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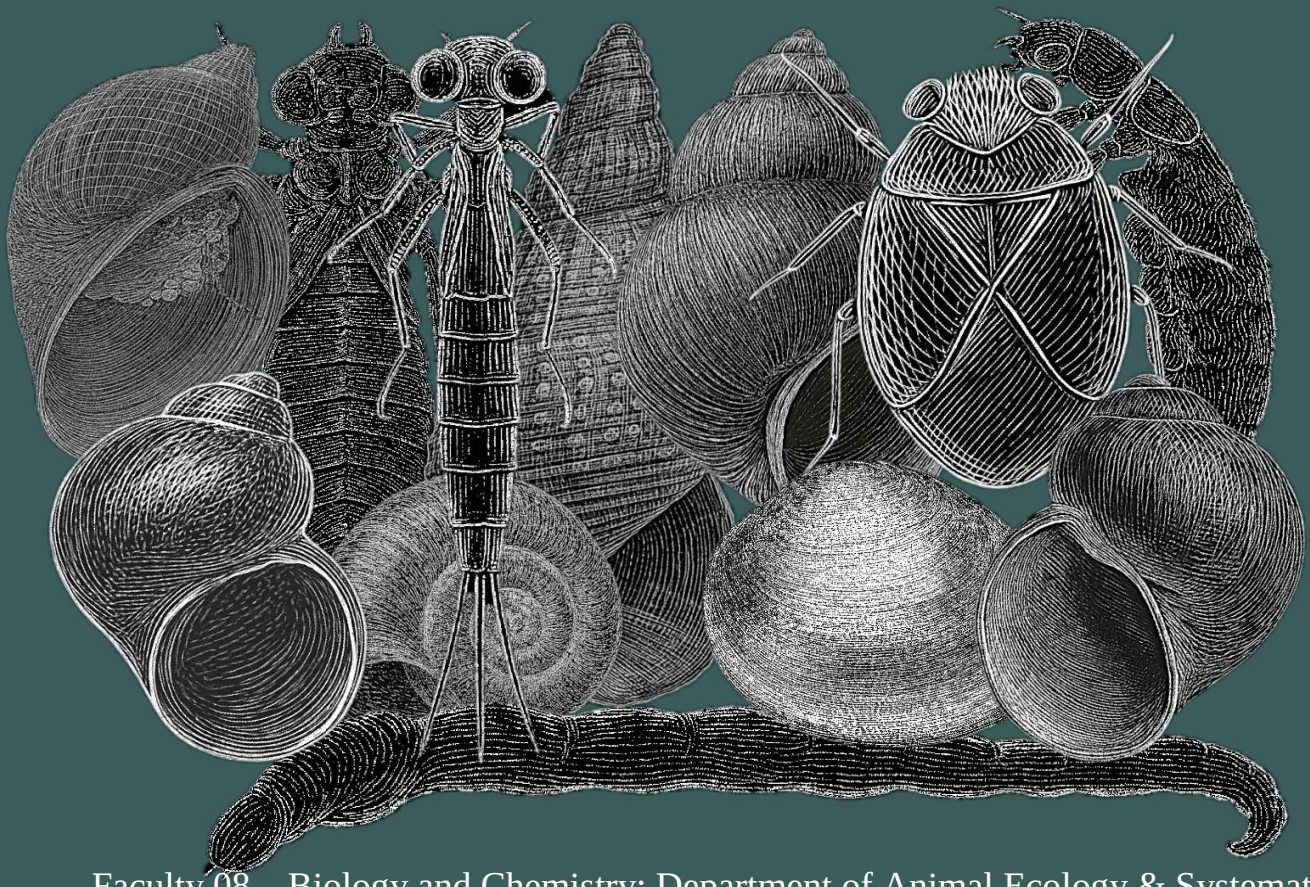
Impact of Anthropogenic and Natural Disturbances on Biodiversity Patterns of Macroinvertebrates at different Taxonomic Levels in African Freshwater Systems



Faculty 08 – Biology and Chemistry; Department of Animal Ecology & Systematics

Dissertation for the award of the degree *Doctor rerum naturalium* (Dr. rer. nat.)

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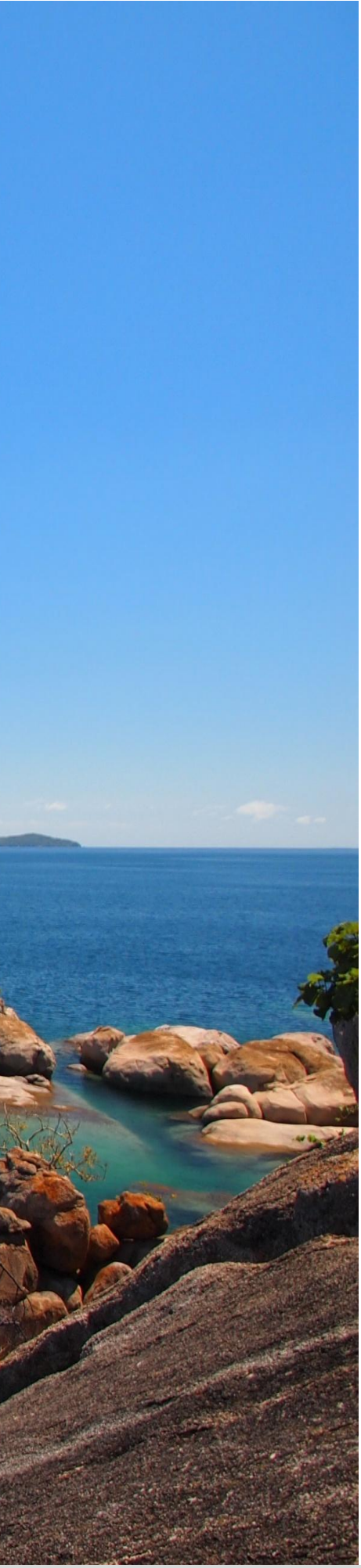

DOCTORAL THESIS

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‘‘Every species is a masterpiece, exquisitely adapted to its place; who are we to destroy or even diminish biodiversity?’’

E.O, Wilson, Biologist

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I. ABSTRACT

Abstract

African freshwater ecosystems are subject to both natural and anthropogenic pressures that affect habitat quality, biodiversity, and ecosystem functioning. Macroinvertebrates have been used in routine bioassessments to detect disturbances for decades. However, it is unclear how accurately they reflect disturbances at different taxonomic levels. This thesis investigates the environmental impact of disturbances in selected East African freshwater ecosystems, and how macroinvertebrates respond to these changes at different temporal and spatial scales. The effects of dam construction and the effectiveness of family-level bioindication for detecting them were tested on the Ruzizi River. Multimetric indices (MMIs) were used in Lake Kivu to assess their ability to identify and distinguish between various environmental pressures. Mollusc community indices were used to assess long-term environmental changes in six Albertine volcanic lakes. Additionally, the freshwater snail *Gabbiella* was tested as a model taxon to reconstruct historical hydrological shifts and water level fluctuations across the African Great Lakes, major connected river systems, and the crater lakes of western Uganda based on phylogenetic and biogeographical analyses. Finally, an eDNA index was used at Lake Albert to test its applicability in biodiversity surveys and use in African freshwater biomonitoring systems. The results showed that on the Ruzizi River, family-level indicators did not adequately capture the impact of the dams, while on Lake Kivu multimetric indices more effectively distinguished between stressed and reference sites. Pollution patterns varied across the lake, with greater impacts in the northern basin than in the central and southern basins. Phylogenetic and biogeography indices proved useful in detecting historical disturbances. The diversification of *Gabbiella* mirrored earlier water-level changes, a trend also reported in other African molluscs and fish groups. A series of vicariance and dispersal events shaped the biogeographic history of *Gabbiella humerosa*, and allowed tracing historical drainage changes during the Pleistocene. However, eDNA analysis yielded limited records of macroinvertebrates, as plankton dominated because only surface water was sampled. The lack of a reference library makes it difficult to accurately assign eDNA sequences. Nevertheless, the results underscore the potential of using aquatic eDNA for taxonomy-free biomonitoring in tropical regions. The results of the historical molluscan biodiversity change in the six volcanic barrier lakes studied in this thesis show that new species have emerged and others have disappeared over time. Invasive taxa were common, with crayfish and non-native snails among those displacing native species. The biogeography of most Albertine volcanic barrier lakes exhibits a Nilotic affinity, except for invasive species and other taxa with wide distributions. The current findings provide a useful basis for conservation efforts and inform strategies to slow the ongoing loss of biodiversity.

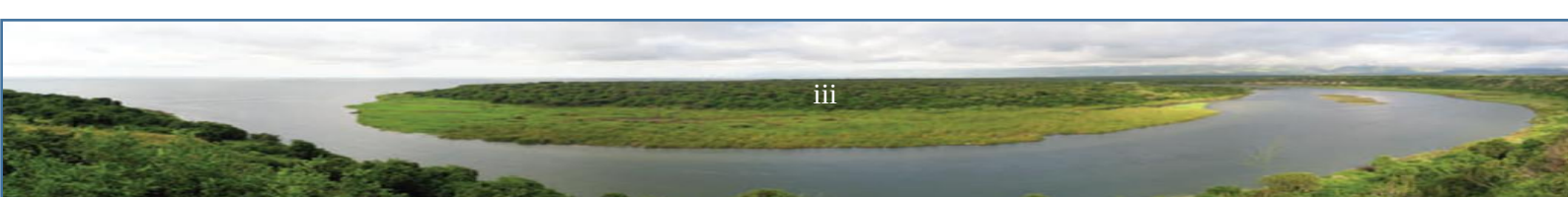
Zusammenfassung

Afrikanische Süßwasserökosysteme sind sowohl natürlichen als auch anthropogenen Belastungen ausgesetzt, die sich auf die Lebensraumqualität, die Biodiversität und die Funktionsweise der Ökosysteme auswirken. Seit Jahrzehnten werden Makroinvertebraten in routinemäßigen Bioassays eingesetzt, um Störungen zu erkennen. Es ist jedoch unklar, wie gut sie Störungen auf verschiedenen taxonomischen Ebenen widerspiegeln. Diese Arbeit untersucht die Umweltauswirkungen von Störungen in verschiedenen (ausgewählten) ostafrikanischen Süßwasserökosystemen und wie Makroinvertebraten auf diese Veränderungen auf verschiedenen zeitlichen und räumlichen Skalen reagieren. Die Auswirkungen des Dammbaus und die Wirksamkeit der Bioindikation auf Familienebene für deren Erkennung wurden am Ruzizi-Fluss getestet. Multimetrische Indizes (MMIs) wurden im Kivu-See angewendet, um ihre Fähigkeit zur Identifizierung und Unterscheidung zwischen mehreren Umweltbelastungen zu bewerten. Indizes der Mollusken-Gemeinschaft wurden verwendet, um langfristige Umweltveränderungen in sechs vulkanischen Seen des Albertine-Rifts zu bewerten. Darüber hinaus wurde die Süßwasserschnecke *Gabbiella* als

Modelltaxon getestet, um historische hydrologische Veränderungen und Wasserstandsschwankungen in den afrikanischen Großen Seen, den wichtigsten verbundenen Flusssystemen und den Kraterseen im Westen Ugandas auf der Grundlage phylogenetischer und biogeografischer Analysen zu rekonstruieren.

Schließlich wurde ein eDNA-Index am Albertsee verwendet, um seine Anwendbarkeit in Biodiversitätserhebungen und seine Verwendung in afrikanischen Süßwasser-Biomonitoringsystemen zu testen. Die Ergebnisse zeigten, dass am Ruzizi Fluss die Indikatoren auf Familienebene die Auswirkungen der Dämme nicht angemessen erfassten, während am Kivu-See multimetrische Indizes effektiver zwischen belasteten und Referenzstandorten unterschieden. Die Verschmutzungsmuster variierten über den See hinweg, wobei die Auswirkungen im nördlichen Becken größer waren als im zentralen und südlichen Becken. Phylogenetische und biogeografische Indizes erwiesen sich als nützlich für die Erkennung historischer Störungen. Die Diversifizierung von *Gabbiella* spiegelte frühere Wasserstandsänderungen wider, ein Trend, der auch bei anderen afrikanischen Mollusken- und Fischgruppen beobachtet wurde. Eine Reihe von Vikarianz- und Ausbreitungsereignissen prägte die biogeografische Geschichte von *Gabbiella humerosa* und ermöglichte es, historische Veränderungen der Entwässerung während des Pleistozäns nachzuvollziehen. Die eDNA-Analyse lieferte jedoch nur begrenzte Detektion von Makroinvertebraten, da Plankton dominierte, vermutlich weil nur Oberflächenwasser beprobt wurde. Das Fehlen einer Referenzbibliothek erschwert zudem die genaue Zuordnung von eDNA-Sequenzen. Dennoch unterstreichen die Ergebnisse das Potenzial der Verwendung von aquatischer eDNA für taxonomiefreies Biomonitoring in tropischen Regionen. Die Ergebnisse der historischen Veränderung der Mollusken-Biodiversität in den sechs vulkanischen Barriere-Seen, die in dieser Arbeit untersucht wurden, zeigen, dass im Laufe der Zeit neue Arten eingewandert und andere verschwunden sind. Invasive Taxa waren weit verbreitet, darunter Krebse und nicht heimische Schnecken, die einheimische Arten verdrängten. Die Biogeografie der meisten vulkanischen Barriere-Seen im Albertine-Gebirge weist eine nilotische Affinität auf, mit Ausnahme invasiver Arten und anderer Taxa mit weit verbreiteter Verbreitung.

Die Erkenntnisse dieser Forschungsarbeiten bilden eine nützliche Grundlage für Naturschutzbemühungen und liefern Informationen für Strategien zur Verlangsamung des anhaltenden Verlusts der biologischen Vielfalt.



II. SYNTHESIS

1. Introduction

1.1. Major anthropogenic and natural stressors on African freshwater biodiversity

Freshwater systems across Africa are home to exceptional biodiversity and play a vital role in ecological processes (Salzburger et al., 2014; Cohen et al., 2019). They also sustain millions of people who rely on these waters for food, income, and daily needs (Pettinotti et al., 2018; Lawrence et al., 2023). Today, these ecosystems are under severe pressure and many are in decline (Cohen et al., 2019; Lawrence et al., 2023). Tectonic events and climatic fluctuations shaped many African freshwater regions (Beadle, 1981; Van Damme & Pickford, 2003; Salzburger et al., 2014; Fouchy et al., 2019). The African Great Lakes (AGL), which formed through tectonic activities are thus susceptible to various natural hazards affecting their ecological health (Balagizi et al., 2018; Ross et al., 2014). Furthermore, climate change, driven by both natural processes and human activities, has severely impacted the AGL, resulting in declining lake water levels and in some cases, the complete drying up of some lakes (Danley et al., 2012; Lyons et al., 2015; Beverly et al., 2022). Climatic cycles of drought and moisture during the Pleistocene therefore reshaped the topography, periodically influencing the diversification processes, speciation and extinction of species (Salzburger et al., 2014; Meier et al., 2023). In particular, volcanic and seismic activity have significantly affected Lake Kivu (Ross et al., 2014; Balagizi et al., 2018). The lava flow from the Nyiragongo and Nyamulagira volcanoes have repeatedly invaded the northern basin of the lake in Kabuno Bay, affecting organisms in the littoral zone (Balagizi et al., 2018; Dusabe et al., 2024). Lake Kivu is also vulnerable to seismic events and degassing incidents, which can precipitate limnic overturns (Balagizi et al., 2018; Rouwet et al., 2021). Due to its permanent stratification and large stores of dissolved gases, such as CO_2 and methane, Lake Kivu poses risks similar to those of the limnic eruptions recorded in Cameroon's Nyos and Monoun lakes (Schmid et al., 2005; Ross et al., 2014; Balagizi et al., 2018; Rouwet et al., 2021). On the other hand, the discovery and subsequent exploration of oil and gas deposits in lakes Edward and Albert has raised environmental concerns, particularly regarding the potential for oil leaks and their impact on aquatic fauna (Verheyen, 2016; Nakiyende et al., 2023). This highlights the interaction between natural and human-induced pressures on biodiversity. Human impacts are visible at multiple levels. At a local scale, for example, they may result from land use practices within catchments, whereas at a broader level, they are linked to global drivers such as climate change (Salzburger et al., 2014). Among the most pressing stressors are climate variability, habitat change, different forms of pollution, and the spread of invasive species (Mpopetsi et al., 2025). A clear example is the introduction of the North American crayfish *Procambarus clarkii* into volcanic and crater lakes of western Uganda and northern Rwanda. Its high reproductive capacity and strong predatory habits have disrupted native communities, allowing it to outcompete and displace local species (Twardochleb et al., 2013; Madzivanzira et al., 2020).

Furthermore, many East African rivers have been dammed to supply growing local population with electricity (Amasi et al., 2021). These dams often alter the rivers' hydrological regimes, destroy habitats, and hinder bidirectional gene flow, thereby harming biodiversity (Martinez et al., 2013; Wang et al., 2019). On the Ruzizi River, for instance, there is a series of dams (Figure 1), almost all of which were constructed without an environmental impact assessment (Muvundja et al., 2022). Moreover, the riparian habitats of many African water bodies are being destroyed by pollution, constant municipal and industrial discharges, and other human activities observed along the shores (Wronski et al., 2015; Dusabe et al., 2022; Oyege et al., 2024). Such pressures degrade aquatic systems by lowering water quality and availability, altering community composition, and reducing biodiversity overall (Wronski et al., 2015; Dusabe et al., 2022). Yet, despite these challenges, the combined effects of natural and human disturbances on African freshwater biodiversity remain poorly understood. This

thesis examines those impacts in the AGL, their major inflowing rivers, the volcanic barrier lakes of western Uganda and Rwanda, and the crater lakes of western Uganda. By pinpointing the main threats and documenting how aquatic communities respond, the study provides a foundation for refining bioassessment tools (Masese et al., 2023).

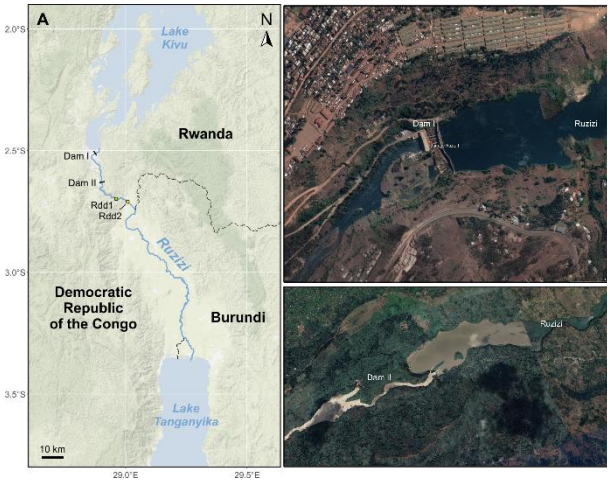


Figure 1. A: Ruzizi River, outflow of Lake Kivu and main tributary of Lake Tanganyika, B: Dam I and C: Dam II (Adapted from Dusabe et al. (2022).

1.2. The use of bioassessments as a means of detecting and quantifying ecological disturbance

Benthic macroinvertebrates are widely used to detect ecological change because they are common, relatively immobile, and closely tied to local conditions. (Dusabe et al., 2019; Masese et al., 2023). Many benthic macroinvertebrates live long enough to reflect changes over time, making them useful for detecting ecological disturbances that chemical analyses may miss (Martinez et al., 2020). Their presence or absence can be translated into tolerance scores, where sensitive taxa are given higher weights and tolerant taxa receive lower ones (Kaaya et al., 2015; Masese et al., 2023). Because of these characteristics, macroinvertebrates are widely used to build indices that measure the ecological integrity of freshwater systems (Ndatimana et al., 2023). Although biotic indices have been applied extensively in African rivers and streams, comparatively little work has been done on using macroinvertebrate-based multimetric indices in lakes (Ndatimana et al., 2023). A range of indices has been applied in these studies, including diversity measures (Arimoro & Keke, 2017; Olawusi-Peters & Ajibare, 2014), country- or region-specific systems such as the South African Scoring System Version 5 (SASS5; Dickens & Graham, 2002), the Tanzania River Scoring System (TARISS; Kaaya et al., 2015), and the Zambian Invertebrate Scoring System (ZISS; Dallas et al., 2018). The use of multimetric indices (MMIs) has become an established practice in ecological research and monitoring over the past decades (Lakew & Moog, 2015; Ndatimana et al., 2023; Gelaw et al., 2024). Their strength lies in combining several ecological measures, which makes them sensitive to different aspects of water quality decline (Edegbene et al., 2019a,b; Masese et al., 2023). Although diversity and biotic indices are widely used at the national or regional level, studies evaluating their effectiveness and applicability are limited (Masese et al., 2023). Even so, most bioassessment approaches still rely on family-level identification (Dallas, 2021; Mezgebu, 2022; Masese et al., 2023). Assessing the effects of disturbances on macroinvertebrate diversity often demands identification at the species level, as closely related taxa may vary in their tolerance to environmental stress (Macher et al., 2016; Mezgebu, 2022). However, separating larvae and pupae of many freshwater groups is challenging, which frequently results in coarse taxonomic assignments or even misidentifications (Sweeney et al., 2011). In this context, DNA barcoding offers a practical way to address these identification challenges (Dusabe et al., 2024; Tumwebaze et al., 2022). Furthermore, indices based on tolerance or biotic integrity may fail to reflect past disturbances, since benthic invertebrate patterns are also influenced by the geology and hydrology of the area (Daniels et al., 2015; Ortiz-Sepulveda et al., 2020; Mahulu et al., 2021).

Phylogenetic relationships and biogeographic indices, however, indicate valuable insights into historical disturbance and the evolutionary history of species (Mahulu et al., 2021; Ortiz-Sepulveda et al., 2020; van der Merwe et al., 2021). Therefore, this study sought to test the efficacy and applicability of family-level diversity indices,

family-level biotic and multimetric indices, species-level mollusc-based indices, species level phylogenetic and biogeographic indices, and eDNA in detecting both natural and anthropogenic disturbances.

1.3. Spatio-temporal trends in macroinvertebrate biodiversity in African freshwater systems

Spatial and temporal trends in macroinvertebrate biodiversity often reflect long-term disturbances in aquatic ecosystems (Dungo et al., 2024; Jiang et al., 2021). These trends are critical for identifying and understanding ecological processes, predicting species distributions, and informing conservation and restoration strategies (Garcia et al., 2024). One of the most important metrics for biodiversity assessment is species richness, because it plays a key role in ecology and biogeography when evaluating changes in biodiversity over spatial and temporal scales (Hillebrand et al., 2018; Sousa-Guedes et al., 2020). According to Wiens (2011) and Knapp et al. (2017), species richness changes over time in response to environmental changes. Changes in habitat and climate can lead to an expansion or contraction of species' ranges, thereby altering the number of species in a region (Van Damme & Pickford, 2003). The composition of freshwater macroinvertebrate communities can change over time due to various species shifts (Bae & Kim, 2024; Rocha Pompeu et al., 2023). Biodiversity patterns change over time. Species may become extinct, new species may become established, and the composition of communities may change as a result (Menezes et al., 2015; Liu et al., 2023). During the early Pleistocene, the uplift of the Rwenzori Mountains divided the palaeolake Obweruka into a northern and a southern basin (Van Damme & Pickford, 2003). This led to the extinction of many species that are no longer found in its successors, lakes Edward and Albert (Van Damme & Pickford, 2003). Similarly, very few mollusc species survived the Pleistocene drought in Lake Malawi (Van Damme & Gotier, 2013). Meanwhile, rising water levels in Lake Rukwa caused it to spill into Lake Tanganyika, allowing an exchange of faunal taxa that had previously been restricted to only one of the lakes (Cohen et al., 2013). In Lake Kivu, species composition in the north may also have shifted following lava flows from the Nyiragongo and Nyamulagira volcanoes, which entered the littoral zone (Balagizi et al., 2018). Non-native species that colonize new habitats may become established and turn out to be invasive (Alpert et al., 2000; Keller et al., 2011). Invasive species often exert strong ecological pressures on native communities, influencing biodiversity across both space and time (Soto et al., 2024). Their effects are thought to be most pronounced in the early stages of an invasion, gradually diminishing as systems adjust over time (Strayer, 2012). However, in African freshwater systems, little is known about how invasions affect the spatial and temporal patterns of macroinvertebrate diversity. Tracking ecosystem condition across different places and through time is important because it can reveal broader ecological trends and indicate points where change becomes critical (Shen et al., 2024; Zhang et al., 2025).

Insights of this kind are vital for developing conservation and management approaches that protect both biodiversity and the ecological processes that support it (Hillebrand et al., 2018). For this research, I focus on a set of lakes Kivu, Bunyonyi, Mutanda, Mulehe, Ruhondo, and Burera as case studies. Looking at these systems side by side makes it possible to see how natural processes and human activities come together to shape biodiversity, and how those effects play out at different scales. The results point to several drivers that stand out as particularly influential, and they also provide a practical basis for suggesting ways to slow biodiversity loss while supporting the long-term resilience of these freshwater ecosystems.

1.4. Implications for the conservation and management of African freshwater systems

The interconnected issues of declining freshwater biodiversity and growing human pressure on the freshwater resources necessitate a comprehensive

approach to reverse environmental degradation and safeguard the essential services provided by these ecosystems (Net et al., 2009; Birnie-Gauvin et al., 2023; Dudgeon & Strayer, 2025). Effective sustainable conservation plans need to strike a balance between human needs and biodiversity conservation (Net et al., 2009). Such plans provide a structured, efficient, and scientifically robust framework for achieving conservation goals, by identifying critical areas and putting the right measures in place (Birnie-Gauvin et al., 2023; Dudgeon & Strayer, 2025). However, conservation assessments of African freshwater systems remain uneven, which can give a misleading picture of how severe certain threats are to habitats and species (Dudgeon & Strayer, 2025). In many cases, aquatic taxa have not been formally described, and for several groups there is too little information on their distribution or abundance to evaluate their conservation status (Dudgeon & Strayer, 2025). Examining past habitat and diversity shifts helps explain current conditions and anticipate future scenarios. (Willis et al., 2007). Nevertheless, African freshwater systems and their associated biota continue to be threatened by natural and human activities (Fouchy et al., 2019). This scenario highlights the urgent need for conservation actions to mitigate the decline in freshwater biodiversity (Tickner et al., 2020; Albert et al., 2021; Dudgeon & Strayer, 2025).

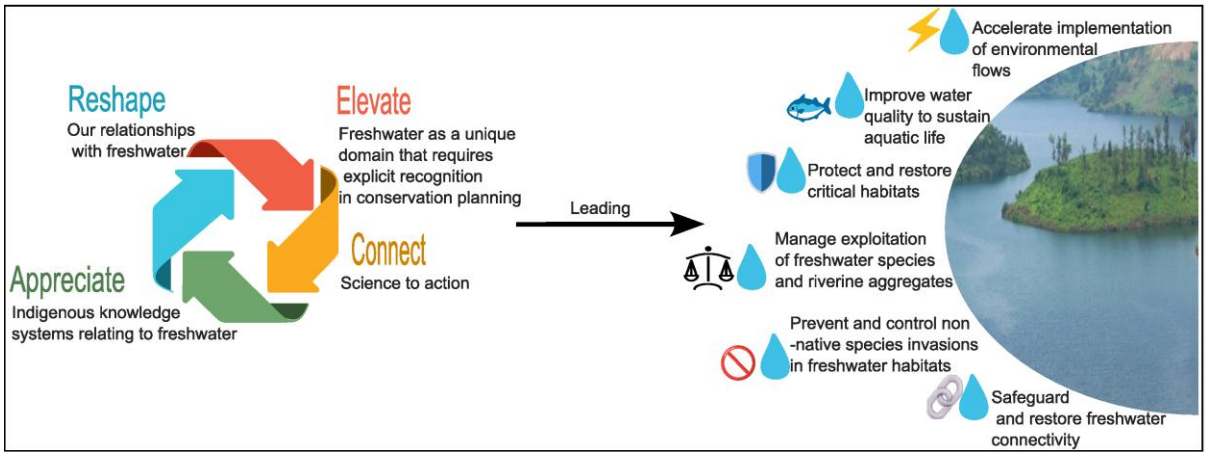


Figure 2. Conservation measures to reduce biodiversity loss. On the left are four actions to build relationships with water (Birnie-Gauvin et al., 2023), which lead to the implementation of the actions on the right. Showing six actions for the recovery of freshwater ecosystems (Tickner et al., 2020).

Freshwater research across the continent faces many challenges. These challenges reduce the quality of scientific studies and make it difficult to develop and implement effective conservation measures (Lawrence et al., 2023). Many African water systems are shared by more than one country. This makes them difficult to manage and protect. There is a lack of relevant data, differences in national governance and legal frameworks, and issues surrounding data and information sharing (Lawrence et al., 2023). In 2020, a global emergency recovery plan was introduced to address the decline of freshwater biodiversity. The plan outlines concrete actions to support restoration (Tickner et al., 2020; see Figure 2). Based on the principles of sustainable water management, the plan includes general guidelines for implementing these actions (Dudgeon & Strayer, 2025). Implementing such measures is considered a means of slowing and potentially reversing biodiversity loss in African freshwater systems (Dudgeon & Strayer, 2025). Furthermore, there is mounting evidence that influencing people's perceptions, values, and behaviors is vital for advancing conservation actions (Maynard et al., 2020; He et al., 2022). A set of four actions was put forward with the aim of raising awareness of freshwater and ensuring that people recognize, value, and conserve water (Birnie-Gauvin et al., 2023; Figure 2). In order to

protect freshwater biodiversity and ecosystem functions in Africa, it is crucial to understand how species react to environmental disturbances. This knowledge is key to effective management and conservation (Dudgeon & Strayer, 2025).

2. Scope & Aims

2.1. Research gap and rationale

The African Great Lakes (AGL) and their surrounding catchments provide critical resources at local, regional, and global levels (Plisnier et al., 2023; Lawrence et al., 2023) and support a rich diversity of species, numbering in the thousands (Salzburger et al., 2014). However, these biologically important resources, are being degraded by natural disturbances and anthropogenic stressors (Wienhues et al., 2024; Dusabe et al., 2024; Dusabe et al., 2022). Tectonic and topographic changes, combined with climate-driven cycles of drought and moisture, have temporarily caused the loss of aquatic habitats and altered the dispersal rates of aquatic organisms in AGL and their catchments (Danley et al., 2012; Ivory et al., 2016; Wienhues et al., 2024). All AGL are subject to water level fluctuations, with some drying up completely, e.g. Lake Victoria (Wienhues et al., 2024), Lake Albert (Karp et al., 2012; Berke et al., 2014) and Lake Malawi, which remained a small pond in the northern catchment area for several years (Johnson et al., 2016; Ivory et al., 2016). While the effects of hydrological changes on fish such as cichlids are well documented, little is known about the macroinvertebrates of the AGL and its catchments. Moreover, although several researchers have labelled Lake Kivu as a species-poor lake due to its exposure to natural disturbances (Sarmento et al., 2007; Snoeks et al., 2012), the impact of these disturbances on macroinvertebrates in the littoral zone of the Kivu basins remains largely unknown. Furthermore, the role of the tributaries in contributing to the lake's biodiversity is not well understood. It remains to be investigated whether their impact depends on the catchment areas to which they belong. The Ruzizi River is influenced by several human activities, including dam construction, and its biodiversity may also be affected by the inflow of saline water from Lake Kivu, though the magnitude of this effect remains uncertain (Muvundja et al., 2022). Gaining insight into the diversification of freshwater biota in the AGL, and their catchments requires examining how both recent and historical environmental changes have contributed to habitat loss and degradation (Salzburger et al., 2014). In this study several volcanic barrier lakes are examined, including Mutanda, Mulehe, Bunyonyi, Burera and Ruhondo. These lakes have been affected by natural disasters and human activities, particularly the introduction of *P. clarki*. Understanding how biological communities respond to changes in aquatic ecosystems is a prerequisite for the developing tools to assess the ecological status of water bodies (Masese et al., 2023). Monitoring programs often depend on biological indicators since, unlike physico-chemical measures, they capture the effects of human disturbances operating at different scales and across both short and long time frames (Garg et al., 2022). Recent trends indicate that most macroinvertebrate-based bioassessment systems rely on family-level identification (Dallas, 2021; Mezgebu, 2022). In this study, the Ruzizi River served as a case example for testing how well family-level bioindication performs in a river with relatively low diversity. Multimetric indices (MMIs) derived from benthic macroinvertebrates are also gaining traction in Africa, though in some cases they are applied without prior testing or validation (Masese et al., 2023; Ndatimana et al., 2023). Lake Kivu was therefore used as a model lake to test the applicability of the MMIs. It is important to understand species-level identity when assessing the influence of natural and human activities on macroinvertebrate communities because even closely related species may differ in their tolerance to stressors (Macher et al., 2016; Mezgebu, 2022). However, species level determination is often very challenging particularly when dealing with larval or pupae. This can reduce taxonomic resolution and lead to misidentifications (Sweeney et al., 2011; Haase et al., 2010), which may produce misleading results.

For molluscs, there is a comparatively well-established literature on identification by morphological methods (see Mandahl-Barth, 1988; Brown, 1994). In combination with DNA barcoding, these methods offer reliable taxonomic identification and a broader tolerance range, making molluscs the ideal indicator group (Alhejoj et al., 2017). In the case of the AGL, major connected rivers and crater lakes in western Uganda, the *Gabbiella* snail was used as an indicator species to study historical natural disturbances. Given that the taxonomic crisis remains a common challenge as many taxa in African waters are still unstudied or unknown. Analyzing whole aquatic communities using eDNA offers a promising approach for assessing ecosystem health, and Lake Albert was used here as a model to demonstrate this concep

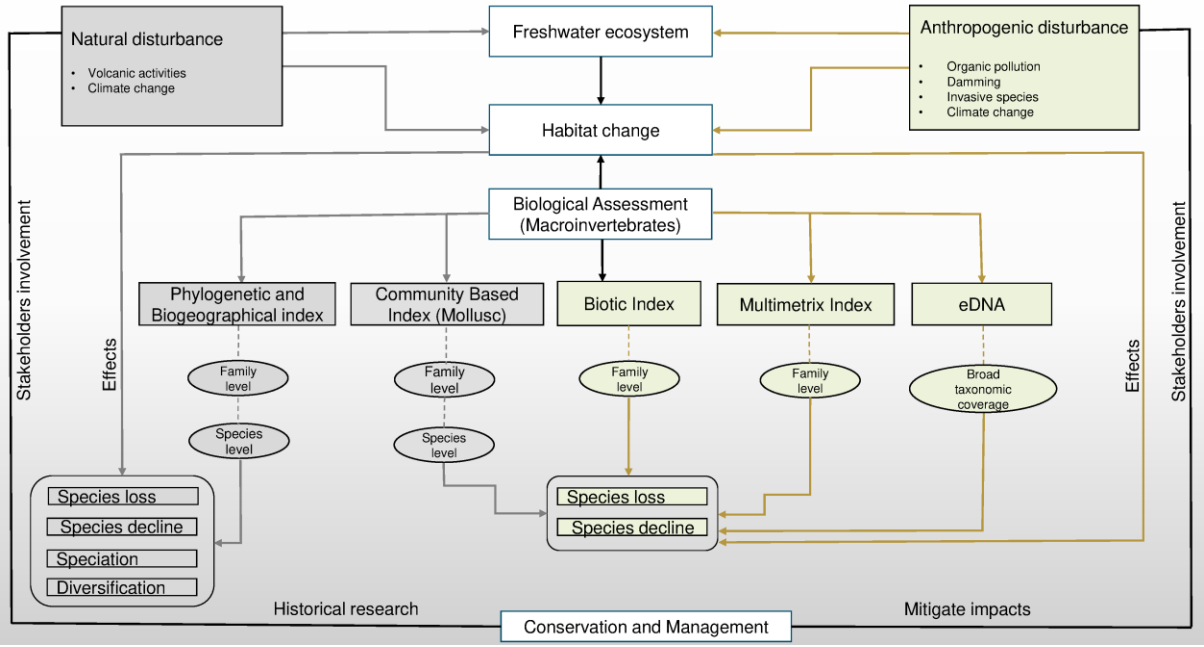


Figure 3. Overview diagram illustrating the core idea of the study, highlighting the natural and anthropogenic disturbances to the freshwater ecosystem, the various bioassessment methods based on macroinvertebrates at different taxonomic levels, and the need to involve stakeholders in conservation.

2.2. Thesis aims

Specifically this thesis aimed to:

2.2.1. Identify the influence of anthropogenic and natural disturbances on macroinvertebrates diversity patterns,

2.2.2. Identify the effectiveness of taxonomic surrogate in detecting disturbances-driven changes in macroinvertebrate biodiversity patterns,

2.2.3. Test the spatial and temporal patterns of macroinvertebrates diversity in relation to varying environmental conditions across different aquatic ecosystems

3. Materials and Methods

In this study, macroinvertebrates were collected from the Ruzizi River (see Figure 4, Dusabe et al., 2022), rivers draining into Lake Kivu on the Rwandan side, and Lake Kivu itself (Hyangya et al., 2022; Dusabe et al., 2024; Ndatimana et al., accepted). Molluscan groups were sampled in volcanic barrier lakes, including lakes Mutanda, Mulehe, Bunyonyi, Burera and Ruhondo (Ssenkuba et al., in final preparation). The type localities of *Gabbiella* spp. were sampled in lakes Tanganyika, Kivu, Edward, Albert, Victoria, Malawi and Turkana; the Semuliki and Victoria Nile Rivers; the Awasi stream; and the western Ugandan crater lakes (Dusabe et al., in revision). The surface water sample for the eDNA was taken from Lake Albert (Bálint et al., 2024). Macroinvertebrates were collected by hand-picking them from stones and rocks, or by scooping samples from soft substrates in various habitats (Dusabe et al., 2022). On the Ruzizi River, samples were collected from sites upstream and downstream of Dams I and II (Dusabe et al., 2022). Sites at Manda/Ngomo, and Kamanyola, which are located further downstream were selected as reference sites because they are considered to be the least impacted by the dams. In other lakes and rivers, specimens were picked by hand from hard substrates in shallow water, such as stones and rocks (Dusabe et al., 2024). For soft substrates, scoop nets and dredges were used. To reach deeper parts of the littoral zone (down to 40 m), an Ekman grab was employed. All specimens were preserved in 80% ethanol. Macroinvertebrates, except molluscs, were identified to the family level (Dusabe et al., 2022; Hyangya et al., 2022; Ndatimana et al., accepted), while molluscs were identified to the genus or species level (see Dusabe et al., 2024; Dusabe et al., in revision). Various biodiversity indices were calculated (Dusabe et al., 2022; Hyangya et al., 2022; Dusabe et al., 2024; Ndatimana et al., accepted) and different multimetric indices were tested to discriminate between levels of site disturbance (see Hyangya et al., 2022; Ndatimana et al., accepted). For molluscs, morphological identification was supplemented with DNA barcoding.

Genetic material was extracted either from whole ethanol-preserved specimens or from foot tissue, using the CTAB protocol (Wilke et al., 2006) and the DNeasy Blood and Tissue Kit following the manufacturer's instructions. Up to five markers, including both mitochondrial and nuclear genes, were then amplified (Dusabe et al., 2024, Bálint et al., 2024, Dusabe et al. (in revision). Sanger sequencing was performed on an ABI 3730xl DNA analyser with the BigDye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, Germany). Additional sequences for all genetic markers were downloaded from the NCBI GenBank database to complement the new sequences. Phylogenetic relationships were reconstructed using maximum likelihood (ML) and Bayesian inference (BI) methods. Prior to the analyses, jModelTest version 2.1.10 (Darriba et al., 2012) was used to select the best-fitting substitution models. Divergence time estimation was performed using BEAST version 1.8.4 (Drummond et al., 2012). The haplotype network was calculated using TCS version 1.21 (Clement et al., 2000; connection limit = 95%). Uncorrected genetic *p*-distances between groups of different *Gabbiella* species and *Gabbiella humerosa* subspecies were calculated using MEGA 11 (Tamura et al., 2021). To infer the biogeographic history of the *G. humerosa* group (see Figure 8), the BioGeoBEARS R package (version 1.1; Matzke, 2013a, b) was used within the R statistical environment (version 3.5.1; R Core Team, 2024). For more details, see Dusabe et al. (in revision). In the eDNA study, the extraction of both DNA and RNA was carried out in parallel from the lysed filter membranes, utilizing the ZymoBIOMICS DNA/RNA Miniprep Kit (Cat. No. R2002, Zymo Research Europe GmbH, Freiburg, Germany) in accordance with the manufacturer's guidelines. DNA extracts were amplified using Euka02 and miCOIint/jgHCO2198 primers with AmpliTaq Gold™ Master Mix (Thermo Fisher Scientific, Waltham, MA, USA). For more details, see Bálint et al. (2024). Sequence variant diversity and community composition were examined to identify differences among sampling sites and between shoreline and pelagic habitats.

