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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).

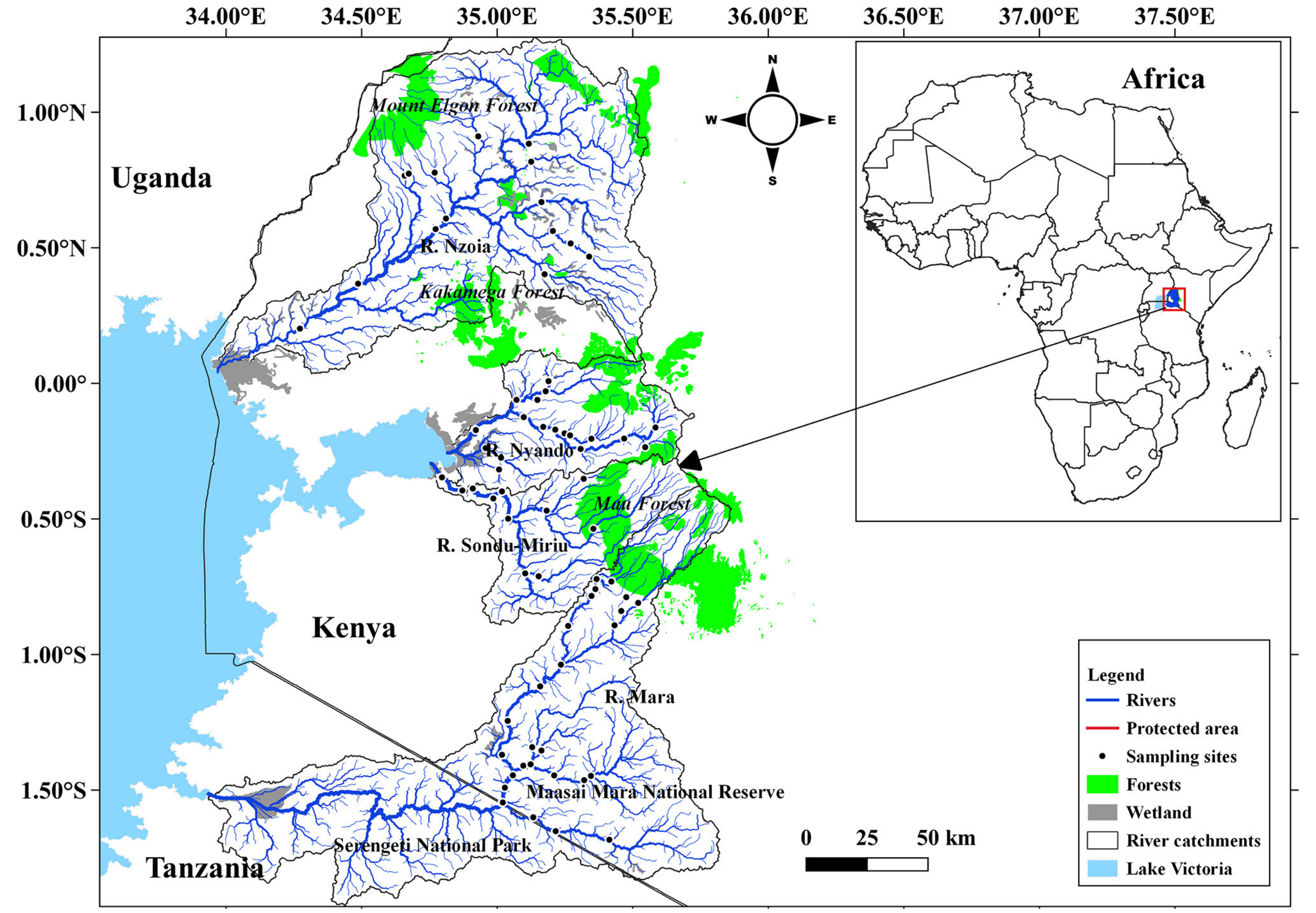

FIGURE 1 | Sampled river catchments and location of sampled stations along Rivers Mara, Nyando, Nzoia, and Sondu–Miriu on the Kenyan side of the Lake Victoria Basin.

