: the biologically essential metals (e.g., Cu, Ni, Zn and Fe) which are important for fish metabolic activities and the non-biologically essential or toxic metals (e.g., Cd, Pb and Hg) which are toxic even in trace concentrations and have no known metabolic roles in fish (Demirezen and Uruc, 2006; Mehana et al., 2020). Among the heavy metals implicated in aquatic pollution in relation to fish, lead, copper, zinc, iron, chromium, and manganese are among the most common (Afshan et al., 2014). Most of these heavy metals are essential for fish metabolism at internationally approved limits, but become very toxic when their concentrations overshoot these limits (Keke et al., 2015; Padrilah et al., 2018). Fish are distinctly in danger of heavy metal insults given that they naturally live and get nourished within the water environment, and as such exhibit certain limitations in avoiding the hazardous effects of heavy metals pollution (Ahmed et al., 2020). Fish ultimately absorb heavy metals directly from water by means of their skin and gills as well as through the intake of food that is polluted with heavy metal contaminants (Vidal-Martínez and Wunderlich, 2017; Hassan et al., 2018). Consequently, heavy metals penetrate the bloodstream of fish and bioaccumulate in their tissues or organs. The bioaccumulated concentrations of heavy metals undergo biological transformations and are either passed out by egestion or are consumed by man, with resultant deleterious outcomes on fish as well as fish consumers (Vidal-Martínez and Wunderlich, 2017), hence the consequence of the resultant deleterious effects of heavy metals on fish can be used in biomonitoring freshwater ecosystems. Among the organs of fish impacted by heavy metals, the muscle is usually preferred given its human-health deductions via consumption (Hassan et al., 2018), and its biomonitoring significance.

Biomonitoring is the use of biological indicators (bioindicators) i.e., aquatic organisms to determine the impact level of human influences on the ecological balance of aquatic ecosystems (Edegbene et al., 2020). Bioindicators are organisms whose presence, absence, or physiological conditions are indicative of environmental quality (Sures, 2003; Arimoro and Keke, 2017). They can either be effect indicators or accumulation indicators. Effect bioindicators reflect alterations in the physiology, molecules, functions, or number of organisms; while accumulation bioindicators (sentinels) are able to effectively accumulate materials from the surroundings to concentrations that are appreciably higher than those in the surroundings without manifesting deleterious outcomes (Sures, 2003; Tellez and Merchant, 2015).

Historically, the assessment of water quality using bioindicator organisms has been through the use of free-living biota such as fish, macroinvertebrates, and plankton (Ortega-Álvarez and MacGregor-Fors, 2011; Tweedley et al., 2014; Keke et al., 2017). Nevertheless, the use of free-living biota as bioindicators is characterized by several constraints; for example: (1) the sampling processes are sophisticated and require loads of funds; (2) sampling routine requires considerable amounts of samples to make any meaningful inference; (3) the composition of the organisms is affected by seasonal and temporal dynamics; (4) the large-scale heterogeneity in interpreting results occasioned by the use of sophisticated analytic tools and methods (Wright, 2010; Vidal-Martínez and Wunderlich, 2017).

Currently, emphasis is shifting to the use of parasites as both effect indicators and accumulation indicators, given that they respond differently to a variety of pollutants in the environment (Vidal-Martínez et al., 2014; Vidal-Martínez and Wunderlich, 2017; Hassan et al., 2018; Al-Hasawi, 2019; Mehana et al., 2020). Helminth parasites of fish (e.g., trematodes, nematodes, cestodes, and acanthocephalans) have been employed as ideal tools for biomonitoring of heavy metal insults in aquatic ecosystem studies (Bush et al., 2001; Sures, 2003; Hassan et al., 2018; Al-Hasawi, 2019). Some of the advantages of using parasites as bioindicators of effects and accumulation include, but not limited to, the following: (1) they do not have complex species richness, particularly in benthic freshwater ecosystem; (2) their taxonomic attributes and life-history are very familiar; (3) each individual host forms a sampling domain with its own group of parasite taxa (Kuris, 1980); (4) given that parasites of high-level predators are regarded as high-level consumers, they inevitably represent ideal indicators of trophic level accumulation (Tellez and Merchant, 2015; Vidal-Martínez and Wunderlich, 2017).

Studies on bioremediating the aquatic environment using host-parasite dynamics as bio-indicators of effects and accumulators of heavy metals insults are still scarce, particularly in the tropics. Due to the strategic location of Chanchaga River, it is believed to be contaminated with heavy metals largely from mining, agriculture, construction works, fishing, domestic and industrial wastes from water processing for municipal supplies (Amadi et al., 2012; Edegbene et al., 2015; Mgbemena et al., 2020). Anthropogenic emissions from crude and artisanal mining activities and metal fetching from underground that are overwhelmingly on the increase around the river would consequently lead to further increase in heavy metals pollution of the river. This present study fills an important gap in the knowledge of the ecotoxicology field by evaluating the possible use of parasites as bio-monitors of heavy metals pollution.

MATERIALS AND METHODS

Study Area, Sampling and Sample Preparation

A total of 195 samples of the most dominant species of fish (72 *Claris gariepinus*, 58 *Tilapia zillii* and 65 *Raiamas nigeriensis*) in Chanchaga River were caught by the use of a trawling net over a period of 24 months (September 2014 and October 2016). Chanchaga River lies between latitude $8^0 43'$N to $9^0 40'$N and longitude $6^0 12'$E to $6^0 47'$E of the equator (**Figure 1**; Edegbene et al., 2015). Chanchaga River is massively polluted given various forms of human-influenced activities ranging from gold mining, crop farming and the presence of Niger State Water Board Authority along the river course (Amadi et al., 2012; Edegbene et al., 2019). Samples of water and fish were collected monthly

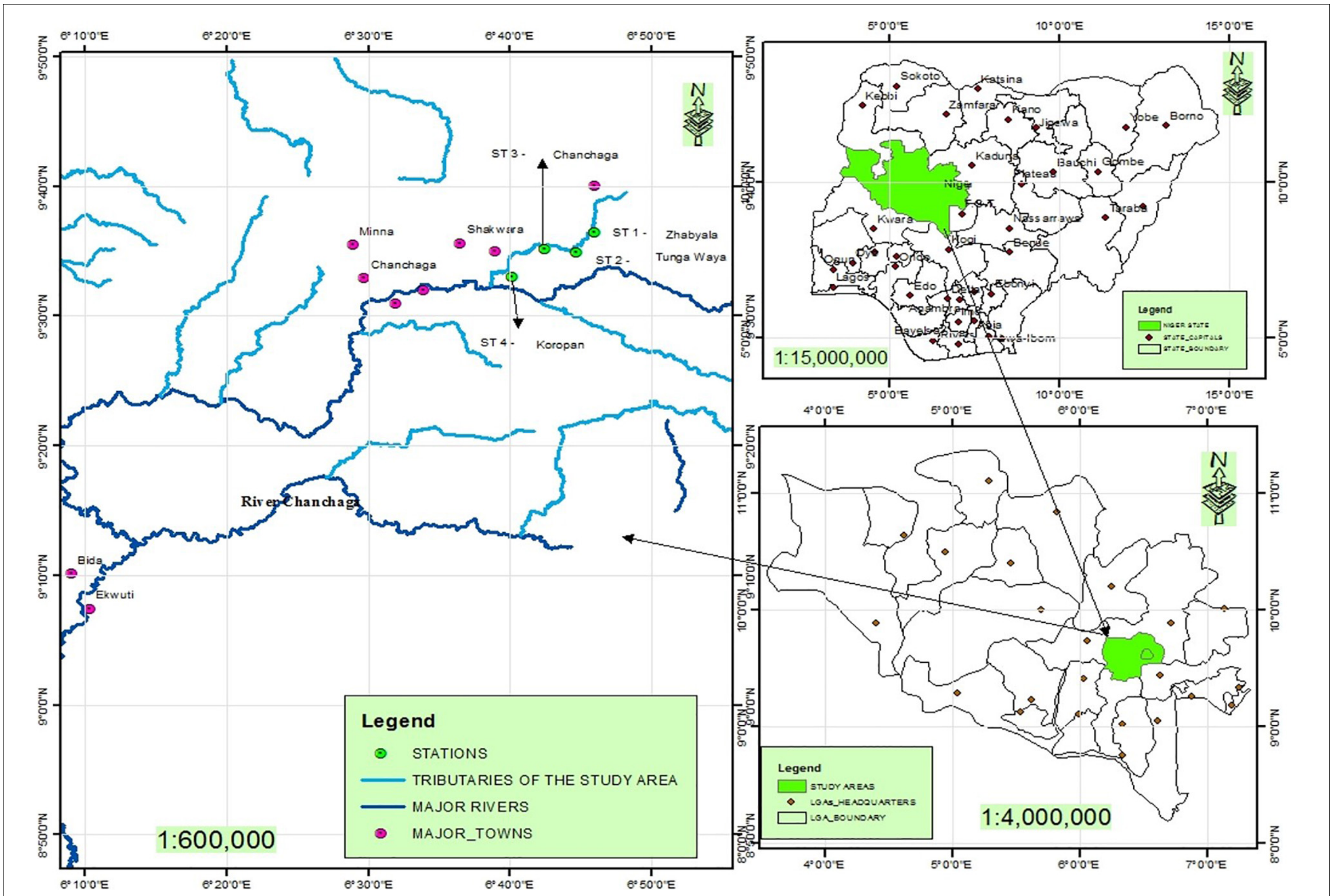

FIGURE 1 | Map of the study area showing the sampling stations, major towns. Source: Remote sensing laboratory, Department of Geography, Federal University of Technology, Minna.

for 24 months from four (4) different locations along the river (named Stations 1 to 4) based on contrasting degrees of anthropogenic disturbances.

Station 1

This station is located at Zhabyala village (latitude $9^0 40'$N and longitude $6^0 46'$E). Human activities are numerous and most intense in this station when compared to other stations. Some of the obvious human activities include welding processes as well as their related discharges, washing of motorcycles and vehicles, fishing by folks, agricultural processing, wood-fetching and cuttings especially of baboon tress, laundry, bathing alongside some small-scale industries like block-making, crude mining, and mechanic workshops. The riparian vegetation is an open vegetation because of the degree of disturbance by locals, and is characterized by mostly bamboo trees *(Bambusa vulgaris*) and some species of epiphytic plants.

Station 2

This station is located at Tunga Waya community of Bosso Local Government Area (latitude $9^0 35'$N and longitude $6^0 39'$E). Human activities here are reduced and not as much as those of Stations 1 and 4, and consist mostly farming of a number of cash crops like melon, cowpea, okra etc., Domestic activities like bathing, washing and defecation are intermittently observed here. The vegetation here is a dense canopy cover with shrubs and grass dominating the surroundings given the limited disturbances by locals.

Station 3

Station 3 is located in Chanchaga area of Minna town close to the Niger State Water Board which is where the name of river originates from (Latitude $9^0 32'$N and Longitude $6^0 34'$E). This site is approximately 4.23 km from Tunga Waya community. Like Station 2, human disturbances are greatly reduced here in comparison to Stations 1 and 4 with intense human disturbances. However, most activities occurring downstream include fishing, laundry, bathing, and passing of human wastes by locals. A canopy cover of mostly mango trees (*Mangifera indica*) is dominant in this station but the area is surrounded by moderately portioned lands. The riparian vegetation here is mostly Rubiaceae *(Nauclea latifolia*).

Station 4

This area is called the Koropan Community (Latitude $9^0 31'$N and Longitude $6^0 32'$E). It is about 1.5 km from the Water Board site. It is a community that harbors quite a number of residential structures and because of this human activities here are relatively high when compared to Stations 2 and 3. Agriculture practices, mining and numerous domestic activities including car washing, laundry and even fishing are also carried out on daily basis near the water body, but not as intense as those of Station 1. The riparian vegetation here is quite sparse or almost absent due to the presence of dense human activities.

Samples of muscles and middle intestines were taken from each fish and kept frozen at −20°C until being processed for heavy metal analysis.

The parasites were initially pooled alongside the fish hosts according to the different stations given that the information would be employed on station-basis for Canonical correspondence analysis (CCA). Carefully dissected fish from each of the stations were examined microscopically for endo-parasites, and helminths parasites found in the intestines of the infected fish were carefully collected using a dropper and washed severally in petri-dishes containing saline solution. Parasites were identified to their taxonomic groups according to their morphological features using relevant taxonomic keys (Hoffman, 1999). Collected helminthic parasites were carefully homogenized into a composite, and kept frozen at −20°C until being processed for heavy metals analysis. Helminth parasitic pool was then made for each of the fish species prior to heavy metals analysis.

Heavy Metals Analysis

Water samples from the four stations were filtered through a 0.45 μm membrane filter and acidified with suprapure HNO_3 to pH < 2, and then analyzed directly for Cu, Pb, Mn, Fe, Zn, and Cr heavy metals by Flame Atomic Absorption Spectrometry (Centre of Genetic Engineering laboratory in Federal University of Technology, Minna Nigeria), using Varian 5-AAS analytic Jena Spectrometer.

Fish muscle samples and parasite tissue samples were analyzed according to the method prescribed by Zimmermann et al. (2001). Firstly, the frozen samples were allowed to thaw, after which 150 mg by wet weight of the homogenized fish tissues or 50 mg of parasites were transferred to a 150 mL perfluoralkoxy (PFA) vessel. A solution containing a mixture of 2 mL HNO_3 (65%, suprapure) and 2.5 mL H2O2 (30%, suprapure) was added to the vessel and heated for 90 minutes at approximately 170°C in a microwave digestion system (CEM GmbH, Kamp-Lintfort, Germany; Model MDS-2000). As soon as digestion was over, the resulting solution was diluted to 5 mL with high-quality deionized water in a volumetric glass flask, and then analyzed for heavy metals (Cu, Pb, Mn, Fe, Zn, and Cr) by Flame Atomic Absorption Spectrometry (Centre of Genetic Engineering laboratory in Federal University of Technology, Minna Nigeria), using Varian 5-AAS analytic Jena Spectrometer. The optimization of the flame wavelength as well as the sample aspiration rate was performed in line with recommendations from manufacturers. The calibration was performed using four aqueous standards with analytical concentrations that are comparable to the linear response extent of the instrument, and also comprising similar concentrations of nitric acid as that of the sample. The analysis of the samples, standards, and blanks were conducted by the use three 10-s integration. The final concentration of heavy metals was achieved by preparing the reagent blank and its value was subtracted (Hassan et al., 2018).

Data Analysis

The range, mean and standard deviation for each parameter and Station were calculated. One-way Analysis of Variance (ANOVA) at 95% level of significance was used to compare means of concentrations of heavy metals among the four collection stations. Significant ANOVAs ($P < 0.05$) were followed by Tukey's *post hoc* HSD tests to identify differences among the means. The range, mean, standard deviation, ANOVA, and *post hoc* were conducted using SPSS software program (version 20). The student t test was used to evaluate significant difference between the heavy metal concentrations of the fish muscle and parasites. t test analysis was performed using PAST statistical package (Hammer et al., 2001).

The bioaccumulation factor (BAF) was calculated for each species of fish with parasites as recommended by Sures et al., 1999 as the ratio of metal concentration in the parasite and the host tissue (C[parasite]/C[host tissue]).

Canonical correspondence analysis (CCA) was used to evaluate the relationships between the parasites abundance (composition) and the heavy metals. CCA was conducted using PAST statistical package (Hammer et al., 2001). Prior to CCA, the test of assumptions of unimodality computed using DCA returned a gradient length of >3.0 (ter Braak, 1995), therefore we performed the ordination using CCA. CCA is a robust mechanism for unraveling complex datasets, and, being a direct gradient analysis, it enables combined analysis of both taxa and environmental data (ter Braak and Smilauer, 2002). The correlation coefficients between the species and the environment provided an estimate of the description of community pattern by individual environmental variables. The significance of the first three canonical axes was tested using a Monte Carlo permutation test with 999 permutations (Jckel, 1986).

RESULTS

The summary of heavy metals concentration from the four study stations in Chanchaga River is shown in **Table 1**. These values are compared with standard values of guidelines of USEPA (2010) for environmental health. As shown in this table, the heavy metals concentrations of the four stations were significantly different ($P < 0.05$) among the stations. The concentrations of Cu, Zn, and Cr were significantly highest at Station 1 while Pb concentration was significantly highest in Station 4. Stations 2, 3 and 4 were generally more similar in terms of heavy metals concentrations. The comparison with USEPA (2010) guideline indicated clear pollution of the river along the stations as Pb, Mn, and Fe were well above the safe limit of USEPA (2010); but Cr, Cu, and Zn values were either lower or equal to the safe limit.

The result of fish species collected from each station is presented in **Table 2**. The lowest numbers of *Clarias gariepinus* and *Tilapia zillii* were collected from Station 1 while the lowest

TABLE 1 | Summary of heavy metals (mg L^{-1}) concentrations of water in Chanchaga River study stations.

	Station 1	Station 2	Station 3	Station 4	*P* Value	USEPA, 2010
Copper*	0.07 ± 0.028^{a} (0.02–0.18)	0.023 ± 0.019^{b} (0–0.12)	0.0465 ± 0.023^{b} (0–0.13)	0.029 ± 0.019^{b} (0–0.12)	0.030	1.3
Lead*	0.48 ± 0.321^{a} (0–1.95)	0.36 ± 0.200^{a} (0–1.07)	0.55 ± 0.180^{b} (0.10–1.02)	0.73 ± 0.227^{c} (0–1.29)	0.018	0.015
Manganese*	0.45 ± 0.266^{a} (0–1.74)	0.42 ± 0.146^{a} (0.02–1.03)	0.14 ± 0.050^{b} (0.06–0.38)	0.14 ± 0.040^{b} (0–0.25)	0.037	0.05
Iron*	0.66 ± 0.146^{a} (0.18–1.19)	0.26 ± 0.113^{b} (0–0.72)	0.73 ± 0.227^{ac} (0.19–1.72)	0.68 ± 0.114^{ac} (0.31–1.00)	0.012	0.3
Zinc*	2.82 ± 0.894^{a} (0–5.78)	1.02 ± 0.234^{b} (0–1.77)	1.34 ± 0.337^{b} (0–2.28)	1.060 ± 0.372^{b} (0–2.30)	0.015	5
Chromium*	0.03 ± 0.006^{a} (0.02–0.05)	0.02 ± 0.005^{b} (0–0.04)	0.01 ± 0.003^{b} (0–0.02)	0.01 ± 0.009^{b} (0–0.04)	0.022	0.02

*Data are the means ± SE derived from monthly values with minimum and maximum values in parenthesis. Different superscript letters in a row show significant differences ($P < 0.05$) indicated by Tukey Honest significant difference (HSD) tests. *Significantly calculated F value detected by ANOVA.*

number of *Raimas nigeriensis* was collected from Station 4. Station 2 contributed the overall highest number of individuals (65) followed by Station 3 with 54, while the lowest number of fishes were collected from Station 1 with 36 individuals. A total of 152 (77.95%) of the 195 different fish species examined were infected with various helminthic parasites. In terms of percentage infection rate by species, 80.6% (58 out of 72 individuals), 74.1% (43 out of 58 individuals), and 78.5% (51 out of 65 individuals) were recorded for *Clarias gariepinus, Tilapia zillii, and Raimas nigeriensis*, respectively. The distribution of the infected fishes as per stations was 44, 41, 32.

A total of 1,310 parasites were encountered and identified, and the majority of these parasites were made up of the class Nematodae, which accounted for 97.3% (1274 parasites) of the entire endo-parasites. Other parasites that made up the remaining 2.7% included the classes Monogenea (*Dactylogyrus* sp.), Cestoda (*Polyonchobothrium clarias*), and Acanthocephala (*Acanthocephalus* sp.), and they contributed only 36 endo-parasites across the study stations. The class Nematoda was represented by *Camallanus* sp., *Capillaria* sp., *Eustrongylides* sp., *Cucullanus* sp. and *Alvinocaris markensis*.

The Fish Parasites and Heavy Metals Relationships

The CCA ordination revealed strong degrees of relationships between fish parasites abundance and measured heavy metals. The first two canonical axes accounted for as considerable as 92.62% of the variation in the parasite data set. An unrestricted Monte Carlo permutation test indicated that the first three canonical axes were significant ($P < 0.05$). As revealed by the convex hulls of the CCA, most of the heavy metals (Pb, Zn, Fe, and Cu) and majority of the parasites were associated to Station 1 that had the highest concentrations of the heavy metals sampled across the stations (**Figure 2**, **Tables 1**, **3**). Axis 1, which was strongly associated with most of the parasites, was mostly explained by Pb, Mn, and Fe. Samples from Station 1 were positioned on the left, close to the center point of the plot. Most of the samples taken from Stations 4 were positioned on the left, whereas those from Stations 2 and 3 were on the right. Axis 2 of the CCA plot was associated mainly with Cu and Mn. *Alvinocaris markensis* was particularly associated with station 2. Station 3 had no very close relationship with majority of the fish parasites. Conversely, many of the fish parasites were found to have very close relationship with station 1 with the highest concentrations of these heavy metals. Similarly, from the CCA ordination plot, species such as *Capillaria* sp., *Acantocephalo* sp., *Dactylogyrus* sp., *Camallanus* sp., *Eustrongylides* sp., and *Cucullanus* sp were characteristic indicators of the highest concentrations of heavy metals at station 1. These species were closely associated with increased heavy metal. The heavy metal, Zn has the closest relationship with the prevalence and abundance of these fish parasites and seemed a perfect indicator for most of the heavy metal insults. In specifics, the fish parasite, *Eustrongylides* sp., is heavily linked to Zn pollution.

TABLE 2 | Fish species distribution across the four sampled stations of Chanchaga River.

	Station 1	Station 2	Station 3	Station 4	Total
Clarias gariepinus	13	20	22	17	72
Tilapia zillii	9	21	17	11	58
Raimas nigeriensis	14	24	15	12	65
Total	36	65	54	40	195

Heavy Metal Concentration and BAF Between Fish Muscle and Parasite of *Clarias gariepinus, Tilapia zillii, and Raimas nigeriensis*

The heavy metal concentrations in fish muscle and parasite of *Clarias gariepinus, Tilapia zillii, and Raimas nigeriensis* are shown in **Table 4**. Generally, Fe, Zn, Mn, Cu, Cr, and Pb concentrations in the parasites of all fishes were clearly higher than those in the muscles of the fish hosts. However, no significant relationships

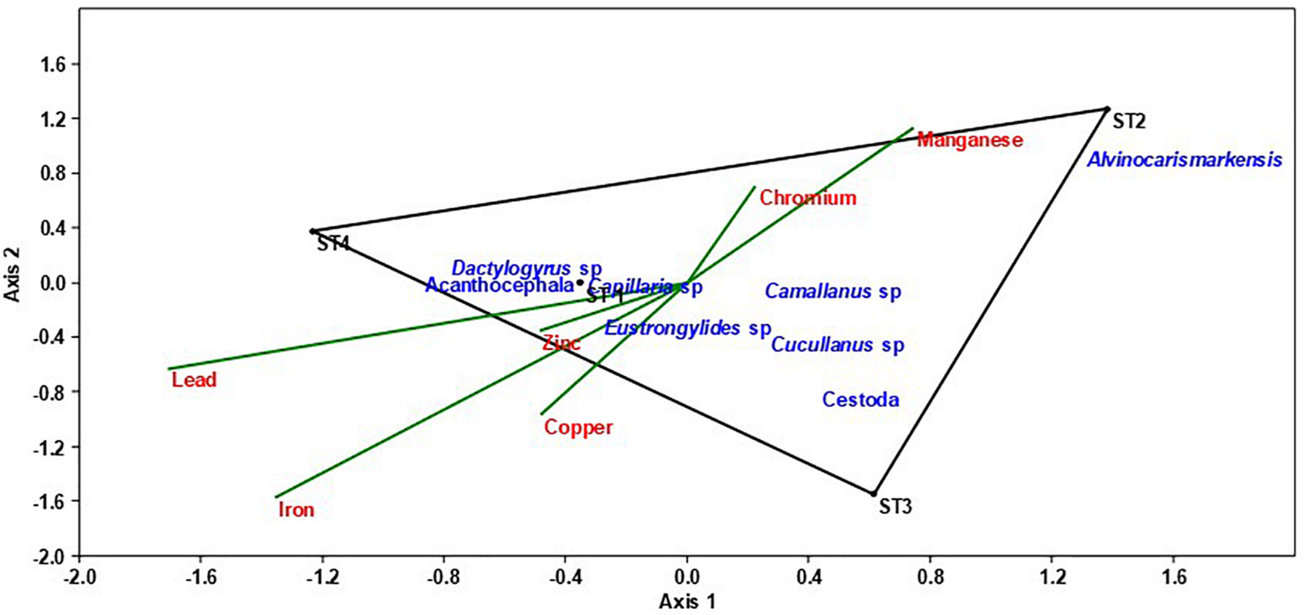

FIGURE 2 | Canonical Correspondence Analysis (CCA) ordination plots of Pb, Cu, Mn, Zn, Fe, and Cr association with helminths parasites metrics of the fish samples collected in Chanchaga River.

($P < 0.05$) were found between the heavy metals of the parasites and those of the muscles of the fish hosts. The metal concentrations in parasites of: *Clarias gariepinus* was in the order of Fe > Zn > Cr > Mn > Pb > Cu; *Tilapia zillii* was in the order of Fe > Zn > Mn > Cu > Cr > Pb; and that of *Raimas nigeriensis* was in the order of Fe > Zn > Cr > Mn > Cu > Pb. Again, for *Raimas nigeriensis*, Pb was not detected in the fish muscle but was detected in the parasites, and this further showed the bioaccumulation capacity of these parasites.

As shown in **Table 5**, the parasites showed relatively high abilities to accumulate heavy metals from the host fishes as the BAF of all metals of all species of fish were high (>1). The overall highest BAF (BAF = Concentration in parasite/Concentration in fish tissues) was obtained for Cu (BAF = 4.60) which presented accumulation ratio between parasites and fish muscle higher than other metals. While Cu (BAF = 4.60) showed highest BAF between parasites and *Tilapia zillii*, Mn (BAF = 2.80) showed highest accumulation factor between parasites and

TABLE 3 | Correlations of importance of the heavy metals in the prevalence of the parasites with the first three axes of canonical correspondence analysis (CCA) in Chanchaga River, Niger State.

Variable	Axis 1	Axis 2	Axis 3
Eigen value	0.1422	0.03479	0.01414
Proportion explained (%)	74.41	18.16	7.385
Copper	−0.2414	**−0.4837**	**0.8651**
Lead	**−0.8547**	−0.3148	−0.3833
Manganese	**0.3724**	**0.5665**	**0.7258**
Iron	**−0.6782**	**−0.7855**	0.1183
Zinc	−0.2435	−0.1773	**0.9731**
Chromium	0.1130	0.3544	**0.9209**

Significance of the axes by the Monte Carlo permutation test is given by F = 6.21 (P < 0.05) All canonical axes were significant. Values in bold indicate a significant difference at P < 0.05.

TABLE 4 | Heavy metal concentration (mg kg^{-1}) in fish muscle and parasite of *Clarias gariepinus, Tilapia zillii, and Raimas nigeriensis*.

Heavy metals	Fish muscle	Parasites
Clarias gariepinus		
Pb	0.071 ± 0.053	0.164 ± 0.015
Cu	0.085 ± 0.025	0.093 ± 0.033
Mn	0.149 ± 0.031	0.417 ± 0.142
Zn	1.395 ± 0.231	1.612 ± 0.281
Fe	1.446 ± 0.181	2.330 ± 0.383
Cr	0.259 ± 0.033	0.437 ± 0.090
Tilapia zillii		
Pb	0.105 ± 0.059	0.188 ± 0.067
Cu	0.121 ± 0.040	0.558 ± 0.069
Mn	0.278 ± 0.044	0.682 ± 0.213
Zn	1.086 ± 0.330	1.394 ± 0.288
Fe	1.830 ± 0.291	3.970 ± 0.513
Cr	0.261 ± 0.055	0.450 ± 0.077
Raimas nigeriensis		
Pb	0 ± 0	0.100 ± 0.068
Cu	0.070 ± 0.130	0.168 ± 0.030
Mn	0.211 ± 0.023	0.319 ± 0.073
Zn	0.550 ± 0.549	2.075 ± 0.131
Fe	1.482 ± 0.114	2.152 ± 0.171
Cr	0.316 ± 0.039	0.457 ± 0.057

Values are expressed in Mean ± Standard error of mean.

TABLE 5 | BAF for the analyzed metals in parasites and fish tissues of *Clarias gariepinus, Tilapia zillii, and Raimas nigeriensis.*

	Pb	Cu	Mn	Zn	Fe	Cr
Clarias gariepinus	2.31 ± 0.034	1.09 ± 0.029	2.80 ± 0.087	1.16 ± 0.256	1.61 ± 0.062	1.69 ± 0.062
Tilapia zillii	1.79 ± 0.063	4.60 ± 0.055	2.45 ± 0.129	1.28 ± 0.309	2.17 ± 0.402	1.72 ± 0.066
Raimas nigeriensis	2.00 ± 0.034	2.40 ± 0.080	1.51 ± 0.048	3.77 ± 0.340	1.45 ± 0.143	1.45 ± 0.048

Values are expressed in Mean ± Standard error of mean.

Clarias gariepinus; and Zn (BAF = 3.77) showed BAF factor between parasites and *Raimas nigeriensis*.

DISCUSSION

Heavy metals are important and acute markers of both fish well-being and the aquatic ecosystem at large (Padrilah et al., 2018). Assuredly, heavy metals being natural components of the aquatic ecosystem are important cofactor for most enzymes that are useful in fish metabolism (Jan et al., 2015). Human-mediated processes such as industrial, mining, and agricultural processes have eventually magnified their concentrations beyond recommended safe limits; with adverse consequences on human health given that the fish consumed by man have the capacity to bioaccumulate these heavy metals (Mehana et al., 2020). In our present study, the heavy metals concentrations revealed obvious pollution of Chanchaga River along the sampled stations – especially at Station 1 with the highest heavy metal values. The increased concentrations of heavy metals in Station 1 with the lowest number of fishes sampled among stations may not be unconnected with the heightened human disturbances in this station occasioned by the presence of artisanal miners, welding works, agricultural activities, among others from locals at this station. Apart from Cr, Cu, and Zn the values of all other heavy metals measured in this present study (Pb, Mn, and Fe) were well above the recommended safe limits of USEPA (2010) for environmental health and this could be attributed to a wide range of anthropogenic activities within the river, being a municipal stream. The consequences of this polluted status of the river is the impaired use of the water and its resources by both man and other aquatic biota – especially fish loss and eventual human-health implications. This could therefore be important in aquatic biomonitoring exercise as pollution effect indicators. Similarly, concentrations of As, Mn, Zn, Cu, Cr, Pb, Cd, and Fe above recommended safe limits have been reported recently in Chad Bath region, Jeddah coast, and elsewhere as ideal effect indicators of fish ecosystem degradation attributed to shipping industry, agricultural discharges, mining and other anthropogenic activities (Oumar et al., 2018; Rajeshkumar and Li, 2018; Mehana et al., 2020). Furthermore, the study of Kim et al. (2018) had reported higher concentrations of heavy metals in fish-based meals than its poultry-based counterpart.

The CCA ordination also showed that the fish parasites were significantly related with the heavy metals of Chanchaga River along the different degrees of pollution. Most of the measured heavy metals (Cu, Mn, Zn and Cr) were highest at Station 1. The combination of these variables might be used to identify and describe the multiple-scale stressor. The correlation of many individual heavy metals with the axes were relatively high for CCA but were not statistically significant. Perhaps, these estimated significances may be the results of the unmeasured heavy metals. Station 1 had very close relationship with majority of the fish parasites. The dominance of most parasites (*Capillaria* sp, *Acantocephalo, Dactylogyrus* sp., *Camallanus* sp., *Eustrongylides* sp., and *Cucullanus* sp.) at Stations 1 is indicative of the highest deteriorating biotic and overall ecological health of the river by heavy metals pollution at this station – and could be important in biomonitoring exercise. Zinc has the closest relationship with most of the fish parasites, and therefore, could be used as indicator of fish parasitic infestation. In specifics, the close linkage between *Eustrongylides* sp. and zinc could mean that *Eustrongylides* sp. was an ideal surrogate for zinc pollution, and could serve as early warning signal for possible zinc pollution of aquatic ecosystem. These helminthic parasites (*Eustrongylides* sp., *Capillaria* sp, *Acantocephalo, Dactylogyrus* sp., *Camallanus* sp., and *Cucullanus* sp.) have been employed as ideal effects indicators of heavy metal insults (Vidal-Martínez et al., 2014; Sures et al., 2017; Vidal-Martínez and Wunderlich, 2017; Hassan et al., 2018; Mehana et al., 2020). In like manner, Ashmawy et al., 2018 demonstrated that the elevated values of heavy metals were related to some helminthic parasites, such as Monogena, Nematodes, and Acanthocephala.

Fish parasites of the helminth group support the survival of their host amidst heavy metals pollution by accumulating higher concentrations of these heavy metals, and by that means act as metal sinks (Marcogliese et al., 2006; Eissa et al., 2012). Among the helminth group, the intestinal parasites access added metals in comparison to parasites that inhabit other body areas of the fish (Nachev et al., 2013). In the present study, the BAF for all heavy metals confirmed the high accumulation capacity of parasites given that heavy metal concentrations were higher in parasites of all fish species sampled in comparison to the fish muscles. Furthermore, the fact that Pb values were below detection limit in the muscle of *Raimas nigeriensis* but was found in the parasites inhabiting its intestine gives even more credence to the ability of these parasites to accumulate heavy metals from the fish body and serve as their heavy metal sink. This result also revealed the possibility of parasites accumulating a significant value of heavy metals from their fish hosts, enabling the fish hosts deal with elevated values of pollutants. Heavy metals ingested by the fish (through intestines or their gills) are transported through the blood to the fish liver, where majority of the metals are extracted to manufacture organo-metallic complexes which are conveyed with bile to either the intestine, to continue the liver-intestinal cycles, or taken out of the fish body by egestion (Sures, 2001 and 2003; Al-Hasawi, 2019; Mehana et al., 2020). Intestinal parasites do not have the capacity to manufacture their own cholesterols and fatty acids and, due to lipophilicity, they meddle in the cycle by easily ingesting the organo-metallic-bile complexes from their host, and this results to a reduction in the capacity of the fish host to accumulate metals (Al-Hasawi, 2019; Mehana et al., 2020). This could possibly suffice to define the higher concentrations of heavy metals in parasites in comparisons to the fish muscles in our study; and could also explain the reason Pb was not recorded from the muscle of *Raimas nigeriensis* but was found in the parasites. Our results are implicitly consistent with reports from some previous studies that helminth parasites (nematodes, cestodes, Acanthocephala) are ideal bioindicators of heavy metal insults in the aquatic ecosystem (Khaleghzadeh-Ahangar et al., 2011; Eissa et al., 2012; Mazhar et al., 2014; Tellez and Merchant, 2015; Sures et al., 2017; Vidal-Martínez and Wunderlich, 2017; Hassan et al., 2018). In the quest to achieve fast growth and development, the larva of nematodes absorbs biologically essential metals (Nachev et al., 2013; Hassan et al., 2016). The absence of digestive tract in cestodes necessitates their ability to accumulate more heavy metals relative to their hosts; and utilize the bile salts in reproduction (Hassan et al., 2018). Similarly, Acanthocephalans and nematodes have wide range of capacity to extract bile than the intestine of their host fish (Sures et al., 2017; Al-Hasawi, 2019). Bamidele and Kuton (2016) reported that intestinal nematodes accumulated more metals (Cu, Cr, Ni, Pb and Fe) than their fish host muscle (*Clarias gariepinus* and *Parachanna obscura*) in Lekki lagoon. Hassan et al. (2018) also reported that Cestoda parasites contained much more heavy metals than their fish host tissues.

To synthesize the major outcomes of this study, the result showed that intestinal helminths were able to reduce the concentrations of heavy metals in the fish tissues by accumulating them. Second, it further confirmed the possible application of helminthic parasites as early warning effect indicators of heavy metal pollution, as most of the parasites were associated to the station with the overall highest heavy metal concentrations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Environmental Protection Agency of Nigeria.

AUTHOR CONTRIBUTIONS

UK performed data analysis and wrote the manuscript. FA designed the study and wrote the manuscript. AM and IO designed the study and performed the sample collection and analysis. All authors read and approved the final manuscript.

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A Multivariate Approach to the Selection and Validation of Reference Conditions in KwaZulu-Natal Rivers, South Africa

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The use of reference conditions is essential to the monitoring and management of aquatic ecosystems. We examined existing and potential reference sites through historical data, maps, and field data collected from river sites in KwaZulu-Natal (KZN), South Africa. In our study, we applied nine criteria that best reflect the characteristics of South African rivers on 24 *a priori* selected reference sites. These nine criteria comprised of catchment conditions (flow modification and natural landscape) and site-specific attributes (water quality, human disturbance, river channel, water abstraction, riparian vegetation, riparian zone modification, and instream habitat quality). The *a priori* selected reference sites were subjected to validation using multivariate methods, such as analysis of similarities (ANOSIM), similarity percentages (SIMPER), and non-parametric multidimensional scaling (MDS) based on the macroinvertebrate fauna by applying a SASS5 threshold considered to be an indicator of undisturbed sites in South African rivers. We identified differences in the macroinvertebrate assemblages of the reference conditions for each river group based on their ecoregions, geomorphology and seasonal variations. Ecoregions and river geomorphology were better in the grouping of sites with similar reference conditions than the seasons. Our findings indicated that all of the selected sites could be considered as valid reference sites; however, caution should be taken in applying this method to lowland rivers because of their noticeable seasonal variability and habitat instability which tend to alter their reference states. We recommend that a type-specific reference condition be developed for lowland rivers. Also, statistical validation of reference conditions should be a continuous process in river biomonitoring.

Keywords: reference conditions, macroinvertebrate, multivariate analysis, geomorphology, rivers, biomonitoring, ecoregions

INTRODUCTION

The River Health Programme of South Africa, which recently metamorphosed into the River Eco-status Monitoring Program (REMP) (Department of Water and Sanitation [DWS], 2016) and the Water Framework Directive (WFD) (European Commission, 2000) recognize the importance of biological criteria in the validation of aquatic ecosystem status or quality (Chaves et al., 2006). This is because biological components of an aquatic ecosystem are good indicators of (1) water quality changes, which may be caused by organic pollution, hazardous substances or nutrient enrichment (eutrophication); (2) habitat modifications by physical disturbance, such as dam construction, canalization, dredging or other forms of construction activities; and (3) biological pressures on populations, such as the introduction of alien species (Nixon et al., 2003; Chaves et al., 2006). For example, a decrease in macroinvertebrate diversity and an increase in tolerant taxa are expected in the presence of stressors, which may be indicated using the SASS5 in South African rivers (Dickens and Graham, 2002).

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

The first step in the Ecological Classification process is the determination of RCs for each of the biotic components (diatoms, riparian vegetation, invertebrates, and fish fauna) of the river ecosystem being surveyed (Kleynhans and Louw, 2007). Establishing a RC and specifying ecological class boundaries allows accurate ecological evaluations of each site by comparing data from similar sites with little or no anthropogenic disturbances (Wallin et al., 2003; Bailey et al., 2004; Chaves et al., 2006). The RCs provide the fundamentals of measuring anthropogenic impacts, evaluate biological community potential; and spatial and temporal natural fauna distribution (Reynoldson et al., 1997; Economou, 2002; Wallin et al., 2003; Bailey et al., 2004). All RCs do not necessarily represent entirely undisturbed or pristine conditions, they often include minor anthropogenic disturbances (Chaves et al., 2006). Although low human pressure effects may be allowed in a RC, a high ecological status must always be achieved (Economou, 2002; Wallin et al., 2003; Bailey et al., 2004). Site hydromorphological and physicochemical attributes of a RC should meet the criteria of minimal disturbance for reference biological communities to be obtained (Reynoldson et al., 1997; European Commission, 2000).

Five different approaches or combinations of the approaches are currently being used in creating RCs for biological indices (Barbour et al., 1996; European Commission, 2000; Economou, 2002; Wallin et al., 2003). These are (1) expert judgment, (2) predictive modeling, (3) historical data, (4) extensive spatial surveys, and (5) paleo-reconstruction. Obtaining survey data is a reliable method for establishing a RC, especially in relatively undisturbed or minimally disturbed sites (Barbour et al., 1996; Wallin et al., 2003; Bailey et al., 2004; Nijboer et al., 2004).

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

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

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

MATERIALS AND METHODS

Study Area

This study was conducted in the major rivers of KZN. The study sites were spread across KZN covering 17 rivers and five ecoregions (Kleynhans et al., 2005) rivers (**Figure 1**). The use of

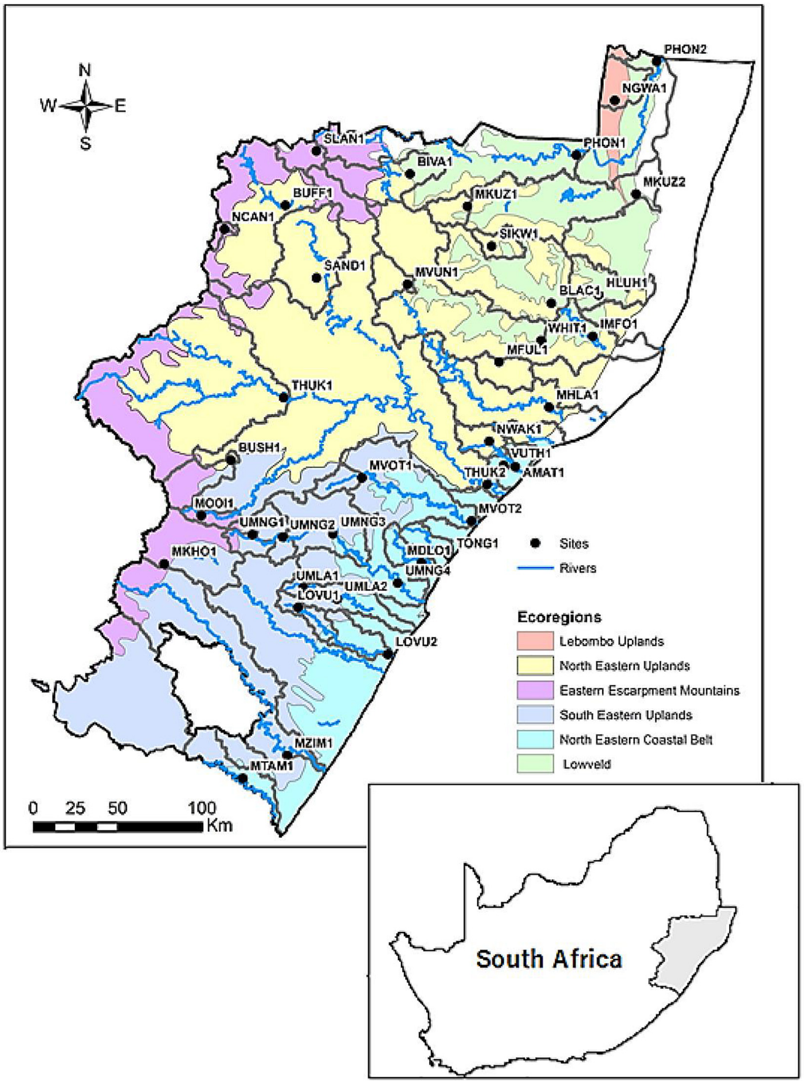

FIGURE 1 | River study sites in KwaZulu-Natal, South Africa for the study from March 2015 to March 2016.

ecoregion characteristics in grouping our study sites was because of their proven efficacy in a variety of applications, which includes ecosystem management, biotic conservation, climate change studies, and sustainable food production strategies (Omernik and Griffith, 2014). The altitudes ranged from 19 to 2098 m a.s.l within a variety of geomorphological zones (Rowntree et al., 2000; Moolman, 2008), ranging from headwater to lowland rivers. Some of the major rivers in this study included the Thukela, uMvoti, uMgeni, Phongolo, uMfolozi, Mooi, Mtamvuna, and Buffalo Rivers. The Thukela River is the longest river in the province, while the uMgeni River has five large dams/impoundments along its course.

Site Selection and Validation

We selected a total of 24 river sites (16 upland sites; 8 lowland sites) situated above major anthropogenic disturbances for this study. We used nine pre-defined criteria, comprising of catchment conditions (flow modification and natural landscape) and site-specific attributes (water quality, human disturbance, river channel, water abstraction, riparian vegetation, riparian zone modification, and instream habitat quality).

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

The South African Scoring System 5 (SASS5) (Dickens and Graham, 2002) was used to assess the macroinvertebrates well-being. The validation process involved the qualitative investigation of macroinvertebrates, habitat quality, and water quality. The minimum *a priori* validation criteria for SASS5 and VEGRAI were values >100 and >70, respectively. Water quality variables (temperature, pH, conductivity, and other related variables) were not considered in the validation process because natural or seasonal hydrogeological differences may cause variations or fluctuations in their measurement (Chaves et al., 2005, 2006; Meinson et al., 2015).

Macroinvertebrates Sampling

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

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

Data Analyses

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

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

RESULTS

Macroinvertebrate Taxa Composition and SASS5

The combined results of the four sampling seasons showed that the macroinvertebrate communities clustered primarily by the river type or geomorphology, with upland streams being approximately 75.5% dissimilar from the lowland rivers of KZN while within-group similarity of the upland sites was 27.1% and the similarity within the lowland sites was 24.1% (**Table 1**). The SIMPER analysis showed that Baetidae had the highest similarity percentage contributions for both upland and lowland groupings at approximately 22.2 and 14.2%, respectively, while Tipulidae contributed the lowest similarity percentage (1.1%) in the upland sites and Notonectidae contributed the lowest similarity percentage (1.2%) in the lowland sites. Atyidae contributed the highest dissimilarity percentage (7.1%) between the upland and lowland sites, while the Athericidae and Tipulidae both contributed the lowest dissimilarity percentage (1.0%) (**Table 1**). For the ecoregions, within-group similarities were 13.8% (LOWV), 27.9% (NEU), 28.7% (SEU), 29% (EEM), and 31.3% (NECB) (**Table 2**). The cut off for low contributing taxa was 90% as calculated from the Bray-Curtis resemblance matrix, which meant that taxa with less than 10% contributions were excluded from the SIMPER analysis. Taxa that contributed to within-group similarity were

TABLE 1 | Dissimilarities in macroinvertebrate taxa between upland and lowland rivers of KwaZulu-Natal, South Africa, from 2015 to 2016.

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

Mean dissimilarity = 75.50%. Diss, dissimilarity; SD, standard deviation.

TABLE 2 | Macroinvertebrate taxa contributing within-group similarities of different river ecoregions of KwaZulu-Natal, South Africa, between 2015 and 2016.

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

SEU, Southeastern uplands; NECB, northeastern coastal belt; EEM, eastern escarpment mountain; NEU, northeastern uplands; LOWV, lowveld; ×, taxa occurrence.

relatively constant for both river types; the upland group had 24 taxa, while the lowland group had 23 taxa (**Table 3**). The SASS indices clearly distinguished between sites, with the upland sites different from the lowland sites (**Figure 2**). The ecoregions also clearly separated from each other. Although there were clear separations between sites and between ecoregions, there were some similarities in taxa composition. The similarities in taxa composition between the upland and lowland sites could have been the reason for their mixed clusters at 40% similarity (**Figure 2**).

Longitudinal Gradients

Longitudinal gradients influenced the macroinvertebrate taxa clusters, although in a mixed selection of both upland and lowland KZN river groups (**Figure 2**, MDS: 2D-stress = 0.18). At 40% similarity, five distinct clusters were formed (**Figure 2**), MDS 2-D Stress = 0.18). Upland and lowland rivers were 75.5% dissimilar, with several taxa differentiating the groups (**Table 2**). Several sensitive taxa that are typical of headwater streams (e.g., Baetidae, Perlidae, Heptageniidae, Psephenidae, and Athericidae) were among the distinguishing taxa. The best predictor variables were SASS score and longitude according to the results of the MDS and distance-based redundancy analysis (dbRDA) plot (**Figure 3**), although the influence of other factors was significant

TABLE 3 | Macroinvertebrate taxa contributing to within-group similarity in the upland (27.13%) and lowland (24.05%) rivers of KwaZulu-Natal, South Africa, between 2015 and 2016.

Species	Upland	Lowland
Aeshnidae	×	
Atyidae	×	×
Baetidae	×	×
Belostomatidae		×
Caenidae	×	×
Chironomidae	×	×
Coenagrionidae	×	×
Corixidae	×	
Elmidae	×	×
Gomphidae	×	×
Heptagenidae	×	×
Hydropsychidae	×	×
Leptoceridae	×	×
Leptophlebiidae	×	×
Libellulidae	×	×
Naucoridae	×	
Notonectidae	×	×
Oligochaeta	×	×
Perlidae	×	×
Philopotamidae		×
Planorbidae		×
Potamonautidae	×	×
Psephenidae		×
Simuliidae	×	
Thiaridae		×
Tipulidae	×	
Tricorythidae	×	×
Veliidae	×	

×, *taxa occurrence.*

among the classification groups. The result of the dbRDA plot showed that SASS scores influenced 59.1% of fitted and 15.1% of the total variation in macroinvertebrate taxa composition, while longitudes influenced 40.9% of fitted and 10.4% of total variation (**Figure 3**). SASS score, number of taxa, ASPT, latitude, longitude, and altitude, were good predictors, while biotopes were not.

Classification Strength

Macroinvertebrate taxa composition within all classification groups of KZN rivers were not significantly different, as indicated by the Global *R*-values (**Table 4**). Hence their reference conditions can be used interchangeably in assessing the rivers between the groups (**Table 4**). All the groups had significant differences ($p < 0.05$) and could not be used as reference sites in assessing the sites between the groups. This showed that all the classification groups had higher within-class similarity than between-class similarity. The ecoregion classification had the largest Global *R*-value. The pairwise results suggested that seven pairs of ecoregions were significantly similar, while the three pairs were different (**Table 4**). Macroinvertebrate taxa compositions were considered homogenous within classification groups, but not between groups (**Figure 3**). The rating, based on the Global *R*-values showed that ecoregions had the highest classification strength, although they were relatively too weak for between-group comparisons. The closer the Global R is to 1, the more positive the result (Clarke, 1993; Clarke and Warwick, 1994, 2001).

DISCUSSION

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

Site Selection and Validation

The identification and selection of undisturbed or minimally disturbed lowland rivers were difficult, and the few included in this study had the best applicable conditions. Sites with incomplete datasets and unstable habitat conditions were excluded from further analyses. Site validation is essential in the determination of RCs because it provides the quantitative measurements of both biotic and abiotic variables that characterize a river system and helps to confirm or refine the pre-selection criteria (Barbour et al., 1996; Chaves et al., 2006). Thus, we adapted the method of Chaves et al. (2006) for site validation, where the biological indices used for validation were the riparian vegetation and macroinvertebrate compositions (**Table 5**).

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

Analysis of Similarity

At the ecoregional scale examined in this study, macroinvertebrate assemblages showed distinct separation, as the percentage dissimilarities were high between ecoregions. The lowest dissimilarity percentage occurred between the

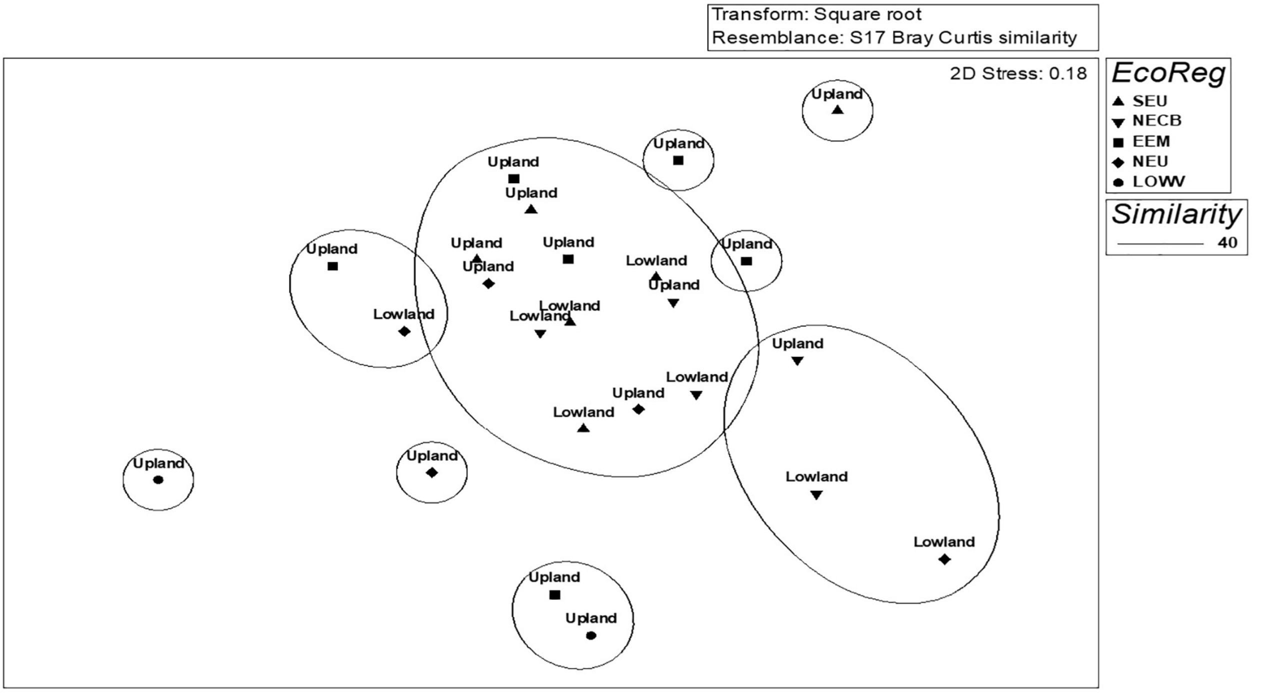

FIGURE 2 | Non-metric multidimensional scaling (MDS) ordination showing the classification of sites based on macroinvertebrate taxa collected in summer 2016 in rivers of KwaZulu-Natal, South Africa. Sites were coded by group geomorphology (upland and lowland) and the shapes represent the ecoregions. SEU, south eastern uplands; NECB, north eastern coastal belt; EEM, eastern escarpment mountains; NEU, north eastern uplands; LOWV, lowveld.

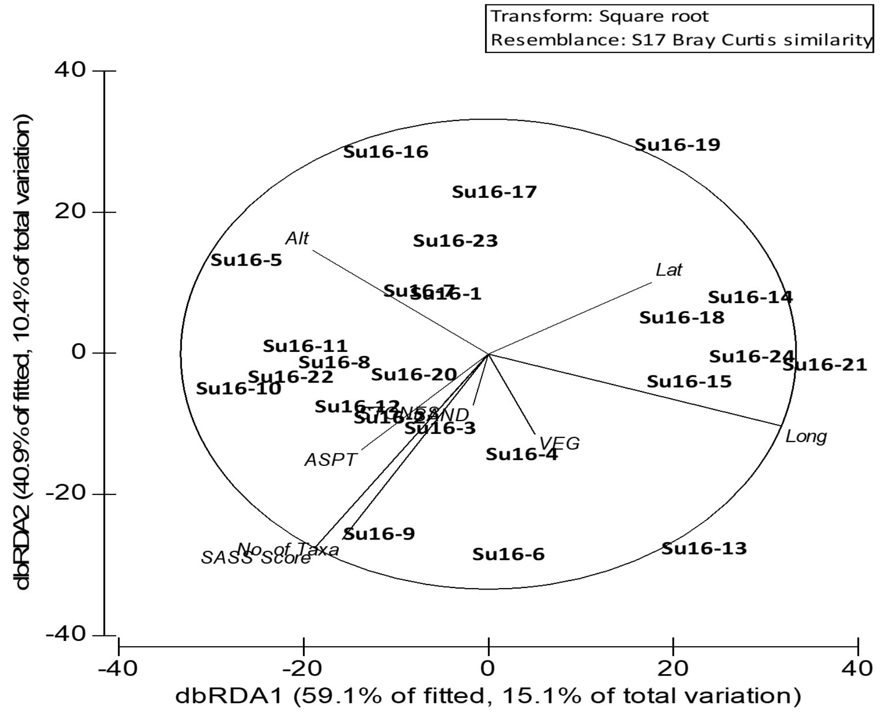

FIGURE 3 | Redundancy Analysis (RDA) of SASS indices, biotope availability and geographic location, using macroinvertebrate abundance in KwaZulu-Natal rivers in summer 2016. Alt, altitude; Lat, latitude; Long, longitude; VEG, vegetation; ASPT, average score per taxon; SASS Score, South African Scoring System score; Su16, summer 2016.

TABLE 4 | Pairwise tests of the analysis of similarity (ANOSIM), indicating the Global R, and Statistic *R*-values in the present study.

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

All tests with $P < 0.05$ were significantly similar. The number of sites (n) in each classification group is given wherever possible.

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

While ecoregional classifications based on macroinvertebrate assemblages are capable of partitioning variability in macroinvertebrate assemblages, an amount of variation in the spatial factors often remains within the classification classes (Dallas, 2004a; Stoddard et al., 2006). These factors may be at the level of river type or other aspects, such as width, depth, substratum, biotope availability, hydrological-type, and canopy cover (Dallas, 2004a; Hawkins et al., 2010). This study revealed that some upland and lowland sites were similar within the same ecoregion, though were partitioned by the longitudinal gradients. Hence, supporting the suggestion of Dallas (2004b) that, longitudinal partitioning may be incorporated into bioassessment in South Africa by separating upland sites from the lowland ones. Many studies have reported distinct differentiations in biotic assemblages between montane and non-montane regions (Tate and Heiny, 1995; Dallas, 2004a); also, that topography and climate are good partitions of biotic variation (Hawkins and Vinson, 2000). Our results showed that river types or geomorphology (upland and lowland river types) have distinct macroinvertebrate assemblages (75% variation), which showed that the RCs of each river types were different in terms

TABLE 5 | Selection criteria for minimally disturbed KwaZulu-Natal river sites (adapted from Chaves et al., 2006).