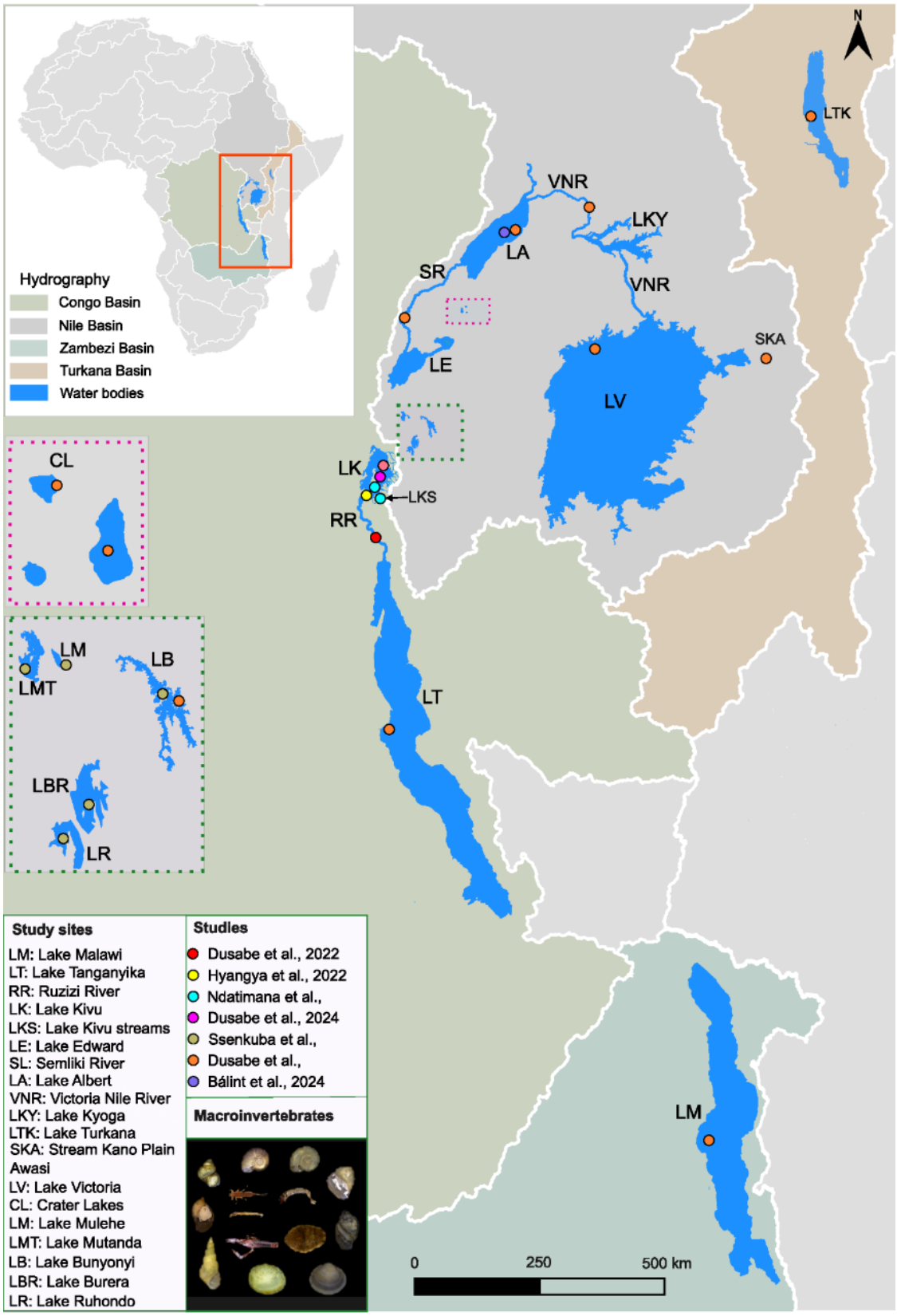


Figure 4. Shows the areas where macroinvertebrates were collected: 1. Ruzizi River (Dusabe et al., 2022), 2. Lake Kivu (Hyangya et al., 2022; Dusabe et al., 2024; Ndatimana et al., accepted), 3. Three western Ugandan volcanic barrier lakes (Lakes Mutanda, Mulehe and Bunyonyi); and two northern Rwandan volcanic barrier lakes (Lakes Burera and Ruhondo): (Ssenkuba et al., in final preparation), 4. Seven African Great Lakes; Lake Bunyonyi; the Victoria Nile River; the Semuliki River; a stream in the Kano Plain in Awasi, Kenya–Kisumu (Dusabe et al., in revision), 5. Lake Albert (Bálint et al., 2024). Each study area is represented by a different colour.

4. Publication outlines

This thesis consists of seven peer-reviewed publications that provide insights into the above objectives and are listed as follows:

Paper 1. Dusabe, M. C., Neubauer, T. A., Muvundja, F. A., Hyangya, B. L., & Albrecht, C. (2022). Family-level bio-indication does not detect the impacts of dams on macroinvertebrate communities in a low-diversity tropical river. *Frontiers in Environmental Science, 10,* 902246. https://doi.org/10.3389/fenvs.2022.902246

This study examines the effects of dam construction on the diversity of macroinvertebrates in the Ruzizi River, both upstream and downstream. Five diversity indices based on the Tanzania Rivers Scoring System (TARISS) were calculated using family-level bio-indication: Shannon-Wiener index, Simpson index, Pielou's Evenness, Rare Family Prevalence (RFP) and Average Score Per Taxa (ASPT). Upstream and downstream of the dams, macroinvertebrate communities showed low species richness, probably linked to limited habitat diversity, fluctuating water levels, changes in temperature and food availability, and additional human pressures. A considerable portion of the river's discharge originates in Lake Kivu, a system that supports comparatively low species richness due to its geological history and episodes of volcanic disturbance.

These same processes continue to shape the lake's limnological characteristics (Jones, 2021). This study found little to no impact of the dams on downstream Ruzizi River's macroinvertebrates, likely because both the upstream and downstream sites are equally disturbed and dominated by taxa with moderate to high pollution tolerance. Community structure at the less impacted downstream sites showed distinct patterns. Diversity was somewhat greater in the vicinity of Dam II compared with Dam I, likely due to the direct effect of proximity to Lake Kivu, located upstream of Dam I, and the fact that Dam II is 30 years younger. For future studies, it is recommended that species-level bio-indicators be used to obtain a more precise, and ecologically relevant assessment, especially in a river with such low diversity.

Contribution of MCD: lead author; I collected data, performed the laboratory identification and drafted the article.

Paper 2. Hyangya, B. L., Kankonda, A. B., Dusabe, M. C., Murhimanya, J. D. K., Kaningini, B. M., & Masilya, P. M. (2022). Choice of benthic macroinvertebrate-based metrics for assessing water quality in the littoral zone under anthropogenic disturbance in southern Lake Kivu (East Africa). *Ecohydrology, 15,* e2468. https://doi.org/10.1002/eco.2468

In this study, 26 parameters were evaluated and organized into four groups: (i) macroinvertebrate community composition and diversity, (ii) measures of disturbance tolerance and pollution sensitivity, (iii) indicators of trophic status, and (iv) biotic indices such as the Average Score Per Taxon (ASPT), the Biological Monitoring Working Party (BMWP), and the Family Biotic Index (FBI). Box-and-whisker plots were then used to assess how well these parameters distinguished reference sites from disturbed sites along the southern shore of Lake Kivu. Six parameters (the number of EPT taxa, the % Diptera, the % Chironomidae, %Ephemeroptera, the % EPT, and the FBI) proved sensitive and could distinguish between reference and disturbed sites. Conversely, metrics based solely on diversity indices, such as taxonomic richness and the Shannon–Wiener and Simpson indices, were unable to distinguish between sites based on disturbance levels. This is similar to what was observed when assessing the impact of dams on the Ruzizi River (Dusabe et al., 2022). This is likely because the differences between the fauna of the two site categories (reference and disturbed sites) are not very pronounced. Therefore, they can be more clearly observed at the lower taxonomic level than at the family level

with regard to the number of taxa present in the reference and disturbed sites. Since metrics derived from benthic macroinvertebrate assemblages are able to differentiate nearshore lake habitats across disturbance gradients, from relatively undisturbed to heavily altered conditions, and thus offer a strong basis for future long-term research.

Contribution of MCD: co-author; I participated in the data collection, analytical discussion and interpretation of the data, and review of the manuscript.

Paper 3. Ndatimana, G., Dusabe, M. C., & Albrecht, C. (2025). Bridging riverine and lacustrine systems: Macroinvertebrate indicators of ecological health in the Rwandan Congo basin. ***Environmental Monitoring and Assessment*** **(accepted).**

This study assessed the ecological status of Lake Kivu by examining macroinvertebrates at various taxonomic levels, molluscs at the species level, and other taxa at the family level. This enabled an innovative, comprehensive comparison of the macroinvertebrate communities in Lake Kivu and its tributaries. Instead of purely taxonomic or sensitivity-based indices, multimeric indices (MMIs), such as the MMIs for Lake Hawassa (MMIH) in Ethiopia (Wondmagegn & Mengistou, 2023), and the macroinvertebrate-based multimetric index of biotic integrity (MMIBI) for the tributaries of Lake Victoria in Kenya (Aura et al., 2021), were used. The study revealed different macroinvertebrate community compositions in Lake Kivu compared to its tributaries, reflecting habitat specificity and ecological affinity. In the rivers, poor ecological status was found in the northern Kivu basin, while moderate conditions were found in the central and southern regions, as indicated by the Ecological Quality Ratio (EQR). As for the lake itself, the central area was found to be more polluted than the northern and southern areas. Additionally, the river system was more polluted than the adjacent lake areas. Overall, the mollusc species enriched the ecological knowledge, while EQR assessments revealed pollution levels that are important for conservation planning. Using MMIs for biomonitoring proved effective and provided a more comprehensive approach than traditional methods. The research also emphasised the value of studying river and lake systems together to provide insight into their interactions. However, no clear causal links could be established between the lake and the rivers, suggesting that further research into their connectivity is required. Expanding the use of MMIs is recommended to improve monitoring accuracy.

Contribution of MCD: co-author; I collected data, performed the laboratory identification, participated in analytical discussion, and reviewed the manuscript.

Paper 4. Dusabe, M. C., Kalinda, C., Clewing, C., Hyangya, B. L., Van Bocxlaer, B., & Albrecht, C. (2024). Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu. ***Journal of Great Lakes Research, 50,*** **102339. https://doi.org/10.1016/j.jglr.2024.102339.**

This study uses molluscs as a model to assess the extent to which historical anthropogenic and natural disturbances have affected biodiversity in Lake Kivu. For the first time, the biodiversity of freshwater molluscs of Lake Kivu (Democratic Republic of Congo and Rwanda) and its tributaries (Rwanda only) was investigated using genetic characterisation. A combined morphological and genetic approach was used to identify new species that had never previously been recorded in Lake Kivu (*Sphaerium* cf. *hartmanni*), as well as updating the taxonomic status of *Afrogyrorbis kigeziensis*. However, some taxa that had been collected previously could not be found during this study. The Shannon-Wiener index and Pielou's evenness were used to evaluate the diversity of the molluscs, and heatmaps illustrated their distribution in the lake. This study revealed that the malacofauna of Lake Kivu, while admittedly poorer than that of other large African lakes, is significantly more diverse than previously reported. The molluscs of Lake Kivu exhibit a Nilotic biogeographic relationship,

probably due to past hydrographic connectivity. This study observed differences in species richness and diversity between the northern and southern basins. The complete absence of living molluscs at most of the sites in the north may be related to Lake Kivu's Holocene history (Ross et al., 2014), during which lava from the Virunga volcanoes repeatedly invaded the lake.

Interestingly, both species richness and abundance decreased with depth due to a sharp decrease in oxygen levels, a change in salinity and a decrease in habitat diversity. Thus, the ability of species-poor molluscan communities to indicate both anthropogenic and natural disturbances suggests their potential for bioindication in littoral zones.

Contribution of MCD: lead author; I collected data, performed the laboratory identification, contributed to the DNA data analysis and drafted the article.

Paper 5. Ssenkuba, F., Rwibutso, M., Dusabe, M. C., Tumusiime, J., & Albrecht, C. (2025). Invaders taking over – mollusc faunal change in volcanic barrier lakes of the Albertine Rift biodiversity hotspot. (in final preparation).

In this paper molluscs are used as a model to study the impact of anthropogenic and natural disturbances on biotic communities in volcanic barrier lakes. These lakes, which were formed by volcanic events in the late Pleistocene around 18 Ka, are lakes Mutanda, Bunyonyi and Mulehe in southwestern Uganda, and lakes Burera and Ruhondo in northern Rwanda.

An integrated approach combining morphological analysis, DNA barcoding, and ecological analyzes was used to assess mollusca diversity, biogeographical affinities and the ecological drivers of distribution. Surprisingly, the results revealed a greater malacological fauna than in the larger lakes in the region. The mollusc communities mainly showed Nilotic affinities but were dominated by widespread species. The results underscore the ongoing homogenisation of macroinvertebrate communities caused by invasive species, such as the North American crayfish (*Procambarus clarkii*), *Physella acuta*, and the Asian lineage of *Melanoides tuberculata*. The results also indicate that several species and subspecies of mollusc previously described by Mandahl-Barth (1954) are becoming increasingly rare. Additionally, new species were discovered that had not previously been found in this system, including *M. tuberculata* and several species of Sphaeriidae. These species may have been overlooked in previous, less comprehensive surveys that focused mainly on the littoral zone or some may have been misidentified. The observed shifts in the mollusc community's composition can be attributed to dramatic changes in the region's ecosystem and limnology. This study highlights the significance of giving these often-overlooked habitats the priority they deserve in regional biodiversity conservation strategies.

Author contributions: Contribution of MCD: co-author. I contributed to the data analysis, analytical discussion and interpretation of the data, as well as the review of the manuscript.

Paper 6. Dusabe, M. C., Clewing, C., Kagoro, G. R., Rwibutso, M., Tumusiime, T., Ndeo, W. O., & Albrecht, C. (2025). Historical drainage evolution and water level fluctuations in the African Great Lakes shaped phylogeny and biogeography of *Gabbiella* gastropods. *Journal of Biogeography*. (in revision).

This paper looks at the phylogeny and biogeography of the gastropod genus *Gabbiella* of the family Bithyniidae from the African Great Lakes (AGL), their major connecting rivers as well as crater lakes from western Uganda. Using *Gabbiella* as a model genus, the historical drainage evolution and water level fluctuations were investigated. While most *Gabbiella* species are restricted to the AGL, the evolutionary links between these lakes remain unclear, particularly with regard to whether lacustrine species evolved from widespread riverine ancestors or dispersed between lakes.

To investigate the phylogenetic relationships and phylogeography of *Gabbiella*, a multi-locus molecular phylogenetic approach combining two

mitochondrial and two nuclear DNA markers was employed. A dated phylogeny was constructed using maximum likelihood (ML) and Bayesian inference (BI) approaches, as well as maximum parsimony haplotype networks, in conjunction with genetic *p*-distance analysis. To investigate the biogeography of the *G. humerosa* subspecies, ancestral ranges were reconstructed using the DIVALIKE model. The results revealed three highly divergent clades, which are consistent with taxonomic classifications rather than geographic distribution. These results largely agree with the conclusions of previous studies based solely on morphological, anatomical and radular characters within the first clade. They also suggest that *Gabbiella* diversified firstly in the Miocene (between 17.31 and 14.35 Ma) and then in the Pliocene (3.36 Ma) and Pleistocene (1.5 Ma). This diversification is consistent with the effects of rifting, which led to changes in the hydrological cycle and fluctuations in water levels. These are thought to have shaped the phylogeographic pattern of *Gabbiella* observed in the AGL today. Rather than extinction, it is thought that colonisation determined the distribution patterns, which highlights the high ecological tolerance of *Gabbiella* spp. Phylogenetic inferences suggest that the *G. humerosa* subspecies evolved in the Kivu-Tanganyika region during the late Pleistocene. While the Lake Tanganyika subspecies evolved in isolation, the Lake Kivu subspecies is the MRCA of the other Nilotic *G. humerosa* subspecies. A series of vicariance and dispersal events determined the current diversity and distribution of *G. humerosa* subspecies in the AGL during the Pleistocene. Consequently, *G. humerosa* is regarded as one of the most valuable models for studying paleohydrological changes and water level fluctuations in the AGL during the Pleistocene. Furthermore, the results of this study highlight similarities and differences in the dispersal patterns of other invertebrate and fish species.

Author contributions: Contribution of MCD: lead author; I collected data, performed laboratory identifications, performed phylogenetic and phylogeographic analyses, prepared maps, and drafted the article.

Paper 7. Bálint, M., Tumusiime, J., Nakintu, J., Baranski, D., Schardt, L., Romahn, J., Dusabe, M.C., Tolo, C.U., Kagoro, G.R., Ssenkuba, F. and Junginger, A., & Albrecht, C. (2024). Environmental DNA barcoding reveals general biodiversity patterns in the large tropical rift Lake Albert. *Science of the Total Environment, 957*, 177308. https://doi.org/10.1016/j.scitotenv.2024.177308

This study evaluates the potential of eDNA for assessing broad biodiversity patterns in large tropical lakes. Lake Albert was selected as a model system, with surface water sampled from three sites: close to the southern inflow of the Semliki River, at the lake's center, and near the northern inflows of the Victoria Nile and Albert Nile. DNA was then extracted and amplified using Euka02 and miCOIint/jgHCO2198 primers. The diversity and community structure at different sites across the lake were analysed to examine the ecological patterns. These were then compared with the patterns found in littoral areas and open-water zones. The results showed that the communities were different in each of the three places. Cyclopoid copepods emerged as the dominant group, aligning with previous morphological observations. However, the number of uniquely benthic taxa represented by the eDNA reads was small. This is to be anticipated given that solely surface water was tested. It is reasonable to conclude that the majority of the DNA fragments should be of pelagic origin. Community composition was strongly shaped by distance from the shoreline, reflecting expected gradients in nutrient and oxygen availability, as also noted by Dusabe et al. (2024) in Lake Kivu. The data confirmed previous findings that nutrient input from rivers is a major factor affecting planktonic microorganisms in the lake. A key issue that arose in this work was the lack of a complete reference sequence database, which affected the precision of taxonomic classification. Nevertheless, the study shows how eDNA met barcoding could be a useful tool for exploring the biodiversity of tropical freshwater

ecosystems that are not well studied. There is an urgent need to expand reference libraries with sequences from tropical taxa to improve the reliability of eDNA for monitoring and conservation purposes. The findings also demonstrate eDNA's capacity to reveal ecological patterns across entire communities, emphasising the importance of conducting broad-based, taxonomically-free monitoring in tropical freshwater ecosystems.

Contribution of MCD: co-author; I was involved in data collection, DNA extraction, and writing and revising the manuscript.

5. Results & Discussion

This thesis comprises seven articles. The articles examine freshwater macroinvertebrates that are good indicators of freshwater habitat quality to assess the impact of natural and human-induced disturbances on African freshwater ecosystems at different taxonomic levels. Various taxonomic indices were evaluated to detect environmental disturbances (see Figure 3) in selected African aquatic systems (see Figure 4). The historical trend in freshwater mollusc richness was primarily investigated in Lake Kivu and then extended to volcanic barrier lakes (see Figure 7). These analyses illustrate the biogeographic relationships of mollusc species and the effects of disturbance over time. Phylogenetic and biogeographic analyses of the genus *Gabbiella* and *G. humerosa* subspecies, respectively, illuminate the historical evolution of drainage and water level fluctuations in the AGL and their associated rivers. These analyses establish *Gabbiella* as the most effective indicator of historical disturbance in the AGL and its catchment area.

Together, the studies provide a more comprehensive understanding of how macroinvertebrates can be used to detect disturbance. They also highlight the impact of historical disturbances on molluscs in different geographical locations and over time.

5.1. Impact of natural and anthropogenic disturbance on macroinvertebrate diversity

Both natural and human-induced disturbances to African freshwater systems significantly impact freshwater macroinvertebrates, altering their distribution, abundance and diversity (Van Damme & Gautier, 2013; Dusabe et al., 2022; 2024). Historical hydrological and climatic changes are among the most important factors thought to influence landscape evolution and contribute to the diversification of freshwater macroinvertebrates (Mahulu et al., 2021; Daniels et al., 2015). This was particularly evident in the study of the AGL, major connected rivers and crater lakes in western Uganda, where the *Gabbiella* snail was studied. Rifting, which triggered paleohydrological changes and water level fluctuations, contributed to the evolution, diversity and distribution of this genus (Dusabe et al., in revision). The study of the molluscs of Lake Kivu shows the negative impact of natural disturbances on mollusc communities and reveals that the malacofauna of Lake Kivu is comparatively poor compared to other AGL (Dusabe et al., 2024). Additionally, sub-lacustrine volcanic eruptions have occurred repeatedly over the last 1,000 years (Ross et al., 2014), temporarily and locally altering limnological conditions. The significant littoral deposits of subfossil shells, particularly along the northern shoreline of the lake, may have been caused by these events (Dusabe et al., 2024). Several researchers working on different organisms have reported the low diversity of Lake Kivu (Salzburger et al., 2014; Snoeks et al., 2012). This low diversity likely reflects the instability of the environment due to natural phenomena such as earthquakes and degassing events as well as the anthropogenic activities of the large neighboring cities (Ross et al., 2014; Balagizi et al., 2018; Dusabe et al., 2024). The north-south differences in species richness in the Lake Kivu basin (see Figure 5) and the lack of molluscs at certain northern sites can be attributed to the history of Lake Kivu during the Holocene period (Dusabe et al., 2024; Ross et al., 2014).

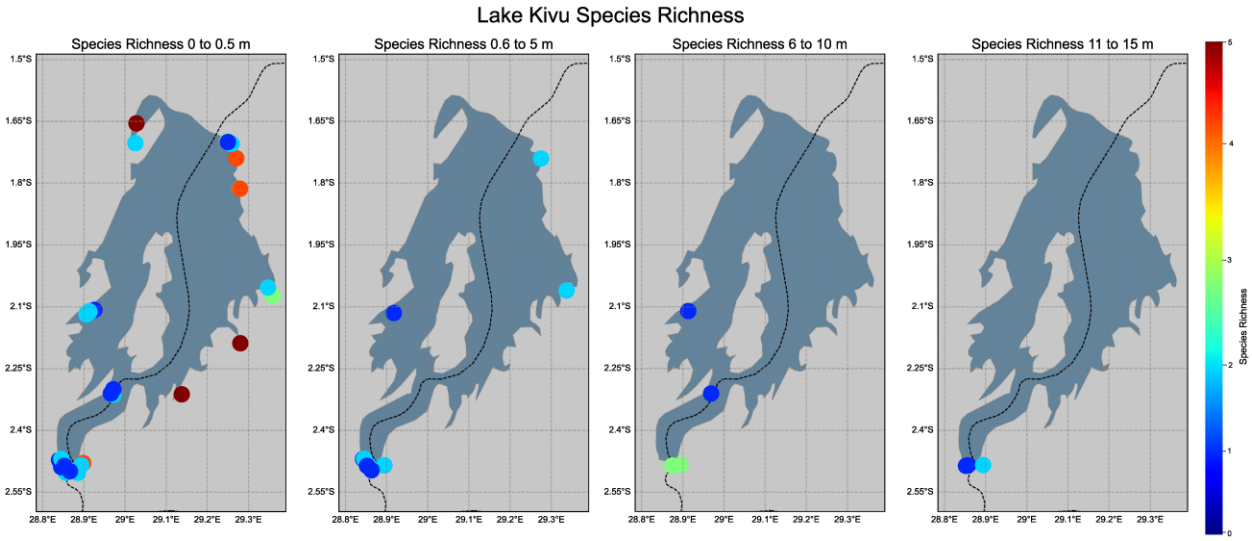


Figure 5. Heatmap visualization of the species richness of molluscs in Lake Kivu with subplots for the different sampling depths, A: 0 to 0.5 m; B: 0.6 to 5 m; C: 5 to 10 m; D:11 to 15 m. The color bar shows the range of species richness at the different locations and depths (Adopted from Dusabe et al. (2024).

During this time, the lake was repeatedly filled with lava from the Virunga volcanoes in the northern basin. These intrusions gave rise to underwater heat waves and caused the complete overflow of large parts of the littoral substrates, with the potential to wipe out entire benthic communities (Dusabe et al., 2024; Ross et al., 2014).These recent volcanic eruptions in combination with the unique physico-chemical properties of the lake, and ongoing anthropogenic activities may have significantly influenced the current composition, distribution and diversity of macroinvertebrates (Dusabe et al., 2024). The Ruzizi River, the outlet of Lake Kivu, experiences frequent releases and retention of water below the dams. Nevertheless, low macroinvertebrate diversity (between 0.63 and 1.08) was found both upstream and downstream of the dams. The low diversity upstream and downstream is likely due to the fact that Ruzizi River water originates from Lake Kivu, which is species-poor due to its geological history and catastrophic volcanic events (Dusabe et al., 2022; Jones, 2021). Consequently, the impact of the dams was imperceptible due to the limited biodiversity in the areas upstream and downstream. A minor variation in the diversity of macroinvertebrates was detected between sites near Dams I and II. This discrepancy is presumably due to Dam I's proximity to Lake Kivu, whereas the water near Dam II is diluted by tributaries (Muvundja et al., 2022). The least disturbed sites further downstream of the Ruzizi River also resulted in higher diversity (between 1.81 and 2.39) than the highly disturbed sites or sites close to Lake Kivu (Dusabe et al., 2022). In addition, the introduction of invasive species such as the North American crayfish (*P. clarkii*) has been observed in the lakes of the Albertine Rift volcanic barrier lakes (Ssenkuba et al., in final preparation). Well-established populations were found in lakes Bunyonyi, Ruhondo and Burera, and the early stages of establishment were observed in lakes Mulehe and Mutanda (Ssenkuba et al., in final preparation). This has significantly affected the mollusc community composition (Ssenkuba et al., in final preparation). Other invasive mollusc species have become established, such as *M. tuberculata* in Lake Mulehe and *P. acuta* in lakes Ruhondo and Burera (Ssenkuba et al., in final preparation). These invasive species pose a potential threat to mollusc diversity by displacing native species and homogenizing communities (Ssenkuba et al., in final preparation). This phenomenon has already been observed in Lake Malawi due to the invasive *M. tuberculata* (Van Bocxlaer & Albrecht, 2015; Van Bocxlaer et al., 2015). These results show that natural disturbances such as rifting, volcanic activity and climate change can cause significant changes in biodiversity, including species evolution, distribution, speciation, and extinction. They can even

lead to the spread of new species. In contrast, human-induced disturbances often lead to a decline in biodiversity.

5.2. Use of taxonomic surrogate to detect both natural and anthropogenic disturbance

In this section, I compare the performance of five bioassessment indices applied at different taxonomic levels of macroinvertebrates. These included the biotic and multimetric indices at family level, a mollusc community index at species level, phylogenetic and biogeographical indices also at species level, and an eDNA-based index designed to capture broader taxonomic groups (Figure 3; Dusabe et al., 2022; Hyangya et al., 2022; Ndatimana et al., accepted; Dusabe et al., 2024; Bálint et al., 2024; Dusabe et al., in revision). However, the biotic index (e.g., TARISS and the Average Score Per Taxa at the family level), and the other four diversity indices (e.g., the Shannon-Wiener Index, the Simpson Index, Pielou's evenness and the Rare Family Prevalence Index), could not detect the impact of dams on the diversity of macroinvertebrates (Dusabe et al., 2022). A plausible explanation is that the sites both upstream and downstream were already highly disturbed and contained insensitive taxa, adding to the usual low diversity of the Ruzizi River due to its saline water originating from Lake Kivu. As these indices do not take into account the sensitivity of species to pollution, a change in community composition does not necessarily lead to a change in diversity indices. Several studies have shown that dams often exert only limited or inconsistent effects on macroinvertebrate assemblages (Xiaocheng et al., 2008; Vaikasas et al., 2013). A broader review by Mbaka & Wanjiru (2015) further highlighted that more than 70% of small dams impact macroinvertebrates either favorably or unfavorably resulting in alterations in quantity and diversity downstream (Martinez et al., 2013; Wang et al., 2013). These findings indicate that dam impacts are context-dependent rather than uniformly negative, with outcomes shaped by factors such as dam size, river characteristics, and the existing level of biodiversity (Dusabe et al., 2022; Gezie et al., 2023; Brown et al., 2024). In line with these observations, the present study also found that diversity indices were weak indicators and could not effectively separate stressor gradients, a pattern previously reported for the Mara River (Masese et al., 2023) and Lake Kivu (Hyangya et al., 2022). In Lake Kivu, multimetric indices (MMIs) distinguished well between undisturbed reference sites and those affected by human activity (Hyangya et al., 2022; Ndatimana et al., accepted). Of the tested measures, six stood out as the most useful: the number of EPT taxa, the proportions of Diptera, Chironomidae, and Ephemeroptera, the overall % EPT, and the Family Biotic Index (FBI). Similar sets of metrics have been applied successfully in other regions (Camargo, 2019; Gabriels et al., 2010). The analysis also revealed that rivers in the northern basin of Lake Kivu are more degraded than those in the middle and southern basins (Ndatimana et al., accepted), a result that agrees with earlier assessments based on biotic indices (Wronki et al., 2015) and mollusc communities (Dusabe et al., 2024). A dated phylogeny was inferred using molecular clock analysis, with the divergence time corroborating historical disturbances (Dusabe et al., in revision). The colonization of *Gabbiella* in the African region (see Figure 6) predates the geological evidence for the closure of the Tethys Seaway (18–20 Ma; Okay et al., 2010), while the split of *Gabbiella stanleyi* and *Gabbiella walleri* (clade 3) from the Asian *Hydrobioides* (Myanmar) coincided with a period of significant climatic and geological changes in Africa during the Middle Miocene (Zachos et al., 2001).

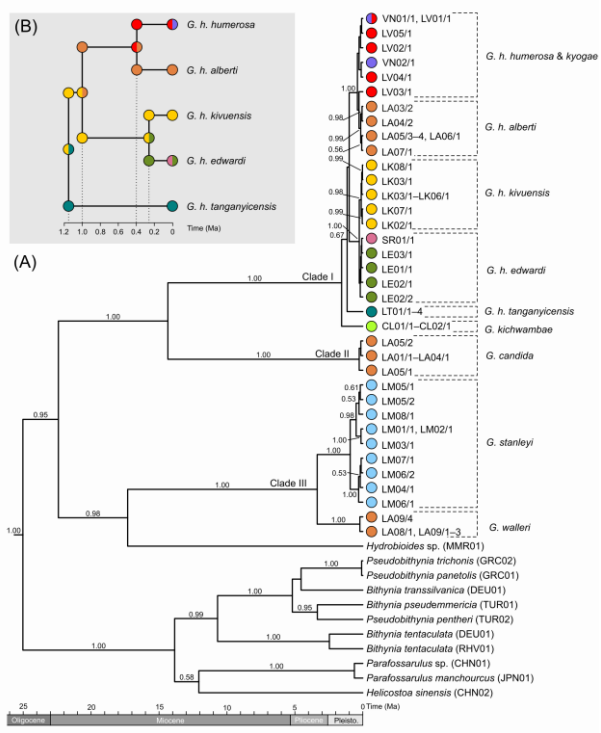


Figure 6. Maximum clade credibility (MCC) tree derived from the BEAST analyses including 95% HPD. The Bayesian posterior probabilities (PP) are indicated at the nodes. Historical biogeography of Gabbiella humerosa subspecies, ancestral area reconstruction from BioGeoBEARS, derived from the Bayesian chronogram, the best-fit model was DIVALIKE. Pie charts represent the probabilities of ancestral states at each node (B). Color codes represent each (sub)species (Adopted from Dusabe et al.(in revision).

The estimated initial diversification of *Gabbiella stanleyi* in Lake Malawi during the Middle Pleistocene (930 ka ago, 95% highest posterior density (HPD) interval = 0.48–1.46 Ma) coincides with the diversification of other gastropods, such as *Lanistes* (Schultheiß et al., 2009; Mahulu et al., 2021) and *Bellamya* (Schultheiß et al., 2011, 2014). The estimated initial diversification of *Gabbiella stanleyi* in Lake Malawi during the Middle Pleistocene (930 ka ago, 95% highest posterior density (HPD) interval = 0.48–1.46 Ma) coincides with the diversification of other gastropods, such as *Lanistes* (Schultheiß et al., 2009; Mahulu et al., 2021) and *Bellamya* (Schultheiß et al., 2011, 2014). This corroborates the 'founder flush' pattern identified by Schultheiß et al. (2009), which occurred after periods of drought between 1.6 and 0.8 Ma (Lyons et al., 2015; Ivory et al., 2016). Furthermore, ancestral area reconstruction was applied to *G. humerosa* to trace its biogeographical history, providing insight into drainage changes during the Pleistocene (Dusabe et al., in revision; details in the biogeography section). This analysis confirms that phylogenetic and biogeographical approaches can reveal signatures of past disturbances (Dusabe et al., in revision). In parallel, environmental DNA was tested at Lake Albert as a tool for biodiversity surveys and disturbance assessment (Bálint et al., 2024). The method, however, recovered only a handful of macroinvertebrate taxa, each represented by very few reads (Bálint et al., 2024). Most of the taxa recovered with the two genetic markers, COI and 18S, were planktonic, which is not surprising given that only surface water was collected (Bálint et al., 2024). Both markers exhibited clear, statistically significant changes in community composition across the gradient from the shoreline to the open waters of the lake (Bálint et al., 2024). A similar pattern has been noted for molluscs in Lake Kivu, where richness decreases with depth (Dusabe et al., 2024). Although these findings underline the challenges of assigning eDNA reads to species because of limited reference databases, they also point to the promise of eDNA as a practical, taxonomy-independent tool for monitoring biodiversity in tropical freshwaters. On this basis, an urgent priority is the development of a continent-wide, eDNA-based monitoring framework for African freshwater system.

5.3. Historical change of mollusc diversity driven by change in freshwater ecosystem functioning

Databases of species records are now widely used to examine biodiversity patterns across space and time (Baselga et al., 2013; Chandler et al., 2017). Yet, their reliability can be limited by spatial biases (Bowler et al., 2022), and recent work has proposed methods to reduce these issues (Johnston et al., 2020; 2023). In this context, two additional sources of bias deserve attention: the patchy spatial coverage of earlier records and shifts in spatial bias through time.

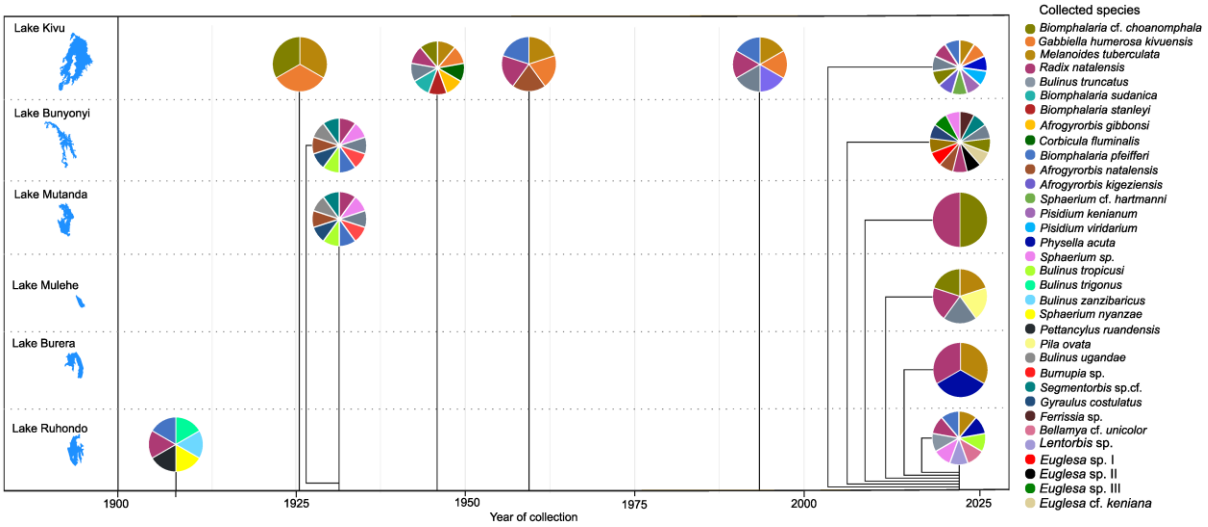


Figure 7. Historical trends of molluscs in six volcanic barrier lakes: Lake Kivu, Lake Bunyonyi, Lake Mutanda, Lake Mulehe, Lake Burera and Lake Ruhondo, collected over a number of years. Lake Kivu: Pilsbry & Bequaert (1927), Dartevelle & Schwetz (1947), Gillet et al. (1960), Brown (1994) and Dusabe et al. (2024). Lake Bunyonyi: Worthington (1932); Ssenkuba et al in final preparation; Lake Mutanda: Worthington (1932); Ssenkuba et al., in final preparation; Lake Mulehe: Ssenkuba et al., in final preparation; Lake Burera: Ssenkuba et al., in final preparation; Lake Ruhondo: Thiele (1911); Ssenkuba et al., in final preparation; Species names are colour-coded to show shifts within different years.

These issues have implications for assessing changes in biodiversity using occurrence data collected without a coordinated sampling design. The 2022 Lake Kivu biodiversity survey contains an extensive list of historical species from previous surveys (see Figure 7). New findings are presented (e.g. *Sphaerium* cf. *hartmanni*), but also findings resulting from taxonomic changes (e.g. *Afrogyrorbis kigeziensis*). Some taxa that had previously been sampled (e.g. *Biomphalaria sudanica* and *Corbicula fluminalis* in 1947) could not be found in any other survey (see Figure 7). Although the sampling in 2022 was relatively thorough, some rare species of snail, such as small planorbids, may have been overlooked.

However, since morphological identification was supplemented by genetic identification, particularly in *Biomphalaria*, as detailed by Dusabe et al. (2024), the presence of cryptic but divergent genetic lineages is unlikely. Moreover, the absence of living *Corbicula fluminalis* and fossil *Mutela* sp. as recorded by Dartevelle & Schwetz (1947), is suspicious, as no bivalve shell or fossil were recorded in the other surveys. New findings from the 2023 survey of Lake Bunyonyi (e.g. *Biomphalaria choanomphala* and *M. tuberculata*) may have been overlooked because the 1931 survey was less extensive. Alternatively, *B. choanomphala* may have been misidentified morphologically and labeled as *Biomphalaria pfeifferi*. *Ferrissia* sp. may have been overlooked in the 1931 survey or it may have colonized the lake at a later date, as it was first recorded in this region by Mandahl-Barth in 1954. Other species recorded in 1931 (e.g. *Bulinus tropicus*, *Bulinus ugandae*, *Burnupia* sp., *Sphaerium* sp.) may have become extinct due to various forms of disturbance, or they may have been overlooked given that they are not very common species. The catchment area of Lake Mutanda is affected by significant anthropogenic disturbance. It is dominated by cultivated hills, with buffer zones of the lake impacted by agricultural activities and several hotel constructions (Tibihika et al., 2016). The area is dominated by tolerant taxa, indicating a high level of pollution (Tibihika et al., 2016). Given this, it is not surprising that the original fauna recorded in 1931 has become extinct, except for *Radix natalensis* (see Figure 7), which can likely adapt to environmental changes (Brown, 1994). Another newcomer *B. choanomphala*, was likely misidentified based on morphological identification alone. The absence of certain species, such as *Bulinus zanzibaricus*, *Pettancylus ruandensis* (*Ferrissia*), and *Sphaerium nyanzae*,

in the 2023 collection of Lake Ruhondo could be due to their extinction. This extinction is likely the results of various factors, including the construction and repeated repairs of the Ntaruka power plant, the presence of invasive species such as *P. clarkii* that may have displaced the native species, and other anthropogenic disturbances in the area. The new installation and repair of the Ntaruka power plant likely have facilitated the dispersal of many more invasive species, such as *P. acuta* and *M. tuberculata*, found in 2023 collection. Meanwhile, *Lentorbis* sp., *Sphaerium* sp. and *Bellamya* cf. *unicolor* either colonized the lake at a later stage or were previously overlooked. On the other hand, *Sphaerium* sp. may have been misidentified as *Sphaerium nyanzae*, since earlier studies relied only on shell morphology, whereas the present study incorporated DNA analysis. Despite the biased spatial sampling, these results provide information on the changes in the mollusc community over time. Therefore, monitoring at a higher spatial and temporal resolution is crucial to accurately tracking species occurrences and understanding rapid changes in habitat conditions.