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;

$$\mathrm{NB} = \frac{\sqrt{\frac{\sum_{i=1}^{n}\left(\mathrm{PC}_{\,i} - \mathrm{PC}_{\,\mathrm{mean}}\right)^{2}}{n}}}{\mathrm{PC}_{\,\mathrm{eigenvalue}}}$$

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

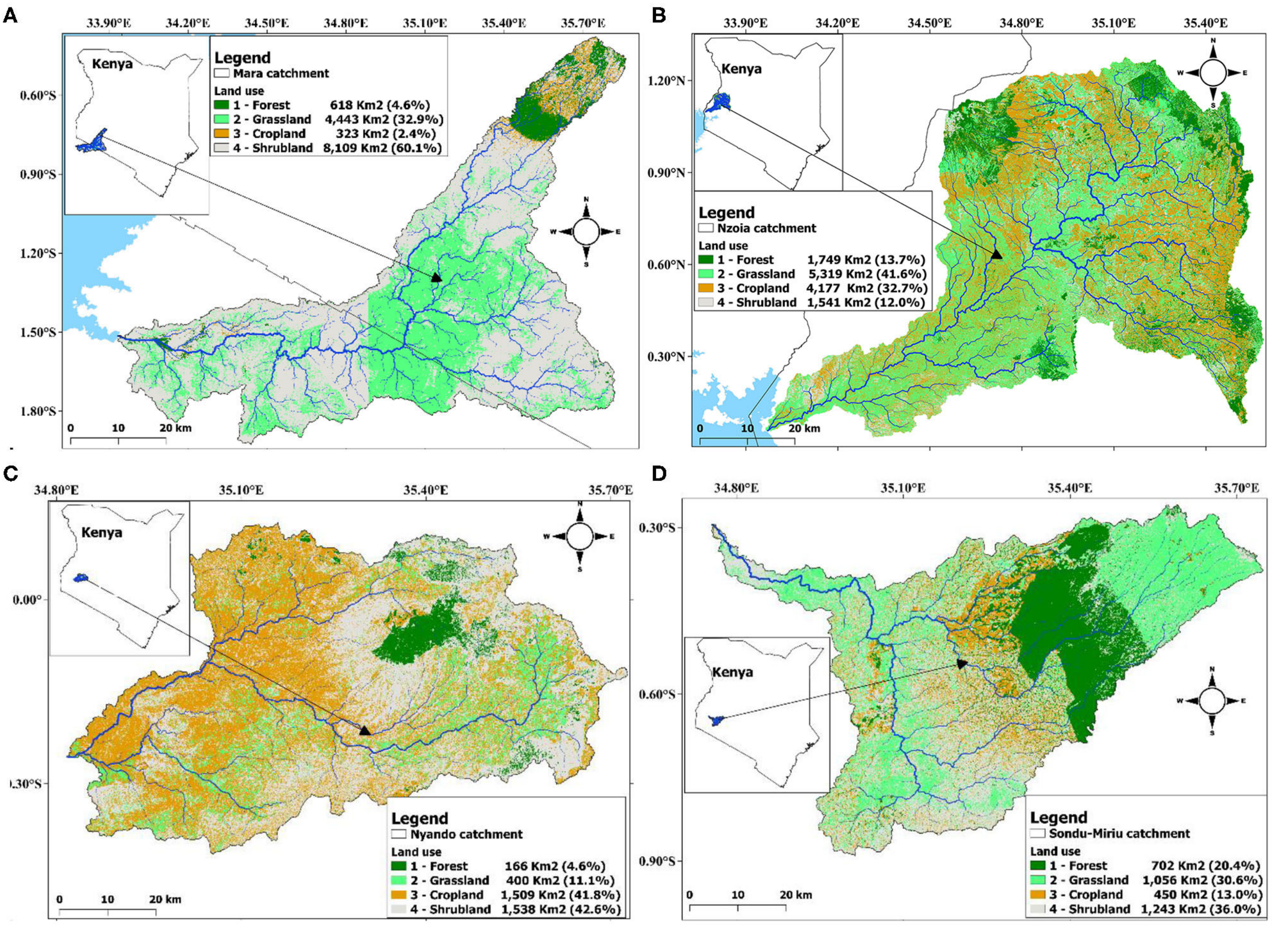

FIGURE 2 | Area in km^2 and percent forest, grassland, cropland and shrubland within the river catchments, **(A)** River Mara, **(B)** River Nzoia, **(C)** River Nyando and **(D)** River Sondu–Miriu of the Lake Victoria Basin.