Criteria	Spatial scale	Description	References
1. Water quality	Site	Visual inspection of the water quality based on color, clarity, odor, and oil film	Hughes, 1995; Barbour et al., 1996
2. Human disturbance	Site	Assessment of the presence of garbage, sewage pipes, industrial effluents pipes, and livestock grazing	Hughes, 1995; Barbour et al., 1996; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009
3. Flow modification	Catchment	Presence of dams higher than 20 m was considered to disturb the natural flows of the sites irrespective of the distance to the sampling site	Hughes, 1995; Barbour et al., 1996; Muhar et al., 2000; Ehlert et al., 2002; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009
4. Natural landscape	Catchment	The level of natural use of the site's drainage area; the degree of usage should be as low as possible for the reference site: <10% of urban and industrial use and <30% of agricultural use	Barbour et al., 1996; Hering et al., 2003; Sánchez-Montoya et al., 2009
5. Natural channel	Site	Presence of bank and bed fixation, artificial channels, and small transversal ditches	Hughes, 1995; Barbour et al., 1996; Ehlert et al., 2002; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009
6. Water abstraction	Site	Presence of hydropeaking, irrigation canals, and water withdrawal for reservoirs, domestic water supply, etc.	Hughes, 1995; Muhar et al., 2000; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009
7. Riparian vegetation	Site	Riparian vegetation cover; ideally should be in near-natural condition, most river types should have total cover and presence of trees in the pristine situation, however, temporary or very high-altitude streams can have different cover levels.	Ehlert et al., 2002; Sánchez-Montoya et al., 2009
8. Riparian zone modification	Site	Presence of recreational facilities, industries or other buildings, such as warehouses, croplands, and tarred roads (spatial disturbances); it should be covered with natural unmanaged vegetation	Hughes, 1995; Muhar et al., 2000; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009
9. Instream habitat quality	Site	Presence of snags, roots, wood logs and dead overhanging vegetation; substrates: boulders and stones in upper reaches, cobble and pebbles in middle stretches and sand, clay, and lime in lower regions; also assess the sediment retention level	Hughes, 1995; Barbour et al., 1996; Ehlert et al., 2002; Hering et al., 2003; Nijboer et al., 2004; Sánchez-Montoya et al., 2009

of taxa composition. Our study showed macroinvertebrate taxa composition within all classification groups of KZN rivers were not significantly different, as indicated by the Global *R*-values. This means that sites that fall within the same groups can be used in the comparing of impaired sites in bioassessment. All groups having significant between-group differences ($p < 0.05$) cannot be used as reference sites in assessing each other.

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

CONCLUSION

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

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

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

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

OA conceived the manuscript with CD and GO'B. OA collected and analyzed the data and wrote the manuscript. CD and GO'B supervised the study and contributed valuable comments to the manuscript. All authors contributed to the article and approved the submitted version.

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Ecological Risk of Water Resource Use to the Wellbeing of Macroinvertebrate Communities in the Rivers of KwaZulu-Natal, South Africa

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

Keywords: bayesian networks, ecological risk, macroinvertebrates, multiple stressors, habitat, relative risk model, risk assessment

INTRODUCTION

Water as a natural resource is essential to life, the environment, industrial growth, development, food production, hygiene, sanitation and power generation (Rast, 2009; DWA, 2010). River systems also provide many goods and services upon which society depends, such as maintaining the habitat and integrity of aquatic organisms, transportation of sediment, recreational and eco-tourism centres (DWA, 2010). The river systems also serve as disposal sites for industrial effluent and solid wastes (DWA, 2010). Global use of freshwater increased by 10% from 2000 to 2010 because of an increase in population growth and economic development (Vörösmarty et al., 2010).

The anthropogenic demands on freshwater ecosystems cause enormous threats to biodiversity around the world (Dudgeon et al., 2006), through various contaminants which may be chemical, physical, radioactive or pathogenic, and maybe from multiple sources, including industrial effluents, agricultural run-off, domestic sewage, construction and mining activities (Alves et al., 2014; Nitasha and Sanjiv, 2015).

Risk assessment is a method used to calculate the probability of the impacts of an unwanted effect on a set of predefined assessment endpoints over a period (Suter, 1993; Walker et al., 2001; Landis and Wiegers, 2007; Hines and Landis, 2014). Ecological Risk Assessment (EcoRA) is a systematic method of describing and explaining scientific facts, laws and relationships to provide a sound basis for developing adequate protection measures for the environment (USEPA, 2000). A relative risk model (RRM) is a cause and effect model used in the calculation of risks to assessment endpoints because of multiple stressors having impacts on the endpoints of a system or habitat (Landis and Wiegers, 2005). The RRM methodology is an improved and expanded version of the traditional three-phase risk assessment method which involves problem formulation, risk analysis and risk characterization. Landis and Wiegers (1997) developed a framework called the regional-scale ecological risk model for ranking and comparing the risks associated with multiple stressors, and this is a useful tool for describing and comparing risks to valued resources (endpoints) within a catchment or region (O'Brien et al., 2018). Risk assessment at a regional scale involves the assessment of multiple habitats with multiple sources of multiple stressors affecting multiple endpoints at a relatively large spatial coverage (Hunsaker et al., 1989; Landis and Wiegers, 1997). While the traditional risk assessment often has only one endpoint, the regional risk methodology usually has multiple endpoints (Walker et al., 2001). Various stressors impinge on the quality of the environment within any region, and the assessment of these stressors may be incomplete if there is no objective framework for the evaluation of the risks associated with the stressors (Linkov et al., 2006). At the regional scale, considerations of multiple sources of stressors affecting various endpoints are allowed (Landis and Wiegers, 2005), because there may be many sources for a single stressor (Liu et al., 2010). Also, a regional scale risk assessment allows for landscape characteristics which may affect the risk estimates of a region (Landis and Wiegers, 2005). However, it is difficult to measure, test, model or assess all the components of the environment at a regional scale and the difficulty arises from the high degree of spatial and temporal variability of regional components (Suter, 1993). The typical impacts considered in risk assessment are mortality, chronic physiological impacts and reproductive defects of the target species or humans (Walker et al., 2001; Mommaerts et al., 2010; Nordberg et al., 2018).

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

Our study aimed to conduct a regional ecological risk assessment of stressors in the rivers of KwaZulu-Natal (KZN) Province, South Africa, to macroinvertebrate biodiversity and ecosystem protection (endpoints) using the BN-RRM approach. We established three objectives in this study. Our first objective was to develop a RRM to estimate the relative contribution of risk from stressors to the selected ecological endpoints. Our second objective was to determine which regions and endpoints were at high risk from anthropogenic activities. Our third objective was to simulate one hundred percent (100%) low risk to the endpoints (representing pristine condition or before urbanization and industrial development) into the model to evaluate the relative risk impacts of the sources and habitats to the selected endpoints. We expected this study to give an insight into the threats from the land use types of KZN, reveal their probable risk and lay the foundation for regional ecological risk assessments of the freshwater resources of KZN.

Our study was conducted on a large scale, making use of all the national indices of river health (fish, vegetation, diatoms, and macroinvertebrate) in South Africa. The risks associated with each of these indices have been addressed through different publications and reports. The specific focus of this study was the risk to macroinvertebrate index, which is made possible by the fact that each of the indices can be used independently of the others. Also, the risk scenarios used in this article were simulations of the risks that could occur at the risk regions (i.e., each sampling point), except for the "Current Scenario" which was obtained from our field results. The 100% low-risk simulation represented the resource management goal for South African rivers.

METHODS

Study Area

KZN Province of South Africa was selected for this study and is located within the eastern escarpment catchment of South Africa, containing four of the 22 primary drainage regions of South Africa, either wholly or partially (Midgley et al., 1994). The mean annual rainfall (MAR) range across the province is ∼616–936 mm (South African Weather Service, 2020) and is drained by the major river systems in the province. Each of the major rivers flows through distinct longitudinal patterns, although they typically exhibit a distinct escarpment zone, with flatter mid-slopes and steep eastern coastal regions (Rivers-Moore et al., 2007). At a scale of 1:500,000, drainage densities for each primary catchment within KZN ranged from 0.03 to 0.51 km of river per km^2, with a mean density of 0.240 km^2 and an average coefficient of variation of 38.6% (Rivers-Moore et al., 2007).

In this study, we chose a total of 39 KZN river sites, and each site represented a risk region (RR) (**Figure 1**), based on their sub-quaternary catchments, proximity to risk sources, habitat characteristics and ecological endpoints. The highest (5th) river order in KZN is the Thukela; other long river systems (4th order streams) are the Phongolo, Buffels and Mzimkhulu Rivers (Rivers-Moore et al., 2007). The uMvoti and Mhlatuze catchments have the highest drainage densities, the southern KZN regions (Mzimkhulu, Mkomazi and uMgeni catchments) also have relatively high drainage densities, while the northern coastal Zululand regions (Mkuze River and Phongola catchment) have the lowest drainage densities (Rivers-Moore et al., 2007). The uMgeni River catchment, spanning 4,418 km^2 is reputed to be one of the most reliable (providing sufficient water supply for human use) large rivers of South Africa (Van Der Zel, 1975) and it has five large dams located on its course for domestic water supplies. Our study was conducted using the relative risk model (RRM), which is made up of three main phases: problem formulation, risk analysis and risk characterization (Landis and Wiegers, 1997, 2005).

Data Collection

For the biodiversity endpoint, macroinvertebrate data were collected from three distinct biotopes grouped into stone, vegetation and GSM (gravel, sand and mud) using a kick net according to the South African Scoring System 5 (SASS5) protocol (Dickens and Graham, 2002). The sampling protocol involves collecting only one sample per biotope group, but care was taken to ensure that all the available biotopes were qualitatively sampled. We sampled each biotope separately (i.e., one sample per biotope), and the macroinvertebrates were preserved in 80% ethanol for taxonomic resolution and taxa abundance counts in the laboratory. The biotopes are stones-in-current (SIC) represented by pebbles and cobbles (2–25 cm), and boulders (25 cm); Stones-out-of-current (SOOC) including pebbles and cobbles, and boulders in pools of water; Marginal vegetation including vegetation growing on fringes and edges of the rivers, while aquatic vegetation was that mostly growing (may

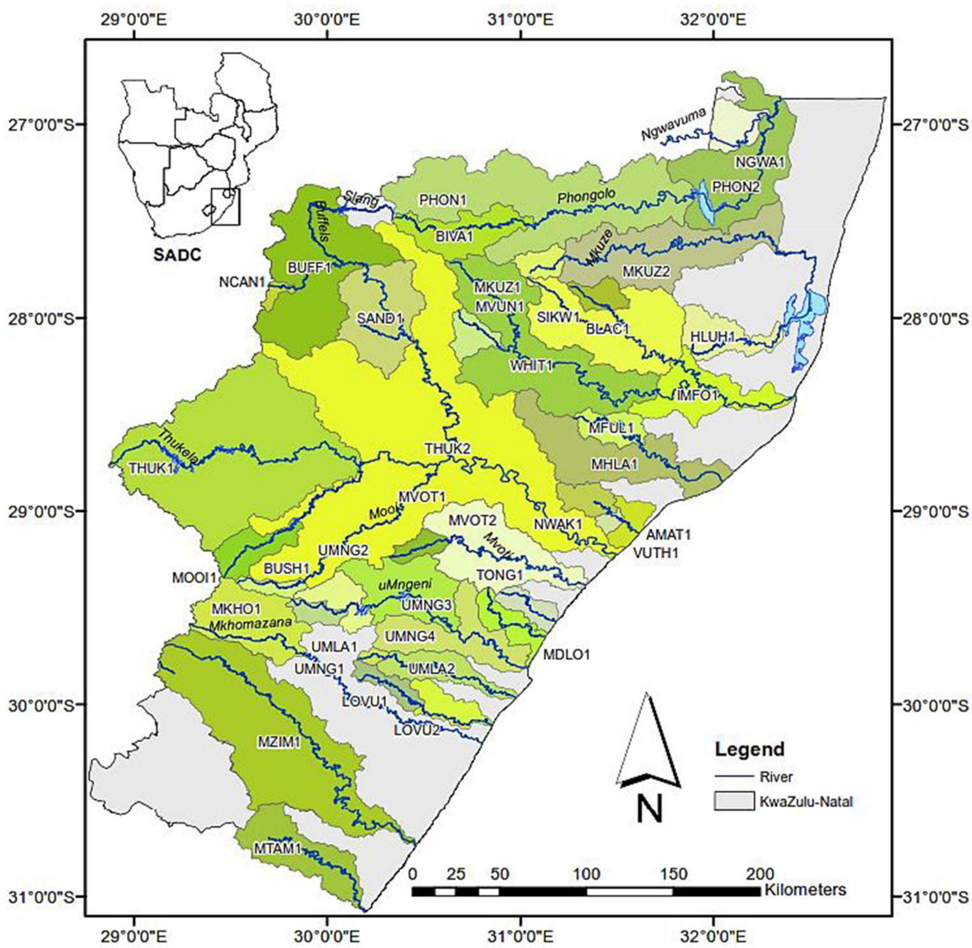

FIGURE 1 | Risk assessment regions of KwaZulu-Natal, South Africa, from 2015 to 2016 [Inset: map of Southern Africa Development Countries (SADC)].

or may not be submerged) inside river channel. Gravel was small stones usually < 2 cm in diameter, while sand and mud were smaller than 2 and 0.06 mm, respectively.

The SASS5 data interpretation is based on the calculation of the SASS5 score (the sum of the sensitivity weightings for taxa present at a site) and average score per taxon (ASPT). The ASPT is the ratio of the SASS score and the number of taxa (Dickens and Graham, 2002; Dallas, 2004). SASS5 data were used in generating MIRAI (Macroinvertebrate Response Assessment Index) scores (Thirion, 2016).

For the ecosystem endpoint, habitat quality and water quality data were used. We measured basic *in situ* water quality parameters (temperature, dissolved oxygen, pH and electrical conductivity) at each site on every sampling occasion using the YSI model 556 MPS handheld multi-probe water quality meter. Habitat data were assessed according to their abundance and quality in supporting the macroinvertebrate abundance and richness.

Problem Formulation

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

Conceptual Model

Our conceptual model describes the hypothesized relationships between the chosen risk sources, stressors, habitats, receptors and impacts to endpoints selected for the study (O'Brien et al., 2018) (**Figure 2A**). A source is an entity that releases single or multiple stressors to the environment (e.g., industrial waste of effluent) or the action that produces stressors (USEPA, 2000), while stressors are the physical, chemical or biological substances that can cause an adverse effect (USEPA, 2000). We chose 10 sources of risks impacting on the rivers of KZN for this study (**Figure 2A**). These ecological risk sources relating to the rivers of KZN were grouped into five major categories to describe the effects of their water resource utilization on the selected risk regions. The categories were industrialization (manufacturing, mining and forestry), agriculture (sugarcane, commercial and subsistence farming), natural vegetation, settlements (rural and urban), and construction (roads, rails and dams).

The stressors evaluated in this study were water quality alteration/abstraction, habitat alteration and flow alteration. These stressors were the resultant synergistic effects or interactions of the risk sources as are being influenced by the anthropogenic activities and natural events within the study area (Hua et al., 2017). The various synergistic interactions of the risk sources linked to each stressor are shown in **Figure 2A**. Each source of risk or threat to our endpoints has varying degrees of stress being exerted on the risk regions (Liu et al., 2010; Bednarek et al., 2014; Lu et al., 2015; Mekonnen et al., 2016).

The instream habitat was selected to represent water quality, flow and habitat stressor states of the risk regions, while riparian vegetation was selected to represent the physical habitat structure and the vegetation response assessment index (VEGRAI) of the risk regions (**Figure 2A**). Habitat was included in the conceptual model of this study because each of the stressors have either direct or indirect effects on the habitat quality of the risk regions and the habitat quality also determines the well-being of the target organisms or receptors (Obery and Landis, 2002; Villeneuve et al., 2018).

A receptor is a biological or ecological component that is exposed to the stressor, while the attribute is the important characteristic of the ecological component to be protected (Hua et al., 2017). Macroinvertebrates are the receptors in this study, while the attributes were ecoregions and river health (**Figure 2A**). Macroinvertebrates well-being could be influenced by a combination of the sources of risk, stressors and habitat quality within the risk region. Ecoregions represent the potential for habitat quality, which determines the increase or decrease in the diversity of macroinvertebrates; while river health (i.e., MIRAI) provides the indications of existing responses of macroinvertebrates to the drivers of the ecosystem or stressors. The ecoregion and river health were used as attributes of ecosystem threat because they both have impacts on macroinvertebrate species composition or well-being (Thirion, 2016). Ecoregion attribute was combined with ecosystem threat to assess the biodiversity endpoint because ecoregions determine the diversity of macroinvertebrates in South African rivers; while the river health (MIRAI) attribute was combined with ecosystem threat to assess the risk to the ecosystem endpoint because river health is a good indicator of ecosystem impairment or quality/sustainability; especially because macroinvertebrate species abundance and diversity are factors calculating the MIRAI data.

Assessment endpoints can be made up of a receptor and an attribute (e.g., macroinvertebrate biodiversity as in this study) (USEPA, 2000). The assessment endpoints should not only be the characteristics of the receptors and aims of the assessment, but they should also be quantitative measurements of the possible degrees of the impacts to the receptors (Hua et al., 2017). For this study, we chose biodiversity and ecosystem well-being as the risk endpoints. This is because a viable biodiversity and quality ecosystem will ensure the sustainable ecological integrity of the risk regions. Ecosystem threat was used to represent the receptor (macroinvertebrate) in the BN-RRM because it better helps in visualizing the effects of the attributes (ecoregion and river health) on the ecological integrity of the risk endpoints (**Figures 2B–D**).

Risk Calculation and Simulation

The evidence used in our assessment was obtained from field assessments between September 2014 and March 2016. The RRM was used to develop a conceptual model, which was used to represent the hypothetic relationships between the sources of stressors, stressors, the ecological components (habitats and receptors) and their associated endpoints (USEPA, 2000; Landis and Wiegers, 2005) (**Figure 2A**). The conceptual model was

A

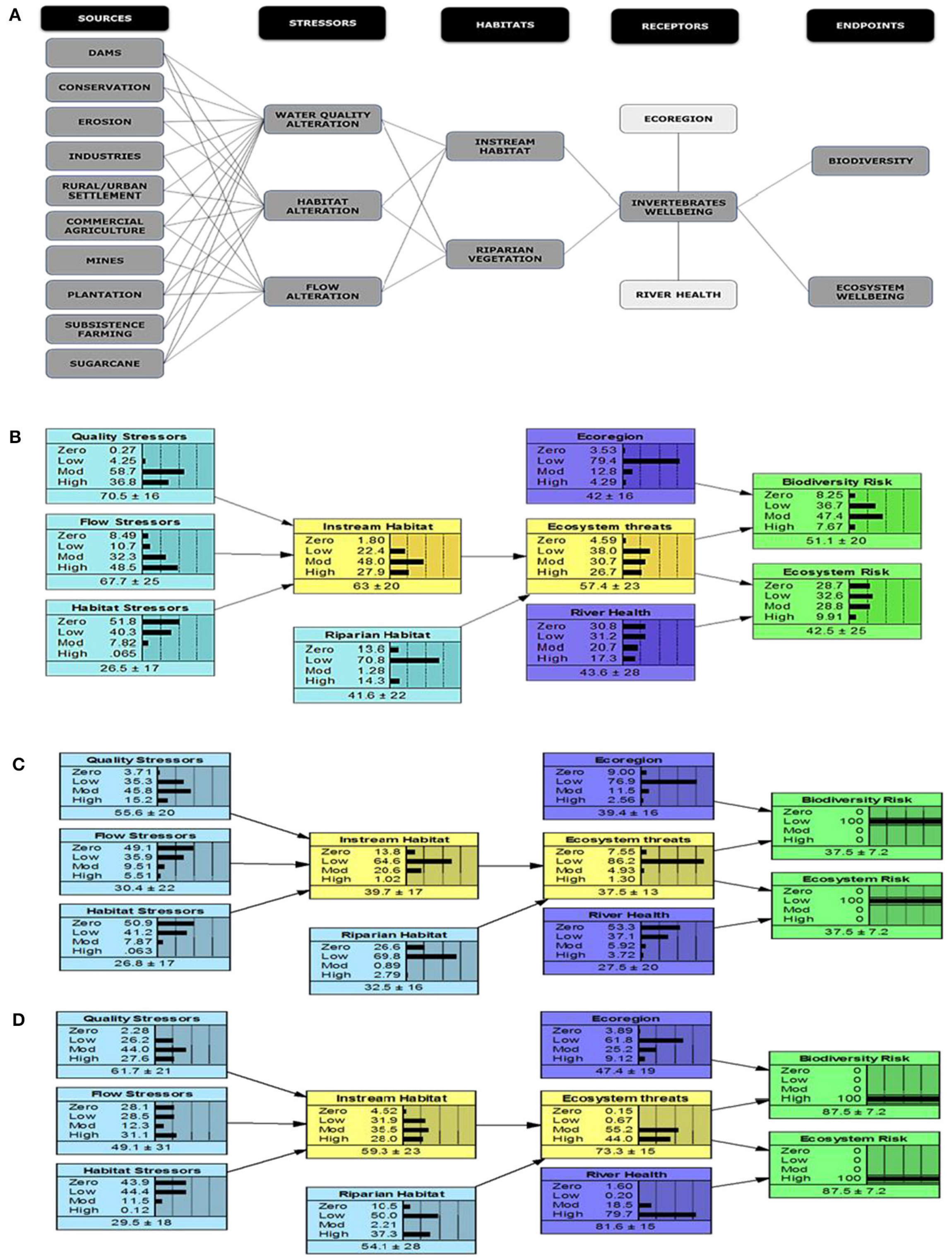

FIGURE 2 | (A) conceptual model showing linkages between sources, stressors, habitats, receptors and assessment endpoints, while **(B)** Bayesian Network Relative Risk Model, using AMAT1 risk region as an example, **(C)** 100% low risk to endpoints simulation, and **(D)** 100% high risk to endpoints simulation.

used as the template for developing the BN-RRM using Netica software (Norsys Software Corporation, 2014) (**Figure 2B**). Our RRM was based on a ranking of the stressors and the habitats to generate possible outcomes of their impacts on the ecological receptors and the assessment endpoints (Landis and Wiegers, 2005). Our ranking was based on the relative risk magnitude or impact of each stressor and habitat using the quantitative and qualitative data obtained during the study period. The risk ranks were expressed as percentages of the impacts of each stressor, from 1% being the least risk or least impact of a stressor; while 100% rank is the highest risk of a stressor to the endpoint. The ranks were zero (1–25%), low (26–50%), moderate (51–75%) and high (76–100), respectively. The rank for each stressor and their justifications are detailed in the Supplementary Information (**Supplementary Table 1**).

The ranks are defined as:

- Zero risk: This describes a pristine or reference state, with no impact or risk.
- Low risk: This represents a mostly natural state with low impact or risk. It is believed to still be within an ideal state for sustainable ecosystem use.
- Moderate risk: This state describes a moderately modified state or moderate impact or risk. It represents the threshold of potential concern or alert.
- High risk: This state represents significant alteration or impairment, with high impacts or risks.

After calculating the risks for the current scenario in the thirty-nine risk regions, three alternative scenarios were proposed, and the risks were calculated for each scenario, endpoint and risk region. The alternate risk scenarios used were:

- Scenario 1: represented a low flow situation. The low flow was simulated because of the climatic situation of South African rivers which are often affected by periodic drought conditions and low annual precipitation.
- Scenario 2: represented impacts of limited or degraded habitat. Habitat degradation or impairment is a big problem in South African rivers as a result of anthropogenic activities (e.g., sand mining activities) and natural disasters (e.g., drought).
- Scenario 3: represented a situation of high-water quality degradation. Water quality degradation is mostly from effects of industrialization (e.g., effluent discharge from industries) and urbanization (e.g., household wastes)
- 100% low risk: This represented the desired risk level with minimal impact to our endpoints. This simulation helped to characterize the impact of each stressor input on the risk endpoints for each region.

Uncertainty Analyses

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

RESULTS

Risk Calculation and Distribution Patterns

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

Our preliminary analysis of the risk sources data showed three regions had high risks of water quality stressors (AMAT1, BUSH1 and SIKW1), with BUSH1 having the lowest score (26%) and SIKW1 had the highest score (50%). For the flow stressor, ten regions had high risks (BIVA1, BLAC1, BUFF1, HLUH1, IMFO1, LOVU1, MDLO1, MKHO1, MVOT12, and TONG1), with BUFF1 having the lowest score (42%) and TONG1 had the highest score (66%). For the habitat stressor, 29 regions (LOVU2, MFUL1, MHLA1, MKUZ1, MKUZ2, MOOI1, MTAM1, MVOT1, MVUN1, MZIM1, NCAN1, NGWA1, NWAK1, PHON1, PHON2, SAND1, THUK1, THUK2, UMLA1, UMLA2, UMNG1, UMNG2, UMNG3, UMNG4, VUTH1, WHIT1) had high risks; PHON2 had the lowest risk (35%), while PHON1 and WHIT1 had the highest score (56%) (**Figure 3**).

The risk distributions for each endpoint in the 39 risk regions were generated from the BN output using Netica software (**Figures 4**, **5**). Often, various distributions may have similar mean values; therefore, it is more important to compare the distributions rather than focus on the mean scores because distributions reflect the actual frequencies from the model calculations (Landis et al., 2017). Risk scores suggest general trends, while risk distributions give specific information about the patterns of relative risk and help to compare differences in risk by region (Landis et al., 2017). The biodiversity endpoint generally displayed low-moderate risk distribution in our current scenario, except AMAT1, BUSH1, and PHON2, which displayed a zero-low risk distribution and a few other sites showing a high risk. Alternative scenario 1 skewed toward moderate risk at all the study sites for the biodiversity endpoint, the scenario 2 showed a generally high risk at most sites, with a few lowland sites being in a moderate risk. The alternative scenario 3, which represented a high deterioration of water quality because of poor mitigation

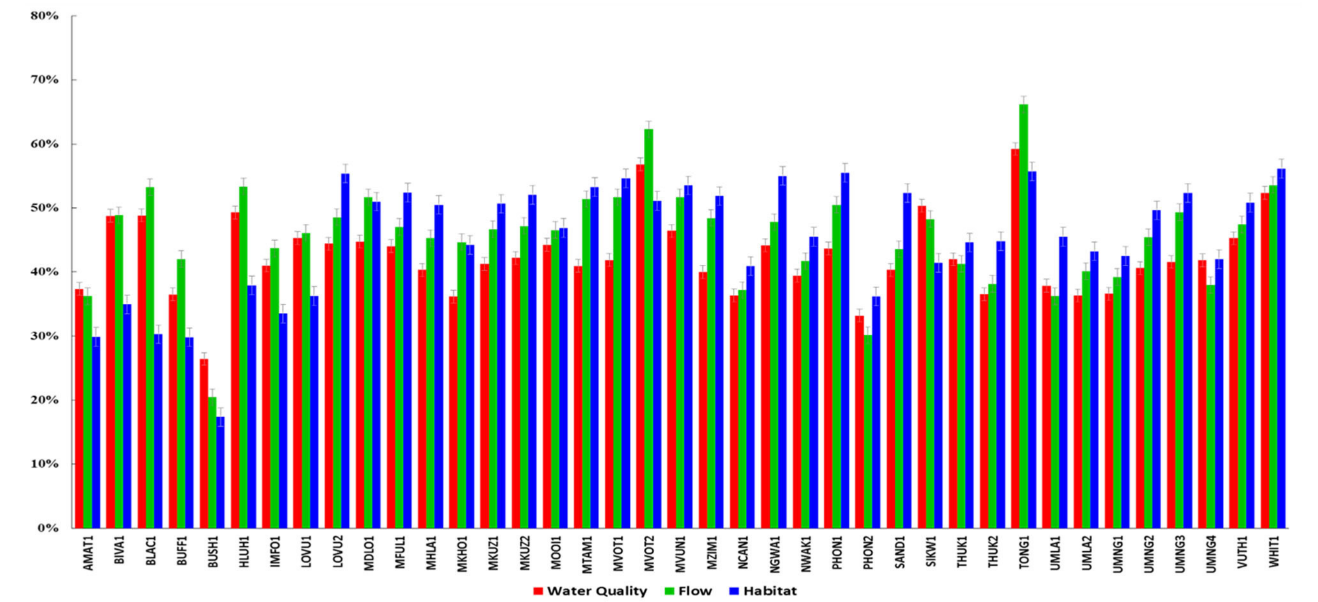

FIGURE 3 | Preliminary analysis of the risk sources for rivers of KwaZulu-Natal, South Africa, from 2015 to 2016.

or management, displayed high-risk patterns (**Figure 4**). The ecosystem risk distribution patterns displayed a zero-low risk distribution in the majority of the regions, while some regions (e.g., HLUH1, MVOT2, and TONG1) displayed a medium-high risk pattern. Scenario 1 generally displayed low-moderate-high risk patterns, while scenario 2 and scenario 3 had a fairly even distribution of medium to high risk (**Figure 5**).

Risk to the Endpoints

For the biodiversity endpoint, the lowest and highest risk scores were obtained in the BUSH1 and MVOT2, respectively, in the current risk scenario. In scenario 1, the lowest (45.1%) and highest (48.3%) risk scores were obtained from BUSH1 and LOVU2, respectively. In scenario 2 had the lowest risk score (48.5%) and the highest risk score (53.9%) from MVOT2 and BUSH1, respectively. For ecosystem endpoint, the BN estimates showed lowest risks at MHLA1 (25.7%), LOVU2 (51%), HLUH1 (47.2%), and LOVU2 (48%) for the current scenario, scenario 1, scenario 2 and scenario 3, respectively. The highest risk scores obtained from the BN estimates were from MVOT2 (69.2%), HLUH1 (60%), MOOI1 (56.3%), and BUSH1 (56.8%) for the current scenario, scenario 1, scenario 2 and scenario 3, respectively. The final risk to biodiversity was shown in **Figure 6**, while the final risk to the ecosystem was shown in **Figure 7**. Sites within the industrial and urban areas were mostly at moderate risk in the current scenario for the two endpoints, while the sites within conserved areas had zero to low risks (**Figures 6A**, **7A**). At the alternative scenario 1 (low flow risk), the biodiversity endpoint had moderate risk at all the sites (**Figure 6B**), while the ecosystem scenario indicated a generally high risk at all sites (**Figure 7B**). At the alternative scenario 2 (high flow risk), both endpoints had predominantly high risks, with very few lowland river sites being at moderate risk (**Figures 6C**, **7C**). For the alternative scenario 3 (water quality risk), biodiversity endpoint was predominantly high with only a few sites being at moderate risk (**Figure 6D**), while all the sites were at high risk for the ecosystem endpoint (**Figure 7D**).

Low-Risk Simulation

An advantage of the BN model is that it can be directly used as an adaptive management tool by setting the state of an endpoint to the desired level and essentially solving the model "backwards" (Ayre and Landis, 2012). For this study, we set our endpoints to 100% low risk. The 100% low-risk simulation represented the resource management goals for South African rivers (DWA, 2012). Using AMAT1 region, our 100% low-risk simulation altered the risk distributions in the BN model and also gave insights into the input parameters posing the highest risk to the endpoints (**Figure 8A**). Water quality stressors posed the highest risk (55.6%) to the biodiversity endpoint, while river health [measured as the macroinvertebrate response assessment index (MIRAI)] posed the highest risk (81.6%) to the ecosystem endpoint. Habitat stressors posed the lowest risk to both biodiversity (27.8%) and ecosystem (29.5%) endpoints (**Figure 8B**).

All the input parameters skewed toward zero or low risk in the low-risk simulation, except water quality stressors that skewed toward moderate risk (**Figure 8A**). The habitat stressors skewed toward zero risks in the low-risk simulation (**Figure 8A**). The flow stressors, riparian habitat, ecosystem threats and instream habitat had higher scores at the current risk scenarios than at the low-risk simulation (**Figure 8B**). The ecoregion, habitat stressors and water quality stressors were fairly the same for both the current scenario and low-risk simulation, but river health input

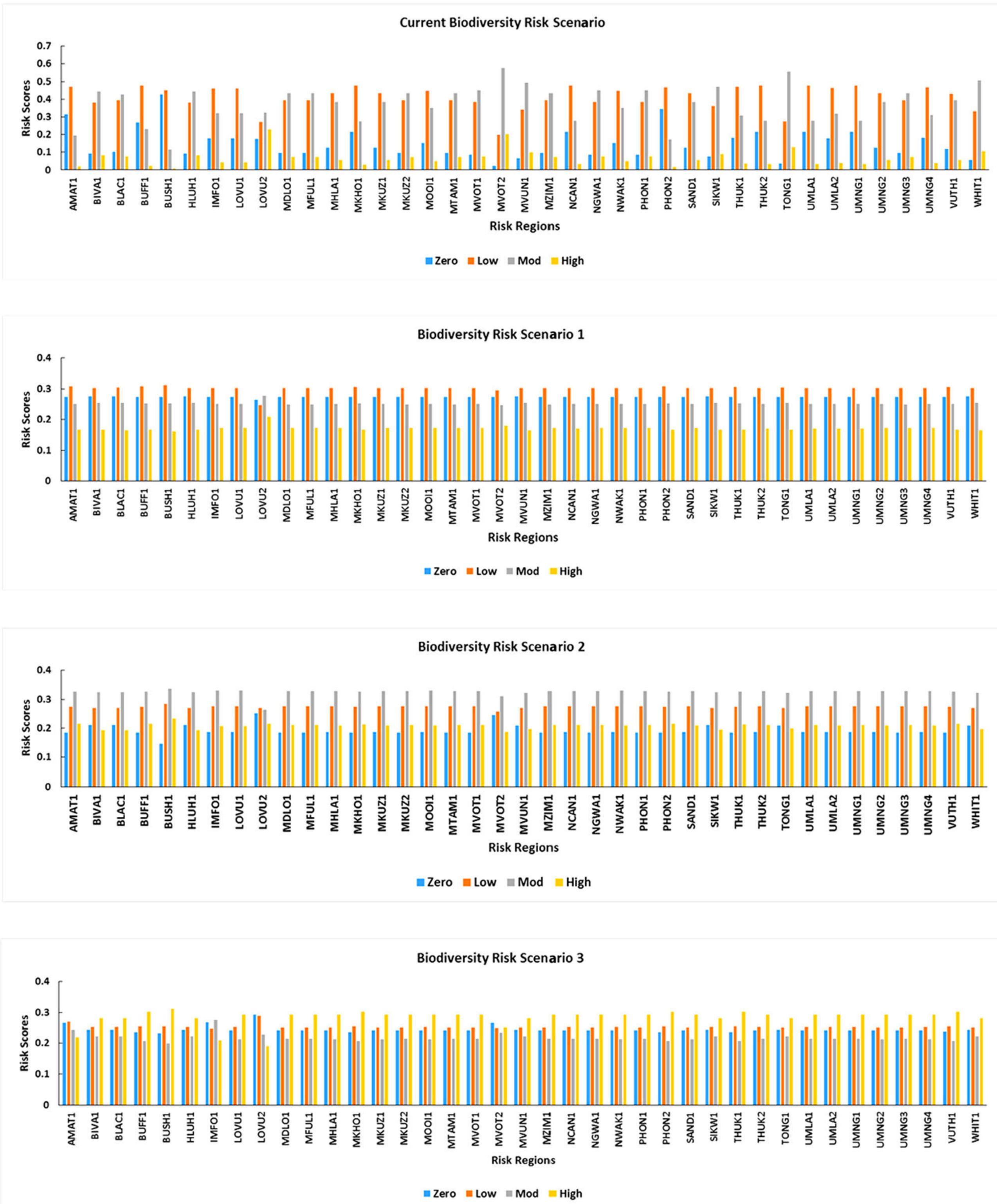

FIGURE 4 | Bayesian network risk distributions across the risk regions and in all scenarios of the biodiversity endpoint.

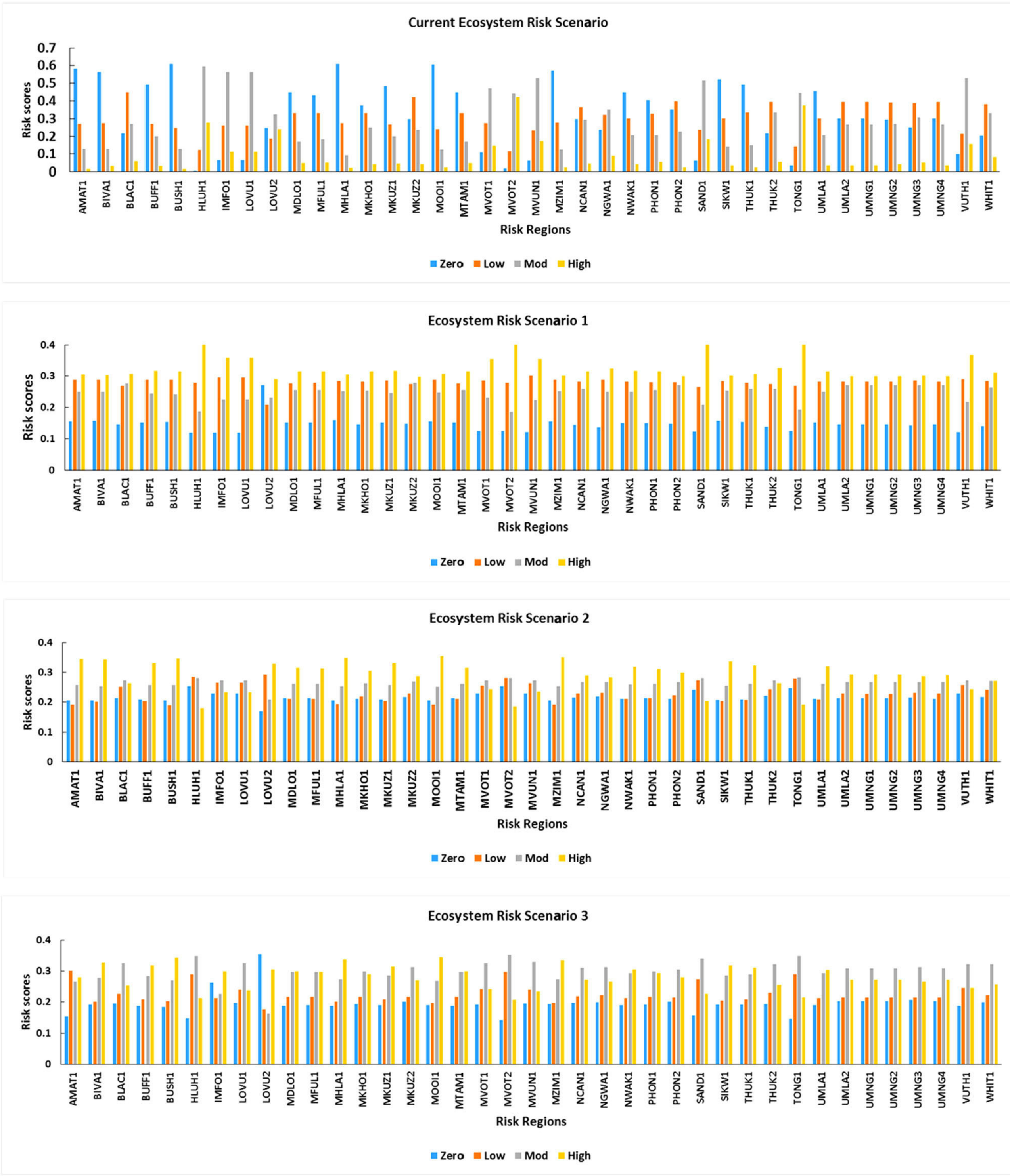

FIGURE 5 | Bayesian network risk distributions across the risk regions and in all scenarios of the ecosystem endpoint.

had lower scores for the current scenario than at the low-risk simulation (**Figure 8B**). The habitat stressors were fairly stable in both the low and current risk scenarios (**Figure 8B**). As the 100% low-risk simulation represented the expected ideal situations for our endpoints, the stressors that are at comparable risk levels in the current and low-risk scenarios gave an indication of

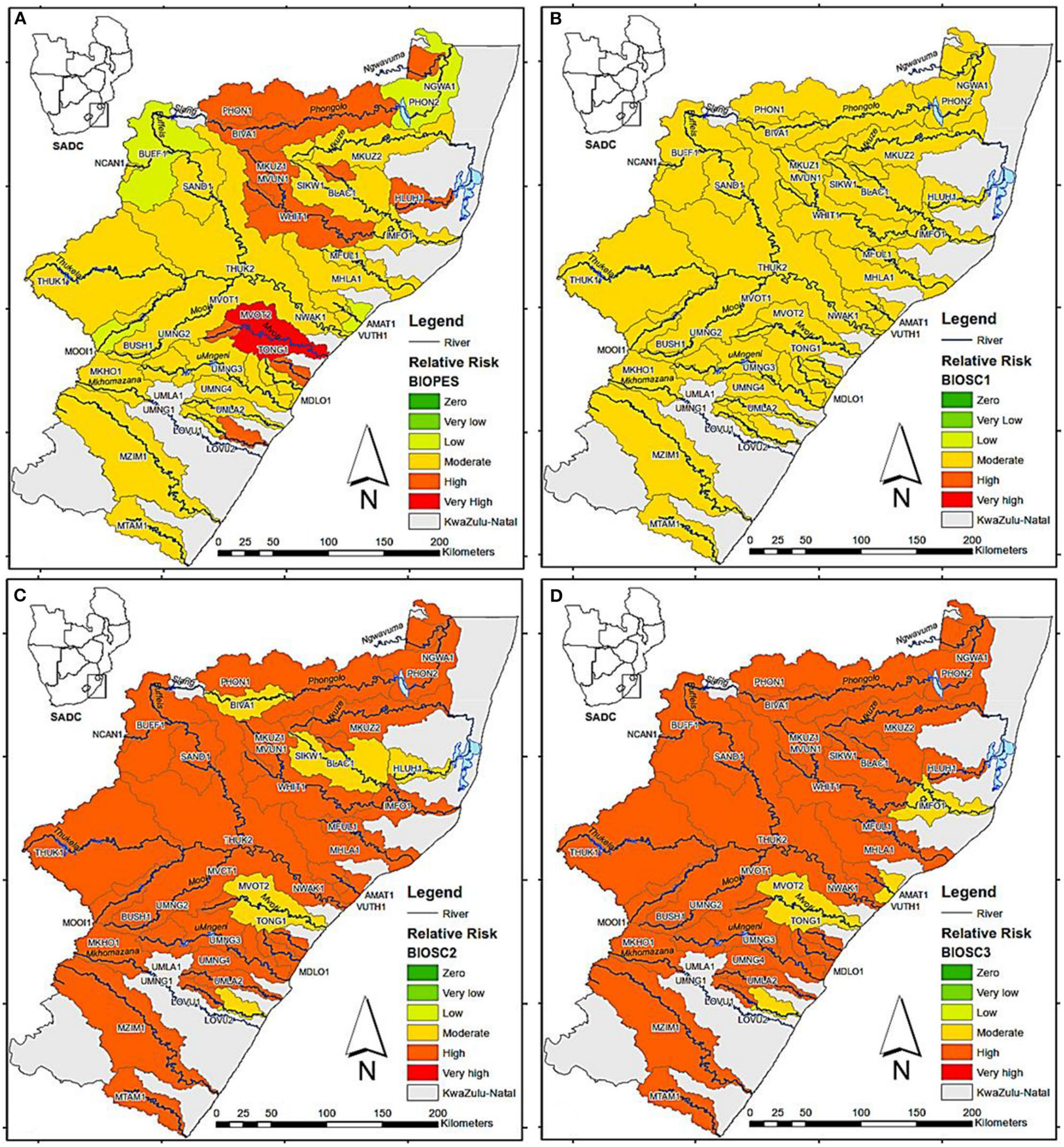

FIGURE 6 | Final biodiversity risk classifications of KwaZulu-Natal rivers studied from 2015 to 2016 based on the present ecological state **(A)**; risk associated with low flow **(B)**; risk associated with limited or degraded habitat **(C)**; and risk associated with poor water quality **(D)**.

acceptable levels of risks in achieving the national management goals for the rivers in this study.

Uncertainty

Our sensitivity analysis indicated that ecosystem threats were the highest contributor to the overall risk to biodiversity, while river health was the highest contributor to the overall risk to the ecosystem and the lowest contributor to both endpoints was habitat stressor (**Table 1**). As expected, there was generally a high probability of endpoints to be at high risk during scenario 3 and the high-risk simulation, but those risk probabilities were reduced in the low-risk simulation.

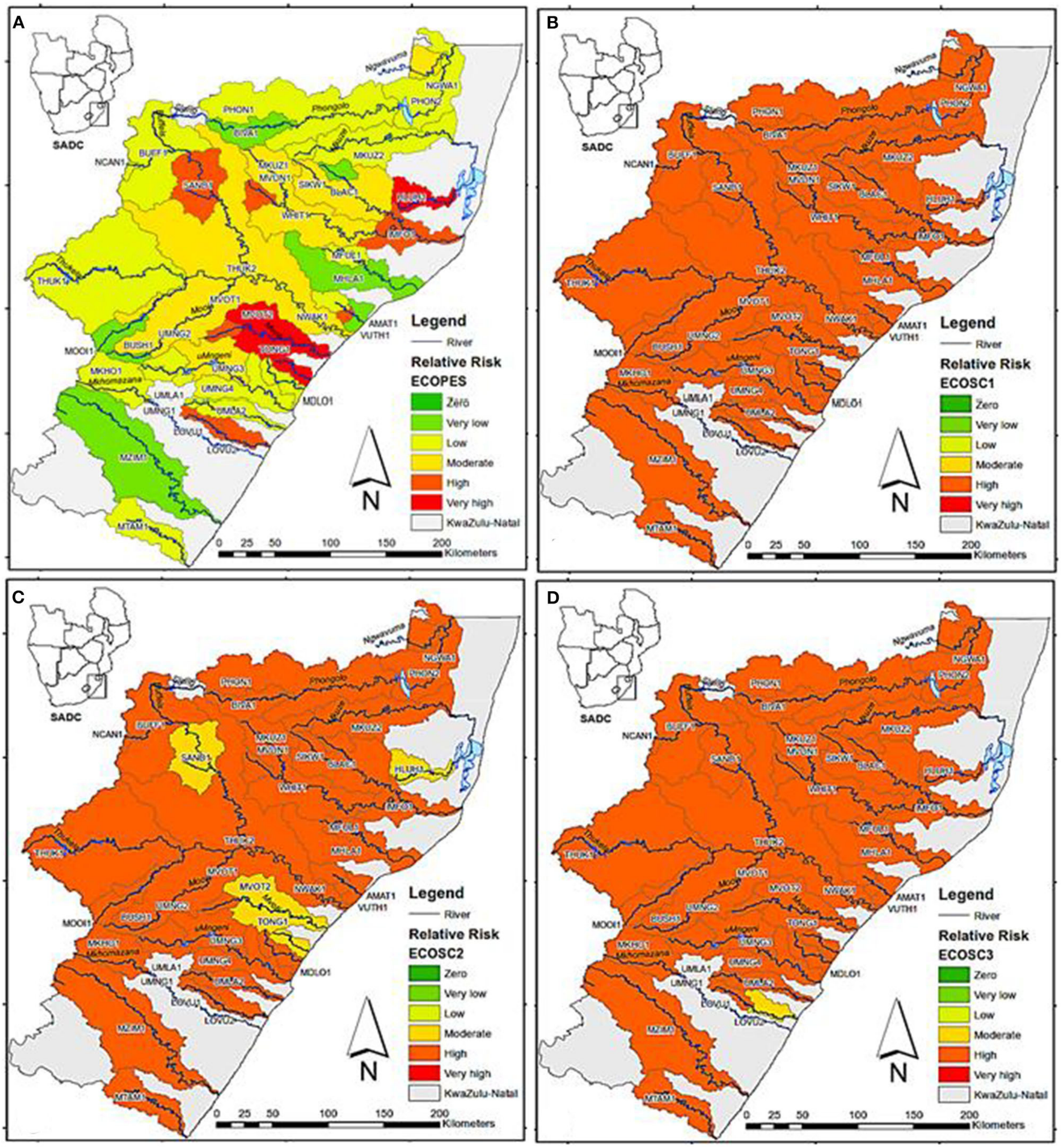

FIGURE 7 | Final ecosystem risk classifications of KwaZulu-Natal rivers studied from 2015 to 2016 based on the present ecological state **(A)**; risk associated with low flow **(B)**; risk associated with limited or degraded habitat **(C)**; and risk associated with poor water quality **(D)**.

DISCUSSION

The purpose of our study was to apply the BN-RRM in assessing the impacts of multiple stressors on the well-being of KZN rivers using macroinvertebrates as our indicator species and incorporating different management alternatives into the models. As demonstrated in this study, BN can be used as an adaptive management tool for ecological risk assessments of multiple stressors, whether they are from chemical or non-chemical sources (Landis et al., 2017). Bayesian Network models can be used interactively to visually communicate responses of endpoints to variables, compare risk regions and can be used

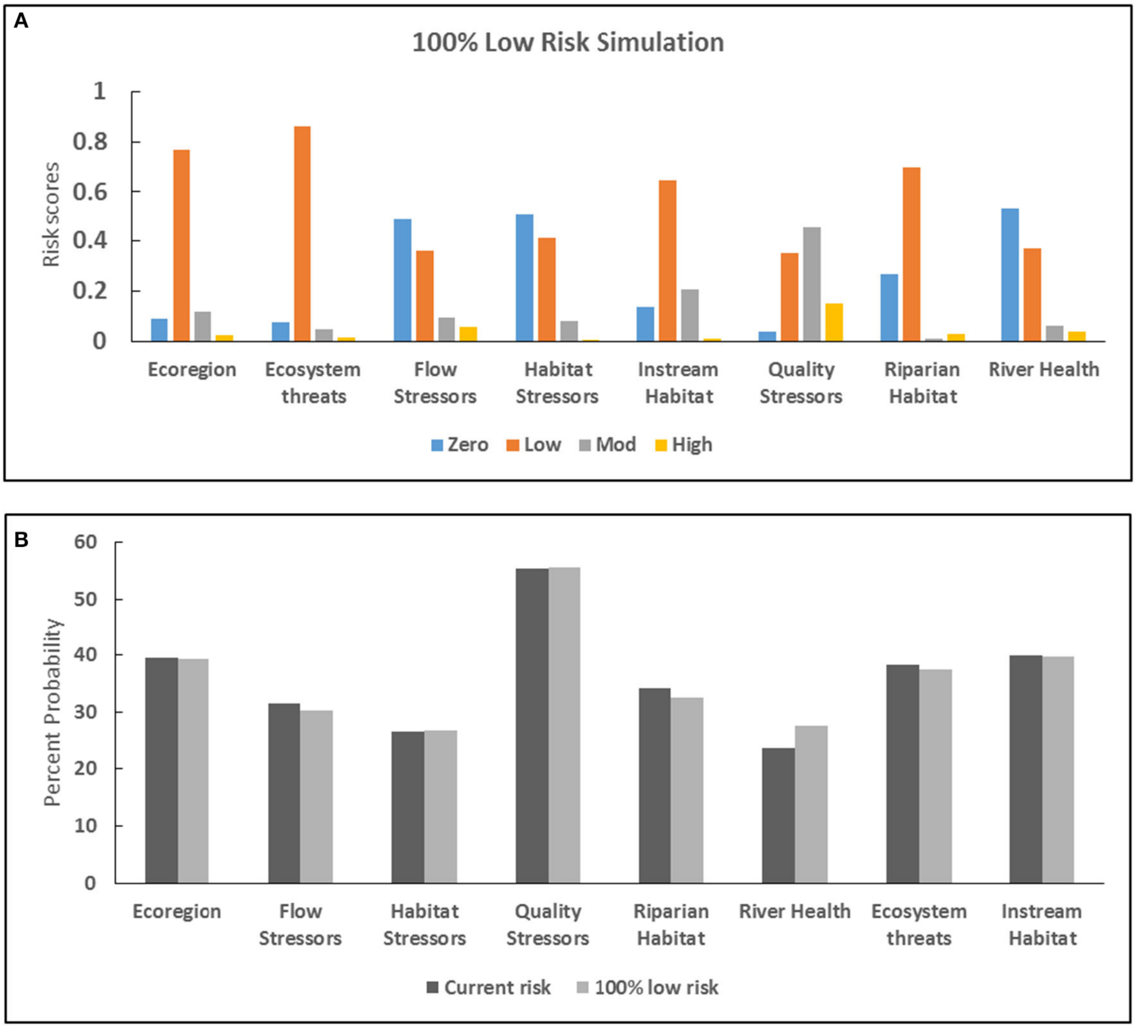

FIGURE 8 | Low risk simulation of KwaZulu-Natal rivers; **(A)** risk distribution for the low risk simulation, and **(B)** comparison between low risk and current risk scenarios.

as a risk communication tool to compare risk under theoretical scenarios (Landis et al., 2017). Our BN succeeded in calculating the overall risk to the two endpoints selected for this study and identified ecosystem threats and river health as the most influential contributors to the risk to biodiversity and ecosystem, respectively, in the study area; while habitat stressors had the lowest risk contribution to both endpoints. The development of risk models and calculation of current risk within the study area was the initial step in assessing the risk to the macroinvertebrate biodiversity and ecosystem well-being. We obtained region-specific data during our extensive sampling program for the model parameters, and these data were used in the calculation of risk to the endpoint in our current scenario.

With the BN model, we were able to account for potential synergistic effects of variables and the effects of ecosystem threats through the conditional probability tables (CPTs), which allowed for complex ecological interactions to be incorporated into the model's complexity (Maxwell et al., 2015; Landis et al., 2017). For example, the CPT for Instream Habitat was selected in this study to represent the integrated variable for water quality (quality stressors), flow stressors, habitat stressors and determinants of physical habitat (Davies and Day, 1998). The CPTs were established using Netica ratio equations whereby when water quality is observed in a high-risk rank state, the relative importance of flow and habitat was hypothesized to be at lower risk states. Thereafter when the flow is in a high-rank state, the other variables are weighted lower and such was done to habitat when it is in a high-rank state. When variables were in a zero to moderate risk state, they were all weighted equally. It is these synergistic effects that may explain why ecosystem threat was the disturbance that most strongly influenced the level of the potential risk to biodiversity endpoint (Landis et al., 2017). Also, input parameters and CPTs can easily be refined or updated to reflect current knowledge of the river sites, thereby reducing uncertainty in the data which may be caused by incomplete data and sampling errors (Marcot et al., 2006; Landis et al., 2017). Also, it is possible for new data to be added to BN risk models to reflect new knowledge of the system (Fuster-Parra et al., 2016). Thus,

TABLE 1 | Sensitivity analysis for endpoints, showing the percent of calculated entropy for each endpoint attributed to input nodes.

Parameters	Risk to biodiversity	Risk to ecosystem
Ecosystem threats	40.6	5.34
Instream habitat	11.5	2.22
Flow stressors	4.72	1.02
Riparian habitat	6.89	0.98
Quality stressors	1.03	0.19
Ecoregions	0.6	NA
Habitat stressors	0.53	0.09
River health	NA	30.6

Percentage is expressed relative to the calculated entropy for each endpoint.
NA, the parameter was not an input parameter to the endpoint.

access to new data will greatly reduce uncertainty and reflect a more accurate risk evaluation (Landis et al., 2017).

Evaluating uncertainties is necessary for policy or management decision making, but care has to be taken as such information may easily be misused (Aven and Krohn, 2014). It is difficult to predict future risk characteristics, therefore, not properly addressing risks and its associated uncertainties may lead to short term solutions, which could be insufficient in the long term (Refsgaard et al., 2013, 2014). Decision support models help a decision-maker to evaluate the consequences of various management alternatives (Holzkämper et al., 2012). However, awareness of the various sources of uncertainty may help to ascertain justified decisions (Uusitalo et al., 2015). Thus, a useful model should include information about the uncertainties related to each of the decision options, because the certainty of the desired outcome may be a central criterion for the selection of the management policy (Uusitalo et al., 2015). Uncertainty in BN risk model results reflects in the risk distributions for each node; where uncertainty increases as the risk distribution increases (Holt et al., 2014).

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

Flowing water is the defining characteristic of rivers (Nadeau and Rains, 2007), with important influence on aquatic biota (Bunn and Arthington, 2002). Flow alteration in rivers is often the most severe and continuing threat to their ecological sustainability and associated floodplain wetlands (Pringle, 2001). However, water resource managers often have difficulty in assessing the flow velocity a river needs to maintain its ecosystem, while still enabling water abstraction for other uses (Vörösmarty et al., 2010). Natural flows periodically include low flow periods as a result of precipitation deficits. Low flows are seasonal but may also be induced by anthropogenic activities which cause a deviation from the natural flow regime (Al-Faraj and Scholz, 2014). Artificial flow reductions are those created by human activities, such as dam closure, groundwater abstraction and water diversion (Adams et al., 2016). Demand for water gets to the peak during dry periods of the year when streams have naturally low flows, which are worsened by water abstraction (Mishra and Singh, 2010). Flow alteration exerts a direct physical influence on aquatic biota and indirectly influences substrate composition, water chemistry, nutrient availability, organic substances, as well as in-stream habitat availability and suitability (Dewson et al., 2007).

Habitat structure affects biota community composition in freshwater ecosystems, with species diversity and abundance often influenced by structural complexity and heterogeneity (Tews et al., 2004). Previous studies have shown that macroinvertebrates can be influenced by both complexity and heterogeneity (Barnes et al., 2013). Hence, structural features of their habitats have consequently become a central focus in river management (Feld et al., 2011). During low flows, there may be adverse effects of habitat heterogeneity as a result of fragmentation, which disrupts essential biological processes such as dispersal and resource acquisition (Saunders et al., 1991). However, not all species in an ecosystem are equally affected by spatial structures in either heterogeneous or fragmented state (Steffan-Dewenter and Tscharntke, 2000). The severity of reduced flow has an important influence on invertebrate responses because it determines the magnitudes of changes in the environment, habitat diversity, sedimentation and availability of food resources (e.g., periphyton) (Lake, 2000). During our study, there were limited habitat diversity and connectivity in the lowland streams as a result of drought (low flow), while a diverse range of suitable microhabitats remained available in the upland rivers. As observed in this study, reduced flows in perennial rivers may cause decreases in taxonomic richness (Poff and Zimmerman, 2010). A loss of taxonomic richness in the upland sites may be attributed to the loss of habitat types (e.g., fast flows or rapids) during the low flows, hence resulting in the generally low-moderate risk to the endpoints of this study in the current scenario, and a resultant high risk in the alternative scenarios. Also during the low flow scenario, changes in macroinvertebrate biodiversity (community composition and taxa richness) could probably result in increased habitat suitability for some species and decreased suitability for others (Gore et al., 2001); hence this will result in high risks to biodiversity and ecosystem well-being as demonstrated in our alternative scenarios. Furthermore, the drift behavior of macroinvertebrates enables them to leave a stream reach or seek refuge in more favorable patches of the river in events of unsuitable low flow conditions (Verdonschot et al., 2014). This drift behavior enables organisms to escape unfavorable conditions, either actively or passively (James et al., 2008). Studies have shown that passive drift decreases during low flow conditions, while other studies have shown that active drift increases during periods of low flow (Naman et al., 2016). Active drifts during low flow are often caused by insufficient water velocities to meet nutritional, physiological and habitat requirements (Brooks and Haeusler, 2016). Active drift may also be a predator avoidance behavior, and this may increase if predator density increases during the low flow (Naman et al., 2016). Active drifts may, therefore, cause a reduction in biodiversity as demonstrated by our alternative risk scenarios.

