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Application of phytoplankton community structure for ranking the major riverine catchments influencing the pollution status of a lake basin

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Abstract

The present study demonstrates the application of a multi-metric Phytoplankton Index of Biotic Integrity (PIBI) approach for ranking of major river catchments in the Kenyan part of Lake Victoria on the basis of their pollution status. The index utilizes water quality and zooplankton data, phytoplankton diversity, abundance and attributes, as well as literature information. The rivers were sampled from 2016 to 2018 during the wet season (March) and dry season (July). The separation power of the Mann-Whitney U test ($p < .05$) qualified eight discriminant metrics for phytoplankton samples into a scoring system of 1, 3 and 5, based on high, fair and slight deviation from the best site, respectively, in development of the final PIBI. The Kuja and Sondu-Miriu rivers had the highest PIBI, signifying least pollution influence on the lake. In contrast, the Yala and Nzoia rivers exhibited the lowest PIBI, representing the catchments with a higher pollution influence on the lake. The fair to poor integrity classes for the major river catchments in the region signified a deteriorating lakescape. The present study presents the preliminary results of using phytoplankton metrics for development of the Index of Biotic Integrity (IBI) approach in the region as a decision-making support tool for the effective management and sustainable use of water resources in the lake basin.

KEYWORDS

bioindicator, catchment, Lake Victoria, management, Zooplankton

1 | INTRODUCTION

The development of bioindicator criteria for assessing the integrity of aquatic systems is based on concepts of regionalization (Omernik, 1995), the use of a multi-metric approach (Karr, Fauch, Angermeier, Yant, & Schlosser, 1986) and the establishment of control or reference conditions (Hughes, 1995). These considerations enable resource users to apply these criteria as benchmarks in both

numeric and narrative evaluations of regional aquatic conditions (Griffith et al., 2005). Several renowned world agencies, including the US Environmental Protection Agency (USEPA, 1990), have promoted the development of biological assessment criteria for assessing water resources, which enables development of numeric and narrative standards based on legally enforceable biological criteria and, as such, play a significant role in water resource management efforts.

To this end, global and regional studies are adopting the use of the Index of Biotic Integrity (IBI) as a biological assessment technique for measuring the ecological health or pollution status of rivers, lakes and other aquatic systems. The use of IBI is a widely accepted bioassessment method that uses a multi-metric indicator for such regional scale assessments (Karr & Chu, 1997; Karr & Dudley, 1981). The methodology has been adapted for a variety of regions, including the United States of America (Karr & Chu, 1997; Simon & Lyons, 1995) and Africa (Aura, Raburu, & Herrmann, 2010; Aura, Kimani, Musa, Kundu, & Njiru, 2017; Masese, Muchiri, & Raburu, 2009). However, in these studies, most of these researchers used macroinvertebrates and fish as the main taxonomic groups, however, examples being Barbour, Gerritsen, Snyder, and Stribling (1999), Bailey, Norris, and Reynoldson (2001) and Masese, Omukoto, and Nyakeya (2013), with minimal or no comparisons with phytoplankton attributes.

Only a few researchers (Barbour et al., 1999; Griffith et al., 2005; Kane, Gordon, Munawar, Charlton, & Culver, 2008) have considered the use of phytoplankton, mainly periphyton, with little information on this approach available from developing and tropical countries in the Great Lakes region in the development of IBI. Indicators such as plankton, however, have been used in some studies (Kundu et al., 2017; Schindler, 1987) to assess the extent of aquatic pollution and degradation, and to examine the relationships between anthropogenic stressors and the condition of biological resources. Plankton also are ideal for offshore water quality monitoring for large lake ecosystems, as was done in the present study, mainly because this approach is inexpensive for collecting samples and also highly sensitive to environmental changes (Schindler, 1987). Further, all components of lake ecosystem functions are influenced by plankton dynamics (Kane et al., 2008).

Phytoplankton are important bioindicators of metals in aquatic ecosystems, having the capacity to accumulate and store these pollutants (Kundu et al., 2017). They are also primary producers and a vital food organism for invertebrates and fish. Thus, they provide a base in the aquatic food web that is the most important factor for the production of organic matter. The interplay of physical, chemical and biological properties of water results in phytoplankton production, with their assemblage (composition, abundance, diversity, distribution) also structured by these factors. The extent of these influences in tropical rivers and streams is of interest because of their high seasonal flow variations, channel heterogeneity, and responses to processes and influences from catchment activities (Sitoki, Kurmayer, & Rott, 2012).

Phytoplankton have considerable potential value as water quality indicators (Murugan, Murugavel, & Kodarkar, 1998). They are good indicators of environmental quality in both lakes and rivers. They also exhibit worldwide distribution, with their species composition and community structure being sensitive to changes in such environmental factors as nutrient enrichment (Jha & Barat, 2003) and pollution levels (El-Bassat, 2007). Expanding on previous efforts to apply and adapt IBIs in various regions and taxonomic groups, therefore, the need to further consider the use of plankton for this purpose in the African Great Lakes region merits much greater attention.

Previous studies (Butcher, Stewart, & Simon, 2003) have provided insights mainly into the effects of land-use changes and anthropogenic activities on aquatic ecosystem integrity. There is very little existing information, however, on the ranking of the major river basins influencing the pollution contributions or water quality of a lake. River catchments, for example, play a pivotal role in determining the increasing eutrophication of a lake attributable to increased nutrient inflows. The most important plant nutrients (nitrogen and phosphorus) are the usual causes of nutrient over-enrichment problems (Campbell, Balirwa, Dixon, & Hecky, 2004). Excessive in-lake nitrogen and phosphorus concentrations also can originate from increased organic and inorganic fertilizers run-off from intensive agricultural activities, municipal waste (e.g. sewage) from cities and livestock wastes (Longgen, Zhongjie, Ping, & Leyi, 2009).

Accordingly, the presents study documents the development of a Phytoplankton Index of Biotic Integrity (PIBI) for ranking the pollution status of major river catchments in the Kenyan portion of Lake Victoria. The possibility of replicating the results of the present study in other Great Lakes regions around the world also is examined.

2 | MATERIALS AND METHODS

2.1 | Study area

Lake Victoria is one of the most unique lakes in the world. With a water surface area of 68,000 km², it is the second largest of all freshwater lakes, with a drainage area of over 193,000 km² traversing five East African countries (Twesigye, Onywere, Getenga, Mwakilila, & Nakiranda, 2011). The lake's quality and quantity are maintained by its discharge through the Nile River, combined with recharging from numerous inflowing rivers, including the Nzoia, Yala, Sondu-Miriu and Kuja from Kenya. The lake is a shared resource between Kenya, Uganda and Tanzania, supporting ~30 million people within a catchment population density of 170 people/km² (Njiru et al., 2012). Most of the basin population relies on agriculture, livestock and fishing as the basis for their livelihoods (Odada, Olago, Kulindwa, Ntiba, & Wandiga, 2004).

The lake and its inflows are also sources of water, employment, transport, hydroelectric power and recreation. The vitality of the lake has been adversely affected, however, by waste discharges from various activities around the lake (Njiru et al., 2012). The impacts of pollution from agricultural, municipal and industrial discharges, for example, are visible in some of the rivers draining into the lake and along its shoreline, especially at Port Victoria in Busia and Winam Gulf in Kisumu (Twesigye et al., 2011). Cyanobacteria blooms also are currently becoming an increasingly common phenomenon near the shores of Lake Victoria (Lung'ayia, M'Harzi, Tackx, Gichuki, & Symoens, 2000).