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%

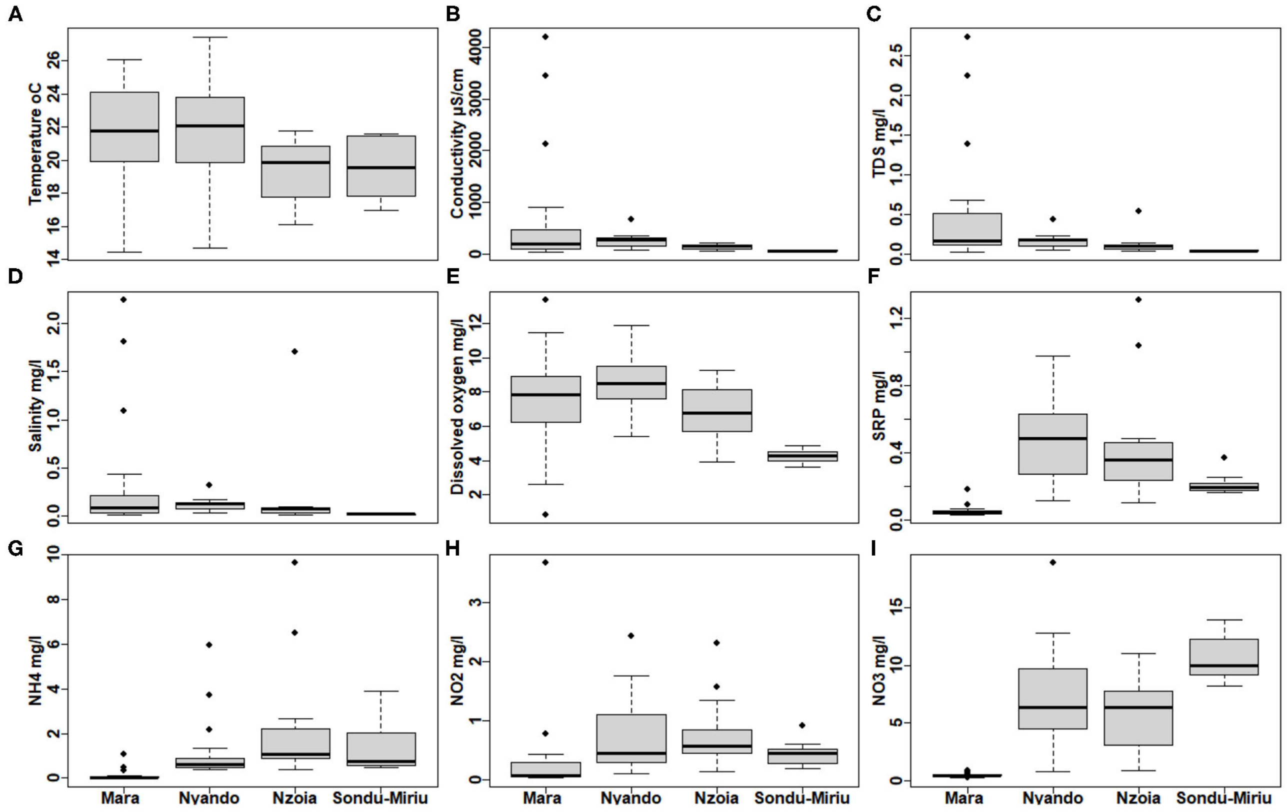

FIGURE 3 | Variation in water quality parameters at the four catchments within the Lake Victoria Basin. **(A)** Temperature, **(B)** conductivity, **(C)** total dissolved solids (TDS), **(D)** salinity, **(E)** dissolved oxygen concentration, **(F)** soluble reactive phosphorus (SRP), **(G)** ammonia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