5.4. Mollusc biogeography and faunal origin

The similarities in the molluscan fauna of lakes Kivu and Edward suggest a Nilotic biogeographical connection (Dusabe et al., 2024; Dusabe et al., in revision). These connections are likely due to the past hydrographic connection between the two lakes (Beadle, 1981; Ross et al., 2014). This indicates that the fauna has remained stable over long periods of time, although the hydrological environment has changed considerably. Surprisingly, the existing drainage pattern, whereby Lake Kivu flows into the Congo River via Lake Tanganyika, is not reflected in any single species. The historical connection between Lake Kivu and the other Niolotic lakes is further supported by the distribution and dispersal route of *G. humerosa* (Dusabe et al., in revision). The lakes Kivu and Tanganyika area was identified as the home of the most recent common ancestor of *G. humerosa* during the early Pleistocene, around 1.50 Ma (95% HPD = 0.94–2.26 Ma). As rifting developed, a vicariance event most likely blocked the hydrographic connection between the two lakes around 1.17 Ma (95% HPD = 0.76–1.59 Ma). This period coincided with rising aridity and climate shifts across Africa, as reported by Trauth et al. (2021). According to Bibi and Kissling (2015) and Cohen et al. (2022), faunal changes likely occurred more widely in Africa around 2.0 to 1.75 Ma and 0.75 to 0.50 Ma, respectively. The vicariance event resulted in the emergence of the solitary Lake Tanganyika subspecies and the ancestors of all Nilotic subspecies (*G. humerosa kivuensis*, *G. humerosa alberti*, *G. humerosa humerosa*, *G. humerosa kyogae* and *G. humerosa edwardi*). Over the course of 1.17–1 Ma, the ancestral range of all Nilotic subspecies expanded from the Lake Kivu region to the Lake Albert area through anagenetic migration (see Figure 8). Another vicariance event occurred around 1.00 Ma (95% HPD = 0.64–1.4 Ma) and separated the ancestral populations in the Lake Kivu and Lake Albert regions. This event was likely triggered by the cooling and drying of the climate in East Africa between 1.7 and 1.0 Ma (Peter, 2004; Hoag et al., 2017), and likely led to genetic isolation and ecological fragmentation. Through anagenetic dispersal, the populations subsequently spread from Lake Kivu to Lake Edward (between 1 Ma and 300 ka) and from Lake Albert to Lake Victoria (between 1 Ma and 400 ka) (Dusabe et. al., in revision). The two ancestral areas, Albert +Victoria and Kivu + Edward were further subdivided by vicariance events around 400 ka (95% HPD = 0.20–0.61 Ma) and 300 ka (95% HPD = 0.12–0.4 Ma), respectively, giving rise to all current known Nilotic *G. humerosa* subspecies. The separation of the Albert and Victoria regions occurred around the time of Lake Victoria's formation (Danley et al., 2012; Salzburger et al., 2014), and coincided with the mitochondrial divergence time of Lake Victoria cichlids (Schedel et al., 2019). The divergence time between Kivu and Edward regions, on the hand, coincides the Middle Pleistocene lava flows (300–350 ka), which diverted water from the Kivu Rift into the Nile system (Holzförster & Schmidt, 2007). Subsequently, with the formation of the Semliki and Victoria Nile, the *G. humerosa* subspecies from Lake Victoria expanded into the

Victoria Nile, while the *G. humerosa* subspecies from Lake Edward spread into the Semliki River.

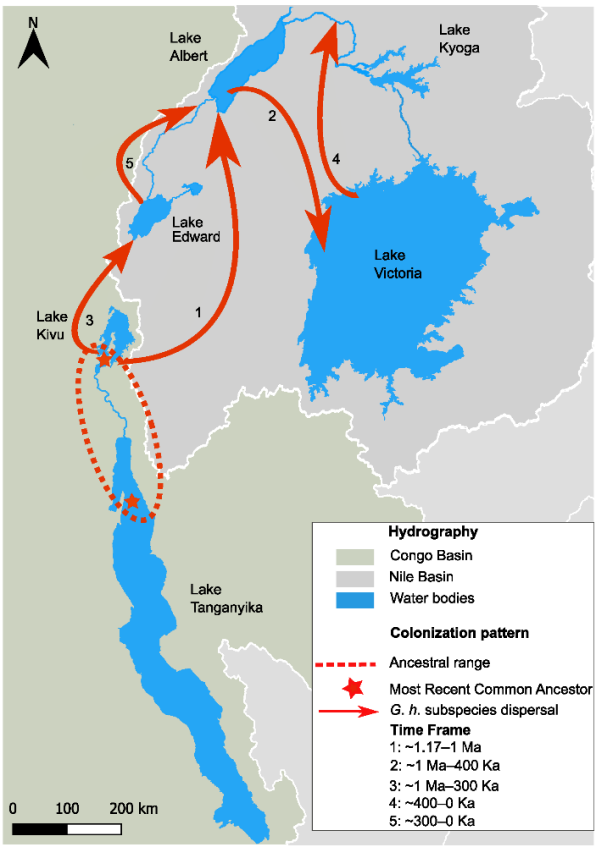


Figure 8. Biogeographical history of the Gabbiella humerosa subspecies from the Early Pleistocene to the present day. Map showing the location of the African Great Lakes and the rivers within their respective hydrological basins where Gabbiella humerosa samples were found. The dotted ellipse shows the distribution area of the ancestor (red star), while the red arrow indicates the origin of the subspecies' dispersal. The time frame indicating the range of dispersal is also shown (Adopted from Dusabe et al. (in revision).

According to Ssenkuba et al., (in final preparation) most molluscs in the volcanic barrier lakes of the Albertine Rift exhibit a predominantly Nilotic affinity. The most likely colonization route is direct, via the connected rivers. However, some species have a wide Afrotropical distribution (Ssenkuba et al., in final preparation). A notable example is *B. truncatus*, which occurs throughout Africa, including North Africa and the Congo Basin (Brown, 1994; Tumwebaze et al., 2019; Dusabe et al., 2024). Colonization can occur from connected water bodies or, more rarely, through long-distance dispersal. Similarly, *B. pfeifferi* is widely distributed in tropical Africa (Brown, 1994), while *Sphaerium* sp. and *Euglesa keniana* are widely distributed in East Africa (Mandahl-Barth, 1988; Clewing et al., 2022). The latter extends its range from Ethiopia to Zambia (Mandahl-Barth, 1988; Clewing et al., 2022). *Pila ovata* is distributed from Egypt across large parts of Africa, including regions with the Zaire Basin (Brown, 1994). The *Segmentorbis angustus*, which is distributed from Sudan to Natal and from Ethiopia to the lower Zaire Basin (Danish Bilharziasis Laboratory, 1987), is found in the large African lakes such as Lake Malawi, Lake Tanganyika, Lake Turkana, Lake Victoria and Lake Albert (Brown, 1994). Another widespread species is *B. tropicus*, which occurs throughout East Africa from Ethiopia to South Africa (Danish Bilharziasis Laboratory, 1987; Brown, 1994). *Radix natalensis* is widespread in Africa and may have an Asian affinity (Brown, 1994). The invasive mollusc species *M. tuberculata* and *P. acuta* have been detected in the molluscs of Lake Mulehe (*M. tuberculata*) and lakes Burera and Ruhondo (*P. acuta*). *Melanoides tuberculata* has proliferated extensively throughout Africa, including the lakes Malawi and Tanganyika (Van Bocxlaer et al., 2015), while *P. acuta*, an invasive lineage originating from the USA, has recently been identified in the tributaries of Lake Kivu in the Albertine Rift, but not in the lake itself (Dusabe et al., 2024), as well as in Lake Naivasha (Albrecht et al., 2025). Nevertheless, *P. acuta* is predicted to continue its global invasion, including in Africa (Albrecht et al., 2025b). As these two invasive species are currently widespread in Africa, long-distance dispersal via connected rivers is possible.

6. Concluding Remarks & Future outlooks

This study highlights the various natural and anthropogenic disturbances affecting African freshwater systems, impacting both biodiversity and the livelihoods of the communities that depend on them. It shows that anthropogenic and natural disturbances strongly influence macroinvertebrate biodiversity in these ecosystems, and that the scale at which these communities are assessed strongly influences the

ability to detect change. The study demonstrate the effectiveness of taxonomic surrogates in assessing freshwater biodiversity and highlights that the appropriate tool depends on the target. For example, a biodiversity index at the family level failed to identify the impact of dams on macroinvertebrates downstream in the Ruzizi River. This highlights the importance of using a species-level index to gain reliable ecological insights at a fine scale. Combining morphological identification with DNA barcoding significantly improved taxonomic resolution. This approach has led to the discovery of species that were previously considered cryptic or overlooked. Multimetric indices effectively distinguished between disturbed and undisturbed sites. However, careful regional calibration is necessary for the accurate application and interpretation of these indices. In addition, the molluscan community-based index performed very well in Lake Kivu, distinguishing the most historically disturbed basin from the others. Moreover, the species-level phylogenetic and biogeographic index using *Gabbiella* species corroborated historical disturbances, such as hydrological changes and water level fluctuations, in African freshwater systems. The timing of *Gabbiella*'s diversification in the African Great Lakes is similar to that of other molluscs and various fish taxa, suggesting that common factors influence diversification. Furthermore, the eDNA used proved to be a promising complementary biomonitoring tool, allowing more species to be detected than with other sampling methods. A major challenge is the lack of reference data, hindering coordinated efforts to expand genetic databases with sequences of different taxa from tropical freshwater systems. Looking to the future, conservation and management strategies need to integrate high-resolution taxonomy, region-specific multimetric indices, and advanced molecular tools to robustly and early detect biodiversity change. Future monitoring should include long-term, extensive spatial surveys; systematic calibration of bioassessment tools against local environmental gradients; and improved eDNA protocols. Conserving freshwater biodiversity under growing anthropogenic and climatic pressures requires the implementation of holistic, watershed-level strategies that address pollution, preserving environmental flows around dams, controlling and preventing invasive species, and safeguarding climate refugia like floodplain wetlands and deep pools. African freshwater systems can be efficiently monitored, conserved, and protected by combining traditional ecological knowledge with molecular innovation, and region-specific management to address both current and new threats.

8. References

Ahmed, S. F., Kumar, P. S., Kabir, M., Zuhara, F. T., Mehjabin, A., Tasannum, N., Hoang, A. T., Kabir, Z., & Mofijur, M. (2022). Threats, challenges and sustainable conservation strategies for freshwater biodiversity. *Environmental Research*, *214*, 113808. https://doi.org/10.1016/j.envres.2022.113808

Albert, J. S., Destouni, G., Duke-Sylvester, S. M., Magurran, A. E., Oberdorff, T., Reis, R. E., Winemiller, K. O., & Ripple, W. J. (2021). Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio*, *50*, 85–94. https://doi.org/10.1007/s13280-020-01318-8

Albrecht, C., Clewing, C., Seebens, H., Chibwana, F. D., Da Silva, E. L., Leal, M. F., Lingofo Bolaya, R., Marwoto, R. M., Odaibo, A., & Pinheiro, T. G. (2025). When One Global Invasion Hides Another—Cryptic Interspecific Invasion in Freshwater Gastropods. *Diversity and Distributions*, *31*, e13958. https://doi.org/10.1111/ddi.13958

Albrecht, C., Kipyegon, J. K., & Junginger, A. (2025). Returners and New Arrivals After the Crash : Intermediate Hosts and Global Invaders Dominate Gastropod Fauna of Lake Naivasha, Kenya. *Diversity*. 17–265. https://doi.org/https://doi.org/ 10.3390/d17040265

Alpert, P., Bone, E., & Holzapfel, C. (2000). Invasiveness, invasibility and the role of environmental stress in the spread of non-native plants. *Perspectives in plant ecology, evolution and systematics*, *3*, 52–66. https://doi.org/10.1078/1433-8319-00004

Alhejoj, I., Bandel, K., Salameh, E. (2017). Aquatic Mollusks: Occurrences, identification and their use as bioindicators of environmental conditions (Salinity, trace elements and pollution Parameters) in Jordan . In: Abdalla, O., Kacimov, A., Chen, M., Al-Maktoumi, A., Al-Hosni, T., Clark, I. (eds) Water Resources in Arid Areas: The Way Forward (pp. 295–318). Springer Water. Springer, Cham. https://doi.org/10.1007/978-3-319-51856-5_17

Amasi, A., Wynants, M., Blake, W., & Mtei, K. (2021). Drivers, Impacts and Mitigation of Increased Sedimentation in the Hydropower Reservoirs of East Africa. *Land*, *10*, 638. https://doi.org/10.3390/land10060638

Arimoro, F. O., & Keke, U. N. (2021). Stream biodiversity and monitoring in North Central, Nigeria: the use of macroinvertebrate indicator species as surrogates. *Environmental science and pollution research*, *28*, 31003–31012. https://doi.org/10.1007/s11356-021-12922-w

Bae, M. J., & Kim, E. J. (2024). Temporal beta diversity and community concordance in freshwater fish and benthic macroinvertebrates on a national scale: Does biodiversity change at the same rate?. *Global Ecology and Conservation*, *56*, e03278. https://doi.org/10.1016/j.gecco.2024.e03278

Baker, N. J., & Greenfield, R. (2019). Shift happens: Changes to the diversity of riverine aquatic macroinvertebrate communities in response to sewage effluent runoff. *Ecological indicators*, *102*, 813–821. https://doi.org/10.1016/j.ecolind.2019.03.021

Balagizi, M. C., Kies, A., Kasereka, M. M., Tedesco, D., Yalire, M. M., & McCausland, W. A. (2018). Natural hazards in Goma and the surrounding villages, East African Rift System. *Natural Hazards*, *93*, *31–66*. https://doi.org/10.1007/s11069-018-3288-x

Bálint, M., Tumusiime, J., Nakintu, J., Baranski, D., Schardt, L., Romahn, J., Dusabe, M.C., Tolo, C.U., Kagoro, G.R., Ssenkuba, F. and Junginger, A., & Albrecht, C. (2024). Environmental DNA barcoding reveals general biodiversity patterns in the large tropical rift Lake Albert. *Science of the Total Environment*, *957*, 177308. https://doi.org/10.1016/j.scitotenv.2024.177308

Baselga, A., Fujisawa, T., Crampton-Platt, A., Bergsten, J., Foster, P. G., Monaghan, M. T., & Vogler, A. P. (2013). Whole-community DNA barcoding reveals a spatio-temporal continuum of biodiversity at species and genetic levels. *Nature communications*, *4*, 1892.

Beadle, L. C. (1981). The inland waters of tropical Africa: An introduction to tropical limnology (2nd. ed.). Longman Group Limited, London, UK.

Berke, M. A., Johnson, T. C., Werne, J. P., Livingstone, D. A., Grice, K., Schouten, S., & Damsté, J. S. S. (2014). Characterization of the last deglacial transition in tropical East Africa: Insights from Lake Albert. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *409*, 1–8. https://doi.org/10.1016/j.palaeo.2014.04.014

Beverly, E. J., White, J. D., Peppe, D. J., Faith, J. T., Blegen, N., & Tryon, C. A. (2020). Rapid Pleistocene desiccation and the future of Africa's Lake Victoria. *Earth and Planetary Science Letters, 530,* 115883.https://doi.org/10.1016/j.epsl.2019.115883

Birnie-Gauvin, K., Lynch, A. J., Franklin, P. A., Reid, A. J., Landsman, S. J., Tickner, D., Dalton, J., Aarestrup, K., & Cooke, S. J. (2023). The RACE for freshwater biodiversity: Essential actions to create the social context for meaningful conservation. *Conservation Science and Practice, 5,* e12911. https://doi.org/10.1111/csp2.12911

Borges, P. P., Dias, M. S., Carvalho, F. R., Casatti, L., Pompeu, P. S., Cetra, M., Tejerina-Garro, F. L., Suarez, Y. R., Nabout, J. C., & Teresa, F. B. (2020). Stream fish metacommunity organisation across a Neotropical ecoregion: The role of environment, anthropogenic impact and dispersal-based processes. *PLoS One, 15*(5), e0233733. https://doi.org/10.1371/journal.pone.0233733

Bowler, D. E., Callaghan, C. T., Bhandari, N., Henle, K., Benjamin Barth, M., Koppitz, C., Klenke, R., Winter, M., Jansen, F., Bruelheide, H., & Bonn, A. (2022). Temporal trends in the spatial bias of species occurrence records. *Ecography, 2022,* e06219. https://doi.org/10.1111/ecog.06219

Brown, D. S. (1994). Freshwater Snails of Africa and their Medical Importance (2nd. ed.). Taylor and Francis, London, UK.

Brown, R. L., Charles, D., Horwitz, R. J., Pizzuto, J. E., Skalak, K., Velinsky, D. J., & Hart, D. D. (2024). Size-dependent effects of dams on river ecosystems and implications for dam removal outcomes. *Ecological applications, 34,* e3016. https://doi.org/10.1002/eap.3016

Camargo, J. A. (2019). Positive responses of benthic macroinvertebrates to spatial and temporal reductions in water pollution downstream from a trout farm outlet. *Knowledge & Management of Aquatic Ecosystems,* 420, 16. https://doi.org/10.1051/kmae/2019010

Chandler, M., See, L., Copas, K., Bonde, A. M., López, B. C., Danielsen, F., Legind, J. K., Masinde, S., Miller-Rushing, A. J., & Turak, E. (2017). Contribution of citizen science towards international biodiversity monitoring. *Biological conservation, 213*, 280–294. https://doi.org/10.1016/j.biocon.2016.09.004

Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology, 9*, 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x

Clewing, C., Geertz, T., Rassam, H., Woldekiros, T. H., & Albrecht, C. (2022). Freshwater diversity at a biogeographic edge zone: the high-mountain pea-clams of Ethiopia. *Systematics and Biodiversity, 20*, 1–15. https://doi.org/10.1080/14772000.2021.2005706

Cohen, A. S., Kaufman, L., & Ogutu-Ohwayo, R. (2019). Anthropogenic Threats, Impacts and Conservation Strategies in the African Great Lakes: A review. In T. C. Johnson & E. O. Odada (Eds), Limnology, climatology and paleoclimatology of the East African Lakes (pp. 575–624). Routledge. https://doi.org/10.1201/9780203748978-33

Cohen, A. S., Van Bocxlaer, B., Todd, J. A., McGlue, M., Michel, E., Nkotagu, H. H., Grove, A. T., & Delvaux, D. (2013). Quaternary ostracodes and molluscs from the Rukwa Basin (Tanzania) and their evolutionary and paleobiogeographic implications. *Palaeogeography, Palaeoclimatology, Palaeoecology, 392*, 79–97. https://doi.org/10.1016/j.palaeo.2013.09.007

Dallas, H. F. (2021). Rapid bioassessment protocols using aquatic macroinvertebrates in Africa–considerations for regional adaptation of existing biotic indices. *Frontiers in Water, 3,* 628227. https://doi.org/10.3389/frwa.2021.628227

Dallas, H. F., Lowe, S., Kennedy, M. P., Saili, K., & Murphy, K. J. (2018). Zambian Invertebrate Scoring System (ZISS): A macroinvertebrate-based biotic index for rapid bioassessment of southern tropical African river systems. *African Journal of Aquatic Science, 43,* 325–344. https://doi.org/10.2989/16085914.2018.1517081

Daniels, S. R., Phiri, E. E., Klaus, S., Albrecht, C., & Cumberlidge, N. (2015). Multilocus phylogeny of the Afrotropical freshwater crab fauna reveals historical drainage connectivity and transoceanic dispersal since the Eocene. *Systematic Biology, 64*, 549–567. https://doi.org/10.1093/sysbio/syv011

Danish Bilharziasis Laboratory. (1987). *A Field guide to African freshwater snails* (2nd ed.). World Health Organization.

Danley, P. D., Husemann, M., Ding, B., DiPietro, L. M., Beverly, E. J., & Peppe, D. J. (2012). The impact of the geologic history and paleoclimate on the diversification of East African cichlids. *International Journal of Evolutionary Biology*, *2012*, 574851. https://doi.org/10.1155/2012/574851

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*, 772–772. https://doi.org/10.1038/nmeth.2109

Dartevelle, E., & Schwetz, J. (1947). Contribution `a l'´etude de la faune malacologique des grands lacs africains: les lacs Albert, ´Edouard et Kivu. Memoires Institut Royal Colonial Belge. *14*, 1–47.

Dickens, C. W., & Graham, P. M. (2002). The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. *African Journal of Aquatic Science*, *27*, 1–10. https://doi.org/10.2989/16085914.2002.9626569

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973. https://doi.org/10.1093/molbev/mss075

Dudgeon, D., & Strayer, D. L. (2025). Bending the curve of global freshwater biodiversity loss: what are the prospects?. *Biological Reviews*, *100*, 205–226. https://doi.org/10.1111/brv.13137

Dusabe, M. C., Clewing, C., Kagoro, G. R., Rwibutso, M., Tumusiime, T., Ndeo, W. O., & Albrecht, C. (2025). Historical drainage evolution and water level fluctuations in the African Great Lakes shaped phylogeny and biogeography of *Gabbiella* gastropods. Journal of Biogeography. (in revision).

Dusabe, M. C., Kalinda, C., Clewing, C., Hyangya, B. L., Van Bocxlaer, B., & Albrecht, C. (2024). Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu. *Journal of Great Lakes Research*, *50*, 102339. https://doi.org/10.1016/j.jglr.2024.102339

Dusabe, M. C., Neubauer, T. A., Muvundja, F. A., Hyangya, B. L., & Albrecht, C. (2022). Family-level bio-indication does not detect the impacts of dams on macroinvertebrate communities in a low-diversity tropical river. *Frontiers in Environmental Science*, *10*, 902246. https://doi.org/10.3389/fenvs.2022.902246

Dusabe, M. C., Wronski, T., Gomes-Silva, G., Plath, M., Albrecht, C., & Apio, A. (2019). Biological water quality assessment in the degraded Mutara rangelands, northeastern Rwanda. *Environmental Monitoring and Assessment*, *191*, 139. https://doi.org/10.1007/s10661-019-7226-5

Edegbene, A. O., Arimoro, F. O., & Odume, O. N. (2019). Developing and applying a macroinvertebrate-based multimetric index for urban rivers in the Niger Delta, Nigeria. *Ecology and Evolution*, *9*, 12869–12885. https://doi.org/10.1002/ece3.5769

Edegbene, A. O., Elakhame, L. A., Arimoro, F. O., Osimen, E. C., & Odume, O. N. (2019). Development of macroinvertebrate multimetric index for ecological evaluation of a river in North Central Nigeria. *Environmental Monitoring and Assessment*, *191*, 274. https://doi.org/10.1007/s10661-019-7438-8

Fouchy, K., McClain, M. E., Conallin, J., & O'Brien, G. (2019). Multiple stressors in African freshwater systems. In S. Sabater, A. Elosegi, & R. Ludwig (Eds.), *Multiple stressors in river ecosystems: Status, impacts and prospects for the future* (pp. 179–191). Elsevier. https://doi.org/10.1016/B978-0-12-811713-2.00010-8

Gabriels, W., Lock, K., De Pauw, N., & Goethals, P. L. (2010). Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica-Ecology and Management of Inland Waters*, *40*, 199–207. https://doi.org/10.1016/j.limno.2009.10.001

Garcia, N., Campos, J. C., Alírio, J., Duarte, L. B., Arenas-Castro, S., Pôças, I., Teodoro, C, A., & Sillero, N. (2025). Assessing spatial and temporal trends over time in potential species richness using satellite time-series and ecological niche models. *Biodiversity and Conservation*, *34*, 429–446. https://doi.org/10.1007/s10531-024-02979-7

Garg, A., Yadav, B. K., Das, D. B., & Wood, P. J. (2022). Improving the assessment of polluted sites using an integrated bio-physico-chemical monitoring framework. *Chemosphere*, *290*, 133344. https://doi.org/10.1016/j.chemosphere.2021.133344

Gatti, R. C. (2025). Ecological Peace Corridors: A new conservation strategy to protect human and biological diversity. *Biological Conservation*, *302*, 110947. https://doi.org/10.1016/j.biocon.2024.110947

Gelaw, T. W., Mengistou, S., & Barker, P. A. (2024). A new diatom-based multimetric index (MMI-D) for ecological health monitoring in the Tropical Rift Valley Lake (Lake Hawassa, Ethiopia). *African Journal of Aquatic Science*, *49*, 330–343. https://doi.org/10.2989/16085914.2024.2417995

Gezie, A., Goshu, G., & Tiku, S. (2023). Ecological effect of a small dam on the macroinvertebrate assemblage and water quality of Koga River, Northwest Ethiopia. *Heliyon*, *9*.e17285. https://doi.org/10.1016/j.heliyon.2023.e17285

Gillet, J., Bruaux, P., Wolfs, J., 1960. Study of the depths at which molluscs occur in Lake Kivu and of the survival of Biomphalaria in deep water. *Annales de la Societe de Medecine Tropicale*. *40*, 643–649.

Guan, Q., Kang, Y., He, F., Li, Z., Xue, Z., & Wu, H. (2025). Diversity patterns and community assembly of aquatic macroinvertebrates in permanent and temporary inland saline-alkaline wetlands. *Biodiversity and Conservation*, *34*, 571–588. https://doi.org/10.1007/s10531-024-02987-7

Haase, P., Pauls, S. U., Schindehütte, K., & Sundermann, A. (2010). First audit of macroinvertebrate samples from an EU Water Framework Directive monitoring program: human error greatly lowers precision of assessment results. *Journal of the North American Benthological Society*, *29*, 1279–1291. https://doi.org/10.1899/09-183.1

He, S., & Wei, Y. (2022). Measuring the conservation attitudes of local farmers towards conservation easements in the Qianjiangyuan National Park. *Global Ecology and Conservation*, *36*, e02123. https://doi.org/10.1016/j.gecco.2022.e02123

Hillebrand, H., Blasius, B., Borer, E. T., Chase, J. M., Downing, J. A., Eriksson, B. K., Filstrup, C. T., Harpole, S.W., Hodapp, D., Larsen, S., Lewandowska, A. M., Seabloom, E. W., Van de Waal, D.B., & Ryabov, A. B. (2018). Biodiversity change is uncoupled from species richness trends: Consequences for conservation and monitoring. *Journal of applied ecology*, *55*, 169–184. https://doi.org/10.1111/1365-2664.12959

Hoag, C., & Svenning, J. C. (2017). African environmental change from the Pleistocene to the Anthropocene. *Annual Review of Environment and Resources*, *42*, 27–54. https://doi.org/10.1146/annurev-environ-102016-060653

Hyangya, B. L., Kankonda, A. B., Dusabe, M. C., Murhimanya, J. D. K., Kaningini, B. M., & Masilya, P. M. (2022). Choice of benthic macroinvertebrate-based metrics for assessing water quality in the littoral zone under anthropogenic disturbance in southern Lake Kivu (East Africa). *Ecohydrology*, *15*, e2468. https://doi.org/10.1002/eco.2468

Ivory, S. J., Blome, M. W., King, J. W., McGlue, M. M., Cole, J. E., & Cohen, A. S. (2016). Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. *Proceedings of the National Academy of Sciences*, *113*, 11895–11900. https://doi.org/10.1073/pnas.1611028113

Johnson, T. C., Werne, J. P., Brown, E. T., Abbott, A., Berke, M., Steinman, B. A., Halbur, J., Contreras, S., Grosshuesch, S., Deino, A., Lyons, R. P., Scholz, C. A., Schouten, S., & Damsté, J. S. (2016). A progressively wetter climate in southern East Africa over the past 1.3 million years. *Nature*, *537*, 220–224. https://doi.org/10.1038/nature19065

Johnston, A., Matechou, E., & Dennis, E. B. (2023). Outstanding challenges and future directions for biodiversity monitoring using citizen science data. *Methods in Ecology and Evolution*, *14*, 103–116. https://doi.org/10.1111/2041-210X.13834

Johnston, A., Moran, N., Musgrove, A., Fink, D., & Baillie, S. R. (2020). Estimating species distributions from spatially biased citizen science data. *Ecological Modelling*, *422*, 108927. https://doi.org/10.1016/j.ecolmodel.2019.108927

Jones, N. (2021). How Dangerous Is Africa's Explosive Lake Kivu?Nature, 597, 466–469. doi:10.1038/d41586-021-02523-5

Kaaya, L. T., Day, J. A., & Dallas, H. F. (2015). Tanzania River Scoring System (TARISS): a macroinvertebrate-based biotic index for rapid bioassessment of rivers. *African Journal of Aquatic Science*, *40*, 109–117. https://doi.org/10.2989/16085914.2015.1051941

Karp, T., Scholz, C. A., & McGlue, M. M. (2012). Structure and stratigraphy of the Lake Albert Rift, East Africa: Observations from seismic reflection and gravity data. in O. W. Baganz, Y. Bartov, K. Bohacs, and D. Nummedal, eds., Lacustrine sandstone reservoirs and hydrocarbon systems: AAPG Memoir 95, p299–318. https://doi.org/10.1306/13291394M952903

Keller, R. P., Geist, J., Jeschke, J. M., & Kühn, I. (2011). Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe*, *23*, 23. https://doi.org/10.1186/2190-4715-23-23

Kilonzo, F., Masese, F. O., Van Griensven, A., Bauwens, W., Obando, J., & Lens, P. N. (2014). Spatial–temporal variability in water quality and macro-invertebrate assemblages in the Upper Mara River basin, Kenya. *Physics and Chemistry of the Earth, Parts A/B/C*, *67–69*, 93–104. https://doi.org/10.1016/j.pce.2013.10.006

Knapp, S., Winter, M., & Klotz, S. (2017). Increasing species richness but decreasing phylogenetic richness and divergence over a 320-year period of urbanization. *Journal of Applied Ecology*, *54*, 1152–1160.https://doi.org/10.1111/1365-2664.12826

Lakew, A., & Moog, O. (2015). A multimetric index based on benthic macroinvertebrates for assessing the ecological status of streams and rivers in central and southeast highlands of Ethiopia. *Hydrobiologia*, *751*, 229–242. https://doi.org/10.1007/s10750-015-2189-1

Lawrence, T. J., Achieng, A. O., Chavula, G., Haambiya, L. H., Iteba, J., Kayanda, R., Kaunda, E., Ajode, M. Z., Muvundja, F. A., Nakiyende, H., Nyamweya, C., Obiero, K., Denis Plisnier, P., Phiri, H., Sibomana, C., & Smith, S. (2023). Future success and ways forward for scientific approaches on the African Great Lakes. *Journal of Great Lakes Research*, *49*, 102242. https://doi.org/10.1016/j.jglr.2023.102242

Lyons, R. P., Scholz, C. A., Cohen, A. S., King, J. W., Brown, E. T., Ivory, S. J., Johnson, T. C., Deino, A. L., Reinthal, P. N., McGlue, M. M. & Blome, M. W. (2015). Continuous 1.3-million-year record of East African hydroclimate, and implications for patterns of evolution and biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*, *112*, 15568–15573. https://doi.org/10.1073/pnas.1512864112

Macher, J. N., Salis, R. K., Blakemore, K. S., Tollrian, R., Matthaei, C. D., & Leese, F. (2016). Multiple-stressor effects on stream invertebrates: DNA barcoding reveals contrasting responses of cryptic mayfly species. *Ecological Indicators*, *61*, 159–169. https://doi.org/10.1016/j.ecolind.2015.08.024

Madzivanzira, T. C., South, J., Wood, L. E., Nunes, A. L., & Weyl, O. L. F. (2020). A Review of Freshwater Crayfish Introductions in Africa. *Reviews in Fisheries Science & Aquaculture*, *29*, 218–241. https://doi.org/10.1080/23308249.2020.1802405

Mahulu, A., Stelbrink, B., Van Bocxlaer, B., Riedel, F., & Albrecht, C. (2021). Going with the flow? Diversification of gastropods reflects drainage evolution in Africa. *Journal of Biogeography*, *48*, 1579–1593. https://doi.org/10.1111/jbi.14096

Mandahl-Barth, G. (1954). The Freshwater Mollusks of Uganda and Adjacent Territories. Musée royal du Congo belge.

Mandahl-Barth, G. (1988). *Studies on African freshwater bivalves*. Danish Bilharziasis Laboratory, Charlottenlund.

Martínez, A., Larranaga, A., Basaguren, A., Pérez, J., Mendoza-Lera, C., & Pozo, J. (2013). Stream regulation by small dams affects benthic macroinvertebrate communities: from structural changes to functional implications. *Hydrobiologia* ***711***, *31–42*. https://doi.org/10.1007/s10750-013-1459-z

Martínez, Y., Gutiérrez, D., Álvarez-Troncoso, R., & Garrido, J. (2020). Impact of small-scale hydropower stations on macroinvertebrate communities for regulated rivers. *Limnetica*, *39*, 317–334. https://doi.org/10.23818/limn.39.21

Masese, F. O., Wanderi, E. W., Nyakeya, K., Achieng, A. O., Fouchy, K., & McClain, M. E. (2023). Bioassessment of multiple stressors in Afrotropical rivers: Evaluating the performance of a macroinvertebrate-based index of biotic integrity, diversity, and regional biotic indices. *Frontiers in Environmental Science*, *11*. https://doi.org/10.3389/fenvs.2023.1015623

Matzke, N. J. (2013a). BioGeoBEARS: BioGeography with Bayesian (and Likelihood) evolutionary analysis in R scripts. University of California, Berkeley, CA.

Matzke, N. J. (2013b). Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography*, *5*, 242–248. https://doi.org/10.21425/F55419694

Maynard, L., Monroe, M. C., Jacobson, S. K., & Savage, A. (2020). Maximizing biodiversity conservation through behavior change strategies. *Conservation Science and Practice*, *2*, e193. https://doi.org/10.1111/csp2.193

Mbaka, J. G., & Wanjiru Mwaniki, M. (2015). A global review of the downstream effects of small impoundments on stream habitat conditions and macroinvertebrates. *Environmental Reviews*, *23*, 257–262. https://doi.org/10.1139/er-2014-0080

Meier, J. I., McGee, M. D., Marques, D. A., Mwaiko, S., Kishe, M., Wandera, S., Neumann, D., Mrosso, H., Chapman, L. J., Chapman, C. A., Kaufman, L., Taabu-Munyaho, A., Wagner, C. E., Bruggmann, R., Excoffier, L., & Seehausen, O. (2023). Cycles of fusion and fission enabled rapid parallel adaptive radiations in African cichlids. *Science*, *381*, eade2833. https://doi.org/10.1126/science.ade2833

Mezgebu, A. (2022). A review on freshwater biomonitoring with benthic invertebrates in Ethiopia. *Environmental and Sustainability Indicators*, *14*, 100174. https://doi.org/10.1016/j.indic.2022.100174

Mpopetsi, P. P., Dondofema, F., Mangadze, T., Masese, F. O., Munyai, L. F., & Dalu, T. (2025). Anthropogenic threats. *Afrotropical Streams and Rivers: Structure, Ecological Processes and Management*, 637–668. https://doi.org/10.1016/B978-0-443-23898-7.00025-7

Mulanda Aura, C., Nyamweya, C. S., Njiru, J. M., Omondi, R., Manyala, J., Musa, S., Owiti, H., Guya, F., Ongore, C., Ogari, Z., & Mwamburi, J. (2021). Using the Multi-metric Index of Biotic Integrity methodological approach to determine the major river catchment that most pollutes a lake. *Aquatic Ecosystem Health & Management*, *24*, 12–20. https://doi.org/10.14321/aehm.024.01.04

Muvundja, A. F., Mulungula, P. M., Kisekelwa, T. K., Muhigirwa, B.C., Pasche, N., Hyangya, L. B., Mudakikwa, R. E., Akonkwa, B. D., Nahayo, D., Migeni, A. Z., Smith, S., Wüest, A., & Lawrence, T. (2022). Current status and strategic way forward for long-term management of Lake Kivu (East Africa). *Journal of Great Lakes Research 49*, 102024. https://doi.org/10.1016/j.jglr.2022.04.004

Nakiyende, H., Basooma, A., Nyeko, J. I., Okello, W., Rugadya, R., Albrecht, C., Lawrence, T., Van Steenberge, M., Smith, S., Muderhwa, N., Matunguru, J., Mulongaibalu, M., & Ajode, M. Z. (2023). Limitations for informed decision making and better management of the transboundary Lake Albert fisheries resources. *Journal of Great Lakes Research, 49, 102165*. https://doi.org/10.1016/j.jglr.2023.02.006

Ndatimana, G., Dusabe, M. C., & Albrecht, C. (2025). Bridging riverine and lacustrine systems: Macroinvertebrate indicators of ecological health in the Rwandan Congo basin. *Environmental Monitoring and Assessment* (accepted).

Ndatimana, G., Nantege, D., & Arimoro, F. O. (2023). A review of the application of the macroinvertebrate-based multimetric indices (MMIs) for water quality monitoring in lakes. *Environmental Science and Pollution Research*, *30*, 73098–73115. https://doi.org/10.1007/s11356-023-27559-0

Okay, A. I., Zattin, M., & Cavazza, W. (2010). Apatite fission-track data for the Miocene Arabia-Eurasia collision. *Geology*, *38*, 35–38. https://doi.org/10.1130/G30234.1

Olawusi-Peters, O. O., & Ajibare, A. O. (2014). Species richness, diversity and abundance of some Decapod Crustaceans in coastal waters of Ondo State, South West, Nigeria. *International Journal of Fauna and Biological Studies*, *1*, 44–51.

Ortiz-Sepulveda, C. M., Stelbrink, B., Vekemans, X., Albrecht, C., Riedel, F., Todd, J. A., & Van Bocxlaer, B. (2020). Diversification dynamics of freshwater bivalves (Unionidae: Parreysiinae: Coelaturini) indicate historic hydrographic connections throughout the East African Rift System. *Molecular Phylogenetics and Evolution*, *148*, 106816. https://doi.org/10.1016/j.ympev.2020.106816

Oyege, I., Katwesigye, R., Kiwanuka, M., Mutanda, H. E., Bosco Niyomukiza, J., Kataraihya, J., Kica, S., & Egor, M. (2024). Temporal trends of water quality parameters, heavy metals, microplastics, and emerging organic pollutants in Lake Victoria and its basin: knowns, knowledge gaps, and future directions. *Environmental Nanotechnology, Monitoring & Management*, *22*, 100962. https://doi.org/10.1016/j.enmm.2024.100962

Peter, B. D. (2004). African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters*, *220*, 3–24. https://doi.org/10.1016/S0012-821X(04)00003-2

Pettinotti, L., de Ayala, A., & Ojea, E. (2018). Benefits from water related ecosystem services in Africa and climate change. *Ecological Economics*, *149*, 294–305. https://doi.org/10.1016/j.ecolecon.2018.03.021

Pilsbry, H. A., & Bequaert, J. (1927). The aquatic mollusk of the Belgian Congo, with a geographical and ecological account of Congo malacology. *Bulletin of the American Museum of Natural History*. *53*, 69–602.

Plisnier, P. D., Kayanda, R., MacIntyre, S., Obiero, K., Okello, W., Vodacek, A., Cocquyt, C., Abegaz, H., Achieng, A., Akonkwa, B., Albrecht, C., Balagizi, C., Barasa, J., Bashongwa, R. A., Bishobibiri, A. B., Bootsma, H., Borges, A. V., Chavula, G., Dadi, T., & Lawrence, T. (2023). Need for harmonized long-term multi-lake monitoring of African Great Lakes. *Journal of Great Lakes Research*, *49*, 101988. https://doi.org/10.1016/j.jglr.2022.01.016

R Core Team. 2024. R: A language and environment for statistical computing. https://www.R-project.org. R Foundation for Statistical Computing, Vienna, Austria.

Ross, K. A., Smets, B., De Batist, M., Hilbe, M., Schmid, M., & Anselmetti, F. S. (2014). Lake-level rise in the late Pleistocene and active subaquatic volcanism since the Holocene in Lake Kivu, East African Rift. *Geomorphology*, *221*, 274–285. https://doi.org/10.1016/j.geomorph.2014.05.010

Rouwet, D., Németh, K., Tamburello, G., Calabrese, S., & Issa. (2021). Volcanic lakes in Africa: the VOLADA Africa 2.0 Database, and implications for volcanic hazard. *Frontiers in Earth Science*, *9*, 717798. https://doi.org/10.3389/feart.2021.717798

Sarmento, H., Leitao, M., Stoyneva, M., Compere, P., Coute, A., Isumbisho, M., & Descy, J. P. (2007). Species diversity of pelagic algae in Lake Kivu (East Africa). *Cryptogamie-Algologie*, *28*, 245–269.

Schmid, M., Halbwachs, M., Wehrli, B., Wüest, A., Schmid, M., Halbwachs, M., Wehrli, B., & Wüest, A. (2005). Weak mixing in Lake Kivu: New insights indicate increasing risk of uncontrolled gas eruption. *Geochemistry, Geophysics, Geosystems*, *6*, 7009. https://doi.org/10.1029/2004GC000892

Schultheiß, R., Van Bocxlaer, B., Wilke, T., & Albrecht, C. (2009). Old fossils–young species: Evolutionary history of an endemic gastropod assemblage in Lake Malawi. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 2837–2846. https://doi.org/10.1098/rspb.2009.0467

Schultheiß, R., Van Boxlaer, B., Riedel, F., von Rintelen, T., & Albrecht, C. (2014). Disjunct distributions of freshwater snails testify to a central role of the Congo system in shaping biogeographic patterns in Africa. *BMC Evolutionary Biology*, *14*, 1471–2148. https://doi.org/10.1186/1471-2148-14-42

Schultheiß, R., Van Boxlaer, B., Riedel, F., von Rintelen, T., & Albrecht, C. (2014). Disjunct distributions of freshwater snails testify to a central role of the Congo system in shaping biogeographic patterns in Africa. *BMC Evolutionary Biology*, *14*, 1471–2148. https://doi.org/10.1186/1471-2148-14-42

Schultheiß, R., Wilke, T., Jørgensen, A., & Albrecht, C. (2011). The birth of an endemic species flock: demographic history of the *Bellamya* group (Gastropoda, Viviparidae) in Lake Malawi. *Biological Journal of the Linnean Society*, *102*, 130–143. https://doi.org/10.1111/j.1095-8312.2010.01574.x

Shen, W., Sun, Y., Li, J., Zhang, H., Ding, Z., & Tang, X. (2024). Monitoring spatio-temporal dynamics of multi-dimensional karst ecosystem quality in Southwest China by integrating multi-source data. *International Journal of Digital Earth*, *17*, 2356119. https://doi.org/10.1080/17538947.2024.2356119

Snoeks, J., Kaningini, B., Masilya, P., Nyina-Wamwiza, L., & Guillard, J. (2012). Fishes in Lake Kivu: Diversity and Fisheries. In: Descy, JP., Darchambeau, F., Schmid, M. (eds), Lake Kivu: Limnology and biogeochemistry of a tropical great lake, 127–152. Aquatic Ecology Series, vol 5. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-4243-7_8

Soto, I., Macêdo, R. L., Carneiro, L., Briski, E., Kouba, A., Cuthbert, R. N., & Haubrock, P. J. (2024). Divergent temporal responses of native macroinvertebrate communities to biological invasions. *Global Change Biology*, *30*, e17521. https://doi.org/10.1111/gcb.17521

Sousa-Guedes, D., Arenas-Castro, S., & Sillero, N. (2020). Ecological niche models reveal climate change effect on biogeographical regions: The Iberian Peninsula as a case study. *Climate*, *8*, 42. https://doi.org/10.3390/cli8030042

Ssenkuba, F., Rwibutso, M., Dusabe, M. C., Tumusiime, J., & Albrecht, C. (2025). Invaders taking over – mollusc faunal change in volcanic barrier lakes of the Albertine Rift biodiversity hotspot. (in final preparation).