The lower reaches of the Nzoia, Yala, Sondu-Miriu and Kuja rivers formed the basis of this methodological approach since they represent the major river catchments and most notable biodiversity

hotspots around Lake Victoria (Figure 1). The Nzoia and Yala rivers constituted the northern section of the area studied in the present study, with the Kuja and Sondu-Miriu rivers being the southern components. These rivers constitute over 45% of the total influx to the lake (Twesigye et al., 2011). In addition to their rich biodiversity, 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 (Graham, 1929). They are threatened, however, by catchment activities that include conversion of wetlands into farms, urban developments, poor management of domestic and industrial wastes and the leaching of agrochemical residues. These activities result in decreased forest cover and increased soil erosion and river pollution (Balirwa et al., 2003). They also have the potential to compromise primary productivity (e.g., phytoplankton growth) and river water quality, which may

affect fish community structure and human health, both within and downstream of the lake.

2.2 | Phytoplankton Index of Biotic Integrity (PIBI) approach

The PIBI development is based on the presence or absence of phytoplankton data (Appendices A, B). A schematic representation of the criteria used in development of the PIBI within the present study is provided in Figure 2. The schematic representation is comprised of field and laboratory procedures (i.e., data collection of physicochemical parameters and plankton as pollution indicators), PIBI development, and validation using phytoplankton and zooplankton comparisons.

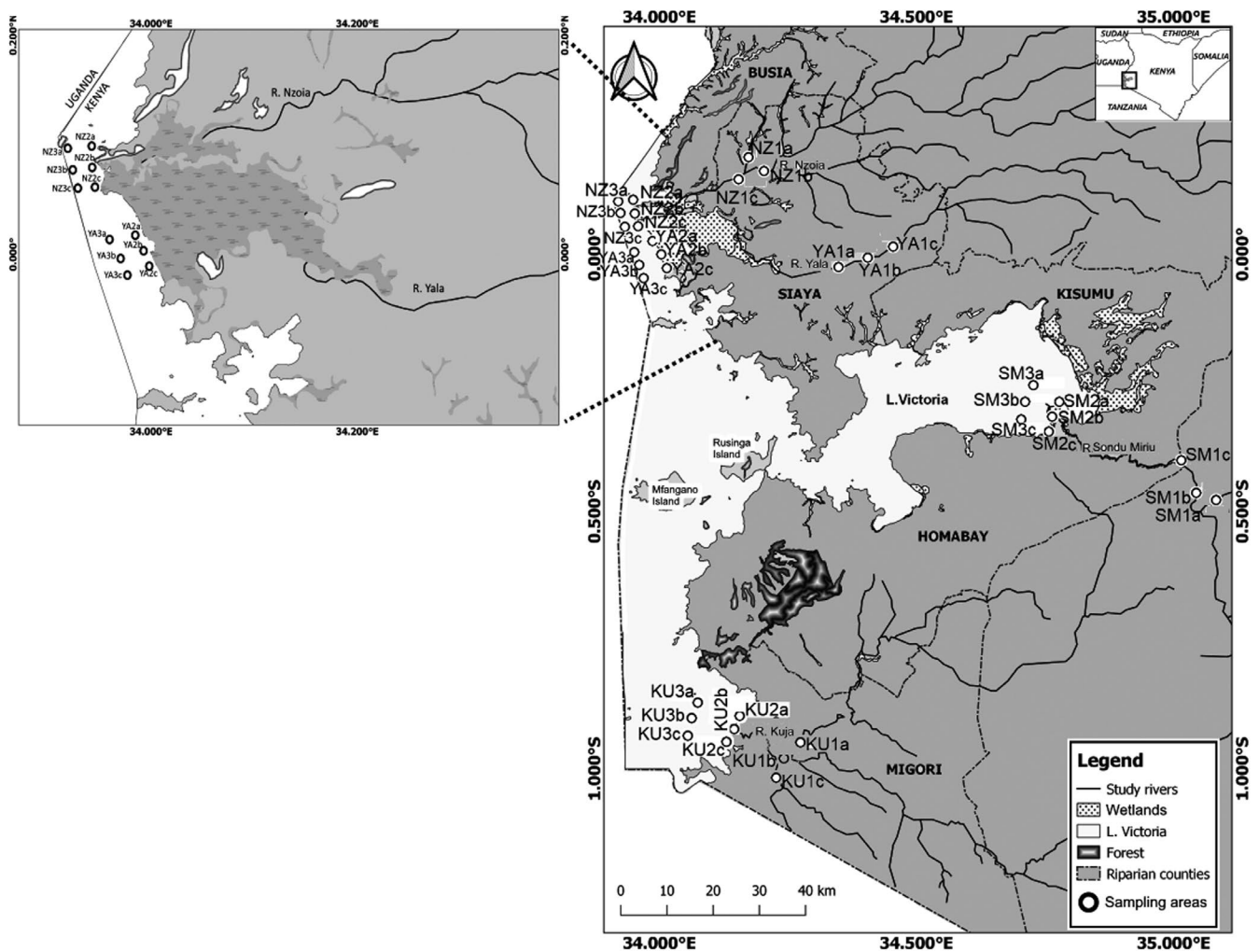


FIGURE 1 Location of study sites for (a) Kuja River (KU), (b) Sondu-Miriu River (SM), (c) Yala River (YA) and (d) Nzoia River (NZ)

Note: sampling sites with at least three replicates as representative of microhabitats included Kuja River: [KU1a, b, c—Kuja River upstream channel; KU2a, b, c—Kuja river mouth before discharge; KU3a, b, c—Kuja river mouth after discharge]; Sondu-Miriu River: [SM1a, b, c—Sondu-Miriu River upstream; SM2a, b, c—Sondu-Miriu river mouth before discharge; SM3a, b, c—Sondu-Miriu river mouth after discharge]; Yala River: [YA1a, b, c—Yala River upstream; YA2a, b, c—Yala river mouth before discharge; YA3a, b, c—Yala river mouth after discharge]; and Nzoia River: [NZ1a, b, c—Nzoia River upstream; NZ2a, b, c—Nzoia river mouth before discharge; NZ3a, b, c—Nzoia river mouth after discharge]