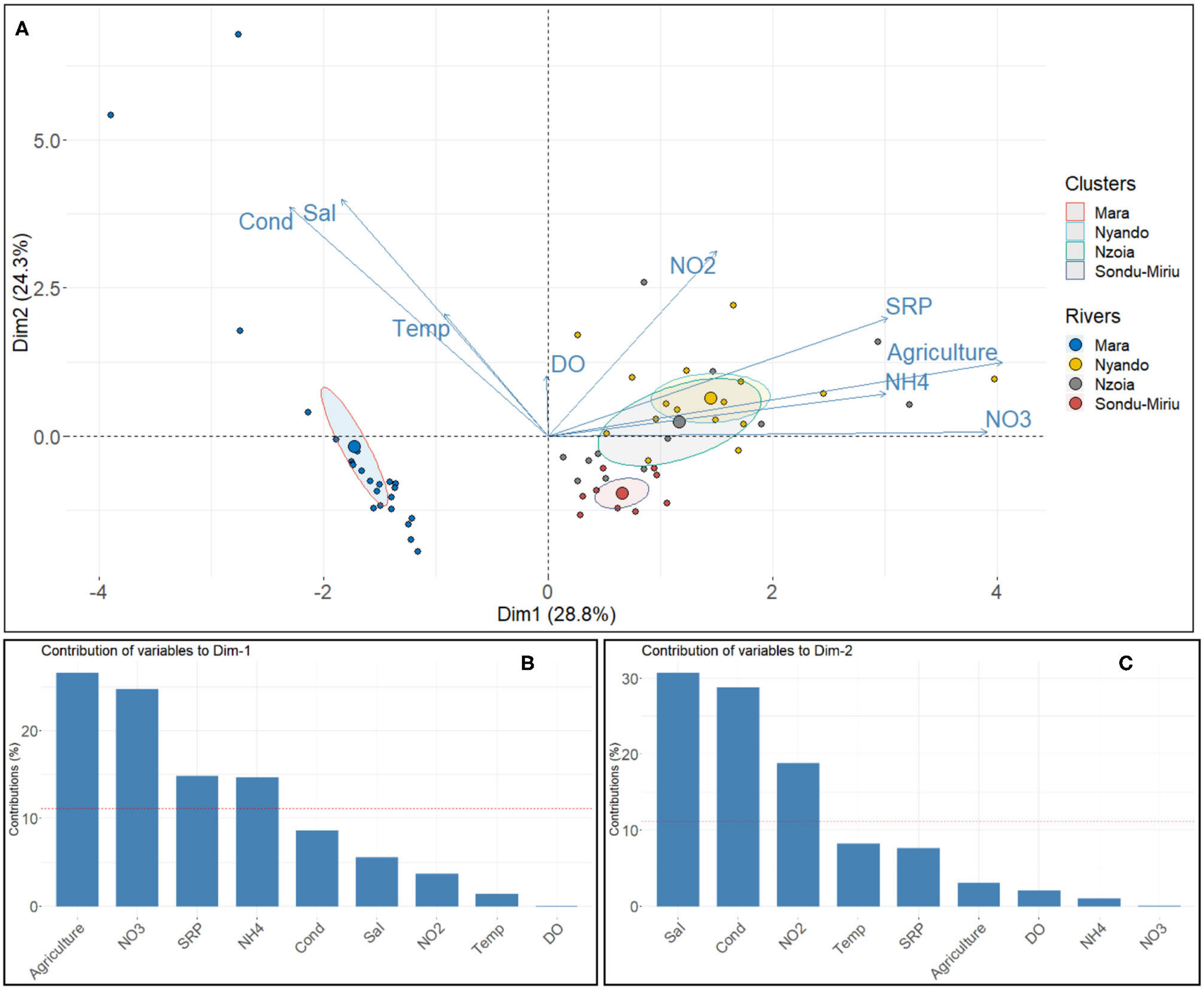

FIGURE 4 | (A) Principal component analysis (PCA) of the influence of water quality parameters on the variability in the river basins, and the contribution of the water quality variables to **(B)** component 1 and **(C)** component 2 of the PCA.