Strayer, D. L. (2012). Eight questions about invasions and ecosystem functioning. *Ecology letters*, *15*, 1199–1210. https://doi.org/10.1111/j.1461-0248.2012.01817.x

Sweeney, B. W., Battle, J. M., Jackson, J. K., & Dapkey, T. (2011). Can DNA barcodes of stream macroinvertebrates improve descriptions of community structure and water quality?. *Journal of the North American Benthological Society*, *30*, 195–216. https://doi.org/10.1899/10-016.1

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*, 3022–3027. https://doi.org/10.1093/molbev/msab120

Thiele, J. (1911). Mollusken der deutschen Zentral-Afrika-Expedition. Wissenschaftliche Ergebnisse der deutschen Zentral-Afrika-Expedition, 1907–1908. Klinkhardt & Biermann.

Tibihika, D. M., Okello, W., Barekye, A., Mbabazi, D., Omony, J., & Kiggundu, V. (2016). Status of Kigezi minor Lakes: A limnological survey in the Lakes of Kisoro, Kabale and Rukungiri Districts. International Journal of Water Resources and Environmental Engineering. *80*, 60–73. https://doi.org/10.5897/IJWREE2016.0645

Tickner, D., Opperman, J. J., Abell, R., Acreman, M., Arthington, A. H., Bunn, S. E., Cooke, S. J., Dalton, J., Darwall, W., Edwards, G., Harrison, I., Hughes, K., Jones, T., Leclère, D., Lynch, A. J., Leonard, P., Mcclain, M. E., Muruven, D., Olden, J. D., Ormerod, S.J., Robinson, J., Tharme, R. E., Thieme, M., Tockner, K., Wright, M., & Young, L. (2020). Bending the curve of global freshwater biodiversity loss: an emergency recovery plan. *BioScience*, *70*, 330–342. https://doi.org/10.1093/biosci/biaa002

Tumwebaze, I., Clewing, C., Chibwana, F. D., Kipyegon, J. K., & Albrecht, C. (2022). Evolution and biogeography of freshwater snails of the genus *Bulinus* (Gastropoda) in afromontane extreme environments. *Frontiers in Environmental Science*, *10*, 902900. https://doi.org/10.3389/fenvs.2022.902900

Tumwebaze, I., Clewing, C., Dusabe, M. C., Tumusiime, J., Kagoro-Rugunda, G., Hammoud, C., & Albrecht, C. (2019). Molecular identification of Bulinus spp. intermediate host snails of *Schistosoma* spp. in crater lakes of western Uganda with implications for the transmission of the *Schistosoma haematobium* group parasites. *Parasites & vectors*, *12*, 565. https://doi.org/10.1186/s13071-019-3811-2

Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*, 1367–1382. https://doi.org/10.1899/12-203.1

Vaikasas, S., Palaima, K., & Pliūraitė, V. (2013). Influence of hydropower dams on the state of macroinvertebrates assemblages in the Virvyte river, Lithuania. *Journal of Environmental Engineering and Landscape Management*, *21*, 305–315. https://doi.org/10.3846/16486897.2013.796956

Van Bocxlaer, B., & Albrecht, C. (2015). Ecosystem change and establishment of an invasive snail alter gastropod communities in long-lived Lake Malawi. *Hydrobiologia*, *744*, 307–316. https://doi.org/10.1007/s10750-014-2093-0

Van Bocxlaer, B., Clewing, C., Mongindo Etimosundja, J. P., Kankonda, A., Wembo Ndeo, O., & Albrecht, C. (2015). Recurrent camouflaged invasions and dispersal of an Asian freshwater

gastropod in tropical Africa. *BMC Evolutionary Biology*, *15*, 33. https://doi.org/10.1186/s12862-015-0296-2

Van Damme, D., & Gautier, A. (2013). Lacustrine mollusc radiations in the Lake Malawi Basin: Experiments in a natural laboratory for evolution. *Biogeosciences*, *10*, 5767–5778. https://doi.org/10.5194/bg-10-5767-2013

Van Damme, D., & Pickford, M. (2003). The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. *Hydrobiologia*, *498*, 1–83. https://doi.org/10.1023/A:1026298512117

Van Damme, D., & Pickford, M. (2003). The late Cenozoic Thiaridae (Mollusca, Gastropoda, Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and evolution of the Tanganyikan thalassoid malacofauna. *Hydrobiologia*, *498*, 1–83. https://doi.org/10.1023/A:1026298512117

Van der Merwe, P. D. W., Cotterill, F. P., Kandziora, M., Watters, B. R., Nagy, B., Genade, T., Flügel, T. J, Svendsen, D., & Bellstedt, D. U. (2021). Genomic fingerprints of palaeogeographic history: The tempo and mode of rift tectonics across tropical Africa has shaped the diversification of the killifish genus *Nothobranchius* (Teleostei: Cyprinodontiformes). *Molecular Phylogenetics and Evolution*, *158*, 106988. https://doi.org/10.1016/j.ympev.2020.106988

Verheyen, E. (2016). Oil extraction imperils Africa's Great Lakes. *Science*, *354*, 561–562. https://doi.org/10.1126/science.aal1722

Wang, J., Ding, L., Tao, J., Ding, C., & He, D. (2019). The effects of dams on macroinvertebrates: Global trends and insights. *River Research and Applications*, *35*, 702–713. https://doi.org/10.1002/rra.3431

Wang, X., Cai, Q., Jiang, W., & Qu, X. (2013). Assessing impacts of a dam construction on benthic macroinvertebrate communities in a mountain stream. *Fresenius Environmental Bulletin*, *22*, 103–110.

Wienhues, G., Temoltzin-Loranca, Y., Vogel, H., Morlock, M. A., Cohen, A. S., Anselmetti, F. S., Bernasconi, S. M., Jaggi, M., Tylmann, W., Kishe, M. A., King, L., Ngoepe, N., Courtney-Mustaphi, C. J., Muschick, M., Matthews, B., Mwaiko, S., Seehausen, O., Tinner, W., & Grosjean, M. (2024). From desiccation to wetlands and outflow: Rapid re-filling of Lake Victoria during the latest Pleistocene 14–13 ka. *Journal of Great Lakes Research*, *50*, 102246. https://doi.org/10.1016/j.jglr.2023.102246

Wiens, J. J. (2011). The causes of species richness patterns across space, time, and clades and the role of "ecological limits". *The Quarterly review of biology*, *86*, 75–96. https://doi.org/10.1086/659883

Wilke, T., Davis, G. M., Qiu, D., & Spear, R. C. (2006). Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: Another case of ancestral polymorphism? *Malacologia*, *48*, 143–157.

Willis, K. J., Araujo, M. B., Bennett, K. D., Figueroa-Rangel, B., Froyd, C. A., & Myers, N. (2007). How can a knowledge of the past help to conserve the future? Biodiversity conservation and the relevance of long-term ecological studies. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*, 175–186. https://doi.org/10.1098/rstb.2006.1977

Wilson, E. O. (2021). *Every species is a masterpiece*. Penguin UK.

Wondmagegn, T., & Mengistou, S. (2023). Development of macroinvertebrate based multimetric index for ecological health monitoring in Lake Hawassa, Ethiopia. *Environmental and sustainability indicators*, *18*, 100242. https://doi.org/10.1016/j.indic.2023.100242

Worthington, E. B. (1932). A Report on the Fisheries of Uganda, investigated by the Cambridge Expedition to the East African Lakes, 1930-31. *The Geographical Journal*, *81*, 269. https://doi.org/10.2307/1784052

Wronski, T., Dusabe, M.C., Apio, A., Hausdorf, B., & Albrecht, C. (2015). Biological assessment of water quality and biodiversity in Rwandan rivers draining into Lake Kivu. *Aquatic Ecology*, *49*, 309–320. https://doi.org/10.1007/s10452-015-9525-4

Xiaocheng, F., Tao, T., Wanxiang, J., Fengqing, L., Naicheng, W., Shuchan, Z., & Qinghua, C. (2008). Impacts of small hydropower plants on macroinvertebrate communities. *Acta Ecologica Sinica*, *28*, 45–52. https://doi.org/10.1016/S1872-2032(08)60019-0

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science*, *292*, 686–693. https://doi.org/10.1126/science.1059412

Zhang, R., Liu, H., Xu, Z., Zhao, X., Wen, L., Zhuo, Y., & Wang, L. (2025). Ecological problem identification based on spatial and temporal dynamics of ecosystem quality: A case study. *Ecological Indicators*, *170*, 112987. https://doi.org/10.1016/j.ecolind.2024.112987

III. PUBLICATIONS

Paper 1: Dusabe et al., 2022

Family-level bio-indication does not detect the impacts of dams on macroinvertebrate communities in a low-diversity tropical river.

Dusabe, M. C., Neubauer, T. A., Muvundja, F. A., Hyangya, B. L., & Albrecht, C. (2022). *Frontiers in Environmental Science*, *10*, 902246. https://doi.org/10.3389/fenvs.2022.902246

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BRIEF RESEARCH REPORT
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Family-Level Bio-Indication Does not Detect the Impacts of Dams on Macroinvertebrate Communities in a Low-Diversity Tropical River

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The Ruzizi River, the outlet of Lake Kivu in the Albertine Rift, flows into Lake Tanganyika and is important for hydropower generation and irrigation. The impacts of 2 dams in the Ruzizi River on macroinvertebrate community composition and diversity were surveyed every 3 months from December 2015 to October 2017. Macroinvertebrate samples were collected at sites upstream and downstream and additionally at two sites further downstream of the dams, in both comparatively pristine and highly disturbed areas. Several indices (Shannon-Wiener index, Simpson index, Pielou's evenness, Rare Family Prevalence, and Average Score Per Taxa) were used to determine the alpha diversity and evenness of macroinvertebrates at the family level. Our results showed little to no immediate effect of the dams on macroinvertebrate diversity. Macroinvertebrate composition differed slightly below the dams compared to upstream. Communities near Dam II had slightly higher diversity compared to Dam I, probably because the vicinity to Lake Kivu has an immediate effect on diversity upstream of the first dam and likely because Dam II is 30 years younger than Dam I. This study suggests the importance of using species-level indices to better understand the ecological impacts of dams on macroinvertebrate diversity of tropical rivers with low species diversity.

Keywords: Ruzizi River dams, macroinvertebrates, biodiversity indices, pollution, environmental flows, hydropower

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INTRODUCTION

Increasing demand for electricity creates an urgent need to build hydropower plants for renewable energy and as a valuable source of revenue (IEA: International Energy Agency, 2019), especially in tropical regions where the world's largest rivers are located (Latrubesse et al., 2005; Sinha et al., 2012). The demand for hydropower in such regions has even led to a global "megadam mania" (Gross, 2016). In addition to hydropower, dams provide many benefits to societies such as flood control, irrigation, and water level regulation (Altinbilek, 2002). Despite the great importance of dams, they alter rivers and their ecosystems significantly (Poff and Matthews, 2013; Zarfl et al., 2015; Couto and Olden, 2018; Grill et al., 2019) by changing the flow

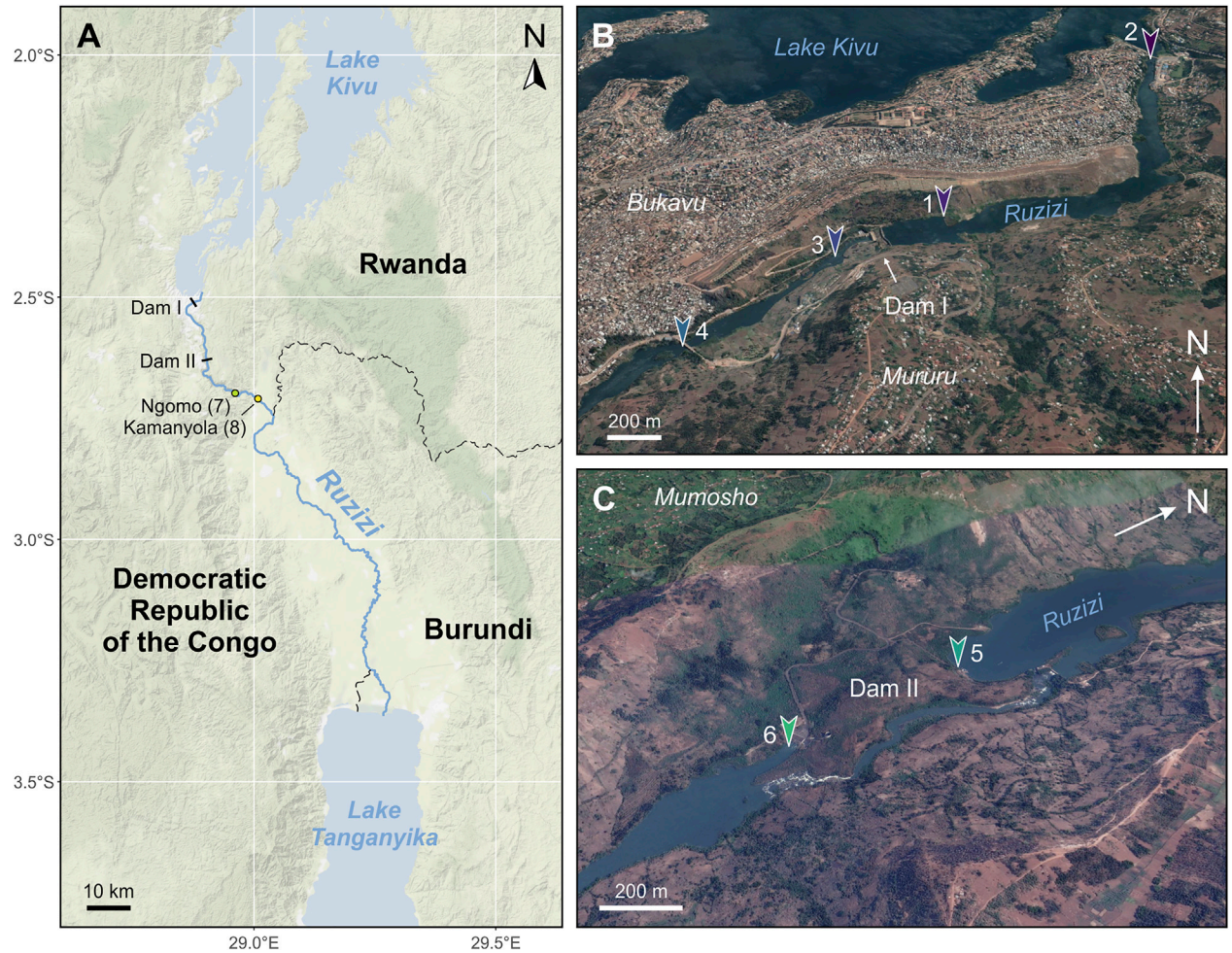

FIGURE 1 | Location of the Ruzizi River, the dams, and collecting sites. See **Table 1** for detailed information on the localities. **(A)** Ruzizi River with Dam I and Dam II and further downstream sites. **(B, C)** Sampling site locations downstream and upstream of Dam I (between Mururu and Bukavu) and Dam II (in Mumosho). The map in **(A)** was created with the R package ggmap 3.0.0 (Kahle et al., 2019). **(B, C)** were created in Google Earth Pro 7.3.4.8248, map data © 2022 Google/Maxar Technologies.

regime, e.g., reducing river connectivity, altering temperature and nutrient status (Bunn and Arthington, 2002; Renöfält et al., 2010; Reid et al., 2019; Kuriqi et al., 2019, 2020). These hydrological changes and habitat fragmentation are mainly related to loss of sediment connectivity, resulting in significant changes in downstream sections and affecting macroinvertebrate communities (Mueller et al., 2011; Martínez et al., 2013). In addition to the mentioned threats, dams can facilitate the establishment of invasive species that could further drive the loss of other aquatic organisms (Dick et al., 2002; Johnson et al., 2008).

Aquatic macroinvertebrates are the most popular organisms used to assess freshwater biological quality (Wright, 2010; Kaaya et al., 2015; Wronski et al., 2015; Dusabe et al., 2019), especially in the stream and river assessments (Dallas, 2021). Macroinvertebrates are recognized as good indicators for monitoring habitat quality (Greenwood et al., 1999; Carter et al., 2017) as well as many different anthropogenic stressors, including changes in flow regime, pollution, eutrophication, and biological invasions (Bonada et al., 2006; Fornaroli et al., 2018; Guareschi and Wood, 2019; Mellado-Díaz et al., 2019).

The effects of dams on downstream macroinvertebrate diversity and abundance have been documented repeatedly (Cortes et al., 1998; Santucci et al., 2005; Sharma et al., 2005; Xiaocheng et al., 2008; Bredenhand and Samways, 2009; Vaikasas et al., 2013; Serrana et al., 2018; Wang et al., 2019). Species richness and abundance of sensitive and tolerant taxa have been the most commonly used metrics to determine the ecological impacts of macroinvertebrates below dams (Martínez et al., 2013). Using biotic indicators to detect the effects of dams on the macroinvertebrate community requires a profound knowledge of the species identities because even closely related species may have different tolerances to environmental stressors (Macher et al., 2016; Mezgebu, 2022). However, accurate identification of freshwater macroinvertebrates to the species level can be difficult, especially for larval or subadult specimens, and often results in low taxonomic resolution or misidentification (Haase et al., 2010; Sweeney et al., 2011).

This in turn reduces the accuracy of the approach and can lead to inaccurate biological assessments and eventually even misguided management (Stein et al., 2014).

A promising alternative to morphological identification is DNA-based identification (Elbrecht et al., 2017), which has been shown to be reliable in non-tropical regions (Stein et al., 2013; Elbrecht and Leese, 2015). Nevertheless, most available bio-indication systems are nowadays based on the family taxonomic level (Dallas, 2021; Mezgebu, 2022). The objective of this research is to evaluate whether a family-level bio-indication can determine the impact of dams on macroinvertebrates in a low-diversity tropical river.

MATERIALS AND METHODS

Study Area

The Ruzizi River, also known as Rusizi, flows from Lake Kivu into Lake Tanganyika (**Figure 1**). It is the only outlet of Lake Kivu and one of the most important tributaries of Lake Tanganyika in the Congo Basin and lies between the Democratic Republic of Congo (DRC) and Rwanda on the one hand and DRC and Burundi on the other (Descy et al., 2012). The Ruzizi River has an average long-term annual flow of about 86 m^3/s (Muvundja et al., 2014; ABAKIR, 2020). For the first 50 km from Lake Kivu to the village of Kamanyola (headwaters), the river lies between the steep, heavily deforested, and barren watersheds (upper Ruzizi) of South Kivu in the D.R. Congo and Rusizi District in Rwanda. After crossing the escarpment, the river drops from an elevation of 1,450 m to 962 m (ABAKIR, 2020). Numerous waterfalls make it a potential hydropower source. After the escarpments (Ngomo in DRC and Nzahaha in Rwanda), the Ruzizi River extends into a vast plain and gradually drops from an elevation of 962–770 m with a low average gradient before entering Lake Tanganyika. The river provides important habitat for a variety of aquatic species (Hughes and Hughes, 1992). The Ruzizi water at the first dam has a high salt concentration (~1.1 g/L or 1,200 μS/cm electrical conductivity), being as is as salty as the surface waters of Lake Kivu due to dissolution of volcanic ashes in most rivers of North Kivu and subaquatic discharge of underground hydrothermal springs. Towards the Lower Ruzizi in Kiliba, the river water freshens considerably (~0.5 g/L or 650 μS/cm) due to further freshwater inputs from the watershed (Muvundja et al., 2022). The Ruzizi River is of outstanding importance to the African Great Lakes Region (DRC, Rwanda, and Burundi) because of hydroelectric power generation. There are two active hydropower dams on the river: the first dam built in 1959 is located 3 km downstream of the outlet of Lake Kivu at Mururu at an altitude of 1,460 m a.s.l. It has an installed capacity of 28 MW (TRACTIONEL and RRI, 1980; Fichtner Gmbh und co, 2008). The second dam was built in 1989 and is located about 16 km from Bukavu in Mumosho at an altitude of 1,393 m a.s.l, with a capacity of 44 MW (**Figure 1**) (TRACTIONEL and RRI, 1980; Fichtner Gmbh und co, 2008; ABAKIR, 2020). Two more dams are planned: Dam III (147 MW) and Dam IV, to be built downstream of Dam II and between dams II and III, respectively (Fichtner Gmbh und co, 2008; Dombrowsky et al., 2014; ONEC-BAD, 2015; ABAKIR, 2020).

Macroinvertebrates Sampling

We sampled the macroinvertebrate community at eight stations from December 2015 to August 2017 (**Supplementary Table S1**). Samples were collected every 3 months to cover both wet and dry seasons. Each collection site was sampled eight times to account for a potential variation in community composition over time. Samples were collected at sites upstream and downstream of Dam I and Dam II. Additional samples were collected further downstream of Dam II at Manda/Ngomo and Kamanyola as reference sites that are unlikely to be impacted by the dams. We collected macroinvertebrates in various habitats by kicking, hand picking, or hand scooping samples for leaf litter and sapropel (Dusabe et al., 2019). We collected the samples on the banks of the Ruzizi River in different substrates (sand, stones, rocks, and macrophytes) by using hand tweezers on stones and rocks and scoop nets (diameter: 20 cm, mesh size: 1 mm) in sands and macrophytes and on the water surface as well as in the water column. Organisms were separated by taxonomic groups, sorted using featherweight tweezers.

At each sampling site, the samples were collected within 50 m along the river shoreline. Some sites were accessible by foot, others by boat. Latitude, longitude, and elevation were recorded at each sampling site using a Garmin GPS IV (**Table 1**). Sampling lasted 60 min and was conducted by three individuals. Samples were preserved with 70% ethanol. All macroinvertebrates were identified to family level using predominantly keys developed for the southern African sub-region (Cape Province to northern Zambia; Day et al., 1999, 2001a, 2001b, 2002; Day and de Moor, 2002; de Moor and Day, 2002; Stals and de Moor, 2007; de Moor et al., 2003). Oligochaeta and Polychaeta were determined to order level due to the lack of identification keys for the region.

Data Analyses

We calculated five different diversity indices: Shannon-Wiener index, Simpson index, Pielou's Evenness, Rare Family Prevalence (RFP) index, which indicates the proportion of families at each station represented by single individuals (Emberton et al., 1997), and Average Score Per Taxa (ASPT) based on TARISS (Tanzania Rivers Scoring System; Kaaya et al., 2015) to categorize the sensitive and tolerant taxa (Kaaya et al., 2015). The ASPT scores of macroinvertebrate groups were categorized as follows: Low sensitivity (1–5), moderate sensitivity (6–10), and high sensitivity (11–15) (Gerber and Gabriel, 2002).

In order to test for an effect of the sampling position with respect to dams (upstream vs. downstream) on each of the five diversity indices we ran linear mixed effects models. We divided the analyses into two batches, one including only the samples upstream and downstream of Dams I and II, respectively, the second one including the samples taken further downstream at Ngomo and Kamanyola (**Figure 1**) as a separate category. The rationale behind the second approach was to compare upstream/downstream samples to communities less impacted by dam

TABLE 1 | Locality information with the villages where Dam I and Dam II are located and coordinates of sampling sites upstream and downstream of the dams.

Village	Dams	Loc. #	Localities	Latitude N	Longitude E
Mururu/Bukavu	Dam I	1	Ruzizi I upstream site 1	−2.507755	28.878461
		2	Ruzizi I upstream site 2	−2.491257	28.892775
		3	Ruzizi I downstream site 1	−2.510530	28.873312
		4	Ruzizi I downstream site 2	−2.515492	28.867921
Mumosho	Dam II	5	Ruzizi II upstream	−2.628099	28.901870
		6	Ruzizi II downstream	−2.633562	28.902669
Ngomo	Planned Dam III	7	Planned Ruzizi III upstream	−2.700285	28.964059
Kamanyola		8	Planned Ruzizi III downstream	−2.709655	29.009136

building. The communities at Ngomo and Kamanyola are located several kilometers downstream of Dam II and are distinct from the communities downstream and upstream of Dam I and Dam II. This could be due to the streams that join the Ruzizi River upstream of the Ngomo site and therefore bring new macroinvertebrate communities. Kamanyola is disturbed by several anthropogenic stressors because the site is located on the Congo-Rwanda border in an area with a high population density, causing the river to be used heavily for domestic purposes and agriculture.

We tested for the influence of two different types of random effect structures, one including the sampling period (month/year), the other including sampling period and sampling area (Dam I, Dam II, and Ngomo/Kamanyola). In all cases we applied random intercept models. Preliminary analyses with more complex random effect designs yielded distinctly less supported or singular models.

We used Akaike Information Criterion (AIC) to select the best model, in each case comparing a candidate model with the corresponding null model lacking fixed effects but having the same random structure. Following Harrison et al. (2018) we used maximum likelihood estimation to compare models with the same random structure, but restricted maximum likelihood to estimate variance components of random effects and model parameters as well as to compare models with different random structure. We applied the classical ΔAIC cutoff of 2 after Burnham and Anderson (2004) to evaluate significant difference among models, but we are aware of the problems associated with such an assumption (compare Harrison et al., 2018) and critically discuss this issue below.

Models that were found significantly better than the corresponding null models were further examined for model adequacy and model fit. We assessed model residuals through simulations that transform them to a standardized scale, tested for potential dispersion issues, and inspected autocorrelation function plots to detect a possible influence of temporal autocorrelation. Marginal R^2 and adjusted intraclass correlation coefficient (ICC) were calculated to assess the variances explained by the fixed and random effects, respectively (Nakagawa et al., 2017; Harrison et al., 2018).

Finally, to compare communities among sampling sites and periods, we ran a nonmetric multidimensional scaling based on a Bray-Curtis dissimilarity matrix for each sampling period. To account for strong variation in abundances, both Wisconsin double standardization and square-root transformation were applied.

All analyses were carried out in R vs. 4.0.3 (R Core Team, 2020) using the packages DHARMa 0.3.3.0 (Hartig and Lohse, 2020), ggeffects 1.0.1 (Lüdecke et al., 2020a), lme4 1.1–26 (Bates et al., 2020), performance 0.6.1 (Lüdecke et al., 2020b), and vegan 2.5–7 (Oksanen et al., 2020).

RESULTS

A total of 4,439 individuals from 13 macroinvertebrate orders were detected across all sampling sites and periods (**Supplementary Table S1**). Mollusca formed the dominant group (40.9%), followed by Odonata (17.8%) and Diptera (13.4%). Other groups found in this study were Trichoptera (7.7%), Ephemeroptera (5.9%), Heteroptera (5.5%), Decapoda (3.9%), Coleoptera (2.2%), Hirudinea (1.2%), Plecoptera (0.72%), and Oligochaeta (0.2%). The composition of the macroinvertebrate community slightly varied across sites. Almost all groups were represented at all sites, but Trichoptera, Ephemeroptera, and Plecoptera dominated at the control site (Ngomo) of the Ruzizi River.

In general, the Shannon-Wiener index was very low, ranging from 0.63 to 2.39. Most families at the sites were represented by more than one individual. The Rare Family Prevalence (RFP) score was low at most sites before and after the dams. At one site from Dam I downstream (loc. 4), a very low RFP value of 0.8 was recorded in December 2015. The highest RFP value (60) was recorded in October 2016 at Kamanyola, where most of the taxa collected were represented by single individuals. The evenness was high when RFP increased, meaning that the number of individuals in a community was fairly constant when many families were represented by single individuals. The ASPT ranged from 2.5 to 6.8, and almost all sites were represented by tolerant and moderately tolerant taxa, except for the Ngomo site, which had more sensitive taxa.

The nonmetric multidimensional scaling analyses for the eight sampling periods yielded stress values between 0.016 and 0.097, suggesting a good fit for each of the ordinations. The plots indicate variation among communities as well as through time (**Supplementary Figure S1**). The plots showed that the least sensitive taxa were represented both upstream and downstream of the 2 dams. Belostomatidae, Libellulidae, Lymnaeidae, and

TABLE 2 | Linear mixed effects models that are better than their corresponding null models, with indication of random effects structure (random intercept) and model fit. See **Supplementary Table S2** for the complete list and **Supplementary Results S1** for model diagnostics. AIC, Akaike Information Criterion; ASPT, average score per taxon; ICC, intraclass correlation coefficient; RFP, rare family prevalence.

Index	Ngomo/Kamanyola	ΔAIC to Corresponding Null Model	Random Effects Structure		Model Fit	
			Sampling area	Sampling period	Marginal R^2	Adjusted ICC
Pielou's Evenness	excluded	4.225	x	x	0.068	0.478
RFP	excluded	2.936	-	x	0.096	0.005
RFP	excluded	3.195	x	x	0.089	0.160
ASPT	included	16.675	-	x	0.268	0.032
ASPT	included	3.191	x	x	0.241	0.120

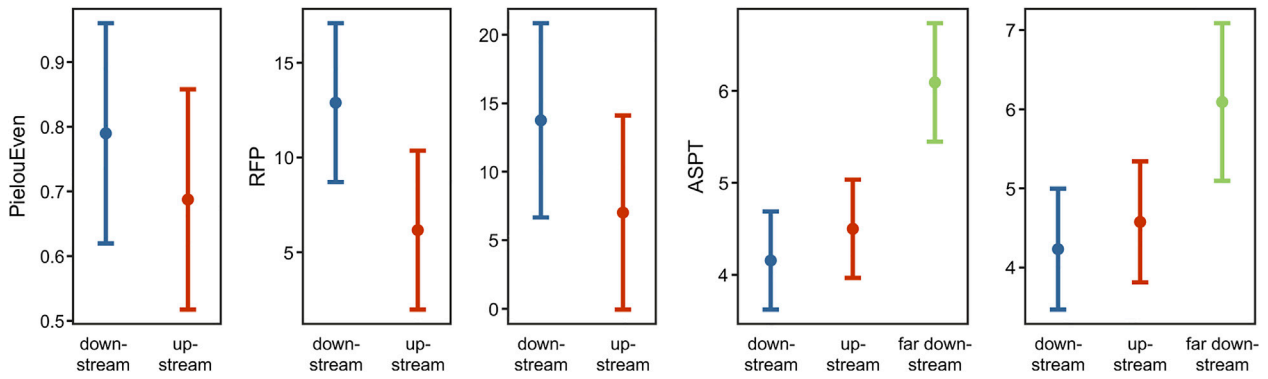

FIGURE 2 | Predicted values of the fixed effects of the five significant linear mixed effects models. The fixed effects of the ASPT models show that the variance in both models is mostly contributed by the communities sampled further downstream of Dam II. ASPT, average score per taxon; RFP, rare family prevalence.

Bithyniidae dominated upstream of Dam I, while Planorbidae, Lymnaeidae, and Atyidae dominated downstream (**Supplementary Figure S1**). Sites at Dam II were more represented with taxa moderately sensitive to pollution and disturbance. Corduliidae and Chlorocyphidae dominated upstream of Dam II, while Hydraenidae and Belostomatidae dominated downstream of Dam II (**Supplementary Figure S1**). Community composition differed at sites further downstream. The Ngomo site was dominated by sensitive taxa of Trichoptera (Hydropsychidae), Plecoptera (Perlidae), and Ephemeroptera (Baetidae). The Kamanyola site, further downstream, was dominated by taxa that are both tolerant and moderately tolerant to pollution and disturbance, such as Nepidae, Libellulidae, and Gomphidae.

For most of the linear mixed effects models between each of the five diversity indices and sampling position (upstream vs. downstream), a null model yielded a better or equally good (ΔAIC <2) fit (**Supplementary Table S2**). Moreover, despite the generally simple structures of the models, several of them had a singular fit (**Supplementary Table S2**), suggesting an overfitting of the model or insufficient data. Since the structure of the models cannot be further reduced reasonably, these models will not be further considered. Only five models were found better than their corresponding null models (**Table 2**). However, ΔAIC values for four out of the five model are below 5, suggesting only a weakly better fit than a null model. Upstream/downstream position had significant but weak effects on Pielou's Evenness and Rare Family Prevalence (RFP) when including only Dams I and II. However, most of the variation detected by the models is due to the random effects structure, particularly the sampling area. Conversely, a moderate effect of upstream/downstream position on ASPT was found when including the communities further downstream of Dam II (**Table 2**; **Figure 2**). As in the other models, sampling area contributed more to the variation of the models than sampling period. Residual and dispersion checks found no violation of model assumptions in any of the five models (**Supplementary Results S1**). Only minor signs of temporal autocorrelation were detected for the two models with ASPT (**Supplementary Results S1**).

DISCUSSION

The studied river system provided an opportunity to examine whether family-level biotic indices should be used to assess the ecological impacts of dams, particularly in a low-diversity river. We found low-diverse macroinvertebrate communities both upstream and downstream of the dams. Our analyses indicated general differences in the community compositions through space and time (**Supplementary Figure S1**), but upstream/downstream communities were found significantly different only for few selected indices (**Table 2**; **Figure 2**).

There are several potential causes for the low macroinvertebrate diversity in the Ruzizi River. Generally,

factors contributing to low taxon richness can be low habitat diversity, unstable water levels, altered thermal regime, and altered food supply (Munn and Brusven, 1991). Moreover, macroinvertebrate assemblages below dams often have lower taxon richness and are typically dominated by certain species (Bona et al., 2008; Takao et al., 2008). In the specific case of the Ruzizi River, the hydrological and geographical setting of the dams is also relevant. The water predominantly comes from Lake Kivu, which is a species-poor lake because of its geological history and catastrophic volcanic events that affect also its current limnology (Jones, 2021). The lake's environmental condition affects the Ruzizi River, especially at Dam I close to the outlet. We found that the habitat is disturbed and degraded by high water release and retention downstream of dams, leading to hydropeaking events (Tonolla et al., 2017; Muvundja et al., 2022). Sometimes water is retained upstream, dramatically altering the river's habitat to the point of complete dry-up downstream. This kills many small organisms such as macroinvertebrates due to their low mobility (Bruder et al., 2016). Conversely, habitat alteration due to flooding is the proposed cause of low macroinvertebrate diversity in the Ruzizi River just upstream of the dams (Hyangya et al., 2014).

The slight difference in macroinvertebrate diversity between sites near dams I and II could be due to the proximity of Dam I to Lake Kivu, which carries the same saline water and therefore has lower diversity than communities around Dam II, whose water is diluted by tributaries (Muvundja et al., 2022). The age difference in their existence could be another factor contributing to the lower diversity at Dam I compared to Dam II, which was constructed 30 years later. For the benthic macroinvertebrates, the impacts may be more noticeable in the short and medium-term due to their reduced capacity for movement and their affinity for the bottom substrates that constitute their living environment (Bhandari et al., 2018; Min and kong, 2020). Our result, however, is consistent with a study that showed that dams of different ages can affect downstream organisms differently, with older dams having a greater impact than those recently built along the same river (Wang et al., 2020).

Additionally to richness differences, we found differences in the community compositions. This especially concerns the relative abundance of sensitive and tolerant taxa. In general, at the sites near Dam I and Dam II, between December 2015 and October 2017, we observed an increase of families with low TARISS scores (representing species that tolerate pollution and disturbance), particularly Mollusca, Diptera (strongly represented by Chironomidae), and Heteroptera (compare Kaaya et al., 2015), while species that are highly sensitive to pollution (such as Trichoptera, Ephemeroptera, and Plecoptera; Kaaya et al., 2015) were rare both upstream and downstream of the dams. We observed considerable variation in flow rate and water level over the studied time interval. Such hydropeaking and drying events usually cause changes in macroinvertebrate communities and decrease the number of sensitive taxa in impacted areas (Wang et al., 2013).

Another factor causing the rarity of highly sensitive taxa could be river bank disturbance and domestic pollution observed upstream and downstream of the dams. Macroinvertebrates are known to be differentially sensitive to water quality degradation (Bonada et al., 2006; Arimoro and Muller, 2010; Fouche and Vlok, 2010). Their presence is therefore considered an indicator of the state of water quality and aquatic health of the environment in which they live. The disturbance and domestic utilization of water at the shores of sites of Dam II were minimal compared to the sites of Dam I. Dam II sites contained more taxa moderately sensitive to pollution and disturbance. Taxa tolerant or moderately tolerant to disturbance dominated at sites further downstream in Kamanyola on the Rwanda-Congo border. The low abundance of sensitive taxa at this site, which is not directly impacted by dams and related flow fluctuations, may have been caused by further anthropogenic activities (such as swimming, washing), agriculture, nearby irrigation, and pollution of the on-site bank. In contrast, the Ngomo site is isolated, and we witnessed little anthropogenic activities and no disturbance, making it a suitable habitat for taxa that do not tolerate pollution.

The surprisingly little effect of the dams on upstream vs. downstream community composition contrasts several previous studies (Cortes et al., 1998; Santucci et al., 2005; Xiaocheng et al., 2008; Bredenhand and Samways, 2009; Serrana et al., 2018; Wang et al., 2019). Recently, a global analysis of the ecological impacts of small hydropower dams generally showed negative ecological effects (Kuriqi et al., 2021) affecting macroinvertebrate communities downstream due to changes in flow velocity (Mcintosh et al., 2002; Sharma et al., 2005; Martínez et al., 2013). However, there are a number of studies that found similarly weak or no immediate impact of dams on macroinvertebrate communities (Ambers, 2007; Xiaocheng et al., 2008; Vaikasas et al., 2013). A global review (Mbaka and Wanjiru, 2015) reports that more than 70% of small dams have either a positive or negative impact on macroinvertebrates by causing either a decrease or an increase in macroinvertebrate abundance and richness downstream of the dams (Mueller et al., 2011; Martínez et al., 2013; Wang et al., 2013).

The reason for this contrast could be the different methods used (Wang et al., 2020). Some studies look at specific taxonomic groups of macroinvertebrates, while others include the entire community. Other factors include differences in the climatic and geomorphological conditions at dam sites (Carr et al., 2019; Turgeon et al., 2019), downstream distance from the dam (Ruhi et al., 2018), and dam size (Poff and Hart, 2002). Additionally, using family-level identifications could potentially obscure relevant information. Most rapid assessment systems for impacts on rivers are based on family-level data (Dallas, 2021; Mezgebu, 2022). However, different macroinvertebrate species within the same family may have very different pollution tolerances (Arimoro and Ikomi, 2008) or ecosystem functions (Baulechner et al., 2020). Lumping them in a single unit can severely bias assessments and eventually be the result of the apparently lacking impact of dams on biodiversity in the Ruzizi River.

Africa has a very high demand for energy supply and hydropower facilities will play an important role in the near future, especially in the Congo River system (Winemiller et al., 2016). At the same time, there is still very little knowledge about the impact of dams on environmental flows and biodiversity in these drainage systems. The

outlook for freshwater biodiversity near dams requires a more organized assessment for predicting, restoring, and managing the resulting changes in river ecosystems (Rolls et al., 2018; Turgeon et al., 2019). Changes in the management of environmental flow regimes can assist the protection and restoration of the aquatic fauna and maintaining river ecosystems downstream of dams in order to maintain ecosystem functioning (Poff and Schmidt, 2016; Kuriqi et al., 2019). This could also help increase the poor macroinvertebrate fauna of the Ruzizi River, and suggestions for altering flow practices have already been made (Muvundja et al., 2022; see also Bruder et al., 2016). Our study examining the macroinvertebrate communities in the low-diversity tropical Ruzizi River shows a weak impact of dams on downstream macroinvertebrates when using family-level bio-indications. We recommend that future studies focus on species level identifications to deliver more precise and ecologically relevant assessment. For such an approach to work, we urgently require more profound baseline studies on the species compositions of freshwater macroinvertebrate communities in Africa.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

MD collected data, performed laboratory identification, and wrote the draft article; TN performed data analyses and contributed to writing the manuscript; FM contributed to the conceptualization of the topic, data collection and writing the manuscript; BH contributed to data collection; CA contributed to the conceptualization of the study and writing the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.902246/full#supplementary-material

REFERENCES

ABAKIR (2020). *Baseline Study for the Basin of Lake Kivu and the Ruzizi River (Report No: 20.214.5-022.00)*. Available at: https://uploads.water-energy food.org/resources/1.ABAKIR_SCR16_WP1_WP2_A4_EN_20210312.pdf

Altinbilek, D. (2002). The Role of Dams in Development. *Water Sci. Technol.* 45 (8), 169–180. doi:10.2166/wst.2002.0172

Ambers, R. K. R. (2007). Effects of a Small, Century-old Dam on a Secondorder Stream in the Virginia Piedmont. *Southeast. Geogr.* 47 (2), 181–201. doi:10.1353/sgo.2007.0018

Arimoro, F. O., and Ikomi, R. B. (2008). Response of Macroinvertebrate Communities to Abattoir Wastes and Other Anthropogenic Activities in a Municipal Stream in the Niger Delta, Nigeria. *Environmentalist* 28 (2), 85–98. doi:10.1007/s10669-007-9033-8

Arimoro, F. O., and Muller, W. J. (2010). Mayfly (Insecta: Ephemeroptera) Community Structure as an Indicator of the Ecological Status of a Stream in the Niger Delta Area of Nigeria. *Environ. Monit. Assess.* 166 (1), 581–594. doi:10.1007/s10661-009-1025-3

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., et al. (2020). *lme4: Linear Mixed-Effects Models Using 'Eigen' and S4. R Package Version 1.1-26*. Available from http://CRAN.R-project.org/package=lme4.