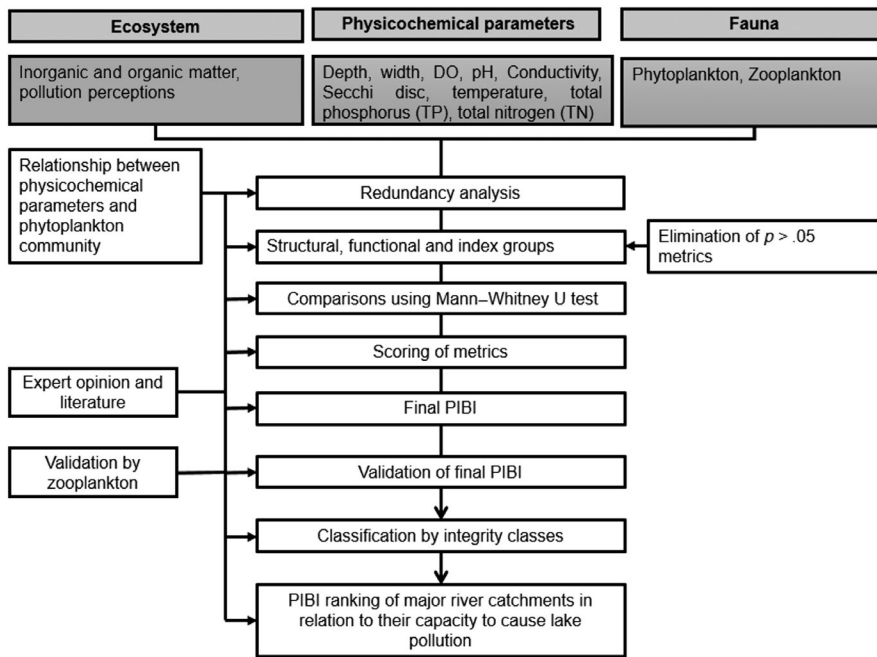


FIGURE 2 Schematic representation of development of a multi-metric Phytoplankton Index of Biotic Integrity (PIBI) for lower reaches of Kenyan portion of Lake Victoria ecosystem

2.2.1 | Field and laboratory procedures

Sampling was conducted in July for the dry season and March for the wet season, from 2016 to 2018. Each sampling expedition took an average of ten days during either July or March for each year from 2016 to 2018, with the involvement of various experts in water quality, plankton and aquatic sciences. Plankton and physicochemical parameter sampling followed a longitudinal transect from an upstream point in each river channel located above and below after a major land-cover or land-use activity (Raburu, Masese, & Aura, 2009) at the lake–river interface before and after discharges (Masese et al., 2013). Various microhabitats, including riffle, pool, run and open waters, were sampled in triplicate (Aura et al., 2010). General environmental observations about each site were noted before each sampling event, including maximum depth, sampling time, weather conditions, site characteristics and global positioning system (GPS) location.

Selected physicochemical parameters were measured using standard methods for in situ data collection and sampling (APHA, 2005). The main physical and chemical parameters (temperature [°C]; dissolved oxygen [DO] concentration; pH; electrical conductivity) were measured with portable electronic water quality meters, with water transparency measured with a standard Secchi disc.

Water samples for nutrient analyses were collected and analysed according to APHA (2005) procedures. The samples were collected directly from the sampling sites using pre-treated 1 L polyethylene sample bottles. They were individually labelled, filled and preserved using sulphuric acid, and stored in cool boxes for laboratory analysis using photometric methods for total nitrogen (TN) and total phosphorus (TP).

Phytoplankton collection and laboratory analytical methods employed by Cocquyt and Vyverman (1994) and Sitoki et al. (2012) were used in the present study. Samples for phytoplankton analyses were

collected from the water surface, with a 25 ml portion of the sample preserved using acidic Lugol's solution. A 2 ml phytoplankton sub-sample was placed in an Utermöhl sedimentation chamber and allowed to settle for at least three hours. Phytoplankton species were identified and counted using a Zeiss Axioinvert 35 Inverted Microscope at 400X magnification. Ten fields of view were counted for the very abundant coccoid cyanobacteria, and 12.42 mm² transects were counted for all other phytoplankton. The entire bottom area of the chamber was examined for the big and rare taxa under low (100×) magnification. Taxa abundances were estimated as biovolumes.

Zooplankton samples were collected and analysed as per methods of Pennak (1953), Edmondson (1959) and Mwebaza-Ndawula (1994), being used to validate phytoplankton occurrences. Zooplankton is the central trophic link between phytoplankton and fish, especially young-of-year fish (Tatrai et al., 1997). This is attributable to the zooplankton peak being observed shortly after that of the phytoplankton (Kundu et al., 2017). Zooplankton sample collection involved using a 1 m long Nansen type plankton net of 60 µm mesh size, with a mouth opening of a 30 cm diameter. The net was hauled vertically through the water column at the river mouths, noting the depth. Water in the flowing rivers was collected with a bucket of known volume and filtered through a 60 µm mesh size. The zooplankton samples were preserved in 5% formalin. Each sample was then filled to a known volume with distilled water in the laboratory, thoroughly shaken to give a uniform distribution, with sub-samples subsequently taken and placed in a counting chamber. Estimates of crustacean zooplankton abundance were made from counts of sub-samples under a Leica dissection microscope using a 25× magnification. Zooplankton densities were determined by taking into account the sample volume, the number of organisms in the sub-sample and the volume of the river/lake water filtered to provide presence–absence data for zooplankton abundances (Appendix A).

2.2.2 | PIBI development

Before developing the phytoplankton metrics, redundancy analysis (CANOCO 4.5 software; Ter Braak & Smilauer, 2002) was used to detect relationships between the physicochemical parameters and the phytoplankton community. This facilitated pin-pointing the physicochemical parameters that may have influenced the lake, as well as explaining the PIBI variations. The strength of the Pearson correlation coefficients between physicochemical parameters and phytoplankton densities of $r \geq 0.7$ was considered to highly likely influence pollution or water quality status of the lake (Raburu et al., 2009).

Classifications into phytoplankton metrics for interpreting community responses to different types of stressors in the lake region consisted of species richness and composition. Tolerance was based on literature from previously studied aquatic ecosystems around the world (Griffith et al., 2005; Lange-Bertalot, 1979, 1980; Ludwig & Reynolds, 1988) and from results on the distribution of taxa in the study area. The selection of metrics for the lake catchment was also based on previous analyses of their relationships with the main environmental gradients and lack of redundancy (Griffith et al., 2005) in response to perturbations.

The collected physicochemical data were compared, using the Kruskal–Wallis one-way ANOVA to examine uncertainty and spatial variations through pair-wise comparisons with other sites. This was done because the data were not normally distributed, and attempts to normalize the data by transformations were unsuccessful. The reference site varied per comparison to accommodate variations of high faunal and floral diversity attributable to the environmental conditions of the adjacent land use in relation to the pollution of the impaired sites (Masese et al., 2013). Data were pooled because of a lack of seasonal variations ($p > .05$) in physicochemical parameters, phytoplankton and zooplankton occurrences, although sampling sites before and after land-use changes were singly considered for analysis.

The PIBI development used the methods suggested by Aura et al. (2010), Aura et al. (2017), Griffith, Kaufmann, Herlihy, and Hill (2001), Griffith, Hill, Herlihy, and Kaufmann (2002), and Griffith et al. (2005). The phytoplankton metrics used in the present study were divided into groups of species richness, composition and tolerance (Table 1). The present study explored the ability of phytoplankton metrics to separate each sampled site from the pair-wise comparison, using Mann–Whitney U non-parametric tests to assess the uncertainty of impaired classification differences with the control classification (Raburu et al., 2009). Potential metrics for PIBI scoring were identified when the tests exhibited significant differences ($p < .05$) in more than two sampled sites in pair-wise comparisons between site groups.