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

The BN-RRM model's intrinsic flexibility makes it a powerful tool for resource management because alternative management scenarios can easily be evaluated for desired objectives (Landis et al., 2017). Moreover, the graphic interface of the model results makes it a valuable tool for collaborative resource management (Carriger et al., 2016). This study provides the foundation for assessing the effects of multiple stressors in rivers of KZN using macroinvertebrate biodiversity and ecosystem as assessment endpoints over a regional spatial scale and incorporating site-specific information. Our study lays the foundation for future risk assessment for the rivers of KZN. The model created in this research also provides a foundation for assessing the impacts of adaptive management strategies, and these models may be adapted to the evaluation of risk changes for best management practices in the rivers of KZN.

Specific chemicals or ecological stressors should be integrated into this risk framework for future studies in KZN; for example, the effects of invasive alien biota or chemicals on biological endpoints can be investigated using this model. Rivers of KZN are being impacted by pollution from different anthropogenic land uses across longitudinal gradients. These anthropogenic sources include effluents from domestic wastes, industrial effluents from the paper and sugar mills, agricultural practices and water abstraction. All these anthropogenic impacts pose risks to the endpoints of the rivers if not properly regulated or managed. Hence the river systems will continue to deteriorate. Deteriorated river systems will consequently not be able to meet their ecological functions.

CONCLUSIONS

Our study has demonstrated that subtle changes in environmental management may result in large changes in the risk distribution of sensitive endpoints and that the BN-RRM risk assessment plays a critical role in adaptive management schemes (Carriger et al., 2016). Strict adherence to environmental laws on the treatment and discharge of wastewater by industries should be enforced, as this will help to improve the water quality of the high-risk regions (e.g., MVOT2 and TONG1).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

OA conceived paper with CD and GO'B. OA collected, analyzed data, and wrote the paper. GO'B also assisted OA to develop the risk models. CD and GO'B supervised the study and contributed valuable comments to the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frwa.2020.584936/full#supplementary-material

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Citizen Science for Bio-indication: Development of a Community-Based Index of Ecosystem Integrity for Assessing the Status of Afrotropical Riverine Ecosystems

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The use of socioeconomic and cultural parameters in the assessment and biomonitoring of ecological health of aquatic ecosystems is still in its nascent stages. Yet, degradation of aquatic ecosystems has elicited concerns because of its bearing on social and economic development of communities consisting of marginalized and vulnerable groups, as well as the expenses and technical knowhow involved in biomonitoring approaches. In this study we developed a Citizen-based Index of Ecological Integrity (CIEI) for assessing and monitoring the ecological status of vulnerable African riverine ecosystems in Lake Victoria Basin, Kenya. The hypothesis is that the citizen-led socioeconomic and cultural metrics provides a more cost-effective broad view of ecosystems than other biomonitoring methods in the assessment of water resources in the developing countries. Selected rivers in the southern part of Lake Victoria (Rivers Kuja and Sondu-Mirui) recorded the highest CIEI than their northern counterparts (Rivers Yala and Nzoia) that had moderate to poor ecosystem integrities. The study demonstrates the usefulness of this approach to elucidate the source of impairment, the extent of impacts and provide a justifiable rationale to advice policy makers on developing guidelines for conservation and management of aquatic ecosystems. We recommend for adoption and promotion of the CIEI perspective in areas where such approaches appear defensible for the assessment of catchment-wide practices in areas with robust indigenous knowledge to provide a broad-view of the ecological health of the aquatic ecosystem.

Keywords: citizen science, community-based monitoring, ecological integrity, water pollution, catchment management

INTRODUCTION

In working toward the protection of freshwater ecosystems, development of decision-support tools for monitoring changes in water quality and biological communities over time has been given a priority in many parts of the world (Statzner et al., 2001; Aura et al., 2020; Ko et al., 2020). Biological communities have especially become common indicators of change, based on the premise that the presence or absence of certain species or groups of species at a given site reflects its environmental quality (Barbour et al., 1999; Dallas et al., 2010). By monitoring how species respond to specific stressors in their environment and developing species-environmental relations along gradients of human influence, our understanding of how human disturbances can shape the structure and functioning of ecosystems has tremendously increased over the last 4 decades (Karr, 1981; Karr and Chu, 2000; Masese et al., 2009; Friberg, 2014). Continuous development and refinement of these approaches have yielded solid theoretical grounds upon which bioindication has flourished and operational biomonitoring programs have been developed (Dickens and Grahm, 2002; Kaaya et al., 2015).

Biomonitoring and bioassessment data and information is particularly important for aquatic systems management, because population growth, migration, and sociocultural activities are contributing to greater rates and extents of watershed development and impairment (Angel et al., 2011; Seto et al., 2011). These altered conditions negatively affect water quality, aquatic life, and functions of stream ecosystems (Smucker and Detenbeck, 2014) and have adverse socioeconomic consequences as well (Pickett et al., 2011). As a result, the proportion of impairment has become an important factor in urban planning and watershed management, because it is strongly associated with development intensity and stressors, and it can be readily quantified and regulated (Bellucci, 2007; Schueler et al., 2009).

Characterizing relationships between watershed conditions and water quality could help identify future priorities for monitoring and restoration (Faghihimani, 2012). However, the methods for data collection and study design could have consequences for interpreting results and for decision making (Carlsson and Berkes, 2005). Various methods of monitoring and assessment have been employed in characterization of aquatic relationships (Lozano et al., 2013). Monitoring actions traditionally focused on one aspect of ecological integrity that involved the determination of pollution from point sources which involves use of chemical and physical water quality, with regulatory efforts aimed at controlling individual parameters (Roux, 1997). With the failure of the chemical and physical water quality to provide information on the overall condition of the aquatic system, the use of biomonitoring approach emerged that was more integrated and holistic (Cairns, 2003). However, both physical-chemical and biological approaches require skills and knowledge as well as costs for their implementation (Masese et al., 2013), which is a major hindrance to continuous monitoring of vulnerable ecosystems in developing countries.

A number of factors determine the choice of a program for assessment and monitoring of ecosystems, such as research costs, human resources, and data needs (Wren et al., 2000). For African systems, biomonitoring of aquatic ecosystems lags behind other regions because of limited financial devotion, lack of technical capacity, and limited guides on biomonitoring. Despite these hindrances, a number of regional or country-specific indices and programs have been developed for biomonitoring (e.g., Dickens and Grahm, 2002; Aschalew and Moog, 2015; Kaaya et al., 2015). Most of these indices and programs, however, are based on biological communities and the physical and chemical parameters of the environment that can be measured using standard methods.

Despite their wide appeal and adoption, traditional ecological data collection methods, and biomonitoring programs are limited in the amount of data that can be gathered across large spatial and temporal scales (Pocock et al., 2017; Achieng' et al., 2020). As a result of these limitations, new approaches to environmental monitoring have been explored, and citizen-science (Conrad and Hilchey, 2011; Theobald et al., 2015; Chandler et al., 2017; Pocock et al., 2017) has emerged as one of the methods, which involve volunteer participation by community members in providing or collecting information following a protocol provided, designed and or validated by experts in the field. This is aligned to co-management approaches that give stakeholders a platform for sustainable management of aquatic resources (Obiero et al., 2015). The approach has among others factors such as cost led to the push toward the inclusion of citizens in stewardship and monitoring of the status of natural resources (Brooks et al., 2005; Mochizuki and Yarime, 2016). While African countries have made a lot progress in this regard, especially on the involvement of communities in top-down decision making, and monitoring and management of natural resources, such as fisheries (Imende et al., 2005; Etiegni et al., 2017), forestry (Crocker et al., 2020), water resources (Bannatyne et al., 2017) and effects of climate change (Tesfahunegn and Gebru, 2020), the development of citizen science as an environmental assessment and monitoring approach is quite limited, and in most cases, it is at its nascent state (Requier et al., 2020).

Managers and non-governmental organizations are increasing their use of citizen volunteers to enhance their ability to monitor and manage natural resources, through tracking of species at risk and conserving protected areas (Conrad and Hilchey, 2011; McKinley et al., 2017; Crocker et al., 2020). However, in many places a comprehensive approach has not been developed on the use of socio-cultural knowledge and experiences in the assessment of the status of aquatic ecosystems (Reid et al., 2010). Social value systems are transient and transitory, as is the environment in which they operate, and are by no means laws of nature (Stephenson et al., 2020). Several fundamental social and cultural values are associated with basic needs, determined by the biology of biota, thus being less subject to modification (Hjalte et al., 1977). Well-noted examples are human interactions with aquatic ecosystems that stimulate such physical surroundings (Tol, 1995). Thus, the indigenous knowledge of individuals who have long interaction with aquatic ecosystems can be utilized in understanding of ecosystem integrity.

In most African countries, biomonitoring, and bioassessment of aquatic ecosystem is often confounded by a lack of, or inadequate and incomplete, data and monitoring initiatives by professional scientists and government agencies (Masese et al., 2013; Mangadze et al., 2019). To fill the void, non-professionals and citizen organizations have emerged the world over to track trends and work toward effective and meaningful planning, management, and stewardship. This is because, data collection requires local inputs that are accompanied by the

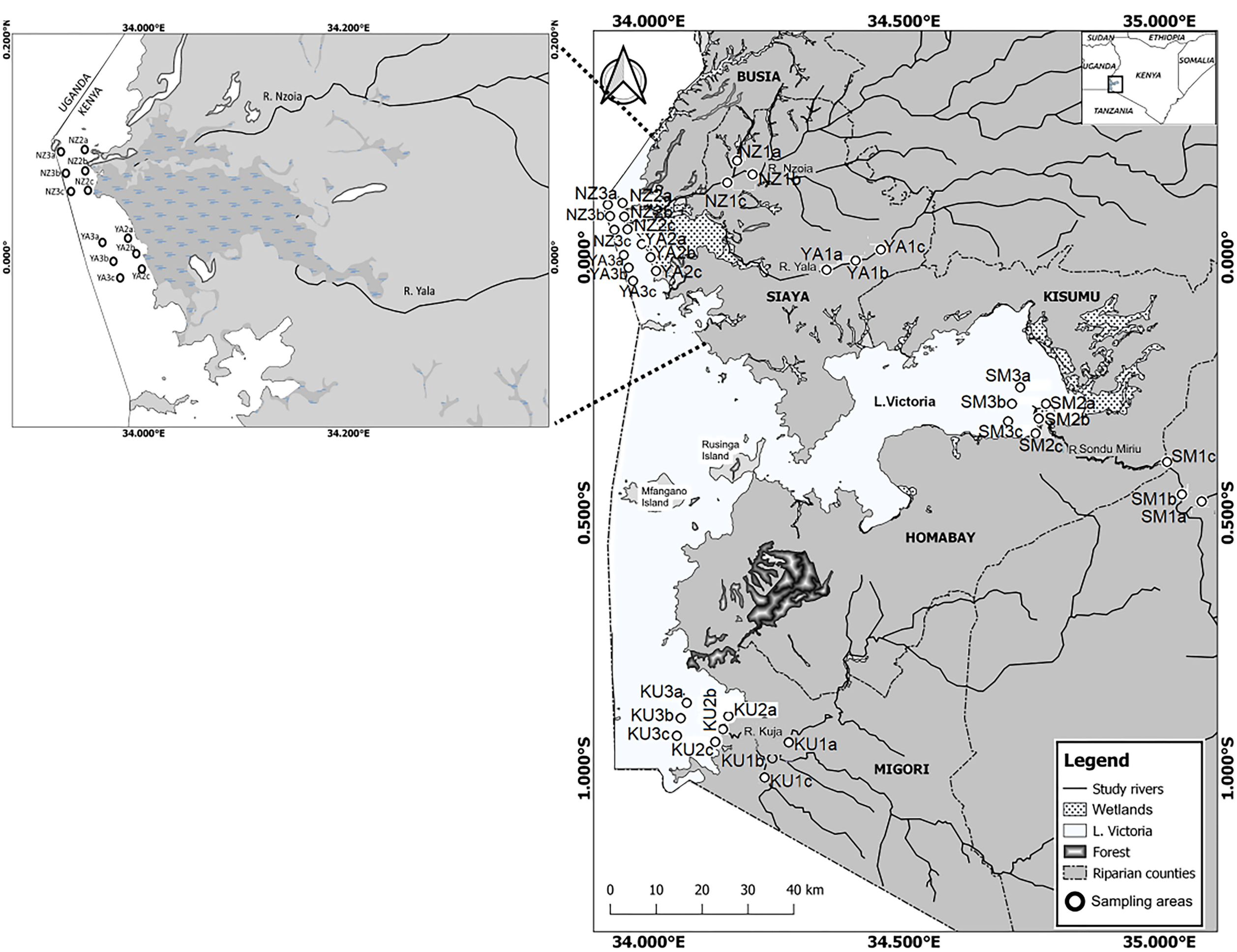

FIGURE 1 | Location of the study sites within the stations of (a) River Kuja (KU), (b) River Sondu-Miriu (SM), (c) River Yala (YA), and (d) River Nzoia (NZ). Sampling sites with at least three replicates as representative of microhabitats included River Kuja: KU1a, b, c—River Kuja upstream channel; KU2a, b, c—Kuja river mouth before discharge; KU3a, b, c—Kuja river mouth after discharge. Sondu–Miriu: SM1a, b, c—River Sondu-Miriu upstream; SM2a, b, c—Sondu-Miriu river mouth before discharge; SM3a, b, c—Sondu-Miriu River mouth after discharge. River Yala: YA1a, b, c—River Yala upstream; YA2a, b, c—Yala river mouth before discharge, YA3a, b, c—Yala river mouth after discharge. River Nzoia: NZ1a, b, c—River Nzoia upstream, NZ2a, b, c—Nzoia river mouth before discharge, NZ3a, b, c—Nzoia river mouth after discharge. The labels (a–d) represents sampled replicates of each site.

equitable participation of data users, including local communities which can lead to better monitored results and sustainability (Stephenson et al., 2020). One such approach is the application of sociocultural perspectives that uses indigenous knowledge and parameters in the creation of indices. More so, by integrating a large number of stakeholders, citizen science has the potential to directly connect scientists to the public and shares the importance of their work (Crocker et al., 2020). This could crack the challenge of communicating the value of scientific research to the public that is increasingly important yet in present world researchers typically have little support for outreach and education activities.

In this study we explored the use of sociocultural and economic perspectives, knowledge, and experiences to determine the pollution status of major rivers in the Lake Victoria Basin, Kenya. We used indigenous knowledge as one of the items in the toolbox of citizen science to develop a multimeric approach to bioassessment based on people's experiences and perspectives forged from living with and interacting with rivers in their localities. The approach has a potential for application in the assessment and monitoring the condition of vulnerable riverine ecosystems in Africa.

MATERIALS AND METHODS

Study Area

This study was done in the lower reaches of major rivers draining the Kenyan part of the Lake Victoria Basin (LVB), specifically Rivers Nzoia, Yala, Sondu-Miriu, and Kuja (**Figure 1**). These rivers constitute over 45% of the total discharge to the lake (Twesigye et al., 2011). The rivers represent the major river catchments with a gradient of disturbance, and most notable biodiversity hotspots around Lake Victoria (Masese et al., 2020). These rivers support an artisanal fishery, particularly during the rainy seasons (Balirwa et al., 2003) and act as a source of water for livestock, irrigation, industries, and domestic uses. They are threatened by catchment activities such as conversion of wetlands into farms, urban developments, poor management of domestic, and industrial wastes and the leaching of agrochemical residues. These activities cause decrease in forest cover, increases in soil erosion and rivers pollution (Balirwa et al., 2003).

Rivers Nzoia and Yala constitute the northern section of this study and Rivers Kuja and Sondu-Mirui are the southern representatives. The Lake Victoria Basin delivers important ecosystem services to more than 40 million people in the three riparian countries. These include fisheries, transport, and water for domestic, agricultural and industrial uses (Aura et al., 2013). The Kenyan part of the lake includes the Winam Gulf (Kavirondo Gulf or Nyanza Gulf), which is joined to the main lake by the Rusinga channel with major river discharges that have been believed to influence pollution status of the lake (Kundu et al., 2017; Aura et al., 2019). Nutrient enrichment in the Winam Gulf of Lake Victoria (Gikuma-Njuru and Hecky, 2005; Guya, 2013) has been attributed to increased extrinsic nutrient loadings associated with changes in land-use activities within its catchment (Verschuren et al., 2002). Due to the combined effects of human population growth, land use and land cover changes in the catchments of major rivers, elevated sedimentation have been reported to occur in the rivers over the years (Masese and McClain, 2012).

Study Design

The participation of riparian communities situated within 5 km of the river were selected on the basis of their involvement in the riverine changes and land use that foster the utilization and dependency on riverine products and their alternatives in this study. This underscores the application of citizen science into perspective. The riverine community benefits from a river site in their neighborhood in terms of anthropogenic activities such as fishing, water abstraction, waste disposal, and other socioeconomic and cultural activities that can be quantified into a measurement of a parameter or value, herein referred to as a metric (Aura et al., 2017; Masese et al., 2020). As a result of these practices and changes, this study aims to determine the role of the riparian communities in assessing the status of riverine systems.

Criteria for Sample Size Determination

The target population in the lower reaches of the selected major river catchments was identified from riparian communities. This consisted of local residents living within 5 km from the boundary of the river and the leaders of the community riverine associations. The northern riparian catchment had a total population of 102,321 people while the southern part had a total population of 110,321 people (National Population Census, 2019). The sample size was arrived at using the equation below by Cochran (1963):

$$n = \frac{z^2 pq}{d^2} \tag{1}$$

Where:

n = the desired sample size (if the population is >10,000)

z = the standard normal deviate at the required confidence level

p = the proportion in the target population estimated to have had characteristics being measured (0.15)

q = 1-p

d = the level of statistical significance set at (0.05)

By using the above equation 400 people were sampled with each river having 100 participants from the entire population to be used for the study. In this case, the sample size method was chosen in order to ensure evenness in the distribution of the targeted sample population.

Criteria for Participants Selection in Interviews

The data were collected through interviews with community members, literature review, and field observations using transect walks to ensure evenness and appropriate representation of the target population. Qualitative data were collected using interviews that were administered to participants. Sampling (riparian community) was done within 5 km because it has been shown that natural systems' use reduces beyond this distance (Ewel, 1999). In the study, one among the elderly members in each household was randomly selected and interviewed for representation.

Data were collected during the wet season (March) and dry season (July) of 2018 and 2019. The participants were interviewed with the help of a local assistant who acted as an interpreter in cases where the participant could not respond in English. Before the interviewers and interpreter were trained on the contents of the questions, and this made it easier for him/her to understand the concept and the research. Closed and open-ended questions were used to extract relevant information from participants and also understand their views. The interview questions consisted of both one response and multi-response questions.

The questions were divided into four sections representing socioeconomic and cultural perspectives classified into structure, scale, pattern, and network (Zhou et al., 2019) in relation to the local situations. The participant's perspectives sought under aforesaid sections on structure and scale and pattern and network metrics are presented in **Table 1** under the socioeconomic and cultural metrics.

Before conducting the interviews, Key informant interviews were conducted among community leaders in the riverine regions. A total of 16 Key Informant Interviews (KIIs) were conducted, with each major riverine site having 4 KIIs who were mainly organizational heads and leaders. The responses of ≥ 50% choice of a reference site helped in the determination of a control site as well as the appropriate method of collecting community perceptions in each of the studied rivers during the scoring process. Furthermore, before interviews, consent of the participants was sought. If they chose not to participate, the next household member was picked. The interview questions was pre-tested on 40 participants (10 from each of the riverine systems) who were not part of the 400 interviewees. Following the pre-testing, a few changes were made to the structure and wording of the questions for clarity.

TABLE 1 | Socioeconomic and cultural metrics and definitions that were evaluated for the development of the Citizen Index of Ecological Integrity (CIEI) for major rivers in the lower reaches of Lake Victoria Basin, Kenya and their predictive responses to increased levels of perturbation.

Socioeconomic and cultural metric	Metric definition	Predicted response/ assumptions to increased perturbation
Structure		
Water color/clarity	Value of increased eutrophication/ sedimentation	Increase
Water odor	Value of increased pollution/ eutrophication	Increase
Average number of individuals of fish per trip	Value of productivity	Decrease
Fish species diversity changes over time	Value of productivity	Decrease
Scale		
Use of river as waste disposal site	Value of increased pollution load	Increase
Forest size per site	Value at ≤ 5 km radius from the river	Increase
Number of bathing/swimming/fishing areas	Value of increased pollution load	Increase
Number of livestock access points	Value at ≤ 5 km radius from the river	
Pattern		
Number of industries per site	Value at ≤ 5 km radius from the river	Increase
Number of farmlands per site	Value at ≤ 5 km radius from the river	Increase
Number of urban areas per site	Value at ≤ 5 km radius from the river	Increase
Number of settlements per site	Value at ≤ 5 km radius from the river	Increase
Network		
Number of conservation groups	Value at ≤ 5 km radius from the river	Decrease
Number of sites with cultural rites	Value for ecosystem friendly rites per river	Decrease
Number of roads/transport network	Value at ≤ 5 km radius from the river	Increase
Level of inhabitants' education	High literacy	Decrease

The participants were purposively chosen with special regard to proximity of residence from the river-line, involvement in socioeconomic and cultural activities related to the river-system and the duration of stay in proximity to the riverine system (Zhang et al., 2018). Those who stayed in close proximity to the river, depended on the river economically and socially, and had at least 20 years of continuous residence were chosen for interviews. This was based on the assumption that they could answer more accurately on the observed changes that the river systems have undergone over time. Snow-ball sampling technique was used when more information was required (Aura et al., 2018).

Field Observations

To assess and identify water quality status for supplementation and validation with the Citizen Index of Ecological Integrity (CIEI) developed, physico-chemical observations were conducted. In this case, the field parameter measurements would show the scientific and verifiable output of the ecosystem which would be compared with the CIEI in assessing the degree of appropriateness. Each sampling expedition took an average of 10 days in July and March for each year from 2016 to 2018 with specific experts in water quality. This was done to add to the information collected through questionnaires. Microhabitats including riffle, pool, run, and open water were sampled in triplicate (Aura et al., 2010). General environmental observations about each site such as the maximum depth, time of sampling, site characteristics, and Global Positioning System (GPS) location were noted before sampling for physico-chemical characteristics as they could have an influence on physico-chemical comprehensions.

The selected physico-chemical parameters that were measured using standard methods for *in situ* data collection and sampling included temperature (°C), dissolved oxygen (DO, mg L^{-1}), pH, and conductivity (μS cm^{-1}). These physico-chemical parameters were measured using portable electronic water quality meters. Water transparency was measured with a standard Secchi disk (APHA, 2005). The water samples were further collected directly from the sampling sites using pre-treated 1 L polyethylene sample bottles for nutrient analyses. The bottles were individually labeled, filled, preserved using sulphuric acid and stored in cool boxes, for laboratory analysis using photometric methods for total nitrogen (TN, μg L^{-1}) and total Phosphorus (TP, μg L^{-1}) (APHA, 2005).

Citizen Index of Ecological Integrity (CIEI)

Figure 2 shows the flow chart used in the attainment of the CIEI. The index is developed using socioeconomic and cultural attributes and validated using physic-chemical parameters and a previous index developed for the region. This is because hydromorphological changes, socioeconomic and cultural shifts within the major river catchments of Lake Victoria have modified the ecology of most of these rivers from a desirable to less desirable condition (Masese and McClain, 2012; Guya, 2019). Thus, the community perceptions on the changes in socioeconomic and cultural perspectives that were classified into structure, scale, pattern, and network were used to develop a citizen-based index for assessing the pollution status of major rivers draining into Lake Victoria, Kenya (**Figure 3**).

The feedback loop of the metrics (**Table 1**) in comparison with increased levels of perturbations was assigned community perception scores of either low (assigned a score of 1), medium (assigned a score of 3) or high (assigned a score of 5) as illustrated in **Table 2**. During the KIIs, 98% of them disagreed with the use of a continuous Likert-scale of 1–10 as it was deemed to be long and difficult to differentiate one score to another due to the closeness of the numbers (data not provided). Thus, although the criterion of 5, 3, and 1 has been heavily criticized by various authors such as Stoddard et al. (2008), it was deemed relevant for this study (**Table 2**). The 5, 3, and 1 system has also commonly been

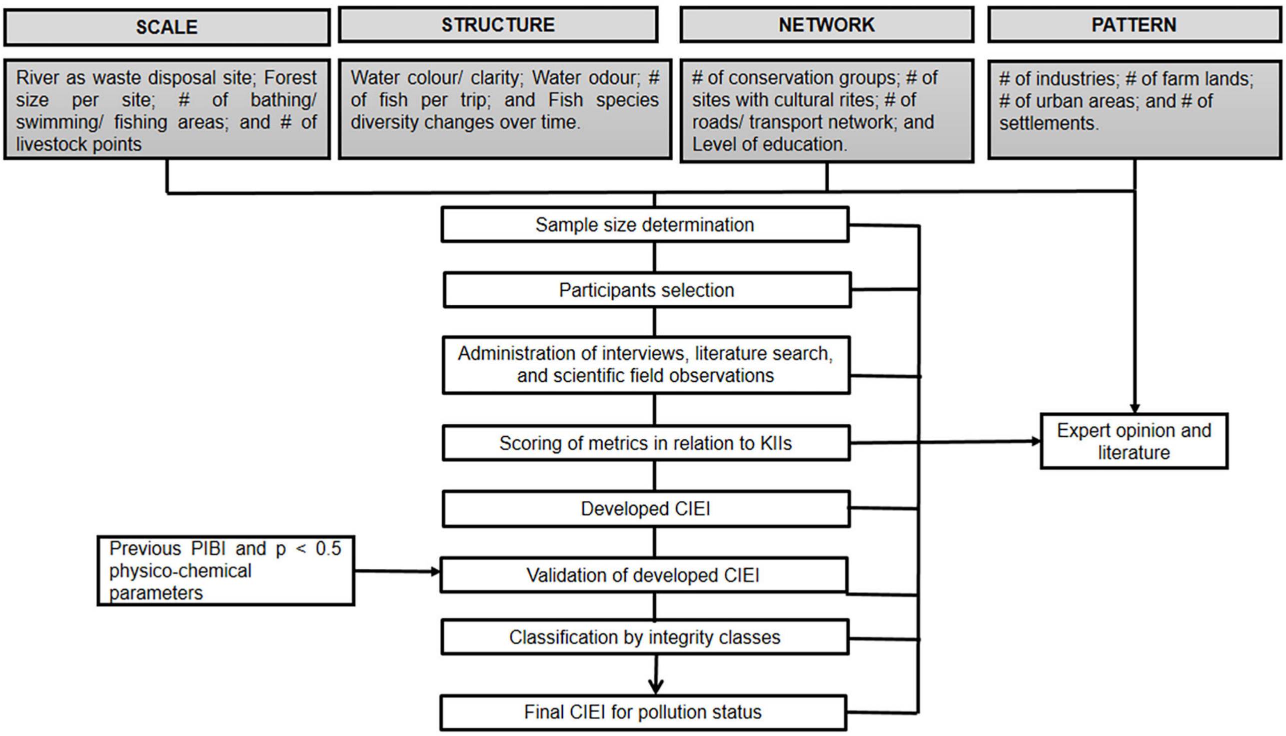

FIGURE 2 | Flow chart showing the steps involved in the development of the Citizen Index of Ecological Integrity (CIEI) novelty for assessing the pollution status of major river catchments in Lake Victoria, Kenya.

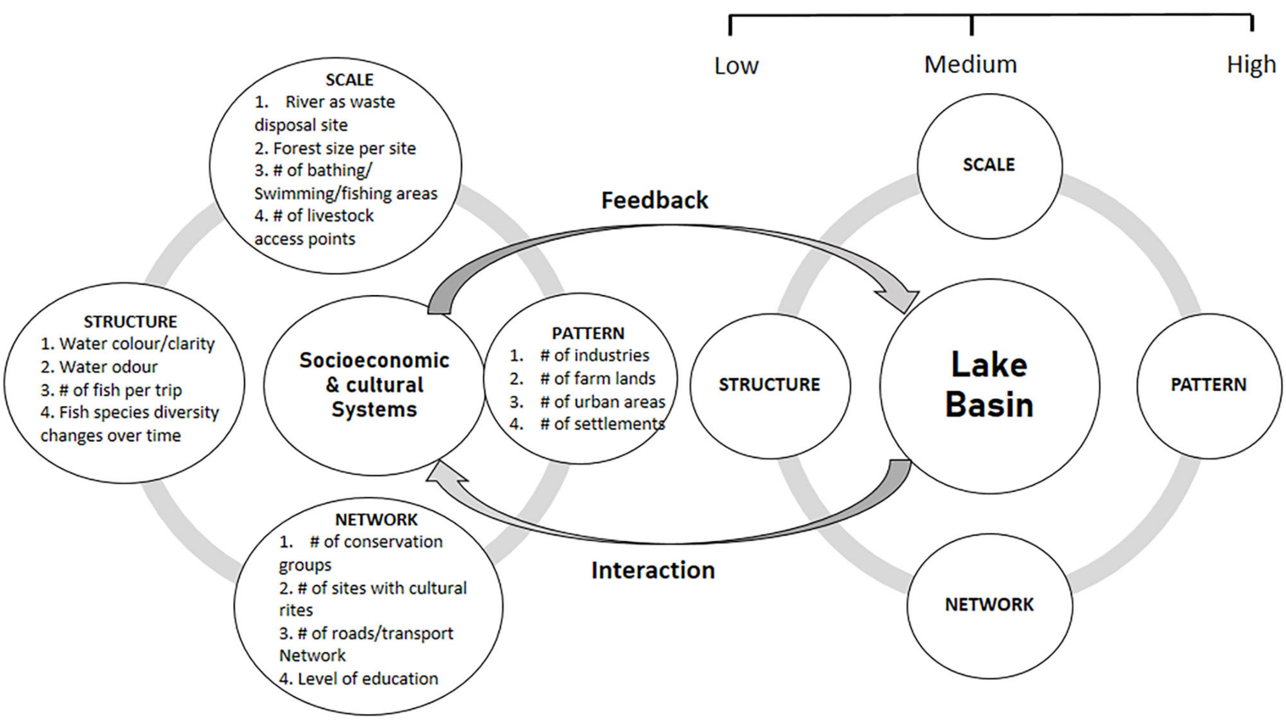

FIGURE 3 | Schematic representation of the socioeconomic and cultural metrics for the development of the Citizen Index of Ecological Integrity (CIEI) novelty for assessing the pollution status of major river catchments in Lake Victoria, Kenya.

TABLE 2 | Developed Citizen Index of Ecological Integrity (CIEI) for ranking of major river catchments in relation to community perceptions on the pollution status in the lower reaches of Lake Victoria, Kenya.

Metrics	River Nzoia			River Yala			River Sondu-Mirui			River Kuja			Scoring criteria			Notes/Assumptions
	NZ1	NZ2	NZ3	YA1	YA2	YA3	SM1	SM2	SM3	KU1	KU2	KU3	5	3	1	
Water color	5	3	3	5	5	3	5	5	3	5	5	5	≥Colorless	Brown-Green	≥Black	≥Black denotes highly polluted water
Water odor	5	3	3	5	3	3	5	5	3	5	5	3	No smell	Musty	≥Pagent	No smell denotes good water quality
Average number of fish per trip	1	1	3	1	1	3	3	3	3	3	3	5	≥10	6–10	≤5	High number denotes good integrity
Fish species diversity changes over time	3	3	5	3	3	5	5	5	5	5	5	5	≥30	15–29	≤15	High proportion denotes good integrity
Use of river as waste disposal site	1	1	3	1	1	3	3	3	3	3	3	3	≤5	4–20	≥20	Low loading denotes better integrity
Forest size per site	1	1	3	3	3	5	3	3	5	3	5	5	≤10	11–29	>30	Low number denotes less pollution loading
Number of bathing/swimming areas per site	3	3	5	1	5	5	5	3	5	3	3	5	≤3	4–5	>5	Low number denotes less pollution loading
Number of livestock access points per site	3	3	5	3	1	5	3	3	5	1	1	5	≤3	4–5	>5	Low number denotes less pollution loading
Number of industries per site	1	1	5	3	1	5	3	3	5	3	3	3	0	1–2	>2	Low number denotes less pollution loading
Number of farmlands per site	3	3	5	3	1	5	3	3	5	1	1	5	≤10	11–29	≥30	Low proportion denotes less pollution loading
Number of urban areas per site	3	3	5	3	3	3	3	3	5	5	3	5	≤1	2–3	>3	Low proportion denotes less pollution loading
Number of settlements per site	1	1	3	3	3	5	3	3	5	3	5	5	≤1	2–3	>3	Low proportion denotes less pollution loading
Number of conservation groups	1	1	3	1	1	3	3	1	3	3	1	5	≥2	1	0	High proportion provides increased integrity awareness
Number of sites with cultural rites	3	1	3	1	1	3	1	1	1	1	1	3	≥2	1	0	High proportion provides increased integrity awareness
Number of roads/transport network	3	1	3	1	3	3	3	1	5	3	3	3	≤1	2–3	≥4	Low proportion denotes less pollution loading
Level of inhabitants education	5	5	3	3	3	3	5	3	3	5	5	3	≥Tertiary	High school	≤Elementary	High literacy promotes conservation
Total CIEI	**42**	**34**	**60**	**40**	**38**	**62**	**56**	**48**	**64**	**52**	**52**	**68**				
Catchment/River CIEI		**45**			**47**			**56**			**57**					

NZ, Nzoia (NZ1, NZ2, NZ3= replications); YA, Yala (YA1, YA2, YA3 = replications); SM, Sondu-Mirui (SM1, SM2, SM3 = replications); KU, Kuja (KU1, KU2, KU3= replications); 1, low; 3, medium; 5, high.

TABLE 3 | Suggested threshold values of ecosystem integrity classes for final Citizen Index of Ecological Integrity (CIEI) development showing the classification level and ranges for ranking pollution levels in the lower reaches of Lake Victoria, Kenya during the study period (Adopted and modified from Aura et al., 2010).

Class of integrity	SCIEI ranges
Good Good water quality; slight pollution characteristics and some degradation (No human activity within 50 m of the riparian zone; bottom substrate dominated by very coarse and coarse, and vegetal materials; clear water with visible substrate)	≥ 55
Fair Moderate water quality; significant pollution levels and degradation (Riparian zone >20 m wide with minimal human activity; natural vegetation maintained along the reach with low instream cover <30%; substrate mainly of coarse and fine material)	47–54
Poor Poor water quality; major/heavy pollution and degradation (Riparian zone <20; collapsed and eroded sites; human activity include, agriculture, water abstraction, urbanization and deforestation; bottom dominated by sand and organic materials; water very turbid)	≤ 46

used for metrics biomonitoring and assessment (e.g., Aura et al., 2010, 2017), and its similar use could give better comparisons with other indices. For each socioeconomic and cultural metric that was expected to decrease with increased pollution levels in relation to the control site, the community awarded a score of 1, because the metrics showed the greatest deviation from the reference KIIsparticipants. Those sites were deemed to be close to the control site by the community were scored as 3, and values above the control site were scored as 5. The scoring by the community was reversed for socioeconomic and cultural metrics expected to increase with pollution levels. The scores for each metric were summed to arrive at the final CIEI value for each sampling site.

Based on the CIEI final scores, the study used integrity classes of good, fair, and poor as quantitative levels to come out with a scenario that could easily be interpreted by the policy makers (Aura et al., 2010) that were based on site visits and field records. The highest and lowest threshold ranges of > 45 and < 57 points were used to avoid a large deviation from all the CIEI final values.

Data Analysis

The interviews were examined to ensure that they were completely and consistently filled. The interview questions were then coded and summary tables and figures produced for the various responses. In order to ascertain the degree of accuracy of the CIEI in determining the pollution status of the sampled ecosystem, previously developed Phytoplankton Index of Biotic Integrity (PIBI) values in similar study sites were used for the validation of the novelty approach (Aura et al., 2020). The similarity of the study sites would help establish the degree of performance and robustness of the CIEI as a citizen-led tool in relation to the previous PIBI developed as a scientific-led output. The physico-chemical data were not normally distributed and attempts to normalize the data by transformations were unsuccessful. The physico-chemical data was compared spatially and temporarily using the non-parametric Kruskal-Wallis one-way ANOVA to examine the uncertainty of values and variations through pair-wise comparisons. The CIEI was correlated with significant ($p < 0.05$) physico-chemical parameters to further validate and strengthen the results of the final index. The study employed the use of SPSS version 21 (SPSS Inc., Chicago, IL, USA) and R version 3.5.0 (R Core Team, 2014) for statistical analyses. Significant differences for all analyses were determined at $p < 0.05$.

RESULTS

The participants in the study fell within the age group of between 55 and 70 years olds depicting participants' good experience. Though unquantified in the study, majority of the participants hesitated in answering questions on the scale metrics. The easiness to answer interview questions were in the order of structure, pattern, network, and scale from the least hesitant to the most hesitant metrics.

Table 2 shows the calculated final CIEI scores of the lower reaches of Lake Victoria, Kenya. In the final CIEI, River Kuja emerged with the highest average CIEI (57 points), while River Nzoia recorded the lowest CIEI (45 points) (**Table 3**) portraying good and poor ecosystem integrity accordingly. Thus, CIEI was in the order of Rivers Kuja, Sondu-Mirui, Yala, and Nzoia, from the highest to the lowest, respectively.

The validation and strengthening of the final CIEI scores showed significant but weak relationship ($R^2 = 0.37$; $p = 0.02$) with the PIBI scores (**Figure 4**). Notably, the CIEI depicted a similar trend with the previous index from North of Lake Victoria to the South. The only difference was the highest scores recorded in CIEI due to use of high number of metrics.

In the field observations, only conductivity, TP and TN levels varied across the sites (Kruskal-Wallis ANOVA; $p < 0.05$) with no temporal variations (**Table 4**). Further validation of the CIEI in relation to physico-chemical parameters with spatial significant variations showed a negative relationship ($p > 0.05$; $R < 0.5$) between the CIEI scores and the physic-chemical parameters.

DISCUSSION

Just like other scientific indices, the evaluation in the use of citizen-led tool for bioindication has been found to have several assumptions and drawbacks. Citizens or communities who have lived side by side with the natural environment for millennia are often believed to have a comprehensive understanding of the ecosystem integrity, including function and structure (Zhang et al., 2018). However, this is not always the case. This is because different inhabitants could have different interests and socioeconomic valuations. Furthermore, knowledge on the integrity, structure and functioning of natural ecosystems such as rivers that is passed down over generations can be harvested in a distorted manner and end up to have no benefit on management and conservation. Notably, the classification of merits and attributes for citizen-based indices are expert-dependent (Masese et al., 2013). The expatriate point of view

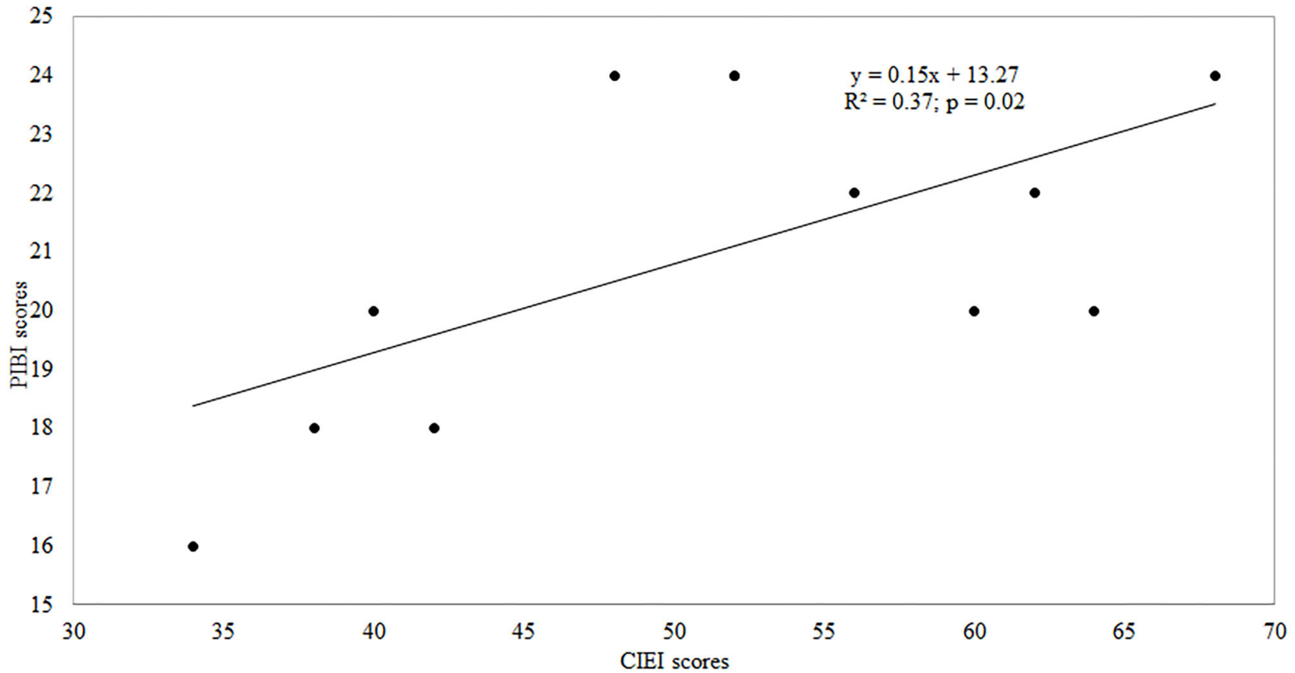

FIGURE 4 | A correlation plot showing the validation of the Citizen Index of Ecological Integrity (CIEI) novelty performance using previously developed Phytoplankton Index of Biotic Integrity (PIBI).

could therefore hinder objectivity and uniformity of the index output and thereby misinterpret conservation priority areas. This is especially the case in Africa where standard methods and tools for bioindication have not been fully developed for use by resource managers and conservationists. Additionally, other assumptions and premises are study dependent due to variability in geographical characteristics (Mangadze et al., 2019).

For example, the current study was based on the premise that pollution of major riverine catchments due to anthropogenic activities can cause longitudinal changes in water quality and habitat conditions that influence a lake basin. Community perceptions were noted to have indiscriminately determined the studied riverine catchments integrity variations as reflected in the spatial variability of metrics used as indicators of degradation. Metric variability and response of metrics to impaired sites indicated CIEI to have corresponded to the changes in the riverine ecosystem based on the physicochemical parameters reported in the study. However, the metrics used were not indiscriminately measured for redundancy and for elimination since each parameter was given a chance to contribute to the final index to ascertain the value and relevance of every participant (Zhou et al., 2017). This meant that each participant input was given a chance to contribute to the final CIEI to ensure full representation and to minimize bias. However, hesitation to answer interview questions on the scale metrics and as per the order of hesitation could be attributed to the riparian communities' activities that were directly linked to their relationships with contamination. Such behavioral responses from participants could be examined and quantified further in future citizen science studies that are related to pollution and environmental degradation.

However, understanding how socioeconomic and cultural perceptions of aquatic systems vary spatially has important implications for researchers, policy makers, environmental organizations aiming to reduce, or minimize pollution of such systems (Zhou et al., 2019). The findings from this study highlight the need for not only future socioeconomic and cultural studies on aquatic systems indices and models, but for outreach and educational efforts to occur in marginalized neighborhoods to increase the trust in the use of such systems and their safety. The improved trust will reduce the economic burden of conserving them and will also address the environmental sustainability issues surrounding pollution in aquatic systems.

The reported participants' age class of 55–70 years signified their longer residence time at the study areas, indicative of significant and valuable responses (Bachetti et al., 2008). This is because the CIEI was purely based on responses participantobtained during the interviews using semi-structured questionnaires and participant observation. This makes CIEI a major bottom-up tool that can be extensively employed to represent and explain ecosystem priority issues of the riparian community such as land use systems, pollution and water resource management.

Along the major river catchments studied herein, over-use of the riparian areas, sewage discharges and agriculture affected the quality of the riparian zones, banks and substrate quality; these were reflected in the developed CIEI (**Tables 2**, **3**) and water quality at the sampling stations (**Table 4**). The order

TABLE 4 | Spatio-temporal mean (± SD) values of selected physico-chemical variables in Rivers Nzoia, Yala, Sondu-Mirui, and Kuja, Kenya.

	River Nzoia			River Yala			River Sondu-Mirui			River Kuja			*p*
Parameters	**NZ1**	**NZ2**	**NZ3**	**YA1**	**YA2**	**YA3**	**SM1**	**SM2**	**SM3**	**KU1**	**KU2**	**KU3**	**Site**
Temperature (°C)	22.0 ± 0.0	22.5 ± 0.0	23.76 ± 1.2	20.9 ± 0.0	22.2 ± 0.0	24.7 ± 0.0	21.7 ± 1.2	20.5 ± 0.0	22.6 ± 0.0	23.0 ± 0.2	24.9 ± 0.0	24.6 ± 0.8	0.07
Conductivity ($\mu S\ cm^{-1}$)	100.0 ± 0.0	100.0 ± 0.0	101.0 ± 1.9	80.0 ± 0.0	81.2 ± 8.8	95.0 ± 4.0	104.0 ± 32.0	79.0 ± 0.0	133.0 ± 32.0	162.0 ± 3.0	126.0 ± 1.0	125.8 ± 5.0	0.02*
Oxygen ($mg\ L^{-1}$)	7.4 ± 0.1	7.5 ± 0.01	7.2 ± 0.5	7.5 ± 0.2	6.0 ± 0.3	6.0 ± 1.1	6.2 ± 0.3	6.4 ± 0.2	5.7 ± 0.5	5.7 ± 0.1	5.3 ± 0.0	5.4 ± 0.1	0.05
Secchi depth (m)	0.9 ± 0.1	0.8 ± 0.0	0.7 ± 0.1	0.9 ± 0.3	0.9 ± 0.5	0.8 ± 0.1	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.0	1.0 ± 0.3	1.0 ± 0.0	0.05
TP ($\mu g\ L^{-1}$)	182.5 ± 14.5	183.0 ± 15.7	170.1 ± 54.1	107.2 ± 3.6	65.3 ± 7.3	63.4 ± 15.5	104.9 ± 8.2	64.9 ± 9.4	122.0 ± 10.1	60.6 ± 4.9	67.7 ± 6.1	69.1 ± 3.3	0.03*
TN ($\mu g\ L^{-1}$)	1533.9 ± 127.9	1442.4 ± 41.6	1504 ± 10.0	1721.8 ± 383.8	1030.9 ± 82.0	1139.9 ± 59.5	1606.8 ± 411.0	2056.9 ± 124.3	1513.3 ± 107.1	1218.7 ± 334.1	911.5 ± 12.9	1586.2 ± 322.2	0.04*

NZ, Nzoia; YA, Yala; SM, Sondu-Mirui; KU, Kuja. [*] *refers to significant p level of Kruskal-Wallis ANOVA, $p < 0.05$.*

of index scoring of rivers from Kuja, Sondu-Mirui, Yala, and Nzoia was from the highest to the lowest, respectively. Observed inclination illustrated the gradient of influence on the pollution status of the lake basin from north to south. Thus, selected northern rivers are highly threatened by catchment activities. The main ones being conversion of wetlands into farms such as the Dominion Farm around River Yala, urban developments, poor management of domestic, and industrial wastes from industries such as Pan Paper Industry along River Nzoia, and leaching of agrochemical residues causing river pollution, decreased forest cover, and increased soil erosion. Conversely, selected southern rivers Sondu-Mirui and Kuja are influenced mainly by agricultural farms, domestic wastes, and urbanization (Balirwa et al., 2003). The variation in the ecosystem integrity could therefore be due to the varying degree of multiple sources of pollutants from agricultural fields, industries, and domestic sources in studied ecosystems (Kundu et al., 2017). Similarly, several authors have recorded differences in space for biota, nutrient variations, pollution levels, water quality, and indices (Lung'ayia et al., 2001; Gikuma-Njuru and Hecky, 2005; Aura et al., 2010; Haande et al., 2011; Masese et al., 2013).

The validity of the CIEI developed was also reinforced by the insignificant weak relationship with conductivity and nutrient concentrations (**Figure 5**), suggesting that the variation exhibited some level of congruent with habitat and water quality of the studied rivers. In this regard, the CIEI was to some extent, able to identify sources of impairment and to assess the level of degradation arising from human activities. This could be because no site in the studied rivers scored the maximum values for all metrics, it can be adduced that even areas considered to be relatively unimpaired were already experiencing the effects of degradation (Aura et al., 2010).

For the management of the Lake Victoria Basin, the metric scores and integrity classes are indicative of a changing environment under the influence of human activities. With increasing human population in the major riverine catchments, the situation is likely to be exacerbated. The challenge is to mitigate the current trend and to improve the CIEI scores at the sites and stations by improving both water and habitat quality. This can be achieved by riparian zone restorations, which have been found to be useful in improving riverine integrity (Kasangaki et al., 2008). Therefore, there is need to adopt this index for monitoring the Afrotropical riverine systems, because it is reliable not only for identifying sources of impairment, but also for assessing restoration successes. The CIEI developed herein in the current form or if further strengthened and reviewed, could offer a potential candidate decision support tool for the management of the lake basin at spatial and temporal level.

CONCLUSIONS

This study explores the preliminary use of socioeconomic and cultural parameters or index in the assessment of natural aquatic ecosystem integrity. The CIEI developed showed a robust approach to ascertain the various levels of ecosystem integrity using community metrics. In this case, southern ecosystem of

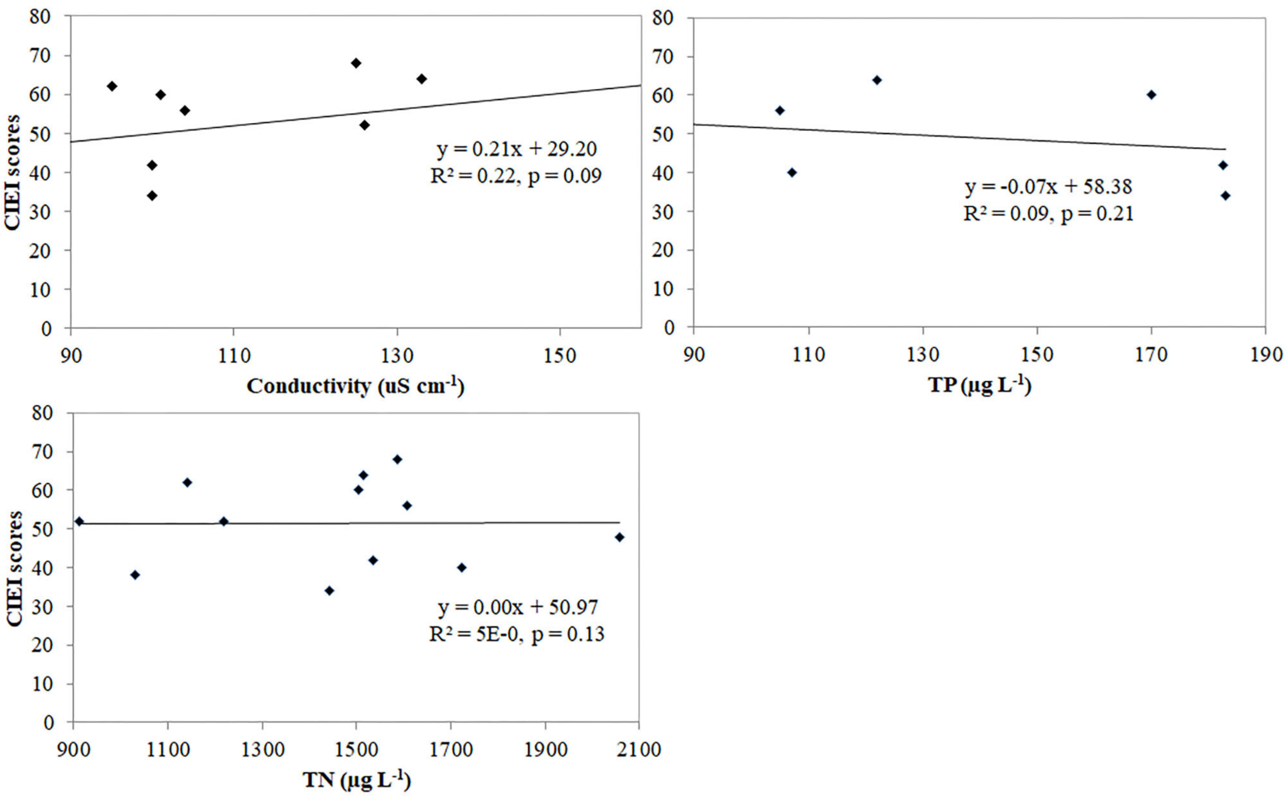

FIGURE 5 | Correlation plots showing further validation and strengthening of the Citizen Index of Ecological Integrity (CIEI) novelty performance for assessing the pollution status of major river catchments in Lake Victoria, Kenya.

Lake Victoria was ranked well than the northern counterpart in terms of pollution status of the lake basin. Thus, CIEI novelty herein could be used to show areas where conservation may be prioritized, with a broader applicability for snapshot studies in marginalized areas and with minimal expenses. There are some deficiencies in the current study that need to be improved in future research. Because the research area is a quite complex system, data with high spatio-temporal accuracy is required for better simulation. More adjustments for the CIEI may be required in the future to compare the upper sections of the catchments with the lower reaches as well as revision and up-scaling of socioeconomic and cultural metrics used.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

CA drafted the manuscript with support from CN, HO, CO, SM, JN, KN, and FM. All authors contributed to the final version of the manuscript.

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Assessment of the Ecological Health of Afrotropical Rivers Using Fish Assemblages: A Case Study of Selected Rivers in the Lake Victoria Basin, Kenya

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Streams and rivers are globally threatened ecosystems because of increasing levels of exploitation, habitat degradation and other anthropogenic pressures. In the Lake Victoria Basin (LVB) in East Africa, these threats are mostly caused by unsustainable land use; however, the monitoring of ecological integrity of river systems has been hampered by a lack of locally developed indices. This study assessed the health of four rivers (Nzoia, Nyando, Sondu–Miriu and Mara) on the Kenyan side of the LVB using physicochemical water quality parameters and a fish-based index of biotic integrity (IBI). Fish tolerance ranking was derived from principal component analysis of water quality parameters, and the concept of niche breadth (NB). The relationship between fish species and water quality parameters was examined with canonical correspondence analysis, whereas community metrics and stressors were evaluated through Pearson network correlation analysis. Fish species richness, trophic structures, taxonomic composition and species tolerance were used to generate the metrics for fish-based IBI. NB showed that most of the fish species were moderately tolerant to poor water. Moderately tolerant and intolerant fish species were negatively correlated with a high level of organic loading in the Mara River. Fish-based IBI scores for the rivers ranged from 26 to 34, with Sondu–Miriu scoring the lowest. Our results show that the cumulative effect of stressors can adequately rank fish species tolerance according to the disturbance gradients and further develop regional metrics to assess river health. Despite the fact that fish communities are declining, continual management and enforcement of environmental regulations are important, with conservation and management of headwaters and low-order streams being essential while they are still species rich.

Keywords: afrotropical rivers, niche breadth, fish index of biotic integrity, trophic level, species sensitivity, multivariate analysis

INTRODUCTION

River catchments are some of the most vulnerable ecosystems through being increasingly exposed to multiple anthropogenic stressors, including habitat degradation, flow alteration, increased water demand, urbanization and agricultural intensification (Dudgeon et al., 2006; Mamun and An, 2020). This has resulted in a loss of hydrological connectivity, increases in nutrient and sediment load, exposure to invasive species and biodiversity loss, most of which occur as multiple interacting factors affecting structure and function of riverine ecosystems (Stevenson and Sabater, 2015; Shao et al., 2019). Predicting river responses to human activities is challenged by the diversity of stressors and habitat alterations associated with them and therefore a quantitative or objective assessment of global river ecosystem health remains a major challenge (Zhao et al., 2019). Nonetheless, predicting the effects of human activities can be improved by recognizing similarities in sets of stressors within classes of human activities and in how different stressors affect rivers, as well as distinguishing the effect stressors have on direct vs. indirect regulation of ecosystem services (Stevenson and Sabater, 2015). Traditional methods assessed the effects of these stressors using physicochemical water quality parameters and their variation compared to a reference condition (Karr and Chu, 1999; Cairns, 2003); however, advanced methods have integrated hydrological variables and response of biological communities when developing multimeric indices to assess the health of aquatic ecosystems (Arman et al., 2019; Ruaro et al., 2020).

The use of biological communities in the assessment of riverine ecosystem health within the Afrotropical region has generally lagged behind equivalent studies in other regions (Ruaro et al., 2020). Although there are many aquatic organisms that can be included in the evaluation of river health (Herman and Nejadhashemi, 2015), regional indices have widely focused on macroinvertebrates (Dickens and Graham, 2002; Thirion, 2007; Masese et al., 2013, 2020c) that are rarely identified to species level and have some inaccuracy due to ecological and physiographical diversity between regions (Hering et al., 2010). Despite this constraint, these approaches are applied to studies within the Afrotropical region; however, the identification keys and indices are typically developed elsewhere. For instance, the Zambia Invertebrate Scoring System (Lowe et al., 2013), Tanzania River Scoring System (Kaaya et al., 2015) and macroinvertebrate based biotic score system (Aschalew and Moog, 2015) have all been modeled around the South African Scoring System (Chutter, 1998; Dickens and Graham, 2002). A few studies on river health have used other organisms, such as macrophytes (Achieng et al., 2014; Kennedy et al., 2016) and phytoplankton (Oberholster, 2011; Ngodhe et al., 2013). Surprisingly, the Fish Response Assessment Index that was developed more than a decade ago (Kleynhans, 2007) has not been widely adopted in the Afrotropical region, yet fish communities have significantly declined.

The Lake Victoria Basin (LVB) of East Africa has an estimated population of 40 million, with a density of more than 500 persons per km^2, and is largely rural and highly dependent on land, forests and river catchments (World Bank, 2016; Sayer et al., 2018a; Olaka et al., 2019). It is dominated by agricultural activities, with 85% of the population dependent on agriculture as their major economic and livelihood activity (Lake Victoria Basin Commission, 2007). These range from small- and medium-scale cultivations to mechanized large-scale cultivation systems, characterized by the high use of fertilizers, pesticides and herbicides, as well as supplementary irrigation (Lake Victoria Basin Commission and GRID-Arendal, 2017). The LVB has experienced a rapid decline in biodiversity, with up to 76% of endemic species threatened with extinction, yet there is a dearth of basic information on the distribution and status of many freshwater species (Sayer et al., 2018a,b). Unsustainable changes in land use that significantly influence ecosystem structure and functioning (Lambin et al., 2003; Turner et al., 2003; De Groot et al., 2010) have impacted river catchments (Ochola, 2006; Odada et al., 2009; Masese et al., 2013; van Soesbergen et al., 2019; Nyilitya et al., 2020), affecting the distribution of fish species in river networks within the LVB (Achieng et al., 2020). Previous studies on the impact of human activities on riverine fish species distribution and biological characteristics within the LVB focused on lower reaches of the basin or were limited to specific rivers (Whitehead, 1959; Corbet, 1961; Masese et al., 2020a). They pre-date some of the rapidly changing uses of land and environmental conditions, and do not capture the state of current fish communities and overall ecosystem health status (Masese and McClain, 2012; Masese et al., 2020a). To develop fish indices that will reliably assess the health of riverine ecosystems in the LVB, it is necessary to consider fish communities that reflect the period of disturbance, as disturbance gradients are associated with losses of sensitive or intolerant species and increases in tolerant species (Vázquez and Simberloff, 2002; Davies and Jackson, 2006). As a result, species that are considered generally sensitive or tolerant to human disturbances are commonly used as indicators of healthy ecosystems or ecosystem deterioration respectively (Segurado et al., 2011; Zeni et al., 2017; Brejão et al., 2018).