Baulechner, D., Jauker, F., Neubauer, T. A., and Wolters, V. (2020). Convergent Evolution of Specialized Generalists: Implications for Phylogenetic and Functional Diversity of Carabid Feeding Groups. *Ecol. Evol.* 10 (20), 11100–11110. doi:10.1002/ece3.6746

Bhandari, B., Shah, R. D. T., and Sharma, S. (2018). Status, Distribution and Habitat Specificity of Benthic Macro-Invertebrates: a Case Study in Five Tributaries of Buddhiganga River in Western Nepal. *J. Inst. Sci. Tech.* 23 (1), 69–75. doi:10.3126/jist.v23i1.22198

Bona, F., Falasco, E., Fenoglio, S., Iorio, L., and Badino, G. (2008). Response of Macroinvertebrate and Diatom Communities to Human-Induced Physical Alteration in Mountain Streams. *River Res. Applic.* 24 (8), 1068–1081. doi:10.1002/rra.1110

Bonada, N., Prat, N., Resh, V. H., and Statzner, B. (2006). Developments in Aquatic Insect Biomonitoring: a Comparative Analysis of Recent Approaches. *Annu. Rev. Entomol.* 51, 495–523. doi:10.1146/annurev.ento.51.110104.151124

Bredenhand, E., and Samways, M. J. (2009). Impact of a Dam on Benthic Macroinvertebrates in a Small River in a Biodiversity Hotspot: Cape Floristic Region, South Africa. *J. Insect Conserv.* 13 (3), 297–307. doi:10.1007/s10841-008-9173-2

Bruder, A., Tonolla, D., Schweizer, S. P., Vollenweider, S., Langhans, S. D., and Wüest, A. (2016). A Conceptual Framework for Hydropeaking Mitigation. *Sci. Total Environ.* 568, 1204–1212. doi:10.1016/j.scitotenv.2016.05.032

Bunn, S. E., and Arthington, A. H. (2002). Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environ. Manag.* 30, 492–507. doi:10.1007/s00267-002-2737-0

Burnham, K. P., and Anderson, D. R. (2004). Multimodel Inference. *Sociol. Methods Res.* 33 (2), 261–304. doi:10.1177/0049124104268644

Carr, M., Li, L., Sadeghian, A., Phillips, I. D., and Lindenschmidt, K. E. (2019). Modelling the Possible Impacts of Climate Change on the Thermal Regime and Macroinvertebrate Species of a Regulated Prairie River. *Ecohydrology* 12 (6), pe2102. doi:10.1002/eco.2102

Carter, J. L., Resh, V. H., and Hannaford, M. J. (2017). "Macroinvertebrates as Biotic Indicators of Environmental Quality," in *Methods in Stream Ecology* (Academic Press), 293–318. doi:10.1016/B978-0-12-813047-6.00016-4

Cortes, R. M. V., Ferreira, M. T., Oliveira, S. V., and Godinho, F. (1998). Contrasting Impact of Small Dams on the Macroinvertebrates of Two Iberian Mountain Rivers. *Hydrobiologia* 389 (1), 51–61. doi:10.1023/A:1003599010415

Couto, T. B., and Olden, J. D. (2018). Global Proliferation of Small Hydropower Plants - Science and Policy. *Front. Ecol. Environ.* 16 (2), 91–100. doi:10.1002/fee.1746

Dallas, H. F. (2021). Rapid Bioassessment Protocols Using Aquatic Macroinvertebrates in Africa-Considerations for Regional Adaptation of Existing Biotic Indices. *Front. Water* 3, 628227. doi:10.3389/frwa.2021.628227

Day, J. A., and De Moor, I. J. (2002). *Guides to the Freshwater Invertebrates of Southern Africa. Non-arthropods (the protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera, Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and Hirudinea)*. WRC Report No. TT 167/02, 5. Pretoria: Water Research Commission, 304.

Day, J. A., de Moor, I. J., Stewart, B. A., and Louw, A. E. (2001a). *Guides to the Freshwater Invertebrates of Southern Africa, Volume 3. Crustacea 2: Ostracoda, Copepoda and Brachiura*. WRC Report No. TT140/08. Pretoria: Water Research Commission, 177.

Day, J. A., Harrison, A. D., and de Moor, I. J. (2002). *Guides to the Freshwater Invertebrates of Southern Africa, Volume 3. Diptera*. WRC Report TT201/02. Pretoria: Water Research Commission, 200.

Day, J. A., Stewart, B. A., De Moor, I. J., and Louw, A. E. (1999). *Guides to the Freshwater Invertebrates of Southern Africa: Volume 2, Crustacea I – Notostraca, Anostraca, Conchostraca and Cladocera*. WRC Report No. TT 121/00. Pretoria: Water Research Commission, 126.

Day, J. A., Stewart, B. A., de Moor, I. J., and Louw, A. E. (2001b). *Guides to the Freshwater Invertebrates of Southern Africa, Volume 4. Crustacea 3: Bathynellacea, Amphipoda, Isopoda, Spelaeogripacea, Tanaidacea and Decapoda*. WRC Report TT 141/00. Pretoria: Water Research Commission, 141.

De Moor, I. J., Day, J. A., and De Moor, F. C. (2003). *Guides to the Freshwater Invertebrates of Southern Africa: Volume 7 Insecta I—Ephemeroptera, Odonata and Plecoptera*. WRC Report No. TT 207/03. Pretoria: Water Research Commission, 288.

de Moor, I. J., and Day, J. A. (2002). *Guides to the Freshwater Invertebrates of Southern Africa. Arachnida and Mollusca*. WRC Report No. TT182/02, 6. Pretoria: Water Research Commission, 141.

Descy, J.-P., Darchambeau, F., and Schmid, M. (2012). "Lake Kivu Research: Conclusions and Perspectives," in *Lake Kivu. Aquatic Ecology Series*. Editors J. P. Descy, F. Darchambeau, and M. Schmid (Dordrecht: Springer), 5, 181–190. doi:10.1007/978-94-007-4243-7_11

Dick, J. T. A., Platvoet, D., and Kelly, D. W. (2002). Predatory Impact of the Freshwater Invader *Dikerogammarus villosus* (Crustacea: Amphipoda). *Can. J. Fish. Aquat. Sci.* 59 (6), 1078–1084. doi:10.1139/F02-074

Dombrowsky, I., Bastian, J., Däschle, D., Heisig, S., Peters, J., and Vosseler, C. (2014). International and Local Benefit Sharing in Hydropower Projects on Shared Rivers: the Ruzizi III and Rusumo Falls Cases. *Water Policy* 16 (6), 1087–1103. doi:10.2166/wp.2014.104

Dusabe, M.-C., Wronski, T., Gomes-Silva, G., Plath, M., Albrecht, C., and Apio, A. (2019). Biological Water Quality Assessment in the Degraded Mutara Rangelands, Northeastern Rwanda. *Environ. Monit. Assess.* 191 (3), 1–13. doi:10.1007/s10661-019-7226-5

Elbrecht, V., and Leese, F. (2015). Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass-Sequence Relationships with an Innovative Metabarcoding Protocol. *PloS One* 10 (7), e0130324. doi:10.1371/journal.pone.0130324

Elbrecht, V., Vamos, E. E., Meissner, K., Aroviita, J., and Leese, F. (2017). Assessing Strengths and Weaknesses of DNA Metabarcoding-based Macroinvertebrate Identification for Routine Stream Monitoring. *Methods Ecol. Evol.* 8 (10), 1265–1275. doi:10.1111/2041-210X.12789

Emberton, K. C., Pearce, T. A., Kasigwa, P. F., Tattersfield, P., and Habibu, Z. (1997). High Diversity and Regional Endemism in Land Snails of Eastern Tanzania. *Biodivers. Conserv.* 6 (8), 1123–1136. doi:10.1023/A:1018384101789

Fichtner Gmbh und Co, K. G. (2008). *Etudes préalables pour l'aménagement d'un site hydroélectrique communautaire Ruzizi III sur la Rivière Ruzizi et le Développement du volet Energie des activités de la Communauté Economique des Paysdes Grands lacs (CEPGL). Lot 1 et Lot 2: Etude de faisabilité pour l'aménagement hydroélectrique de Ruzizi III, Etude de préfaisabilité du site SISI 5 sur la Ruzizi et du réaménagement de l'exutoire du lac Kivu à Cyangugu (Topographie), Rapport de Topographie 5761P21. Energie des Grands lacs (EGL)-Union Européenne(UE)*. Décembre.

Fornaroli, R., Ippolito, A., Tolkkinen, M. J., Mykrä, H., Muotka, T., Balistrieri, L. S., et al. (2018). Disentangling the Effects of Low pH and Metal Mixture Toxicity on Macroinvertebrate Diversity. *Environ. Pollut.* 235, 889–898. doi:10.1016/j.envpol.2017.12.097

Fouche, P., and Vlok, W. (2010). Water Quality Impacts on Instream Biota of the Shingwedzi River, South Africa. *Afr. J. Aquat. Sci.* 35 (1), 1–11. doi:10.2989/16085914.2010.466644

Gerber, A., and Gabriel, M. J. M. (2002). *Aquatic Invertebrates of South African Rivers Field Guide*. Pretoria: Institute of water quality studies, department of water affairs and forestry, 149.

Greenwood, M. T., Bickerton, M. A., Gurnell, A. M., and Petts, G. E. (1999). Channel Changes and Invertebrate Faunas below Nant-y-Môch Dam, River Rheidol, Wales, UK: 35 Years on. *Riv. Res. Appl.* 15 (1-3), 99–112. doi:10.1002/(SICI)1099-1646(199901/06)15:1/3<99::AID-RRR530>3.0.CO;2-I

Grill, G., Lehner, B., Thieme, M., Geenen, B., Tickner, D., Antonelli, F., et al. (2019). Mapping the World's Free-Flowing Rivers. *Nature* 569 (7755), 215–221. doi:10.1038/s41586-019-1111-9

Gross, M. (2016). A Global Megadam Mania. *Curr. Biol.* 26 (17), R779–R782. doi:10.1016/j.cub.2016.08.050

Guareschi, S., and Wood, P. J. (2019). Taxonomic Changes and Non-native Species: An Overview of Constraints and New Challenges for Macroinvertebrate-Based Indices Calculation in River Ecosystems. *Sci. Total Environ.* 660, 40–46. doi:10.1016/j.scitotenv.2019.01.008

Haase, P., Pauls, S. U., Schindehütte, K., and Sundermann, A. (2010). First Audit of Macroinvertebrate Samples from an EU Water Framework Directive Monitoring Program: Human Error Greatly Lowers Precision of Assessment Results. *J. North Am. Benthol. Soc.* 29 (4), 1279–1291. doi:10.1899/09-183.1

Harrison, X. A., Donaldson, L., Correa-Cano, M. E., Evans, J., Fisher, D. N., Goodwin, C. E. D., et al. (2018). A Brief Introduction to Mixed Effects Modelling and Multi-Model Inference in Ecology. *PeerJ* 6, e4794. doi:10.7717/peerj.4794

Hartig, F., and Lohse, L. (2020). *DHARMa: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models*. R package version 0.3.3.0. Available from http://CRAN.R-project.org/package=DHARMa

Hughes, R. H., and Hughes, J. S. (1992). *A Directory of African Wetlands*. IUCN, UNEP, and WCMC, Gland, Nairobi and Cambridge

Hyangya, L., Munini, M., Mushagalusa, M., Kisekelwa, T., Masilya, M. P., Isumbisho, M. P., et al. (2014). *Inventaire systématique des macroinvertébrés du lac de barrage de Mumosho sur La rivière Ruzizi (Bukavu, RDC)*. Bukavu: Cahiers du CERUKI, Nouvelle Série, Numéro spécial Professeur Alphonse Byamungu, 51–62.

IEA: International Energy Agency (2019). Southeast Asia Outlook 2019. Available at: https://webstore.iea.org/download/direct/2887?filename=southeast_asia_energy outlook_2019.pdf (Accessed Mar 31, 2019).

Jellyman, P., and Harding, J. (2012). The Role of Dams in Altering Freshwater Fish Communities in New Zealand. *N. Z. J. Mar. Freshwater Res.* 46 (4), 475–489. doi:10.1080/00288330.2012.708664

Johnson, P. T., Olden, J. D., and Vander Zanden, M. J. (2008). Dam Invaders: Impoundments Facilitate Biological Invasions into Freshwaters. *Front. Ecol. Environ.* 6 (7), 357–363. doi:10.1890/070156

Jones, N. (2021). How Dangerous Is Africa's Explosive Lake Kivu? *Nature* 597 (5877), 466–469. doi:10.1038/d41586-021-02523-5

Kaaya, L., Day, J., and Dallas, H. (2015). Tanzania River Scoring System (TARISS): a Macroinvertebrate-Based Biotic Index for Rapid Bioassessment of Rivers. *Afr. J. Aquat. Sci.* 40 (2), 109–117. doi:10.2989/16085914.2015.1051941

Kahle, D., Wickham, H., Jackson, S., and Korpela, M. (2019). *Ggmap: Spatial Visualization with ggplot2. R Package Version 3*. Available from http://CRAN.R-project.org/package=ggmap.

Kuriqi, A., Pinheiro, A. N., Sordo-Ward, A., Bejarano, M. D., and Garrote, L. (2021). Ecological Impacts of Run-Of-River Hydropower Plants-Current Status

and Future Prospects on the Brink of Energy Transition. *Renew. Sust. Energ. Rev.* 142, 110833. doi:10.1016/j.rser.2021.110833

Kuriqi, A., Pinheiro, A. N., Sordo-Ward, A., and Garrote, L. (2019). Flow Regime Aspects in Determining Environmental Flows and Maximising Energy Production at Run-Of-River Hydropower Plants. *Appl. Energy* 256, 113980. doi:10.1016/j.apenergy.2019.113980

Kuriqi, A., Pinheiro, A. N., Sordo-Ward, A., and Garrote, L. (2020). Water-energy-ecosystem Nexus: Balancing Competing Interests at a Run-Of-River Hydropower Plant Coupling a Hydrologic-Ecohydraulic Approach. *Energy Convers. Manag.* 223, 113267. doi:10.1016/j.enconman.2020.113267

Latrubesse, E. M., Stevaux, J. C., and Sinha, R. (2005). Tropical Rivers. *Geomorphology* 70 (3-4), 187–206. doi:10.1016/j.geomorph.2005.02.005

Lessard, J. L., and Hayes, D. B. (2003). Effects of Elevated Water Temperature on Fish and Macroinvertebrate Communities below Small Dams. *River Res. Applic.* 19 (7), 721–732. doi:10.1002/rra.713

Lüdecke, D., Aust, F., Crawley, S., and Ben-Shachar, M. S. (2020a). Ggeffects: Create Tidy Data Frames of Marginal Effects for 'ggplot' from Model Outputs. R package version 1.0.1. Available from http://CRAN.R-project.org/package=ggeffects.

Lüdecke, D., Makowski, D., Waggoner, P., Patil, I., and Ben-Shachar, M. S. (2020b). *Performance: Assessment of Regression Models Performance*. R package version 0.6.1. Available from http://CRAN.R-project.org/package=performance.

Macher, J. N., Salis, R. K., Blakemore, K. S., Tollrian, R., Matthaei, C. D., and Leese, F. (2016). Multiple-stressor Effects on Stream Invertebrates: DNA Barcoding Reveals Contrasting Responses of Cryptic Mayfly Species. *Ecological Indicators* 61, 159–169. doi:10.1016/j.ecolind.2015.08.024

Malmqvist, B. r., and Englund, G. r. (1996). Effects of Hydropower-Induced Flow Perturbations on Mayfly (Ephemeroptera) Richness and Abundance in North Swedish River Rapids. *Hydrobiologia* 341 (2), 145–158. doi:10.1007/BF00018118

Martínez, A., Larrañaga, A., Basaguren, A., Pérez, J., Mendoza-Lera, C., and Pozo, J. (2013). Stream Regulation by Small Dams Affects Benthic Macroinvertebrate Communities: from Structural Changes to Functional Implications. *Hydrobiologia* 711 (1), 31–42. doi:10.1007/s10750-013-1459-z

Mbaka, J. G., and Wanjiru Mwaniki, M. (2015). A Global Review of the Downstream Effects of Small Impoundments on Stream Habitat Conditions and Macroinvertebrates. *Environ. Rev.* 23 (3), 257–262. doi:10.1139/er-2014-0080

McIntosh, M. D., Benbow, M. E., and Burky, A. J. (2002). Effects of Stream Diversion on Riffle Macroinvertebrate Communities in a Maui, Hawaii, Stream. *River Res. Applic.* 18 (6), 569–581. doi:10.1002/rra.69410.1002/rra.694

Mellado-Díaz, A., Sánchez-González, J. R., Guareschi, S., Magdaleno, F., and Toro Velasco, M. (2019). Exploring Longitudinal Trends and Recovery Gradients in Macroinvertebrate Communities and Biomonitoring Tools along Regulated Rivers. *Sci. Total Environ.* 695, 133774. doi:10.1016/j.scitotenv.2019.133774

Mezgebu, A. (2022). A Review on Freshwater Biomonitoring with Benthic Invertebrates in Ethiopia. *Environ. Sustain. Ind.* 14, 100174. doi:10.1016/j.indic.2022.100174

Min, J.-K., and Kong, D.-S. (2020). Distribution Patterns of Benthic Macroinvertebrate Communities Based on Multispatial-Scale Environmental Variables in the River Systems of Republic of Korea. *J. Freshw. Ecol.* 35 (1), 323–347. doi:10.1080/02705060.2020.1815599

Mueller, M., Pander, J., and Geist, J. (2011). The Effects of Weirs on Structural Stream Habitat and Biological Communities. *J. Appl. Ecol.* 48 (6), 1450–1461. doi:10.1111/j.1365-2664.2011.02035.x

Munn, M. D., and Brusven, M. A. (1991). Benthic Macroinvertebrate Communities in Nonregulated and Regulated Waters of the Clearwater River, Idaho, U.S.A. *Regul. Rivers Res. Mgmt.* 6 (1), 1–11. doi:10.1002/rrr.3450060102

Muvundja, F. A., Walumona, R. J., Dusabe, M. C., Alunga, L. A., Kankonda, A. B., Albrecht, C., et al. (2022). The Land-Water-Energy Nexus at the Ruzizi River Dams (Lake Kivu Outflow, African Great Lakes Region): Status, Challenges, and Perspectives. *Front. Environ. Sci. (Accepted)*. doi:10.3389/fenvs.2022.892591

Muvundja, F. A., Wüest, A., Isumbisho, M., Kaningini, M. B., Pasche, N., Rinta, P., et al. (2014). Modelling Lake Kivu Water Level Variations over the Last Seven Decades. *Limnologica* 47, 21–33. doi:10.1016/j.limno.2014.02.003

Nakagawa, S., Johnson, P. C. D., and Schielzeth, H. (2017). The Coefficient of Determination R^2 and Intra-class Correlation Coefficient from Generalized Linear Mixed-Effects Models Revisited and Expanded. *J. R. Soc. Interface.* 14 (134), 20170213. doi:10.1098/rsif.2017.0213

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2020). vegan: Community Ecology Package. R package version 2.5-6. 2019. Available from http://CRAN.R-project.org/package=vegan.

Onec-Bad (2015). *Groupe de la Banque Africaine de Développement Projet : centrale hydroélectrique de Ruzizi III (147 MW) Pays : Multinational (Burundi-Rd Congo-Rwanda). ONEC-2/ONE*. Abidjan: Banque Africaine de Développement.

Poff, N. L., and Hart, D. D. (2002). How Dams Vary and Why it Matters for the Emerging Science of Dam Removal. *BioScience* 52 (8), 659–668. doi:10.1641/0006-3568(2002)052[0659:hdvawi]2.0.co;2

Poff, N. L., and Matthews, J. H. (2013). Environmental Flows in the Anthropocence: Past Progress and Future Prospects. *Curr. Opin. Environ. Sustain.* 5 (6), 667–675. doi:10.1016/j.cosust.2013.11.006

Poff, N. L., and Schmidt, J. C. (2016). How Dams Can Go with the Flow. *Science* 353 (6304), 1099–1100. doi:10.1126/science.aah4926

R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available from https://www.r-project.org/.

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., et al. (2019). Emerging Threats and Persistent Conservation Challenges for Freshwater Biodiversity. *Biol. Rev.* 94 (3), 849–873. doi:10.1111/brv.12480

Renã–Fã"Lt, B. M., Jansson, R., and Nilsson, C. (2010). Effects of Hydropower Generation and Opportunities for Environmental Flow Management in Swedish Riverine Ecosystems. *Freshw. Biol.* 55 (1), 49–67. doi:10.1111/j.1365-2427.2009.02241.x

Rolls, R. J., Heino, J., Ryder, D. S., Chessman, B. C., Growns, I. O., Thompson, R. M., et al. (2018). Scaling Biodiversity Responses to Hydrological Regimes. *Biol. Rev.* 93 (2), 971–995. doi:10.1111/brv.12381

Ruhi, A., Dong, X., McDaniel, C. H., Batzer, D. P., and Sabo, J. L. (2018). Detrimental Effects of a Novel Flow Regime on the Functional Trajectory of an Aquatic Invertebrate Metacommunity. *Glob. Change Biol.* 24 (8), 3749–3765. doi:10.1111/gcb.14133

Santucci, V. J., Jr, Gephard, S. R., and Pescitelli, S. M. (2005). Effects of Multiple Low-Head Dams on Fish, Macroinvertebrates, Habitat, and Water Quality in the Fox River, Illinois. *N. Am. J. Fish. Manag.* 25 (3), 975–992. doi:10.1577/M03-216.1

Serrana, J. M., Yaegashi, S., Kondoh, S., Li, B., Robinson, C. T., and Watanabe, K. (2018). Ecological Influence of Sediment Bypass Tunnels on Macroinvertebrates in Dam-Fragmented Rivers by DNA Metabarcoding. *Sci. Rep.* 8 (1), 1–10. doi:10.1038/s41598-018-28624-2

Sharma, C. M., Sharma, S., Borgstrom, R., and Bryceson, I. (2005). Impacts of a Small Dam on Macroinvertebrates: A Case Study in the Tinau River, Nepal. *Aquat. Ecosys. Health Managem.* 8 (3), 267–275. doi:10.1080/14634980500218332

Sinha, R., Latrubesse, E. M., and Nanson, G. C. (2012). Quaternary Fluvial Systems of Tropics: Major Issues and Status of Research. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 356-357, 1–15. doi:10.1016/j.palaeo.2012.07.024

Stals, R., and de Moor, I. J. (2007). *Guides to the Freshwater Invertebrates of Southern AfricaColeoptera, WRC Report No. TT 320/07*, 10.

Stein, E. D., White, B. P., Mazor, R. D., Jackson, J. K., Battle, J. M., Miller, P. E., et al. (2014). Does DNA Barcoding Improve Performance of Traditional Stream Bioassessment Metrics? *Freshw. Sci.* 33 (1), 302–311. doi:10.1086/674782

Stein, E. D., White, B. P., Mazor, R. D., Miller, P. E., and Pilgrim, E. M. (2013). Evaluating Ethanol-Based Sample Preservation to Facilitate Use of DNA Barcoding in Routine Freshwater Biomonitoring Programs Using Benthic Macroinvertebrates. *PloS One* 8 (1), e51273. doi:10.1371/journal.pone.0051273

Sweeney, B. W., Battle, J. M., Jackson, J. K., and Dapkey, T. (2011). Can DNA Barcodes of Stream Macroinvertebrates Improve Descriptions of Community Structure and Water Quality? *J. North Am. Benthol. Soc.* 30 (1), 195–216. doi:10.1899/10-016.1

Takao, A., Kawaguchi, Y., Minagawa, T., Kayaba, Y., and Morimoto, Y. (2008). The Relationships between Benthic Macroinvertebrates and Biotic and Abiotic Environmental Characteristics Downstream of the Yahagi Dam, Central Japan, and the State Change Caused by Inflow from a Tributary. *River Res. Applic.* 24 (5), 580–597. doi:10.1002/rra.1135

Tonolla, D., Bruder, A., and Schweizer, S. (2017). Evaluation of Mitigation Measures to Reduce Hydropeaking Impacts on River Ecosystems - a Case Study from the Swiss Alps. *Sci. Total Environ.* 574, 594–604. doi:10.1016/j.scitotenv.2016.09.101

TRACTIONELRRI (1980). Projet Définitif D'une Deuxième Centrale Hydroélectrique sur la Ruzizi, Hydrologie. Pièce Contractuelle No. 2A. A.S.B.L. *pour l'Electrification de la Région des Grands Lacs "EGLBujumbura"*. Bruxelles-Dortmund.

Turgeon, K., Turpin, C., and Gregory-Eaves, I. (2019). Dams Have Varying Impacts on Fish Communities across Latitudes: a Quantitative Synthesis. *Ecol. Lett.* 22 (9), 1501–1516. doi:10.1111/ele.13283

Vaikasas, S., Palaima, K., and Pliūraitė, V. (2013). Influence of Hydropower Dams on the State of Macroinvertebrates Assemblages in the Virvyte River, Lithuania. *J. Environ. Eng. Landsc. Manag.* 21 (4), 305–315. doi:10.3846/16486897.2013.796956

Wang, J., Ding, C., Heino, J., Jiang, X., Tao, J., Ding, L., et al. (2020). What Explains the Variation in Dam Impacts on Riverine Macroinvertebrates? A Global Quantitative Synthesis. *Environ. Res. Lett.* 15 (12), 124028. doi:10.1088/1748-9326/abc4fc

Wang, J., Ding, L., Tao, J., Ding, C., and He, D. (2019). The Effects of Dams on Macroinvertebrates: G Lobal Trends and Insights. *River Res. Applic.* 35 (6), 702–713. doi:10.1002/rra.3431

Wang, X., Cai, Q., Jiang, W., and Qu, X. (2013). Assessing Impacts of a Dam Construction on Benthic Macroinvertebrate Communities in a Mountain Stream. *Fresenius Environ. Bull.* 22 (1), 103–110.

Winemiller, K. O., McIntyre, P. B., Castello, L., Fluet-Chouinard, E., Giarrizzo, T., Nam, S., et al. (2016). Balancing Hydropower and Biodiversity in the Amazon, Congo, and Mekong. *Science* 351 (6269), 128–129. doi:10.1126/science.aac7082

Wright, J. (2010). *Biomonitoring with Aquatic Benthic Macroinvertebrates in Southern Costa Rica in Support of Community Based Watershed Monitoring*. Ontario, Canada: MSc Thesis, York University.

Wronski, T., Dusabe, M. C., Apio, A., Hausdorf, B., and Albrecht, C. (2015). Biological Assessment of Water Quality and Biodiversity in Rwandan Rivers Draining into Lake Kivu. *Aquat. Ecol.* 49 (3), 309–320. doi:10.1007/s10452-015-9525-4

Xiaocheng, F., Tao, T., Wanxiang, J., Fengqing, L., Naicheng, W., Shuchan, Z., et al. (2008). Impacts of Small Hydropower Plants on Macroinvertebrate Communities. *Acta Ecol. Sin.* 28 (1), 45–52. doi:10.1016/s1872-2032(08)60019-0

Zarfl, C., Lumsdon, A. E., Berlekamp, J., Tydecks, L., and Tockner, K. (2015). A Global Boom in Hydropower Dam Construction. *Aquat. Sci.* 77, 161–170. doi:10.1007/s00027-014-0377-0

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RESEARCH ARTICLE

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Choice of benthic macroinvertebrate-based metrics for assessing water quality in the littoral zone under anthropogenic disturbance in southern Lake Kivu (East Africa)

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Abstract

Benthic macroinvertebrates are widely used to assess the ecological quality of fresh waters. This is because they are in direct contact with the aquatic environment and respond differently to pollutants and changes in the watershed, which are difficult to assess by toxicological or chemical monitoring alone. This study used benthic macroinvertebrate parameters to assess the quality of the nearshore waters of lake Kivu. Twenty-six metrics covering various aspects of the community were tested using whisker plots to compare their sensitivity in discriminating between reference and disturbed stations. Nine parameters (% EPT taxa, % Diptera taxa, % Chironomid taxa, % Insect taxa; % no Insects taxa, ratio EPT/Chironomid taxa, % moderate tolerant taxa, % very moderate tolerant taxa, Family Biotic Index) were found to be sensitive and were able to discriminate between reference and disturbed stations. All sensitive metrics, with the exception of the percentage of EPT taxa, were positively and/or negatively correlated with the physico-chemical parameters affected by the changes in the littoral zone. The combined values of the three calculated biotic indices (ASPT, BMWP, and FBI) showed that the biological water quality varies from moderate to good in the reference stations and from average to poor in the disturbed stations. It is concluded that metrics based on benthic macroinvertebrates are effective for assessing water quality in the littoral zone of Lake Kivu in the context of the lack of historical water quality databases and specific tools for toxicological assessment. It is suggested to compare the performance of this approach with others currently used in bio-indication.

KEYWORDS
benthic fauna, bioassessment, lakeshore modifications, water management, watershed

1 | INTRODUCTION

Human activities (rapid urbanization, dams, and irrigation) alter aquatic ecosystems, ecological integrity, and water quality (Ntislidou et al., 2018; Tsanis et al., 2006). As this phenomenon has become a major concern for many states, several approaches, including physico-chemical (Calmuc et al., 2020; Pandey et al., 2018; Saal et al., 2020) and biological (Baptista et al., 2007; Barinova & Mamanazarova, 2021; Pissaridou et al., 2021; Pont et al., 2021) approaches, have been developed to monitor and to assess the impacts of pressures from anthropogenic and natural stressors on surface and groundwater qualities (Pont et al., 2021; Saal et al., 2020).

In 2000, the implementation of the European Water Framework Directive (DCE: Commission européenne, 2000), which aimed to define methods for assessing the ecological status of lakes, motivated the development of several methods for assessing anthropogenic pressures to support the assessment of ecological quality status or to define reference conditions (Akay & Dalkıran, 2019; Guimaraes et al., 2009; Odountan et al., 2019). In Europe, the United States, Canada, and Australia and New Zealand in addition to several national standardized methods for assessing impairment, several indices have been developed and proposed for adoption (Ozoliņš et al., 2021; Vitecek et al., 2021). To date, there is still a growing need for methods, which are widely accepted by the scientific community and which quantify habitat quality and human pressures in order to achieve sustainable environmental management objectives (Odountan et al., 2019; Poikane et al., 2016).

In Central Africa, notably in DR Congo, few studies have been devoted to assessing the ecological quality of aquatic ecosystems using parameters based on biological communities, despite the increasing pressures and impacts on aquatic systems (Odada et al., 2003). This is all the more true as the Lake Kivu basin is under continuous anthropogenic pressure resulting from the demographic growth of the large cities bordering the lake, such as Bukavu and Goma in DR Congo and Gisenyi, Kibuye, and Nyamasheke in Rwanda (Kaningini et al., 1999; Lina, 2016). But only a few studies are focusing on the water quality of Lake Kivu in relation to the increasing pressures of human activity. To date, knowledge is still scarce, and we are far from an understanding of the water use policy and ecological sustainability of the natural environment of the lake (Basima et al., 2006). This justifies the use in this study of the benthic macroinvertebrate metric approach to assess water quality in the littoral zone of the lake.

White et al. (2008) highlighted the sensitivity of macroinvertebrates to environmental factors compared with macrophytes and fish species, indicating the highly responsive nature of macroinvertebrates to changes in environmental variables and their usefulness in assessing associated effects on water bodies (Akay & Dalkıran, 2019; Guimaraes et al., 2009; Odountan et al., 2019; Ozoliņš et al., 2021; Vitecek et al., 2021). The method used is specifically based on the estimation of different metrics representing structural and functional aspects of the macrobenthic community considered as a community with some taxa being pollution tolerant and others not. It is also important to note that the approach based on benthic fauna metrics is the very first step used in many studies aiming to develop multimetric indices for macroinvertebrate-based water quality assessment (Odountan et al., 2019; Ozoliņš et al., 2021; Poikane et al., 2016; Vitecek et al., 2021). It is therefore a complementary method to the physico-chemical approach but with the advantages of having rapidly integrated results of several aspects of the environment and fauna, at lower cost (Lévêque, 1994).

This study aimed to fill the gap on how Lake Kivu responds to the combined impacts of natural and other effects on the water quality of the southern shoreline using benthic macroinvertebrate metrics and targeted two specific objectives: (i) to select potential metrics that can accurately contribute to the development of a multimeric index of ecological integrity for the littoral zone of Lake Kivu and (ii) to use the selected benthic macroinvertebrate metrics to assess the biological water quality of the lake.

2 | MATERIAL AND METHODS

2.1 | Study area: Lake Kivu

This study was carried out in Lake Kivu, one of the great lakes of the Albertine Rift Valley. It is located in East Africa, south of the equator, between S 1°34′–2°30′ and E 28°50′–29°23′ (see Figure 1). Lake Kivu is the smallest (2370 km^2), the least bulky (580 km^3), and the highest (1463 m above sea level) of the large lakes of the East African Rift Valley (Kaningini, 1995; Tietze et al., 1980), and it forms a natural border between the Democratic Republic of the Congo in the west and Rwanda in the east (see Figure 1) (Descy et al., 2012; Verbeke, 1957).

Four sampling stations were selected in the littoral zone (<1 m depth) in Bukavu sub-basin. One station was considered as a reference (undisturbed area) and named Bukavu 1 (BK1) and three altered stations: Bukavu 2 (BK2), Bukavu 3 (BK3), and Bukavu 4 (BK4) (Table 1, Figure 1). An additional four sites were selected in Ishungu sub-basin, three of which are considered reference sites: Ishungu 1 (IS1), Ishungu 2 (IS2), Ishungu 4 (IS4), and a degraded site, Ishungu 3 (IS3) (Table 1). All stations were 30 m long, and the width of the shoreline at the sampling point was between 5 and 10 m depending on the site configuration, due to the narrow and steep nature of the Lake Kivu shoreline (Beadle, 1981; Verbeke, 1957). They were selected to cover as much as possible the dominant micro-habitats taking into account the morphological characteristics, the vegetation cover up to 10 m from the shore, and the types of substrates dominating the bottom (Table 1).

2.2 | Water sampling and environmental parameter recorded

The environmental parameters were collected monthly from January to December 2018, between 8:30 and 10:30 each time and at the same date as the benthic macroinvertebrate samples. Temperature, dissolved oxygen, and conductivity were measured in situ using a

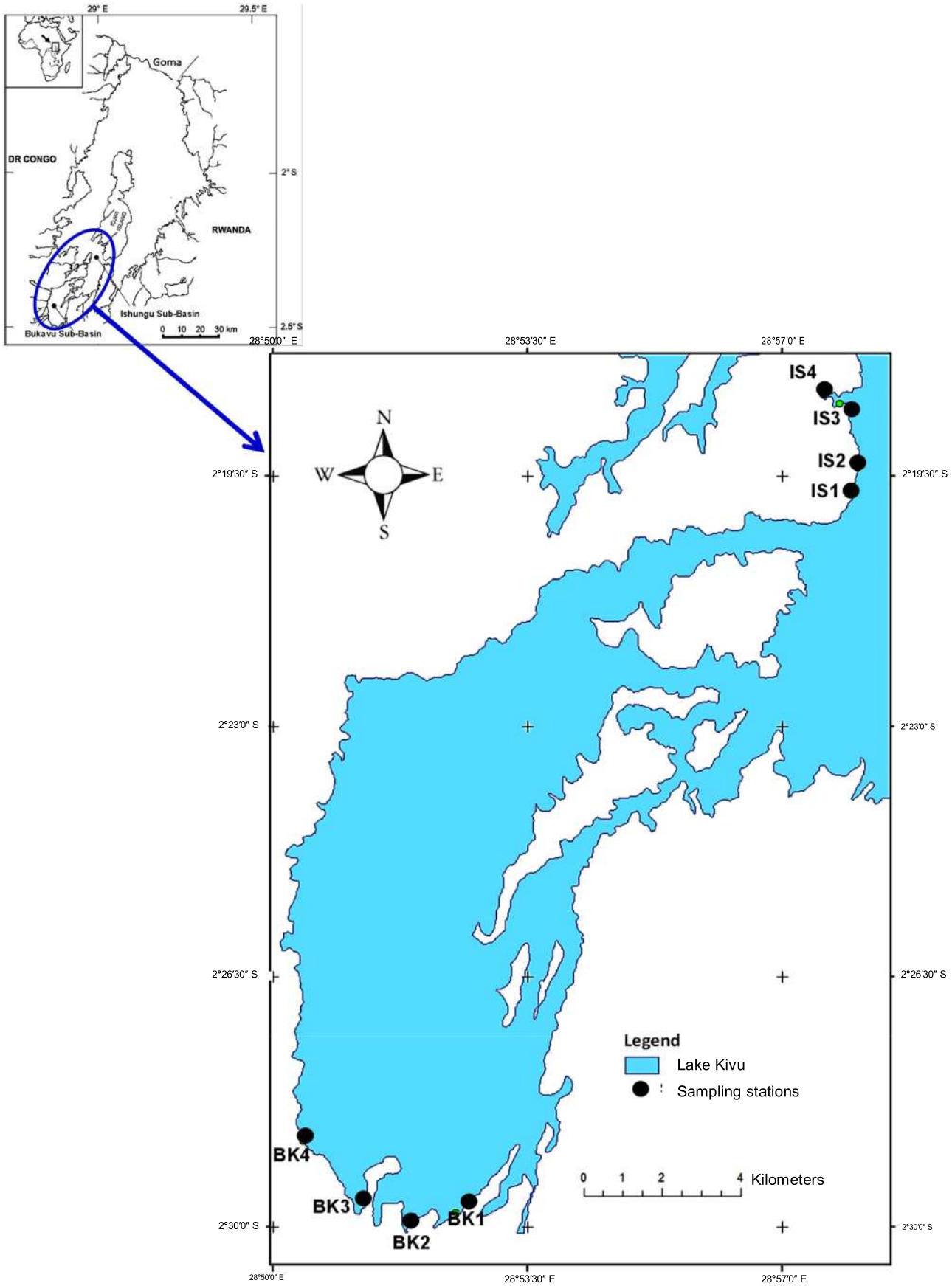

FIGURE 1 Lake Kivu map and location of sampling stations in the Bukavu and Ishungu sub-basins (BK = Bukavu, IS = Ishungu)

TABLE 1 Location coordinates and characteristics of sampling stations (the assessment of the percentage of vegetation cover was done via qualitative visual inspection)

Station	Depth (m)	Coordinates (in DMS)	Nature of banks and major substrates
BK1	0.59 ± 0.13	S 02°29′70″ E 28°52′68″	Reference station located in Nyalukemba Bay. The banks are swamp, with 80% of macrophyte plant cover. The dominant substrate is sandy-silt mixed with organic debris.
BK2	0.47 ± 0.11	S 02°29′95″ E 28°51′84″	Disturbed station with mud fill banks and a residential water channel carrying wastewater. The bottom is gravelly-clay and a 25% vegetation cover dominated by herbaceous plants.
BK3	0.47 ± 0.12	S 02°29′66″ E 28°51′23″	Disturbed station located next to the mouth of the Kahwa River. Sandy-clay bottom, banks flooded with various wastes and less than 5% vegetation cover.
BK4	0.46 ± 0.10	E 02°28′80″ S 28°50′42″	Disturbed station with banks modified by backfilling with a sand, silt and clay. Fifteen percent plant cover dominated by a mixture of grasses and macrophytes.
IS1	0.64 ± 0.07	S 02°19′75″ E 28°57′95″	Reference station with banks mainly composed of rocks, gravel and silt, 10% vegetation cover dominated by herbaceous plants.
IS2	0.60 ± 0.11	S 02°19′64″ E 28°58′94″	Reference station with natural banks having a dominant substrate consisting of rock covered with clays mixed with organic debris. 90% plant cover dominated by macrophytes.
IS3	0.60 ± 0.09	S 02°18′49″ E 28°57′78″	Disturbed station with banks completely barren, 2% vegetation covers by herbaceous plants. The bottom is dominated by rocks and clay.
IS4	0.58 ± 0.41	S 02°18′37″ E 28°57′56″	Reference station located at the outlet of a small stream. Partially natural banks with muddy clay bottom mixed with organic debris and covered with 99% macrophyte plants.

multiparametric field probe (YSI Incor.550 Dissolved Oxygen made in USA); pH with a pH-meter (pH 330/SET-1 made in Germany) and turbidity with a turbidimeter (HACH 21000, Made in Germany). The water depth at each sampling station was determined by a brand echo sounder (Plastimo Echotest II, 59588 made in France).