The scoring of metrics was based on a criterion of 5, 3, or 1, depending on whether its value at a site approximated, deviated slightly from or deviated greatly, respectively, from conditions observed at the reference site (Karr et al., 1986). This criterion has commonly been used for fish and macroinvertebrates taxa, rather than 1, 2 and

3, and its similar use could give better comparisons with other faunal IBI (Raburu et al., 2009). The present study used IBI percentiles to establish scoring thresholds based on subdivisions of observed values (Barbour et al. 1996). Threshold values for each selected metric were established at median ranges of 25th and 65th percentiles for the control site. For each metric expected to decrease with pollution levels, values below the 25th percentile were scored as 1 because they exhibited the greatest deviation from the control site. Values between the 25th and 65th percentiles were scored as 3, since they fell within the median ranges for the control sites. Values above the 65th percentile were scored as 5. The scoring was reversed for the metrics expected to increase with pollution levels. The scores for each metric were summed to arrive at the final PIBI value for each sampling site.

Since eight metrics were used, each with a maximum value of five, the highest possible value of 40 served as a benchmark for the three-class scheme, which comprised eight points per classification based on the range and distribution of PIBI scores, and the need to minimize overrating of integrity attributes (Table 2). The maximum scores were used as the threshold values for the pollution–response relationships (Aura et al., 2017, 2010) of the PIBI scores. Integrity measurement classes of good, fair and poor were used in the present study to assess the influence of the major river catchments on the lake pollution, in relation to previous literature (Karr et al., 1986). The thresholds were assigned integrity classes based on field observations enumerated in Table 2, during sampling and from expert opinions, to avoid much deviation from the actual description of the representative sampled sites. During field observations, variables were recorded with the goal of obtaining a detailed description of the catchments, including local scale characteristics (e.g., average stream size; mean depth; discharge; mineral grain size; inorganic matter; tree litter vegetation type) and catchment-scale characteristics (land cover).

The present study employed the SPSS version 21 (SPSS Inc.) and R version 3.5.0 (R Core team 2014) for statistical analyses, with a significance level set at $p < .05$.

2.2.3 | Circumstantial evidence of PIBI performance

The PIBI was validated to establish the level of robustness of the major river catchment rankings in relation to lake pollution and to strengthen the results of the final PIBI. This was undertaken by comparing phytoplankton and zooplankton abundances, as well as comparing the PIBI value for each site against the occurrence of zooplankton. Spearman rho (r^2) analysis compared phytoplankton and zooplankton abundances. The principal component analysis (PCA) was used to explain the variance between PIBI scores and zooplankton abundances. Zooplankton assemblages in correlation with phytoplankton attributes were considered to be circumstantial evidence of the PIBI as a bioindicator of pollution since both zooplankton and phytoplankton occurrences are coupled to environmental conditions (Frontier, 1973). Contaminants impacting fish, birds and humans, for

TABLE 1 Phytoplankton metrics classification and results of Mann-Whitney U tests (as *p* value) based on discrimination using representatives of impaired sites in lower reaches of major Lake Victoria Kenya river catchments (*p* < .05 indicated in bold for more than two cases of pair-wise comparisons)

Metric	Description	<i>p</i> value	Source/explanation
Richness and composition			
Phytoplankton families richness	Number of phytoplankton divisions	0.003	Number highest with good water quality (Stevenson & Bahls, 1999)
Phytoplankton species richness	Number of phytoplankton species/Le	0.001	Number highest with good water quality (Stevenson & Bahls, 1999)
Non-diatom genera richness	Number of non-diatom genera richness	0.01	Decreases with increased pollution, although pollution may shift algal dominance from diatoms to non-diatom taxa attributable to toxic conditions (Steinman, Mulholland, & Kirschtel, 1991)
Total phytoplankton biovolume	Phytoplankton biovolume in 1 L of water	0.03	Number highest with eutrophic waters (Stevenson & Bahls, 1999)
Relative abundance of non-diatom families	Number of non-diatom families/total number of phytoplankton cells	0.06	Decreases with increased pollution, although pollution may shift algal dominance from diatoms to non-diatom taxa attributable to toxic conditions (Steinman et al., 1991)
Relative abundance of diatoms	Number of diatom cells/total number of phytoplankton cells	0.03	Relative abundance of diatoms may decline with increased pollution due to a shift in dominance from diatoms to non-diatom (e.g. Cyanobacteria or Chlorophyta) (Steinman et al., 1991).
Pielou index	Measure of evenness	0.01	Decreases with increased pollution, although a shift in dominance from diatoms to non-diatom (e.g. Cyanobacteria or Chlorophyta) usually may reduce or increase the evenness (Ludwig & Reynolds, 1988)
Shannon-Wiener diversity index (H')	Shannon-Wiener diversity index (H') to log ₁₀ (Dajoz, 2000)	0.07	Decreases with increased pollution, although affected by number of species and their equitability (Ludwig & Reynolds, 1988).
Whittaker index (β _w)	Evaluate taxonomic similarity between site phytoplankton communities	0.01	Phytoplankton species similarity may increase with pollution (Whittaker, 1972) with a shift in similarity from diatoms to non-diatom (e.g. Cyanobacteria or Chlorophyta)
Tolerance			
Diatom abundance	log ₁₀ (no. of diatom cells + 1)/log ₁₀ (expected no. of diatom cells + 1)	0.05	Diatom abundance to decrease with increasing nutrient enrichment and pollution (Pan, Stevenson, Hill, Herlihy, & Collins, 1996).
Relative abundance of <i>Aulacoseira ambigua</i>	Number of <i>A. ambigua</i> cells/total number of phytoplankton cells	0.06	Cosmopolitan diatom tolerant of certain toxic conditions and very tolerant of low nutrient conditions; relative abundance expected to decrease with increasing nutrient enrichment (Griffith et al., 2002).
Diatom biovolume	Diatom biovolume in 1 L of water	0.02	Expected that biovolume, which decreases for both positive and negative deviations from the regional median cell abundance, to decrease with increasing nutrients, organic matter and toxicants (Talling, 1987).

example, have been previously bioconcentrated by phytoplankton and zooplankton (Bruner, Fisher, & Landrum, 1994).

3 | RESULTS

3.1 | Physicochemical parameters

The sampling sites at the river mouths exhibited the highest TP and TN concentrations (Table 3). The Nzoia and Sondu-Miri river mouths had the highest TP (N22: 183.0 ± 15.7 µg/L) and TN (2056.9 ± 1 24.3 µg/L) concentrations, respectively. The lowest TP concentrations (<70 µg/L) were measured at the upstream

areas of the Yala and Kuja rivers. The electrical conductivity and TP and TN concentrations levels generally varied across the sites (Kruskal-Wallis ANOVA; *p* < .05) exhibiting no temporal variations. These parameters exhibited *r* ≥ 7, compared with phytoplankton densities at each sampling site. The highest mean (±SD) electrical conductivity values were also observed at the river mouths, although the Kuja River upstream site exhibited the highest levels (KU1: 162.0 ± 3.0 µS/cm), while the lowest levels were observed for the Nzoia river mouth (YA2: 81.2 ± 8.8 µS/cm). Marked variations (Kruskal-Wallis ANOVA; *p* < .05) in the mean (±SD) depth and mean (±SD) width were observed, while the organic and inorganic matter, and mineral grain size, exhibited gradual differences in types and sizes going downstream.