Ranking of species tolerance to human perturbations in riverine ecosystems has been based on qualitative professional judgments and/or literature from outside the LVB, usually with little support from empirical, ecological or physiological data (Wang et al., 2018). The tolerance rankings of other species have been based on simple mathematical explorations, which are easy to implement, but do not account for natural or multiple environmental variables (Lenat, 1993; Segurado et al., 2011). With increased computing power and multivariate methods, quantitative evaluation of environmental variability and taxa response along multiple stressor gradients have been possible through evaluation of similarity–dissimilarity or correlation–covariance matrices (Jongman et al., 1995; Hermoso et al., 2009; Achieng et al., 2017). For instance, ranking species tolerance has been possible using principal component analysis (PCA), whereby eigenvalues are used to determine species tolerance along a gradient of a perturbation (Jongman et al., 1995; Segurado et al., 2011). Understanding fish species tolerance to environmental perturbation is essential when formulating community metrics to develop ecological indices for assessing riverine ecosystem health. Only two published studies have developed a fish-based index of biotic integrity (IBI) for rivers (Raburu and Masese, 2012) and wetlands (Naigaga et al., 2011) in the LVB; however, neither quantitatively computed tolerance ranking for species in response to environmental gradients.

In this study, we assessed the health of four river catchments in the LVB in Kenya using fish assemblages and water quality parameters. This was achieved by mapping land use at the river catchments, using cropland as a proxy for agricultural activities, which are known to be a dominant stressor at the basin. We ranked fish tolerance to perturbation through the concept of niche breadth (NB) using multivariate PCA and eigenvalues as the first tolerance ranking in the region. This allowed us to develop fish IBI to assess the health of these rivers in the LVB. Given the recent intensification of land activities in the basin, we predicted that fish communities have largely declined in response to stressors facing these ecosystems and that the responses are basin specific due to variations in stressors and their intensity at different catchments. In addition, we proposed that the cumulative effect of stressors can be used to rank fish tolerance to perturbation and depict river health. This approach is unique in that it is developing specific indices for Afrotropical ecosystems, rather than borrowing and modifying methods from other regions or using species responses to stressors relevant to temperate and subtropical regions.

MATERIALS AND METHODS

Study Area

This study focused on water quality and fish species in four river catchments, Mara, Nyando, Nzoia and Sondu–Miriu, on the Kenyan side of the LVB (**Figure 1**). Of the four rivers, the Mara River is the transboundary between Kenya and Tanzania and the lifeline of the Maasai Mara National Reserve (MMNR) in Kenya and Serengeti National Park (SNP) in Tanzania. All the four rivers originate in the forested western slopes of the Mau Escarpment. In their upper and middle reaches, these rivers drain high potential areas for agricultural production with mean annual rainfall ranging from 1,350 to 2,400 mm (Olaka et al., 2019). Rainfall displays a bimodal distribution with two distinct peaks from March to May (long rains) and October to December (short rains) (Kizza et al., 2009). The rivers are important for domestic, industrial and irrigation water supplies and also support navigation and energy production. They also have exceptional biodiversity resources rich with native and endemic species (Masese et al., 2020a; Pringle et al., 2020).

The four river catchments differ considerably in their disturbance gradients. Although the Mara River catchment has the least land area utilized for crop farming in the headwater and few urban areas in the middle reaches compared to the other catchments, it has undergone significant changes in land use over the last five decades with increased sediment load as a result of pollution (Dutton et al., 2018a). A considerable proportion of the middle and lower reaches are under protection as part of the MMNR and SNP with the nomadic Maasai community grazing large herds of livestock in the area. The livestock and large wildlife (mainly hippopotamus) are major sources of organic matter and nutrients in the Mara River and its tributaries (Subalusky et al., 2015; Dutton et al., 2018b; Iteba et al., under review). In its headwater, the Sondu–Miriu River has large-scale tea plantations by multinationals practicing conservation farming, maintaining riparian zones along streams. However, the river has lost substantial forest cover in the past, and this has been linked with increased levels of sediments and nutrients in the river (Jacobs et al., 2017; Kroese et al., 2020). The river also experiences a number of influences in its middle reaches and lower reaches, such as tea processing factories and growing urbanization, subsistence agriculture and hydropower production. The Nyando catchment has the most varied disturbance gradients with large-scale tea farming upstream, processing factories and agrochemical industries at middle reaches and mixed farming with urbanization downstream. A number of agroprocessing industries, such as the Muhoroni and Chemelil sugarcane processing factories, have been a source of water pollution in the river (Raburu and Masese, 2012). The Nzoia River drains the grain basket of Kenya, and its catchment is dominated by large-scale commercial agriculture in the upper and middle reaches. There is also extensive mixed farming in the middle and lower reaches. Potential sources of pollution in the river include agricultural and urban run-off and wastewater discharges from big cities (such as Eldoret and Kitale), sugarcane processing factories and the Webuye Panpaper Mills, which is currently dormant but has a history of water pollution (Orori et al., 2006; Achieng et al., 2017; K'oreje et al., 2018).

Field Sampling

A total of 68 sites were sampled between September 2018 and February 2020, with 26 sites in Mara, 17 in Nyando, 14 in Nzoia and 11 in Sondu–Miriu (**Figure 1**). Site selection was based on their location on the fluvial continuum to capture point and nonpoint sources of pollution and obvious sources of habitat degradation, such as livestock watering points and hippopotami pools. Site selection also considered catchment size, land use at the catchment and accessibility, with all major tributaries for each river sampled. Dissolved oxygen concentration, temperature, total dissolved solids (TDS), salinity and electrical conductivity (EC) were measured *in situ* using a hydrolab (YSN professional series model; ProtoComm II L/N 12G100510). Water samples were also collected using HDPE bottles for analysis of nutrients [nitrates (NO_3), nitrite (NO_2), ammonium (NH_4) and soluble reactive phosphorus (SRP)] using standard methods (APHA, 2005).

Fish samples were collected by a generator-powered electrofisher (Honda GX240 8 HP; 400 V 10 A) and a backpack shocker (Achieng et al., 2020; Masese et al., 2020a). Sampling was done during daylight hours and stunned fish collected with a scoop net. Captured fish were identified, counted, weighed (0.1 g) and length (cm) measured. Specimens of each species were preserved in 75% ethanol for subsequent confirmation of species identification, with the remaining live fish returned to the river. Identification was done to species level using a number of taxonomic keys (Eccles, 1992; Skelton, 1993). Feeding habits/trophic levels were identified using the FishBase database (Froese and Pauly, 2019).

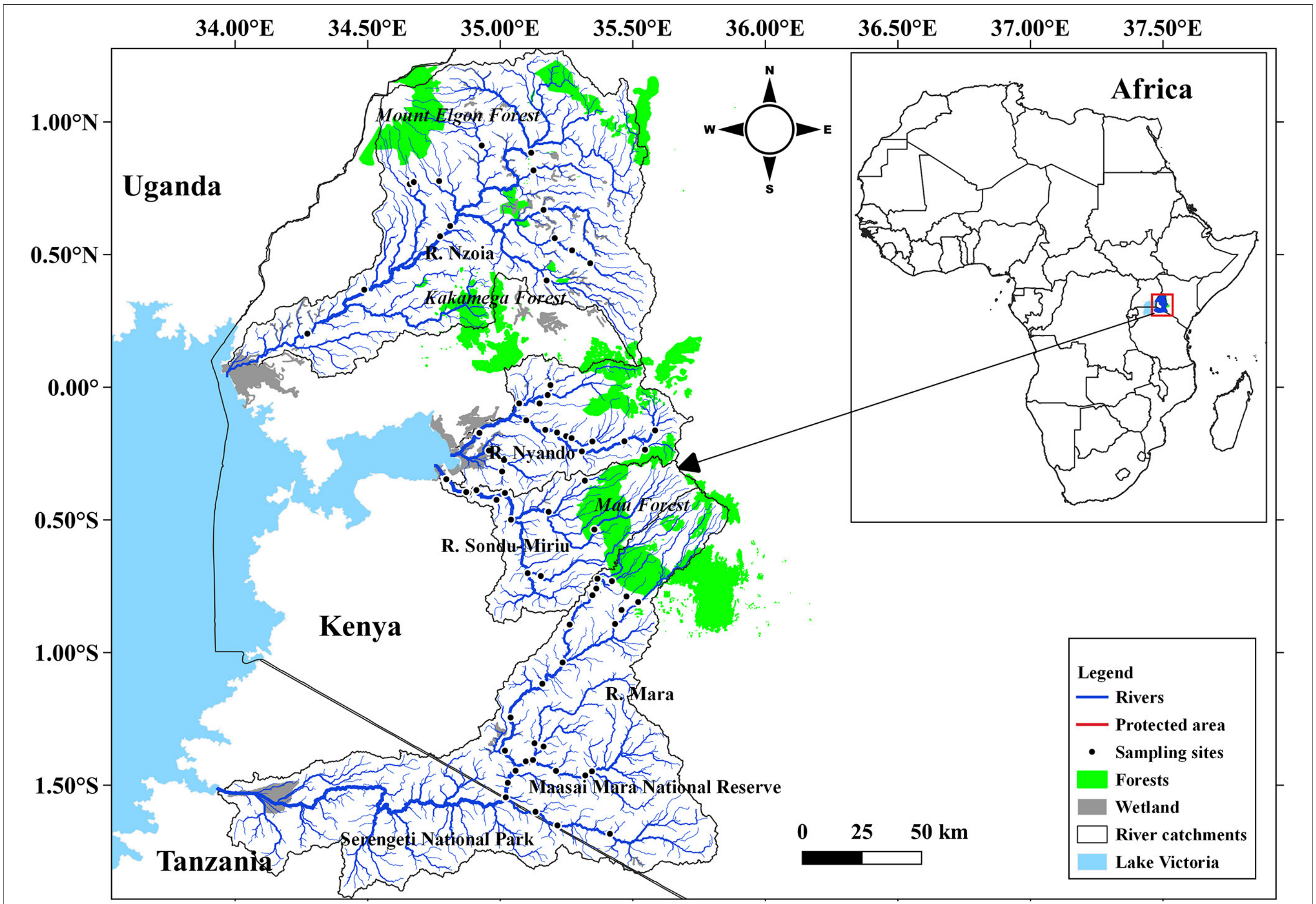

FIGURE 1 | Sampled river catchments and location of sampled stations along Rivers Mara, Nyando, Nzoia, and Sondu–Miriu on the Kenyan side of the Lake Victoria Basin.

Data Analyses

Land Use Classification and Statistical Analysis

Land use classification and catchment maps were generated with QGIS 3.14, using Semi-Automatic Classification plug-in to download sentinel-2 images during the study period (Congedo, 2020a,b). The satellite images were preprocessed and processed for all the band sets, mosaic, clipped and supervised classifications were done for land use/land cover (Akgün et al., 2004; Huth et al., 2012; Congedo, 2016; Herbei et al., 2016) in each of the four catchments, with four categories of land use (forest, grassland, cropland and shrubland). Water quality measurements were explored before further analysis using box-and-whiskers plot to visualize summaries and compare their variation (Williamson et al., 1989; Dekking et al., 2005; Hubert and Vandervieren, 2008) at the catchments. The measurements were then natural log (ln) transformed to satisfy the assumptions for parametric general linear model analysis of variance (GLM-ANOVA), which was used to infer significant difference (Hothorn et al., 2008; Madsen and Thyregod, 2010) in the measured water quality parameters between catchments. Moreover, PCA was used to determine the components that explained most of the variation and identified water quality parameter measurements that contributed to these variations at the catchments (Achieng et al., 2017). Fish assemblage were analyzed with one-way analysis of similarity (ANOSIM) to inferred significant dissimilarity in fish communities at the catchments, while similarity percentages (SIMPER) was used to separate the fish into relative abundance of specific species that contributed to the dissimilarity at catchments (Álvarez et al., 2017; Achieng et al., 2020; Masese et al., 2020a). Canonical correspondence analysis (CCA) was then used to infer significant relationships between fish species and environmental variables (O'Connell et al., 2004; Hoeinghaus et al., 2007; Junqueira et al., 2016) at the four catchments. In addition, Pearson network correlation analysis (PNCA) was used to infer the relationship between environmental variables (water quality parameters and proportional land use as forest and agriculture) and fish community metrics (species richness, trophic structures, taxonomic composition and species tolerance) (Mamun and An, 2020). Finally, fish community metrics were used to develop the

Fish-based IBI. PCA and PNCA were plotted with R software (R Core Team, 2020; RStudio Team, 2020) using packages ggplot2 and qplot (Wickham, 2016) in the R Environment, whereas CCA, ANOSIM and SIMPER were analyzed using PAST software version 4.03 (Hammer et al., 2001).

Tolerance Values Based on the Concepts of Niche Breadth

We estimated the NB of species along the main environmental gradient (Segurado et al., 2011). This measure was assumed to be a surrogate of species tolerance to human-induced pressures, based on an hypothesis that generalist species (wide NB) are more tolerant to pressures than specialist species (narrow NB), according to the specialization–disturbance hypothesis (Vázquez and Simberloff, 2002; Segurado et al., 2011; Slatyer et al., 2013). This indicates that as instream habitats are simplified and homogenized, populations of specialist species often decline or are extirpated, whereas generalist species tend to increase in abundance (Zeni et al., 2017; Brejão et al., 2018). The main environmental gradient was based on the scores of the first and second axes of a PCA calculated from all environmental variables considered in the study. NB was computed as the mean standard deviation of the scores of the two first PCA axes at sites (site scores/loading or vector matrix) where a particular species was present, weighted by their eigenvalues (2.59 and 2.18, respectively) from the PCA as follows;

$$NB = \frac{\sqrt{\frac{\sum_{i=1}^{n} (PC_{\ i} - PC_{\ mean})^2}{n}}}{PC_{\ eigenvalue}}$$

where NB = niche breadth, PC_i = principal component loading for each site where species i is present at all the catchments, PC_{mean} = mean of the principal component loadings for all sites where species i is present, and $PC_{eigenvalue}$ = eigenvalue for the PCA.

IBI Model and Community Similarity

The IBI was computed from 12 metrics, some of which were previously used in the region (Raburu and Masese, 2012). Metrics 1 and 2 represented the total number and percentage of native species respectively. Metrics 3, 4 and 5 represented the number of benthic riffle, benthic pool, and pelagic pool species, respectively. Metrics 6 and 7 considered the number of sensitive species and proportion of tolerant species, whereas metrics 8, 9 and 10 evaluated the proportion of omnivorous, insectivorous, and carnivorous, respectively. Finally, metrics 11 and 12 were computed from Simpson Dominance Index and Margalef Index. Each metric was assigned a value of 5, 3 or 1 (Harris and Silveira, 1999; Raburu and Masese, 2012; Mamun and An, 2020) and river health was determined by adding the value for each metric and categorizing the results as excellent (36–40), good (28–34), fair (20–26), poor (14–18), or very poor (8–13) (Atique and An, 2018; Mamun and An, 2020).

RESULTS

Land Use Change and Water Quality Analysis

The four catchments were 13,493, 12,786, 3,613 and 3,451 km^2 for Mara, Nzoia, Nyando and Sondu–Miriu respectively. There was extensive cover of shrubland (60.1%) and grassland (32.9%) in the Mara catchment (**Figure 2A**), grassland (41.6%) and cropland (32.7%) in the Nzoia catchment (**Figure 2B**), shrubland (42.6%) and cropland (41.8%) in the Nyando catchment (**Figure 2C**) and shrubland (36.0%) and grassland (30.6%) in the Sondu–Miriu catchment (**Figure 2D**). Of the four catchments, forest was proportionately largest in the Sondu–Miriu catchment (20.4%) and smallest in the Mara and Nyando catchments (4.6%), whereas the proportion of cropland was largest in the Nyando catchment (41.8%) and smallest in the Mara catchment (2.4%).

GLM-ANOVA inferred significant differences in all water quality parameters ($p < 0.001$) at catchment scale, except for temperature (**Supplementary Table 1**). This was also confirmed with box-and-whiskers plot (**Figure 3A**). EC, TDS, salinity and dissolved oxygen concentration were significantly lower in the Sondu–Miriu River (**Supplementary Table 1**), but with very high variation at sites in the Mara River, ranged from 44.0 to 4,202 μS/cm, 0.03 to 2.73, 0.02 to 2.24 and 0.84 to 13.37 mg/L, respectively (**Supplementary Table 1**). Three sites in the Talek River tributary of the Mara River, which is seasonal and hosts the largest population of livestock and hippopotami, recorded the highest levels of EC, salinity and TDS (**Figures 3B–D**). Dissolved oxygen varied the most (**Figure 3E**) while SRP, HN_4, NO_2 and NO_3 were significantly lower in Mara River (**Figures 3F–I**; **Supplementary Table 1**), but did not differ between the Nzoia and Sondu–Miriu Rivers. NO_2 concentrations were highest in the Sondu–Miriu River (**Figure 3I**), whereas SRP and NH_4 concentrations were highest in the Nzoia and Nyando Rivers (**Figures 3F,G**).

PCA identified the water quality parameter measurements that contributed to the observed variation in the four catchments, with components 1 and 2 accounting for up to 53% of the variance (**Figure 4A**). Component 1 explained 29% of the variation in the four catchments and showed that Nzoia and Nyando had similar stressors (NO_3, NH_4, SRP) as the major impacts (**Figures 4A,B**), with a high contribution from cropland as a proxy for agricultural activities. Component 2 explained 24% of the variation and highlighted conductivity, salinity, and temperature as key variables influencing variation in the Mara catchment (**Figures 4A,C**).

Fish Composition, Sensitivity, and Abundance

A total of 2,269 fishes, representing 28 species, were sampled in the four catchments. Of these species, 11 were insectivorous, 10 omnivorous, five herbivorous and two carnivorous (**Table 1**). *Labeobarbus altianalis*, an omnivorous feeder, was the most abundant species (combined abundance = 621 individuals), with a relative abundance of 27.4% and the predominant species in the samples from both the Nyando and Mara catchments, with 297 (38.4%) and 216 (33.6%), respectively. The samples

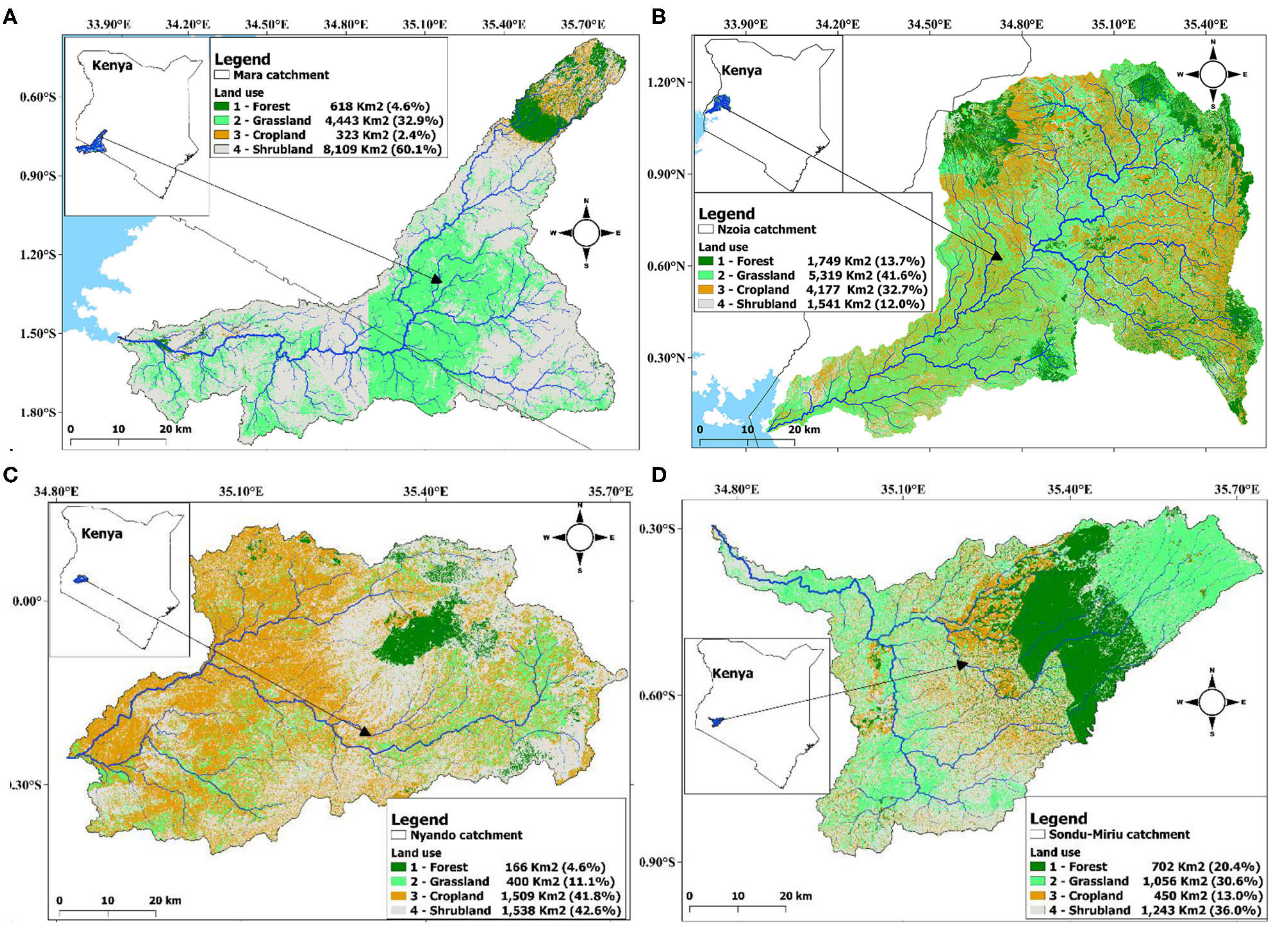

FIGURE 2 | Area in km^2 and percent forest, grassland, cropland and shrubland within the river catchments, **(A)** River Mara, **(B)** River Nzoia, **(C)** River Nyando and **(D)** River Sondu–Miriu of the Lake Victoria Basin.

from Nzoia and Sondu–Miriu catchments were dominated by *Enteromius neumayeri*, an omnivorous feeder, with 263 (42.0%) and 168 (74.3%) respectively. *E. neumayeri* was also the second most abundant species in the total sample (503), with a relative abundance of 22.2%. The remaining fish species each had a relative abundance of <10%. Species sensitivity, calculated from the concept of NB using the PCA loadings of environmental variables at sites where species were present and weighted by their eigenvalues, identified *Labeo victorianus* and *Clarias gariepinus* as tolerant species, *Chiloglanis somereni*, *Clarias theodorae*, *Gambusia affinis* and *Haplochromine* species as intolerant species, whereas 13 species were moderately tolerant (**Table 1**). Species that were sampled in only one river and one site ($n = 9$) could not be used to determine sensitivity.

One-way ANOSIM for fish composition and abundance at the catchment, with the rivers as factors, indicated significant dissimilarity ($R = 0.155$, $p = 0.001$). SIMPER separated the difference in composition of fish species to relative abundance of a few species (**Table 2**). The difference in species composition was as a result of a few species in most of the catchments. For instance, between the Mara and Nzoia catchments, Mara and Nyando catchments, Nyando and Nzoia catchments, Mara and Sondu–Miriu catchments and Nyando and Sondu–Miriu catchments, *L. altianalis* contributed 14.30, 16.23, 20.16, 24.83 and 31.31% of the dissimilarities respectively. The second species that contributed to dissimilarities at the catchments was *E. neumayeri* at 15, 18.46, 19.98, 21.69, and 23.46% between Nyando and Sondu–Miriu, Mara and Sondu–Miriu, Nyando and Nzoia, Nzoia and Sondu–Miriu and Mara and Nzoia catchments respectively. Other species, like *Enteromius nyanzae*, contributed the most dissimilarity in fish composition between the Mara and Nyando catchments (29.26%), whereas *Chiloglanis somerini* and *Amphilius jacksonii* contributed the most dissimilarity (29.68 and 22.83% respectively) in species composition between Nzoia and Sondu–Miriu catchments. The overall dissimilarity at the catchments ranged between 35.22 and 84.35%, with Mara and Nyando catchments having the least dissimilarity.

CCA identified the influence of water quality variables and agricultural activities on fish assemblage as significant ($p = 0.006$) with components 1 (32%) and 2 (20%) providing 52%

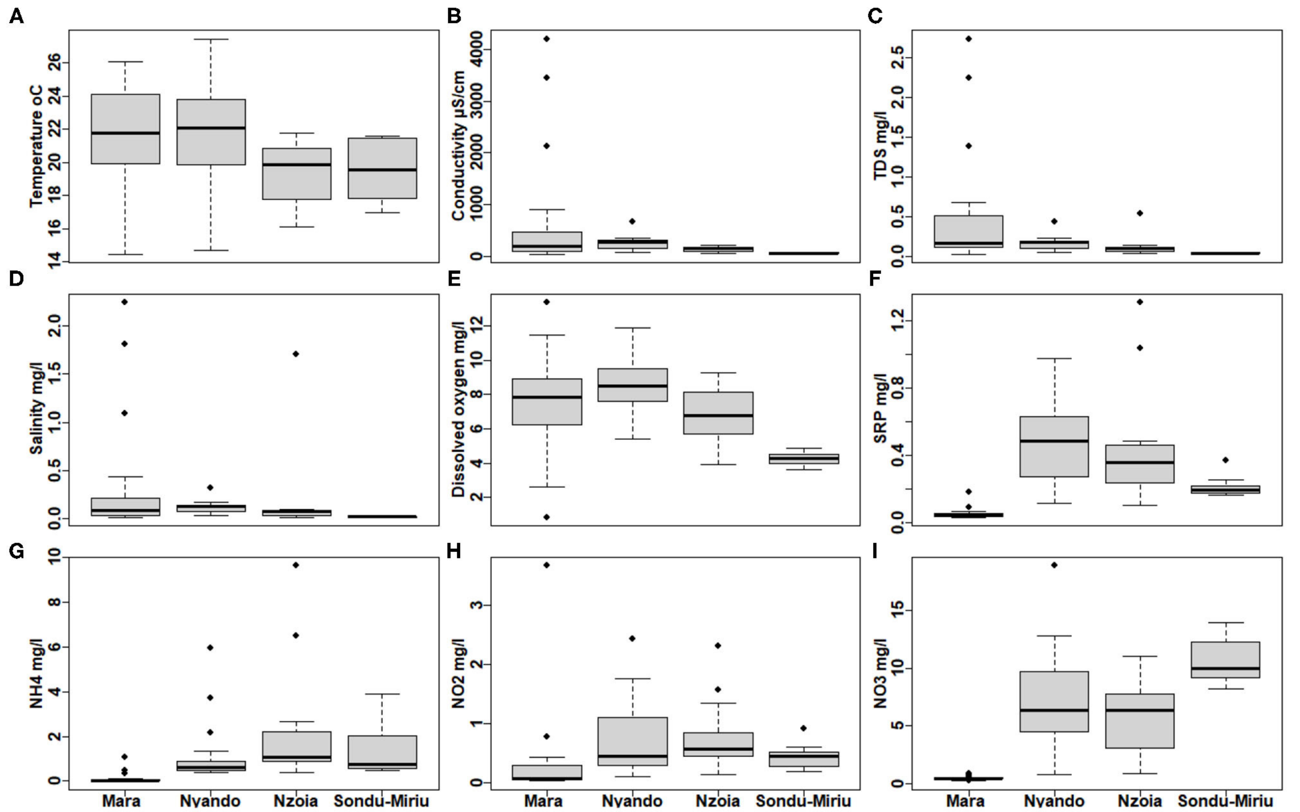

FIGURE 3 | Variation in water quality parameters at the four catchments within the Lake Victoria Basin. **(A)** Temperature, **(B)** conductivity, **(C)** total dissolved solids (TDS), **(D)** salinity, **(E)** dissolved oxygen concentration, **(F)** soluble reactive phosphorus (SRP), **(G)** ammonia (NH_4), **(H)** nitrite (NO_2) and **(I)** nitrates (NO_3).

of the assemblage variability in the four catchments (**Figure 5**). It revealed significant relationships between water quality parameters and fish species at the catchments and sites. Fish species (*C. gariepinus*, *L. victorianus*, *Enteromius amphigramma*, *Enteromius cercops* and *Pseudocrenilabrus multicolor*) at the Mara and Nyando catchments were constrained with high levels of conductivity, salinity, temperature, NO_2 and dissolved oxygen (**Figure 5**), whereas fish species, including *Enteromius yongei*, *Leptoglanis* species, *Haplochromine* species and *C. somereni*, at the Nzoia and Sondu–Miriu catchments were constrained with NH_4, NO_3, SRP and land use of cropland (as a proxy to agriculture) (**Figure 5**).

Correlation Between Environmental Variables, Trophic Levels, Land Use, and Development of Index of Biotic Integrity

The relationships between land use, water quality measurements, trophic level and species sensitivity were evaluated using PNCA (**Figure 6**). The analysis correlated the forested sites with agricultural activities, NH_4, NO_3, SRP and intolerant fish species, giving a correlation coefficient ranging from 0.22 to 0.69. All the tolerant fish species were carnivorous feeders, and were found in sites with high conductivity, TDS, salinity and NO_2, with correlation coefficients ranging from 0.30 to 0.97 (**Figure 6**). Omnivorous, insectivorous and herbivorous fish were categorized as moderately tolerant to the measured water quality parameters, with correlation coefficients from 0.34 to 0.72. The moderately tolerant fish species either had a weak correlation with the two major categories of disturbance gradients (agriculture and organic loading) or had variable correlations (**Figure 6**).

The IBI was computed from 12 metrics categorized into three groups, namely, fish composition and abundance, trophic level and diversity metrics (**Table 3**). There were no ranked sensitive species sampled in the Mara and Nyando catchments, whereas the Nzoia and Sondu–Miriu catchments had two and three intolerant/sensitive species respectively. Benthic riffle, pool and pelagic pool species were in all the catchments but at varying numbers. The Mara catchment had the most benthic riffle species ($n = 5$) and the least benthic pool species ($n = 1$), while the Sondu–Miriu catchment had only one benthic riffle species and two benthic pool species (**Table 3**). Pelagic pool species were relatively abundant, with nine species at the Mara and Nyando catchments and four species at the Sondu–Miriu catchment (**Table 3**). The Mara and Sondu–Miriu catchment also had the highest proportion of tolerant species (13.33 and 14.29% respectively), despite being in the same scoring category (3) as the Nzoia and Nyando catchment. The proportion of carnivorous fish species scored low (1) in all four catchments, whereas the

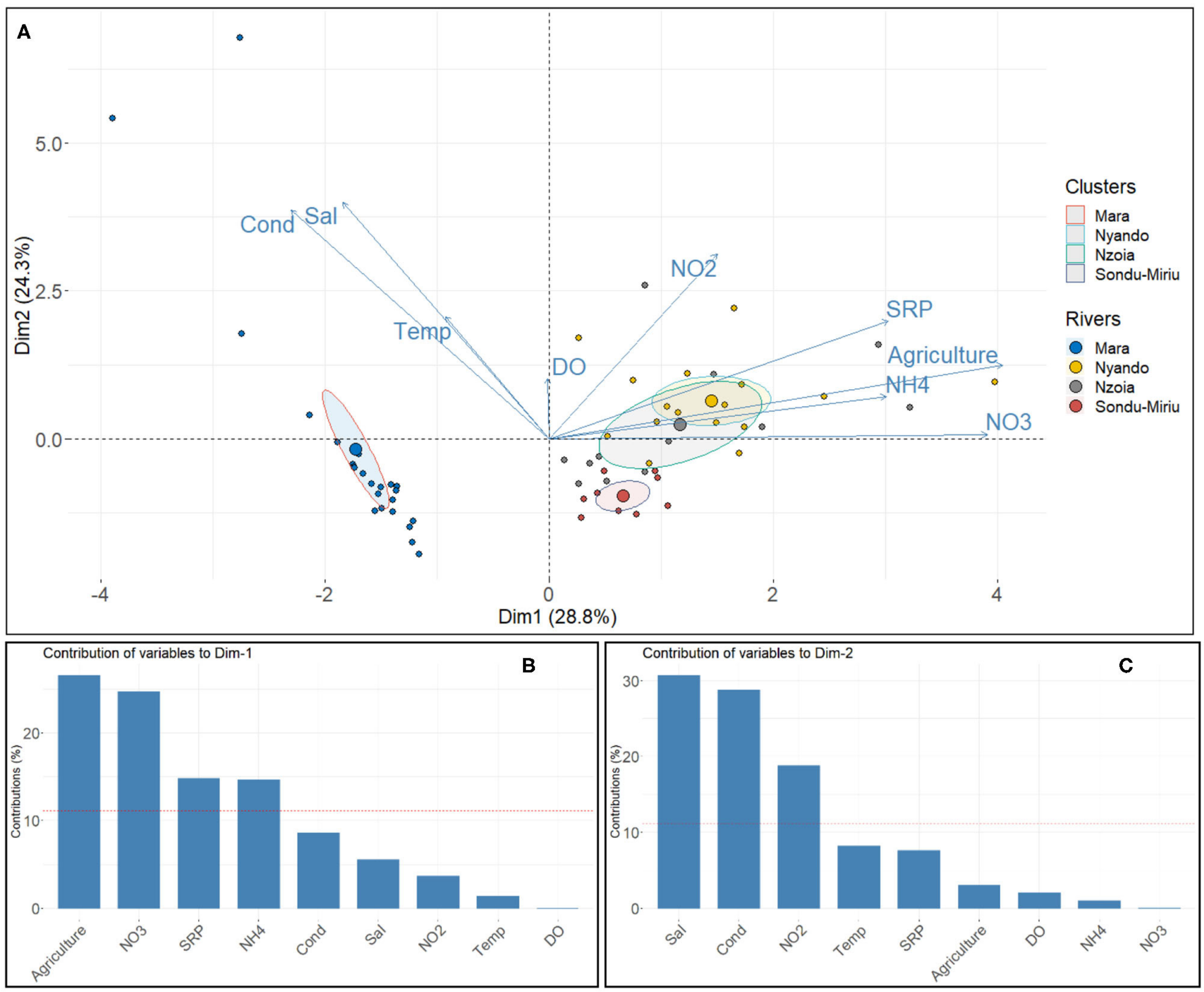

FIGURE 4 | (A) Principal component analysis (PCA) of the influence of water quality parameters on the variability in the river basins, and the contribution of the water quality variables to **(B)** component 1 and **(C)** component 2 of the PCA.

proportion of omnivorous and insectivorous scored lowest in Mara (1) and highest in Nzoia (5). The Simpson dominance index scored highest in the Mara, Nyando, and Nzoai (5) catchments and intermediate (3) at the Sondu–Miriu catchment, whereas the Margalef index scored low in all the catchments (**Table 3**). The IBI, with scores ranging from 12 to 60 and intervals of 12 units, was categorized into very poor (12), poor (12–24), fair (24–36), good (36–48) and very good (48–60). The health of the four catchments were all fair, with Sondu–Miriu being the lowest (26) followed by Mara and Nyando (28) and finally Nzoia (34) (Figure 7).

DISCUSSION

In this study, we set out to develop a fish-based IBI to assess the health of rivers in the LVB of Kenya. The concept of NB adequately ranked fish species tolerant to the environmental gradients and fish community metrics of species richness, trophic structures, taxonomic composition and species tolerance aggregated their response to stressors with a final score as an indication of river health. We showed that the proportion of agricultural land use in the LVB varies across the catchments. Cropland occupied between 13 and 42% of unprotected catchments (Nzoia, Nyando, and Sondu–Miriu) but only ∼2.4% of the Mara catchment, which is extensively a protected area with the MMNR on the Kenyan side and the SNP on the Tanzanian side. Although Mngube et al. (2020) have shown an increase in the proportion of agricultural land use at the Mara catchment within the unprotected area, which could be double our proportional agricultural land use, cropland at the catchment is still less than the other three catchments. This confirms our results that unprotected areas within river catchments of the LVB support large populations that are rural and depend on agriculture as their major economic activity.

TABLE 1 | Distribution, relative abundance, trophic level, and sensitivity or tolerance of fishes in the Mara, Nyando, Nzoia and Sondu–Miriu rivers, Lake Victoria Basin, Kenya.

Species	Sp. Sen	NB	Trop. L	Mara (RA%)	Nyando (RA%)	Nzoia (RA%)	Sondu–Miriu (RA%)	TNI	TRA (%)
Amphilius jacksonii	Mod	0.38	Ins			100 (15.9)		100	4.4
Bagrus docmak			Car	2 (0.3)				2	0.1
Barbus sp.1	Mod	0.34	Ins	53 (8.2)				53	2.3
Barbus sp.2			Ins			130 (20.8)		130	5.7
Chiloglanis somereni	Int	0.16	Her			5 (0.8)		5	0.2
Chiloglanis sp.			Her			1 (0.2)		1	0.1
Clarias alluaudi			Ins		2 (0.3)			2	0.1
Clarias gariepinus	Tol	0.72	Car	55 (8.6)	9 (1.2)	8 (1.3)	3 (1.3)	75	3.3
Clarias liocephalus	Mod	0.48	Omn	84 (13.1)	89 (11.50)	13 (2.2)	1 (0.4)	187	8.2
Clarias theodorae	Int	0.10	Omn				16 (7.1)	16	0.7
Enteromius amphigramma	Mod	0.36	Omn	30 (4.7)	3 (0.4)			33	1.5
Enteromius apleurogramma	Mod	0.36	Ins	5 (0.8)	6 (0.8)			11	0.5
Enteromius cercops	Mod	0.44	Ins	21 (3.3)	70 (9.0)			91	4.0
Enteromius jaksoni			Ins		12 (1.6)			12	0.5
Enteromius kerstenii	Mod	0.51	Omn	16 (2.5)	8 (1.0)			24	1.1
Enteromius magdalenae			Ins	1 (0.2)				1	0.1
Enteromius neumayeri	Mod	0.42	Ins	30 (4.7)	42 (5.4)	263 (42.0)	168 (74.3)	503	22.2
Enteromius nyanzae	Mod	0.34	Ins		146 (18.9)	7 (1.1)		153	6.7
Enteromius yongei			Omn			1 (0.2)		1	0.1
Gambusia affinis	Int	0.24	Ins			3 (0.5)	1 (0.4)	4	0.2
Haplochromine	Int	0.03	Omn				3 (1.3)	3	0.1
Labeo victroianus	Tol	0.89	Her	29 (4.5)				29	1.3
Labeo victorianus	Mod	0.59	Her	79 (12.3)	84 (10.9)			163	7.2
Labeobarbus altianalis	Mod	0.47	Omn	216 (33.6)	297 (38.4)	74 (11.8)	34 (15.0)	621	27.4
Labeobarbus bynni			Omn		2 (0.3)			2	0.1
Leptoglanis sp.			Omn			8 (1.3)		8	0.4
Oreochromis niloticus	Mod	0.38	Her	4 (0.6)	1 (0.1)			5	0.2
Pseudocrenilabrus multicolor	Mod	0.51	Omn	18 (2.8)	3 (0.4)	13 (2.1)		34	1.5
NS				15	15	13	7		
NI				643	774	626	226	2,269	

NS = number of species, species sensitivity (Sp.sen), niche breadth (NB), trophic level (Trop. L), species richness in each catchment (NI = number of individuals), percent relative abundance (RA%), percent total relative abundance (TRA%), and total number of individuals (TNI) of fish species sampled at Mara, Nyando, Nzoia and Sondu–Miriu catchment. Tol, tolerant species; Mod, moderately tolerant species; Int, intolerant species; Omn, omnivorous; Car, carnivorous; Her, herbivorous; Ins, insectivorous.

Cropland was used as a proxy for agricultural activities within the four catchments, with the understanding that ∼85% of the LVB population depends on agriculture, essential to local and national economies (Ochola, 2006), particularly in terms of food security, income generation and employment. Catchment degradation and land use activities, from the rapid population increase, were shown to vary from deforestation and overexploitation of the natural resources to heavily intensified agriculture throughout drainage of the LVB (Verschuren et al., 2002), whether small-scale subsistence or large-scale commercial agriculture with heavy mechanization and use of fertilizers (Lake Victoria Basin Commission and GRID-Arendal, 2017). The highest population densities and agricultural activities occur in the drainages of Kenyan, Rwandan and Burundi rivers that together contribute ∼90% of total river discharge into Lake Victoria (Balirwa and Bugenyi, 1988). Our results confirmed that, except for the protected areas with their restricted access and increased level of monitoring, conservation and management, these catchments are increasingly threatened by human activities. Protected areas are not entirely exempt from other stressors, with rangelands (savannah, grasslands and shrublands) within the Mara catchment shown to have declined from 79% in 1973 to 52% by 2000, and forest areas reducing by 32% within the same period (Oruma, 2017). Other disturbances, such as discharges of municipal and industrial wastewaters from urban centers, deforestation and deterioration of riparian vegetation and introduction of exotic species and livestock, have also impacted on the catchment (Masese and McClain, 2012; Masese et al., 2018).

Variation in water quality parameters is evidence of stressors that have contributed to deterioration in river water quality in many of the LVB rivers (Simonit and Perrings, 2011; Twesigye et al., 2011). Based on the selected water quality parameters, we inferred significant variation in nutrient load (NO_3, SRP,

TABLE 2 | ANOSIM percentage of fish abundance at four rivers catchments in Lake Victoria Basin, Kenya.

Species	Mara vs. Nyando		Mara vs. Nzoia		Mara vs. Sondu–Miriu		Nyando vs. Nzoia		Nyando vs. Sondu–Miriu		Nzoia vs. Sondu Miriu	
	Av. dissim	**Contrib. %**	**Av. dissim**	**Contrib. %**	**Av. dissim**	**Contrib. %**	**Av. dissim**	**Contrib. %**	**Av. dissim**	**Contrib. %**	**Av. dissim**	**Contrib. %**
Amphilius jacksonii	—	—	7.88	**10.07**	—	—	7.14	9.04	—	—	11.74	**22.83**
Bagrus docmak	0.14	0.40	0.16	0.20	0.23	0.27	—	—	—	—	—	—
Barbus sp.1	3.74	**10.62**	4.18	5.34	6.10	7.23	—	—	—	—	—	—
Barbus sp.2	—	—	10.24	**13.09**	—	—	9.29	**11.75**	—	—	15.26	**29.68**
Chiloglanis somerini	—	—	0.39	0.50	—	—	0.36	0.45	—	—	0.5869	1.142
Chiloglanis sp.	—	—	0.08	0.10	—	—	0.07	0.09	—	—	0.1174	0.2283
Clarias alluaudi	0.14	0.40	—	—	—	—	0.14	0.18	0.20	0.2381	—	—
Clarias gariepinus	3.25	9.22	3.70	4.73	5.98	7.09	0.071	0.09	0.60	0.7143	0.5869	1.142
Clarias liocephalus	0.35	1.00	5.60	7.15	9.55	**11.32**	5.43	6.87	8.80	**10.48**	1.408	2.74
Clarias theodorae	—	—	—	—	1.84	2.18	—	—	1.60	1.905	1.878	3.653
Enteromius amphigramma	1.91	5.41	2.36	3.02	3.45	4.09	0.21	0.27	0.30	0.3571	—	—
Enteromius apleurogramma	0.07	0.20	0.39	0.50	0.58	0.68	0.43	0.54	0.60	0.7143	—	—
Enteromius cercops	3.46	9.82	1.66	2.12	2.42	2.87	5.00	6.33	7.00	8.333	—	—
Enteromius jaksoni	0.85	2.41	—	—	—	—	0.86	1.09	1.20	1.429	—	—
Enteromius kerstenii	0.56	1.60	1.26	1.61	1.84	2.18	0.57	0.72	0.80	0.9524	—	—
Enteromius magdalenae	0.07	0.20	0.08	0.10	0.12	0.14	—	—	—	—	—	—
Enteromius neumayeri	0.85	2.41	18.36	**23.46**	15.88	**18.83**	15.79	**19.98**	12.60	**15**	11.15	**21.69**
Enteromius nyanzae	10.30	**29.26**	0.55	0.70	—	—	9.93	**12.57**	14.60	**17.38**	0.8216	1.598
Enteromius yongei	—	—	0.08	0.10	—	—	0.07	0.09	—	—	0.1174	0.2283
Gambusia affinis	—	—	0.24	0.30	0.12	0.1364	0.21	0.27	0.10	0.119	0.2347	0.4566
Haplochromine	—	—	—	—	0.35	0.41	—	—	0.30	0.3571	0.3521	0.6849
Labeo sp.	2.05	5.81	2.29	2.92	3.34	3.96	—	—	—	—	—	—
Labeo victorianus	0.35	1.00	6.23	7.96	9.09	**10.78**	6.00	7.60	8.40	**10**	—	—
Labeobarbus altianalis	5.72	**16.23**	11.19	**14.30**	20.94	**24.83**	15.93	**20.16**	26.30	**31.31**	4.695	9.132
Labeobarbus bynni	0.14	0.40	—	—	—	—	0.14	0.18	0.20	0.2381	—	—
Leptoglanis sp.	—	—	0.63	0.81	—	—	0.57	0.72	—	—	0.939	1.826
Oreochromis niloticus	0.21	0.60	0.32	0.40	0.46	0.55	0.07	0.09	0.10	0.119	—	—
Pseudocrenilabrus multicolor	1.06	3.01	0.39	0.50	2.07	2.46	0.71	0.90	0.30	0.3571	1.526	2.968
Overall average dissimilarity (%)	**35.22**		**78.25**		**84.35**		**79**		**84**		**51.41**	

Significant contributions to dissimilarities are in bold font.

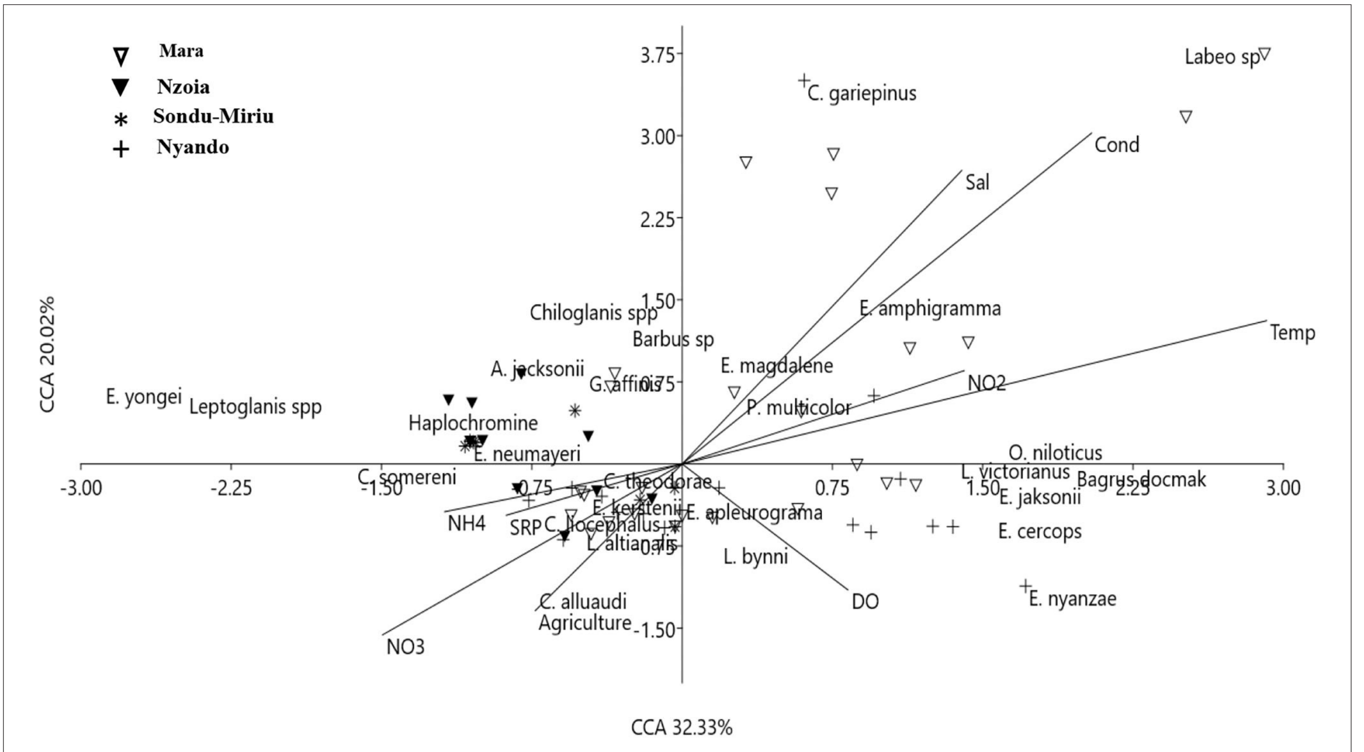

FIGURE 5 | Canonical correspondence analysis of the effects of water quality on fish assemblages at the four catchments in the Lake Victoria Basin.

TABLE 3 | Candidate metrics used in developing the index of biotic integrity for biological assessment of the ecological health of rivers in Lake Victoria Basin, Kenya.

Model metric	Scoring criteria			(Score)			
	5	3	1	Mara	Nyando	Nzoia	Sondu–Miriu
Total number of native species				14 (3)	14 (3)	13 (3)	7 (1)
Percent native fish individuals				99% (5)	99% (5)	100% (5)	100% (5)
Number of benthic riffle species	Expectations of M1–M5 vary with stream size and region			5 (3)	3 (1)	4 (1)	1 (1)
Number of benthic pool species				1 (1)	3 (1)	2 (1)	2 (1)
Number of pelagic pool species				9 (3)	9 (3)	7 (3)	4 (1)
Number of sensitive/intolerant species	>8	4–7	≤3	0 (1)	0 (1)	2 (1)	3 (1)
Proportion of tolerant species	<5%	5–20%	>20%	13.33% (3)	6.6% (3)	7.69% (3)	14.29% (3)
Proportion of omnivores	<20%	20–45%	>45%	56.61% (1)	51.94% (1)	17.41% (5)	23.89% (3)
Proportion of insectivores	>45%	20–5%	<20%	17.42% (1)	35.92% (3)	80.35% (5)	74.78% (5)
Proportion of carnivores	>30%	10–30%	<10%	8.55% (1)	1.163% (1)	1.278% (1)	1.33% (1)
Simpson ($D = \sum[ni(ni-1)/N(N-1)]$)	<0.33	0.33–0.66	>0.66	0.1681 (5)	0.2195 (5)	0.2605 (5)	0.5806 (3)
Margalef ($D = (S-1)/\log 2N$)			< 4	2.165 (1)	2.105 (1)	1.864 (1)	1.107 (1)
Aggregate IBI score				28	28	34	26

In brackets are the index scores using the interval scoring criteria; 1 for lowest score and 5 for highest score.

NH_4, and NO_2) in the catchments and sites, especially within the Nyando and Nzoia rivers, which had the highest proportion of cropland. The Lake Victoria Basin Commission and GRID-Arendal (2017) report showed that of the 40 million people in the LVB, more than 12.5 million reside on the Kenyan side of the basin, with 92% of this a rural population and an average population density of more than 500 people/km^2, with some places exceeding 1,200 persons/km^2 (Olaka et al., 2019). This population density, combined with a predominance toward rural living, equates to a need for greater and enhanced agricultural activities. The growing practice of large-scale cultivation systems characterized by the heightened use of fertilizers, pesticides and herbicides, as well as supplementary irrigation, threatens the environmental well-being of the region (Lake Victoria Basin Commission, 2007, 2012). The situation is particularly critical where demands to meet the needs of the rapidly increasing

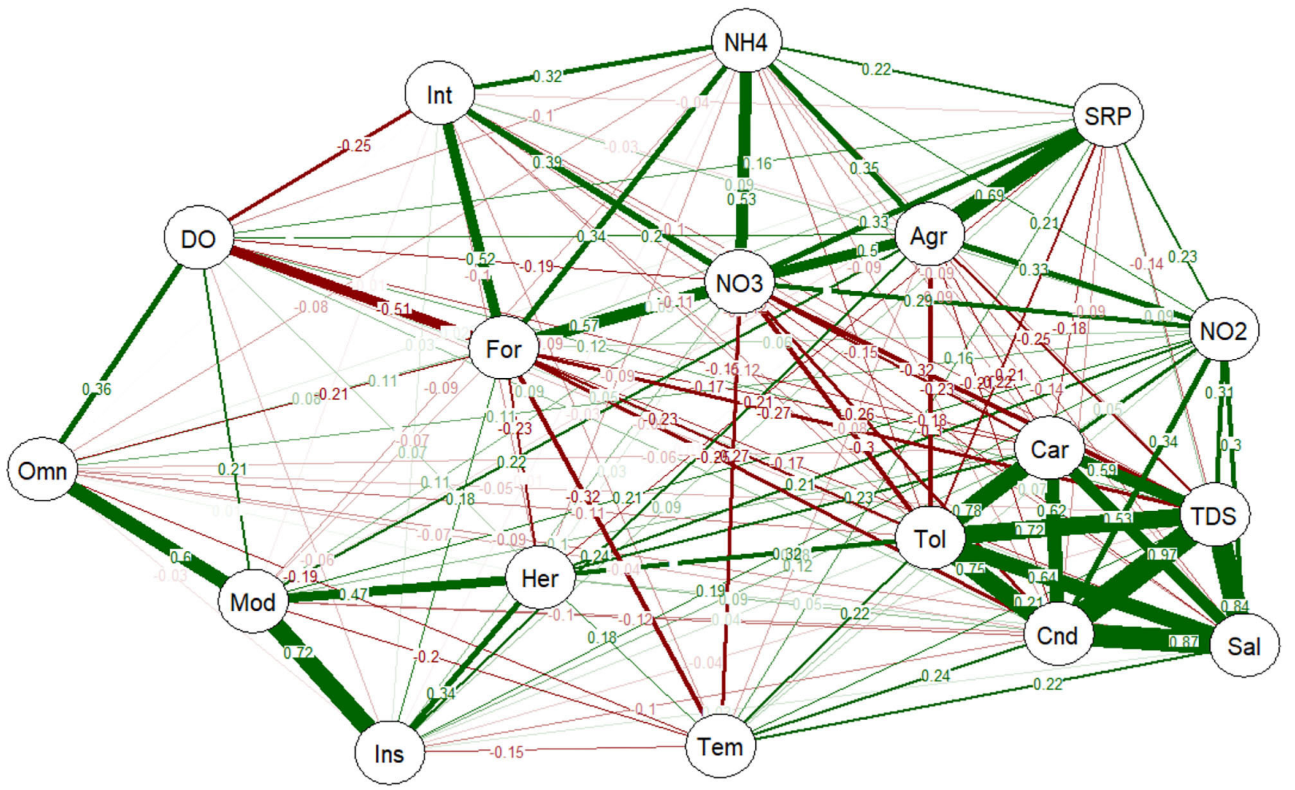

FIGURE 6 | PNCA of environmental gradients with species sensitivity, trophic levels, and land use (forest and agriculture). Thick lines indicate greater value of correlation, whereas narrow lines indicate smaller value of correlation. Green lines are positive correlations, whereas red lines are negative correlation. Omn, omnivorous; Car, carnivorous; Her, herbivorous; Ins, insectivorous; Tol, tolerant species; Int, intolerant species; Mod, moderately tolerant species; For, forested area; Agr, agriculture; DO, dissolved oxygen; Tem, temperature; Cnd, conductivity; TDS, total dissolved solid; Sal, salinity; NO_2, nitrite; NO_3, nitrate; SRP, soluble reactive phosphorus; NH4, ammonium.

human and livestock populations, in the form of space, shelter, food, water, health services and waste disposal, have placed increasing pressure on the resources of the basin (Lake Victoria Basin Commission, 2011).

The Mara River catchment differs from the other three catchments in terms of land use and water quality stressors. The river is also the most hydrologically varied, with tributaries being predominantly seasonal and with high EC. The basin has the smallest proportion of cropland, but the largest population of livestock and wildlife in the protected areas of the MMNR in Kenya and the SNP in Tanzania. Studies have shown that the middle reach rangelands of the catchment contain large herds of livestock, with more than 220,000 cattle estimated to live in the Talek subcatchment (Ogutu et al., 2011), and wildlife, including more than 4,000 *Hippopotamus amphibius* (Kanga et al., 2011). Such high livestock and wildlife numbers collectively contribute to a high deposit of organic matter and nutrients into the river (Subalusky et al., 2015; Dutton et al., 2018b; Masese et al., 2020b), leading to the high conductivity, salinity and TDS data shown in our results. Analysis of the water quality parameters identified two key stressors as nutrient loading from diffused agricultural sources and organic loading, mainly from large herds of domestic and wild grazers in the Mara River (Kanga et al., 2011; Masese et al., 2020b). These stressors were shown to have significant and distinct impacts on fish communities in the rivers. However, in addition to our findings, the basin is impacted by multiple stressors arising from land use and land cover changes, agricultural expansion and intensification (leading to habitat loss/fragmentation) to human intrusions and the more than 40 million inhabitants at the basin (World Bank, 2016). Despite these disturbances and natural system modifications (Makalle et al., 2008; Odada et al., 2009; Twesigye et al., 2011), the rivers are of great socioeconomic value to people and rich with biodiversity, including fish communities.

The previously high biodiversity of species richness and endemism in the LVB (Darwall et al., 2011; Sayer et al., 2019) has drastically reduced, with fish species composition and abundance in river catchments and satellite water bodies, including wetlands, being threatened by catchment activities (Wakwabi et al., 2006; Achieng et al., 2020; Masese et al., 2020a). We found greater species diversity and abundance within the protected areas in the Mara catchment and low-order streams in the Nyando catchment than in other sites within the same catchments. These two catchments also had the highest species composition and richness, but with none of the ranked intolerant/sensitive species. This does not exclude sensitive or intolerant species in these rivers as the methodology used to rank species sensitivity required the species to be sampled in more than one site; hence, some nine fish species were not ranked, and five of these were either in the Mara or Nyando catchment. This included *Bagrus docmak,* which had previously been ranked as sensitive (Raburu and Masese, 2012), and *Enteromius magdalenae* and *Labeobarbus bynni*. The advantage of ranking species sensitivity calculated from the NB concept is that it does not require expert judgment on species response to stressors. It determines species response to the specific environmental gradients measured rather than a generalized response to stressors and therefore can be applied to the different stressor gradients within a catchment to compare how a species responds to different pollution gradients. However, it is not applicable to a species that has a narrow geographical range or is endangered and therefore difficult to sample, with the exception of samples that can be found at varying environmental gradients in the same location. Species composition and abundance was generally lower than previous studies, an indication that catchment management is a critical concern and an immediate consideration, whereas conservation of headwaters and low-order streams that are still species rich will be critical to prevent further loss.