The water samples for nutrient (phosphate, ammonium, nitrite and silica) analyses were taken at each station using a small 5 L polyethylene canister, previously rinsed with distilled water. Immediately after being collected the water sample was filtered using Macherey-Nägel GF/5 filters with a porosity of 0.7 μm, labelled vials were placed in a portable freezer at 4°C and later on stored in a fridge at −20°C before all analyses. The concentrations of nutrients, orthophosphate, ammonium (NH_4^+), nitrites (NO_2^-), and silica (SiO_2), were measured using standardized techniques of UV–Visible spectrophotometric analysis of water samples (APHA, 2005). A spectrophotometer branded *Spectronic ®20*, USA, was used at a wavelength specified according to the nutrient to be measured (Isumbisho Mwapu, 2006; Muvundja, 2009). For the extraction of chlorophyll-*a*, water was filtered through Macherey-Nägel GF/5 filters with a porosity of 0.7 μm, and then the filter papers were kept in labelled vials containing 90% acetone for the extraction of chlorophyll-*a*. For the determination of chlorophyll-*a* concentration, the extract in acetone was sonicated once, kept at 4°C protected from light for 12 h and then sonicated a second time. The algal concentration was finally calculated according to the Lorenzen equation according to APHA (2005).

2.3 | Macroinvertebrate sampling and identification

Macroinvertebrate sampling was semi-quantitative and was taken monthly in the littoral zone from January to December 2018 in order to obtain as much as possible the representativeness of the taxa present in each station (Cañedo-Argüelles & Rieradevall, 2011). Samples were collected using a 250 μm mesh dip net to enable the different subhabitats to have prospected. The standardized effort time was 30 min per replicate, trying to survey all habitats, including sediment, gravel, and macrophytes, in proportion to the area, they occupy at the station (Cañedo-Argüelles & Rieradevall, 2011). Habitats with coarse substrates (stones and deadwood) were carefully sampled by hand, and the substrates were carefully rinsed in a basin and the contents were filtered through a 250 μm mesh sieve. For each sample, two replicates were taken and all post-sampling processing was done according to the XP T90-388 macroinvertebrate processing protocol (AFNOR, 2010).

Maroinvertebrate specimens were sorted into broad taxonomic groups, according to the sampling dates and stations, and stored in 50 ml glass vials containing 70% ethanol for conservation. All fixed samples are kept in the laboratory of the Teaching and Research Unit in Applied Hydrobiology (UERHA) of the Institut Supérieur Pédagogique de Bukavu (ISP-Bukavu) for further analysis. The identification of all the macroinvertebrates were determined up to the family level (Appendix A) using the nomenclature of Tachet et al. (2010), Brown (1994), Hamada et al. (2018), Cumberlidge and Meyer (2011), and Meyer and Cumberlidge (2011).

2.4 | Selection of benthic community metrics

Twenty-six metrics were selected and calculated on the benthic community (Table 2) according to Ntislidou et al. (2018), Odountan et al. (2019), and Vitecek et al. (2021). These metrics were selected and evaluated based on the main characteristics of the benthic macroinvertebrate community including measures of taxonomic abundance and diversity, pollution tolerance and trophic level (Baptista et al., 2007; Camargo, 2019). Thereafter, the selected metrics were

TABLE 2 Twenty-six candidate metrics selected and tested, their definition and expected response to increasing disturbance and pollution

Metrics calculated	Abbreviation	Definition of metrics	Response to perturbation	Reference having used these metrics in previous similar studies
1. Community composition and diversity				
Total taxa on family level	No.Tot.Tax	Number of taxa on family level in the community	Decrease	Ntislidou et al. (2018), Odountan et al. (2019) and Vitecek et al. (2021).
Total number of individuals	No.Tot.Ind	Number of individuals in the community	Variable	
% EPT taxa	% EPT	Percentage of individuals Ephemeroptera-Plecoptera-Trichoptera family	Variable	
% insects no Diptera taxa	%.Ins-Dipt. Tax	Percentage of individuals of insects no Diptera taxa in the community	Decrease	
% Diptera	% Dipt	Percentage of individuals of Diptera families	Increase	
% chironomids	% Chiro	Percentage of individuals of Chironomids family	Increase	
% insects	% Ins	Percentage of individuals of insects families	Increase	
% non-insects taxa	% No Ins. Tax	Percent of individuals of non-insects families	Variable	
Shannon's diversity index	H	Shannon's (1948) index of diversity	Decrease	
Simpson's dominance index	D	Simpson's (1949) index of dominance	Decrease	
Evenness	$e^{H/S}$	Sheldon (1969) index of evenness	Decrease	
Similarity	J	Jaccard (1901) index of Similarity	Increase	
2. Tolerance to perturbation/ sensitivities to pollution				
Ratio EPT/chironomids	EPT/Chiro	Ratio of the number of EPT/chironomid individuals in the community	Decrease	Ntislidou et al. (2018), Odountan et al. (2019) and Vitecek et al. (2021).
% dominance	% Dom.	Percentage of the most dominant family in the community	Increase	
% 3 most dominant taxa	% 3 Dom. Tax	Percentage of the three most dominant family in the community	Increase	
% intolerant taxa (tolerance value <4)	% Int.Tax	Percentage of family intolerant to disturbance (tolerance value <4)	Decrease	
% moderately tolerant taxa (tolerance value 4–6)	% Mod.Tol. Tax	Percentage of family moderately tolerant to disturbance (tolerance value 4–6)	Increase	
% very tolerant taxa (tolerance value >6)	% Ver.Tol. Tax	Percentage of family very tolerant to disturbance (tolerance value >6)	Increase	
3. Functional feeding groups				
% predators		Percentage of individuals in predators	Variable	Ntislidou et al. (2018), Odountan et al. (2019) and Vitecek et al. (2021).
% gathering-collectors		Percentage of individuals in gathering-collectors	Variable	
% filters-collectors		Percentage of individuals in predators filters-collectors	Decrease	
% scavengers		Percentage of individuals in scavengers	Decrease	
% shredders	% Shr	Percentage of individuals in shredders	Decrease	
4. Biotic indices				
Average score per taxon index	ASPT	Average tolerance score of all family within the community	Decrease	

(Continues)

TABLE 2 (Continued)

Metrics calculated	Abbreviation	Definition of metrics	Response to perturbation	Reference having used these metrics in previous similar studies
Biological monitoring working party index	BMWP	Representative of pollution tolerant organisms at the family level	Decrease	Ntislidou et al. (2018), Odountan et al. (2019) and Vitecek et al. (2021).
Family Biotic Index	FBI		Increase	

divided into four groups: (i) metrics related to community composition and diversity, (ii) metrics related to disturbance tolerance and pollution sensitivity, (iii) metrics related to trophic status, and (iv) metrics related to biotic indices.

2.5 | Data analysis

The mean values of the physico-chemical water parameters were calculated for each station.

Due to the absence of specific water standards in DR Congo for the assessment of freshwater quality, the parameter values obtained were compared with the limits of the international standards proposed by ANZECC (2000), CCME (2009), and (USEPA, 1995).

Depending on the case, the one-way ANOVA or Kruskal–Wallis test ($p < 0.05$) was applied to compare the mean values of parameters showing significant differences between stations. A two-way ANOVA was performed to determine the influence of sampling sites grouped into reference (REFER) and disturbed (POOR) stations, and of months on the physico-chemical parameters of the water. The Tukey–Kramer multiple comparison test was applied to compare sites in pairs.

The results of the macroinvertebrates metrics were presented according to the sampling stations. The most sensitive metrics were used to better discriminate the disturbed and reference stations. The sensitivity of each metric was determined by the degree of interquartile overlap in box-and-whisker plots (Baptista et al., 2007; Kashian & Burton, 2000). The box-and-whisker plots allow the visualization of the variation in the metric range between reference stations (BK1, IS1, IS2, and IS4) and disturbed stations (BK2, BK3, BK4, and IS3). The metrics were given one of the following five sensitivity values: a sensitivity score of 3 was assigned if there was no overlap in the interquartile range; a sensitivity score of 2 if there was some overlap in the interquartile range but both medians were outside the interquartile range overlap; a sensitivity score of 1 if there was moderate overlap in the interquartile range but one median was outside the interquartile range overlap; a sensitivity score of 0a if one range completely overlapped the other interquartile range but one median was outside the interquartile range overlap, and a sensitivity score of 0b if both medians were inside the interquartile range overlap area (Figure 2) (Baptista et al., 2007; Kashian & Burton, 2000). A metric was considered sensitive when the comparison between the box-and-whisker plots of the reference stations (REFER) and disturbed stations (POOR) obtained a sensitivity score of 3 (Baptista et al., 2007). As the metrics were mostly expressed as proportions and tend not to be normally distributed, the non-parametric Mann–Whitney test was performed to determine significant differences between the reference and disturbed stations. These statistical comparisons were not used to eliminate metrics but rather to describe the differences between the benthic fauna of the two categories of stations and as a method of validating the potential metrics previously determined by the box-and-whisker plots visualization (Baptista et al., 2007; Kashian & Burton, 2000). The similarity between the sampling stations based on all tested metrics was assessed by hierarchical clustering using the distance provided by the weighted arithmetic mean linkage method (WPGMA) after log transformation (log10 (x + 1)) of the data except for pH values, to eliminate scale bias. The Spearman correlation ($p < 0.05$) was performed to explore the relationship between physico-chemical parameters and macroinvertebrate metrics in the sampling stations. Graphing and statistical analyses were performed using Minitab 17 Statistical Software package.

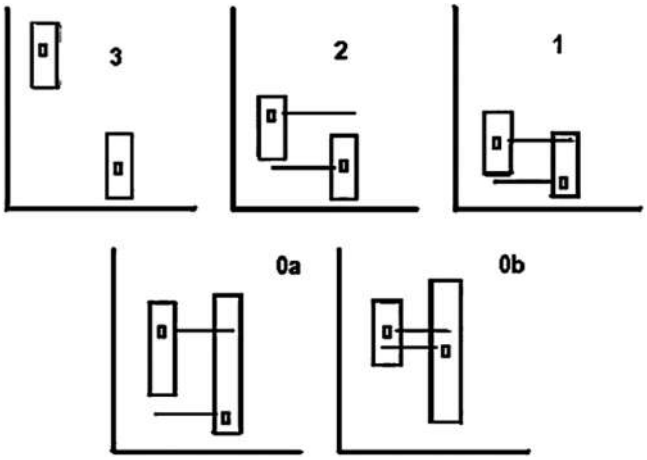

FIGURE 2 Evaluation of metrics sensitivity, according to Barbour et al. (1996) and Baptista et al. (2007). Small squares represent median numbers and boxes represent inter-quartile ranges (25–75% percentiles).

3 | RESULTS

3.1 | Physico-chemical water quality parameters

The values of the physico-chemical water quality parameters measured at different sampling stations and the related descriptive

statistics are presented in Table 3. The one-way ANOVA result showed significant between stations variations for all measured parameters, except TDS, SiO_2 and PO_4^{3-} and NO_2^-. As for the standard norms of the physico-chemical parameters as a function of aquatic fauna (Table 3), PO_4^{3-} values are out of the norm in all stations, whether disturbed or not. Referring to CCME (1999), NO_2^- concentrations are upper limit value 0.02 mg/L at disturbed stations BK2 and BK3 and turbidity exceeded the limit value of 5NTU at the disturbed stations of BK2, BK4, and IS3 and the reference station of IS4.

The two-way ANOVA results for the comparisons of the values of physico-chemical parameters between grouped disturbed and reference stations (Figure 3) showed significant effects of the stations on five parameters, which are EC ($F = 7.04$, $p = 0.009$), TDS ($F = 4.86$, $p = 0.030$), turbidity ($F = 12.66$, $p = 0.001$), PO_4^{3-} ($F = 5.43$, $p = 0.022$), and NO_2^- ($F = 6.65$, $p = 0.011$). No seasonal effect was significant while comparing the mean values of each parameter between the rainy and dry seasons. The effects of the interaction between seasons and sampling stations were also no significant for all parameters except for TDS ($F = 5.12$, $p = 0.026$) (Table 4).

3.2 | Tested metrics

The values obtained per station for the assessed macroinvertebrate metrics (Table 5) show that the proportions of Ephemeroptera-Plecoptera-Tricoptera taxa, three groups considered as indicators of little disturbed environments, did not exceed 0.5% in all the altered stations whereas in the reference stations they are higher than 2%, except for one undisturbed station (BK1) where only 0.84% of them was observed. The group of insects was the most dominant in the disturbed stations with abundances of more than 70% while their proportions were less than 44% in the reference stations. Of this group, Diptera and more particularly the Chironomidae family, a taxonomic group dominating a disturbed environment, were the most abundant in two disturbed stations (BK3 and BK4) where their proportions varied between 47% and 77%, but they did not exceed 15% in all reference stations. The same observation was done for the proportions of highly pollution tolerant taxa, which were above 50% in all disturbed stations, while moderately pollution tolerant taxa showed proportions above 50% in all reference stations. The intolerant taxa did not exceed 8% in all sampled stations (Table 5).

The contributions of functional feeding groups to the trophic structure of the macroinvertebrate community at each sampling station (Table 5) showed that predators are dominant in the disturbed stations BK2 (53.33%) and IS3 (47.29%), and gathering-collectors are dominant in the disturbed stations BK3 (74.93%), BK4 (60.68%), and the reference station IS1 (64.20%). Scavengers are dominant in the disturbed station BK1 (64.98%) and disturbed station IS3 (56.74%). Filters-collectors are only present in one reference station (IS4) while shredders are only present in the only one disturbed station (IS3) with respective station abundances of 7.33% for filters-collectors and 0.16% for shredders.

TABLE 3 Mean and standard deviation (mean ± Sd.) of physico-chemical parameters of all sampling stations in Bukavu and Ishungu sub-basins ($N = 12$) and standard limit values of parameters for the maintenance of aquatic life according to the guidelines of three reference agencies

	Bukavu sub-basin sampling stations				Ishungu sub-basin sampling stations			ANOVA or Kruskal–Wallis		
Parameters	BK1$^+$	BK2^x	BK3 ^{x}	BK4 ^{x}	IS1$^+$	IS2$^+$	IS3 ^{x}	IS4$^+$	*F or H*	*p*	**
Temp. (°C)	24.0 ± 0.9^b	24.1 ± 0.9^b	23.7 ± 0.8^b	24.3 ± 0.7^{ab}	24.5 ± 0.4^{ab}	24.5 ± 0.4^a	25.0 ± 0.6^a	25.0 ± 0.4^a	6.20	0.000	-
DO (mg/L)	**5.3** ± 0.6^b	**5.8** ± 0.6^{ab}	**5.9** ± 0.6^{ab}	6.3 ± 0.6^a	6.0 ± 1.0^{ab}	6.4 ± 0.2^a	**5.8** ± 1.0^{ab}	6.1 ± 0.5^{ab}	3.01	0.007	$6^{1,2,3}$
pH	8.9 ± 0.4^{ab}	8.9 ± 0.5^{ab}	8.6 ± 0.7^b	8.8 ± 0.3^{ab}	9.0 ± 0.1^{ab}	9.0 ± 0.1^{ab}	9.0 ± 0.1^a	8.9 ± 0.4^{ab}	2.26	0.037	$6.5–9^{1,2,3}$
EC (µS·cm^{-1})	1189.1 ± 28.4^{ab}	1203.2 ± 30.5^a	1182.4 ± 10.2^{ab}	1222.1 ± 56.9^a	1192.8 ± 17.0^{ab}	1193.5 ± 5.7^{ab}	1201.0 ± 6.9^a	1158.2 ± 54.1^b	2.78	0.012	1500^3
TDS (ppt)	0.66 ± 0.01^a	0.65 ± 0.01^a	0.66 ± 0.00^a	0.66 ± 0.01^a	0.66 ± 0.01^a	0.67 ± 0.02^a	0.65 ± 0.01^a	0.66 ± 0.02^a	1.53	0.169	1^1
Turbid. (NTU)	4.8 ± 3.1^{cd}	**12.8** ± 9.8^{ab}	4.5 ± 3.3^{cd}	**9.2** ± 5.6^{bcd}	3.0 ± 1.1^d	3.0 ± 1.1^{bcd}	**17.0** ± 6.9^a	**10.4** ± 3.3^{abc}	7.80	0.000	5^1
Chl. *a* (µg/L)	0.50 ± 0.42^{abc}	0.53 ± 0.23^{abc}	0.75 ± 0.49^a	0.53 ± 0.34^{abc}	0.53 ± 0.34^{bc}	0.45 ± 0.30^{abc}	0.23 ± 0.08^c	0.64 ± 0.33^{ab}	2.62	0.017	12^3
SiO_4^{4-} (mg/L)	4.95 ± 2.19^a	5.41 ± 1.55^a	5.41 ± 1.55^a	5.41 ± 1.55^a	5.85 ± 1.39^a	5.44 ± 1.83^a	5.38 ± 1.04^a	5.38 ± 1.04^a	1.95	0.072	-
PO_4^{3-} (mg/L)	**0.23** ± 0.22^a	**0.35** ± 0.33^a	**0.36** ± 0.28^a	**0.34** ± 0.20^a	**0.27** ± 0.27^a	**0.22** ± 0.13^a	**0.22** ± 0.13^a	**0.27** ± 0.23^a	1.10	0.367	0.1^1
NO_2^- (mg/L)	0.01 ± 0.01^a	0.01 ± 0.01^a	**0.06** ± 0.11^a	0.01 ± 0.01^a	0.01 ± 0.01^a	0.01 ± 0.01^a	**0.05** ± 0.03^a	0.01 ± 0.01^a	1.56	0.157	0.02^1
NH_4^+ (mg/L)	0.27 ± 0.13^b	0.29 ± 0.13^{ab}	0.35 ± 0.12^{ab}	0.25 ± 0.06^b	0.25 ± 0.11^b	0.26 ± 0.13^b	0.22 ± 0.18^b	0.50 ± 0.34^a	2.85	0.010	1.37^1

Note: For the same parameter, the stations with the same letter are not different ($^+$: reference stations, ^{x}: Disturbed stations, **: limit value recommended for aquatic fauna: *1*: CCME (1999); *2*: USEPA (1986, 1995); and *3*: ANZECC (2000). Values in bold denote average values of parameters that are outside the limit values for the maintenance of aquatic life).

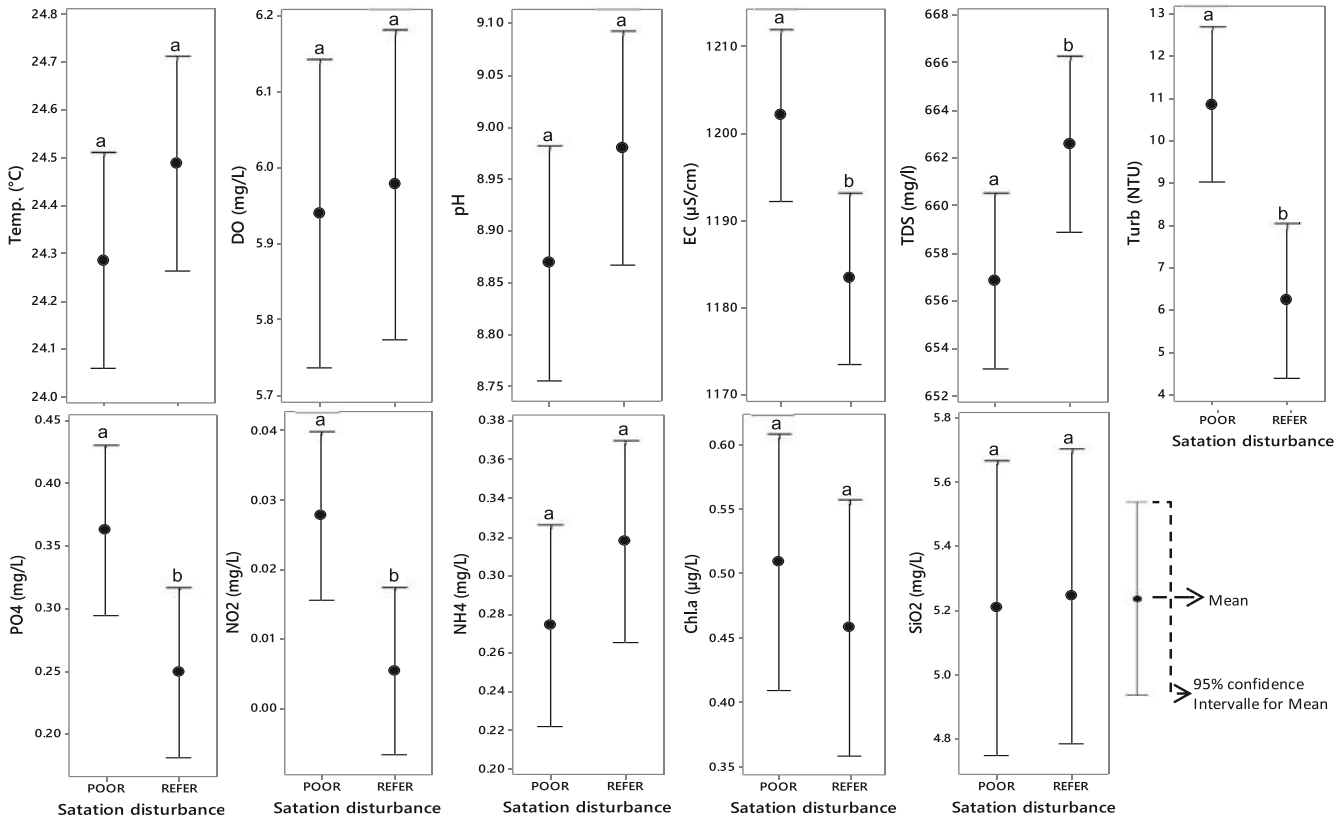

FIGURE 3 Interval plots of physico-chemical parameters according to stations disturbance (The pooled standard deviation was used to calculate 95% confidence interval for the mean)

TABLE 4 Results of the two-way ANOVA for physico-chemical parameters according to sampling stations disturbance and seasons periods

Parameters	Source of variation	DF	SS	MS	*F* value	*P* value
Temp.	Station disturbance	1	0.982	0.982	1.58	0.212
	Seasons	1	0.539	0.539	0.87	0.355
	Station disturbance*seasons	1	0.433	0.433	0.69	0.407
	Errors	92	57.511	0.625		
DO	Station disturbance	1	0.034	0.034	0.07	0.795
	Seasons	1	0.961	0.961	1.92	0.169
	Station disturbance*seasons	1	0.005	0.005	0.01	0.917
	Errors	92	46.525	0.505		
pH	Station disturbance	1	0.297	0.297	1.88	0.174
	Seasons	1	0.020	0.020	0.13	0.718
	Station disturbance*seasons	1	0.284	0.284	1.81	0.182
	Errors	92	14.44	0.156		
EC	Station disturbance	1	8463	8462	7.04	0.009**
	Seasons	1	4	3.99	0.00	0.954
	Station disturbance*seasons	1	1851	1850	1.55	0.217
	Errors	92	109,944	1195		
TDS	Station disturbance	1	789.1	789.1	4.86	0.030*
	Seasons	1	582	582	3.59	0.060
	Station disturbance*seasons	1	796.1	796.1	5.12	0.026*
	Errors	92	14303.7	155.5		

TABLE 4 (Continued)

Parameters	Source of variation	DF	SS	MS	*F* value	*P* value
Turb.	Station disturbance	1	518.37	5.18.37	12.66	0.001**
	Seasons	1	18.74	18.74	0.46	0.500
	Station disturbance*seasons	1	141.83	141.83	3.56	0.062
	Errors	92	3666.75	39.86		
PO_4^{3-}	Station disturbance	1	0.308	0.308	5.43	0.022*
	Seasons	1	0.118	0.118	2.09	0.152
	Station disturbance*seasons	1	0.009	0.009	0.16	0.692
	Errors	92	5.279	0.57		
NO_2^-	Station disturbance	1	0.011	0.011	6.65	0.011*
	Seasons	1	0.004	0.004	2.72	0.102
	Station disturbance*seasons	1	0.002	0.002	1.36	0.246
	Errors	92	0.164	0.001		
NH_4^+	Station disturbance	1	0.044	0.044	1.33	0.253
	Seasons	1	0.013	0.013	0.39	0.533
	Station disturbance*seasons	1	0.008	0.008	0.24	0.628
	Errors	92	3.122	0.033		
Chl. *a*	Station disturbance	1	0.061	0.061	0.51	0.479
	Seasons	1	0.041	0.041	0.34	0.560
	Station disturbance*seasons	1	0.263	0.263	2.18	0.143
	Errors	92				
SiO_2	Station disturbance	1	0.031	0.031	0.01	0.913
	Seasons	1	2.574	0.574	0.99	0.322
	Station disturbance*seasons	1	0.186	0.186	0.07	0.791
	Errors	92	241.450	2.624		

Abbreviations: DF, degree of freedom; MS, mean of squares; SS, sum of squares.
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

TABLE 5 Values of biological metrics and indices at sampling stations in Bukavu and Ishungu sub-basins

Metrics assessed	BK1	BK2	BK3	BK4	IS1	IS2	IS3	IS4
Community composition and diversity								
No.Tot.Tax	16	18	15	22	15	21	16	22
No.Tot.Ind	1896	1774	1552	2004	1109	2580	1108	6020
% EPT	0.84	0.23	0.26	0.20	3.25	2.02	0	8.36
% Dipt	14.98	32.24	77.02	51.90	11.18	15.66	24.19	14.34
% Chiro	14.98	21.42	72.85	47.11	11.18	15.50	23.83	14.34
% Ins	34.39	74.07	83.29	88.22	44.09	38.60	71.48	35.43
% Ins-Dipt.Tax	19.41	41.83	6.27	36.33	32.91	22.95	47.29	21.09
% No Ins.Tax	65.61	25.93	16.71	11.78	55.91	61.40	28.52	64.57
H	1.57	2.12	1.13	1.96	1.66	1.75	1.89	2.03
D	0.34	0.15	0.55	0.26	0.30	0.30	0.20	0.18
$e^{H/S}$	0.30	0.49	0.19	0.34	0.35	0.27	0.41	0.35
J	0.57	0.75	0.41	0.64	0.61	0.57	0.68	0.66
Disturbance tolerance and pollution sensitivity								
EPT/Chiro	0.06	0.01	0	0	0.29	0.13	0	0.58
% Dom.	54.85	21.42	72.85	47.11	49.77	50.08	29.96	33.38
% 3 Dom.Tax	78.48	57.27	87.47	62.08	79.80	77.52	72.56	66.50

(Continues)

TABLE 5 (Continued)

Metrics assessed	BK1	BK2	BK3	BK4	IS1	IS2	IS3	IS4
% Int.Tax	0	1.13	0.52	0,40	0	0	0.36	7.85
% Mod.Tol.Tax	63.08	45.55	18.02	28.14	72.23	68.84	38.63	62.32
% Ver.Tol.Tax	34.81	53.33	80.42	71.06	26.69	29.46	61.01	29.71
Trophic functional groups								
% Prd	18.14	53.33	8.88	30.14	33.27	23.10	47.29	32.60
% Gth	16.24	22.32	74.93	60.88	64.20	19.84	26.35	25.43
% Flt	0	0	0	0.09	0	0	0	7.33
% Scv	64.98	24.35	15.93	8.58	2.52	56.74	25.99	34.25
% Shr	0	0	0	0	0	0.16	0	0
Biotic indices								
ASPT	5^{LN}	3.9^{CM}	4.8^{LM}	4.1^{LM}	4.5^{MM}	4.8^{LN}	4.2^{LM}	5.6^{LN}
BMWP	80^{CI}	67^{MI}	62^{MI}	95^{CI}	77^{CI}	101^{U}	63^{MI}	123^{U}
FBI	6.4^{SP}	6.8^{VSP}	7.5^{SOP}	7.1^{VSP}	5.8^{SP}	6.2^{SP}	6.7^{VSP}	5.7^{FSP}
Overall water quality[a]	Good	Poor	Poor	Fair	Moderate	Good	Poor	Good

Note: For water quality biotic indices ^{CM}: critical modified = very poor quality, ^{SOP}: severe organic pollution likely = very poor quality, ^{LM}: largely modified = poor quality, ^{VSP}: very substantial pollution likely = poor quality, ^{SP}: substantial pollution likely = fairly poor quality, ^{MM}: moderately modified = fair quality, ^{FSP}: fairly substantial pollution likely = fair quality, ^{MI}: moderately impacted = moderate quality, ^{LN}: largely natural with few modifications = good quality, ^{CI}: clean but slightly impacted = good quality, ^{U}: unpolluted or unimpacted = very good quality.
[a]The overall average water quality taking into account the water quality values deduced from the ASPT, BMWP, and IBF indices at each sampled station.

The biotic index values obtained and their significance for the indicated biological water quality showed inter-station variations (Table 5). The overall assessment of the water quality deduced from the values of the calculated three biotic indices (ASPT, BMWP, and FBI) indicated that the water quality varies from moderate to good quality in reference stations and fair to poor water quality in disturbed stations. The water quality was of good quality in the three reference stations named BK1, IS2, and IS4, moderate in one the reference station IS1, fair quality in the disturbed station BK4, while water of poor quality was observed in three disturbed stations known as BK2, BK3, and IS3.

Hierarchical clustering of the sampling stations based on all tested metrics using the distance provided by the weighted arithmetic mean linkage method (WPGMA) also highlighted a clear classification of reference and disturbed stations (Figure 4). Based on the values and significance of the biotic indices, three groups are observed corresponding to unimpacted stations (group 1) (IS4), very slightly impacted stations (group 2) (BK1, IS1, and IS2), and heavily disturbed stations (group 3) (BK2, BK3, BK4, and IS3).

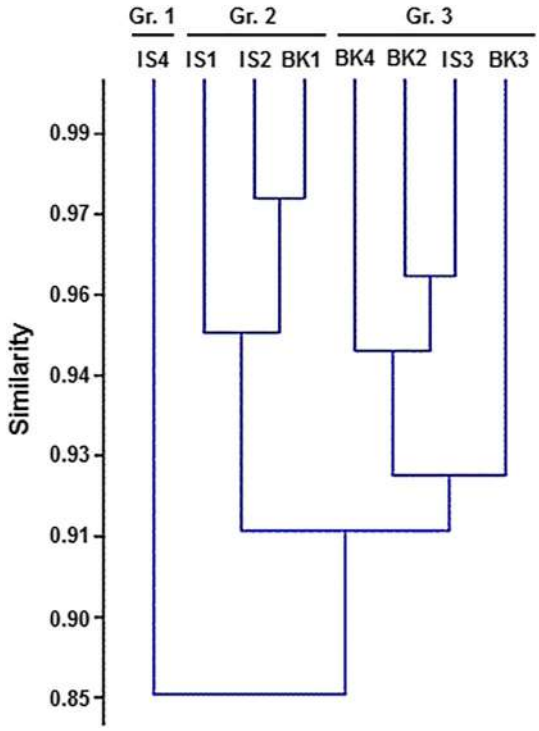

FIGURE 4 Hierarchical clustering of the sampling stations on the basis of all tested metrics was assessed by hierarchical clustering using the distance provided by the weighted arithmetic mean linkage method (WPGMA).

3.3 | Metrics sensitivity tests and correlation between metrics and physico-chemical parameters

Box-and-whisker plots used to determine the sensitivity of the metrics to discriminate between reference and disturbed stations are presented in Figure 5. Based on the sensitivity test applied to the 26 metrics assessed in this study, 9 (% Dipt, % Chiro, % Ins, % Non Ins. Tax, EPT/Chiro, % Mod.Tol.Tax, % Ver.Tol.Tax, and FBI) were considered sensitive with a sensitivity score of 3 between the reference and the degraded sites (Table 6). Only six metrics were statistically different between reference and disturbed stations according to the Mann–

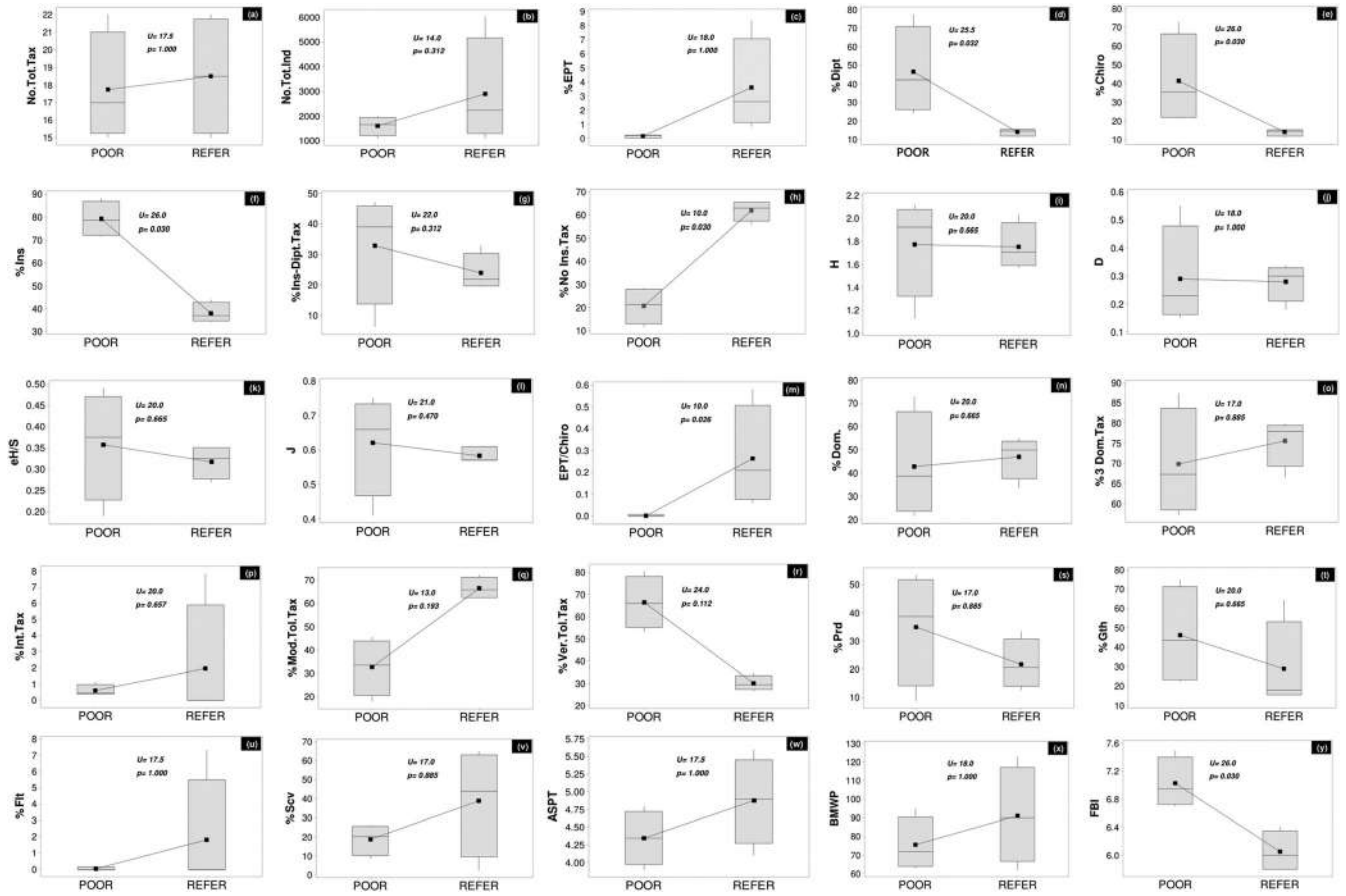

FIGURE 5 Box and whisker plots of all metrics tested for their sensitivity to discrimination of reference stations (REFER) and disturbed stations (POOR). Small squares represent mean values, bars within boxes represent median values, boxes represent interquartile ranges (25–75% percentiles) and whiskers indicate maximum and minimum values of non-outliers.

Whitney U test ($p < 0.05$) and were therefore considered valid for discriminating between reference and disturbed stations (Table 6). That are the percentage of Dipteran (% Dipt) ($U = 25.5$, $p = 0.032$), percentage of Chironomids (% Chiro) ($U = 26$, $p = 0.030$), percentage of Insects (% Ins) ($U = 26$, $p = 0.030$), and percentage of no insects taxa (% No Ins.Taxa) ($U = 10.0$, $p = 0.024$) for metrics related to community composition and diversity and EPT/Chironomids (EPT/Chiro) ($U = 10$, $p = 0.026$) for metrics related to tolerance to disturbance and sensitivities to pollution and Family Biotic Index (FBI) ($U = 26$, $p = 0.030$) for metrics related to biotic indices.

The matrix of correlations (Spearman method) between all metrics and physico-chemical water parameters are presented in Figure 6. The metrics considered sensitive were only significantly correlated with the FBI index among the three calculated biotic indexes that summarize the biological quality of the water (ASPT, BMWP and FBI). The FBI was positively correlated with % Dipt ($r = 0.99$, $p < 0.001$), % Chiro ($r = 0.99$, $p = 0.001$), % Ins ($r = 0.92$, $p = 0.001$), and % Ver.Tol. Tax ($r = 0.94$, $p = 0.001$) and negatively correlated with % EPT ($r = -0.84$, $p = 0.033$), EPT/Chiro ($r = -0.91$, $p = 0.001$), and % Mod.Tol.Tax ($r = -0.94$, $p = 0.001$).

The correlation between the values of the physico-chemical parameters of the water that are the first to be impacted by disturbances of exogenous origin in the lakeshore (Table 7) showed the positive significant correlations between percentage of EPT taxa (% EPT) and NO_2^- ($r = 0.814$, $p = 0.013$), percentage of Diptera taxa and (% Dipt) and PO_4^{3-} ($r = 0.813$, $p = 0.047$), percentage of insects (% Ins) and PO_4^{3-} ($r = 0.885$, $p = 0.003$), percentage of insects no dipteran taxa (%Ins-Dipt.Tax) and pH ($r = 0.804$, $p = 0.016$), percent of insects no dipteran taxa (% Ins-Dipt.Tax) and SiO_4^{4-} ($r = 0.873$, $p = 0.004$), Equitability index (J) and pH ($r = 0.756$, $p = 0.029$), equitability index (J) and SiO_4^{4-} ($r = 0.788$, $p = 0.019$), percentage of moderate tolerant taxa (% Mod.Tol.Tax) and pH ($r = 0.775$, $p = 0.023$), percentage of moderate tolerant taxa (% Mod.Tol.Tax) and NO_2^- ($r = 0.761$, $p = 0.028$), percentage of very tolerant taxa (% Ver. Tol.Tax) and PO_4^{3-} ($r = 0.878$, $p = 0.004$), percent of predators (% Prd) and pH ($r = 0.720$, $p = 0.043$), percent of predators (% Prd) and SiO_4^{4-} ($r = 0.830$, $p = 0.010$), and Family Biotic Index (FBI) and PO_4^{3-} ($r = 0.712$, $p = 0.047$). However, negative significant correlations were observed between percent of EPT taxa (% EPT) and PO_4^{3-} ($r = -0.839$, $p = 0.009$), percentage of dipteran taxa and (% Dipt.Tax) and pH ($r = -0.752$, $p = 0.031$), percentage of insects (% Ins) and NO_2^- ($r = -0.746$, $p = 0.033$), percentage of no insects taxa (% No Ins.Tax) PO_4^{3-} ($r = -0.811$, $p = 0.014$), Simpson dominance index (D) and turbidity ($r = -0.796$, $p = 0.017$), most dominant taxon (%

TABLE 6 Responses from the comparison of metrics between reference and disturbed stations and the results of the Mann–Witney *U* test (*U* values and *p* level)

Metrics assessed	Sensibility test value[a]	*U*	*p* level	Metric validation
No.Tot.Tax	0b	17.5	1.000	-
No.Tot.Ind	0a	14.0	0.312	-
% EPT	3	18.0	1.000	-
% Dipt	**3**	**25.5**	**0.032**	**Valid**
% Chiro	**3**	**26.0**	**0.030**	**Valid**
% Ins	**3**	**26.0**	**0.030**	**Valid**
% Ins-Dipt.Tax	0b	22.0	0.312	-
% No Ins.Tax	**3**	**10.0**	**0.024**	**Valid**
H	0b	20.0	0.665	-
D	0b	18.0	1.000	-
$e^{H/S}$	0b	20.0	0.665	-
J	0b	21.0	0.470	-
EPT/Chiro	**3**	**10.0**	**0.026**	**Valid**
% Dom.	0b	20.0	0.665	-
% 3 Dom.Tax	0a	17.0	0.885	-
% Int.Tax	0b	20.0	0.657	-
% Mod.Tol.Tax	3	13.0	0.193	-
% Ver.Tol.Tax	3	24.0	0.112	-
% Prd	0b	17.0	0.885	-
% Gth	1	20.0	0.665	-
% Flt	0b	17.5	1.000	-
% Scv	0a	17.0	0.885	-
% Shr	-	-	-	-
ASPT	2	17.5	1.000	-
BMWP	0a	18.0	1.000	-
FBI	**3**	**26.0**	**0.030**	**Valid**

[a]Sensibility test value-based box-and-whisker plots observations.

Dom) and turbidity ($r = -0.786$, $p = 0.021$), percentage of moderate tolerant taxa (% Mod.Tol.Tax) and PO_4^{3-} ($r = -0.756$, $p = 0.029$), percentage of very tolerant taxa (% Ver.Tol.Tax) and NO_2^- ($r = -0.829$, $p = 0.010$), percentage % filters-collectors (% Flt.) and electrical conductivity ($r = -0.713$, $p = 0.046$), and Family Biotic Index (FBI) and NO_2^- ($r = -0.750$, $p = 0.031$).