TABLE 2 Suggested ecosystem integrity classes for final multi-metric Phytoplankton Index of Biotic Integrity (PIBI) for interpretation of pollution level rankings in lower reaches of Kenyan portion of Lake Victoria during study period

Class of integrity	PIBI ranges
GOOD Good water quality; slight pollution characteristics; and some degradation (no human activity within 50 m of riparian zone; no intolerant phytoplankton taxa > 20; bottom substrate dominated by very coarse and coarse, and vegetal materials; clear water with visible substrate)	≥35
FAIR Moderate water quality; significant pollution levels; and degradation (riparian zone > 20 m wide with minimal human activity; natural vegetation maintained along river reach with instream cover > 50%; substrate mainly of coarse and fine material)	27–34
POOR Poor water quality; major/heavy pollution; and degradation (riparian zone < 20; collapsed and eroded sites; human activities include agriculture, water abstraction, urbanization and deforestation; >30% phytoplankton tolerant taxa; bottom dominated by sand and organic materials; water very turbid)	≤26

3.2 | Phytoplankton Index of Biotic Integrity (PIBI)

There were 70 different phytoplankton species identified during the wet season (March 2016, 2017 and 2018) and 65 species during the dry season (July 2016, 2017 and 2018) sampling (Appendix B). Diatom species dominated the river mouths in both the wet and dry seasons being mainly comprised of *Synedra cunningtonii* and *Aulacoseira* spp. The composition of the phytoplankton community by species in the sampled rivers was dominated by diatoms, with averages of 60% in the wet and 55% in the dry seasons, respectively, at most sampling sites. The phytoplankton cells also exhibited high densities in the river mouths for both seasons, particularly the Yala, Sondu-Miriu, Kuja and Nzoia rivers, with $> 100 \times 10^6$ cells/L. Cyanobacteria were common in the dry the season, especially *Anabaena* sp. and *Microcystis* sp. Phytoplankton biovolumes were highest at the Yala River lakeside site in March and July 2017 and in the Sondu-Miriu River ($>16 \text{ mm}^3/\text{L}$). In March, the Nzoia river mouth exhibited the highest biovolume of $>80 \text{ mm}^3/\text{L}$ in March. The phytoplankton biovolume at most sites (YA1, NZ2, NZ3, SM2, SM3, KU1) was $<5 \text{ mm}^3/\text{L}$ in July of 2017 and 2018.

Eight of the 12 selected phytoplankton metrics differed significantly ($p < .05$) between sampling sites and seasons (Table 1). The metrics exhibiting discriminative attributes were incorporated in the development of the PIBI. The metrics indicated no significant differences ($p > .05$) including the relative abundance of non-diatom families, diatom abundance, relative abundance of *Aulacoseira ambigua* (diatom) and the Shannon–Wiener diversity index (H').

For the final PIBI, the Kuja River sampling sites were ranked as the least important catchment sites in influencing lake basin pollution or water quality, noting they exhibited the highest average score (30 out of 40 points) as well as fair water quality (Tables 2, 4). The Nzoia River catchment was ranked as having the most influence on lake basin pollution or water quality (average of 24 out of 40 points). This river exhibited an integrity class of poor water quality and associated heavy pollution characteristics with its PIBI of ≤ 26 points. None of the major river catchments exhibited good water quality and only low pollution characteristics. Notably, the southern rivers ranked better than their northern counterparts, with the Kuja,

Sondu-Miriu, Yala and Nzoia rivers exhibiting the highest to the lowest scores, respectively.

The phytoplankton peaks occurred after the zooplankton peak abundances, or coincided with them in most cases, although with a weak negative relationship ($r^2 \leq 0.50$) (Figure 3a). The principal component analysis (PCA) grouped the relationship between PIBI scores and zooplankton abundances into three groups (Figure 3b). One group consisted of those sampling sites having high PIBI scores (e.g., KU3, SM2, KU2, SM1 and KU1), which exhibited a negative relationship with zooplankton. The second group had sites with fair PIBI scores (e.g., SM3 and YA3) exhibiting a positive relationship with Cyclopoda and Copepoda. The third group consisted of sites with lower PIBI scores (e.g., NZ1, YA1, NZ2, YA2 and NZ3), which exhibited a negative relationship with Cladocera. The PCA explained 94% of the variance between PIBI scores and zooplankton abundances.

4 | DISCUSSION

Lake Victoria was used as a case study because of its multiple sources of pollutants from agricultural fields, industries and domestic sources. These pollutants have turned the lake eutrophic with persistent algal blooms and water hyacinth growths, which has severely degraded its water quality (Njiru et al., 2012). Many of the pollutants are increasingly originating from non-point sources (NPS) in river catchments that ultimately end up in the lake. About 65% of these inputs are in river discharges (Aura, Musa, Njiru, Ogello, & Kundu, 2013; Hecky, Mugidde, Ramlal, Talbot, & Kling, 2010). Discharge inputs have also been observed to lower the lake's dissolved oxygen (DO) concentrations, raise its nutrient concentrations and increase its turbidity (Hecky et al., 2010). Thus, in situ physicochemical and biological longitudinal profile measurements of these response variables can provide insights into the water quality from river catchments discharging to the lake (Okely, Imberger, & Antenucci, 2010). Conservation may be prioritized, with a broader applicability to other African Great Lakes, including those with riverine discharges, by ranking the major river catchments influencing the lake pollution.

TABLE 3 Spatio-temporal mean ($\pm$ SD) values of selected physicochemical variables, and environmental data for Nzoia, Yala, Sondu-Miriu and Kuja rivers, Kenya (* refers to significant p level of Kruskal–Wallis ANOVA, $p < .05$; see Figure 1 for names and initials of study sites)

Parameters	Nzoia River			Yala River		
	NZ1	NZ2	NZ3	YA1	YA2	YA3
Temperature ($^{\circ}$ C)	22.0 $\pm$ 0.0	22.5 $\pm$ 0.0	23.76 $\pm$ 1.2	20.9 $\pm$ 0.0	22.2 $\pm$ 0.0	24.7 $\pm$ 0.0
Electrical conductivity (μ S/cm)	100.0 $\pm$ 0.0	100.0 $\pm$ 0.0	101.0 $\pm$ 1.9	80.0 $\pm$ 0.0	81.2 $\pm$ 8.8	95.0 $\pm$ 4.0
Oxygen concentration (mg/L)	7.4 $\pm$ 0.1	7.5 $\pm$ 0.01	7.2 $\pm$ 0.5	7.5 $\pm$ 0.2	6.0 $\pm$ 0.3	6.0 $\pm$ 1.1
pH	7.6 $\pm$ 0.1	7.6 $\pm$ 0.03	8.3 $\pm$ 0.4	7.7 $\pm$ 0.0	7.1 $\pm$ 0.0	8.5 $\pm$ 0.4
Secchi depth (m)	0.9 $\pm$ 0.1	0.8 $\pm$ 0.0	0.7 $\pm$ 0.1	0.9 $\pm$ 0.3	0.9 $\pm$ 0.5	0.8 $\pm$ 0.1
TP concentration (μ g/L)	182.5 $\pm$ 14.5	183.0 $\pm$ 15.7	170.1 $\pm$ 54.1	107.2 $\pm$ 3.6	65.3 $\pm$ 7.3	63.4 $\pm$ 15.5
TN concentration (μ g/L)	1,533.9 $\pm$ 127.9	1,442.4 $\pm$ 41.6	1,504 $\pm$ 10.0	1,721.8 $\pm$ 383.8	1,030.9 $\pm$ 82.0	1,139.9 $\pm$ 59.5
Average width (m)	10.1 $\pm$ 2.5	7.4 $\pm$ 1.6	74.5 $\pm$ 9.5	8.5 $\pm$ 0.40	6.2 $\pm$ 2.0	50.3 $\pm$ 6.6
Mean depth (m)	9.7 $\pm$ 1.3	6.2 $\pm$ 1.6	4.4 $\pm$ 0.2	3.4 $\pm$ 1.1	4.2 $\pm$ 1.0	5.7 $\pm$ 0.4
Inorganic matter	Gravel	Gravel	Mud	Gravel	Sandy	Silt
Organic matter	Very coarse	Very coarse	Fine	Coarse	Coarse	Fine
Vegetation litre	Fallen leaves	Fallen leaves	Detritus	Absent	Fallen leaves	Detritus