Of the 19 fish species ranked in this study, the tolerance of nine species, *A. jacksonii*, *C. gariepinus*, *Enteromius apleurogramma*, *E. cercops*, *Enteromius kerstenii*, *E. nyanzae*, *L. victorianus* and *P. multicolor*, were similar to the previous study by Raburu and Masese (2012) in the same region. Four species, *C. somerini*, *Clarias liocephalus*, *E. amphigramma* and *G. affinis*, found in this study have not previously been reported, and three morphospecies (*Barbus* sp., *Labeo* sp. and *Haplochromis* sp.) were not identified to species level. In this study, the ranking of four species (*C. theodorae*, *E. neumayeri*, *Oreochromis niloticus* and *L. altianalis*), using NB computation, was not in agreement with list generated using the expert judgment method; however, their ranking did not vary considerably (Raburu and Masese, 2012). When comparing the two methodologies, some intolerant species were ranked as moderately tolerant or moderately tolerant species ranked as tolerant by the expert judgment method. This could be as a result of a species-specific response to a particular stressor; however, expert judgment generalized this response to that of multiple stressors.

Differences in fish composition in the catchments could be attributed to variations in the relative abundance of six species, predominantly *L. altianalis, E. neumayeri* and to a lesser extent *A. jacksonii*, *E. nyanzae*, *C. liocephalus* and *L. victorianus*. Moreover, CCA related the tolerant species (*C. gariepinus* and *Labeo* species) with high conductivity from organic load, whereas intolerant species (*G. affinis*, *C. somerini* and *C. theodorae*) had negative relationships with organic load. Moderately tolerant species did not show any strong relationship with either organic load or agriculture. A clear depiction of the relationship between stressors, species sensitivity (tolerant, moderately tolerant and intolerant), and land use (forest and agriculture) was shown with PCNA, confirming that the significant difference in species distribution, abundance and composition at the catchments was a response to stressors, as shown by the CCA. Apart from the stressors measured in this study, it is most likely that the fish assemblages also respond to other stressors, including those that are basin-specific. This suggests that management of riverine fish assemblages will be more effective at basin or subcatchment scales rather than at the larger LVB level (Achieng et al., 2020).

Fish species richness, tropic structures, taxonomic composition, species sensitivity and diversity indices were used as metrics to compute IBI, based on the concept that the values of these metrics change as a response to stressors. Studies have shown them to decline with increasing nutrients, organic matter and ionic material pollution (Kim and An, 2015, Mamun and An, 2020) and therefore an indication of disturbance. Although species richness and composition were high in the Nyando and Mara rivers, the proportions in the categories of trophic structure and number of benthic and pelagic species were quite low. This could be due to the high dominance of two species (*L. altianalis* and *E. neumayeri*), suggesting the apparent species richness and composition are still under threat. With all IBI scores in the four catchments ranging between 26 and 34, they were all evaluated to be in fair health.

CONCLUSION

Riverine fish species richness and composition in the LVB have declined in the past decades in response to increasing complexity and multiple stressors in the catchments of many rivers. This has resulted in the loss of sensitive species, species migration to headwaters, low-order streams and less polluted subcatchments or to protected areas with restricted access and increased levels of monitoring, conservation and management, as observed in the Mara catchment. It is difficult to quantify the number of species lost in the past decades due to a scarcity of data and the lack of regular monitoring. Our results demonstrate that the cumulative effect of stressors can adequately rank fish species tolerance to disturbance gradients and help to further develop regional metrics to assess and monitor river health. Multivariate methods have proven to be reliable in ranking species tolerance and can be used without prior knowledge of species biology and ecology. They can combine the effects of multiple variables and factors into species-specific responses along gradients of degradation, including some intrinsic characteristics, which are not easily observable. Although the measured variables were limited to nutrient and organic loading, which are significant contributors to catchment degradation, it is most likely that the fish assemblages also respond to hydrological variable, such as flow rates and discharge, and other stressors that are basin-specific, indicating that the management of riverine fish populations will be more effective at individual river basin or subcatchment levels rather than at an LVB scale. The fish-based IBI showed that all the catchments were in a fair health, although the evaluation of additional stressors may record different levels of species response and is therefore most likely to provide a more detailed assessment of ecological conditions in the rivers. Ecological conditions could also be evaluated at the site level, so as to eliminate confounding effects caused by upstream–downstream effects of pollutants and other disturbances. We also recommend conservation and management of the catchments with the protection of headwaters and lowland streams, which are still species rich, to prevent further loss of the exceptional biodiversity, which are native and endemic to the LVB.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further enquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by the National Commission for Science, Technology and Innovation.

AUTHOR CONTRIBUTIONS

AA, FM, SA, PR, and BK-A participated in data collection and drafting the manuscript. TC and CF participated in conceptualizing and drafting the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frwa.2020.620704/full#supplementary-material

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REVIEW
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Rapid Bioassessment Protocols Using Aquatic Macroinvertebrates in Africa–Considerations for Regional Adaptation of Existing Biotic Indices

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Benthic macroinvertebrates are commonly used to assess water quality and ecological condition of aquatic ecosystems and they form the basis of several biotic indices. Many of these biotic indices are based on rapid bioassessment protocols (RBP). The first RBP based on macroinvertebrates, developed in Africa in the early 1990s, was the South Africa Scoring System (SASS). Since then SASS has been widely used in southern Africa and beyond, and has formed the basis of several other RBPs developed in Africa. This paper explores the RBPs and associated biotic indices currently used in Africa, primarily those that are rapid, field-based with low taxonomy (mostly family level) and which rely on sensitivity weightings of individual taxa to generate three metrics for interpreting water quality and ecological condition of aquatic ecosystems. Recommendations for future regional adaptation of RBPs, including calibration, validation, and modification of RBPs and biotic indices for new regions are provided. To date, five RBPs have been developed in Africa, while some existing biotic indices have been used outside their intended regional range. Key to the efficacy of any RBP and associated biotic index is the ability to detect a water quality impact, or change in river health. Important considerations when adapting an index for a new region or country include evaluating the suitability of the sampling protocol to local river conditions, evaluating the distribution of aquatic macroinvertebrate taxa in the region, assigning sensitivity weightings to new taxa in the region, evaluating the ability of the biotic index to detect impacts, evaluating within-country spatial and temporal variability in macroinvertebrate assemblages, and developing appropriate data interpretation guidelines based on metric scores and reference conditions. Often several iterations of a biotic index are needed, with improvement in efficacy with each version, following spatially and temporally comprehensive sampling. Future RBPs developed for bioassessment of rivers in Africa will promote the protection, conservation, and management of African riverine ecosystems.

Keywords: biomonitoring, ecological condition, spatial variability, river health, temporal variability, water quality

INTRODUCTION

Benthic macroinvertebrates are commonly used to assess river water quality and form the basis of several biotic indices including those used in the United Kingdom (e.g., Armitage et al., 1983; Wright et al., 1998), Europe (e.g., Alba-Tercedor and Sánchez-Ortega, 1988; Camargo, 1993; Bonada et al., 2006), North America (e.g., Hilsenhoff, 1988; Rosenberg and Resh, 1993; Barbour et al., 1999), South America (e.g., Baptista et al., 2007; Buss and Vitorino, 2010), Asia (e.g., Morse et al., 2007; Hartmann et al., 2010; Blakely et al., 2014), Australia (e.g., Chessman, 1995, 2003; Smith et al., 1999), New Zealand (e.g., Stark, 1993, 1998; Stark and Maxted, 2007), and Africa (e.g., Chutter, 1972, 1998; Dickens and Graham, 2002; Palmer and Taylor, 2004; Ollis et al., 2006; Dallas, 2009; Kaaya et al., 2015; Dallas et al., 2018). Many of these biotic indices are based on rapid bioassessment protocols (RBPs), which provide a reliable, quick, and cost-effective method for evaluating water quality in perennial rivers. Macroinvertebrate-based biotic indices often form the primary tool for management of water quality and river health in riverine ecosystems (Ollis et al., 2006).

RBPs based on macroinvertebrates are typically qualitative, multihabitat (= multibiotope), rapid, field-based methods that derive metrics using sensitivity weightings of individual taxa, which reflect their water quality tolerances (Dallas, 1995, Dallas, 1997). For all RBPs, the associated biotic index generates three metrics, namely Total Score (sum of the sensitivity weightings of taxa recorded at a site: SASS Score, NASS Score, TARISS Score, ZISS Score, OKASS Score), Number of Taxa and Average Score Per Taxon (ASPT = Total Score divided by Number of Taxa). The sampling equipment, habitats or biotopes sampled, time or effort for sampling and processing of samples sometimes differ amongst RBPs (Bonada et al., 2006). In particular, choice of biotope sampled varies from multihabitat to single biotope, and combining samples from similar biotopes based on substrate similarities (e.g., all stone, all vegetation) vs. hydraulic similarities (e.g., all in-current samples). Processing of samples is commonly field-based to family-level, although processing varies amongst protocols, and the advantages of laboratory vs. field-based processing and taxonomic resolution have been argued (Carter and Resh, 2001; Bonada et al., 2006). Numerous biotic indices, especially those reliant on rapid, field-based protocols, use family-level taxonomic resolution because it is easier and less expensive (Bonada et al., 2006).

Although Chutter (1972) developed a biotic index for South African rivers in 1972, it was labor-intensive and thus never gained traction for biomonitoring (Chutter, 1998). Subsequently, a quicker and simpler index was developed, the South African Scoring System (SASS), which constituted the first RBP in Africa (Dallas, 1997; Chutter, 1998; Dickens and Graham, 2002). SASS was derived from the Biological Monitoring Working Party system (BMWP) (Hawkes, 1997) and modified for assessing water quality and condition of South African rivers (Dallas, 1997, Chutter, 1998). SASS5 (version 5) has been applied without modification in other regions of southern Africa, including Zimbabwe (Phiri, 2000; Ndebele-Murisa, 2012; Bere and Nyamupingidza, 2014; Mwedzi et al., 2016), Swaziland (Mthimkhulu et al., 2004), and Kenya (Oigara and Masese, 2017). Bere and Nyamupingidza (2014) confirmed the ability of SASS5 to reflect water quality and ecological health of lotic systems in Zimbabwe, which they attributed to the dominance of widely occurring macroinvertebrate taxa in their study (Bere and Nyamupingidza, 2014). In comparison, studies in more tropical regions such as Kenya, concluded that there is a need for testing and validation of the protocol before extending its use beyond South Africa (Oigara and Masese, 2017). Elias et al. (2014b) recommended that tropical African regions ideally need to develop their own RBP and biotic indices, rather than relying on indices developed from other geographical areas, especially non-tropical regions. SASS has also been used in other sub-Saharan regions of Africa including Ethiopia and West Africa (pers. Comm. R. Palmer).

SASS5 has been regionally modified for bioassessment of rivers in other African countries including Namibia: Namibian Scoring System (NASS; Palmer and Taylor, 2004), Tanzania: Tanzania River Scoring System (TARISS; Kaaya et al., 2015) and Zambia: Zambian Invertebrate Scoring System (ZISS; Dallas et al., 2018). It has also been adapted for non-wadeable, deltaic aquatic biotopes in the Okavango Delta in Botswana: Okavango Assessment System (OKASS; Dallas, 2009). TARISS has been used in Ugandan and Rwandan rivers to assess water quality (Dusabe et al., 2019; Tumusiime et al., 2019), although identifications of macroinvertebrates were done on preserved samples in the laboratory and not in the field, and in the case of Dusabe et al. (2019), the TARISS sampling protocol was not followed as biotopes were combined.

Several other studies have used other indices, including multi-metric indices such as B-IBI (Benthic Index of Biological Integrity, Barbour et al., 1999) for aquatic macroinvertebrates in rivers in Tanzania (Elias et al., 2014b) and Kenya (Masese et al., 2009). In these studies, the strength of the correlations between each metric and water quality allowed for the selection of a sub-set of metrics for inclusion in the final multi-metric index and subsequent calculation of integrity classes and B-IBI Scores. A laboratory-based macroinvertebrate biotic score system (ETHbios) has been developed in Ethiopia for assessing the ecological status of rivers (Aschalew and Moog, 2015). Other countries, including Nigeria, have used various biometric indices including Hilsenhoff's Biotic Index (Hilsenhoff, 1982) to evaluate water quality (Ogbeibu et al., 2013).

While, RBPs and associated biotic indices developed in one country or region may be applied in other countries or regions, it is recommended that they are calibrated, validated, and modified to ensure their effectiveness (Kaaya et al., 2015; Dallas et al., 2018). The aim of this paper is to examine and discuss RBPs and biotic indices currently used in rivers in Africa, in particular those RBPs that are rapid, field-based with low taxonomic resolution (mostly family level) and which rely on sensitivity weightings of individual taxa to generate three metrics for interpreting water quality and ecological condition of aquatic ecosystems. The paper further aims to provide recommendations for regional calibration, validation, and modification of these RBPs and associated biotic indices for application in new regions.

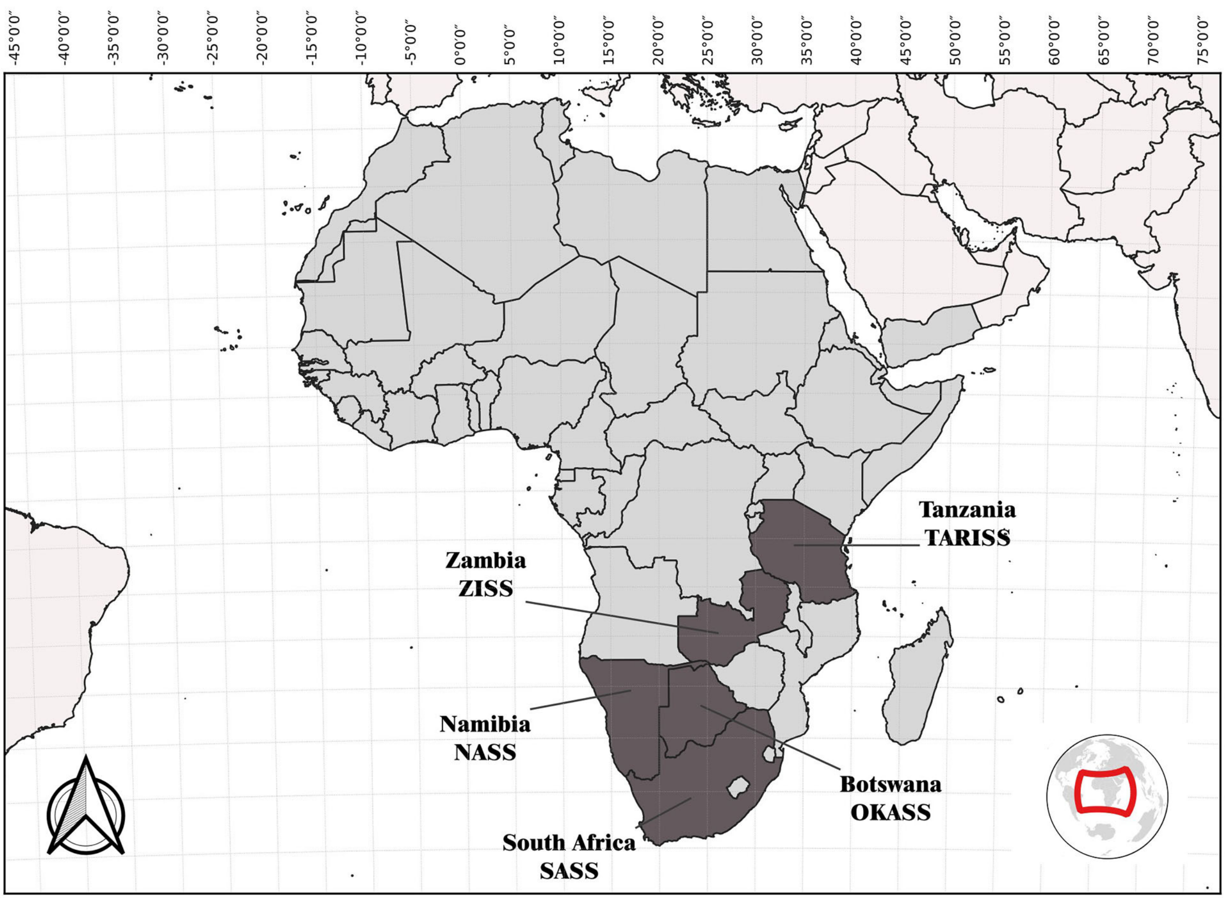

FIGURE 1 | Rapid bioassessment protocols developed in Africa including the South African Scoring System (SASS; Dickens and Graham, 2002), the Namibian Scoring System (NASS; Palmer and Taylor, 2004), the Okavango Assessment System (OKASS; Dallas, 2009), the Tanzania River Scoring System (TARISS; Kaaya et al., 2015), and the Zambian Invertebrate Scoring System (ZISS; Dallas et al., 2018).

METHODS

An extensive literature search was undertaken to source published studies on RBPs based on aquatic macroinvertebrates in African rivers (**Figure 1**). The literature search was conducted by means of Google Scholar, using a combination of the following search criteria: Africa, RBP, aquatic invertebrate, biomonitoring, ecological condition, macroinvertebrate, rapid bioassessment, and river health. Relevant articles were identified based on their titles, abstracts, methods, and results sections. Focus was on rapid, field-based protocols with low taxonomy (mostly family level) including SASS (Dickens and Graham, 2002), NASS (Palmer and Taylor, 2004), TARISS (Kaaya et al., 2015), and ZISS (Dallas et al., 2018), which are all RBPs based on aquatic macroinvertebrates developed for rivers. In addition, the Okavango Assessment System (OKASS; Dallas, 2009) was also included as this was adapted from SASS for use non-wadeable, deltaic aquatic biotopes in the Okavango Delta, Botswana. Each of these RBPs were evaluated to glean information on the sampling protocols used (biotopes sampled, duration/area sampled), sampling equipment used (kick sampling, equipment), aquatic macroinvertebrate taxonomy (taxonomic level, number of taxa, common and unique taxa), distribution and sensitivity weightings (range and actual value); methods for detecting and evaluating impacts (analyses used, human disturbance gradients, water quality correlations); spatial and temporal heterogeneity (spatial distribution of sites and seasonal sampling), and data interpretation guidelines (hierarchical spatial frameworks). In addition, the number of assessments used for the development of each RBP was ascertained. This provided an indication of the regional robustness of each biotic index. The results for each of these aspects are presented and discussed.

RESULTS

Sampling Protocols

The SASS, NASS, TARISS, and ZISS sampling protocols are all applied in wadeable (<1 m depth, <20 m wide) rivers, while the ZISS protocol also includes a method for larger non-wadeable rivers (>20 m wide). These protocols are intended for use in

TABLE 1 | Standardized rapid bioassessment protocol for rivers based on aquatic macroinvertebrates used for South African Scoring System (SASS), Namibian Scoring System (NASS), Tanzania River Scoring System (TARISS), and Zambian Invertebrate Scoring System (ZISS).

SAMPLING IN SMALLER WADEABLE RIVERS (<20 m WATER SURFACE WIDTH) (SASS, NASS, TARISS, ZISS)
Stones (substrate particles with any dimension >1.5 cm): Kick stones in current (SIC) for 2 min if stones are loose, up to a maximum of 5 min if bedrock is present. Ensure that a minimum of six separate SIC areas are sampled, starting downstream, and working upstream. SIC areas sampled should include a range of stone sizes (pebbles, cobble, boulders, and bedrock), depths (shallow to deep), flows (slow to fast), and hydraulic biotopes (riffle, run, rapid, cascade) if available. Kick stones (and bedrock) out of current (SOOC) for 1 min, covering a range of stone sizes and depths if available. A single Stones (S) biotope sample includes samples collected from stones in current and stones out of current.
Vegetation: Sweep marginal vegetation for a total of 2 linear meters (SASS, NASS, TARISS) or 2 min (ZISS), covering several locations to ensure different marginal vegetation growth forms and different flow types are included, if present at the site and accessible. Sweep aquatic vegetation for a total of 1 square meter (SASS, NASS, TARISS) or 1 min (ZISS). A single Vegetation (V) biotope sample includes samples collected from marginal and aquatic vegetation.
Gravel, sand, and mud (substrate particles with largest dimension <1.5 cm): Stir and sweep gravel, sand, mud for 1 min total. Ensure sampling is done both in and out of current, if present at the site. A single Gravel, Sand, and Mud (G) biotope sample includes samples collected from in and out of current.
Hand picking and visual observation: Check stones (different sizes, in and out of current) and observe water surfaces across the site for up to 1 min (SASS, NASS, TARISS) or up to 5 min per biotope (ZISS). Include a shoreline inspection for shells which are often buried in the bank sediment especially in larger rivers. If a new taxon is found that was not recorded in stones, vegetation or gravel/sand/mud, then record in biotope where it was found by circling estimated abundance on score sheet.
SAMPLING IN LARGER RIVERS BY BOAT (>20 m WATER SURFACE WIDTH AND/OR NON-WADEABLE) (ZISS)
Stones: If substrate is accessible, sample as for smaller rivers.
Vegetation: Select four sub-sites positioned with two on each bank preferably at least 50 m apart. If an island is present, one of the four sub-sites should be positioned on the island. Sweep marginal vegetation for 1 min and aquatic vegetation for 30 s at each sub-site to give a total of 4 min for marginal vegetation and 2 min for aquatic vegetation. Ensure that all growth forms and flow types present at the site are included. A single Vegetation (V) biotope sample includes samples collected from marginal and aquatic vegetation.
Gravel, sand, and mud: If substrate is accessible, sample as for smaller rivers.
Hand picking and visual observation: Check stones (different sizes, in and out of current) if accessible and observe water surfaces across the site for up to 1 min (SASS, NASS, TARISS) or up to 5 min per biotope (ZISS). Include a shoreline inspection for shells which are often buried in the bank sediment especially in larger rivers. If a new taxon is found that was not recorded in stones, vegetation or gravel/sand/mud, then record in biotope where it was found by circling estimated abundance on score sheet.
FIELD PROCESSING OF SAMPLES
Collected samples are identified in the field using a processing tray for a maximum of 15 min per biotope. If no new taxon is seen for approximately 5 min, then identification may be stopped. A magnifying glass and photographic identification guide are used to assist with identifications. Identified taxa in each biotope are recorded on the score sheet under the appropriate biotope heading before combining the three columns into a single total (site) column. An abundance estimate is given for each taxon in each biotope and for the site using the following abundance categories: 1, A: 2–10, B:11–100, C: 101–1000, D: >1000.

low to moderate flows and are not to be used during high flow (flood) events, or in lentic systems such as wetlands and impoundments, or in estuaries or ephemeral rivers (Dickens and Graham, 2002). The sampling protocol of these four RBPS are similar since they were all derived from SASS. An overview of the sampling protocols is provided in **Table 1**. Importantly, sampling is undertaken per biotope, namely stones (stones in current and stone out of current), vegetation (marginal and aquatic), and gravel/sand/mud, with effort for each biotope standardized by time or area (**Table 1**). The OKASS sampling protocol was adapted from SASS and is applied in non-wadeable, deltaic aquatic biotopes, with sampling undertaken per biotope including marginal vegetation in current (channel), marginal vegetation out of current (lagoon), floating vegetation, submerged vegetation, and seasonally-inundated floodplain.

Aquatic Macroinvertebrate Taxonomy, Distribution, and Sensitivity Weightings

For existing RBPs, a taxon's tolerance to water quality impairment is reflected in its sensitivity weighting, with highly sensitive taxa assigned a weighting of 15, while highly tolerant taxa have a weighting of one. For all RBPs in Africa, sampled macroinvertebrates are identified to family level (or for a few taxa, e.g., Porifera, to a higher level; or a lower level, e.g., Baetidae and Hydropsychidae) on site using a magnifying glass. The total number of taxa in each RBP are 99 (SASS5), 93 (NASS2), 96 (TARISS1), 90 (ZISS1), and 58 (OKASS1). The number after the RBP name denotes the version, with SASS already in version 5, NASS in version 2, and the others in version 1. When the four RBPs developed for rivers are examined, 81 taxa are common to all (**Table 2**). Regional endemics such as those common in the south-western Cape, South Africa, and tropical taxa resulted in some taxa being absent from one or more RBPs.

SASS sensitivity weightings were initially derived using expert opinion and subsequently validated through field testing and correlation with impact. SASS underwent five revisions (SASS1 to SASS version 5) following extensive testing in several regions within South Africa by a number of river ecologists (Dallas, 1995, 1997; Chutter, 1998; Dickens and Graham, 2002) with more than 1,000 SASS assessments undertaken during this testing phase. This facilitated an iterative approach to the development of SASS, with new taxa added and sensitivity weightings adjusted based on observed data. Since then, more than 12,162 SASS assessments have been undertaken in South Africa (Freshwater Biodiversity Information System (FBIS), 2020) and it forms the backbone of river health assessment in South Africa. In comparison, NASS comprised approximately 50 assessments on the perennial rivers in Namibia (pers comm. R Palmer), TARISS comprised 101 assessments on 85 rivers in four freshwater ecoregions of Tanzania (Kaaya et al., 2015), ZISS comprised

TABLE 2 | Taxa and sensitivity weightings for Rapid Bioassessment Protocols currently used in Africa, including the South African Scoring System (SASS, Dickens and Graham, 2002), Namibian Scoring System (NASS, Palmer and Taylor, 2004), Tanzania River Scoring System (TARISS, Kaaya et al., 2015) and Zambian Invertebrate Scoring System (ZISS, Dallas et al., 2018), and Okavango Assessment System (OKASS, Dallas, 2009).

	SASS	NASS	TARISS	ZISS	OKASS
Version	**5**	**2**	**1**	**1**	**1**
Taxon	**Sensitivity weightings**				
PORIFERA (Sponges)	5	5	5	5	
COELENTERATA (Cnidaria)	1	1	1		
TURBELLARIA (Flatworms)	3	3	3	3	3
ANNELIDA					
Oligochaeta (Earthworms)	1	1	1	1	1
Hirudinea (Leeches)	3	3	3	3	6
CRUSTACEA					
Conchostraca (Clam shrimps)					6
Amphipoda	13		13		
Potamonautidae (Crabs)	3	3	3	3	3
Atyidae (Shrimps)	8	8	8	8	11
Palaemonidae (Prawns)	10	10	10		
HYDRACARINA (Water mites)	8	8	8	8	8
PLECOPTERA (Stoneflies)					
Notonemouridae	14		14		
Perlidae	12	12	12	12	
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp.	4	4	4	4	4
Baetidae 2 sp.	6	6	6	6	
Baetidae > 2 sp.	12	12	12	12	
Caenidae (Squaregills/Craneflies)	6	6	6	6	9
Dicercomyzidae			10	9	
Ephemeridae	15	15	15	15	
Ephemerythidae		6	9	10	
Heptageniidae (Flatheaded mayflies)	13	13	13	9	15
Leptophlebiidae (Prongills)	9	9	9	9	12
Machadorythidae		11		8	
Oligoneuridae (Brushlegged mayflies)	15	15	15	15	
Polymitarcyidae (Pale Burrowers)	10	10	10	10	13
Prosopistomatidae (Water specs)	15	15	15	15	
Teloganodidae SWC	12				
Tricorythidae (Stout Crawlers)	9	9	9	9	9
ODONATA (Dragonflies and Damselflies)					
Calopterygidae (Broad-winged damsels)	10	10	10	10	
Chlorocyphidae (Jewel damselfly)	10	10	10	10	
Synlestidae (Chlorolestidae) (Malachite)	8	8	8		
Coenagrionidae (Sprites and blues)	4	4	4	4	4
Lestidae (Emerald Damselflies)	8	8	8	8	8
Platycnemidae (Brook Damselflies)	10	10	10	10	
Protoneuridae	8	8	8	8	
Aeshnidae (Hawkers and Emperors)	8	8	8	8	8
Corduliidae (Cruisers)	8	8	8	8	8
Gomphidae (Clubtails)	6	6	6	6	6
Libellulidae (Darters)	4	4	4	4	4
LEPIDOPTERA (Aquatic Caterpillars/Moths)					
Crambidae (=Pyralidae)	12	12	12	12	12
HEMIPTERA (Bugs)					
Aphelocheiridae				5	
Belostomatidae (Giant water bugs)	3	3	3	3	3
Corixidae (Water boatmen)	3	3	3	3	3
Gerridae (Pond skaters/Water striders)	5	5	5	5	5
Hydrometridae (Water measurers)	6	6	6	6	6
Naucoridae (Creeping water bugs)	7	7	7	7	10
Nepidae (Water scorpions)	3	3	3	3	6
Notonectidae (Backswimmers)	3	3	3	3	3
Pleidae (Pygmy backswimmers)	4	4	4	4	7
Veliidae/Mesoveliidae (Ripple bugs)	5	5	5	5	8
MEGALOPTERA (Fishflies, Dobsonflies, and Alderflies)					
Corydalidae (Fishflies and Dobsonflies)	8		8		
Sialidae (Alderflies)	6		6		
NEUROPTERA					
Sisyridae (Spongillaflies)		4			
TRICHOPTERA (Caddisflies)					
Dipseudopsidae	10	10	10	10	
Ecnomidae	8	8	8	8	8
Hydropsychidae 1 sp.	4	4	4	4	4
Hydropsychidae 2 sp.	6	6	6	6	
Hydropsychidae > 2 sp.	12	12	12	12	
Philopotamidae	10	10	10	10	10
Polycentropodidae	12	12	12	12	
Psychomyiidae/Xiphocentronidae	8	8	8	11	
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae	11	11	11		
Glossosomatidae SWC	11				
Hydroptilidae	6	6	6	6	6
Hydrosalpingidae SWC	15				
Lepidostomatidae	10	10	10	10	
Leptoceridae	6	6	6	6	9
Petrothrincidae SWC	11				
Pisuliidae	10	10	10	10	
Sericostomatidae SWC	13				
COLEOPTERA (Beetles)					
Mixed beetles					8
Curculionidae (Snout beetle)#		5			
Dytiscidae/Noteridae (Diving beetles)	5	5	5	5	
Elmidae/Dryopidae (Riffle beetles)	8	8	8	8	5
Gyrinidae (Whirligig beetles)	5	5	5	5	
Haliplidae (Crawling water beetles)	5	5	5	5	
Hydraenidae (Minute moss beetles)	8	8	8	8	
Hydrophilidae (Water scavenger beetles)	5	5	5	5	

(Continued)

TABLE 2 | Continued

	SASS	NASS	TARISS	ZISS	OKASS
Version	**5**	**2**	**1**	**1**	**1**
Taxon	**Sensitivity weightings**				
Limnichidae (Minute marsh loving beetle)	10	10	10	8	
Psephenidae (Water Pennies)	10		10	10	
Scirtidae (Marsh beetles)	12	12	12	12	12
DIPTERA (Flies)					
Athericidae (Water snipe fly)	10	10	10	10	
Blephariceridae (Mountain midges)	15		15		
Ceratopogonidae (Biting midges)	5	5	5	5	5
Chironomidae (Midges)	2	2	2	2	2
Culicidae (Mosquitoes)	1	1	1	1	1
Dixidae (Dixid midge)	10		10	10	
Empididae (Dance flies)	6	6	6	6	6
Ephydridae (Shore flies)	3	3	3	3	
Muscidae (House flies, Stable flies)	1	1	1	1	1
Psychodidae (Moth flies)	1	1	1	1	1
Sciomyzidae (Marsh flies)					2
Simuliidae (Blackflies)	5	5	5	5	5
Stratiomyidae (Soldier flies)					2
Syrphidae (Rat tailed maggots)	1	1	1	1	1
Tabanidae (Horse flies)	5	5	5	5	5
Tipulidae (Crane flies)	5	5	5	5	5
GASTROPODA (Snails)					
Ampulariidae (Apple snail)		3		5	3
Ancylidae (Limpets)	6	6	6	6	
Bithyniidae (Faucet snails)		3		3	3
Bulinidae (previously Bulininae)	3	3	3	3	
Hydrobiidae (Mud snails)	3	3	3	3	3
Lymnaeidae (Pond snails)	3	3	3	3	6
Neritidae			4		
Physidae (Pouch snails)	3	3	3		
Planorbidae (Orb snails) (previously Planorbinae)	3	3	3	3	6
Thiaridae (=Melanidae)	3	3	3	3	3
Viviparidae (River snails) ST	5	5	5	5	
PELECYPODA (Bivalves)					
Corbiculidae	5	5	5	5	5
Etheriidae (Freshwater oyster)		6			
Mutelidae				6	
Sphaeriidae (Pills clams)	3	3	3	3	3
Unionidae (Perly mussels)	6	6	6	6	6

For ZISS: Baetidae < 3 sp., Baetidae 3 sp., Baetidae > 3 sp.; SWC, South-Western Cape endemic; OKASS1: Mixed beetles = all beetles e.g., Dytiscidae, Noteridae, Sperchidae; #: NASS, Non-native invasive taxon.

151 assessments on 95 rivers across the country (Dallas et al., 2018), while OKASS comprised 103 assessments on 54 deltaic sites in three regions of the Okavango delta (Dallas, 2009). For all of these RBPs further use of the respective indices has taken place and will most likely be used to further improve the index. When an RBP is used in a new region, as part of the validation process, sensitivity weightings of existing taxa need to be checked and sensitivity weightings of new taxa assigned. Palmer and Taylor (2004) assigned sensitivity weightings to new taxa based on observed data and similarity with existing taxa. Canonical analysis of principal coordinates has been used to predict sensitivity weightings of new macroinvertebrate taxa along the disturbance gradient (Kaaya et al., 2015), as well as correlation of occurrence to impact ratings, evaluation of closely related SASS families, known life-history modes, and anatomical adaptations (Dallas et al., 2018).

Impact Detection and Evaluation

To evaluate the efficacy of the biotic index to demonstrate changes in river water quality, data on anthropogenic activities and ecosystem disturbance need to be collected at each site by assessing catchment, channel and habitat impacts. Both TARISS (Kaaya et al., 2015) and ZISS (Dallas et al., 2018) utilized versions of the site characterization protocol (Dallas, 2005) and Index of Habitat Integrity (Kleynhans, 1996), developed as part of the SASS testing phase in South Africa. This protocol evaluates the quantity and severity of anthropogenic impacts at a site and integrates potential impacts into an index of habitat integrity; in addition to assessing water quality impacts, modification to the channel, condition of the local catchment, and land-use (Dallas, 2005). For TARISS, levels of human disturbance across sites were derived by evaluating local catchment disturbance, instream and riparian habitat integrity (Kaaya et al., 2015). In Dallas et al. (2018) distinction between "impacted" and minimally impacted (or "unimpacted") reference sites were used to generate thresholds of impact for each of the groups of impact. For OKASS, potential anthropogenic disturbances were used to calculate a Human Disturbance Score for each site (Dallas, 2009). To evaluate the ability of the biotic index to detect impacts and disturbance, metrics are correlated with the disturbance gradient to determine how each metric responds to the disturbance gradient, and to test differences among metrics from sites classified as impacted or reference.

Spatial and Temporal Heterogeneity

Dallas (2004a), Kaaya (2015), and Dallas et al. (2018) demonstrated that macroinvertebrate assemblages differed among river types and recommended the inclusion of a hierarchical spatial framework within which bioassessment data is interpreted. SASS, TARISS, and ZISS, all use two-level hierarchical spatial frameworks to offer geographic partitions within which macroinvertebrate assemblages are expected to be similar, thereby assisting in interpretation of bioassessment data (**Table 3**). Level I relate to broad geographic regions, while level II relates to longitudinal zonation of river systems. In South Africa, for Level I, 31 ecoregions were derived from vegetation and terrain, with inclusion of geology, soil, altitude, rainfall, air temperature, and runoff variability (Kleynhans et al., 2005). For Level II, simplified geomorphological zonation was used to differentiate rivers into Upland or Lowland (Dallas, 2007a), based on research that demonstrated macroinvertebrate assemblages are typically divided into upland and lowland assemblages, with

TABLE 3 | Hierarchical spatial frameworks for Rapid Bioassessment Protocols (RBP) currently used in Africa, including the South African Scoring System (SASS, Dickens and Graham, 2002), Namibian Scoring System (NASS, Palmer and Taylor, 2004), Tanzania River Scoring System (TARISS, Kaaya et al., 2015) and Zambian Invertebrate Scoring System (ZISS, Dallas et al., 2018), and Okavango Assessment System (OKASS, Dallas, 2009).

RBP	Level I	Level II	Level III
SASS	South African ecoregions (Level I, version 2005) are derived from vegetation and terrain, with inclusion of geology, soil, altitude, rainfall, air temperature, and runoff variability (Kleynhans et al., 2005) There are 31 Level I ecoregions in SA.	Simplified geomorphological zonation differentiated into Upland or Lowland.	Sampling is undertaken per biotope (stones, vegetation, and gravel/sand/mud).
NASS	None–Namibia only has five perennial rivers.	None–Namibia only has five perennial rivers.	Sampling is undertaken per biotope (stones, vegetation, and gravel/sand/mud).
TARISS	Eleven freshwater ecoregions of the world (Abell et al., 2008) and five climatic zones (Indeje et al., 2000) were used to divide the country into 12 ecoregions based on hydrological (catchment) boundaries and climatic characteristics.	Twelve landform features and three slope classes were used to generate a geomorphologic classification. Validation is required for other ecoregions across the country (Kaaya, 2015).	Sampling is undertaken per biotope (stones, vegetation, and gravel/sand/mud).
ZISS	Freshwater ecoregions of the world (Abell et al., 2008).	Stream order ("high" = stream orders 7–9; "low" = stream order 3–6).	Sampling is undertaken per biotope (stones, vegetation, and gravel/sand/mud).
OKASS	None–regional variation was not significant amongst deltaic regions and macroinvertebrate assemblages were relatively uniform across different areas of the Delta (Dallas and Mosepele, 2007, 2020).	None–longitudinal zonation is not applicable to deltaic systems.	Sampling is undertaken per biotope (marginal vegetation in current (channel), marginal vegetation out of current (lagoon), floating vegetation, submerged vegetation, and seasonally-inundated floodplain).

little differentiation at the finer level, e.g., Mountain Stream vs. Upper Foothill (Dallas, 2004a). In Tanzania, for Level I, Kaaya (2015) used freshwater ecoregions of the world (Abell et al., 2008) and five climatic zones (Indeje et al., 2000) to divide the country into 12 ecoregions based on hydrological (catchment) boundaries and climatic characteristics. For the Level II, 12 landform features and three slope classes were used to generate a geomorphologic classification (Kaaya, 2015). In Zambia, for Level I, freshwater ecoregions of the world (Abell et al., 2008) were used, and for Level II, stream order ("high" = stream orders 7–9 and "low" = stream order 3–6) was used (Dallas et al., 2018). Further partitioning of spatial variability is also included at biotope level, with all RBPs undertaking sampling for each biotope separately, and in some cases also interpreting metrics per biotope (e.g., ZISS). In comparison, examination of spatial variation of aquatic macroinvertebrate assemblages in the Okavango Delta (Dallas and Mosepele, 2007, 2020) showed that assemblages did not vary significantly amongst different region of the Delta, although macroinvertebrate assemblages differed amongst aquatic biotopes (**Table 3**). On this basis, Dallas (2009) proposed preliminary data interpretation guidelines based on two dominant deltaic habitats, namely marginal vegetation in current, and marginal vegetation out of current.

Temporal variability was only examined for SASS (Dallas, 2004b) and OKASS (Dallas, 2009), with the latter focusing on high-water level period (July) and the low-water period (October), which is more appropriate for the deltaic biotopes. The influence of sampling season on macroinvertebrate assemblages, taxon occurrence, and SASS Scores was investigated and seasonal variability was shown to be more prevalent in some regions of South Africa and some biotopes (Dallas, 2004b), with SASS Scores in the stones in current biotope varying seasonally, inter-annually and in response to wet and dry cycles, as well as antecedent flow events, water temperature and abundance of benthic algae (Palmer, 1997). For OKASS, although more taxa were generally recorded during October, the low-water period, compared to July, the high-water period, this was not statistically significant (Dallas, 2009).

Data Interpretation Guidelines

SASS data interpretation guidelines are most advanced for South Africa, with guidelines provided for each "ecoregion-geomorphological zone" using the relationship between SASS Score and ASPT (Dallas, 2007a; Dallas and Day, 2007). Specifically, the number of biotopes sampled was positively correlated with SASS Score and number of taxa, while ASPT was negatively correlated with the number of biotopes (Dallas, 2007b). Data interpretation guidelines were not developed for NASS (Palmer and Taylor, 2004) as Namibia only has five perennial rivers, namely the Orange, Kunene, Okavango, Zambezi, and Chobe, while NASS has only been used in the north-eastern region. Data interpretation guidelines have not yet been developed TARISS (Kaaya et al., 2015). Provisional data interpretation guidelines have been produced for ZISS, although these require further testing and validation (Dallas et al., 2018). ZISS guidelines are based on metric scores (ZISS Score, Number of Taxa and ASPT) generated for reference sites within each "ecoregion-stream order-biotope" combination (e.g., Zambezian Headwaters-low order-stones). Dallas (2009) proposed preliminary data interpretation guidelines based OKASS Score for two deltaic biotopes, namely marginal vegetation in current, and marginal vegetation out of current.

DISCUSSION

Sampling Protocols

The rationale for sampling biotopes separately is to ensure more accurate data interpretation since differences in availability of biotopes at a site may affect macroinvertebrate assemblages, given biotope preferences of some macroinvertebrate taxa (Dallas, 2007b). The availability of biotopes typically varies longitudinally down a river in response to broad geomorphological characteristics, with upper reaches dominated by stones, while lower reaches may only have vegetation and sand (Dallas, 2004a). When an RBP is being considered for use in a region outside the country where it was developed, it is important to consider the river systems prevalent in the region, including the variety of aquatic biotopes across rivers in the region. Biotopes sampled for all RBPs are dependent on which biotopes are present at the site. Where biotopes of the region resemble those of existing RBPs, then the sampling protocol can be adapted without much revision. For example, for ZISS, Dallas et al. (2018) concluded that stones and vegetation biotopes were more reliable than gravel/sand/mud in differentiating impacted from reference sites. Similarly, Dallas (2007b) noted that gravel/sand/mud biotope added very little to the SASS Scores or number of taxa in SASS. The earlier RBPs were limited to wadeable rivers, but since the development of OKASS and ZISS, non-wadeable, larger rivers, and deltaic biotopes may also be sampled. It should be emphasized that if the sampling protocol of an RBP is not followed (as summarized in **Table 1**), then it is not legitimate to assign sensitivity weightings to taxa recorded at a site to generate metrics for interpreting the impact of water quality impairment or deriving an ecological condition for the site. In particular, a result generated from a chemically preserved sample is not a legitimate SASS/NASS/TARISS/ZISS/OKASS result. Typical examples of where a protocol has not been adhered to include laboratory identification of preserved taxa instead of time controlled field-based identification, use of surber or box sampling instead of kick sampling, and only sampling one biotope at a site. The strict adherence to established sampling protocols is to ensure quality control and standardization so that results may be compared (Dickens and Graham, 2002).

Aquatic Macroinvertebrate Taxonomy, Distribution, and Sensitivity Weightings

Variation in geology and climate may influence physico-chemistry of river water, which may affect the distribution and sensitivity of macroinvertebrate assemblages (Day and King, 1995). When calibrating and validating a biotic index for a new region, it is important to undertake extensive sampling of macroinvertebrate assemblages across a range of sites in the region so that the full variety of taxa present in the region may be established. This is often done iteratively as a community of biomonitoring practitioners is formed within a region. Normally, when developing a RBP for a new region, macroinvertebrate samples collected from each biotope using the RBP would be preserved, and the identification of each taxon confirmed in the laboratory. This also facilitates the creation of a reference collection of aquatic macroinvertebrates for the region, which is a particularly useful resource for new biomonitoring programmes. Extensive sampling and taxonomic confirmation enable the detection of new taxa, not previously included in existing RBPs, including regionally endemic taxa, and provides evidence of the absence of other taxa in a region. Unfortunately, the development and adaptation RBPs and biotic indices is often hindered by poorly known taxonomy (e.g., tropical East Africa, Elias et al., 2014a, Ochieng et al., 2019). Fortunately, the RBPs described in this paper are mostly family level, which has greater taxonomic confidence than genus or species. Indeed, the use of predominantly family-level taxonomy in RBPs is a prerequisite since they are field-based.

The full range of taxa present may differ within a region, depending on latitudinal differences and regional endemism. The majority of taxa in the four RBPs developed for rivers were common to all (81 taxa), with a few regional endemics and less common taxa comprising the balance. Some taxa (Dicercomyzidae, Ephemerythidae, Machadorythidae, Sisyridae, Curculionidae, Sciomyzidae, Stratiomyidae, Ampulariidae, Bithyniidae, and Mutelidae) included in NASS, TARISS, and ZISS should ideally be included in future versions of SASS as these taxa have been recorded in the South Africa, and a further revision of SASS is likely. In addition, a recent study in Uganda noted five new taxa to be included in a modified TARISS, namely Chordodidae, Ptilodactylidae, Aspidytidae, Leptopodidae, and Paraecnomidae, which were not included in the TARISS (Tumusiime et al., 2019), although the latter is not a recognized family and is included in Ecnomidae. Importantly, prior to any new taxon being included in a RBP developed for a new region, it is recommended that all identifications be confirmed by a recognized institute and their taxonomic classification be verified on the taxonomic backbone of the Global Biodiversity Information Facility. Taxa excluded from the list of taxa during the validation process for a new region, are normally because they were not sampled in the new region. In some instances, this may be due to limited sampling or because the taxon is rare, or in other cases it may be because the taxon does indeed not occur in the region, such as those taxa endemic to the south-western Cape, South Africa. This emphasizes the importance of undertaking extensive sampling across the region to ensure that all potential taxa are sampled and included in the updated list of taxa for a new region. It is also useful to calculate the frequency with which each taxon is recorded in a region to provide an indication of taxon rarity.

When a RBP and biotic index are developed for a new region, attention should be given to validating the sensitivity weightings assigned to each macroinvertebrate family in the region. Existing indices apply different weightings for two families (Baetidae and Hydropsychidae) based on the number of species within each family sampled and recorded at a site. Families that have many species may exhibit a wider range of within-family tolerance compared to families with few species (Bonada et al., 2006). As part of the validation process, sensitivity weightings of existing taxa need to be checked and sensitivity weightings of new taxa assigned based on correlation with disturbance gradients, sensitivity weightings of closely related families, known life-history modes, anatomical adaptations, and expert knowledge. It is recommended that the RBP be assigned a version to facilitate keeping track of revisions and that an iterative approach be adopted when developing a RBP for a new region.

Impact Detection and Evaluation

Biotic indices should be able to detect changes in water quality and ecological condition of the aquatic ecosystem they are designed to be used in. As part of the regional validation process it is necessary for the index to be applied across a range of sites exhibiting a gradient of impacts. During the testing of the index, and undertaken concurrently with sampling of macroinvertebrates, data on anthropogenic activities, and ecosystem disturbance need to be collected at each site by assessing catchment, channel, and habitat impacts. Strong correlations between the metrics and human disturbance provides confidence in the biotic indices to detect impacts and disturbance. The relationships between metrics and disturbance can be evaluated for each biotope (Dallas et al., 2018) and for the site as a whole (Kaaya et al., 2015). Recently, Tumusiime et al. (2019) showed that macroinvertebrate assemblages differed between test (= impacted) and reference sites, which provided confidence in the ability of the TARISS to distinguish impacted and reference sites, thus validating the efficacy of TARISS in Uganda.

Spatial and Temporal Heterogeneity

Lotic systems are intrinsically heterogeneous with spatial and temporal variability occurring at multiple scales. Spatial variability may result from catchment-scale factors such as altitude, geology, channel slope; site-scale factors such as canopy cover, stream width, stream depth; and habitat-scale factors such as substratum, biotope availability, hydraulics (Dallas, 2007c). These factors may effect macroinvertebrate assemblage structure and an understanding of spatial variation in macroinvertebrate assemblages and the use of biotic indices based on these assemblages, is thus needed. Spatial frameworks are often used to overcome this intrinsic spatial variability and improve the reliability of data interpretation. The hierarchical spatial frameworks developed for SASS, TARISS, and ZISS, facilitated partitioning of intrinsic spatial variability, although further testing has been recommended for TARISS (Kaaya, 2015). The inclusion of biotope level sampling ensures that substrate, which has been identified as an important predictor for classification of macroinvertebrates in rivers (Dallas, 2007c), is considered. During the initial validation of the SASS protocol, the issue arose as to whether to combine samples based on substrate similarities (e.g., all stone, all vegetation) or hydraulic similarities (e.g., all in-current samples), with the former selected for SASS. Subsequent comparison of two RBPs, SASS, and the Iberian Peninsula (IB-protocol), which combined hydraulic biotopes (Prat et al., 2000), confirmed the similarity of the bioassessment results and the ability of each RBP to detect water quality impacts even though the RBPs used different biotope combinations (substrate vs. hydraulic) and had different sampling equipment (mesh diameter), sampling and laboratory processing methods (Bonada et al., 2006).

Temporal variability in macroinvertebrate assemblages may occur in response to seasonal variability in factors such as water temperature (Dallas, 2008), biotope availability (Armitage et al., 1995), and stream flow (McElravy et al., 1989). Given that seasonal variability was prevalent in some regions of South Africa and some biotopes (Dallas, 2004b), Dickens and Graham (2002) recommended that season be factored into SASS data interpretation, as some natural intra- and inter-annual variation in macroinvertebrate assemblages is likely. Kaaya et al. (2015) recognized the potential influence of seasonal variability on TARISS and recommended that it be examined for each river type in Tanzania.

Data Interpretation Guidelines

Developing appropriate data interpretation guidelines is perhaps the most challenging aspect when developing RBPs and biotic indices for a new region. However, it is critical to ensure that impacts reflected in the metrics are real and not merely a consequence of intrinsic spatial and temporal variability of macroinvertebrate assemblages, as this may affect our ability to interpret bioassessment data. An understanding of variability is important to facilitate the establishment of reference conditions, including expected macroinvertebrate assemblages (Dallas, 2004a,b; Dallas and Day, 2007). Reference conditions represent the natural or least-impacted condition for a particular type of river, and are used as a measure with which impacted sites are compared. Highly variable systems may lead to patchiness of taxa, which need to be considered when developing the mechanism for interpreting bioassessment data (Dallas, 2004a,b; Dallas, 2007b; Dallas and Day, 2007). The basis for interpretation of metrics for a particular sample is understanding the "natural" variability of metrics from the site or similar sites (i.e., variability of reference sites) and whether the metric for a test site falls within (unimpacted = reference) or outside (impacted) that variation.

Normally metrics are used for assessing ecological condition, either as tables or graphs showing different ecological categories. Embedding data interpretation within a spatial framework, as for SASS, provides a robust, easy to use system for evaluating change in water quality and ecological condition. Spatial and temporal variability may be accounted for by defining the reference condition as a band (Dallas and Day, 2007). Further, by utilizing the relationship between SASS Score and ASPT, between-site variation in the availability of biotopes is taken into account (Dallas and Day, 2007). The validity of this relationship for metrics derived from other RBPs and biotic indices in other regions, however, would need to be tested. Kaaya et al. (2015), while demonstrating that the validated TARISS technique is a dependable method for rapid bioassessment of rivers in Tanzania, advised that interpretation guidelines for each river type still need to be developed, using the "river type specific reference condition" approach. Since TARISS was only validated in two Tanzanian ecoregions, further validation in the other ecoregions is needed before it qualifies as a national biotic index.

CONCLUSION

This paper outlines important aspects that need to be considered when assessing water quality and river condition using RBPs and associated biotic indices. RBPs developed for new regions, and which are based on existing RBPs and indices, need to be comprehensively tested, calibrated, validated, and modified before they can be used with confidence in a new region. Of the five RBPs currently used in Africa, SASS has been the most widely tested across a range of river types, providing insight into spatial, and temporal variability of the biotic index. It has proven its value as a rapid, cost-effective and reliable tool for assessing river water quality and health. Other RBPs such as TARISS, ZISS, and OKASS require further within-region testing, especially in relation to spatial and temporal variability and interpretation of data. While TARISS1 has been developed within a spatial framework and validated for two regions in Tanzania, Kaaya et al. (2015) recommended further regional expansion and testing. Dallas et al. (2018) demonstrated that ZISS1 could detect moderate to high anthropogenic impacts on water quality and river condition. Dallas et al. (2018) recommended that ZISS only be done at sites with stones and/or vegetation biotope(s) and not at sites with only gravel/sand/mud, as these sites were not suitable for ZISS. Further sampling however is suggested to test this observation as well as the sensitivity of ZISS to a range of pollution types and intensities. Dallas (2009) recommended that further sampling of selected sites be undertaken to allow for the generation of additional data at both reference and monitoring sites, to help test and refine OKASS.

Confidence in the efficacy of an RBP and associated biotic index to assess river water quality and condition, is dependent on adequate calibration and validation of the index, including the development of appropriate data interpretation guidelines. The development of RBPs from other countries have shown that, whilst many RBPs are cost-effective, with less training and equipment requirements compared to more intensive monitoring protocols; the development of RBPs requires large amounts of data, collected iteratively together with testing and adaptation (Wright et al., 1998; Smith et al., 1999). Reliability of data is key. This is achieved by standardizing the RBP and including quality control measures such as practitioner accreditation (Dickens and Graham, 2002). Whilst RBPs currently used in Africa do not incorporate predictive models such as in RIVPACS (Wright et al., 1998) and AusRivAS (Smith et al., 1999), it is not beyond the scope of a calibrated and validation RBP and biotic index, and holistically implemented biomonitoring system, to facilitate the development of predictive models in the future. RBPs developed for bioassessment of rivers in Africa will promote the protection, conservation and management of African riverine ecosystems.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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Can Macroinvertebrate Traits Be Explored and Applied in Biomonitoring Riverine Systems Draining Forested Catchments?

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Trait-based approach (TBA) in recent time has received tremendous attention as complementary tool over taxonomic-based approach in assessing ecological health of riverine systems in developed countries, but in the Afrotropical region the trait-based approach is still in its infancy. No trait-based approach has been developed for riverine systems draining forested catchment in the Afrotropical region. Hence, this study was conducted to explore and apply macroinvertebrates traits as potential biomonitoring tools in assessing ecological health of riverine systems draining forested catchments in the Niger Delta area of Nigeria. Selected physico-chemical variables were sampled together with macroinvertebrates in 18 stations of 10 riverine systems from 2008 to 2012. The 18 stations were classified into three ecological classes namely near natural stations (NNS), slightly disturbed stations (SDS), and moderately disturbed stations (MDS) using physico-chemical-based classification with the aid of principal component analysis (PCA). The results revealed traits such as possessions of hardshell body armouring, preferences for clear and transparent water and opaque water, climbing and crawling mobility mechanisms, large (>20–40 mm) body size, preferences for scrapping, shredding, and grazing feeding habits to be associated with NNS and SDS based on RLQ (R, physico-chemical variables; L, taxa; Q, traits) analysis performed. Thus, these traits were deemed to be sensitive to human impact in forested systems. Also, traits such as tegument/cutaneous respiration, soft and exposed body armouring, burrowing mobility mechanism, spherical body shape, preference for detritus [fine particulate organic materials (FPOM)] food materials, small (>5–10 mm) body size and preference for filter feeding mechanism were associated with MDS. Hence, they were deemed tolerant of human impact in forested systems. A fourth-corner test performed revealed tegumental/cutaneous respiration preference, soft and exposed body armouring and burrowing mobility mode, which were associated with the MDS on the RLQ ordination were also positively correlated to 5 day biochemical oxygen demand (BOD_5); while preference for clear and transparent water, which were positively associated with MDS, were also positively correlated with pH and negatively correlated to dissolved oxygen (DO). Overall, this study affirmed that the TBA can be explored in biomonitoring riverine systems draining forested catchments. Nevertheless, we suggest the trait-based approach to be further explored, with a view to developing trait-informed indices for biomonitoring Afrotropical riverine systems.

Keywords: forestry, trait-based approach, functional feeding groups (FFGs), ecological classes, RLQ and fourth-corner analyses, Niger Delta, Nigeria

INTRODUCTION

Trait-based approach has recently gained attention as complementary to taxonomy-based approach when assessing riverine water quality (Fierro et al., 2017; Krynak and Yates, 2018; Desrosiers et al., 2019). The trait-based approach is engrained on the Habitat Template Concept (HTC) postulated by Townsend and Hildrew (1994). The HTC states that organisms survive in ecosystems where they possess the appropriate traits combinations, allowing them to adapt, and thrive in their external environments (Townsend and Hildrew, 1994). For instance, the possession of rapid reproductive turn-over has been postulated to confer resilience on organism in disturbed riverine systems (Townsend and Hildrew, 1994; Edegbene et al., 2020a). With regard to forested riverine systems receiving allochthonous materials from surrounding riparian vegetation, functional feeding groups (FFGs) such as shredders would dominate such systems providing a non-taxonomy based approach for assessing functional changes along the river length (Vannote et al., 1980; Moares et al., 2014; Brand and Miserendino, 2015).

Vannote et al. (1980) in their popular river continuum concept postulated the proportional distribution of functional feeding groups from forested systems dominated by allochthonous production to open system dominated by autochthonous production, shredders adapted for breaking down coarse particulate organic matter (CPOM) are expected to be dominant in forested system, whereas collector-gatherers and filter-filter feeders, which are adapted for consuming fine particulate organic materials (FPOM) are expected to be dominant in large open river systems (Vannote et al., 1980; Edegbene, 2020).

Although the trait-based approach is being increasingly applied for biomonitoring purposes (Statzner and Beche, 2010; Castro et al., 2018), the taxonomy-based approach is still widely used in the assessment of ecological status of riverine systems (e.g., Tonkin et al., 2016; Arimoro and Keke, 2017; Krynak and Yates, 2018; Edegbene et al., 2019a,b). In this regard, macroinvertebrates taxonomic structures are the most explored due to their important position in aquatic food web, their easy collection, and well-established biomonitoring protocols (Bonada et al., 2006). The taxonomy-based approach takes into account the structural distribution of the abundance, diversity, and composition of aquatic macroinvertebrates in relation to environmental stress (McGill et al., 2006). However, one of the short coming of this approach is that taxonomy is geographically constrained and often requires adaptation when applied across multiple distant geographical spaces (Edegbene et al., 2020a; Odume, 2020). In the Afrotropical context, identification of macroinvertebrates remains a challenge due to scarcity of taxonomic expertise further compounding the utility of the taxonomy-based approach to freshwater biomonitoring. Some authors such as Akamagwuna et al. (2019), Edegbene et al. (2020a,b), and Odume (2020) have thus call for the development of the trait-based approach to complement the taxonomy-based approach in the Afrotropical region.