The use of stepwise multiple linear regression on the dataset showed that three variables relating to biological water quality and benthic fauna diversity were affected by the season. These are ASPT ($r^2 = 0.284$, $p = 0.004$), % insects ($r^2 = 0.96$, $p = 0.0001$), and %EPT ($r^2 = 0.25$, $p = 0.01$).

4 | DISCUSSION

4.1 | Overall physico-chemical characterization of the water

Physico-chemical parameters are the first environmental variables to be impacted by disturbances that may occur in the water body and its watershed (Calmuc et al., 2020; Pandey et al., 2018; Saal et al., 2020). Also, the physico-chemical parameters of waters are considered as the most important factors to be able to influence the species composition, diversity, stability, production and physiological conditions of biological community assemblage in a water bodies (Barinova & Mamanazarova, 2021; Pissaridou et al., 2021). In this study, the results showed that four (DO, Turbidity and the nutrients PO_4^{3-} and NO_2^-) of the 11 measured parameters (Table 3) recorded values above the recommended limits for the sustainability of life in the aquatic environment (ANZECC, 2000; CCME, 1999; USEPA, 1986, 1995).

The comparison of parameters between the disturbed and reference stations (Figure 3) showed differences between parameters such as EC, TDS, turbidity, PO_4^{3-}, and NO_2^-. Except for TDS, the values of all these parameters are significantly higher in the disturbed stations than in the reference stations highlighting the effect of human disturbances in the nearshore of the lake affecting its water quality on a spatial scale. The high values of turbidity are related to the high soil erosion rate reported in the region (Muvundja et al., 2009), sewage discharges, and various waste discharges observed in the disturbed

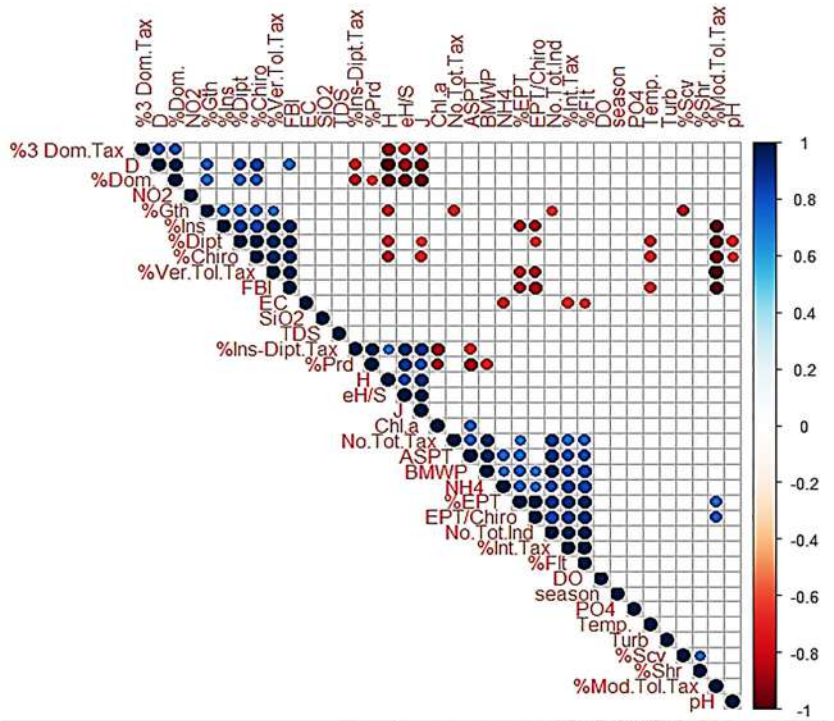

FIGURE 6 The matrix of correlations (Spearman method) between all metrics and physico-chemical water parameters

Correlation Coefficient (r)	Description (Rough Guideline)
+1.0	Perfect positive + association
+0.8 to 1.0	Very strong + association
+0.6 to 0.8	Strong + association
+0.4 to 0.6	Moderate + association
+0.2 to 0.4	Weak + association
0.0 to +0.2	Very weak + or no association
0.0 to -0.2	Very weak - or no association
-0.2 to – 0.4	Weak - association
-0.4 to -0.6	Moderate - association
-0.6 to -0.8	Strong - association
-0.8 to -1.0	Very strong - association
-1.0	Perfect negative association

stations during the sampling period as evidenced by Lina (2016). Turbidity is known to reduce water quality by absorbing heat, increasing water temperature and decreasing dissolved oxygen by reducing the light penetration in the lake (Bilotta & Brazier, 2008). This was the observation at disturbed stations and especially at two disturbed stations named BK3 and IS3 in the lake waters affecting these particular areas. High turbidity levels in waters are also known to hinder aquatic insects, smother fish eggs, reduce growth rates, and disrupt the microhabitats of many other aquatic organisms (CCME, 2001; Kjelland et al., 2015). In this study, turbidity values were negatively correlated with the percentage of the most dominant taxon (% Dom) and the Simpson dominance index (D) indicating their evidenced effects on these organisms and their distribution in the lake littoral waters (Bilotta & Brazier, 2008; Kjelland et al., 2015).

The high value of PO_4^{3-} and NO_2^{-} concentrations obtained in this study highlighted the impact of human activities in the catchment area on the quality of the coastal waters. Such high concentrations would be linked to probable contamination from the immediate catchment area and constitute a source of eutrophication of the lake. Indeed, in an unpolluted lake, phosphorus loading in the lake is consumed almost immediately by organisms without creating a surplus (Kjelland et al., 2015). However, the addition of phosphorus and nitrogen from the catchment directly to the lake by human activities is known to increase the differences in key nutrient concentrations between different parts of a large lake (UNEP/WHO/UNESCO/WMO, 1992). In this case, these nutrients indicate the beginning of enrichment of the lake coastal waters with nitrogen and phosphorus, which would be transported to the nearby coastal waters by rainwater, gullies, and groundwater (Saal et al., 2020). The positive correlation observed between PO_4^{3-} and NO_2^{-} with certain metrics such as % EPT taxa, % Diptera, % Insects, % no insects taxa, % moderate tolerant taxa, % very tolerant taxa, and Family Biotic Index evidenced that the increase in these two nutrients would have influences on both the composition and structure of benthic macroinvertebrates, as underlined by the values of the various biotic indices, which are an expression of the simple assessment of the effects of organic pollutants on benthic macroinvertebrates (Mandaville, 2002).

TABLE 7 Metrics that correlated with individual physico-chemical variables using Spearman's correlation including correlation coefficient (*r*) and significance (*p*)

Metrics	Variable	*r*	*p*
% EPT taxa	PO_4^{3-}	−0.839	0.009**
	NO_2^-	0.814	0.013*
% Diptera	pH	−0.752	0.031*
	PO_4^{3-}	0.713	0.047*
% Chironomids	pH	−0.785	0.021*
% Insects	PO_4^{3-}	0.885	0.003**
	NO_2^-	−0.746	0.033*
% Insects no Diptera taxa	pH	0.804	0.016*
	SiO_4^{4-}	0.873	0.004**
% No insects taxa	PO_4^{3-}	−0.811	0.014*
Simpson dominance index	Turbidity	−0.773	0.024*
Equitability index	pH	0.756	0.029*
	SiO_4^{4-}	0.788	0.019*
% Most dominant taxon	Turbidity	−0.786	0.021*
% Moderate tolerant taxa	pH	0.775	0.023*
	PO_4^{3-}	−0.756	0.029*
	NO_2^-	0.761	0.028*
% Very tolerant taxa	PO_4^{3-}	0.878	0.004**
	NO_2^-	−0.829	0.010*
% Predators	pH	0.720	0.043*
	SiO_4^4	0.830	0.010*
% Filters-collectors	EC	−0.713	0.046*
Family Biotic Index	PO_4^{3-}	0.712	0.047*
	NO_2^-	−0.750	0.031*

*$p < 0.05$. **$p < 0.01$.

The combination of the three water quality indices obtained based on macroinvertebrate communities (ASPT, BMWP, and FBI) (Table 5) confirms the degradation of water quality along a gradient ranging from water of good water quality in the reference stations BK1, IS2, and IS4 to water of poor water quality in the degraded stations BK2, BK3, and IS3 (Table 4). This shows that at each station either disturbed or not, the composition and structure of macroinvertebrates perfectly reflect the water quality of the water at the particular station. For example, Diptera and more particularly the Chironomidae, known to be the most dominant taxonomic groups in altered environments (Mandaville, 2002; Tachet et al., 2010), are most abundant in the altered stations of BK3 and BK4 where the water was of poor quality, a sign of probable severe pollution, whereas their proportions did not exceed 15% in all reference stations where the biological water quality is good, a sign of largely natural stations with little modification. Same observation was done for the proportions of taxa highly tolerant to pollution (Table 5), which are more abundant in all disturbed stations while taxa moderately tolerant to pollution have the highest proportions in all reference stations. The very low proportion of intolerant taxa in almost all the sampled stations (Table 4) would be related to the lack of excellent water quality in all these sites.

The absence of significant differences in physico-chemical parameters between seasons and of interaction between stations and seasons, with the exception of TDS (Table 4), can be explained by the monthly sampling frequency. On the other hand, it could also suggest the quasi-permanent nature of potential sources of disturbance exogenous to the littoral zone of the lake. In both cases, this should be clarified by weekly sampling over a long period and by diversifying the sampling stations on the basis of a disturbance gradient. This partly explains why the parameter analysis discussed in this study is especially spatially oriented.

4.2 | Macroinvertebrate metrics tested

The assessment of water quality using aquatic organisms, also known as biomonitoring, is an approach that is over a century old (Carter et al., 2017; Odountan et al., 2019). It was originally developed for use in lotic systems (Barbour et al., 1996; Mandaville, 2002) and has been progressively extended to lentic systems (lakes, ponds, and reservoirs) (Gabriels et al., 2010; Odountan et al., 2019). Benthic macroinvertebrates are the most valued indicator species because their composition exhibits natural variations due to season, lake depth, and mesoscale habitat structure, as well as biotic effects (competition and predation) (Ligeiro et al., 2020; López-López & Sedeño-Díaz, 2015; Tachet et al., 2010).

In the context of lack historical faunal data and resources for operational modelling of water quality in Lake Kivu, taking into account these biological variables linked to benthic macroinvertebrates would be a good option for assessing the individual and cumulative effects of several sources of disturbance, whether point or diffuse and for monitoring these effects over the medium and long term in qualitative and quantitative terms (Odountan et al., 2019). This is all the more so as these indicators act as a monitoring and warning system that addresses ecological, ethical, and economic concerns (Lévêque, 1994). The fact that most benthic macroinvertebrates are relatively long-lived, with cycles ranging from several months to several years, makes them important indicators of the status of a specific site over time (Ligeiro et al., 2020; López-López & Sedeño-Díaz, 2015; Tachet et al., 2010). In addition, these organisms are sensitive to the cumulative effects of physical and chemical disturbances, to which they respond with a range of sensitivities to many types of stressors (Tachet et al., 2010).

Despite the modifications and effects that the littoral zone of Lake Kivu undergoes (Lina, 2016; Muvundja et al., 2009) and that would have an impact on the macroinvertebrates of this zone, very few data exist on this community so that the choice of stations that are not or only slightly altered (= reference) and severely altered (= test) was paramount to compare metric values between the two groups. However, the identification of the reference state for a specific lake type based on benthic invertebrates is not easy due to natural variations caused by season, lake depth, mesoscale habitat

structure, and biotic effects (competition and predation) (Carter et al., 2017). The use of hierarchical clustering of the sampling stations based on all tested metrics assessed by hierarchical clustering using the distance provided by the weighted arithmetic mean linkage method (WPGMA) (Figure 4). This allowed the grouping of the selected stations into three categories along a disturbance gradient, which supported the station choices made a priori according to the criteria proposed by Jónasson (2004), Pissaridou et al. (2021), Ntislidou et al. (2018), and Vitecek et al. (2021). Community similarity indices (e.g., percentage similarity, hierarchical clustering) are another method for comparing macroinvertebrate composition between stations. They have already been used in several multi-metric and multivariate approaches (Sharifinia et al., 2016). Indeed, most similarity indices compare the composition of two samples on a taxon-by-taxon basis, which allows discriminating between stations based on their taxonomic composition and relative abundances (Weiss & Reice, 2005).

The identification of taxa down to the family level showed clearly how the natural variations and human events influence benthic macroinvertebrate variations linked to the dominance of an isolated species, to which metrics linked to faunal composition such as the percentage of taxa dominating the sample are sensitive based on the results of multivariate analyses (Ozoliņš et al., 2021). According to Warwick (1988), anthropogenic pollution and disturbance theoretically modify community structure at the highest taxonomic level, whereas natural environmental conditions affect the community structure only through replacement at the species level. Hence, Carter et al. (2017) advocate that the study of communities at higher taxonomic levels (family, phylum, …) allows to free oneself from the influence of natural environmental factors.

The examination of the sensitivity of 26 metrics tested based on the box-and-whisker plots method (Baptista et al., 2007; Kashian & Burton, 2000) (Figure 5), selected nine sensitive metrics that best discriminate the reference stations from those disturbed in the lakeshore zone. These are the percentage of EPT taxa (% EPT), percentage of dipteran taxa (% Dipt), percentage of Chironomid taxa (% Chiro), percent of no insect's taxa (% No Ins.Tax), ratio EPT/Chironomid taxa (EPT/Chiro), percent of moderate tolerant taxa (% Mod.Tol.Tax), percent of very moderate tolerant taxa (% Ver.Tol.Tax), and Family Biotic Index (FBI). Comparisons of the sensitivity of these nine metrics resulted in only six (number of EPT taxa, % Dipt, % Chironomidae, % Ephemeroptera or % EPT and FBI) being selected as the metrics that could properly discriminate between reference and disturbed sites as also observed elsewhere (e.g., Camargo, 2019; Gabriels et al., 2010). EPT and Dipteran groups including chironomids showed predictable responses to low oxygen levels and over-enrichment of key nutrients in the water, which explains their use in many studies to assess nutrient enrichment (e.g., Barinova & Mamanazarova, 2021; Serra et al., 2016). In this study, there was correlation between these groups and oxygen, a parameter that did not record very low values (>6 mg/L in four stations and 5.3 to 5.8 mgl/L in four other stations). This observation can be partially explained by the waves, which would constantly have a re-oxygenating effect on the littoral lake waters, especially as this is noted in the stations with and without macrophytes. However, the metrics correlated negatively and/or positively with the nutrient PO_4^{3-} (correlated with % EPT and % Diptera), a variable which was above the interval limits for aquatic life in all stations and the nutrient NO_2^{-} (correlated with % EPT) which had values that exceeded the threshold limits in the disturbed stations of BK3 and IS3.

Metrics based on diversity indices such as taxonomic richness, Shannon–Weaver index, and Simpson index did not discriminate between stations according to their degree of disturbance although they are widely used as tools to measure of disturbance in comparisons between different lake habitats (Odountan et al., 2019). This can be justified by the fact that in this study the faunal differences between the two categories of stations (reference vs. disturbed) seem to be better observed on the level of taxonomic abundances than on the number of family-level taxa present in the reference and disturbed stations. Similarly, the functional feeding groups did not show discriminating differences between the two categories of stations compared. This could partly be explained by the fact that many species exposed to stressors can change or shift their habitat and feeding preferences in response to degraded environmental conditions as observed by Camargo (2019).

Taking into account the effect of seasonal periods, the ASPT, % insects, and % EPT are the three metrics of the macro-benthic community that were found to be influenced by season. As with % insects and % EPT, the influence of season is certain and is thought to be related to the ecology of most of these insects, particularly Chironomidae, Ephemeroptera, Plecoptera, and Trichoptera, which are insects that undergo metamorphosis (Lancaster & Downes, 2013). Within these groups, there is a relative abundance of polyvoltine taxa (organisms that can have several generations per year) with many swarming periods during the year (Lancaster & Briers, 2008). These periods coincide with aerial life, a situation that certainly influences their composition and abundance in the same environment during different seasonal periods (Šporka et al., 2006). As the ASPT is obtained by averaging the BMWP score of the sample, the latter being the sum of the individual scores of the taxa collected, it is obvious that the ASPT is influenced by the season, especially as it does not depend on the richness of the families present but rather indirectly on the abundances within them (Barbour et al., 1999; Mandaville, 2000).

5 | CONCLUSION

This study illustrated that metrics based on benthic macroinvertebrate assemblage are effective in distinguishing nearshore lake habitats along a disturbance gradient ranging from relatively stable to sufficiently impacted areas. Therefore, they can be used as a useful tool to assess the biological quality of littoral lake waters in order to contribute to the general water policy making in DR Congo and the ecological sustainability of the natural environment of Lake Kivu. Overall, the descriptive results of the metric showed that the water quality is in the range of good to fair in the reference sites, while in the disturbed

stations it varies from moderate to poor depending on the case. Within the macrobenthic assemblage, this is reflected in the low representativeness of sensitive taxa and the preponderance of moderately tolerant and highly tolerant taxa. This indicates that the human activities in the watershed of the lake basin impact the water quality in the lake. It would be desirable to assess the performance of the macroinvertebrate-based metrics along a disturbance gradient in the littoral zone and its sub-watershed. The nine metrics that showed sensitivity to station discrimination in the present study can serve as reference metrics that can be refined by long-term sampling and the use of sophisticated multivariate statistical analyses. Given that the basic principle of biomonitoring by macroinvertebrate assemblages is the comparison of disturbed with non-disturbed stations, and recognizing that pristine reference conditions are rarely available in a study, the choice of stations to be sampled remains the most important factor to be considered in using this approach in future studies. Although the results obtained here have practical implications for ecological assessment and management of the littoral zone, it is important to advocate the development of other benthic invertebrate-based multimeric indices of the Lake Kivu littoral zone that are specific to the identified stressors. These stressors should be categorized beforehand to maximize the efficiency of such an approach, which is economically advantageous compared with ecotoxicological or physico-chemical approaches.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

AFNOR. (2010). Qualité de l'eau – Traitement au laboratoire d'échantillons contenant des macro-invertébrés de cours d'eau. XP T90-388, 21 pp.

Akay, E., & Dalkıran, N. (2019). Assessing biological water quality of Yalakdere stream (Yalova, Turkey) with benthic macroinvertebrate-based metrics. *Biologia*, *1-17*, 1347–1363. https://doi.org/10.2478/s11756-019-00387-9

ANZECC. (2000). Australian and New Zealand guidelines for fresh and marine water quality. Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Camberra, 1–103.

APHA. (2005). In A. Eaton, L. S. Clesceri, E. W. Rice, & A. E. Grenberg (Eds.), *Standard methods for the examination of water and waste water, 21st edition* (Centennial ed.). APHA, AWWA and WEF. 69pp

Baptista, D. F., Buss, D. F., Egler, M., Giovanelli, A., Silveira, M. P., & Nessimian, J. L. (2007). A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro state, Brazil. *Hydrobiologia*, *575*(1), 83–94. https://doi.org/10.1007/s10750-006-0286-x

Barbour, M. T., Gerritsen, J., Griffith, G. E., Frydenborg, R., McCarron, E., White, J. S., & Bastian, M. L. (1996). A framework for biological criteria for Florida streams using benthic macroinvertebrates. *Journal of the North American Benthological Society*, *15*(2), 185–211. https://doi.org/10.2307/1467948

Barbour, M. T., Gerritsen, J., Snyder, B. D., & Stribling, J. B. (1999). *Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish* (2nd edition). U. S. Environmental Protection Agency, Office of Water. EPA 841-B-99–002.

Barinova, S., & Mamanazarova, K. (2021). Diatom algae-indicators of water quality in the lower Zarafshan River, Uzbekistan. *Water*, *2021*(13), 358. https://doi.org/10.3390/w13030358

Basima, L. B., Mbalassa, M., Muhigwa, B., & Nshombo, M. (2006). Anthropogenic influences on the littoral zone biota of Lake Kivu, Bukavu Basin, DR Congo. *Internationale Vereinigung für Theoretische Und Angewandte Limnologie: Verhandlungen*, *29*(5), 2283–2288. https://doi.org/10.1080/03680770.2006.11903100

Beadle, L. C. (1981). *The inland waters of tropical Africa, an introduction to tropical limnology* (2nd ed.). Longman. 84pp

Bilotta, G. S., & Brazier, R. E. (2008). Understanding the influence of suspended solids on water quality and aquatic biota. *Water Research*, *42*, 2849–2861. https://doi.org/10.1016/j.watres.2008.03.018

Brown, D. S. (1994). *Freshwater snails of Africa and their medical importance* (Second revised ed.) (p. 609). Taylor & Francis. https://doi.org/10.1201/9781482295184

Calmuc, M., Calmuc, V., Arseni, M., Topa, C., Timofti, M., Georgescu, L. P., & Iticescu, C. (2020). A comparative approach to a series of physico-chemical quality indices used in assessing water quality in the lower Danube. *Water*, *12*(11), 3239. https://doi.org/10.3390/w12113239

Camargo, J. A. (2019). Positive responses of benthic macroinvertebrates to spatial and temporal reductions in water pollution downstream from a trout farm outlet. *Knowledge and Management of Aquatic Ecosystems*, *420*, 16. https://doi.org/10.1051/kmae/2019010

Cañedo-Argüelles, M., & Rieradevall, M. (2011). Early succession of the macroinvertebrate community in a shallow lake: Response to changes in the habitat condition. *Limnologica*, *41*(4), 363–370. https://doi.org/10.1016/j.limno.2011.04.001

Carter, J. L., Resh, V. H., & Hannaford, M. J. (2017). Macroinvertebrates as biotic indicators of environmental quality. In *Methods in stream ecology* (pp. 293–318). Academic Press. https://doi.org/10.1016/B978-0-12-813047-6.00016-4

CCME. (1999). Canadian environmental qualities guidelines, Canadian Council of Ministers of the environment, Manitoba statuary publications, Winnipeg, Canada. Updated in 2000.

CCME. (2001). Canadian water quality guidelines for the protection of aquatic life: Canadian water quality index 1.0 technical report. In Canadian environmental quality guidelines, 1999. Winnipeg, Manitoba.

CCME. (2009). Canadian Council of Ministers of the Environment. Canadian water quality guidelines for the protection of aquatic life: CCME water quality index 1.0. User's manual. In: Canadian environmental quality guidelines, Winnipeg.

Commission Européenne, Parlement européen et Conseil de l'Union Européenne. (2000). Directive 2000/60/CE du Parlement européen et du Conseil du 23 octobre 2000 établissant un cadre pour une politique communautaire dans le domaine de l'eau. *Journal officiel de la Communauté Européenne*, *L327*, 1–72.

Cumberlidge, N., & Meyer, K. S. (2011). The freshwater crabs of Lake Kivu (Crustacea: Decapoda: Brachyura: Potamonautidae). *Journal of Natural*

History, *45*(29–30), 1835–1857. https://doi.org/10.1080/00222933.2011.562618

Descy, J. P., Darchambeau, F., & Schmid, M. (Eds.) (2012). *Lake Kivu: Limnology and biogeochemistry of a tropical great lake* (Vol. 5). Springer Science & Business Media. https://doi.org/10.1007/978-94-007-4243-7

Gabriels, W., Lock, K., De Pauw, N., & Goethals, P. L. (2010). Multimetric macroinvertebrate index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). *Limnologica-Ecology and Management of Inland Waters*, *40*(3), 199–207. https://doi.org/10.1016/j.limno.2009.10.001

Guimaraes, R. M., Facure, K. G., Pavanin, L. A., & Jacobucci, G. B. (2009). Water quality characterization of urban streams using benthic macroinvertebrate community metrics. *Acta Limnologica Brasiliensia*, *21*(2), 217–226.

Hamada, N., Thorp, J. H., & Rogers, D. C. (Eds.) (2018). *Thorp and Covich's freshwater invertebrates: Volume 3: Keys to Neotropical Hexapoda*. Academic Press.

Isumbisho Mwapu, P. (2006). Ecologie du zooplancton du lac Kivu (Afrique de l'Est). Fac.Sci., Université de Namur, Namur, Belgique, 180p.

Jónasson, P. M. (2004). Benthic Invertebrates. *The Lakes Handbook* (Vol. 1, pp. 341–416). https://doi.org/10.1002/9780470999271.ch12

Kaningini, B. (1995). Etude de la croissance, de la reproduction et de l'exploitation de Limnothrissa miodon au lac Kivu, bassin de Bukavu (Zaïre). PhD thesis, University of Namur, Belgium.

Kaningini, M., Micha, J.-C., Vandenhaute, J., Platteau, J.-P., Watongola, H., Melard, C., Wilondja, M.K., Isumbisho, M. (1999). Pêche du Sambaza au filet maillant dans le lac Kivu. Rapport final du projet ONG/219/92/Zaïre.Presses Universitaires de Namur, ETEC, 187p.

Kashian, D. R., & Burton, T. M. (2000). A comparison of macroinvertebrates of two Great Lakes coastal wetlands: Testing potential metrics for an index of ecological integrity. *Journal of Great Lakes Research*, *26*(4), 460–481. https://doi.org/10.1016/S0380-1330(00)70708-8

Kjelland, M. E., Woodley, C. M., Swannack, T. M., & Smith, D. L. (2015). A review of the potential effects of suspended sediment on fishes: Potential dredging-related physiological, behavioral, and transgenerational implications. *Environment Systems and Decisions*, *35*, 334–350. https://doi.org/10.1007/s10669-015-9557-2

Lancaster, J., & Briers, R. A. (Eds.) (2008). *Aquatic Insects: Challenges to populations. Proceedings of the Royal Entomological Society's 24th symposium*. CAB International. x + 332 pp. ISBN 978-1-84593-396-8

Lancaster, J., & Downes B. J. (2013). Aquatic entomology. Oxford Scholarship Online. https://doi.org/10.1093/acprof:oso/9780199573219.001.0001

Lévêque, C. (1994). State of health of aquatic ecosystems: importance of biological variables. *In* État de santé des écosystèmes aquatiques: Les variables biologiques comme indicateurs. Actes du séminaire national Hydrosystèmes, Paris, 2-3 novembre 1994. Cemagref édition, 13-26p.

Ligeiro, R., Hughes, R. M., Kaufmann, P. R., Heino, J., Melo, A. S., & Callisto, M. (2020). Choice of field and laboratory methods affects the detection of anthropogenic disturbances using stream macroinvertebrate assemblages. *Ecological Indicators*, *115*, 106382. https://doi.org/10.1016/j.ecolind.2020.106382

Lina, A. A. (2016). Evaluation des charges polluantes (domestiques et industriels) arrivant au lac Kivu dans la ville de Bukavu, RDCongo. Thèse de doctorat, Faculté des Sciences, Université de Liège, 241p.

López-López, E., & Sedeño-Díaz, J. E. (2015). Biological indicators of water quality: The role of fish and macroinvertebrates as indicators of water quality. In *Environmental indicators* (pp. 643–661). Springer. https://doi.org/10.1007/978-94-017-9499-2_37

Mandaville, S. M. (2000). Limnology-eutrophication and chemistry, carrying capacities, loadings, benthic ecology and comparative data; Project F-1, soil & water Conservation society of Metro Halifax. Synopses 1, 2, 3, 13, and 14, 210 p.

Mandaville, S. M. (2002). *Benthic macroinvertebrates in freshwaters: Taxa tolerance values, metrics, and protocols* (Vol. 128) (p. 315). Soil & Water Conservation Society of Metro Halifax.

Meyer, K. S., & Cumberlidge, N. (2011). A revision of the freshwater crabs (Crustacea: Decapoda: Brachyura: Potamonautidae) of the Lake Kivu drainage basin in central and East Africa. *Zootaxa*, *3011*(1), 45–58. https://doi.org/10.11646/zootaxa.3011.1.5

Muvundja, F. A., Pasche, N., Bugenyi, F. W., Isumbisho, M., Müller, B., Namugize, J. N., & Wüest, A. (2009). Balancing nutrient inputs to Lake Kivu. *Journal of Great Lakes Research*, *35*(3), 406–418. https://doi.org/10.1016/j.jglr.2009.06.002

Ntislidou, C., Lazaridou, M., Tsiaoussi, V., & Bobori, D. C. (2018). A new multimetric macroinvertebrate index for the ecological assessment of Mediterranean lakes. *Ecological Indicators*, *93*, 1020–1033. https://doi.org/10.1016/j.ecolind.2018.05.071

Odada, E. O., Olago, D. O., Bugenyi, F., Kulindwa, K., Karimumuryango, J., West, K., & Achola, P. (2003). Environmental assessment of the east African rift valley lakes. *Aquatic Sciences*, *65*(3), 254–271. https://doi.org/10.1007/s00027-003-0638-9

Odountan, O. H., de Bisthoven, L. J., Abou, Y., & Eggermont, H. (2019). Biomonitoring of lakes using macroinvertebrates: Recommended indices and metrics for use in West Africa and developing countries. *Hydrobiologia*, *826*(1), 1–23. https://doi.org/10.1007/s10750-018-3745-2

Ozoliņš, D., Skuja, A., Jēkabsone, J., Kokorite, I., Avotins, A., & Poikane, S. (2021). How to assess the ecological status of highly Humic Lakes? Development of a new method based on benthic invertebrates. *Water*, *13*(2), 223. https://doi.org/10.3390/w13020223

Pandey, L. K., Lavoie, I., Morin, S., Park, J., Lyu, J., Choi, S., Lee, H., & Han, T. (2018). River water quality assessment based on a multi-descriptor approach including chemistry, diatom assemblage structure, and non-taxonomical diatom metrics. *Ecological Indicators*, *84*, 140–151. https://doi.org/10.1016/j.ecolind.2017.07.043

Pissaridou, P., Vasselon, V., Christou, A., Chonova, T., Papatheodoulou, A., Drakou, K., Tziortzis, I., Dörflinger, G., Rimet, F., Bouchez, A., & Vasquez, M. I. (2021). Cyprus' diatom diversity and the association of environmental and anthropogenic influences for ecological assessment of rivers using DNA metabarcoding. *Chemosphere*, *272*, 129814. https://doi.org/10.1016/j.chemosphere.2021.129814

Poikane, S., Johnson, R. K., Sandin, L., Schartau, A. K., Solimini, A. G., Urbanič, G., Arbačiauskas, K., Aroviita, J., Gabriels, W., Miler, O., Pusch, M. T., Timm, H., & Böhmer, J. (2016). Benthic macroinvertebrates in lake ecological assessment: A review of methods, intercalibration and practical recommendations. *Science of the Total Environment*, *543*, 123–134. https://doi.org/10.1016/j.scitotenv.2015.11.021

Pont, D., Valentini, A., Rocle, M., Maire, A., Delaigue, O., Jean, P., & Dejean, T. (2021). The future of fish-based ecological assessment of European rivers: From traditional EU water framework directive compliant methods to eDNA metabarcoding-based approaches. *Journal of Fish Biology*, *98*(2), 354–366. https://doi.org/10.1111/jfb.14176

Saal, I., Bouchelouche, D., Hamache, C., & Arab, A. (2020). Evaluation of the surface water quality in the Kebir-Rhumel catchment area (Northeast Algeria) using biotic indices and physico-chemical analyses. *Environmental Science and Pollution Research*, *1-15*, 46565–46579. https://doi.org/10.1007/s11356-020-10598-2

Serra, S. R., Cobo, F., Graca, M. A., Doledec, S., & Feio, M. J. (2016). Synthesising the trait information of European Chironomidae (Insecta: Diptera): Towards a new database. *Ecological Indicators*, *61*, 282–292. https://doi.org/10.1016/j.ecolind.2015.09.028

Sharifinia, M., Mahmoudifard, A., Namin, J. I., Ramezanpour, Z., & Yap, C. K. (2016). Pollution evaluation in the Shahrood River: Do physico-chemical and macroinvertebrate-based indices indicate same responses to anthropogenic activities? *Chemosphere*, *159*, 584–594. https://doi.org/10.1016/j.chemosphere.2016.06.064

Šporka, F., Vlek, H. E., Bulánková, E., & Krno, I. (2006). Influence of seasonal variation on bioassessment of streams using macroinvertebrates. *Hydrobiologia*, *566*, 543–555. https://doi.org/10.1007/s10750-006-0073-8

Tachet, H., Richoux, P., Bournaud, M., & Usseglio Polatera, P. (2010). *Invertébrés d'eau douce: systématique, biologie, écologie*. CNRS Editions.

Tietze, K., Geyh, M., Müller, H., Schröder, L., Stahl, W., & Wehner, H. (1980). The genesis of the methane in Lake Kivu (Central Africa). *Geologische Rundschau*, *69*(2), 452–472. https://doi.org/10.1007/BF02104549

Tsanis, I. K., Wu, J., Shen, H., & Valeo, C. (2006). Environmental Hydraulics: Hydrodynamic and Pollutant Transport Modelling of Lakes and Coastal Waters. In *Environmental Hydraulics - Hydrodynamic and Pollutant Transport Modelling of Lakes and Coastal Waters* (pp. vii–360). https://doi.org/10.1016/s0167-5648(06)56001-0

UNEP, WHO, UNESCO, WMO. (1992). *GEMS/water operational guide* (Third ed.). GEMS/W.92.1 UNEP, WHO, UNESCO, WMO. 220p

US EPA. (1986). Ambient Water Quality Criteria for Bacteria - 1986. EPA 440/5–84-002. United States Environmental Protection Agency, January, 1986.

US EPA. (1995). Draft method for the measurement of toxic PCB congeners by isotope dilution HRGC/HRMS, October 4, 1995 draft version. Washington, DC.

Verbeke, J. (1957). Recherches écologiques sur la faune des grands lacs de l'Est Congo Belge. Explor. Hydrobiol. lac Kivu, Edouard et Albert (1952–1954). *Inst. Roy. Sci. Nat. Belg., B 3* (1), 177p.

Vitecek, S., Johnson, R. K., & Poikane, S. (2021). Assessing the ecological status of European Rivers and lakes using benthic invertebrate communities: A practical catalogue of metrics and methods. *Water*, *13*(3), 346. https://doi.org/10.3390/w13030346

Warwick, R. M. (1988). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. *Marine Ecology Progress Series*, *46*, 167–170. http://researchrepository.murdoch.edu.au/id/eprint/23157. https://doi.org/10.3354/meps046167

Weiss, J. M., & Reice, S. R. (2005). The aggregation of impacts: Using species-specific effects to infer community-level disturbances. *Ecological Applications*, *15*(2), 599–617. https://doi.org/10.1890/02-5100

White, M. S., Xenopoulos, M. A., Hogsden, K., Metcalfe, R. A., & Dillon, P. J. (2008). Natural lake level fluctuation and associated concordance with water quality and aquatic communities within small lakes of the Laurentian Great Lakes region. In *Ecological effects of water-level fluctuations in lakes* (pp. 21–31). Springer. https://doi.org/10.1007/978-1-4020-9192-6_4

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APPENDIX A: TABLE OF THE MAIN FAMILIES IDENTIFIED AND THE ABUNDANCES OF MACROINVERTEBRATES BY SAMPLING STATION

Order/family	BK1	BK2	BK3	BK4	Tot BK	IS1	IS2	IS3	IS4	Tot. IS	Tot. Gen
Coleoptera											
Elmidae	8			144	152		4	8	8	20	172
Hydraenidae				4	4						4
Hydrophilidae				24	24		4		12	16	40
Decapoda											
Atyidae	12		4	4	20		4	4	2076	2084	2104
Potamonautidae	4	4			8		4	4	0	8	16
Diptera											
Athericidae		20	8		28						28
Chironomidae	284	380	1116	944	2724	124	400	264	892	1680	4404
Empididae				28	28			4		4	32
Muscidae		160	28	4	192						192
Syrphidae		8	20	52	80						80
Tabanidae		4	8	12	24						24
Tipulidae							4			4	4
Ephemeroptera											
Baetidae			4		4		12		12	24	28
Caenidae	12	4	4		20	36	40		36	112	132
Leptophlebidae									12	12	12
Polymitarcydae									456	456	456
Gastropoda											
Ampullariidae	8	8	36		52		4		336	340	392
Bithynidae	1040	360	188	116	1704	16	1292	208	1168	2684	4388
Planorbidae	164	60	20	44	288	4	84	52	420	560	848
Thiaridae	16	4	0	8	28	8	72	28	8	116	144
Tricoptera											
Hydropsychidae	4				4						4
Heteroptera											
Belostomatidae	4	88		28	120	12	24	12	40	88	208
Naucoridae	92	268		156	516	209	308	128	208	853	1369
Plecoptera											
Perlidae									4	4	4
Odonata											
Aeshnidae								4	16	20	20
Coenagrionidae	144	276	40	248	708	60	128	332	320	840	1548
Cordilluidae	12		4	12	28	12	4	28	48	92	120
Gomphidae	40		16	8	64	12	44		8	64	128
Lestidae		16		16	32						32
Libellulidae	52	90	32	84	258	20	20	12	132	184	442
Protoneuridae						4	4			8	8
Annelida											
Glossiphoniidae						4			4	8	8
Hirudinidae		24		8	32	36	64	4	4	108	140
Lumbiculidae			8	56	64	552	60	16		628	692

Paper 3: Ndatimana et al., (accepted)

Bridging riverine and lacustrine systems: Macroinvertebrate indicators of ecological health in the Rwandan Congo basin.

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Bridging riverine and lacustrine systems: Macroinvertebrate indicators of ecological health in the Rwandan Congo basin

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Abstract

Connectivity between lakes and tributaries is essential for freshwater ecosystem health. However, integrated studies of macroinvertebrate assemblages across these interconnected systems remain limited in tropical Africa. This study examined the structure of the macroinvertebrate community and the ecological health of Lake Kivu and its tributaries using multimetric indices. Macroinvertebrates were collected from 23 lake sites and 53 tributary sites between 2010 and 2022. Diversity indices were quantified, community differences were tested using permutational multivariate analysis (PERMANOVA), whereas Indicator value analysis (Indval) was performed to identify ecosystem-taxa associations. The ecological health status of the lake was assessed using the Multimetric Index for Lake Hawassa (MMIH), and the Macroinvertebrate-Based Multimetric Index for Biotic Integrity (MMIBI) for tributaries. Lake and tributary systems exhibited distinct community compositions, with molluscs dominating the lake and insects prevalent in tributaries. Families Thiaridae, Bithyniidae and Planorbidae were prevalent in the lake, while Baetidae and Chironomidae were common in the tributaries. Indicator species analyses revealed strong habitat affinities of *Melanoides tuberculata* and *Gabbiella humerosa* to the lake, and *Radix natalensis* and *Pisidium kenianum* to the rivers. The diversity indices indicated significantly higher macroinvertebrate diversity in the tributaries ($p<0.001$), whereas molluscan diversity was greater

in the lakes ($p<0.05$). The results classified the lake sites as having “fair” to “good” ecological status, while the tributaries ranged from “poor” to “moderate”, reflecting the varying levels of anthropogenic pressure. These findings highlight the ecological distinctiveness of lacustrine and riverine systems, and demonstrate the effectiveness of using macroinvertebrates for integrated, basin-scale monitoring and management.

Key words: Ecological Quality Ratio (EQR), Multimetric index (MMI), River Continuum Concept (RCC), Lake Kivu, and biomonitoring

INTRODUCTION

The connection between a lake and its catchment is important in shaping the lake characteristics and its responses to catchment changes (Anderson, 2014; Vilbaste et al., 2024). Lakes connect to their catchment mainly by direct inflow through rivers and surface runoff. The catchment influence lakes and rivers differently due to various factors such as topography, human activities and the nature of the riparian zone (Dai et al., 2017). As rivers meander through the catchment, they collect organic and mineral materials, which are washed into the lake. This eventually affects the chemical and ecological dynamics of the receiving ecosystem (Kling et al., 2000; Jones et al., 2010). In fact, systems connecting through agricultural, urbanized and industrialized areas are affected by organic and inorganic pollution (Lei, 2010; Ozdemir et al., 2025). Further, major infrastructures such as dams, diversion for hydropower production, irrigation and municipal water supply significantly deplete water volume in both the rivers and the receiving lake (Unnikrishnan et al., 2020). The fluctuations in water volume affect water quality through changes in temperature, dissolved oxygen, and nutrient concentrations, ultimately affecting habitats stability (Mosley et al., 2012; Zerlin & Henry, 2014). Consequently, lake biodiversity often declines abruptly due to habitat destruction, deteriorating water quality, and increased exposure to extreme environmental conditions in the catchment (Panigrahi, 2007; Akbayeva et al., 2025). However, the extent of these impacts depends on the characteristics of both the tributaries and the receiving lake, such as shape and size (Jones et al., 2010).