Pollution of major riverine catchments from anthropogenic activities can cause longitudinal changes in water quality and habitat conditions that can significantly influence a lake (Masese et al., 2013). Industrial discharges, use of riparian areas, silt and sewage discharges, and agricultural activities have affected the water quality at the sampling sites in the Lake Victoria basin (Aura et al., 2019). The highest TN and TP levels at the river mouths of Lake Victoria that are causing increased nutrient enrichment in the lake are attributable to increased anthropogenic activities in its basin and the nature of the bay (Reynolds, Huszar, Kruk, Naselli-Flores, & Melo, 2002). Scientific evidence of nutrient enrichment

in Lake Victoria basin has been further reported by Lung'ayia et al. (2000), Lung'ayia, Sitoki, and Kenyanya (2001) and Gikuma-Njuru and Hecky (2005).

The strong Pearson correlation coefficients ($r \geq 7$) of TP, TN and electrical conductivity levels, in comparison with the phytoplankton community, illustrated the influence of increased nutrient concentrations and conductivity on the water quality status of the lower reaches of Lake Victoria. This could be attributed to the semi-closed nature of the Winam Gulf, coupled with upstream anthropogenic activities. The gulf has a limited water exchange with the main basin of Lake Victoria (Calamari, Akech, & Ochumba, 1995), which could

TABLE 4 Metrics and scoring criteria (system) for development of multi-metric Phytoplankton Index of Biotic Integrity (PIBI) for ranking major river catchments for pollution status in the lower reaches of Kenyan portion of Lake Victoria (maximum score of 40 points is used; see Figure 1 for names and initials of study sites)

Phytoplankton metrics	Nzoia River			Yala River			Sondu-Miriu River			Kuja River			Scoring criteria		
	NZ1	NZ2	NZ3	YA1	YA2	YA3	SM1	SM2	SM3	KU1	KU2	KU3	5	3	1
Phytoplankton families richness	3	3	3	3	3	3	3	5	3	3	5	3	>5	4–2	<2
Phytoplankton species richness	3	3	3	3	3	3	5	3	3	5	3	5	<20	21–40	>45
Non-diatom genera richness	1	1	1	3	1	1	3	5	3	3	3	5	<10	11–20	>20
Total phytoplankton biovolume	3	3	5	5	3	5	5	5	5	5	5	5	<4.0	4.1–6.5	>6.6
Relative abundance of diatoms	5	3	5	3	3	5	5	3	5	5	5	5	>50	25–50	<25
Pielou index	3	3	3	3	3	3	1	1	3	1	3	3	>3.5	3.4–2.0	<2.0
Whittaker index	3	3	3	3	3	3	3	5	5	3	5	3	<2.0	3.6–2.0	>3.6
Diatom biovolume cells	3	3	3	3	5	5	1	3	1	3	3	1	<6.0	6.1–7.9	>8.0
Site PIBI	24	22	26	26	24	28	26	30	28	28	32	30			
River PIBI	24			26			28			30					

Sondur-Miriu			Kuja River			p
SM1	SM2	SM3	KU1	KU2	KU3	Site
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
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*
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
7.9 ± 0.1	7.7 ± 0.0	7.7 ± 0.5	8.0 ± 0.1	7.9 ± 0.1	7.8 ± 0.2	0.06
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
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*
1,606.8 ± 411.0	2,056.9 ± 124.3	1,513.3 ± 107.1	1,218.7 ± 334.1	911.5 ± 12.9	1586.2 ± 322.2	0.04*
4.5 ± 1.3	4.8 ± 0.8	68.9 ± 7.3	4.2 ± 1.7	5.1 ± 2.0	89.8 ± 10.6	0.03*
1.2 ± 0.0	0.7 ± 0.1	0.9 ± 1.1	1.9 ± 0.9	6.0 ± 2.1	9.0 ± 1.9	0.01*
Sandy	Sandy	Silt	Sandy	Sandy	Mud	
Fine	Fine	Fine	Fine	Fine	Fine	
Detritus	Absent	Detritus	Detritus	Fallen leaves	Detritus	

worsen the water quality in the lake and the basin at large. In most cases, the impacts of eutrophication attributable to phytoplankton blooms are probably diminished by dilution effects resulting from surface seiches (Haande et al., 2011), which distinguishes the Winam Gulf from the numerous more open bays around Lake Victoria.

The nearshore variation in phytoplankton community in relation to nutrient and electrical conductivity levels could also be attributable to variations in river inflows versus concentrations, direct wastewater discharges, deforestation and agricultural activities, among others (Huisman, Matthijs, & Visser, 2005). These factors are beyond the scope of the present study, but merit further analysis, mainly because stream ecology could present mixed findings in regard to phytoplankton occurrence. There is currently much debate among researchers over the suitability of descriptors and indices based on taxonomic recognition of the phytoplankton species (Basset et al., 2012; Gaedke, 1992). Where the phytoplankton are not growing under nutrient limited conditions, for example, flow and dilution could be the main driving forces for phytoplankton species composition and abundance in lotic systems. As such, they may not be useful as indicators of nutrient conditions in the upper catchment. Such factors necessitate the need for a multi-metric index incorporating various phytoplankton, morphology and physicochemical attributes that can affect phytoplankton occurrence in riverine systems, in order to obtain a comparative assessment of nutrient load impacts. Notably, the current study would add to the literature of other studies that have indicated how the abundance distribution of the phytoplankton communities can vary significantly in relation to human-generated or environmental forcings (Sabetta et al. 2008).

Further, the marked variations in mean depths and widths for the selected rivers may very likely have affected the PIBI in the current study. This is because small rivers with a high nutrient concentration may be thought to relate to poor water quality and low PIBI. In contrast, a relatively unpolluted river with low nutrient concentrations

and a high PIBI may be associated with high nutrient loads to the lake and, therefore, have a higher influence on lake water quality than a small river with a high nutrient concentration. Thus, since IBI accommodates all such metrics, it is recommended that nutrient loads be incorporated in future PIBI efforts.

Nevertheless, the present study provides preliminary findings on the use of phytoplankton for classifying major river catchments in the Great Lakes region of Africa in regard to their impact on lake water quality. Phytoplankton communities in the river and in-lake sites responded to catchment variability, as reflected in the spatial variability of functional and structural metrics used as pollution indicators in the present study. Thus, PIBI has potential as a decision support tool that can be adopted and adjusted in the future for lake basin management aimed at changing the pollution status to achieve a sustainable holistic approach to ecosystem conservation.