Globally, the trait-based approach has grown in popularity for assessing and monitoring riverine health (e.g., Statzner and Beche, 2010; Descloux et al., 2014; Kuzmanovic et al., 2017; Serra et al., 2017; White et al., 2017; Berger et al., 2018; Castro et al., 2018; Krynak and Yates, 2018; Milosevic et al., 2018; Desrosiers et al., 2019), but only few studies have attempted to develop and apply the trait-based biomonitoring approach for assessing riverine systems health in the Afrotropical region (e.g., Akamagwuna et al., 2019; Edegbene et al., 2020a,b; Odume, 2020). The studies of traits in the Afrotropical region have focused largely on assessing freshwater systems subject to urban, agricultural, and industrial pollution. For instance, Odume (2020) developed trait-based approach for monitoring a river system in an urbanized and industrialized catchment in South Africa. Apart from the use of FFGs, the trait-based approach has not been applied to forested systems. In the present study, we explore the possibility of using macroinvertebrates traits for biomonitoring riverine systems draining forested catchments in the Niger Delta area of Nigeria.

Most of the riverine systems in the Niger Delta area of Nigeria drain forested catchments with patches of mangrove swamps dominated by red and white mangroves (Adekola and Mitchell, 2011; Edegbene et al., 2019b). The area is internationally recognized as a biodiversity hotspot (Tonkin et al., 2016). Despite the ecological importance of the area, most of the studies conducted in assessing the ecological status of riverine systems within the area is still centered on the use of aquatic biota composition, diversity and abundance (Edegbene and Arimoro, 2012; Arimoro et al., 2015). No study has been conducted to explore the importance of using macroinvertebrates traits in assessing and monitoring the ecological status of the forested riverine systems within the Niger Delta area. Therefore, the question was asked "Can macroinvertebrate traits be explored and applied for biomonitoring riverine systems draining forested catchments?" In the light of this question, the aim of this study was to explore and apply macroinvertebrate traits in biomonitoring riverine systems draining forested catchments in the Niger Delta region of Nigeria.

MATERIALS AND METHODS

The Study Area

The Niger Delta area of Nigeria is a tropical rain forest belt which occupies an area ∼70,000 km^2 in the Southern region of Nigeria (Edegbene et al., 2020a). The area is located within the interception of 5°27′-6°50′N and 5°35′-6°41′E of the equator (Tonkin et al., 2016; Edegbene et al., 2020a). The area is dominated by rainforest, mangrove and freshwater swamps (Tonkin et al., 2016). The forests are characterized by dense

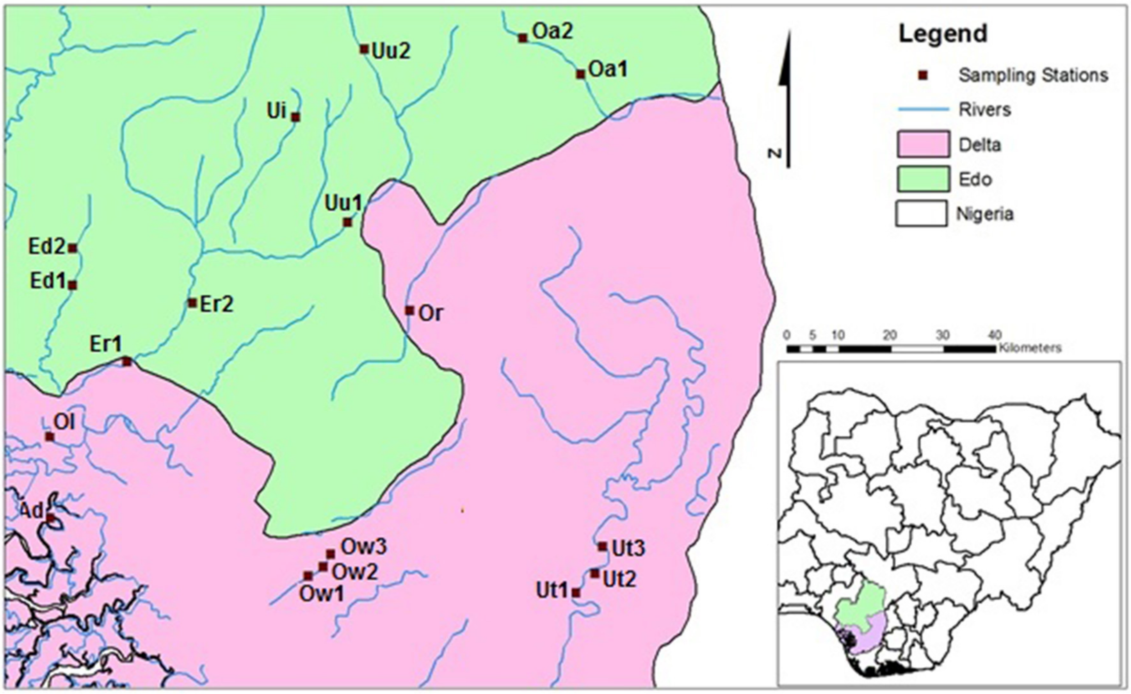

FIGURE 1 | Map of the study area showing the sampled stations.

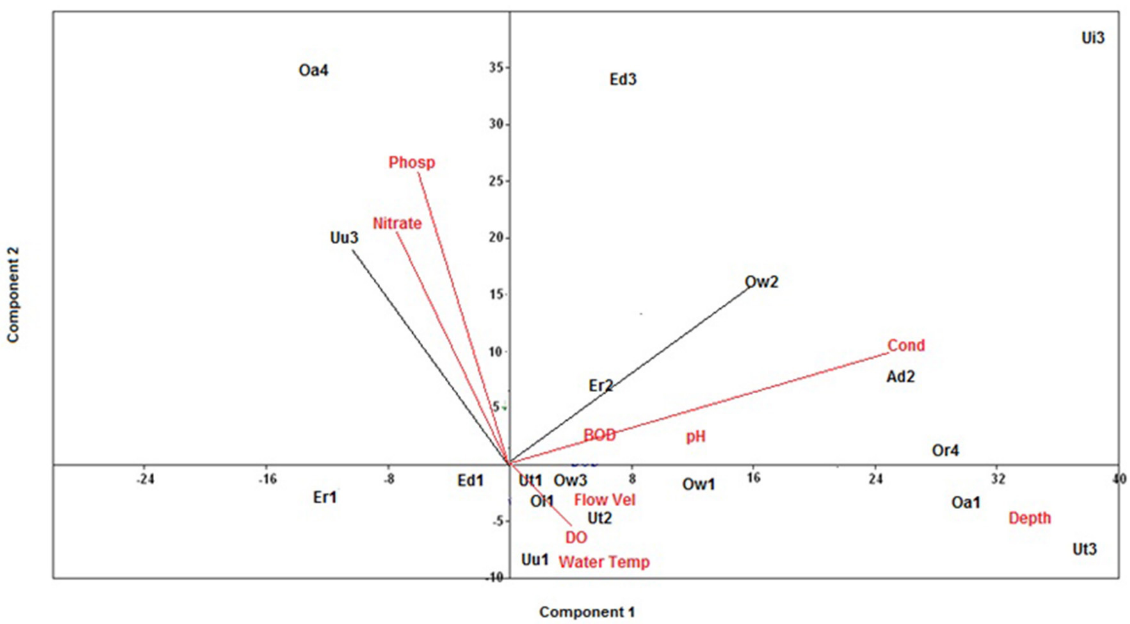

FIGURE 2 | PCA visualizing the 18 stations correlation with selected physico-chemical variables. Water Temp, Water temperature; Flow Vel, Flow velocity; DO, Dissolved oxygen; BOD, 5-day biochemical oxygen demand; Cond, Electrical conductivity; Phosp, phosphate.

canopy of trees which include *Pandanus* spp., *Bambusa* spp., *Mitragyna* spp., and *Elaeis* spp. (Tonkin et al., 2016). Two major seasons characterized the area (wet and dry) seasons. The wet season is between March and September, while the dry season is between October and February (Edegbene and Arimoro, 2012). In-between the dry season month of December and February, a short season called harmattan sets-in with relatively cold weather condition of an average temperature of 10°C.

Study Rivers and Stations

Eighteen (18) sampling sites located in 10 riverine systems draining forested catchments were selected for the study (**Figure 1**). The sampled rivers include Rivers Umu (Uu1, Uu2), Utor (Ut1, Ut2, Ut3), Edor (Ed1, Ed2), Ogbomwen (Ow1, Ow2, Ow3), Orogodo (Or), Oleri (Ol), Adofi (Ad), Eriora (Er1, Er2), Owan (Oa1, Oa2), and Umoni (Ui) Rivers.

Physico-Chemical Variables and Macroinvertebrates Sampling and Analysis

In the course of this study, physico-chemical variables were measured in each station once monthly from 2008 to 2012 (5 years). The physico-chemical variables sampled and analyzed for this study include water temperature, flow velocity, depth, electrical conductivity (EC), dissolved oxygen (DO), 5-day biochemical oxygen demand (BOD_5), pH, nitrate, and phosphate. Details on how physico-chemical variables were measured and analyzed for this study are contained in an earlier publication conducted by Edegbene et al. (2019b).

Macroinvertebrates samples were also collected at each sampling station along with physico-chemical variables for the 5 years period. Collection of macroinvertebrates were done using kick net of 500 µm mesh size as earlier described by Lazorchak et al. (1998). Also details on macroinvertebrates

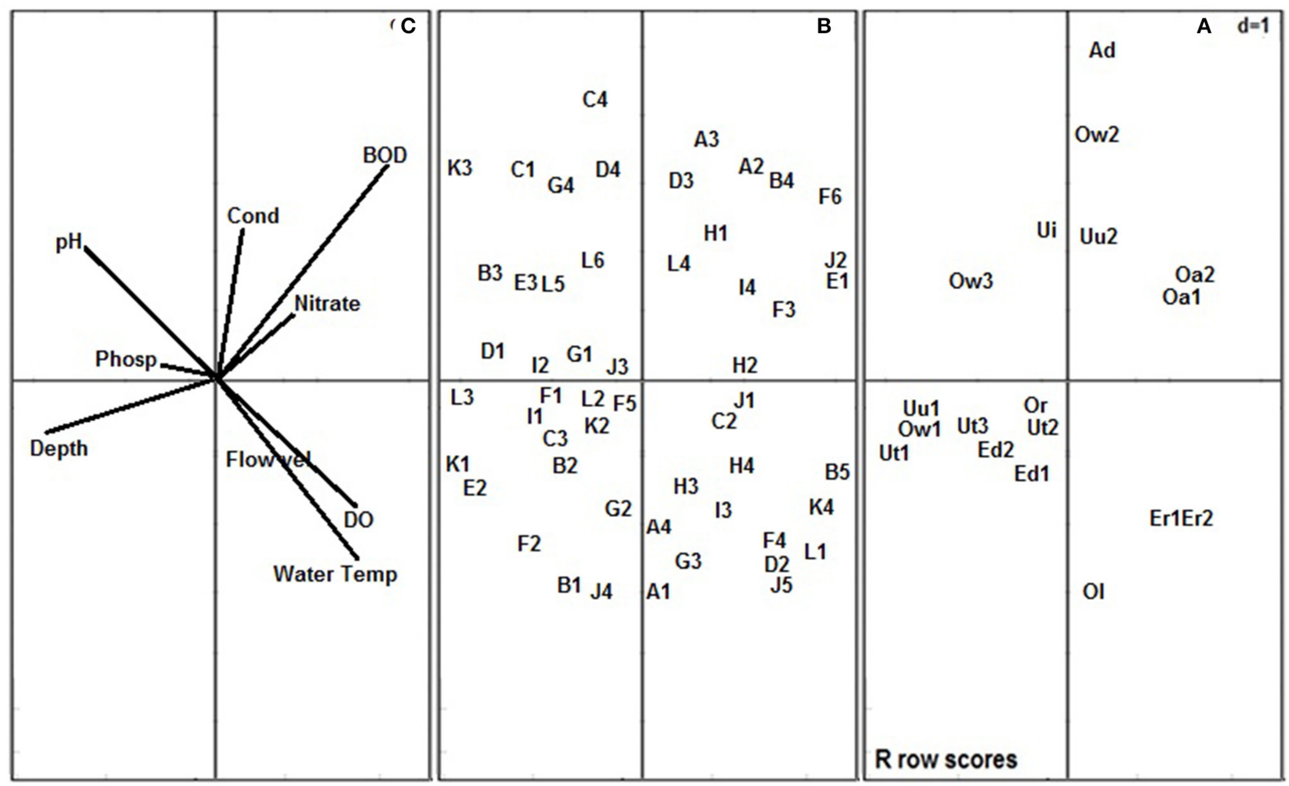

FIGURE 3 | Co-variation of the 18 river stations **(A)**, macroinvertebrate traits **(B)** and physico-chemical variables **(C)** along the first two components of the RLQ. Stations: Uu1, Umu River Station 1; Uu2, Umu River Station 2; Ut1, Utor River Station 1; Ut2, Utor River Station 2; Ut3, Utor River Station 3; Ed1, Edor River Station 1; Ed2, Edor River Station 2; Ow1, Ogbonwen River Station 1; Ow2, Ogbonwen River Station 2; Ow3, Ogbonwen River Station 3; Ol, Oleri River; Or, Orogodo River; Ad, Adofi River; Er1, Eriora River Station 1; Er2, Eriora Station 2; Oa1, Owan River Station 1; Oa2, Owan River Station 2; Ui, Umoni River. Physico-chemical variables: Water Temp, Water Temperature; Flow, Flow velocity; DO, Dissolved oxygen; BOD, 5 day biochemical oxygen demand; Cond, Electrical conductivity; Phosp, Phosphate. Stations ecological classes: NNS, near natural stations; SDS, slightly disturbed stations; MDS, moderately disturbed stations. Macroinvertebrate traits: "A1, Gills; A2, Tegumental/cutaneous; A3, Aerial: spiracle; A4, Aerial/vegetation: breathing tube, strap/other apparatus; B1, Hardshell; B2, Completely sclerotized; B3, Partly sclerotized; B4, Soft and exposed; B5, Cased/tubed; C1, Clear and transparent waters; C2, Silty; C3, Turbid waters; C4, No preference; D1, 1 year (Univoltine); D2, 2 years (Bivoltine); D3, >2 years (Multivoltine); D4, longer than 1 year (Semivoltine); E1, Free-living; E2, Temporary attachment; E3, Permanent attachment; F1, Climbing; F2, Crawling; F3, Sprawling; F4, Swimming; F5, Skating; F6, Burrowing; G1, Streamlined; G2, Flattened; G3, Spherical; G4, Cylindrical/tubular; H1, Detritus (FPOM); H2, Detritus (CPOM); H3, Macrophytes/algae; H4, Animal materials; I1, Highly sensitive to oxygen depletion; I2, Moderately sensitive to oxygen depletion; I3, Moderately tolerant of oxygen depletion; I4, Highly tolerant of oxygen depletion; J1, Very small (<5 mm); J2, Small (>5–10 mm); J3, Medium (>10–20 mm); J4, Large body size (>20–40 mm); J5, Very large body size (>40–80 mm); K1, Egg; K2, Larva aquatic stage; K3, Nymph aquatic stage; K4, Pupa aquatic stage; L1, Predating; L2, Scraping; L3, Grazing; L4, Filter feeding; L5, Deposit feeding; L6, Shredding" (Edegbene et al., 2020b).

collection, processing, sorting, identification, and enumeration are contained in an earlier publication by Edegbene et al. (2019b).

Statistical and Data Analyses

Station Ecological Classification Using Physico-Chemical Variables

The 18 stations sampled during the sampling period were ecologically classified by visualizing the correlation between selected physico-chemical variables and sampled stations using principal component analysis (PCA; **Figure 2**). The PCA ordination was computed using vegan package within the R-programming language (Oksanen et al., 2019) details of the actual station ecological classification was done following earlier method employed by Murphy et al. (2013), Edegbene et al. (2019b, 2020a).

Macroinvertebrate Traits

In this study 12 traits, divided into 53 traits attributes were selected. The 12 traits were those related to body size, body shape, body armouring, respiration, turbidity preference, voltinism, attachment mechanism, mobility, feeding preference, sensitivity to organic pollution, aquatic stages, and feeding habit (see **Figure 3**) for list of selected traits attributes. Most of these traits have been reported to be suitable for assessing various kinds of human disturbances. Traits information were primarily obtained from available literature containing traits information in Nigeria (Edegbene et al., 2020a), and supplemented and confirmed by traits literature information from elsewhere (Odume et al., 2018).

The selected macroinvertebrate traits link with each macroinvertebrate taxon was affirmed by fuzzy coding method (Chevenet et al., 1994). Fuzzy coding method shows the trait affinity with each taxon, and it accounts for any possible functional differences that may occur among species within the same taxon (Odume, 2020). Further, the fuzzy coding method compensates for any variation that would come with the allocation of a given taxon to a trait attribute (Mondy and Usseglio-Polatera, 2014). An affinity score of 0–3 was awarded to describe the taxon affinity to a given trait (Chevenet et al., 1994). A taxon is awarded a score of 0 if it shows no affinity to a given trait, while scores of 1, 2, and 3 was awarded to a taxon if it shows low, moderate and high affinity, respectively to a given trait (Chevenet et al., 1994). In processing the collated trait affinity, each trait score was multiplied by the relative abundance of the macroinvertebrate taxon.

Exploring Macroinvertebrate Traits Distribution

To explore macroinvertebrate traits distribution patterns in the forested riverine systems, an RLQ ordination plot was performed. The RLQ is a multivariate ordination which was developed by Dolédec et al. (1996), and it performs an ordination on three datasets: environmental variables e.g., physico-chemical variables (R), taxa (L), and traits (Q). In this study, the RLQ ordination was used to relate physico-chemical variables (R), macroinvertebrates

TABLE 1 | Stations ecological classification in the present study.

Rivers/station codes	Near natural stations (NNS)	Slightly disturbed stations (SDS)	Moderately disturbed stations (MDS)	Catchment size (km^2)	Land use type
Uu1	X			104	Forestry
Ut1	X			3,598	Forestry
Ut2	X			4,480	Forestry/Urbanization
Ut3		X		4,483	Forestry/Urbanization
Ed1		X		77	Forestry
Ow1		X		525	Forestry
Ow3		X		531	Forestry
Or		X		681	Forestry/Urbanization
Ol		X		431	Forestry
Ad			X	339	Forestry
Ed2			X	530	Forestry
Er1			X	42	Forestry
Er2			X	61	Forestry
Oa1			X	6,184	Forestry/Urbanization
Oa2			X	6,221	Forestry/Urbanization
Ow2			X	511	Forestry
Ui			X	57	Forestry
Uu2			X	839	Forestry
Total number of stations per ecological class	3	6	9		

Stations abbreviations: Uu1, Umu River Station 1; Uu2, Umu River Station 2; Ut1, Utor River Station 1; Ut2, Utor River Station 2; Ut3, Utor River Station 3; Ed1, Edor River Station 1; Ed2, Edor River Station 2; Ow1, Ogbonwen River Station 1; Ow2, Ogbonwen River Station 2; Ow3, Ogbonwen River Station 3; Ol, Oleri River; Or, Orogodo River; Ad, Adofi River; Er1, Eriora River Station 1; Er2, Eriora Station 2; Oa1, Owan River Station 1; Oa2, Owan River Station 2; Ui, Umoni River.

taxa (L), the traits (Q), and sampled stations. The RLQ first two components were tested for statistical significance using the Monte Carlo permutation test at 999 permutations argument ($P = 0.05$).

Macroinvertebrate traits relationships with physico-chemical variables were evaluated using a multivariate test known as fourth-corner test. The fourth-corner test shows the association between traits and physico-chemical variables. The test shows traits that show positive, negative or no association with given physico-chemical variables.

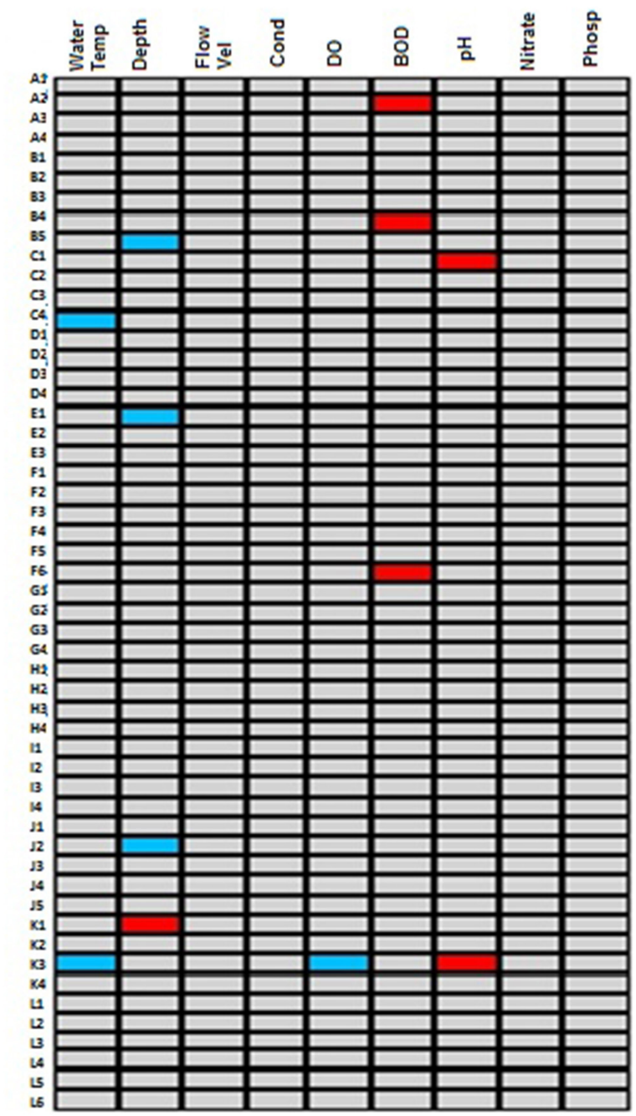

FIGURE 4 | Summary of the fourth-corner test performed for macroinvertebrates traits and physico-chemical variables in the selected riverine systems draining forested catchment. Significant positive relationships are shown in red colored cells while the significant negative correlations are shown in blue colored cells. The gray colored cells represent no-significant relationships.

RESULTS

Stations Ecological Classification Using Physico-Chemical Variables

From the results of the PCA ordination, Component 1 and 2 had Eigen values of 456.2 and 101.9, respectively. Station 1 of Edor and Eriora Rivers were positioned on Component 2 and they had no correlation with any physico-chemical variable (**Figure 2**). Positioned on Component 1 of the PCA were Oleri River, Stations 1 of Ogbonwen, Owan, and Utor Rivers, Station 3 of Rivers Ogbonwen, and Stations 2 and 3 of Utor Rivers which were correlated with DO, flow velocity, water temperature, and depth (**Figure 2**). BOD_5, electrical conductivity (EC), and pH were positively correlated with Adofi River, Stations 2 of Eriora and Ogbonwen Rivers, Edor, Eriora, Utor Rivers, and Orogodo River, and they were positioned on Component 1. Station 2 of Owan and Umu Rivers were correlated with nitrate and phosphate on Component 2 (**Figure 2**).

The classification of stations into ecological classes was undertaken by correlating sampled stations with physico-chemical variables. Initially, stations associating with physico-chemical variables indicating pollution (e.g., EC, BOD_5, nitrate, and phosphate) were classified as disturbed stations, while stations associating with physico-chemical variables indicating good water quality (e.g., DO) were classified as non-disturbed stations. The actual stations ecological classification in this study was done by extracting the coordinate values of each station from the first component of the PCA. Further, the inter-station distances of each station were calculated following the methods earlier employed by Murphy et al. (2013). In adopting the methods employed by Murphy et al. (2013), the 18 stations were classified into three ecological classes, which include near natural stations (NNS), slightly disturbed stations (SDS), and moderately disturbed stations (MDS). The first component of the PCA was adopted for computing station ecological classes because it explained the highest percent variation 79% compared to the second component which explained 17.7% percent variation of the PCA (Murphy et al., 2013). Similar methods have recently been used by Edegbene et al. (2019b, 2020a) to classify sites into ecological categories along urban and agricultural pollution gradients. The stations classes are shown in **Table 1**.

Exploring the Distribution Patterns of Macroinvertebrate Traits

The NNS (Station 1 of Umu and Utor Rivers and Station 2 of Utor River) and SDS (Station 1 of Edor and Ogbonwen, Station 3 of Utor and Orogodo Rivers) were associated with Component 1 while all the MDS except Stations 2 of Edor River were associated with Component 2 on the RLQ ordination plane (**Figure 3**). Positioned at the center of the RLQ biplot were Oleri and Umoni Rivers. Stations classified as NNS and SDS at Component 1 were positively correlated with pH, phosphate and water depth, while stations classified as MDS which were associated with Component 2 and were positively correlated with decreased concentration of BOD_5, EC, and nitrate (**Figure 3**).

Traits that were associated with the NNS and SDS include the possessions of hardshell, complete sclerotization of the body, partial sclerotization of the body, preferences for clear and transparent waters and turbid waters, 1 year (univoltinism), longer than 1 year (semivoltinism), preferences for temporary and permanent attachment, climbing and crawling, flattened body shape, cylindrical/tubular body shape, preferences for a high and moderate sensitivity to oxygen depletion, large (>20–40 mm) body size, preferences for scrapping, shredding, and grazing feeding habits and nymph aquatic stage (**Figure 3**). Thus, signifying their sensitivity to impact in forested systems. Further, traits that were associated with the MDS include tegumental/cutaneous respiration, soft and exposed body, a preference for free-living, burrowing, spherical body shape, a preference for detritus (FPOM) as food materials, small body size (>5–10 mm), and a preference for filter feeding (**Figure 3**). Hence, proving their tolerance to impact in forested systems.

The Eigen values of the first two components of the RLQ were 3.89 and 1.39, respectively, and the RLQ Component 1 explained 65.74% variation, while Component 2 explained 22.93%. A projected total inertia of 6.057 was recorded while a variance for physico-chemical variables for Components 1 and 2 were 3.39 and 5.42, respectively and the traits variance for Component 1 was 19.60 and Component 2 was 12.21. There was no statistically significant difference between the macroinvertebrates traits and physico-chemical variables ($P > 0.05$) as revealed by a Monte-Carlo test at 999 permutations.

To further confirms traits sensitivity to and tolerant of impact in forested systems, a fourth-corner test was performed after the RLQ ordination in a bid to further explore the traits correlation with physico-chemical variables. The result revealed that a preference for tegumental/cutaneous respiration, soft and exposed body and burrowing, which were associated with the MDS on the RLQ ordination were also positively correlated with BOD_5 on the fourth-corner test (**Figure 4**). This further confirms their tolerance of human impact in forested systems owing to their positive relationship with BOD_5 a pollution indicating physico-chemical variable. On the other hand, preference for clear and transparent waters and nymph aquatic stage which were positively associated with MDS on the RLQ ordination were also positively correlated with pH on the fourth-corner test (**Figure 4**), while only preference for clear and transparent waters was negatively correlated to DO.

DISCUSSION

The present study explored the possibility of biomonitoring riverine systems draining forested catchments in the Niger Delta area of Nigeria using macroinvertebrate trait-based approach. The results revealed traits such as preferences for clear and transparent water, univoltinism, semivoltinsim, preferences for climbing and crawling, flattened body shape, cylindrical/tubular body shape, preferences for high and moderate oxygen depletion, large body size (>20–40mm), preferences for scrapping, grazing and shredding to be associated with near natural stations (NNS), and slightly disturbed stations (SDS). The distributions of these traits were defined by decreased pH, phosphate, BOD_5, EC, and nitrate concentration, suggesting that they are sensitive to impact in forested systems. Most of the traits associated with NNS and SDS have been reported to be positively correlated with decreased pH (Moyo and Richoux, 2017), and decreased concentrations of some pollution-indicating physico-chemical variables such as BOD_5, EC, nitrate, and phosphate (Edegbene et al., 2020a). Thus, these traits are overall sensitive to increasing human impacts in forested systems and their disappearance should thus serves as a warning signal. The results pertaining to the distribution of these traits are similar to those reported by Guilpart et al. (2012), Pallottini et al. (2017), and Milosevic et al. (2018) indicating that traits such as shredding and scrapping were associated with riverine stations close to natural condition. Functional feeding groups (FFGs) are commonly used for assessing disturbances in forested systems (Stepenuck et al., 2002; Mondy and Usseglio-Polatera, 2014). As with other studies, the results in this study suggest that shredders and scrappers are sensitive to impact in forested systems. Shredders are particularly sensitive to changes in the type and quality of leaf litters due to human impact on forest (Stepenuck et al., 2002; Mondy and Usseglio-Polatera, 2014). Fierro et al. (2017) and Kuzmanovic et al. (2017) had reported forested riverine systems receiving allochthonous materials from surrounding riparian vegetation to be dominated by organisms that are shredders, scrappers and collector-gatherers. Therefore, from a biomonitoring perspective, relative change of shredders and scrappers can serve as good indicators of water quality impact in forested systems. This is particularly so because these organisms are sensitive to changes in allochthonous inputs (Vannote et al., 1980; Moares et al., 2014; Brand and Miserendino, 2015). This finding showed that functional traits can be used to assess whether riverine systems within the Niger Delta area of Nigeria are near to natural conditions or disturbed. Furthermore, owing to the continuous urbanization of the Niger Delta area, the outlined functional traits would serves as gauge to separate rivers that are perturbed from those that are unperturbed.

Macroinvertebrate taxa with large body size (>20–40mm) were associated with NNS and SDS on the RLQ ordination. This finding in the present study is in line with the prediction of the habitat template concept (HTC), which affirms that organisms with large body size associates more with sites that are less disturbed (Townsend and Hildrew, 1994). Organisms with large body size have been reported to possess a reduced surface area to volume ratio, which makes them vulnerable to disturbances (Townsend and Hildrew, 1994; Edegbene et al., 2020a).

Traits such as tegumental/cutaneous respiration, soft and exposed body, burrowing, possession of a small body size (>5–10 mm), preferences for free-living and filter feeding were associated with moderately disturbed stations (MDS) on the RLQ ordination and they were positively correlated with decreasing dissolved oxygen concentrations. Organisms that exhibit tegumental/cutaneous are less sensitive to depletion in dissolved oxygen because of their efficiency in gaseous exchange with their external environment. These organisms are often reported in disturbed sites (Lamouroux et al., 2004; Tomanova and Usseglio-Polatera, 2007; Tomanova et al., 2008; Desrosiers et al., 2019). Similarly, specialist burrowers are also able to tolerate fluctuation in vertical DO saturation, enabling them to cope in systems that are depleted in dissolved oxygen. Overall, the use of tegumental/cutaneous for respiring as well as a preference for burrowing seems to be tolerant of human impact in forested systems. These suggestions are in line with those of Lamouroux et al. (2004), Tomanova and Usseglio-Polatera (2007), and Tomanova et al. (2008) who had earlier reported these traits to be associated with impacted river systems.

Small body size (<5 mm) organisms were also associated with MDS in the RLQ ordination. This finding is also in line with the habitat template concept (HTC) which states that small body sized organisms favorably associate with stations that are disturbed as they are resilient to disturbance (Townsend and Hildrew, 1994). The resilience of small body sized organisms results from their ability to reproduce many offspring in one reproductive cycle, as well as their possession of large surface area to volume ratio (Townsend and Hildrew, 1994; Poff et al., 2006; Edegbene et al., 2020a), and these features possibly support the non-vulnerability of taxa that possesses small bodies to perturbed ecological health conditions.

CONCLUSION

The observed results suggest the differential distribution of traits in forested systems. Traits such as tegumental/cutaneous respiration, soft and exposed body, a preference for free-living, burrowing, spherical body shape, FPOM, and small body size were associated with the moderately disturbed stations, suggesting that they are tolerant of human impact in forested systems. Traits such as the possessions of hardshell and complete sclerotization, preferences for clear and transparent waters, univoltinism, semivoltinism, preferences for temporary and permanent attachment, climbing and crawling, flattened body shape, cylindrical/tubular body shape, preferences for a high and moderate sensitivity to oxygen depletion, large body size, nymph aquatic stage, preferences for scrapping, shredding, and grazing which were observed at the near natural stations and slightly disturbed stations, but not at the moderately disturbed stations seemed to be sensitive to human impact in forested systems. The differential distribution of traits generally indicates their value for biomonitoring. It is suggested that the trait-based approach can be further explored, with a view to developing trait-informed indices such as the proportion of sensitive invertebrate (PSI) (Extence et al., 2017) and species at risk (SPEAR) (Verberk et al., 2013) models for Afrotropical riverine systems.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AE and OO conceptualized and designed the work. AE and FA collected and analyzed physico-chemical variables and macroinvertebrate data. AE performed the statistical/data analyses and wrote the initial draft of the manuscript. FA, OO, EO, and UK reviewed and edited the manuscript draft and the manuscript was finalized by AE. All authors contributed to the article and approved the submitted version.

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Using the Biological Condition Gradient Model as a Bioassessment Framework to Support Rehabilitation and Restoration of the Upper Tana River Watershed in Kenya

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The biological condition gradient (BCG), a scientific framework that describes the change in ecosystem characteristics in response to human-induced levels of stressors, was modified and used to characterize watershed habitats in the Upper Tana River watershed, Kenya. The inbuilt utilities of BCG, including its simplicity, versatility, and its robust nature, allowed its use by seven taxonomic groups of macroinvertebrates, diatoms, fish, herpetofauna (amphibians and reptiles), plants, macrofungi, and birds to assess and monitor landscape conditions in both terrestrial and aquatic habitats. The biological data were described using taxa abundance distribution measures followed by multivariate analyses to determine their relationship with water or soil quality and thereafter assessment of taxa tolerant levels in response to environmental stress and disturbances. Preliminary findings reported that the taxonomic groups complemented each other, with each taxonomic group reliably assessing ecological conditions to a certain degree that supported assigning all 36 sampled sites into BCG tiers. The BCG models developed for all taxonomic groups assisted in the identification and selection of taxa indicating varying levels of landscape conditions. These taxa, referred to as flagship or indicator taxa, assist in simplifying the BCG model and, hence, are possible for use by parataxonomists or ordinary citizens to assess and monitor the ecological health of habitats under consideration. Furthermore, the capability of BCG models to assess landscape conditions shows how they can be used to identify important habitats for conservation, direct investment for restoration, and track progress.

Keywords: aquatic biodiversity, bioassessment, biomonitoring, watersheds, conservation

INTRODUCTION

The development of a scientific-based environmental assessment and biomonitoring framework that is simple and robust enough for use by the general public remains a major limitation for sustainable management of ecosystems (Graham et al., 2004). Such a framework should be usable by the general population to monitor the ecological health of the environment, including having clear sustainable management targets to be achieved during rehabilitation and restoration initiatives (Leigh et al., 2019). The history of development and use of biological indicators show their use started with the Saprobien System (SS) concept in the early 1900s, which employed benthic macroinvertebrates and planktonic plants and animals as indicators of organic loading and low dissolved oxygen in aquatic ecosystems (Beck, 1954; Pantle and Buck, 1955; Vollenweider, 1968; Davis, 1995). The SS described the trophic state of a lake by classifying the ecological condition status along a disturbance response gradient due to pollution from human and natural influences. Today, it has evolved into indices of biotic integrity (IBIs) that are based on indices including community structure, richness, dominance and abundances as measures of pollution effects (e.g., Wilhm and Dorris, 1966; Karr, 1981; Davis 1995; Hawkins, 2006), and multivariate indices that combine weighted effects of the variates to predict membership in different water quality classes (e.g., Davies et al., 2016). Both multimetric and multivariate models use knowledge of species' natural history traits to express ecosystem changes in response to increasing levels of stressors. These ecosystem gradient models use undisturbed or pristine habitat conditions as a reference point against arrays of habitats or sites experiencing varying levels of disturbances and are validated by actual measurement of environment variables.

One biomonitoring framework that can be simplified and used for the assessment of ecological conditions is the United States Environmental Protection Agency's Biological Condition Gradient (BCG; Davies and Jackson, 2006; USEPA, 2016). The BCG is grounded on the concepts of stress ecology articulated by Odum et al. (1979), Odum (1985), Rapport et al. (1985), and Cairns et al. (1993). The BCG starts by describing the biological condition in natural or minimal disturbed habitats and the expected changes in biological conditions along a stressor gradient caused by human-induced environmental changes. Along this disturbance gradient, assemblages of taxa are selected and used to describe ecological conditions for the sites. The original BCG was developed based on common patterns of biological response to stressors observed empirically by aquatic biologists and ecologists from different geographic areas in the United States (Courtemanch et al., 1989; Courtemanch, 1995; Yoder and Rankin, 1995; Davies and Jackson, 2006). Using the practical experience of a diverse group of aquatic scientists from different biogeographic areas and independent of specific methods of assessment, the BCG is a heuristic model based on generalized observable changes in the aquatic community that can be used as a common language expressing habitat condition and change (Davies and Jackson, 2006; USEPA, 2016). The USEPA has extended the use of the BCG concept to other aquatic habitats including coral reefs, estuaries, and mangroves.

The initial use of SS, IBIs, and BCG was primarily for the monitoring and assessment of aquatic ecosystems. However, the strong links that exist between watersheds and water quality in wetlands (Masese et al., 2012; Minaya et al., 2013) suggest ecological conditions in terrestrial catchments can be used to describe the health of wetlands. As in aquatic habitats, changes in terrestrial ecological components of species composition, diversity, and ecosystems are likely to be reflected in ecosystem condition changes due to increasing levels of stress and disturbances. Therefore, the frameworks of using biological indices in monitoring and assessment of wetlands can be extended and applied in associated watersheds through using terrestrial biological components to describe ecosystems' health. The approach of integrating both aquatic and terrestrial conditions is expected to promote holistic and integrated sustainable management of landscapes that is presently being promoted as a river to the basin to the ecological network (Ishiyama et al., 2017).

METHODS

To monitor ecological conditions of aquatic and their linked terrestrial ecosystems in the Upper Tana River (UTR) watershed, this research settled on adapting the BCG framework, which has utilities that can be used to overcome challenges of obtaining and interpreting complex scientific data for sustainable management of waters resources (Davies et al., 2016). First, the six BCG tiers were categorized and then linked to the expected set of taxonomic group responses using a subset of the BCG attributes. This was followed by the collection of biological data along disturbance gradients in both aquatic and terrestrial ecosystems. Data collected were used to describe ecological conditions of studied sites for aquatic and terrestrial taxonomic groups of macroinvertebrates, diatoms, fish, herpetofauna, vegetation, macrofungi, and birds. Relevant attributes used to develop the BCG tiers in UTR were 1) historically documented, sensitive, long-lived, or regionally endemic taxa, 2) sensitive (intolerant) taxa, 3) intermediate tolerant taxa, 4) tolerant taxa, and 5) presence of non-native species. Thereafter, collected biological data were used to assess the ecological conditions for each study site and then appropriate sustainable management interventions were proposed.

Study Area

The UTR watershed is the upper part of the Tana River Catchment and is situated in Central Kenya. It lies between latitudes 0°30′N and 2°30′S and longitudes 37°00′E and 41°00′E. This study was undertaken in selected major rivers in Nyeri and Murang'a counties in the UTR (**Figure 1**). Both counties have an area of 4,919 km^2 (with Nyeri having an area of 2,361 km^2 and Murang'a with 2,558 km^2) and a human population of 1.82 million with densities of 228 and 429 persons per km^2 in Nyeri and Murang'a, respectively (KNBS Kenya National Bureau of Statistics, 2019). A large percentage of

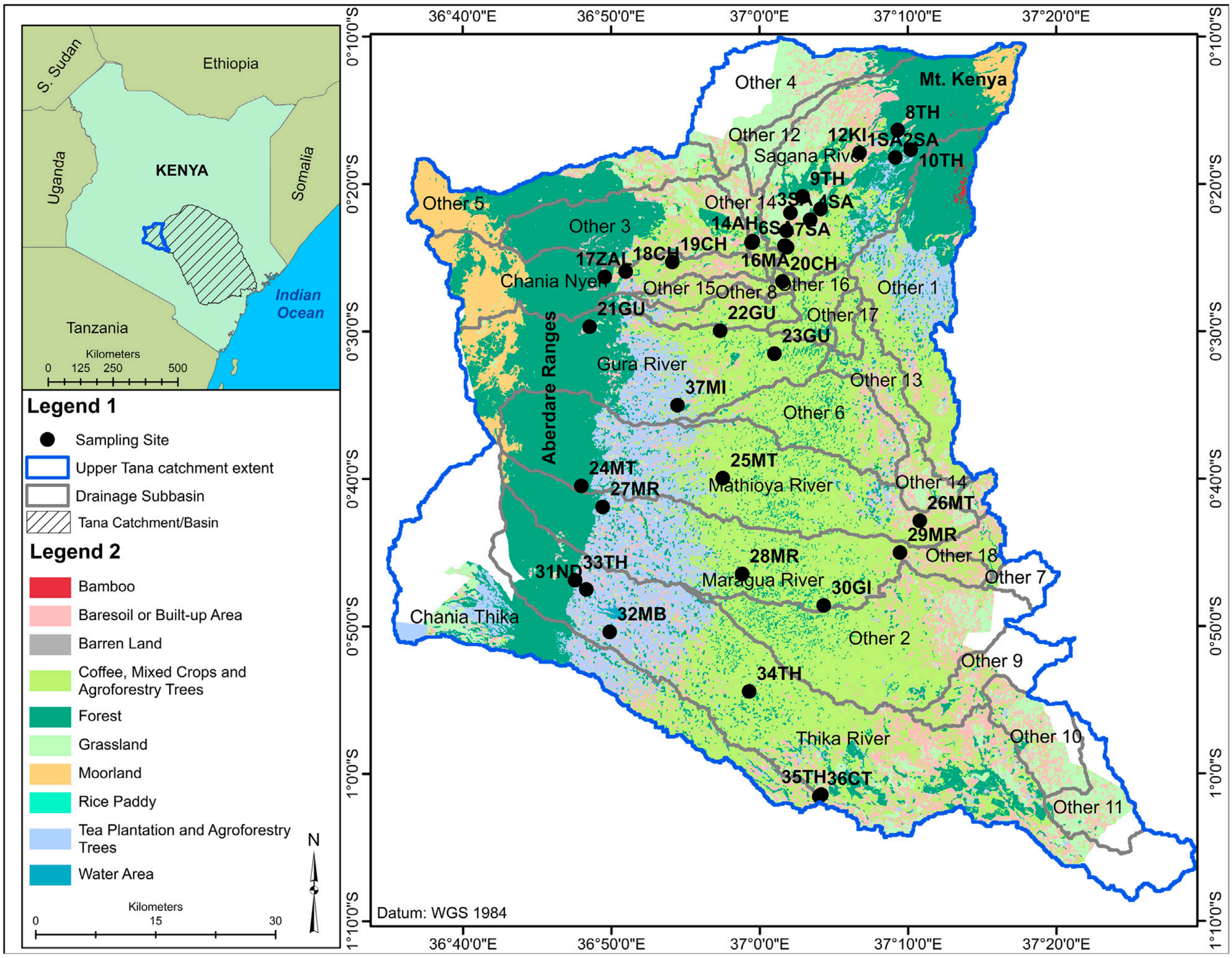

FIGURE 1 | A map of the Upper Tana River watershed showing sampling sites and the six major sub-catchments of rivers Sagana, Chania (in Nyeri), Gura, Mathioya, Maragua, and Thika. Land use and cover represent levels of disturbances with forests, and tea is the least disturbed area, coffee and mixed agriculture are moderately disturbed areas, while intensive horticulture and livestock farming indicate severely disturbed areas. For full names of sampling sites' abbreviations, see **Table 1**.

the land area lies between 914 and 3,000 m ASL, though a small portion comprises the high-elevation areas of Mount Kenya (3,000–5,199 m) and Aberdare Range (3,000–3,999 m). The area has an equatorial climate with two wet seasons (March–May and October–December) separated by dry seasons. The mean annual rainfall in low-lying areas is 790 mm, mid-elevations is 1,500 mm, and high-elevations is 2000 mm, while monthly mean temperature ranges from 16–20°C. Both rainfall and temperatures are functions of elevations, and the area has three major agroecological zones (AEZs) that are defined by climate, landform and soils, and land cover (FAO, 1996). The AEZ 1 represents alpine zones in the high-elevation areas, and AEZ 2 represents high-potential zones and AEZ 3 represents medium-potential zones, which are both found in the mid-elevation to low-lying areas. For instance, the AEZ 1 was confined to mountains and immediate surroundings acting as a watershed, whereas AEZ 2 was found in high-potential areas supporting forestry, tea, livestock rearing, and tourism, while AEZ 3 supported agricultural activities of coffee, food crops, horticulture, and livestock rearing. Approximately 80% of the population depends on subsistence agriculture for livelihoods and a majority of households have small- to medium-sized farm (0.5–2 ha). Agriculture is mainly rain-fed, but recently irrigation farming is becoming more practiced due to erratic and unreliable rains. The mountainous settings are a good source of fast-flowing rivers that have created topography with steep slopes and deep valleys that influence local climate and types of crops grown. The steep topographies are prone to gulley erosion and landslides. The major rivers flowing from Mt Kenya are Sagana, Thegu, and Nairobi and those from Aberdare Ranges are Honi, Muringato, Chania (in Nyeri County), Gura, Mathioya, Maragua, Thika, and Chania (bordering Murang'a and Kiambu counties). The area geology is of volcanic rocks of the Pleistocene age and Achaean type that have weathered to give fertile soils suitable for agriculture activities. The exploitation of land and water resources has resulted in the degradation of the UTR, and the consequences are an environmental crisis that is responsible for the water insecurity issues that now threaten the social, political, and economic systems in the region (MEMR, 2012). In addition, the area is endowed with small- to medium-sized wetlands that are experiencing different levels of human use that are associated with their degradation and loss (Sakané et al., 2011).

Sampling Design

Data on soil, water quality, and biological components were collected in the six major sub-catchments of Rivers Sagana, Chania (in Nyeri), Gura, Mathioya, Maragua, and Thika in the UTR catchment (**Figure 1**). For each sub-catchment, a minimum of three sampling sites were established along a disturbance gradient to represent undisturbed and then moderately to severely human-impacted areas. Overall stratified random sampling was used to select 36 permanent sites (9 sites in undisturbed, 19 in moderately disturbed, and 9 in severally disturbed areas). Sampling sites in undisturbed areas were located in the river headwaters, which had minimal human activities, and major land uses were natural forests and adjacent tea plantations. Moderately disturbed areas were found in midsections of the sub-catchments, and major types of land use were tea, coffee, zero-grazing of livestock, and moderate horticulture. Severely disturbed areas were found in the lower section of the sub-catchments, and major human activities were intensive horticultural farming and grazing of livestock in lowland floodplains and wetlands. The location coordinates of each sampling site were made using portable GPS and later aided in the mapping of species distribution and water parameters. Sampling was carried out during the end of the dry season (August–October, 2018). During this sampling regime, it was not possible to collect data for biological, water, and soil components in all sites due to logistical constraints especially time. However, efforts were made to have all components sampled in all three different land-use/-cover zones. For instance, all 36 sites were sampled for water quality and diatoms, fish were sampled in 30 sites, macroinvertebrates were sampled in 24 sites, soil parameters and macrofungi were sampled in 21 sites, vegetation was sampled in 17 sites, herpetofauna was sampled in 16 sites, and birds were sampled in 10 sites (**Table 1**).

Water Quality and Flow Regimes

Electric conductivity (EC) and turbidity were measured *in situ* using portable meters (Hanna Instruments models, i.e., HI 93703d and HI 99300, respectively). Total nitrogen (TN), total phosphates (TP), and chemical oxygen demand (COD) were determined using gravimetric methods using UV-spectroscopy and natural organic matter (NOM) with a UV-light emitting diode. Water flow velocity was measured using the float method (Harrelson, 1994; Davids et al., 2019). The selection of sampled water quality parameters determined was informed by our research experience in similar habitats in tropics on those known to be affected by effects of land-use and -cover changes (Ndaruga et al., 2004; Ndiritu et al., 2006).

Macroinvertebrates

Samples were collected in the riffles and runs using a 500-μm mesh size dip net. The runs and riffles' biotopes were preferred because they are the most abundant and productive biotopes in the rivers' headwaters and are known to support a great diversity of stress-sensitive taxa such as the mayflies, caddisflies, and stoneflies (Dallas, 2021). Also, it is common for rapid assessment protocols to go for a single or few biotopes. Samples were taken by dipping, jabbing, and sweeping upstream for 2 min (Barbour, 1999). Collected samples were sorted in the field, beginning with the removal of large debris, then picking all available specimens using forceps, and putting them in vials with 70% ethanol. In each site, a final simple random sampling was done from any other available biotopes (microhabitats), and the sample was combined in a separate vial for the development of a checklist. Taxonomic identification was carried out in the Invertebrate Zoology Laboratory in the National Museums of Kenya (NMK) based on morphospecies concept using standard monographs and identification keys (Day and de Moor, 2003; De Moor et al., 2003; Stals and Moor, 2007; Griffiths et al., 2015). Identification was done to order and family levels that are acceptable levels of taxonomic resolution for rapid macroinvertebrates' assessment purposes (Graham et al., 2004). Thereafter, representative specimens were vouchered and stored permanently at the Invertebrates' Collection in NMK.

Diatoms

Samples were collected from cobbles (boulders) that were close to the riverbank from riffles (Ndiritu et al., 2006). The flowing water at the edge of the mainstream (littoral zone) is assumed to be of the same physical and chemical quality as that in the mainstream. Streams and rivers considered were from order 1–3 and most had high flow velocities that are known to reduce water quality variabilities within the cross section of a given river reach (Rode and Suhr, 2007). Diatom samples were collected from all sites where water quality variables were collected.

Fish

Different methods were applied to sample fish depending on the suitability of the river habitats. These included hooks and line, monofilament gillnets, dip nets, and direct observations (Côté and Perrow, 2013). More information on fish was obtained by interviewing the local community within the study sites as well as checking fish collections and records at the Ichthyology Section of the NMK.

Herpetofauna: Amphibians and Reptiles

A timed limited search (TLS) method for a one-person hour was carried out in different amphibian and reptile microhabitats in the 16 sampled sites (Malonza et al., 2011). Sampling was done during the daytime, which means amphibians especially frogs that are active at night were relatively underestimated. However, interviews were conducted in the form of questions about amphibians and reptiles, especially those that are easy to identify and are found in the area, and this aided in increasing the body of data gathered. Local individuals were asked to describe the species and later showed the herpetofauna species pictures available in guide books (Spawls et al., 2002; Channing and Howell, 2006).

Vegetation

Vegetation data were collected in 17 sites targeting forest remnants along the valleys and riverine systems, pine and eucalyptus woodlots, and agricultural areas. In each site, three plots of 10 m × 10 m with 300 m intervals were laid out perpendicular to both sides of the river. The data were enriched with opportunistic surveys along with road networks in farms as well as in areas outside the established study plots. Data were collected on all different plant life forms present including tree, shrubs, lianas, herbs, climbers, and epiphytes, as well as estimations of their cover, abundance, and density. Human impacts and levels of disturbances were also captured. Special attention was also given to the invasive and alien species. Vegetation identification was done using the standard floras and books including Flora of Tropical East Africa (FTEA) for various families and relevant books (Beentje, 1994; Dalitz et al., 2011; Agnew, 2013).

Fungi

Macrofungi assemblages were sampled in riparians zones and watersheds adjacent to established permanent sampling sites. In each of the permanent sampling site, three plots 20 m × 20 m were systematically sampled along a belt transect 300 m × 20 m such that the distances between plots were approximately 100 m, with the first plot located near the river. The collection of macrofungi samples involved the use of standard sampling methods (Mueller et al., 2004). Encountered macrofungi were photographed *in situ*, and data were recorded on their densities and diversities. Representative fruit bodies were carefully collected, stored, and transported to Mycology Laboratory in the NMK for curation and preservation. The specimen was identified up to genera and species levels according to morphospecies concept using standard monographs (e.g., Harkonen et al., 2003; Ndong, et al. 2011).

Soil Parameters

Soil samples were collected in the same sites as macrofungi and analyzed for soil organic carbon, soluble phosphorus, soil texture, and pathogenic fungi taxa of *Phytophora*, *Fusarium*, and *Phythium*. Three 30 cm deep soil cores were collected in each of the 300 m bell transects and then mixed thoroughly to obtain a composite sample. Soil samples were analyzed following standard methods for tropical soils as described by Anderson and Ingram (1993). Soil electric conductivity was determined in water using a 1:2.5 soil solution ratio. Available phosphorus was measured using the modified Olsen's method with pH at 8.5, and soil organic carbon was measured according to the routine colorimetric dichromate oxidation method. The soil pathogens *Phytophora*, *Fusarium*, and *Phythium* were isolated from the soil samples and then identified using morphological characteristics of colony motif, shape, and sporangium size scanned under a light microscope at ×400 magnification.

Birds

Three avian sampling methods, namely, point counts, mist netting, and timed species lists, were used. At least 10 point counts were conducted at intervals of 200 m along a 2 km transect (Bennun and Howell, 2002) in each sampling site. Mist netting was used in forested sites to capture the presence of skulking lower canopy and undergrowth species. The timed species counts were used to quickly build a comprehensive species list for each site as well as map species distributions following protocols of the Kenya Bird Map initiative for long-term monitoring of environmental changes at the landscape level (http://kenyabirdmap.adu.org.za/). We examined bird species richness along the condition gradient categorized as undisturbed (tiers 1 and 2), moderately disturbed (tiers 3 and 4), and severely degraded (tiers 5 and 6; Davies and Jackson, 2006). The data were further examined using a simple classification system for East African Forest birds developed by Bennun et al. (1996). The system goes further than a simple species list and detects subtle differences between forest avifauna in both space and time by classifying them based on their forest dependency. Thus, the classification system has three categories. 1) Forest specialists, which are the "true" forest birds' characteristic of undisturbed forest. 2) Forest generalists, which may occur in undisturbed forest but are also regularly found in forest strips, edges, and gaps. They are likely to be relatively more common there and in the secondary forest than in the interior of intact forest. 3) Forest visitors, which are often recorded in the forest but are not dependent upon it. They are almost always more common in nonforest habitats, where they are most likely to breed. We used proportions of birds in each category in the classification system to develop indices that indicate various forest conditions (see Furness and Greenwood, 1993; Bennun et al., 1996; Bryce et al., 2002).

Data Synthesis and Presentation

To examine patterns in the composition of taxa assemblages in relation to ecological conditions, multivariate approaches of principal component analysis (PCA), detrended correspondence analysis (DCA), and canonical correspondence analysis (CCA) were carried out (ter Braak and Šmilauer, 1999). Before PCA, DCA, and CCA analyses, the distribution model exhibited by all set of data was checked by running a DCA by default to determine whether the data had monotonic or unimodal forms of distribution. Data sets with monotonic distribution have a total length of gradients of less than four, whereas those with more than four are treated as being unimodal. All species data sets displayed unimodal types of distribution and were analyzed using either DCA or CCA, while soil and water quality had monotonic distribution and were analyzed using PCA. The indirect PCA method was used to detect changes in soil and water quality by grouping sites according to environmental conditions. The DCA is unconstrained indirect gradient analysis and grouped sites according to taxa similarities.

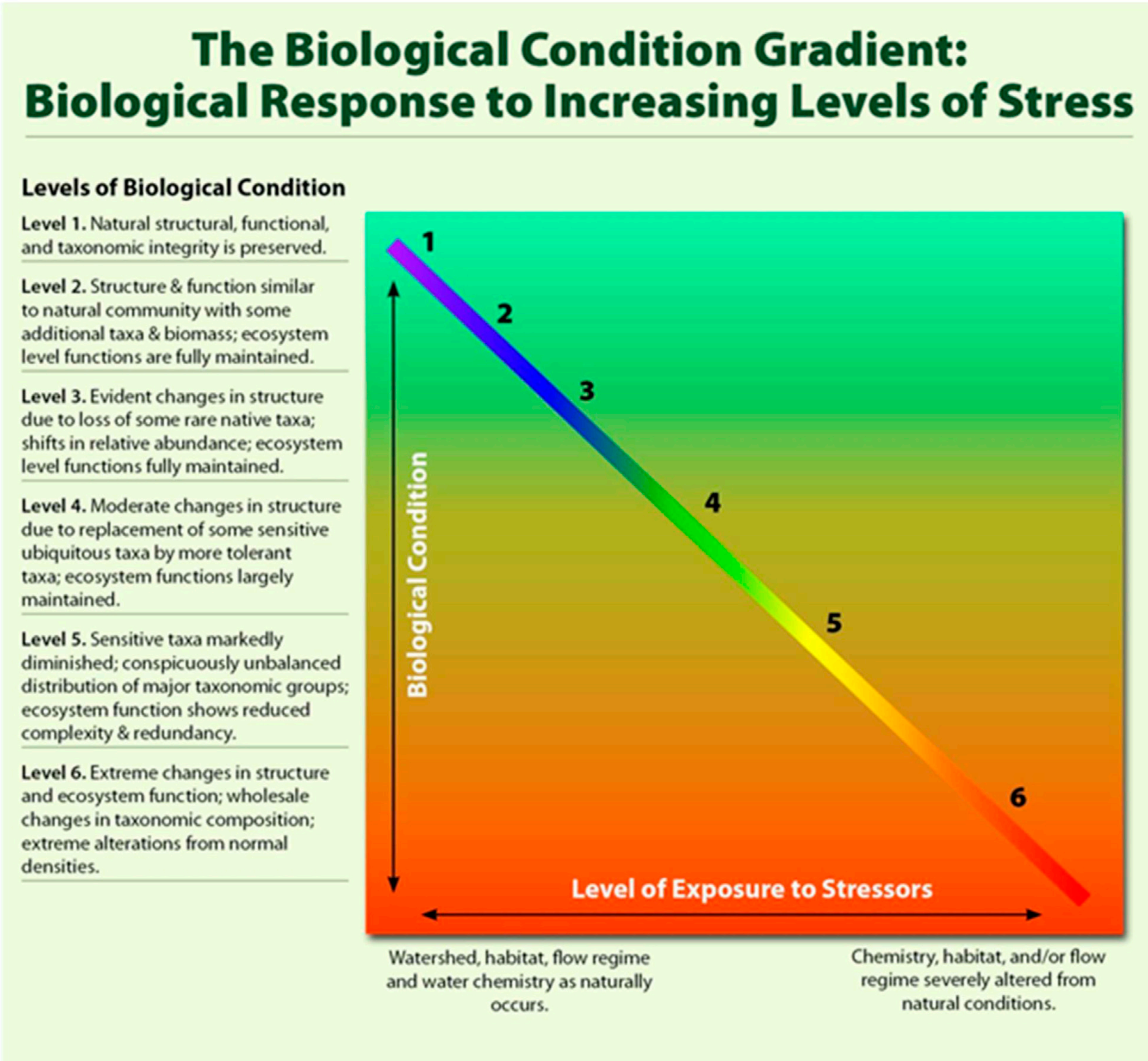

FIGURE 2 | Conceptual model of the BCG. Although in reality, the relationship between stressors and their cumulative effects on the biota is likely nonlinear, and the relationship is presented as such to illustrate the concept (adapted from USEPA, 2016).