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.

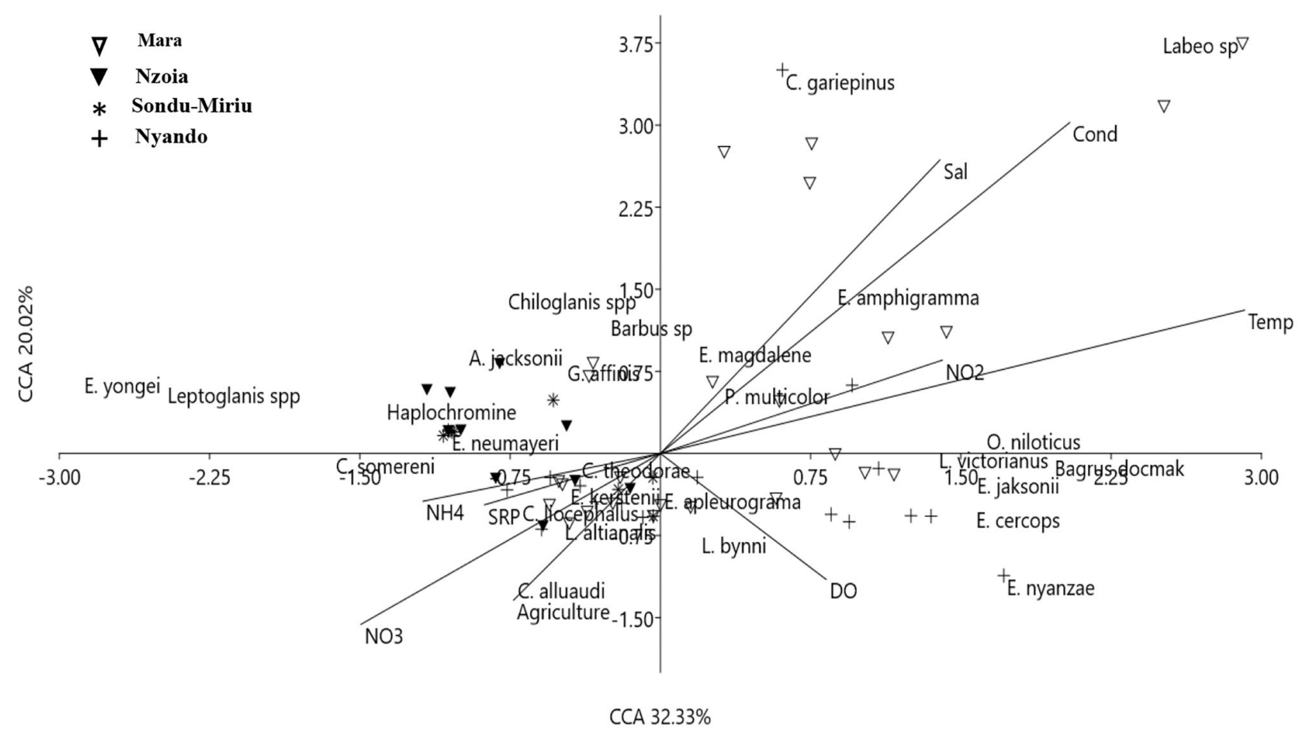

FIGURE 5 | Canonical correspondence analysis of the effects of water quality on fish assemblages at the four catchments in the Lake Victoria Basin.