Statistically similar phytoplankton metrics, which included relative abundance of non-diatom families, diatom abundance, relative abundance of *A. ambigua* (diatom) and Shannon–Wiener diversity index, highlighted the indiscriminate longitudinal occurrence of such attributes from upstream to the river mouths. Non-diatom families consisted of chlorophyceae (*Scenedesmus* spp., *Botryococcus* sp., *Ankistrodesmus falcatus* and *coelomonol* spp.), Zygnematophyceae (*Cosmarium* and *Straurastrum paradoxum*) and Euglenophyceae (*Phacus* spp., *Euglena* spp., *Trachelemonous* and *Strombomonous* spp.), which were most frequently encountered in the present study, being consistent with the results of Talling (1987), who noted such phytoplankton species dominated the Lake Victoria basin.

Similar to the present study, however, several researchers also reported differences in space and time for diatoms and phytoplankton species, richness and biovolume, which are linked to nutrient variations, pollution levels and water mixing (Gikuma-Njuru & Hecky,

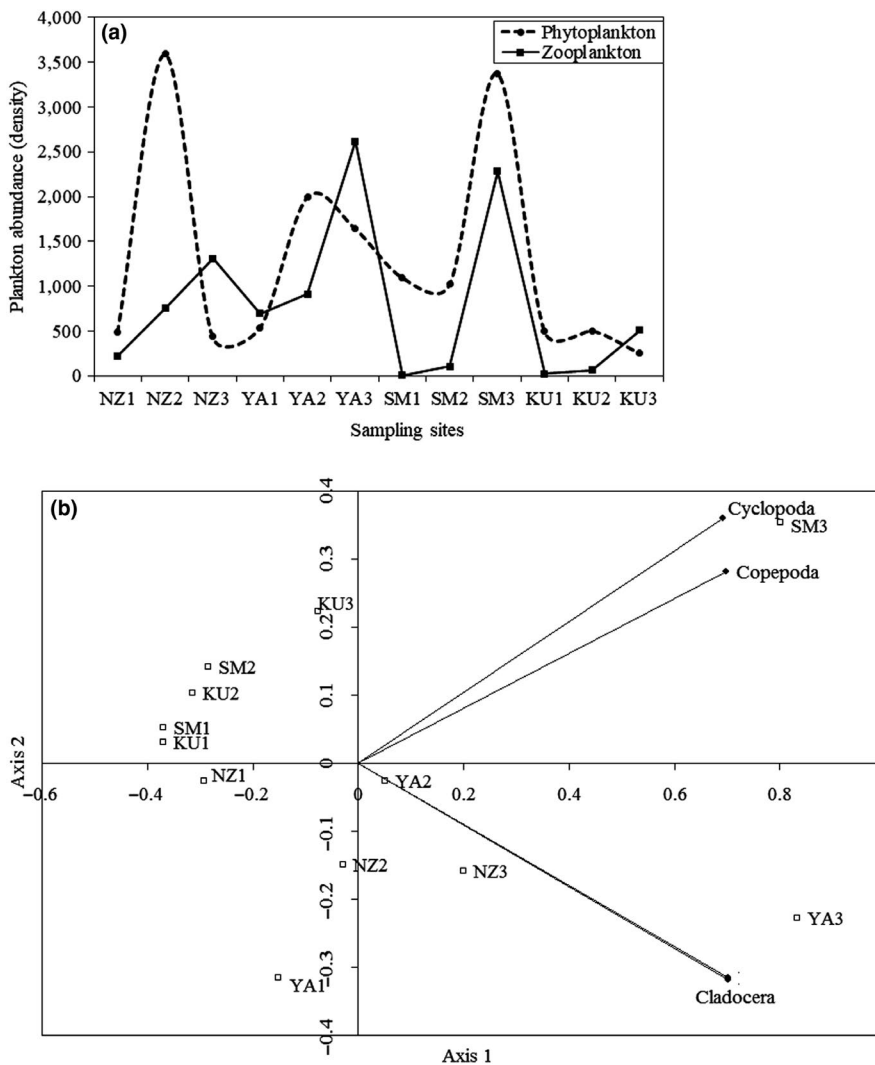


FIGURE 3 Validation of multi-metric Phytoplankton Index of Biotic Integrity (PIBI) scores based on phytoplankton and zooplankton relationships in lower reaches of Kenyan portion of Lake Victoria catchment, using (a) correlation between phytoplankton and zooplankton abundance, and (b) biplots for sampling sites PIBI and zooplankton abundances

2005; Haande et al., 2011; Lung'aya et al., 2000, 2001). The phytoplankton biovolume was found to be relatively low, compared to observed nutrient concentrations (Haande et al., 2011). As a result of the robustness of such findings, the order of PIBI values of major river catchments of the Kuja, Sondu-Miriu, Yala and Nzoia rivers from the highest to the lowest scores, respectively, illustrates the gradient of influence on the pollution status of the lake basin from north to south. The lack of good water quality in the integrity classes indicates the lake basin is deteriorating because of anthropogenic activities on a longitudinal scale. This is because, with an index of suitability descriptions and scores such as those recorded in the present study, any slight differences in the scores could indicate a large variation in ecosystem status across the basin (Raburu et al., 2009).

Peaks in phytoplankton and zooplankton abundances were only weakly associated (Figure 3a), seemingly suggesting zooplankton occurred as a trophic link between phytoplankton and fish (El-Bassat, 2007; Mwebaza-Ndawula, 1994). The PIBI scores and zooplankton abundance groups in the PCA were dependent on the highest, fair to lowest scores, as per the rankings of the sampling sites. The 94%

proportion of the PCA explanation of variance between PIBI scores and zooplankton abundances provided circumstantial evidence of the phytoplankton approach.

5 | CONCLUSIONS

The present study provides preliminary results for development of an Index of Biotic Integrity approach in the region based on phytoplankton attributes. The PIBI developed ranked the order of the Kuja, Sondu-Miriu, Yala and Nzoia rivers from the highest to lowest, respectively. The PIBI corresponded closely with the coupling of water quality, phytoplankton attributes and anthropogenic influences. Further, the findings of the integrity classes for the major river catchments in the region ranged from fair in the upper reaches to poor water quality in the lower reaches of the Lake Victoria drainage system. Thus, the present study offers a potential candidate decision support tool for more effective management of the lake basin.

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APPENDIX A

Zooplankton list for major catchments of Kenyan portion of Lake Victoria basin (+, presence; blank, absence during sampling period)

Order	Species	Nzoia River			Yala River			Sondur-Miriu River			Kuja River		
		NZ1	NZ 2	NZ 3	YA1	YA 2	YA 3	SM1	SM2	SM3	KU1	KU2	KU3
Copepoda	<i>Copepod nauplius</i>	+	+	+		+	+	+	+	+	+	+	+
	<i>Calanoida</i>					+							
Cyclopoida	<i>Cyclopoida</i>	+	+	+	+	+	+	+	+	+	+	+	+
Cladocera	<i>Diaphanosoma excisum</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Moina micrura</i>	+	+	+	+	+	+		+	+			+
	<i>Daphnia barbata</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Daphnia lumhortzi</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Bosmina longirostris</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Ceriodaphnia cornuta</i>					+	+		+	+			
	<i>Chydorus sp</i>									+			
Rotifer	<i>Brachionus calyciflorus</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Brachionus angularis</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Brachionus caudatus</i>	+	+	+	+	+	+		+	+	+	+	+
	<i>Brachionus patulus</i>					+	+			+			+
	<i>Brachionus falcatus</i>	+		+	+		+			+			+
	<i>Trichocerca sp</i>	+								+			+
	<i>Filinia sp</i>	+		+	+	+	+			+			+
	<i>Asplanchna spp</i>	+		+	+	+	+			+			+
	<i>Polyathra spp</i>	+		+	+	+	+			+			+
	<i>Lecane spp</i>				+					+			