The biplots are interpreted indirectly by attributing the grouping of species and sites to some environmental factors. The CCA is a constrained direct gradient analysis and elucidates the relationships between biological assemblages of species and their environment (ter Braak et al., 1995). In summary, CCA is multiple linear regression that corrects for spatial autocorrelation by incorporating conditional autoregressive models and randomization test options during analyses. During these analyses, randomized 499 Monte Carlo unrestricted permutation tests under full model were performed to determine which water quality or soil variables exerted significant influences on the distribution of various biological components at $p < 0.05$, using conditional automatic forwarding options (Lepš and Šmilauer, 2003). Also, the significance of the first ordination axis and all four axes together was tested. During DCA and CCA analyses, rare taxa were excluded (i.e., those that were recorded from only a single site). Meanwhile, some biological components were described using taxa abundance distribution measures of diversities and richness (Magurran 2004).

BCG Development

The development of BCGs for the UTR commenced with the compilation and analysis of available biological data for the seven taxonomic groups. Following the USEPA's method for BCG development (USEPA, 2016), knowledge and recommendations of experts were compiled during a

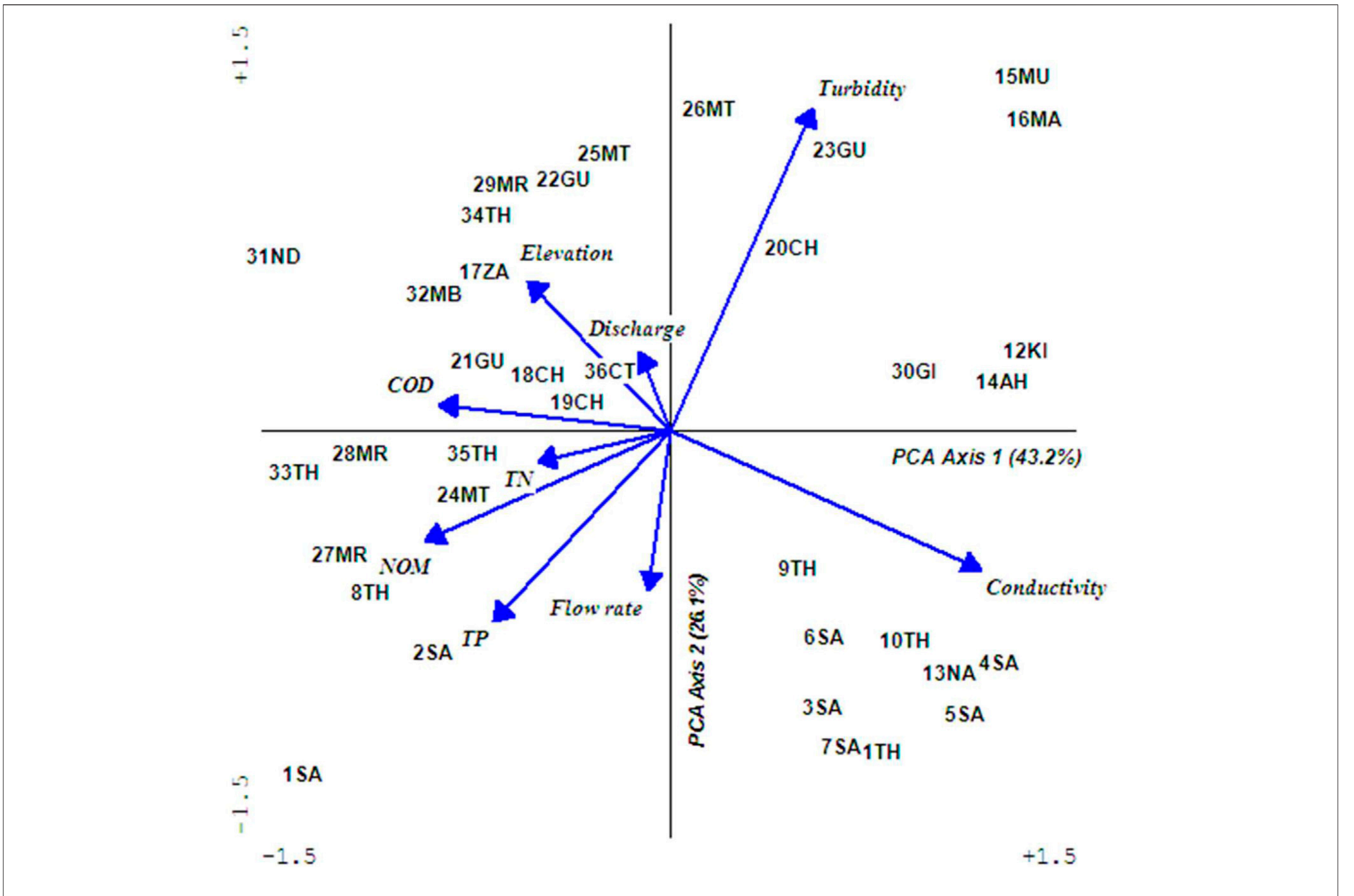

FIGURE 3 | A PCA biplot showing the grouping of sampling sites according to water conditions. Abbreviated labels represent TP: total phosphates (mg/L), TN: total nitrogen (mg/L), COD: chemical oxygen demand (mg/L), and NOM: natural organic matter (mg/L). Units used to measure conductivity were (mS/cm), flow rate (m/sec), and discharge (m^3/sec). The full names of abbreviated sampling sites is given in **Table 1** and original water quality is provided as **Supplementary Table S1**.

workshop held in August 2018 during which experts familiar with each of the seven taxonomic groups were tasked to assess and define each of the species' preferred ecological conditions (Ndiritu et al., 2018). In this workshop, the experts used five taxonomic characteristics or attributes of taxonomic composition and community structure to develop the BCG, which included historically documented, sensitive, long-lived, or regionally endemic taxa, sensitive (intolerant) taxa, intermediate tolerant taxa, tolerant taxa, and non-native or intentionally introduced species (Davies and Jackson, 2006 in USEPA, 2016). The final phase was a field trial collection of data and observations for each of the seven taxonomic groups at some or all of the 36 established permanent sampling sites, and then experts assigning various sampling sites to the six BCG tiers using the species data collected (Davies et al., 2016). The BCG is a descriptive model that interprets changes in ecological conditions in response to human disturbance in six tiers of 1) natural or native condition, 2) minimal changes in the structure of the biotic community and minimal changes in ecosystem function, 3) evident changes in the structure of the biotic community and minimal changes in ecosystem function, 4) moderate changes in the structure of the biotic community and minimal changes in ecosystem function, 5) major changes in the structure of the community, and 6) severe changes in the structure of the biotic community and major loss of ecosystem functions (**Figure 2**). And to the best of our knowledge, this is the first initiative where the BCG model has been used to assess ecological conditions of habitats outside the USA.

RESULTS

Bioassessment of Habitats in the UTR Watershed

Characterization of Sites Using Water Quality

Examination of ordination results revealed that water in the Upper Tana River was strongly influenced by conductivity, elevation, turbidity, and discharges (**Figure 3**). Rivers in the drier zones of Sagana catchments had low discharges but high conductivity, a situation attributed to high water abstraction, drier environments, and land-use changes. Those in Chania, Gura, Mathioya, and Maragu'a catchments were characterized

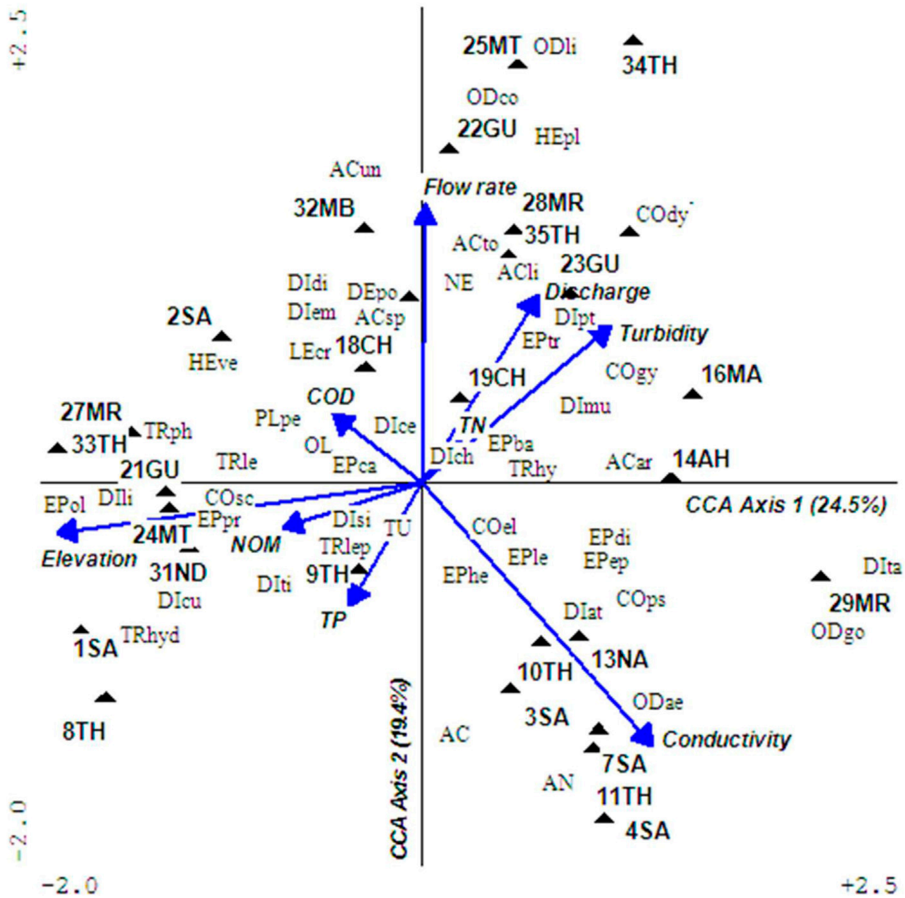

FIGURE 4 | A triplot of macroinvertebrates assemblages and water quality during the dry season. The first four CCA axes explained significant relationships between macroinvertebrates and water quality (p = 0.05). Arrow represents water quality parameters and filled triangle sampling sampled sites. The abbreviated four-letter labels signify macroinvertebrates' taxa with the first two-uppercase letters standing for order and the next two lower case letters for family. Full macroinvertebrates' taxa names are provided as **Supplementary Table S2**.

by high turbidity and water discharges in the lower sections, while the small streams in intensively farmed areas had elevated levels of TN, TP, and NOM. The headwater rivers originating from forests in Aberdare were found in higher elevations and with relatively clean water.

Effects of Water Quality on Macroinvertebrates

The CCA analyses found most significant factors influencing macroinvertebrates were elevation (F = 2.77, p = 0.01), conductivity (F = 1.94, p = 0.01), and discharge (F = 1.84, p = 0.04). The CCA grouped sampling sites along ecological condition gradients (**Figure 4**). All four CCA axes had a strong relationship between macroinvertebrates and water quality parameters (r = 0.87–0.94) with the first axis having remarkable interaction with elevation and conductivity, the second axis with conductivity and flow rate, and the third axes with water discharges in rivers. A total of 51 macroinvertebrates' taxa collected from 28 sites were used to determine ecological conditions in rivers and produced interesting findings. Macroinvertebrates associated with undisturbed sites (1SA, 2SA, 8TH, 21GU, 24MT, 27MR, 31IN, and 33TH) representing BCG tiers 1 and 2 were Scirtidae, Limoniidae, Oligoneuridae, Prosopistomatidae, Philopomatidae, and Hydroptilidae (**Table 1**). Macroinvertebrates somehow separated tiers 3 and 4, with tier 3 comprising sites 18CH, 22GU, 25MT, and 32MB that were characterized by high flow rates, moderate discharges, and turbidity. Indicator taxa were Sperchonidae, Unionicolidae, Potamonautidae, Dixidae, Empididae, Ceratopogonidae, Caenidae, Crambidae, Pleidae, Perlidae, Coenagrionidae, Libellulidae, and Oligochaetes, while tier 4 was represented by sites 14AH, 16MA, 19CH, 23GU, 34TH, and 35TH with high turbidity and discharges, and representative taxa were Arrenuridae, Dytiscidae, Gyrinidae, Muscidae, Ptychopteridae, Baetidae, and Trichorythidae. The lower sections of River Maragua site MR29 was classified as tier 5 with high turbidity and representative taxa were Tabanidae, Gomphidae, and Psephenidae. The mid and lower sections of Rivers Sagana, Thegu, and Nairobi had extremely low water discharge due to water abstraction that caused high conductivity levels in all the sites (3SA, 4SA, 7SA, 10TH, 11TH, and 13NA) and were placed in tier 6. Indicator taxa were Acarina, Ancylini, Psephenidae, Athericidae, and

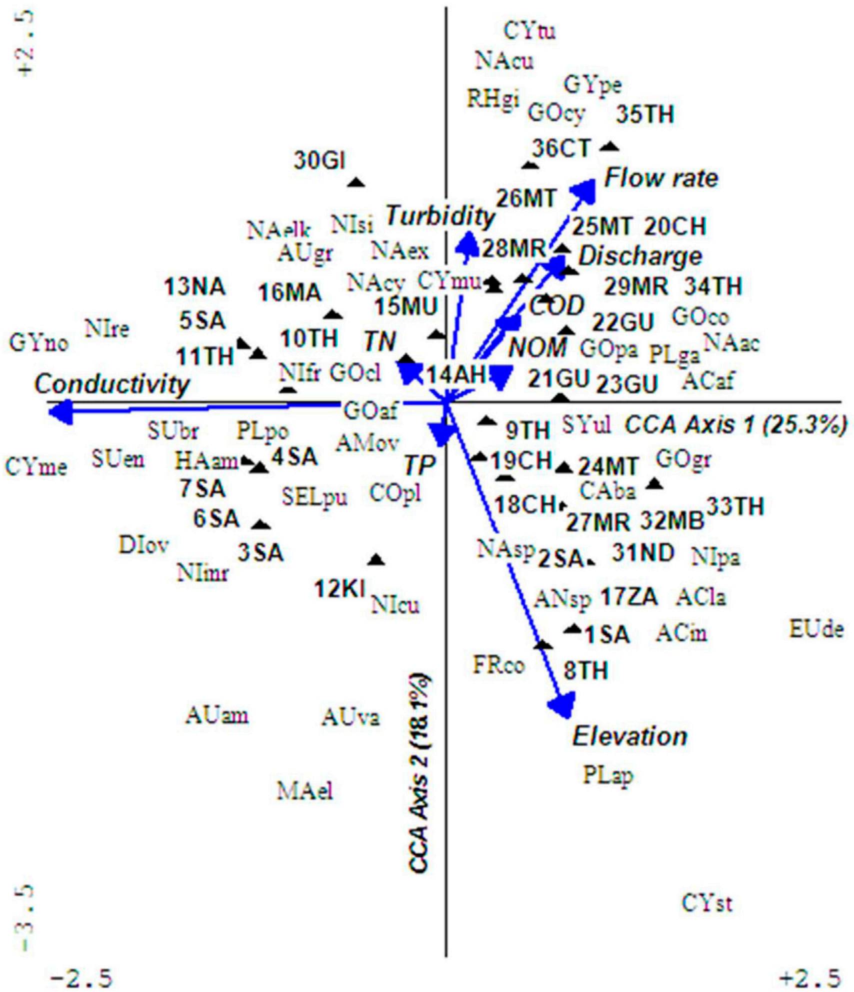

FIGURE 5 | A triplot of diatom assemblages found in cobble microhabitat and water quality during the dry season. The first axis and all axis tests returned significant relationships, that is, F = 2.37, *p* = 0.03 and F = 1.43, *p* = 0.01, respectively, implying variation of diatoms explained by water quality on the triplot was strongly significant. Arrow represents water quality parameters and filled triangle sampled sites. The abbreviated four-letter labels signify diatoms with the first two upper case letters standing for genus and the next two lower case letters for species. Full diatom species names are provided as **Supplementary Table S3**.

Aeshnidae. Meanwhile, other taxa displayed cosmopolitan distribution and had occasional to frequent abundances in the UTR such as Elmidae, Chironomidae, Simuliidae, Baetidae, Caenidae, Dicercomyzidae, Ephemerythidae, Heptageniidae, Leptophlebiidae, Hydropsychidae, and Leptoceridae.

Effects of Water Quality on Diatoms

The CCA found a significant relationship between diatom assemblages and water quality parameters of conductivity (F = 2.92 *p* = 0.01), elevation (F = 1.92, *p* = 0.01), and mildly with turbidity (F = 1.64, *p* = 0.07). Tests of significance of the first and all canonical axes were significant at F = 2.37, *p* = 0.03 and F = 1.43, *p* = 0.01, respectively. The interaction between diatoms and water quality axes in the triplots were strong ranging from r = 0.82–0.92. As predicted, diatom assemblages and water parameters grouped sampling sites along ecological condition gradients depending on land-use types, from undisturbed sites in headwaters to severely disturbed in the lower river sections (**Figure 5**). It was possible to identify flagship diatom species relating to certain key water quality parameters that changed and affected ecological conditions in rivers such as conductivity, elevation, and turbidity. Changes in conductivity and turbidity were attributed to human influences on watersheds, whereas elevation was due to natural influences primarily relating to temperatures. Preliminary findings show that diatoms associated with undisturbed sites representing BCG tiers 1 and 2 were *Diploneis ovalis*, *Cymbella muereli*, and *Placoneis*, moderately disturbed for BCG tiers 3 and 4 were *Aulacoseira granulata*, *Achnathes affinis*, *A. hungarica*, *Coconeis placentula*, *Diatoma elangatum*, *Gomphonema affinis*, *G. clevei*, *G. gracile*, *G. parvulum*, *Melanosira varians*, *Nitzschia sigma*, *Pleurosira laevis*, and *Synedra ulna*, while those in severely disturbed sites in BCG tiers 5 to 6 were *Achnanthes hungarica*, *Amphora ovalis*, *Navicula cyptocephala*, *N. elkab*,

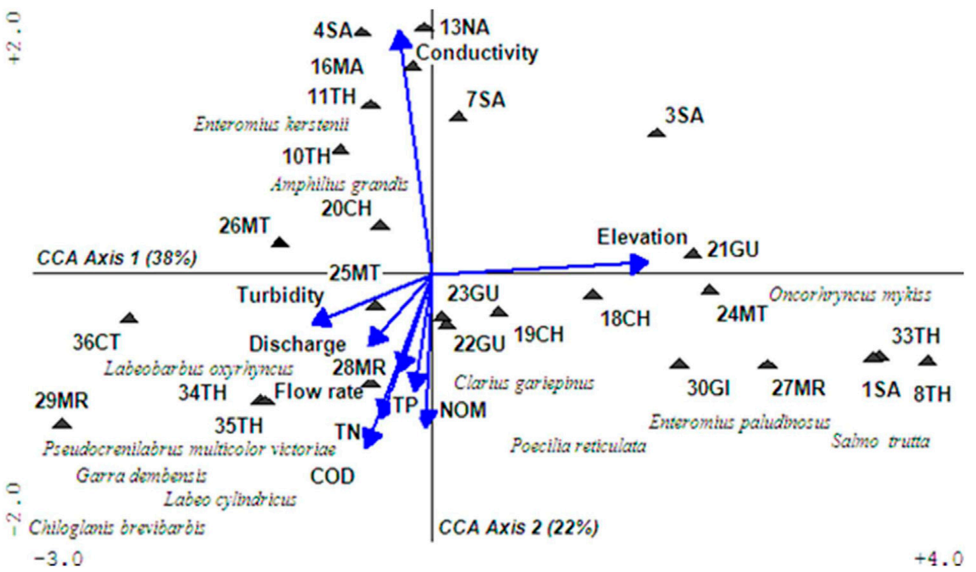

FIGURE 6 | CCA triplot of fish and water quality in Upper Tana River Watershed.

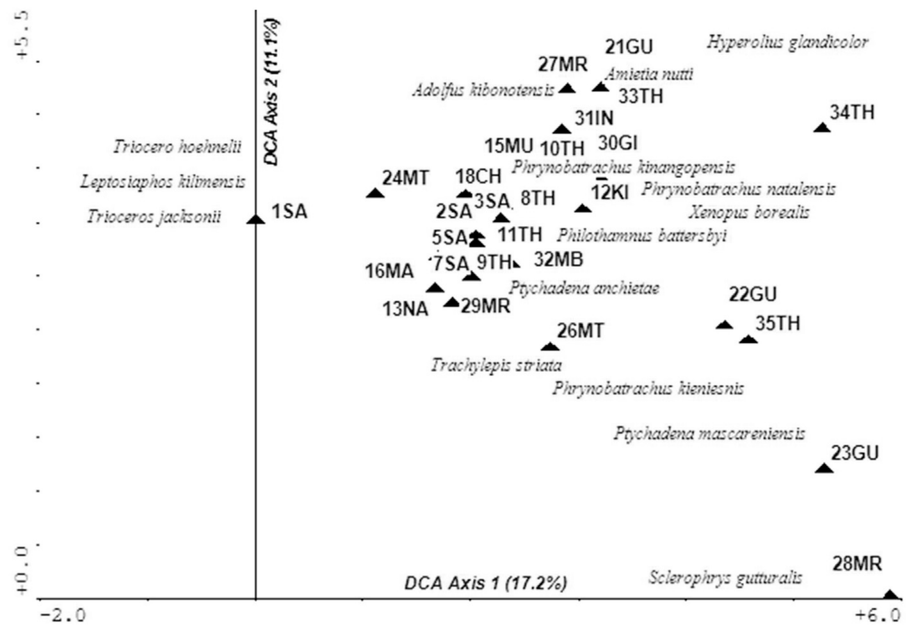

FIGURE 7 | DCA biplot of herpertofauna of the Upper Tana River watershed.

Nitzschia frustulum, and *N. palea*. A diatom summary of the description and categorization of sampled sites into the BCG tiers is provided in **Table 1**.

Fish

A total of 12 fish taxa were recorded from 30 sampling sites. The most common fish taxa were *Amphilius grandis* that was found in 12 sites, followed by *Enteromius kerstenii* found in 10 sites, *Garra dembensis* found in 7 sites, and *Oncorhyncus mykiss* found in 6 sites, whereas *Labeobarbus oxyrhyncus* was found in 5 sites and *Poecilia reticulata* was moderately abundant in 4 sites. The other six species were occasional and in low populations. The CCA grouped sites into several groups in response to ecological conditions and were significantly influenced by elevation ($F = 3.8$, $p = 0.00$) and conductivity ($F = 2.31$, $p = 0.03$), **Figure 6**. Undisturbed sites in high-elevation areas consisted of 1SA, 8TH,

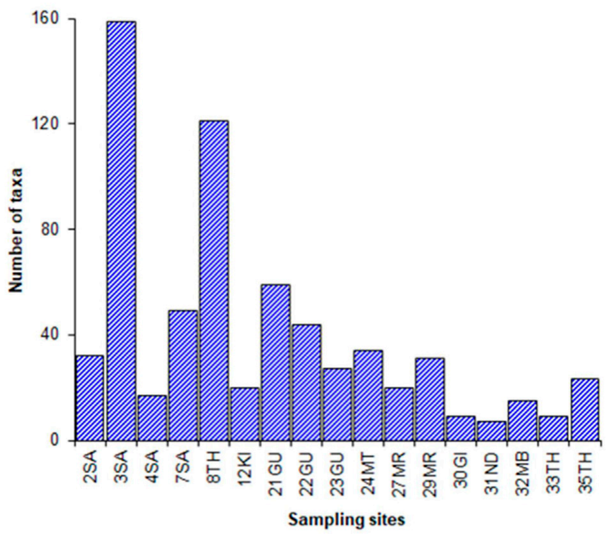

FIGURE 8 | The number of vegetation taxa recorded per site in the UTR watershed.

18CH, 21GU, 24MT, 27MR, and 33TH, and indicator species were *Enteromius paludinosus*, *Oncorhryncus mykiss*, and *Salmo trutta* (the latter two are introduced species). These sites were classified as tiers 1 and 2. Moderately disturbed sites were found in the mid-elevations where agriculture was the main type of land use and water ecological conditions were primarily influenced by turbidity and nutrients. Sites in this zone were 19GU, 20CH, 22GU, 23GU, 25MT, 26MT, 28MR, 29MR, 35TH, and 36CT, and fish supported were *Amphilius grandis*, *Clarius gariepinus*, *Chiloglanis brevibarbis*, *Garra dembensis*, *Labeo cylindricus*, *Pseudocrenilabrus multicolor victoriae*, *Labeobarbus oxyrhyncus*, and *Poecilia reticulata*, and sites were categorized as belonging to tiers 3 and 4. Sites 3SA, 4SA, 7SA, 10TH, 13NA, and 16MA were significantly influenced by conductivity, and the most abundant species found here (*Enteromius kerstenii* and *Amphilius grandis*) were infested with ectoparasites. The sites were categorized as severely degraded and were classified as belonging to tiers 5 and 6.

Herpetofauna

A total of 15 herpetofauna taxa were found in 28 sites. This was a low number when compared to over 30 known taxa from the area, a situation attributed to the dry season when this group of organisms is generally dormant. In addition, no night searches were done for amphibians when they are most active. Analysis of the DCA biplot indicates that the distribution of taxa was influenced by elevation, land use, and cover (**Figure 7**). Taxa associated with undisturbed forests sites were found in 1SA, 21GU, 27MR, 31IN, and 33TH which were *Adolfus kibonotensis*, *Amietia nutti*, *Trioceros hoehnelii*, *T. jacksonii*, and *Leptosiaphos kilimensis.* Moderately disturbed sites such as 22GU, 23GU, 26MT, 28MR, and 35TH supported occasional to rare species such as *Ptychadena mascareniensis*, *Phrynobatrachus kieniensis*, *Scherophrys gutturalis*, *Trachylepis striata*, *Xenopus borealis*, and *Phrynobatrachus natalensis.* *Ptychadena anchietae* and *Philothamnus battersbyi* were the most abundant and widespread species, dominating the most environmentally stressed sites in the UTR watershed of 3SA, 4SA, 7SA, 10TH, 11TH, and 13NA.

Vegetation

Approximately 600 species were recorded from 17 sampling areas in UTR (**Figure 8**). A relatively high number of species were found in forest areas experiencing mild to severe disturbances in Mts Kenya and Aberdare, which implied species richness was an unreliable measure of forest ecological health. Meanwhile, the ecological conditions were evaluated using taxa composition. Both mountains supported primary and mature secondary forests, were protected, and had minimal human disturbances. Plant taxa recorded were indicators of undisturbed forest conditions and were closed canopy species such as *Impatiens tinctoria*, *Impatiens fischeri*, *Arisaema mildbraedii*, *Polystachya cutriformis*, *Begonia meyeri-johannis*, *Asplenium rutifolium*, *Asplenium theciferum*, *Asplenium hypomelas*, *Cyathea manniana*, *Eulophia horsfallii*, and *Lobelia* sp. Most of the trees in closed forests were covered by bryophytes, ferns, and orchids, another good indicator of an undisturbed environment. These sites (1SA, 8TH, 21GU, 24MT, 27MR, and 33TH) are classified as tiers 1 and 2. The midsection sites (22GU, 27MR, and 32MB) occurred in tea and coffee zones, categorized as moderately disturbed belonging to tiers 3 and 4. In addition, dominant taxa found along the riverine were represented by individuals that thrive well in secondary forests such as *Neonotonia wightii*, *Bridelia micrantha*, *Polystachya cultriformis*, and *Croton alienus.* The lower regions of UTR were dominated by farmlands, pastures, and floodplains that supported weedy species of *Achyranthes aspera*, *Commelina benghalensis*, *Vernonia lasiopus*, *Biden pilosa*, *Ageratum conyzoides*, and *Tithonia diversifolia.* The trees and shrubs included *Rhus vulgaris*, *Maytenus arbutifolia*, *Croton macrostachyus*, and *Erythrina abyssinica*, whereas the swampy areas were dominated by *Typha domingensis*, *Cyperus dichrostachyus*, *Ludwigia stolonifera*, *Lemna gibba*, *Centella asiatica*, and *Rumex bequertii.* Alien and invasive species were recorded in the disturbed areas and these included *Ricinus communis*, *Verbena bonariensis*, *Lantana camara*, and *Datura stramonium.*

Macrofungi Community and Soil Parameters

Results of PCA using relevant soil parameters grouped sampling sites according to their conditions with SOC and soluble phosphate decreasing downstream (**Figure 9A**). Similarly, *Phytophora* and *Fusarium* abundances increased in degraded agricultural soils, while *Phythium* was associated with soils with minimal disturbances. A total of 88 genera of macrofungi were recorded from 21 samples in UTR. Further analysis of CCA using 55 genera (that were occasional to abundant in occurrences) found that elevation ($F = 1.53$, $p = 0.01$) and soluble phosphorus ($F = 1.4$, $p = 0.05$) significantly interacted with macrofungi with elevation mildly correlating with CCA axis 1 ($r = -0.5$) and soluble phosphorus strong with CCA axis 2 ($r = 0.7$). However, no clear patterns were observed and macrofungi

TABLE 1 | Characterizing sampling sites into tiers using water quality, soil parameters, and various taxonomic groups (–) = signify no data or inadequate data.

Sampling sites	Site name	Water quality	Soil parameters	Macroinvertebrates	Diatoms	Fish	Herpetofauna	Vegetation	Macrofungi	Birds
1SA	Sagana hatchery	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	–
2SA	Sagana KFS	1–2	–	1–2	1–2	1–2	–	1–2	–	–
3SA	Sagana mid	5–6	–	6	4–6	5–6	5–6	–	–	–
4SA	Sagana lower	5–6	–	6	5–6	5–6	5–6	4–6	–	–
5SA	Sagana/Thegu	5–6	–	–	4–6	–	–	–	–	–
6SA	Sagana/Thegu/ Nairobi 1	5–6	–	–	4–6	–	–	–	–	–
7SA	Sagana/Thegu/ Nairobi 2	5–6	3–4	6	4–6	5–6	5–6	4–6	4–5	–
8TH	Thegu upper	2–3	1–3	1–2	3–4	1–2	–	2–3	1–2	1–2
9TH	Thegu mid 1	4–5	–	–	4–5	–	–	–	–	–
10TH	Thegu mid 2	5–6	4–6	6	5–6	5–6	5–6	–	3–5	–
11TH	Thegu lower	5–6	4–5	6	5–6	5–6	5–6	–	3–5	–
12KI	Kimahuri	4–5	4–5	–	4–5	–	-	4–6	3–4	–
13NA	Nairobi	5–6	–	6	4–5	5–6	5–6	–	–	–
14AH	Amboni/Honi	4–5	–	4	4–5	4	–	–	–	–
15MU	Muringato	4–5	4–5	–	4–5	–	–	–	5	–
16MA	Muringato/Amboni	4–5	3–4	4	4–5	4–5	–	–	5	–
17ZA	Zaina	1–2	–	–	1–2	–	–	–	–	–
18CH	Chania upper	2–3	–	3	2–3	1–2	–	–	–	–
19CH	Chania mid	3–4	3–4	4	3–4	3–4	–	–	3–4	–
20CH	Chania lower	4–5	–	–	4–5	3–4	–	–	3–4	
21GU	Gura upper	1–2	1–2	1–2	1–2	1–2	1–2	1–2	2–4	1–2
22GU	Gura mid	2–3	3–5	3	3–4	3–4	3–4	3–4	2–4	3–4
23GU	Gura lower	3–4	3–5	4	4–5	3–4	3–4	3–4	2–4	3–4
24MT	Mathioya upper	1–2	2–4	1–2	1–2	1–2	–	1–2	2–3	–
25MT	Mathioya mid	3–4	4–5	3	3–4	3–4	3–4	–	3–4	–
26MT	Mathioya lower	4–5	–	–	4–5	3–4	–	–	–	–
27MR	Maragua upper	1–2	2–4	1–2	1–2	1–2	1–2	1–2	2–4	–
28MR	Maragua mid	3–4	–	–	3–4	3–4	3–4	–	–	–
29MR	Maragua lower	4–5	4–5	5	5–6	3–4	–	4–5	3–5	–
30GI	Githambara	4–5	3–4	–	4–5	-	–	3–4	2–4	3–4
31ND	Ndiara	2–3	2–3	1–2	2–3	1–2	1–2	3–4	–	3–4
32MB	Mbuguti	2–3	–	3	2–3	3		3–4	–	3–4
33TH	Thika upper	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2	1–2
34TH	Thika mid	4–5	–	4	3–4	4	–	–	–	–
35TH	Thika lower	4–5	4–5	4	5–6	3–4	3–4	5–6	4–5	–
36CT	Chania (Thika) lower	4–5	4–5	–	5–6	3–4	–	5–6	4–5	–

distribution seemed to be influenced by both micro- and macroenvironmental factors relating to habitats (**Figure 9B**). The undisturbed sites supported *Agaricus*, Cuphophyllus, *Hygrocybe*, *Hygrophorus*, and *Marasmius*, while moderately impacted areas had higher abundances of ectomycorrhiza species of *Laccaria* spp. It was evident that macrofungi abundances and diversity decreased downstream as a response to decreasing SOC. The majority of macrofungi community recorded in UTR were saprophytic (90%), colonizing dead wood, litter, and soil organic matter.

Birds

Birds were studied at 10 sites and richness was highest in tier 2 sites, gradually declining across the moderately disturbed sites (tiers 3 and 4) to the lowest in tiers 5 and 6 sites (**Figure 10**). Kenrick's starling, *Poeoptera kenricki*, a regional endemic (Kenya and Tanzania) bird was recorded in the undisturbed forest sites (tiers 1 and 2). However, two local endemics Hinde's babbler, *Turdoides hindei*, and Hunter's cisticola, *Cisticola hunteri*, were recorded at tiers 3, 4, 5, and 6 sites. Species occurrence indices were not significantly higher at tiers 1 and 2 sites than at the other sites (tier 2 index = 5.95; tiers 3 and 4 index = 5.87; tiers 5 and 6 index = 5.16, $p > 0.05$). Severely modified sites (tiers 5 and 6) had the lowest average occurrence indices. Yellow-whiskered greenbul, *Eurillas latirostris* (forest generalist F), gray apalis, *Apalis cinerea* (a true forest specialist FF), and tropical boubou (a forest visitor f) were the most commonly encountered species at tier 2 sites, while at tiers 5 and 6 site, yellow-whiskered greenbul (F), gray-backed Camaroptera, *Camroptera brachyura* (f), and common bulbul, *Pyconotus barbatus* (f) were the most common. Baglafecht weaver, *Ploceus baglafecht*, red-eyed dove, *Streptopelia semitorquata*, and northern fiscal, *Lanius humeralis*, all forest visitors (f) were the most common in the severely modified sites. Predictably, the diversity of forest specialist species was higher in the tier 2 sites and completely absent in sites in tiers 3 to 6 (**Figure 11**). Conversely, the diversity of forest visitor species was highest in tiers 5 and 6 sites.

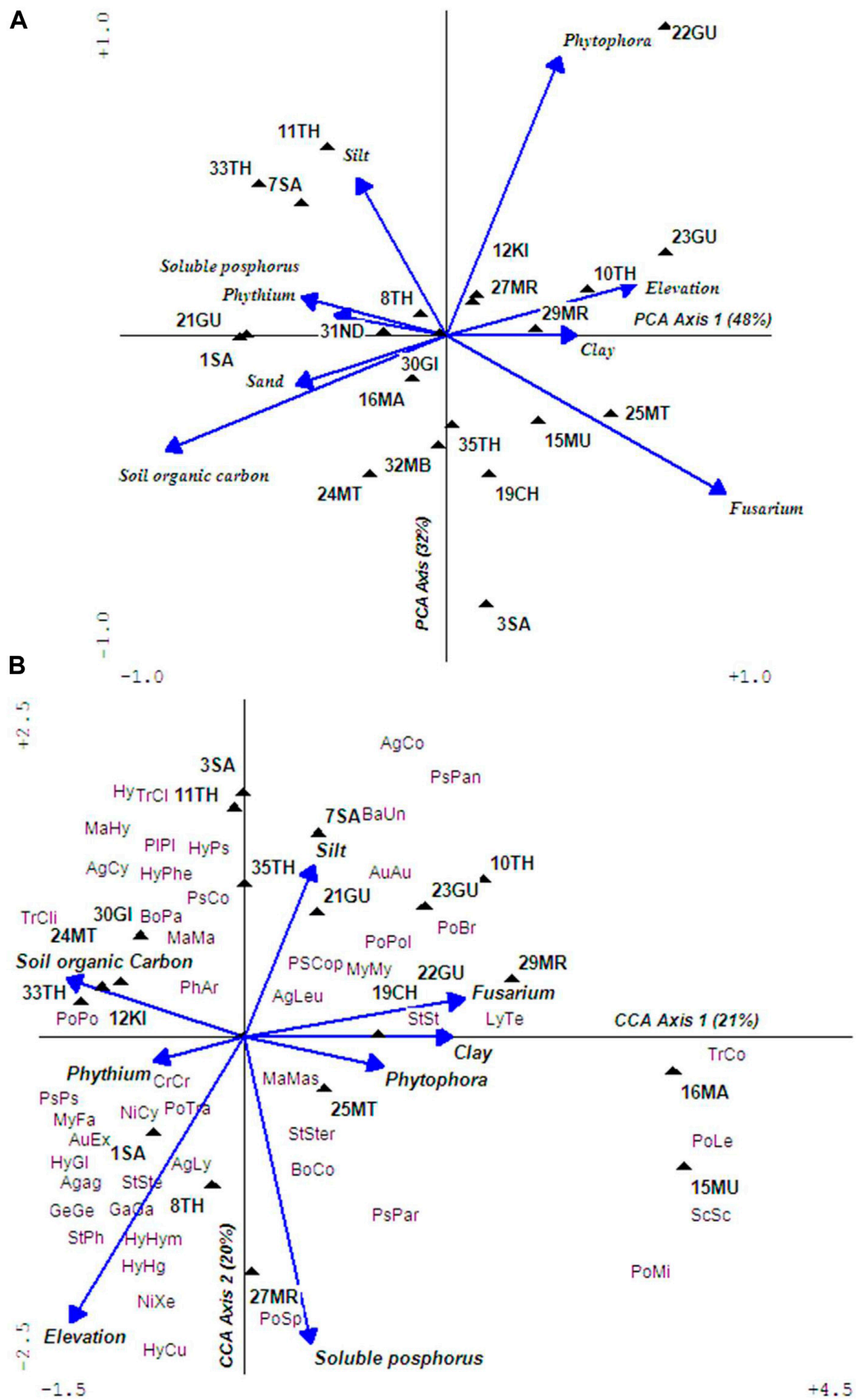

FIGURE 9 | Ordination plots of PCA **(A)** and CCA **(B)** showing ordination of sites according to environmental variables and species compositions in the UTR watershed. The abbreviated four-letter labels signify macrofungi with the first two uppercase letters standing for family and the next two lowercase letters genus. Full taxon names are provided as **Supplementary Table S4**. Soil parameters used to prepare PCA are provided as **Supplementary Table S5**.

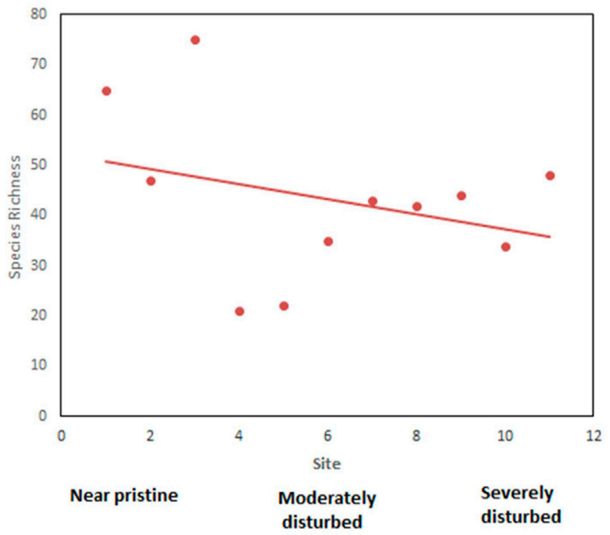

FIGURE 10 | The relationship between species richness and habitat disturbance species richness declined progressively from undisturbed sites (1–3), through moderately disturbed sites (4–8) to severely disturbed sites (9–12).

Characterization of Sampled Sites into BCG Tiers

All data collected during this study were individually used to assign all 36 permanent sampled sites to various BCG tiers (**Table 1**). As predicted sites in undisturbed areas represented ecological conditions in tiers 1 and 2, moderately disturbed (tiers 3 and 4), and severely human-impacted areas were classified as tiers 5 and 6. The taxonomic groups were found to complement each other with each taxonomic group assessing ecological conditions of their habitats to a certain degree correctly. The use of multivariate analysis and examinations of taxa abundance and distributions assisted in the identification and selection of biological components (taxa), water, and soil quality parameters that were indicators of varying levels of landscape conditions. As predicted, ecological conditions deteriorated downstream in response to agricultural intensification.

DISCUSSION

As predicted, analyses of water and soil parameter as well as biological data from both terrestrial and aquatic habitats assisted in placing sampled sites into BCG tiers (**Table 1**). Measurements of water and soil parameters validated changes in ecological conditions and more specifically the establishment of undisturbed reference sites. The decision to consider both terrestrial and aquatic taxa, followed by determination of their interactions with associated water or soil parameters assisted in identifying environmental stressors in UTR. More important was the identification of indicator species both in terrestrial and aquatic habitats that can be used to monitor and assess ecological conditions by parataxonomists or ordinary citizens, and the results can be appreciated at all levels of expertise.

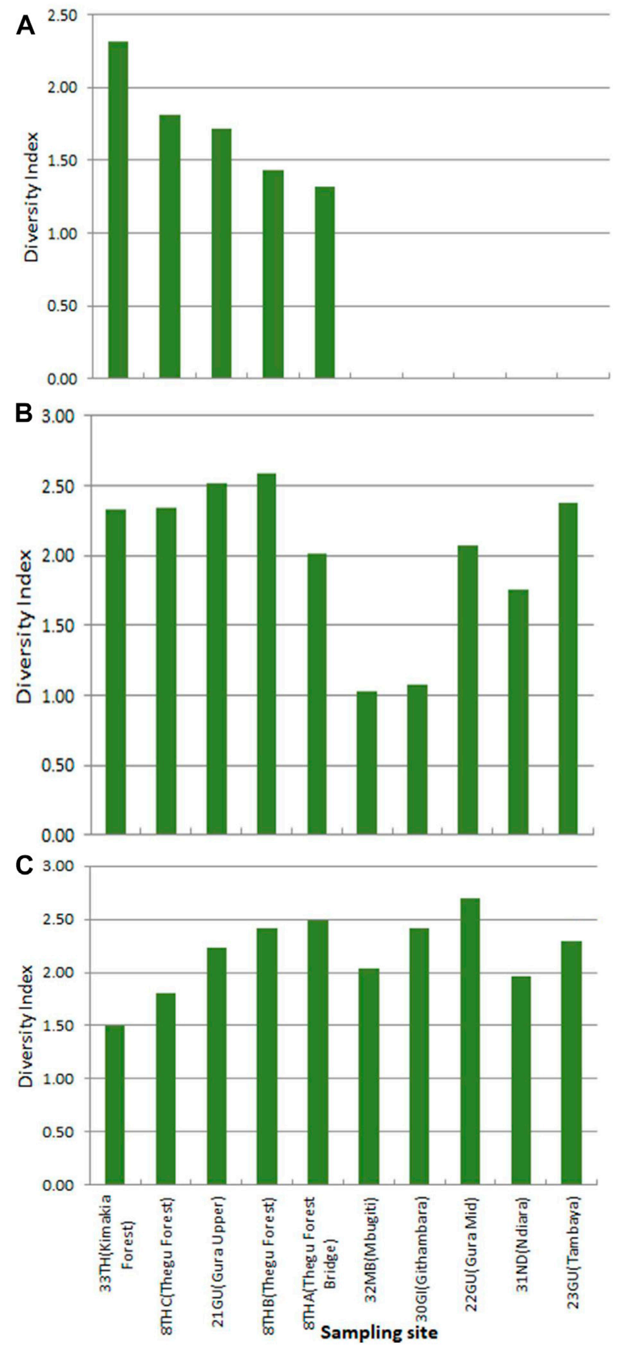

FIGURE 11 | Bird species diversity of **(A)** forest specialists, **(B)** forest generalists, and **(C)** forest visitors at each sampling site.

This study revealed that all the seven taxonomic groups responded significantly to ecological condition changes in the UTR watershed. Both natural and anthropogenic factors influenced taxa abundances and distributions. Elevation, a natural factor, was one of the factors that interacted significantly with all taxonomic groups. Many of the undisturbed sites are found at higher elevation particularly in the headwaters draining protected lands. The elevation is also known to be a surrogate measure of temperature (McGuire et al., 2012). Global increments of temperatures are a consequence of climate change and land use driven by human activities such as impacts of land-cover changes that are found to be both intensive and widespread in parts of the watershed (Vitousek, 1994). Not surprisingly, land-use change was also found to affect water quality parameters in rivers such as conductivity, turbidity, and discharge. Land-cover change in watersheds of Rivers Thegu, Sagana, and Nairobi is suspected to be the major contributor of low water flow as well as increasing temperature. Also, uncontrolled water abstraction for irrigation agriculture contributes to high conductivity levels that are found to adversely affect most aquatic taxonomic groups. The high turbidity levels in mid to lower sections of rivers are also attributed to land-use cover changes and improper farming practices.

The overall goal of this study was to understand watershed degradation in UTR in order to restore and sustain its capacity to support and provide ecosystem services of water and biodiversity conservation (MEMR 2012). Specifically, the initiative was aimed at laying down a strong foundation for the establishment of a bioassessment framework to support rehabilitation of the UTR watershed under the auspices of the Upper Tana Nairobi Water Fund (UTNWF) initiative. A primary motivation for this study was to develop bioassessment tools that could reliably track condition and change attributable to activities of the NWF in a way that could be easily understood, communicate to the UTNWF and their cooperating partners, government agencies, academic institutions and the affected communities in the watershed, each of which has varying knowledge and expertise in interpreting biological condition. One of the major impediments to the implementation of successful rehabilitation activities is the lack of an official and structured biomonitoring network to gather and evaluate ecological data. A recent global analysis of the ecological conditions of rivers observed that most of them have poor waters, a situation attributed to lack of monitoring programs due to economic constraints, technical limitations, and limited knowledge of abiotic and biotic components, as well as poor awareness by decision makers (Feio et al., 2021). Also, insufficient citizen understanding and involvement contribute to reduced success and sustainability of rehabilitation initiatives.

The approach by UTNWF and NMK to develop a pilot BCG framework for a comprehensive bioassessment of rivers in the UTR watershed using seven taxonomic groups yielded promising results. The BCG assessment utilities were modified to accommodate terrestrial taxonomic groups, including vegetation, macrofungi, herpetofauna, and birds, which allow better incorporation of the river to the basin to ecological network biomonitoring. This approach has been generally ignored in the overall rehabilitation of rivers. There must be a long-term and extensive monitoring approach that considers ecological health that includes the suite of ecological components in watersheds or catchments (Buss et al., 2015). Many of the terrestrial biological components used to describe ecological conditions that have been used elsewhere including the use of bird feeding guilds, abundance, and distribution to indicate various forest conditions (Furness and Greenwood 1993; Bennun et al., 1996; Bryce et al., 2002), how vegetation is important in supporting ecological processes and functions in landscapes (Handa et al., 2012; Riis et al., 2020), the strong interactions that exist between macrofungi and their environments (Mangan et al., 2010; Segnitz et al., 2020), and herpetofauna are very sensitive sentinels of environmental change due to their permeable thin skin and complex life cycle that makes them vulnerable to chemical and physical changes in both terrestrial and aquatic habitats (Bell and Donnelly, 2006). A program with several biomonitoring taxa is likely to bring on board many stakeholders and in doing so, assist in overcoming challenges that bewilder biomonitoring programs such as poor knowledge of biodiversity especially in developing countries, as well as increasing public awareness on the importance of river ecosystems and ecosystems services as such the need for motivating landscapes rehabilitation projects (Leigh et al., 2019). These taxa are widespread and relatively well-known locally and so easy to popularize among the residents for monitoring.

As expected, the aquatic taxonomic groups of macroinvertebrates, diatoms, and fish grouped sampled sites into various tiers consistently and similarly to those of terrestrial taxa. The response of macroinvertebrates to water quality was consistent with other studies carried in Kenya (Cumberlidge, 1981; Mathooko and Mavuti, 1992; Mathooko, 2002; Dobson et al., 2002; Mwaura et al., 2002; Smart et al., 2002; Ndaruga et al., 2004; Muli, 2005; Kibichi et al., 2007; Kundu e t al, 2017; Ochieng et al., 2019; Raburu et al., 2009; Masese et al., 2009; Masese et al., 2012; Nyakeya et al., 2009; Aura et al., 2010, 2017; Ojunga et al., 2010; Raburu and Masese, 2012; Minaya et al., 2013; Ngodhe et al., 2013; Kilonzo et al., 2014; Mbaka et al., 2014a; Mbaka et al., 2014b; M'Erimba et al., 2014b; Odhiambo and Mwangi 2014; Gichana et al., 2015, Orwa et al., 2015; Minoo et al., 2016; Kundu et al, 2017). The availability of this huge body of literature offers opportunities to strengthen the use of BCG, especially overcoming problems of determining undisturbed ecological conditions as well as the knowledge needed during pre- and post-status of ecosystems during rehabilitation programs. Only a few studies exist on fish bioassessment (Raburu and Masese, 2012; Achieng et al., 2021), and we recommend their continued promotion because they are sensitive to effects of poor agricultural practices and deforestation, which cause sedimentation and loss of habitat diversity and heterogeneity (Hocutt et al., 1994; Ganasan and Hughes, 1998; Toham and Teugels, 1999). Similarly, the use of diatoms in bioassessment of rivers is still at a nascent stage in Kenya (Ndiritu et al., 2006; Triest et al., 2012); however, findings of this study found them to be very reliable in assessing river conditions, especially the effects of climate on water quality.

The application of multivariate analyses of PCA, DCA, and CCA assisted in identifying water quality parameters adversely compromised as well as grouped sampling sites according to their taxa similarities. Moreover, the analyses ordinated taxa and sampling sites along an elevation-environment stress gradient that assisted in identifying and placing all 36 permanent sampling sites in any of the six BCG tiers (**Table 1**). Agboola et al. (2020) employed a multivariate approach in the selection and validation of reference condition in monitoring and management in South Africa. By using the BCG, it was possible to prioritize sites requiring rehabilitation in the UTR watershed, nature of restoration, and to achieve specific ecological conditions. For instance, rivers draining dry sub-catchments of Thegu, Nairobi, Sagana, Honi, and Amboni were experiencing low water flow discharges during dry seasons which caused elevated levels of conductivity, consequently causing stress on aquatic taxa. Those rivers draining the Aberdare watershed were primarily affected by improper land-use practices that affected discharges and turbidity.

Going forward, there is an urgent need for countries to establish national bioassessment frameworks to increase the body of knowledge to support river rehabilitation programs. Rivers are major sources of water, and their protection is crucial if countries are to meet United Nations Sustainable Development Goals' targets that depend on secured water future. Just like in other parts of the world, river monitoring initiatives in Kenya are limited by 1) coordination across institutions and between stakeholders, 2) scientific gaps, and 3) insufficient resources (Feio et al., 2021). The current attempts by UTNWF in the UTR to have bioassessment to support rehabilitation programs is laudable and should extend to other major watersheds (Crafter et al., 1992; MEMR 2012). The UTR watershed with an area of 8,340 km^2 is experiencing environmental degradation due to poor land and water management practices, and the consequences are reduced surface water qualities and quantities resulting in environmental crises that now threaten the social, political, and economic systems in the region (WRMA Water Resources Management Authority, 2014). Crucially, this watershed is a major source of water for domestic, agricultural, and industrial sectors for a human population of 4.87 million in UTR and another 95% of 4.4 million people living in Nairobi City (County). Also, waters from UTR generate 60% of hydropower energy used in Kenya, with sedimentation being a major concern on the longevity of dams.

CONCLUSION

The development of BCG models using multiple taxa groups as a bioassessment framework helped describe ecological conditions in rivers and landscapes (watersheds) in UTR. The initial BCG metrics were developed based on aquatic taxonomic groups of fish, macroinvertebrates, and diatoms to support the objectives of restoring and maintaining the chemical, physical, and biological integrity of the USA's water resources (Clean Water Act; CWA, 1972). However, it was discovered during this exercise that there was a strong case that the BCG framework can also be applied in terrestrial ecosystems using associated taxa such as birds, amphibians, macrofungi, and vegetation. The strong relationship between landscape and aquatic ecosystems demonstrated through this study informs this idea (Buss et al., 2015). Analyses of the relationship between all the seven taxonomic groups and environmental variables using analytical techniques of multivariate, species abundance, and distribution measures of richness, diversities and indicator species assisted in the grouping of 36 sites according to six BCG tiers. Indeed, the seven taxonomic groups complemented each other, with each taxonomic group reliably assessing the ecological conditions of each site. In building different BCG models, it was possible to identify taxa indicating various ecological conditions that in the long term can be proposed as indicator flagship taxa in the UTR. In most cases, flagship taxa are known by local communities and citizens and this makes it easier for them to contribute to data collection and bioassessment programs, which is one way to promote public or citizens' environmental awareness, education, and participation (Graham et al., 2004; Leigh et al., 2019). The aim is to strengthen this long-term biomonitoring program as well as make it compatible with other taxa assessment programs such as national mapping of birds under the Kenya Bird Map (http://www.birdmap.africa/coverage/country/Kenya/; Wachira et al., 2015). In summary, the capacity of the BCG models to assess landscape conditions meant that they can be used to identify important habitats for conservation, direct investment for restoration, and track progress using measurable biological and environmental parameters targets under a long-term biomonitoring program.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The research and findings presented in this manuscript were obtained under auspices of National Museums of Kenya (NMK) which is governed by its collection and research policy framework that abides by national and international research standards and mandated by an Act of Parliament, The National Museums and Heritage Act of 2006 (https://www.museums.or.ke/biodiversity-database/). Thus, NMK is a Kenyan government institution and its core mandate is to undertake biodiversity research and manage all biological collections in Kenya.

AUTHOR CONTRIBUTIONS

GN conceived the manuscript with TT, PN, and DC and then supervised the study and contributed valuable comments. GN, TT, PN, VM, EN, GK, LN, PK, PM, MM, JG, and DO collected data. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2021.671051/full#supplementary-material

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Benthic Macroinvertebrates as Ecological Indicators: Their Sensitivity to the Water Quality and Human Disturbances in a Tropical River

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Macroinvertebrate metrics are helpful tools for the assessment of water quality and overall aquatic ecosystem health. However, their degree of sensitivity and the most reliable metrics for the bioassessment program development are very poorly studied in Togo. This study aimed to test the sensitivity of metrics calculated at the family and genus levels. A total of 21 water quality parameters and macroinvertebrates' data were collected during three periods at 20 sampling sites within the Zio River. The canonical correspondence analysis (CCA), factor analysis (FA), and Spearman's correlation analysis were conducted on water quality parameters and macroinvertebrates' data. The results reveal that macroinvertebrate structure and composition were affected by water quality parameters related to human disturbances. In this study, three groups of macroinvertebrate communities were identified including sensitive taxa such as Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO) taxa; the resistant or resilient taxa such as Oligochaeta, Hirudinea, Diptera, and Pulmonates (OHDP) taxa; and tolerant taxa such as Prosobranchia, Bivalvia, Lepidoptera, Heteroptera, and Coleoptera (PBLHC). All the 13 macroinvertebrate-based metrics were found to be sensitive in the detection of water quality and human disturbance gradients. However, metrics related to EPTO and the tolerance measure [multimetric index of the Zio River basin (MMIZB), Average Score per Taxon (ASPT), and Biological Monitoring Working Party (BMWP)] are the most robust in discrimination of pressure gradients. This study reveals that macroinvertebrates are sensitive and can be used for the bioassessment program development at the order, family, or genera taxonomic level.

Keywords: sensitivity, metrics, ecological indicators, water quality, macroinvertebrates, taxonomic level

INTRODUCTION

Rivers are important ecosystems with high ecological value (Nguyen et al., 2018), and the health of these ecosystems is important for the human societies that depend on them (Dickens et al., 2018). Rivers are an important source of renewable water supply for human beings and freshwater ecosystems (Vorosmarty et al., 2010) and provide many goods and services such as domestic uses, navigation, recreational activities, and nursing grounds and food for many organisms (Berger et al., 2016). Until the late 1960s, the overriding interest in water has been on the available amount for consumption. Except when undesirable water quality conditions persist, the available water was considered acceptable for consumption. Only during the last three decades of the 20th century has water quality been deteriorated to the point where it is considered as important as managing for the availability of water (Abbasi and Abbasi, 2012).

Globally, human impact is changing the availability of freshwater (Rodell et al., 2018). Among freshwater ecosystems, streams and rivers are the most influenced or threatened by a range of anthropogenic stresses (Allan, 2004; Best and Darby, 2020). In the same line, Dudgeon et al. (2006) grouped the major threats to freshwater species under five interacting categories: overexploitation; water pollution; flow modification; destruction or degradation of habitat; and invasion by exotic species. The degree of these stressors led to a geographical variation in the threat to freshwater species with almost one in three freshwater species threatened with extinction worldwide (Collen et al., 2013), while estimates suggest that at least 10,000–20,000 freshwater species are extinct or at risk of extinction worldwide (Balian et al., 2008; Vorosmarty et al., 2010). For example, 1,116 freshwater migratory fish species are threatened and with 102 additional extinctions (Hogan, 2011). In tropical regions, particularly in Sub-Saharan countries, rivers are under pressure due to human activities that deteriorate water quality, limiting water availability for drinking and other uses (Traoré et al., 2016; Kaboré et al., 2018; Tampo, 2018; Chetty and Pillay, 2019; Agboola et al., 2020a). Furthermore, the river pollution problem, related to human disturbances through anthropogenic activities and urbanization (Azrina et al., 2006; Faridah et al., 2012; Edegbene et al., 2021), has many adverse impacts on river ecosystems that have required managers to increase water assessment efforts (Clifford and Tariro, 2005). Many African countries have concerns about the ecological status of their rivers and are increasing their investments on the restoration of degraded rivers (Smith et al., 2009; Fayiga et al., 2018; Wantzen et al., 2019).