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

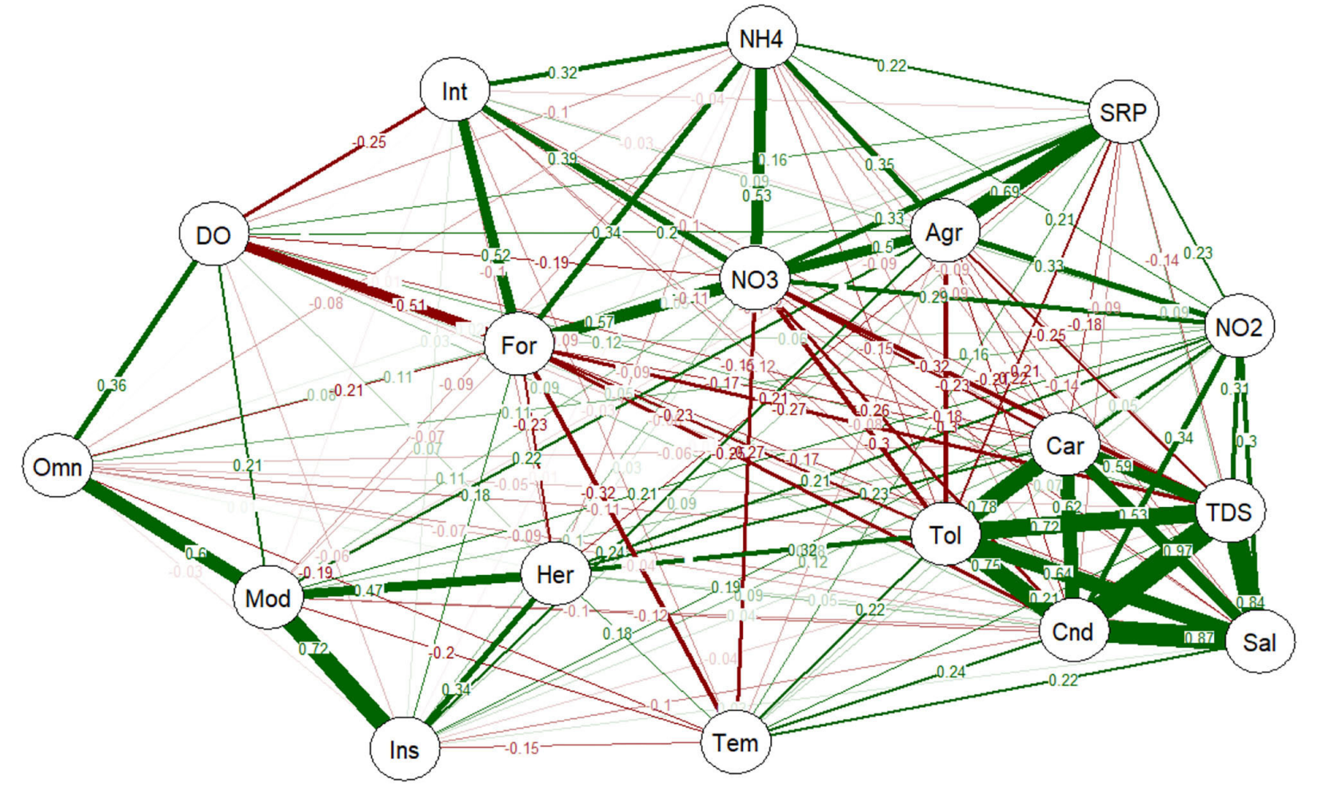

FIGURE 6 | PNCA of environmental gradients with species sensitivity, trophic levels, and land use (forest and agriculture). Thick lines indicate greater value of correlation, whereas narrow lines indicate smaller value of correlation. Green lines are positive correlations, whereas red lines are negative 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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