APPENDIX B

Phytoplankton list for major catchments for Kenyan portion of Lake Victoria basin (+, presence; blank, absence during sampling period)

Family	Genus	River Kuja			River Sondur-Miriu			River Yala			River Nzoia		
		KU1	KU 2	KU3	SM1	SM2	SM3	YA1	YA2	YA3	NZ1	NZ2	NZ3
Chlorophyceae	<i>Ankistrodesmus falcatus</i>			+	+			+		+		+	
	<i>Botryococcus braunii</i>		+		+				+			+	
	<i>Coelastrum microphorum</i>	+	+	+							+		
	<i>Coelomon merostoides</i>									+			
	<i>Coelomon regularis</i>		+	+		+	+			+			
	<i>Coelomon vestitus</i>									+			
	<i>Crucigenia sp</i>		+		+		+		+	+			+
	<i>Crucigenia tetrapedia</i>								+	+			
	<i>Crucigenia triangulare</i>			+						+			
	<i>Crucigenia quadrata</i>									+			
	<i>Crucigenia excarata</i>									+			+
	<i>Crucigenia meneghiana</i>							+		+			
	<i>Kirchnella contrata</i>									+			+
	<i>Kirchnella lunaris</i>				+	+	+			+		+	
	<i>Pediastrum tetras</i>					+				+			
	<i>Scenedesmus acuminatus</i>	+	+	+	+	+				+			+

(Continues)

TABLE 1 (Continued)

Family	Genus	River Kuja			River Sondu-Miriu			River Yala			River Nzoia		
		KU1	KU 2	KU3	SM1	SM2	SM3	YA1	YA2	YA3	NZ1	NZ2	NZ3
	<i>Scenedesmus incrassatulus</i>					+	+	+	+	+		+	
	<i>Scenedesmus obliquus</i>					+	+	+		+		+	
	<i>Scenedesmus rusboskii</i>					+		+		+			
	<i>Scenedesmus maximus</i>					+				+		+	+
	<i>Schroederiella africana</i>		+			+				+			
	<i>Schroederia setigera</i>					+				+			+
	<i>Tetradron arthromisforme</i>					+		+	+	+			
	<i>Tetradron muticum</i>		+			+				+	+		
	<i>Tetradron trigonum</i>					+				+		+	
	<i>Tetredron arthromisforme</i>					+				+			
	<i>Oocystis burgei</i>					+				+			
	<i>Oocystis sp</i>					+				+			
	<i>Anabaena cirinalis</i>		+							+			
	<i>Anabaena flos-aqua</i>		+			+							
	<i>Anabaenopsis taganyikae</i>					+	+					+	
	<i>Aphanocapsa pulchra</i>							+		+			
	<i>Aphanocapsa rivularis</i>												
	<i>Aphanothece sp</i>					+	+	+	+				
	<i>Chroococcus dispersus</i>	+	+		+	+	+	+	+		+		
	<i>Chroococcus limnetica</i>	+			+	+	+					+	
	<i>Chroococcus turgidus</i>	+	+		+							+	
	<i>Cylindrospermopsis africana</i>					+				+	+	+	
	<i>Merismopedia tenuissimma</i>							+	+	+	+	+	
	<i>Microcystis aeruginosa</i>				+								
	<i>Microcystis wasenbergii</i>	+			+	+		+	+		+		
	<i>Plankolyngbya tallingii</i>	+				+	+						
	<i>Planktolingbya circumcreta</i>		+				+					+	+
	<i>Planktolingbya contrata</i>		+										+
	<i>Planktolingbya limnetica</i>		+					+		+			
	<i>Romeria ankensis</i>						+	+	+	+			
	<i>Romeria elegans</i>						+		+			+	+
Bacillariophyceae (diatoms)	<i>Amphora ovaris</i>	+						+	+	+			+
	<i>Amphora sp</i>	+			+			+	+		+		+
	<i>Aulacoseira ambigua</i>		+		+			+	+	+	+		+
	<i>Aulacoseira nyansensis</i>			+				+	+	+			+
	<i>Aulacoseira schroederia</i>							+	+		+		+
	<i>Characium pyriforme</i>	+						+	+			+	+
	<i>Chodatella armata</i>			+				+	+	+			+
	<i>Cyclotella kutzingiana</i>	+						+	+	+			+
	<i>Cymbella cistula</i>		+	+	+	+		+	+				+
	<i>Cymbella sp</i>							+	+				+
	<i>Diatoma hemiale</i>	+	+	+	+								+
	<i>Navicula granatum</i>	+							+		+		+
	<i>Navicula lancenolata</i>	+									+		+
	<i>Navicula muticum</i>									+		+	+
	<i>Navicula salicuta</i>					+				+			+
	<i>Navicula sp</i>		+	+	+	+	+			+			+

(Continues)

TABLE 1 (Continued)

Family	Genus	River Kuja			River Sondu-Miriu			River Yala			River Nzoia		
		KU1	KU 2	KU3	SM1	SM2	SM3	YA1	YA2	YA3	NZ1	NZ2	NZ3
	<i>Nitzschia sub-acicularis</i>			+								+	+
	<i>Nitzschia palea</i>		+	+									+
	<i>Nitzschia recta</i>		+				+						+
	<i>Stephanodiscus athiopica</i>												+
	<i>Stephanodiscus sp</i>								+		+	+	+
	<i>Surillella affins</i>	+											+
	<i>Surillella effusa</i>					+						+	+
	<i>Surillella linearis</i>	+											+
	<i>Surillella ovaris</i>	+											+
	<i>Surillella bisenata</i>		+										+
	<i>Synedra cunningtonii</i>		+						+				+
	<i>Synedra ulna</i>		+	+									+
	<i>Pinnularia gibba</i>		+		+								+
	<i>Fragillaria pinnatta</i>	+											+
	<i>Fragillaria aethiopica</i>												+
	<i>Fragillaria sp</i>				+				+				+
	<i>Fragillaria sublienata</i>					+		+		+	+	+	+
Dinoflagellates	<i>Glenoridinium pernardii</i>				+				+		+		
Euglenophyceae	<i>Eugena acus</i>			+	+	+	+	+			+		+
	<i>Euglena viridis</i>									+	+	+	
	<i>Phacus longicauda</i>		+					+		+		+	
	<i>Phacus pleuronectes</i>	+											
	<i>Phacus sp</i>	+	+			+	+	+		+	+		
	<i>Strombonous sp</i>	+				+							
	<i>Trachelemonous armata</i>						+	+		+	+	+	
	<i>Trachelomonas volvocina</i>						+	+					
Zygnematophyceae	<i>Closterium navicula</i>								+				
	<i>Cosmarium menenghiana</i>	+								+	+		
	<i>Cosmarium regnesi</i>	+		+		+				+		+	
	<i>Cosmarium sp</i>												+
	<i>Straurastum paradoxum</i>	+			+			+			+		+