In many studies, biological methods are valuable for determining natural and anthropogenic influences on water resources and habitats because biota responds to stressors from multiple spatial or temporal scales (Weigel and Robertson, 2007; Resende et al., 2010; He et al., 2020; Kurthen et al., 2020). In addition, the use of aquatic organisms in ecological studies has proven more effective than using environmental variables alone, because the aquatic community integrates structural and functional characteristics and reflects the health of the studied streams (Bonada et al., 2006; He et al., 2020). Accordingly, there is an increasing interest in the application of ecological thresholds for natural resource management (King and Baker, 2010; Forio et al., 2018). Several metrics and biotic indices are developed and used across the world (Barbour et al., 1996; Hering et al., 2006; Agboola et al., 2019; He et al., 2020; Ko et al., 2020. Gonçalves and Menezes (2011) argued that the use of biotic indices as a tool for river quality assessment was more useful in evaluating river health than the conventional national water quality assessment standard practices in many countries. Metcalfe (1989) and Alba-Tercedor (1996) on the other hand pointed at the disadvantage of the physicochemical assessment, which measures the water quality only at the time of sampling.

Among the river components, aquatic macroinvertebrates are the most sensitive to anthropogenic pressures (Nessimian et al., 2008; Mereta et al., 2012; Agboola et al., 2020a,b; Ko et al., 2020). Macroinvertebrate responses to the change in aquatic ecosystem condition is universally recognized, and their responses are used in indices to monitor freshwater ecosystem for integrity, aiding in decision making in management (Richter et al., 2003; Kaboré et al., 2016; Agboola et al., 2019; Tampo et al., 2020; Edegbene et al., 2021). Macroinvertebrates are commonly used in assemblages for biomonitoring because they integrate various desirable characteristics, such as ubiquity, different levels of tolerance to perturbations, and sampling cost-effectiveness (Mary, 1999; Dickens and Graham, 2002; Hering et al., 2006; Resh, 2008; Kenney et al., 2009; Li et al., 2012; Kaboré, 2016; Agboola et al., 2019; Ko et al., 2020).

The freshwater macroinvertebrate taxa vary in response to organic pollution; and thus, their diversity and composition have been used to make inferences about pollution loads (Kenney et al., 2009; Wan Abdul Ghani et al., 2018; Tampo et al., 2020). Generally, in natural pristine rivers, high abundances and richness of species could be found (Barbour et al., 1996, 1999). However, high impact due to human activities caused many changes of the assemblages and biodiversity of the river fauna (Wright et al., 1993; Pinel-Alloul et al., 1996). Despite the development and application of a variety of biotic indices, scores, and metrics based on macroinvertebrates for water quality and ecosystem health assessment in America and Europe, the literature provides little information on biological assessment and monitoring tools of freshwater ecosystems in Sub-Saharan Africa especially in West Africa (Adams, 1993; Dallas, 1997; Kaboré et al., 2016). In Togo, there is paucity of information on bioassessment of riverine ecosystems health, except for some works on hydrochemistry, macroinvertebrates as indicators, and multimetric index of the Zio River basin (MMIZB) carried out by Tampo et al. (2015, 2020) and Tampo (2018). The measure of macroinvertebrate sensitivity at different taxonomic scales is however very important for bioassessment (Bailey et al., 2001; Schmidt-Kloiber and Nijboer, 2004; Marshall et al., 2006; Metzeling et al., 2006; Jones, 2008; Costas et al., 2018) and essential for developing a biomonitoring program in Togo. Therefore, the aim of this study was to test the effectiveness and the robustness of a set of macroinvertebrate-based metrics in the Zio River.

MATERIALS AND METHODS

Study Area and Sampling Sites

This study was undertaken in the main stem of the Zio River. It is located in a tropical climate between the latitude $6°5'$ and $7°18'$N (**Figure 1**) and includes three of the five ecoregions of Togo. The agriculture and other human activities described previously (Tampo et al., 2015, 2020; Tampo, 2018) are more intensive in the lower reaches of the Zio River. The river crosses some villages and

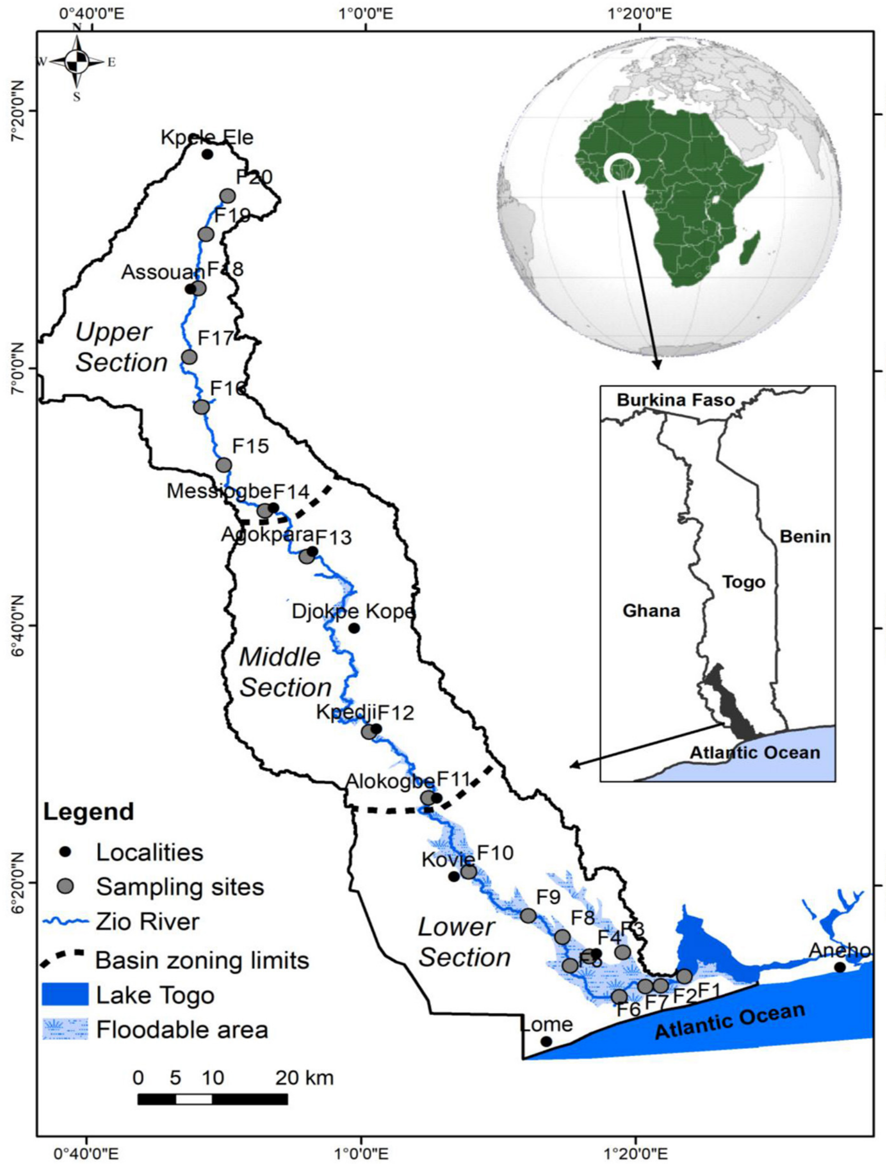

FIGURE 1 | The study area and sampling site localities for the present study.

the outskirts of the city of Lomé; and its water is the major source for domestic and agricultural purposes in rural and semi-urban areas. Moreover, the main activity in the basin is agriculture (maize, cassava, bean, yams, rice, etc.), fishing, and few industrial activities such as gravel washing and sand extraction (Bawa et al., 2018). According to Tampo (2018), the river can be subdivided into three sections, such as the upper section, the middle section, and the lower section, which is influenced by seawater during the low flow. Twenty sampling sites were selected across the study area, which included a range of impaired and unimpaired ecological states within the Zio River following anthropogenic disturbances (Tampo et al., 2020).

Sampling and Water Parameter Analyses

Twenty sites were sampled three times between 2013 and 2014 during different seasons (the rainy season, the dry season, and a transition period between the rainy season and dry season). Therefore, 60 samples were collected and 21 water quality parameters measured. At each site, the water quality parameters such as temperature, electrical conductivity (EC), dissolved oxygen (DO), and pH were measured *in situ* using Wissenschaftliche Technische Werkstätten (WTW) multipurpose water quality probe. After the *in situ* measurement, 1.5 L of water was taken in a plastic bottle and stored in a cool environment for analysis of chemical parameters in the

TABLE 1 | Selected metrics and their definition.

Categories	Metrics	Definition
Taxonomic richness	TNF	Total number of family taxa in benthic macroinvertebrate
	TNG	Total number of genus taxa in benthic macroinvertebrates
	EPTF	Number of family taxa in order of Ephemeroptera, Plecoptera, and Trichoptera
	EPTG	Number of genus taxa in order of Ephemeroptera, Plecoptera, and Trichoptera
	ETOF	Number of family taxa in the order of Ephemeroptera, Trichoptera, and Odonata
	ETOG	Number of genus taxa in the order of Ephemeroptera, Trichoptera, and Odonata
Tolerance measure	MMIZB	Multimetric Index of the Zio river Basin (Togo)
	BMWP	Biological Monitoring Working Party System (England)
	ASPT	Average Score per Taxon
Diversity indices	Sha_H_F	Shannon's diversity index at family resolution
	Sha_H_G	Shannon's diversity index at genus resolution
	Marg_F	Margalef diversity index at family resolution
	Marg_G	Margalef diversity index at genus resolution

laboratory within 48 h after collection. For microbiological analysis, samples were collected in borosilicate glassware of 500 ml. The chemical oxygen demand (COD) was determined by the potassium permanganate method, and biological oxygen demand (BOD) was determined by the 5 days' test according to respirometry method. Major and minor ions were determined by titration method (Ca^{2+}, Mg^{2+}, HCO_3^-, and Cl^-) and by UV-spectrophotometric method (SO_4^2, NO_3^-, PO_4^{3-}, total iron, Mn^{2+}, and NH_4^+), while K^+ and Na^+ were determined by flame emission spectrophotometer. Total suspended solid (TSS) was determined by gravimetric method (dried at 105°C). All these parameters were measured in the Laboratory of Applied Hydrology and Environment (LAHE) of the Université de Lomé (Togo) with an accuracy ranking from 1 to 2% according to the standard methods as prescribed by AFNOR (1997) and Rodier et al. (2009).

Macroinvertebrate Collection and Identification

Benthic macroinvertebrates were sampled using a dip net (circular opening, 33 cm of diameter; mesh size, 320 μm) in lentic habitats and a Surber Sampler (rectangular opening, 20 cm × 25 cm; mesh size, 320 μm) for lotic habitats. At each site, substrate samples were taken and combined to one composite sample following protocol described by AFNOR (1997) and Rodier et al. (2009). All animals were identified at the family level and at the genus level for mollusk gastropod, Annelida, and insects using taxonomic manuals and keys (Durand and Levêque, 1981; Merritt and Cummins, 1996; Tachet et al., 2010).

Metric Selection

In this study, 13 metrics classified in three categories were used to assess the ecological status of the Zio River and to test their sensitivities (**Table 1**). These metrics were calculated using macroinvertebrate features (number of taxa, diversity indices, abundance, and tolerance score). The selection of these metrics was based on their simplicity and reliability for assessing the water quality of the river as well as their suitability to detect anthropogenic disturbances (Raburu et al., 2009; Jun et al., 2012; Nguyen et al., 2014; Kaboré et al., 2016; Agboola et al., 2019; Tampo et al., 2020). **Table 1** indicates the metrics used and their definition.

Statistical Analysis

The canonical correspondence analysis (CCA) was applied in order to establish the relationship between water quality parameters and macroinvertebrate abundance and to identify water quality parameters affecting macroinvertebrate community. The analysis was performed under PAST software (version 3.0) based on dataset of water quality parameters and a dataset of abundance of macroinvertebrate community at phylum–class, subclass–order, and family and genus levels. In addition, we assessed the potential of macroinvertebrate taxa and metrics detected in this study to serve as bioindicators for the river's environmental condition investigated. Therefore, factor analysis (FA) and Spearman's correlation analysis between macroinvertebrate data and water quality variables were used, and with expert's consensus following Kaboré et al. (2016) and Tampo et al. (2015). The FA was performed using principal components as factor extraction method without any rotation. The factor loadings were considered for the explanation of correlations among variables and the detection of reliable metrics as indicators of water quality. Spearman's correlation and FA were computed using STATISTICA (version 7.0) for Windows.

RESULTS

Status of Water Quality in the Zio River

The descriptive analysis gives an overview on the variation of water quality parameters in the Zio River during the three sampling periods (**Table 2**). EC expresses the degree of water mineralization and salinity. It varies in this study from 10,506 to 38.10 μS/cm, with high standard deviations (±1,755.07

TABLE 2 | Descriptive statistics of water quality parameters.

Categories	Variables	Mean	Medi	Min	Max	25 P	75 P	SD
General parameters	pH	7.07	7.15	6.34	7.8	6.725	7.375	0.403
	EC (μS/cm)	540.12	126.5	38.1	10,506	72.65	368.5	1755.07
Global pollution parameters	DO (mg/L)	7.19	7.5	0.6	12.8	5	9.75	3.36
	TSS (mg/L)	108.6	70	12	320	47	158	86.08
	COD (mg/L)	8.68	4.9	4	30.7	4	11.9	6.43
	BOD (mg/L)	4.88	2	2	20	2	7.5	4.21
	COD/BOD	1.92	2.00	1.16	4.30	1.70	2.00	0.44
Bacteriological parameters	TC (Cfu/100 ml)	84.41	40	8	710	20	101	118.48
	FC (Cfu/100 ml)	22.38	8	0	177	2	22.5	38.80
Major ions	HCO_3 (mg/L)	106.63	88.45	24.4	325.74	58.8	138.55	69.35
	Ca (mg/L)	13.37	9.6	3.2	56	8	17.6	10.02
	Mg (mg/L)	7.04	5.44	1.44	28.8	3.84	9.6	5.11
	Na (mg/L)	84.02	15	2.6	1,860	6.15	39.5	313.72
	Cl (mg/L)	104.29	7.95	1	2,803.08	3.5	26.365	447.59
	SO_4 (mg/L)	3.87	3.2	0.05	11.6	1	6.5	3.36
	K (mg/L)	5.99	3	0.8	71	1.95	4.55	11.98
Minor ions	NO_3 (mg/L)	1.46	1.09	0.12	7.8	0.76	1.6	1.30
	NH_4 (mg/L)	0.34	0.06	BDL	5.6	0.03	0.4	0.79
	PO_4 (mg/L)	0.05	0.03	BDL	0.36	0.02	0.05	0.06
	Mn (mg/L)	0.23	0.04	BDL	2.57	0.03	0.05	0.59
	Fe (mg/L)	1.54	1.10	BDL	6.53	0.79	1.9	1.43

Min, minimum value; Max, maximum value; 25 P, 25th percentile value; 75 P, 75th percentile value; SD, standard deviation value; BDL, below detection limit; Medi, median; EC, electrical conductivity; DO, dissolved oxygen; TSS, total suspended solid; COD, chemical oxygen demand; BOD, biological oxygen demand; TC, total coliforms; FC, fecal coliforms.

μS/cm) indicating the large variability of EC from upstream to downstream of the Zio River. The pH ranged from 6.4 to 7.8, with the mean value varying from neutral toward a state of alkaline. The variation trends of most of major ions such as Ca^{2+}, Mg^{2+}, Na^+, K^+, HCO_3^-, Cl^-, and SO_4^{2-} are similar to EC and in the range of natural water quality. The concentration of minor ions such as NH_4^+, NO_2^-, Fe, PO_4^{3-}, and Mn^{2+} was below the detection limits in some sites and very low in other sites. The COD and the BOD are indicators of organic pollution in the water. COD values ranged from 4 to 30.7 mg/L with a mean of 8.68 mg/L, while BOD ranged from 2 to 20 mg/L with a mean value of 4.88 mg/L. The ratio COD/BOD is also an indicator of river pollution in terms of domestic or industrial effluents and biodegradability. This ratio ranges from 1.16 to 4.30 with a mean value of 1.92. The DO is one of the common parameters used to assess water quality and aquatic ecosystems' health. In the present study, the DO value varied from 0.6 to 12.8 mg/L. Many values recorded are high or close to 7 mg/L, showing that water in the Zio River can be qualified as good-to-excellent DO quality according to surface water standards except for a few polluted sites at the lower section of the river. The TSSs are defined as solids in the water including organic and inorganic materials that can be trapped by a filter. TSS ranged from 12 to 320 mg/L with a mean value and 75th percentile value of 108.6 and 158 mg/L, respectively.

Among microbiological parameters, total coliforms (TC) and fecal coliforms (FC) are often used as indicators of bacterial contamination in the water. Their presence is an indication of fecal contamination in water. In the present study, TC and FC range from 8 to 710 Cfu/100 ml and from 0 to 177 Cfu/100 ml, respectively. These values can express a risk of a bacterial contamination of water in the Zio River.

Sensitivity of Macroinvertebrate Community to the Water Quality

Sensitivity at Phylum–Class Level

The CCA indicates the environmental parameters that affect macroinvertebrate community at phylum–class level (**Figure 2A**). **Table 3** shows the key indicator taxa and the total taxa identified at different taxonomic levels. The **Supplementary Data** in **Appendix A** indicate Spearman's correlation between water quality parameters and phylum–class abundance. From **Figure 2A**, phylum Annelida is affected by BOD, COD, and NH_4^+. **Appendix A** indicates that Annelida abundance is significantly and positively correlated with these parameters ($r > 0.60$; $p < 0.05$) and negatively correlated with DO ($r < -70$; $p < 0.05$). The significant correlation ($r > 0.60$; $p < 0.05$) of these parameters with TC and FC suggests that they can affect microbiological quality in the Zio River water. From **Figure 2A**, the class Crustacea is affected by EC and Cl and Na contents, which are core salinity parameters. Moreover, their abundance is significantly correlated with EC, Na, and Cl ($r > 0.60$; $p < 0.05$) and can suggest the degree of crustacean tolerance to the water salinity. From the results of CCA (**Figure 2A**) and the relationship (Spearman's correlation between water quality parameters and taxon abundance), this study identified at

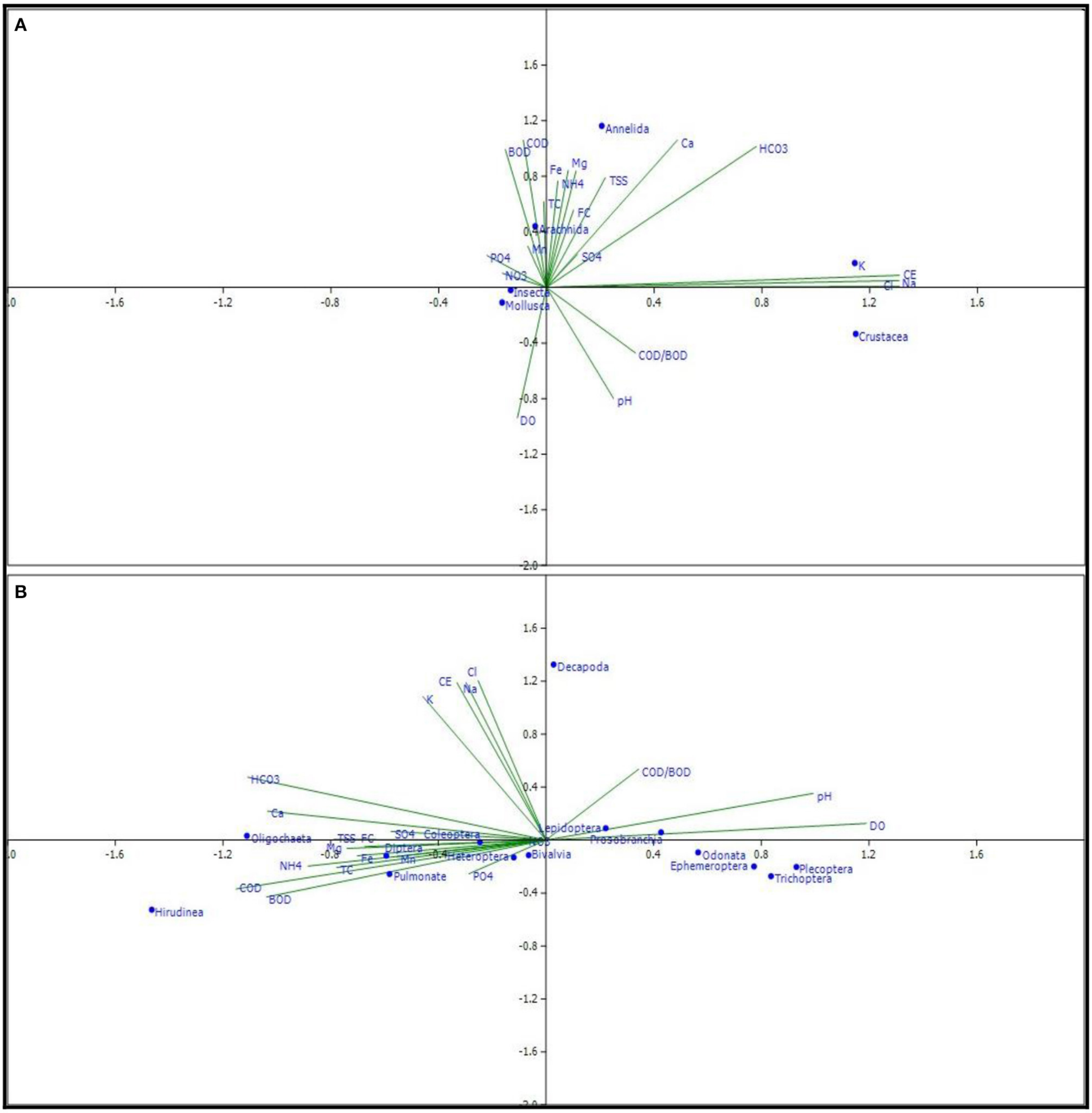

FIGURE 2 | A canonical correspondence analysis (CCA) biplot showing the association between water quality parameters and abundance of macroinvertebrate phylum–class **(A)** and subclass–order **(B)** resolution.

phylum–class level five taxa as indicators including three taxa as tolerant, one taxon as resistant, and one taxon as an indicator of water salinity (**Table 3**).

Response at Subclass–Order Level

The CCA indicates the environmental parameters that affect macroinvertebrate community at subclass–order level (**Figure 2B**). The **Supplementary Data** in **Appendix B** indicate Spearman's correlation between water quality parameters and subclass–order composition. The orders such as Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO) are significantly influenced by DO with a significant and positive correlation ($r > 0.60$; $p < 0.05$). Their abundances are negatively influenced by BOD, COD, TSS, and NH4 ($r < -0.60$; $p < 0.05$). The subclasses such as Hirudinea (Leeches) and Oligochaeta are positively affected by BOD, TSS, and NH4 ($r > 0.60$; $p < 0.05$)

TABLE 3 | Key taxa as indicated by their respective response groups for their specific taxonomic levels.

Response groups*	Phylum–class		Subclass–order		Family		Genera	
	Key taxa	**Total taxa**	**Key taxa**	**Total taxa**	**Key taxa**	**Total taxa**	**Key taxa**	**Total taxa**
Sensitive taxa	Not identified	0	Odonata; Epheroptera Trichoptera Plecoptera	4	Gomphidae	24	*Leste*	53
					Coenagriinidae		*Paragomphus*	
					Chlorocyphidae		*Gomphidia*	
					Corduliidae		*Lestinogomphus*	
					Perlidae, Leptoceridae, Hydroptilidae, Ecnomidae, Heptagniidae, Polymitarcyidae		*Neurogomphus*	
					Oligoneuriidae, Letophlebiidae		*Phyllomacromia*	
					Philopotamidae		*Afronurus*	
					Tricorythidae		*Ephoron*	
							Tricorythus	
							Elassoneuria	
							Notonurus	
							Thraulus	
							Perla	
							Leptocerus	
							Ceraclea	
							Macronema	
							Hydroptila	
							Orthotrichia	
							Chimara	
							Sapho	
Tolerant taxa	Mollusca	3	Prosobranchia Bivalvia	10		30	*Chironom*	68
	Insecta		Lepidoptera		Baetidae		*Culicoides*	
	Arachnida		Heteroptera		Libellulidae		*Dixa*	
			Coleoptera		Hydropsychidae		*Simulium*	
			Pulmonate		Pyralidae		*Stenochironomus*	
					Athericidae		*Cryptochironomus*	
					Dixidae		*Atherix*	
					Ceratopogonidae		*Urothemis*, *Pseudagrion*, *Pantala*, *Zygonyx*, *Olpogastra*	
					Haliplidae		*Potadoma*, *Cleopatra*, *Melania*	
					Gyrinidae		*Pseudocloeon*, *Cloeon*, *Caenodes*, *Eatonica*, *Ephemera*	
					Hydrophilidae		*Ceriagrion*	
					Elmidae			
					Naucoridae			
					Notonectidae			
					Veliidae			
					Mutelidae			
					Pilidae			
					Thiaridae			

(Continued)

TABLE 3 | Continued

Response groups*	Phylum–class		Subclass–order		Family		Genera	
	Key taxa	Total taxa	Key taxa	Total taxa	Key taxa	Total taxa	Key taxa	Total taxa
							Erystalis, *Orthocladius*, *Tanytarsus*, *Tanypus*, *Cricotopus*, *Ablabesmyia*, *Procladius*,	
							Aedes, *Culex*	
Resistant taxa	Annelida	1		3	Syrphydae, Psychodidae, Culicidae	11	*Limnaea*, *Ceratophallus*, *Biomphalaria*, *Bulinus*, *Ceratophallus*	20
					Chironomidae			
					Hirundinidae			
			Hirudinea		Lymnaeidae			
			Oligochaeta		Bulinidae			
			Diptera		Tubificidae			
					Naididae			
Salinity sensitive	Crustacean	1		1		2		4
			Decapoda		Palaemonidae		*Macrobrachium*	
All taxa	5	18	67	145				

**Sensitive taxa: The value in indicator taxa decreases with the increasing anthropogenic disturbances. Tolerant taxa: The value in indicator taxa does not follow substantially the variation of anthropogenic disturbances. Resistant or resilient: The value in indicator taxa increases with the increase of anthropogenic disturbances.*

but negatively correlated with DO ($r < -0.65$; $p < 0.05$). The Pulmonates mollusks and Diptera are significantly correlated with FC and TC ($r > 0.60$; $p < 0.05$). From **Figure 2B**, EPTO are in an opposite trend in comparison with Oligochaeta, Hirudinea, Diptera, and Pulmonates (OHDP). Decapoda is significantly and positively correlated with EC, Na, K, and Cl and then positively correlated with the salinity. The other subclass–order such as Prosobranchia, Bivalvia, Lepidoptera, Heteroptera, and Coleoptera (PBLHC) do not reveal a significant association with water quality parameters. From **Figure 2B** and Spearman's correlation, at subclass–order resolution, four groups of macroinvertebrates can be distinguished as indicator taxa with different sensitivity to water quality parameters. The first group is composed of EPTO, which are sensitive to the decrease of DO, and to the increase of BOD, COD, TSS, and NH4. The second group is composed of OHDP, which has the opposite response in comparison with the first group. The third group is represented by Decapoda, which seems to be influenced by the salinity parameters (EC, Na, Cl, and K). The fourth group is composed of PBLHC, which does not show significant correlation with water quality parameters. The key indicator taxa and total number of taxa in each group at subclass–order resolution are mentioned in **Table 3**.

Response at Family Level

The CCA indicates the environmental parameters that affect macroinvertebrate community at the family level (**Figure 3A**). The **Supplementary Data** in **Appendix C** indicate Spearman's correlation between water quality parameters and families composition. In **Figure 3A**, except some few families (Baetidae, Caenidae, Libellulidae, and Hydropsychidae), most of the families are from EPTO orders, and other families such as Atyidae from Decapoda, and Elmidae and Gyrinidae from Coleoptera are also strongly affected by water quality parameters. The abundance of these families increases with the increasing DO but decreases with the increasing BOD, COD, NH_4, TC, and FC concentrations. Spearman's correlation confirmed this trend by a significant correlation between the abundance of these families and DO ($r > 0.50$; $p < 0.05$), BOD, COD, NH_4, FC, and TC ($r < -0.50$; $p < 0.05$). Therefore, the families such as Atyidae, Elmidae, and Gyrinidae and those from EPTO can be classified as sensitive taxa (**Table 3**). In contrast, the abundance of Syrphidae, Psychodidae, Culicidae, and Chironomidae from Diptera order; Hirudinidae from Hirudinea; Lymnaeidae and Bulinidae from Pulmonates; and Tubificidae and Naididae from Oligochaeta increases when BOD, COD, NH_4, TC, and FC concentrations increase and decreases when DO concentration increases. The abundances of these taxa are significantly correlated with DO ($r < -0.50$; $p < 0.05$) and with BOD, COD, NH_4, TC, and FC ($r > 0.50$; $p < 0.05$). These relationships suggest that the families such as Syrphidae, Psychodidae, Culicidae, Chironomidae, Hirudinidae, Lymnaeidae, Bulinidae, Tubificidae, and Naididae can be classified as resistant or resilient taxa (**Table 3**). According to **Figure 3A**, the abundance of Palaemonidae is influenced positively by EC, Na, and Cl and is significantly and positively correlated with these parameters ($r > 0.55$; $p < 0.05$). This family from Decapoda can be considered as an indicator taxon of water salinity. The remaining families' taxa mainly from PBLHC;

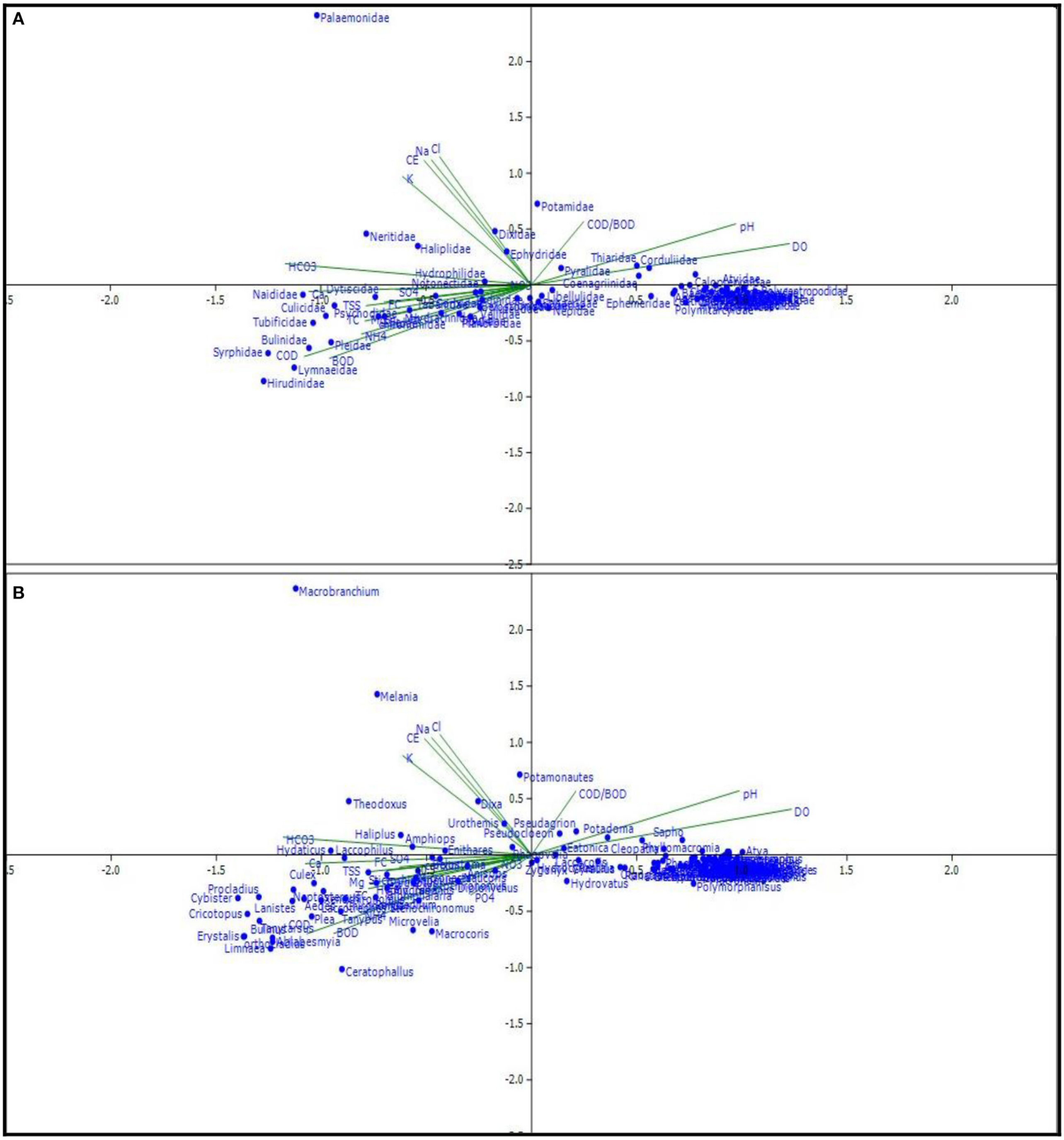

FIGURE 3 | A canonical correspondence analysis (CCA) biplot showing the association between water quality parameters and abundance of macroinvertebrate family resolution **(A)** and genera resolution **(B)**.

some families' taxa from Diptera and Decapoda; and also the few families from EPTO did not reveal a significant relationship with water quality variables ($r < |0.50|$; $p < 0.05$). These taxa were classified as tolerant or indifferent taxa as indicated in **Table 3**.

Response at Genus Level

The CCA indicates the environmental parameters that affect macroinvertebrate community at the genus level (**Figure 3B**). From **Figure 3B** and **Table 3**, it is seen that at the genus level, there is increase of sensitive taxa number for each

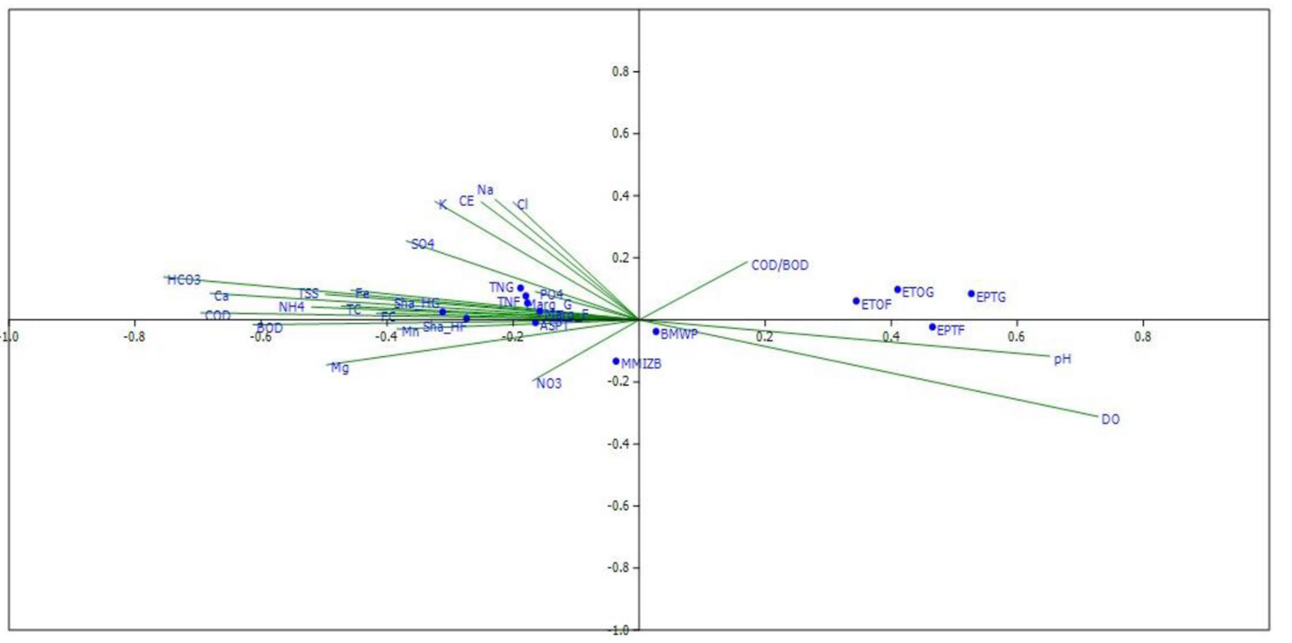

FIGURE 4 | A canonical correspondence analysis (CCA) biplot showing the association between water quality parameters and macroinvertebrate metrics.

group. Most of the genera from EPTO; genera *Atya* (Atyidae) from Decapoda, *Limnius* and *Elmis* (Elmidae) and *Orectogyrus* and *Aulonogyrus* (Gyrinidae) from Coleoptera; and some genera from Heteroptera such as *Ranatra* (Nepidae) and *Hydrometra* (Hydrometridae) were identified as sensitive taxa (**Table 3**). However, some genera from Ephemeroptera such as *Pseudocloeon*, *Cloeon* (Baetidae), *Caenodes* (Caenidae), *Eatonica*, and *Ephemera* (Ephemeridae) and others from Odonata such as *Urothemis*, *Zygonyx*, *Olpogastra*, *Pantala* (Libellulidae), and *Pseudagrion* (Coenagriinidae) are found to be tolerant taxa in this study. Some genera from Diptera are also identified as tolerant taxa (*Chironomus*, *Culicoides*, *Dixa*, *Simulium*, *Stenochironomus*, *Cryptochironomus*, *Atherix*, etc.), while other genera from Diptera are identified as resistant or resilient taxa (*Erystalis*, *Orthocladius*, *Tanytarsus*, *Tanypus*, *Cricotopus*, *Ablabesmyia*, *Procladius*, *Aedes*, *Culex*, etc.). The genera from Pulmonate (*Limnaea*, *Ceratophallus*, *Biomphalaria*, *Bulinus*, and *Ceratophallus*) were observed as resilient or resistant taxa, while the genera from Prosobranchia were distributed among tolerant taxa (*Potadoma*, *Cleopatra*, and *Melania*) and resilient taxa (*Lanistes*, *Pila*, and *Theodoxus*). Most of the genera from Coleoptera and Heteroptera identified here belong to tolerant and resistant taxa, with more genera from Heteroptera found in the tolerant group (**Table 3**). The genus *Macrobrachium* from Palaemonidae is positively influenced by salinity parameters and positively correlated with these parameters. In this study, it can be considered as an indicator taxon of water salinity (**Table 3**). The tolerant taxa were the most represented in terms of total number and at the different levels of identification. At the family level, 30 tolerant taxa, 24 sensitive taxa, 11 resistant taxa, and two taxa influenced by water salinity (salinity sensitive taxa) were identified. At the genus level, the same trend was observed, with 68 tolerant taxa, 53 sensitive taxa, 20 resistant taxa, and four salinity indicator taxa recorded (**Table 3**).

Response of Macroinvertebrate Metrics

The CCA indicates the association between water quality parameters and metrics (**Figure 4**). **Table 4** presents the correlation coefficients (factor loadings) between observed variables (raw water quality parameters and macroinvertebrate metrics) and the latent or underlying variables (factors). The **Supplementary Data** in **Appendix D** indicate Spearman's correlation between water quality parameters and metrics. From **Figure 4**, three groups of metrics can be distinguished according to their relationship with water quality parameters. First, the metrics for taxonomic richness are composed of ETOF, ETOG, EPTF, and EPTG. These metrics are positively and significantly correlated with DO ($r > 0.65$, $p < 0.05$), but they are negatively and significantly correlated with EC, BOD, COD, TC, FC, Cl, Na, and NH_4 ($r < -0.60$, $p < 0.05$). Then, the tolerant metrics are composed of ASPT and Biological Monitoring Working Party (BMWP) including MMIZB. These metrics are positively correlated with DO ($r > 0.65$, $p < 0.05$) and negatively correlated with EC, BOD, COD, TC, FC, and NH_4 ($r < -0.55$, $p < 0.05$). Finally, the diversity indices are represented by Marg_G, Marg_F, Sha_H_F, and Sha_H_G, as well as the total number of family and genera that reveal a strong positive association with DO ($r \leq 0.5$, $p < 0.05$) and significant negative correlation with EC, TC, Cl, Na, and NH_4 ($r \geq -0.50$, $p < 0.05$). From **Table 4**, the first factor is significantly and positively correlated with TSS, COD, BOD, TC, and NH_4, but a significant negative association was observed with pH and DO. Therefore, the first factor can represent the

TABLE 4 | Correlation between the first four factors and observed variables (factor loadings).

Variables	Factor 1	Factor 2	Factor 3	Factor 4
pH	**−0.62496**	0.371382	0.310675	0.024158
EC	0.27342	**0.931603**	−0.14976	0.077719
DO	**−0.70289**	0.093536	**0.540439**	0.103397
TSS	**0.55742**	−0.14142	0.034907	**0.598913**
COD	**0.69588**	−0.29253	−0.4957	−0.00347
BOD	**0.64649**	−0.36051	−0.43668	0.073614
COD/BOD	−0.21656	0.373366	0.058845	−0.30288
TC	**0.52832**	−0.19976	−0.18145	0.410132
FC	0.44941	−0.08155	−0.00426	**0.580457**
HCO_3	**0.77794**	0.287358	−0.07091	0.001782
Ca	**0.70414**	0.086488	−0.12011	−0.03126
Mg	0.5541	−0.13333	0.103874	−0.16566
NO_3	0.23357	−0.22256	0.344777	−0.3927
NH_4	**0.50038**	−0.11696	**−0.53631**	−0.24402
PO_4	0.23801	−0.11469	−0.12556	−0.37408
Mn	0.34649	−0.18103	−0.40382	−0.3859
Fe	0.47418	−0.19232	0.056711	0.406136
SO_4	0.37718	0.097633	**−0.56601**	−0.42466
Cl	0.2263	**0.948174**	−0.12543	0.079061
Na	0.24987	**0.942503**	−0.13753	0.078978
K	0.34218	**0.894504**	−0.18144	0.058124
EPTF	**−0.96613**	−0.01489	−0.04152	0.024452
EPTG	**−0.93517**	−0.01647	−0.1529	0.041739
ETOF	**−0.96038**	0.047505	−0.07334	0.051604
ETOG	**−0.94462**	0.02463	−0.13799	0.04554
TNF	**−0.87114**	0.10523	−0.36039	0.076465
TNG	**−0.76167**	0.090138	**−0.53994**	−0.05993
Sha_HF	**−0.71879**	−0.13893	−0.32013	0.318758
Sha_HG	**−0.5764**	−0.2348	**−0.62323**	0.220572
Marg_F	**−0.91595**	0.017118	−0.24944	0.154508
Marg_G	**−0.83037**	0.011699	−0.49225	0.01772
BMWP	**−0.95862**	0.012813	−0.06363	0.008768
ASPT	**−0.88283**	0.099968	0.156796	−0.16431
MMIZB	**−0.83721**	−0.19106	0.207458	−0.18571
Total variance	43.9%	13.0327%	9.4995%	6.2442%

EC, electrical conductivity; DO, dissolved oxygen; TSS, total suspended solid; COD, chemical oxygen demand; BOD, biological oxygen demand; TC, total coliforms; FC, fecal coliforms; BMWP, biological monitoring working party; ASPT, average score per taxon; MMIZB, multimetric index of the Zio River basin. Bold value means significant correlation.

water quality deterioration due to anthropogenic pressures. There is a positive association between the second factor and EC, Na, Cl, and K. This factor can represent the water salinity degree due mainly to mineralization processes or seawater. All the metrics tested in this study displayed a significant negative association with the first factor, but they did not reveal a significant association with the second factor. This result means that all metrics tested in this study are sensitive to water quality deterioration or anthropogenic disturbances affecting water quality.

DISCUSSIONS

Water Quality Parameters and Their Interpretation

Knowledge about water quality parameters is an important part of environmental monitoring and determining the condition of habitats. When water quality is poor, it affects not only aquatic life but also the aquatic ecosystem health. This section details with some parameters that affect water quality and aquatic ecosystem health. The values of EC show that more than 75% of water samples were in the range of natural freshwater, which varies from 0.5 to 1,500 μS/cm (Rodier et al., 2009). The high values of EC can affect freshwater organisms such as macroinvertebrate communities (Environmental Protection Agency of Ireland, 2001) as found in the present study; it is revealed that EC affects Palaemonidae taxon particularly the genus *Macrobrachium*. As a result, this taxon can detect the salinity or EC in the water of the Zio River. The pH has been considered as an important parameter in the ecology of aquatic macroinvertebrates (Thomsen and Friberg, 2002; Yuan, 2004). Benthic macroinvertebrates are sensitive to pH variation, and values below 5 or >9 are considered harmful (Yuan, 2004). The pH value found in the present study is in the range of natural water pH 6.5–8.5 according to some standards [U.S. Environmental Protection Agency (USEPA), 1980; Environmental Protection Agency of Ireland, 2001; Rodier et al., 2009; WHO, 2011]. However, according to Thomsen and Friberg (2002), the low pH values are associated with lower diversity of benthic macroinvertebrates and cause decreasing emergence rates of this community (Hall et al., 1980). The range of pH found in the present study is associated with high diversity of some macroinvertebrates such as EPTO taxa. The DO is an excellent indicator of water quality and one of the most sensitive to water quality degradation and therefore indicates the degree of water body self-purification (WHO, 1996; Sanchez et al., 2006; Makhoukh et al., 2011), whereas BOD indicates the quantity of biodegradable substances that mainly originate from organic matters into the water body due to human activities. In this study, the mean DO value observed in the range of saturated water (6–7 mg/L) with the 75th percentile value around 9.75 mg/L can indicate that most of the water samples can be classified from good to excellent quality according to international standards (Environmental Protection Agency of Ireland, 2001; Rodier et al., 2009). In the same vein, our study showed that DO and pH affect EPTO taxa found in the present study as sensitive taxa. Therefore, EPTO taxa or related metrics are able to detect variables such as DO and pH in the water of the Zio River.

The BOD value increases when DO value decreases and often indicates an organic pollution. We found that in the pristine rivers, this value is below 2 mg/L; and in moderately polluted rivers, it can range from 2 to 8 mg/L, although the rivers above 8 mg/L may be considered as severely polluted (Rejsek, 2002; Rodier et al., 2009). The COD is an indicative measurement of oxygen consumed by reducing substances (organic, nitrite, sulfide, ferrous salts, and others) in the water. It is also an important parameter of water quality assessment (Rejsek, 2002).

The 75th percentile values and the mean values of BOD and COD indicate that the waters of this study can be considered as moderately polluted. The ratio COD/BOD is a meaningful parameter used to assess biodegradation in natural water. It is also used in environmental monitoring to assess the self-purification power of a river, which is the suitability for a river containing organic matter to transform this organic matter into mineral matter by natural processes (Mara and Pearson, 1999; Mara, 2004). A ratio of COD/BOD ranging from 1.5 to 2.5 indicates a good biodegradability and self-purification power (Mara et al., 2007; Rodier et al., 2009). Accordingly, the Zio River can be considered as a suitable river for self-purification process.

The TSS in water may cause pathogen problems, as the growth of anaerobic bacteria attached to suspended material can increase the risk of disease outbreaks and a significant portion of toxic organics (Rosewarne et al., 2014; Rono, 2017). In addition, the trace elements can be adsorbed onto organic matter present in water as suspended materials (Rono, 2017; Helmecke et al., 2020). TSS can also cause ecosystem disturbance through reduced water clarity, limiting photosynthesis and asphyxiation of some gill-breathing organism by clogging gills (Rodier et al., 2009; Rosewarne et al., 2014; Swinkels et al., 2014). The high levels of suspended solids can be considered as a form of pollution, which will have the effect of reducing the quality of the habitat for cold-water organisms (Rejsek, 2002; Rodier et al., 2009). All sampling sites within the Zio River contain acceptable concentration of TSS for aquatic ecosystem functioning life. Ammonium usually reflects a process of anaerobic degradation of nitrogenous organic matter and can be a good indicator of river pollution by urban effluents (Chapman and Kimstach, 1996). The range of NH_4^+ values is near to natural water, but the standard deviation indicates a slight punctual source pollution of the study river. The present study revealed that COD, BOD, TSS, and NH_4^+ affect OHDP taxa found here as resistant taxa. Thus, the findings from this study suggest that OHDP taxa or related metrics are able to detect COD, BOD, TSS, and NH_4^+ in the water of the Zio River. Chloride is frequently associated with wastewater; and it is often included in assessments as an indicator of possible fecal contamination or as a measure of the amount of dispersion of wastewater discharges into the natural environment (WHO, 1996). In pristine freshwater, chloride concentrations are generally less than 10 mg/L and sometimes less than 2 mg/L (WHO, 1996; Rodier et al., 2009). Sodium concentration is often related to the nature of contact rock, evaporation, and seawater intrusion phenomenon. In this latter case, sodium content is often correlated with chloride content. The 75th percentile and mean values of sodium and chloride indicate that about 75% of water samples were not affected by sea intrusion, while 25% were affected as reported by Tampo (2018) at the lower reaches. TC and FC are parameters that indicate the possible fecal contamination of water. The recommended values in freshwater should be below 10 Cfu/100 ml. The values recorded here reveal a risk of fecal contamination at some sites. The Zio River water quality trend follows a gradient of pristine-to-poor water quality from the upper reaches to the lower reaches with a mosaic of good and poor water quality in the lower reaches based on proximity to anthropogenic pressure and saltwater intrusion (Tampo, 2018).

Response of Macroinvertebrates at Different Taxonomic Levels

In the present study, some phylum–class taxa could effectively indicate the ecological integrity associated with water quality. This could be explained by the fact that these phylum–classes were dominated by taxa with the same or very few different lifestyles that could point out a single category of pollution (Tachet et al., 2010). From this study, crustaceans were found to be good indicators of the freshwater salinity. Indeed, crustaceans were dominated by two families (Palaemonidae and Atyidae) and well known to be influenced by EC or water salinity (Attrill et al., 1999; Cuesta et al., 2006; N'Zi et al., 2008; Collocott et al., 2014). At the genus level, it was found that *Macrobrachium* from Palaemonidae was the most affected by salinity gradients and can be considered as the key indicator of water salinity. This corroborates the findings of other studies, which indicate that the life cycle or style of *Macrobrachium* is mainly related to estuary or water salinity (N'Zi et al., 2008; Collocott et al., 2014; Gangbe et al., 2016; Chen et al., 2018; Adam et al., 2019). Meanwhile, Mollusca taxon is associated with water eutrophication due to organic pollution. The findings revealed that phylum Annelida could be considered as generalists with high tolerance to stream degradation. At subclass–order level, the identified group of EPTO was found to be a sensitive group due to high number of sensitive taxa belonging to this group. This sensitivity of EPTO taxa is well known and described by earlier authors (Hilsenhof, 1988; Rodier et al., 2009; Tachet et al., 2010; Ko et al., 2020). The high abundance and diversity of EPTO taxa indicate good or excellent water quality with good ecological conditions (Tampo et al., 2015, 2020; Kaboré, 2016). Many metrics and multimetric indices calculated through EPTO taxa are widely used in biomonitoring programs as reported by many authors (Rodier et al., 2009; Mereta et al., 2012, 2013; Tampo et al., 2015, 2020; Kaboré et al., 2016). The results support other studies that found that the high abundance of Oligochaeta, Hirudinea, Pulmonates, and some Diptera often indicates organic pollution as seen in the present study. Globally, this has been seen with possible fecal contamination and substantial anthropogenic pressures (Hilsenhof, 1987; Rodier et al., 2009; Tachet et al., 2010; Tampo et al., 2020). This resistance to pollution of some macroinvertebrate groups can be explained by their lifestyle and the feeding strategy. For example, the Oligochaeta feed on detritus, bacteria, and dead remains of other aquatic organisms. Pulmonates are not only detritivores but also the intermediate host of many parasites (Tachet et al., 2010). According to Rodier et al. (2009), Oligochaeta and Leeches are indicators of polluted water and deteriorated habitat. Their number increase with the increase of eutrophication, which affects the overall ecosystem health (Kazanci et al., 2015). Within the insect fauna, Hilsenhof (1987) has demonstrated that Libellulidae, Lestidae, and Coenagrionidae from Odonata order are tolerant taxa. Furthermore, Rodier et al. (2009) showed that in a biomonitoring program based on a standardized global biotic index, Baetidae (algae grazer) and Caenidae from Ephemeroptera are resistant taxa, while Mary (1999) has reported some sensitive taxa in Heteroptera families. Here, we found within the EPTO order a notable diversity, with several families observed in good habitat conditions. This could be explained by the presence of families less tolerant or even sensitive to pollution. At the genus level, more taxa emerged as sensitive with specific indications. Some studies reported this increase of the sensitivity degree and the specific indication of macroinvertebrates when moving from phylum–class to species taxonomic level (Bailey et al., 2001; Jones, 2008; Menezes et al., 2011). However, according to the review paper of Jones (2008), many experts showed that the family level is sufficient or may be better in bioassessments even if genus and species taxonomic levels are required. The present study agrees with this consensus and shows that the family level is sufficient and better in the detection of nutrient pollution, organic pollution, and human disturbances and which pressures may drive shifts when using indices or metrics in macroinvertebrate communities. In addition to this, the present study showed that subclass and order levels are less sufficient of specific pollution but better for rapid evaluation of pollution at the global scale. These findings also agreed with those of other studies carried out in Africa (Dickens and Graham, 2002; Mereta et al., 2013; Elias et al., 2014; Kaboré et al., 2016; Dalu and Chauke, 2020; Ochieng et al., 2020; Tampo et al., 2020; Edegbene et al., 2021) in Asia (Blakely et al., 2014; Nguyen et al., 2014) and Europe (Poquet et al., 2009; Rodier et al., 2009; Costas et al., 2018) where these studies suggested the use of macroinvertebrates at the family level for the development of biomonitoring programs. The findings of the present study highlight the importance of using the family level for biomonitoring program in Togo and even elsewhere in Africa because of cost-effectiveness and the lack of systematic knowledge on macroinvertebrates.

Macroinvertebrate-Based Metrics

According to the results of the ordination (CCA and FA) and Spearman's correlation analysis, the metrics calculated at the family level and those calculated at the genus level were strongly correlated ($r \geq 0.80$) and with the first factor ($r \geq |0.75|$). This strong correlation is a redundant association between metrics calculated at family and genus levels. Thus, these two taxonomic levels can translate the same or common information. Our results also indicate that the taxonomic detail (family or genus level) does not substantially affect metrics used in bioassessment. These results are near the consensus that the lowest taxonomic level (species) is not always required for bioassessments (Jones, 2008). For example, in the case of differentiating impacted from unimpacted sites, some researchers showed that species-level identification was not always the best, but total richness and EPT richness performed better at family than at species, and family-level diversity measures also performed well (Schmidt-Kloiber and Nijboer, 2004; Schmidt-Kloiber et al., 2006). The negative correlation observed in the present study with all metrics confirmed the fact that they appear to be highly sensitive in the variation of water quality variables linked to the degree of human pressures. This sensitivity of macroinvertebrate metrics to the water quality and anthropogenic pressures is widely reported (Hering et al., 2006; Jun et al., 2012; Helson and Williams, 2013; Lakew and Moog, 2015; Kaboré et al., 2016; Aura et al., 2017; Tampo et al., 2020). The degree and the type of sensitivity of each one can explain the three groups of metrics identified by the CCA. In this way, reporting the sensitivity of EPTO group, many authors found that the diversity and abundance of this group increase with the increase of DO and with the decrease of nutrients, BOD, COD, and TSS (Mereta et al., 2013; Tampo et al., 2015; Kaboré et al., 2016). Thus, the EPTO group is the most suitable in the evaluation of water quality and aquatic ecosystems health and is used worldwide in bioassessments and monitoring programs (Armitage et al., 1983; Hilsenhof, 1987; Barbour et al., 1996, 1999; Schmidt-Kloiber et al., 2006; Rodier et al., 2009). The metrics related to tolerance measure (MMIZB; ASPT and BMWP) are also highly correlated with the first factor and some water quality parameters. This result revealed their sensitivity in water quality variation and mostly in the detection of anthropogenic pressures. Furthermore, in the previous studies, MMIZB revealed its sensitivity by discriminating impaired sites and unimpaired sites (Tampo et al., 2020). The two metrics ASPT and BMWP were also used in many studies as a single metric or integrated in a multimetric index for the monitoring of watersheds (Ferreira et al., 2011) for assessing water quality (Nguyen et al., 2014) and for assessing ecological integrity (Solimini et al., 2007; Lakew and Moog, 2015). That is likely because of their robust sensitivity and high discrimination power; the tolerance measure metrics are mostly recommended for ecological status evaluation under many conditions (Barbour et al., 1996; Hering et al., 2006). The total taxonomic number and the diversity indices were also calculated at family and genus levels. The results show that for the same metric of this group calculated, using family-level and genera-level data did not reveal a big difference about their relationship with water quality parameters and the first factor. Indeed, in bioassessments, richness is used to summarize biological condition, not to generate exhaustive taxon checklists, so coarse taxonomic resolution is often sufficient. For example, Marshall et al. (2006) reported only a 6% information loss when benthos data were rolled up to family from species. These results agree with those of several authors who found that correlations between ordination site-scores and environmental variables are slightly affected by taxonomic detail (Hewlett, 2000; Waite et al., 2000; Metzeling et al., 2006). In the same vein, Metzeling et al. (2006) reported that reducing taxonomic detail from species to family had little effect on ordination plots and gave similar principal axis correlations with environmental variables. Stronger evidence was provided by Hewlett's (2000) classification and ordination-based study of Australian stream sites. Even if the different taxonomic resolutions are known to influence the responses of macroinvertebrates through several mechanisms, our findings suggest that the use of macroinvertebrate metrics approach based on the family level has proven useful for Zio River's aquatic ecosystem monitoring.

CONCLUSION

In many developing countries across Africa such as Togo, rivers are threatened by intense agriculture (using fertilizers and pesticides), urbanization, and severe pollution, leading to habitat loss and water quality and negatively affecting aquatic organisms. With ongoing multiple pressures, the urgent need of biomonitoring tools is crucial for ensuring valuable biodiversity and water resource conservation. Along the Zio River gradient including several types of pressures, it has been demonstrated that macroinvertebrate indices or metrics have proven their sensitivity to water quality variation and human disturbance from order-level identification up to the genus level. Specifically, those related to EPTO taxa and tolerance were suitable in detection of water quality and human disturbances. The findings of this study confirm the importance of maintaining the family level for bioassessment and biomonitoring programs development in developing countries, especially in Sub-Saharan Africa, due to its cost-effectiveness and the lack of systematic knowledge on macroinvertebrates. However, the genus and species taxonomic levels are needed to improve the understanding of responses on the family level and the detection of specific pollution. We, therefore, recommend the use of the family level in metric and index formulation to monitor the Zio River.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Materials**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Wendengoudi Guenda, University of Joseph KI-ZERBO, Laboratory of Animals Biology and Ecology, 03 BP 7021 Ouagadougou 03, Burkina Faso.

AUTHOR CONTRIBUTIONS

LT conceived the manuscript and wrote the paper with IK and EHA. LT and IK collected and analyzed the data. AO assisted LT in the laboratory analyses. LMB and GD-B supervised the laboratory analyses. EHA corrected the English language. LMB and GD-B supervised the study and contributed valuable comments to the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/frwa.2021.662765/full#supplementary-material

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