Riverine biodiversity is shaped by the structure and composition of riparian zones (Masese et al., 2014a). It also varies along a longitudinal gradient described by the River Continuum Concept (RCC), which integrates biotic and abiotic factors from headwater to mouth (Vannote et al., 1980). Key abiotic variable such as sediment composition, temperature, light availability, flow regime,

and water chemistry play key roles in shaping community assemblages (Bækkelie et al., 2017; Shuman et al., 2020). Macroinvertebrate communities shift in structure along the river continuum in response to changes in resource availability and habitat conditions, reflecting the RCC's framework of ecological integration (Benjamin et al., 2025). At the river–lake interface, a distinct ecotone forms where riverine and lacustrine systems interact, supporting higher macroinvertebrate diversity than found in lakes alone (Hillbricht-Ilkowska & Węgleńska, 2003). This zone is shaped by flow dynamics and hydro-morphological conditions (Jones et al., 2010; Pennock et al., 2020), as the mixing of chemically and physically distinct waters promotes organic matter deposition and reduces flow velocity (Tian et al., 2020). Here, the convergence of upstream allochthonous inputs and lake backflows further enhances nutrient retention, biomass, and overall ecological productivity (Lin & Guo, 2016; Tian et al., 2020). Due to their ecological sensitivity and well-defined traits, macroinvertebrates are widely used as indicators in assessing the condition of riverine and lacustrine ecosystems (Masese et al., 2014a; Assie et al., 2025).

Several approaches have employed macroinvertebrates as bioindicators to evaluate ecological health of riverine and lacustrine systems (Fierro et al., 2017; Koperski, 2011; Kaaya et al., 2015; Brown & Williams, 2016; Ndatimana et al., 2023a). In Africa, biomonitoring using macroinvertebrate-based indices are biased in riverine ecosystems (Odountan et al., 2019, Ndatimana et al., 2023b). Traditionally, taxa presence/absence and diversity indices such as SASS (Dickens & Graham, 2002) and TARISS (Kaaya et al., 2015) were developed to guide the assessment of rivers in African regions. These Sensitivity-based indices provide insights into the significance of macroinvertebrates in ecosystem monitoring and conservation (Tampo et al., 2021). Combining various metrics, including traditional ecological indices (e.g., alpha diversity), macroinvertebrate traits such as functional feeding groups (FFGs), and composition, multimetric indices (MMIs) were developed (e.g., Alemneh et al., (2019); Kaboré et al., (2022); Wondmagegn & Mengistou, (2023) and Ndatimana et al., (2023a). This approach accounts for various aspects of habitat and reduces errors in sensitivity scores of certain taxa (Chen et al., 2019). In African regions where there are no specific indices, water quality assessment relies on indices from ecologically similar areas, as well as generalized sensitivity-based indices. For instance, the application of macroinvertebrate-based assessments in Rwanda have employed the TARISS (e.g., Dusabe et al., 2019, and Nsengimana et al., 2025), alongside generalized indices such as ASPT, The Baur biotic Score, BMWP and other traditional ecological indices (e.g.: Wronski et al., 2015).

Lake Kivu, Rwanda's largest water body (Olapade & Omitoyin, 2012; Nsabimana et al., 2020), has garnered considerable attention from researchers for various reasons. Its deep layers contain dissolved carbon dioxide (CO_2) and methane (CH_4), making it potentially susceptible to explosive gas release (Hirslund & Morkel, 2020). Moreover, besides being deep (Ross et al., 2014) and at high altitude (Degens et al., 1973), it was also claimed to have relatively low diversity, compared to other African great lakes (Sarmento et al., 2007; Dusabe et al., 2024). Lake Kivu is prone to pollution and disturbances due to land use and land cover changes in its catchment (Olapade & Omitoyin, 2012; Bagalwa et al., 2015; Hahirwabasenga et al., 2024). The urbanization increases around the lake basin with towns like Rubavu, Karongi and Rusizi in Rwanda (Bundervoet et al., 2017; Turatsinze et al., 2024), and Goma and Bukavu in DR Congo (Bayumbasire et al., 2021) with a population of 5.7 million people (Muvundja et al., 2023). The increasing agricultural activities in the basin contribute to nutrient loading into the lake from used fertilizer and pesticides, which are carried through rivers (Muvundja et al., 2009; Bagalwa et al., 2015). This compromises water quality and survival of inhabiting organisms. As the risk of pollution increases, the growing need for water quality assessment is needed to safeguard the ecological integrity of the ecosystem. Indeed, on the Rwandan side of Lake Kivu basin, water pollution assessed using physico-chemical indices was reported in some parts of the lake and its tributaries (Nsabimana et al., 2020; Mupenzi et al., 2017). However, studies on the application of macroinvertebrates in assessing the water quality of the Kivu basin are still limited. The available studies in this region focused on rivers or the lake separately (Wronski et al., 2015; Hyangya et al., 2022; Dusabe et al., 2024), with minimal attention to their connectivity. Consequently, there is limited information on macroinvertebrate assemblages of Lake Kivu and its tributaries, their associated water quality, and the overall ecological health of these interconnected yet distinct systems within the basin.

This study aimed at assessing the ecological integrity of Rwandan part of the Congo Basin using macroinvertebrates, specifically: (1) comparing the riverine macroinvertebrate community structure to that in Lake Kivu. (2) testing the ecological health status of Lake Kivu and its tributaries, by applying the selected macroinvertebrate-based MMIs available in the region. Understanding macroinvertebrate patterns as bioindicators in the lake and its tributaries is vital for assessing the interactions of interconnected systems, supporting effective water resource management and conservation in the basin.

METHODS

Study area

The study was carried out in the Lake Kivu drainage system in Rwanda, including both the lake and its tributaries (rivers) (Fig. 1). Lake Kivu forms part of the western branch of the East African rift valley system (Ross et al., 2014, Dusabe et al., 2024). It is situated at an elevation of 1,463 m above sea level (Degens et al., 1973) and is shared between Rwanda and Democratic Republic of the Congo with a catchment area of 5097 km^2 (Muvundja et al., 2023). It has a surface area, volume and a maximum depth of 2,370 km^2, 549 km^3 and 486 m, respectively (Ross et al., 2014; Muvundja et al., 2023). The region receives an average annual precipitation of 1,404 mm, and is characterized by a humid climate with a bimodal precipitation pattern. The rainy season extends from September to May, while the dry season occurs from June to August. The rainy season is further divided into a long rainy period from February to May and a short rainy period from October to December (Muvundja et al., 2014; Dusabe et al., 2024). Precipitation is the primary contributor to the lake's water budget, accounting for 49% of the total inflow. Riverine input from more than 100 small tributaries constitutes 32%, while sub-aquatic springs contribute the remaining 20% (Muvundja et al., 2014; Muvundja et al., 2023). Lake Kivu has two primary outflow pathways; Rusizi River, accounts for approximately 54% of the total outflow, and evaporation which accounts for the remaining 46% (Muvundja et al., 2014).

The Rwandan part of Kivu basin, cover 33% of Rwanda's total area and receive 10% of the total national water through various rivers (REMA, 2009; Mupenzi et al., 2017). The lake catchment through which its rivers meanders mainly in hills with some wetlands and dominated by agricultural activities. Moreover, the Industrial activities such as breweries and other agri-based processing, are particularly done in the cities adjacent to the lake (Wronski et al., 2015). Land cover in the catchment comprises 65% dryland, cropland, and pasture, 17% evergreen broadleaf forest, 11% shrubs, and 7% other land types (Muvundja et al., 2009). Over time, much of the 17% evergreen forests, which buffered numerous rivers examined in this study, have been gradually replaced by farmland, pasture, and exotic tree species like Australian Acacia and Eucalyptus (Wronski et al., 2015). In the increase rate of deforestation coupled with steep terrain of the catchment area has increased the risk of soil erosion and landslides (Karamage et al., 2016).

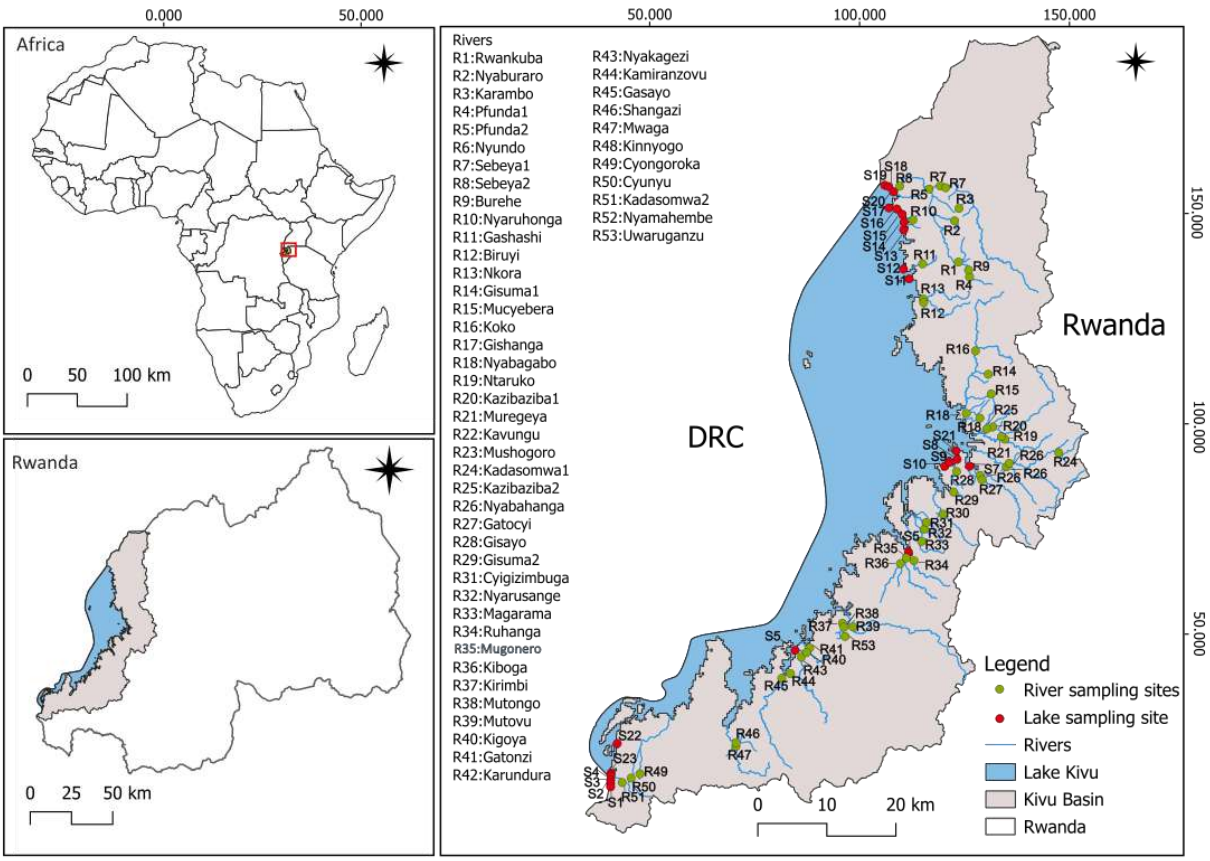


Fig. 1. Map showing the study sites within the Rwandan part of the Congo Basin, including sampling points for Lake Kivu and its tributaries (shown on the right). Insets of the map of Africa (top left) and Rwanda (bottom left) are also presented for geographic context.

Macroinvertebrates sampling

Macroinvertebrates were collected from Lake Kivu between October and November 2022, covering the northern, middle, and southern parts of the Rwandan catchment. Further, the study incorporated samples previously collected in 2010, 2018, and 2019. Samples were collected from 23 points, of which ten, six and seven points were from the northern, middle and southern part, respectively (Fig 1, Table 1b). Macroinvertebrates were sampled from the littoral zones of the lake using a 20 cm diameter scoop net (1 mm mesh), and an Ekman grab to collect specimen from soft sediment, while a Surber net with 250 µm mesh size was employed to sample hard substrates. Meanwhile, 53 tributaries from 29 catchments sampled by Wronski et al., 2015 were reused in this study, covering 13, 23, and 17 rivers from northern, middle and southern part respectively (Fig.1,

176 Table 1a). Each river was sampled once between December 2013 to May 2014. The selection of
177 the sampling points was based on river habitats and substrates (Wronski et al., 2015).
178 Macroinvertebrate collection was done using two methods, kick sampling and hand net sampling.
179 After rinsing, the collected macroinvertebrates were pooled together, sorted, and placed into plastic
180 containers.

181 All macroinvertebrates' samples (from both lake and rivers) were preserved in 70% Ethanol and
182 transported to the University of Giessen Systematics and Biodiversity collection (UGSB), Giessen
183 for further processing and identification. Due to lack of detailed macroinvertebrate identification
184 guides for genera and species in the region, the identification was mainly done to family level
185 using keys by Day et al. (1999, 2001a&b, 2002); Day and de Moor (2002); de Moor and Day
186 (2002); de Moor et al. (2003); Stals and de Moor (2007). However, molluscs were identified to
187 the species level whenever possible using identification key by Brown (1994). In cases where a
188 shell-based morphological approach limited identification to the species level, DNA barcoding
189 was used to improve taxonomic resolution. The tissue samples were used for DNA extraction, the
190 standard barcoding fragment of the COI gene was amplified, sequenced, then the resulting
191 sequences were compared with reference GenBank to confirm species identity.

192 Table 1a. The list and location of the sampled rivers with their respective basin section

S/N	Latitude	Longitude	Altitude	Date	River	Catchment
Northern section						
R1	S01.80151	E029.35048	2028	Feb 2014	Rwankuba	Sebeya
R2	S01.74616	E029.34263	2047	Feb 2014	Nyaburaro	Sebeya
R3	S01.73303	E029.34458	1989	Feb 2013	Karambo	Sebeya
R4	S01.82026	E029.35886	2072	Dec 2013	Pfunda1	Sebeya
R5	S01.70939	E029.30865	1683	Dec 1013	Pfunda2	Sebeya
R6	S01.70724	E029.32755	1814	Dec 2013	Nyundo	Sebeya
R7	S01.70251	E029.32708	1795	Dec 1013	Sebeya1	Sebeya
R8	S01.70494	E029.26190	1472	Feb 2014	Sebeya2	Sebeya
R9	S01.81956	E029.35815	2096	Dec 2013	Burehe	Burehe
R10	S01.74711	E029.28240	1434	Dec 2013	Nyaruhonga	Nyaruhonga
R11	S01.80420	E029.29734	1606	Feb 2014	Gashashi	Gashashi
R12	S01.85009	E029.29918	1470	Feb 2014	Biruyi	Nkora
R13	S01.85009	E029.29918	1470	Feb 2014	Nkora	Nkora
Middle section						
R14	S01.95159	E029.38412	1910	April 2014	Gisuma1	Koko
R15	S01.97529	E029.39048	1994	April 2014	Mucyebera	Koko

R16	S01.91596	E029.36395	1629	April 2014	Koko	Koko
R17	S02.00057	E029.35705	1434	April 2014	Gishanga	Gishanga
R18	S02.00250	E029.37395	1622	April 2014	Nyabagabo	Nyabagabo
R19	S02.03045	E029.39901	1527	April 2014	Ntaruko	Muregeya
R20	S02.02013	E029.39095	1631	Feb 2014	Kazibaziba1	Muregeya
R21	S02.02994	E029.39874	1529	April 2014	Muregeya	Muregeya
R22	S02.06699	E029.41105	1581	April 2014	Kavungu	Mushogoro
R23	S02.06406	E029.41335	1582	April 2014	Mushogoro	Mushogoro
R24	S02.05043	E029.47610	2136	April 2014	Kadasomwa1	Mushogoro
R25	S02.01986	E029.39097	1625	Feb. 2014	Kazibaziba2	Muregeya
R26	S02.08145	E029.37558	1478	April 2014	Nyabahanga	Nyabahanga
R27	S02.08257	E029.37512	1479	April 2014	Gatocyi	Nyabahanga
R28	S02.07200	E029.34411	1490	April 2014	Gisayo	Gisayo
R29	S02.10213	E029.33362	1467	April 2014	Gisuma2	Kamaramaka
R30	S02.13135	E029.32529	1561	April 2014	Kiraro	Kiraro
R31	S02.14219	E029.30565	1562	April 2014	Cyigizimbuga	Cyigizimbuga
R32	S02.14613	E029.30117	1553	April.2014	Nyarusange	Nyarusange
R33	S02.16687	E029.29632	1533	April 2014	Magarama	Magarama
R34	S02.19228	E029.28585	1470	April 2014	Ruhanga	Ruhanga
R35	S02.19117	E029.27752	1463	April 2014	Mugonerp	Mugonero
R36	S02.19349	E029.27254	1476	April.2014	Kiboga	Kiboga
Southern section						
R37	S02.27201	E029.19338	1482	May 2014	Kirimbi	Kirimbi
R38	S02.28011	E029.19550	1479	May 2014	Mutongo	Kirimbi
R39	S02.28086	E029.20691	1486	May 2014	Mutovu	Kirimbi
R40	S02.30781	E029.15141	1478	May 2014	Kigoya	Kigoya
R41	S02.30724	E029.15289	1479	May 2014	Gatonzi	Kigoya
R42	S02.31111	E.029.1430 3	1474	May 2014	Karundura	Karundura
R43	S02.31135	E029.14361	1471	May 2014	Nyakagezi	Karundura
R44	S02.34159	E029.11832	1463	May 2014	Kamiranzovu	Kamiranzovu
R45	S02.34417	E029.09553	1510	May 2014	Gasayo	Gasayo
R46	S02.43086	E029.05773	1527	May 2014	Shangazi	Cyongoroka
R47	S02.43086	E029.05773	1527	May 2014	Mwaga	Cyongoroka
R48	S02.446.83	E028.96992	1675	May 2014	Kinnyogo	Gisuma
R49	S02.47096	E028.93147	1649	May 2014	Cyongoroka	Cyunyu
R50	S02.47425	E028.92357	1627	May 2014	Cyunyu	Cyunyu
R51	S02.48265	E028.90944	1574	May 2014	Kadasomwa 2	Kadasomwa
R52	S02.444.03	E028.97657	1659	May 2014	Nyamahembe	Gisuma
R53	S02.28043	E029.19753	1482	May 2014	Uwaruganzu	Kirimbi

193 R stands for river

194 Table 1b. The location of sampling sites on the lake with their respective basin sections.

S/N	Latitude	Longitude	Date
Northern section			
S11	-1.81336	29.2795	October 2022
S12	-1.813	29.27903	October 2022
S13	-1.74814	29.27814	October 2022
S14	-1.74014	29.27461	November 2022
S15	-1.74006	29.27511	November 2022
S16	-1.73939	29.27028	November 2022
S17	-1.73847	29.27542	November 2022
S18	-1.70307	29.25801	June 2010
S19	-1.69798	29.24905	May 2019
S20	-1.70423	29.25916	June 2010
Middle section			
S6	-2.18839	29.28014	November 2022
S7	-2.07361	29.35914	November 2022
S8	-2.06222	29.34678	November 2022
S9	-2.06086	29.34578	November 2022
S10	-2.06036	29.33725	November 2022
S21	-2.05329	29.34718	June 2010
Southern section			
S1	-2.48436	28.89408	November 2022
S2	-2.48408	28.89364	November 2022
S3	-2.48322	28.89428	November 2022
S4	-2.47922	28.89847	November 2022
S5	-2.31239	29.13775	November 2022
S22	-2.43384	28.90397	June 2010
S23	-2.4753	28.89877	June 2010

Data analysis

Macroinvertebrate community structure

Macroinvertebrate community patterns and characteristics were investigated across the region (lake and tributaries). All analyses were performed using R software (R core team, 2024). Clustered heatmaps (pheatmap package; Kolde, 2019) were generated to visualize similarities among sites and macroinvertebrate taxa for both rivers and lakes. This was done to understand the similarities among sampling stations for both rivers and lakes. To handle skewness, the raw data (abundance) were log (x+1) transformed for further analysis (Heino, 2008). The difference in assemblage composition between the lake and its tributaries, were performed using the permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis distance

matrix through “adonis2” function of Vegan package, with 999 Permutations (Oksanen et al., 2022). A similar analysis was applied to the molluscan dataset (identified at species level).

Indicator species analysis (Indval) was applied to different taxonomic resolutions. The overall data were assessed at the family level, whereas molluscan species were analyzed at the species levels to identify the taxa distinguishing the lake from the tributaries. This analysis evaluates the association of taxa (in this case, either families or species) with sample groups (here defined as water bodies, including Lake Kivu and tributary assemblages) by analyzing occurrence data and the relative abundances of individual taxa. Based on the results, the algorithm calculates indicator values (Indval) statistics, which range from 0 to 1 for non-indicator to the best indicator taxa respectively, along with their associated probabilities. The indicator analysis was conducted using the “indicspecies” package (Cáceres & Legendre, 2009), with 999 permutations and a significance level of $\alpha = 0.05$. The Indicator Value (IndVal) method identifies species that are strongly associated with particular habitats based on their frequency and exclusivity (Dufrêne & Legendre, 1997). The assessment of diversity, four major indices were selected to inform diversity patterns of macroinvertebrates. Shannon-Wiener index, Simpson (dominance), Margalef (richness), and Pielou (evenness) were computed. To investigate the diversity variation between lake and river, the diversity indices were tested and visualized using two-tailed Wilcoxon test and box plots respectively. The biodiversity indices were computed and visualized using packages “Vegan” and “tidyverse” (Wickham et al., 2019; Oksanen et al., 2022).

Ecological health status of water bodies

The study area was divided into three sections: the north (13 rivers, 10 lake sites), the center (23 rivers, 6 sites), and the south (17 rivers, 7 sites) (Table 1) to assess ecological variability across the basin. River and site selection was based on accessibility, habitat representativeness and historical monitoring data. The ecological health status of the lake and rivers was assessed through the application of macroinvertebrate-based indices available in the region. Macroinvertebrate-based approaches suitable for assessing and comparing the water bodies in the basin were critically selected. Although presence-absence indices are developed and applied for rivers in the region (Dickens & Graham, 2002; Kaaya et al., 2015), they were excluded in the analysis, since no such index is documented for lakes. To ensure effective comparison between lake and its tributaries, multimetric indices (MMIs) developed in the region were used. Indeed, MMIs factors in aspects

beyond presence-absence, such as diversity, composition, and traits (Chen et al., 2019; Alemneh et al., 2019). The multimetric index for Lake Hawassa (MMIH), Ethiopia (Wondmagegn and Mengistou, 2023), and macroinvertebrate-based multimetric index for biotic integrity (MMIBI) for Lake Victoria tributaries, Kenya (Aura et al., 2021) were applied for the lake and rivers, respectively. During the application, 10 metrics for MMIH were computed based on the lake dataset, while 11 metrics for MMIBI were computed on rivers (Aura et al., 2021). Ecological Quality Ratio (EQR) was calculated by dividing the observed ecological quality value for each habitat by respective reference conditions. This enabled comparison of the water quality between the lake and rivers. Indeed, EQR is essential for standardizing and calibrating the assessment of ecological status, ensuring effective comparison across various index scales (Navarro et al., 2009; Bennett et al., 2011).

RESULTS

Macroinvertebrate composition and diversity

A total of 2362 individuals from 16 orders and 27 families were collected from Lake Kivu (Appendix 1). Littorinimorpha, Hygrophila, and Odonata orders dominated with 68.33%, 12.61%, and 4.57%, respectively of the total abundance. The most represented families in lake were Bithyniidae (68.33%) and Planorbidae (9.1%), followed by Hirudinidae (3.38%) and Naucoridae (3.09%) (Appendix 1). Among molluscs, 1894 individuals belonging to nine species were recorded in the lake, with *Gabbiella humerosa* being the dominant species (85.21%), followed by *Biomphalaria pfeifferi* (8.23%) (Appendix 3). On the other hand, 4204 individuals (17 orders and 48 families) were collected from rivers (Appendix 2). Odonata (36.13%), Ephemeroptera (16.15%), Hygrophila (13.79%), and Diptera (13.77%) were the dominant orders while Baetidae (10.94%), Chironomidae (9.06%), Lymnaeidae (8.18%), and Sphaeriidae (7.20%) dominated the families (Fig. 2). Moreover, 878 molluscan individuals recorded in rivers were represented by four species i.e., *Radix natalensis* (39.06%), and *Pisidium kenianum* (34.51%), *Biomphalaria pfeifferi* (21.41%) and *Physella acuta* (5%) (Appendix 4).

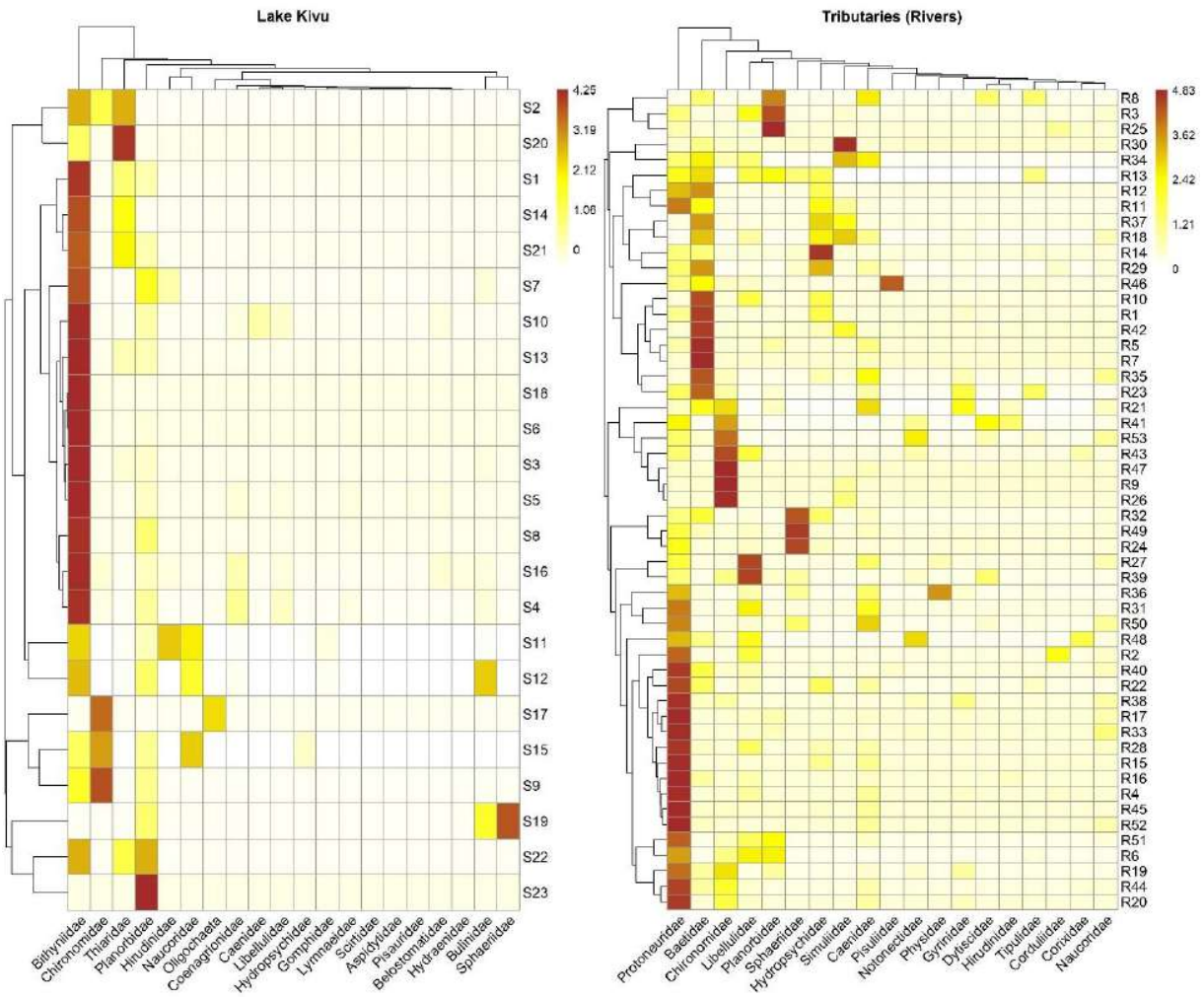


Fig. 2. Heatmaps showing the distribution and relative abundance of macroinvertebrate families across sampling sites in Lake Kivu and tributary rivers. Color intensity indicates relative abundance, with hierarchical clustering applied to highlight ecological similarity.

Lake macroinvertebrate composition differs significantly from that of tributaries (PERMANOVA, $p < 0.001$, Perms=999). Further, the species composition of molluscs was different in the two habitats (PERMANOVA, $p < 0.001$, Perms=999). The indval analysis (significance, $p < 0.05$) showed 5 families with a strong affinity to the lake, including Thiaridae (p=0.001), Coenagrionidae (p=0.002), Bulinidae (p=0.01), Atyidae (p=0.004), and Gomphidae (p=0.039). Further, three species, *Melanoides tuberculata* (p=0.001), *Afrogyrorbis kigeziensis* (p=0.001), and *Gabbiella humerosa* (p=0.001) were found to be significantly associated with the lake, indicating their strong affinity for its environmental conditions. Meanwhile, the rivers were significantly associated with families Protoneuridae (p=001), Gyrinidae (p=0.004), Caenidae (p=0.005), Hydropsychidae

(p=0.01), Libellulidae (p=0.022), and Baetidae (p=0.029). Specifically, two molluscan species; *Radix natalensis* (p=0.001) and *Pisidium kenianum* (p=0.003) were associated with the tributaries, with the latter occurring exclusively in these systems.

With reference to diversity indices (Shannon, Simpson, Pielou, and Margalef), there were higher taxa diversity and dominance in tributaries than the lake as indicated by Shannon index ($p<0.001$), and Simpson index (p=0013), respectively (Fig. 3). However, there was no significant difference in taxa richness and evenness between the lake and tributaries based on Margaref index (p=0.46) and Pielou evenness (p=0.52) (Fig. 3).

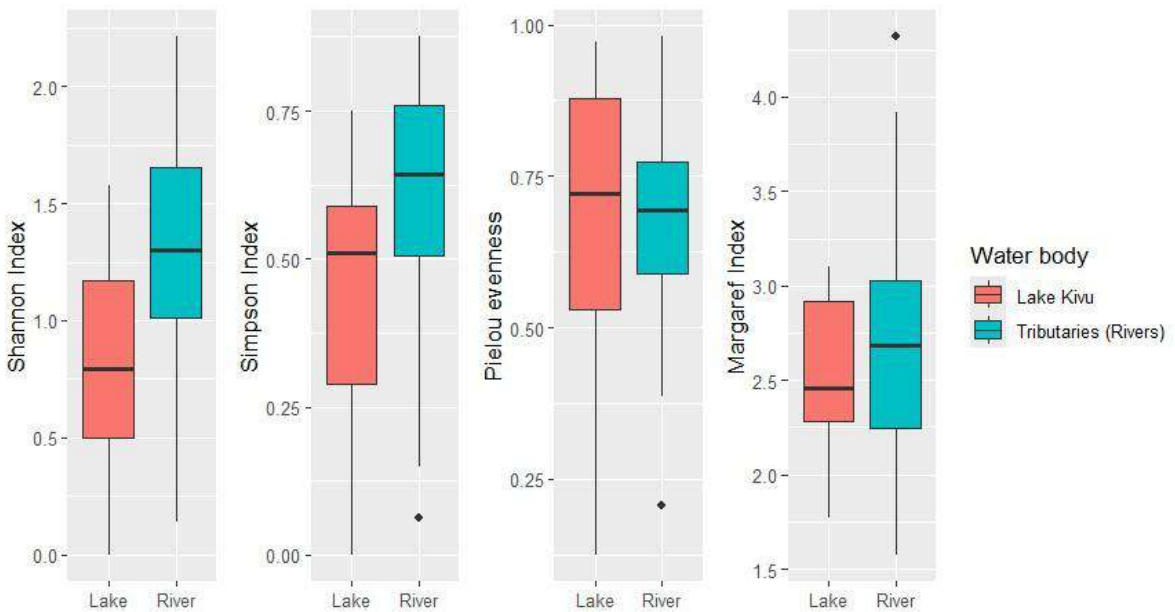
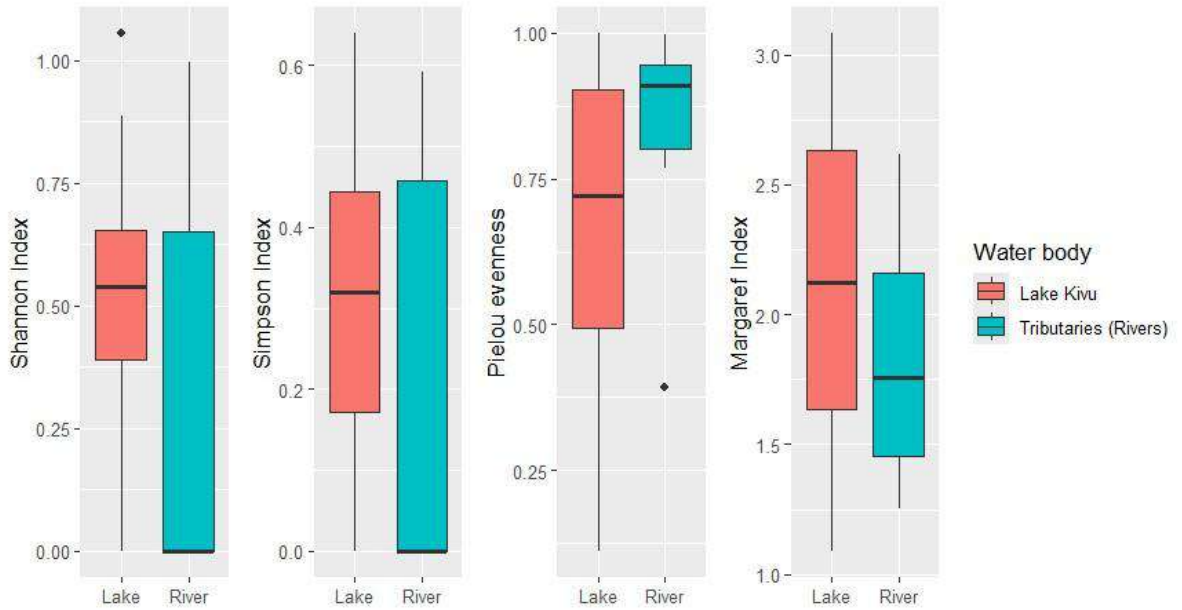


Fig. 3. Diversity indices of macroinvertebrate families from Lake Kivu (N=2363) and its tributaries (N= 4204).

Diversity indices (Shannon, Simpson, Pielou, and Margalef) were also applied to molluscan species to assess variations in the community structure across the two water systems. The Wilcoxon test showed a significantly higher diversity in the lake than rivers as shown by the Shannon index (p=0.02) and Simpson index (p=0.03) (Fig. 4). However, the species' evenness and richness did not significantly differ between the lake and rivers, as indicated by Pielou (p=0.06) and Margalef index (p= 0.21; Fig. 4).



293

294 Fig. 4. Diversity indices of molluscan species from Lake Kivu (N=1894) and its tributaries (N=
295 878).

296 ***Ecological health status of the water bodies***

297 The values of ten metrics-MMIH applied to the three sections of the Lake (Table 1) range between
298 28 and 32 out of 50 possible score (Table 2). The northern and southern sections expressed the
299 same index score (32) whereas the middle section had a slightly lower score (28). Thus, based on
300 this index, these scores fall into the water quality category II (Northern and Southern), and category
301 III (for middle section). This implies that the ecological status of Northern and Southern section is
302 "good", whereas the middle section reflected the conditions of "fair". On the other hand, the rivers
303 were classified in similar ways, applying MMIBI accordingly. Eleven metrics were used to score
304 for macroinvertebrates (Table 2). The MMIBI values ranged from 29 to 31, whereby southern,
305 middle, and northern parts scored 33, 31 and 29, respectively of the maximum score of 55 (Table
306 2). The MMIBI results showed that the Southern and Middle parts of catchment have moderate
307 integrity class, where there is significant pollution levels and degradation. However, the northern
308 part portends poor health status, with lower water quality characterized by the heavy pollution and
309 degradation.

310

311 Table 2. Computed metrics from the applied multimetric indices, and their respective values
312

Lake Kivu			
MMIH	**Northern**	**Middle**	**Southern**
%Eph-Trich	0.71	0.61	0.23
%intolerant	12.64	1.59	2.93
%Trichoptera	0.28	0	0.11
%Diptera	4.31	1.35	0.93
%Chironomidae	4.31	1.35	0.93
% ref.abun	29.46	34.41	36.11
% ref.rich	70.37	59.25	55.55
Ratio-Eph &Trich to Chiro	0.16	0.45	0.25
Ratio-intol/toler	0.14	0.016	0.03
HBI	7.025	6.43	6.38
MMIH	32	28	32
Lake Kivu tributaries			
MMIBI _Metrics	**Northern**	**Middle**	**Southern**
N-Ephemeroptera	172	308	199
N-Trichoptera	51	161	56
N-Hemiptera	2	45	91
Shannon-div index	2.02	2.46193	2.33
% Hemiptera	0.28	2.7	4.94
%Trichoptera	7.28	9.67	3.04
%Tolerant taxa	42.71	42.36	46.63
%Dominant taxon	24.71	25.96	35.59
%scraper	51.71	24.09	15.38
%Shredder	1.571	5.46	17.44
%filters	8.71	16.34	3.48
MMIBI	**29**	**31**	**33**

313 Abbreviations: MMIH, Multimetric index for Lake Hawassa; HBI, Hilsenhoff Biotic Index; MMIBI,
314 Macroinvertebrate-based multimetric index for biotic integrity; intol, Intolerant; toler, Tolerant; abun,
315 abundance; Rich, Richness; N-, Number of; Ref, Reference.

316 The health status of the lake and rivers were compared using the Ecological Quality Ratio (Fig 5).
317 The lake expressed higher EQR in northern and southern sections than to their adjacent rivers with
318 0.64 to 0.52, and 0.64 to 0.6, respectively. However, the EQR did not differ in the middle section
319 (Fig. 5). Although some difference occurred throughout the sections, the water quality of the lake
320 and rivers is classified under moderate ecological health class (Fig. 5).

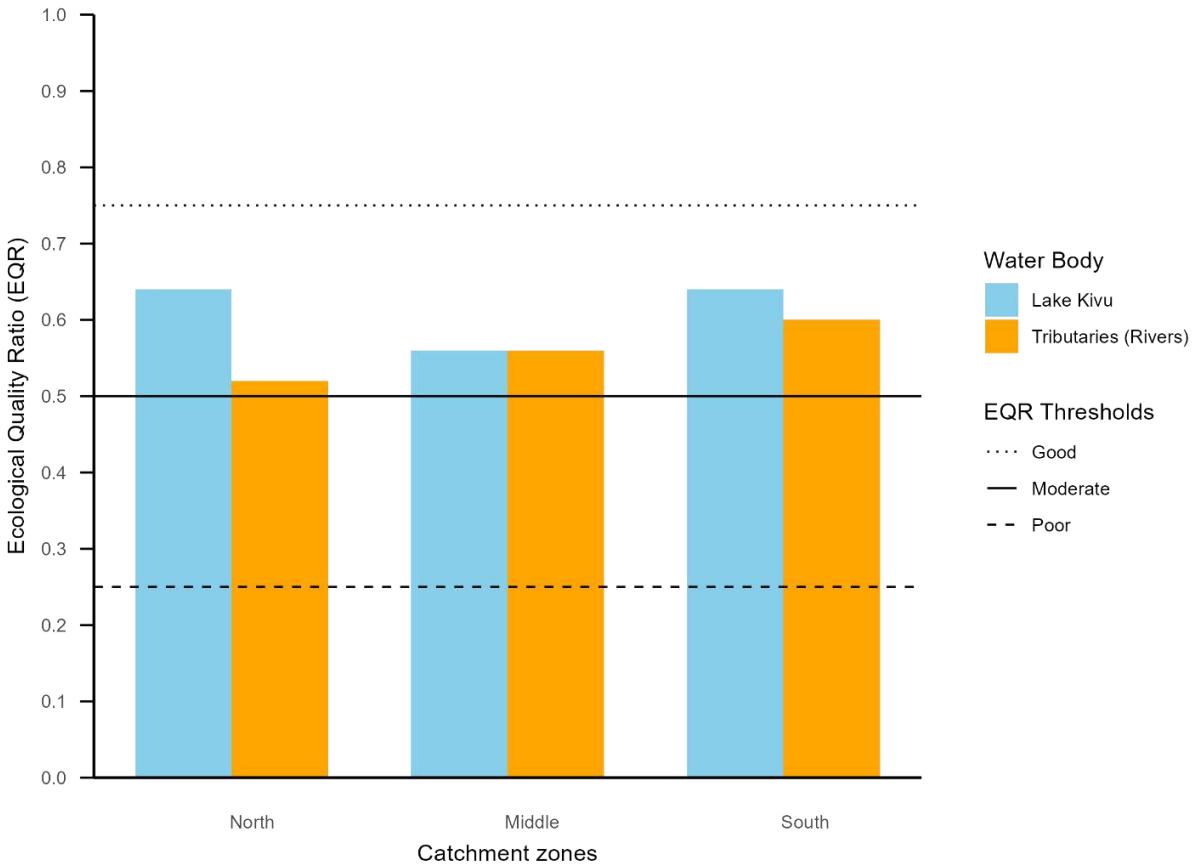


Fig. 5. The Ecological Quality Ratio (EQR) for Lake Kivu and its tributaries across catchment zones, based on MMIH and MMIBI indices.

DISCUSSION

Macroinvertebrate composition in Lake Kivu and its tributaries

Macroinvertebrate composition showed clear ecological differentiation between lake and tributary habitats, with molluscs being dominant in the lake, whereas insects dominated rivers. The lake was dominated by molluscs, particularly Bithyniidae and Planorbidae, which are well adapted to calm, nutrient-rich waters with stable substrates. Their dominance is consistent with previous findings that many gastropods favor lentic systems where biofilms and organic matter accumulate (Jakubik et al., 2014; Coelho et al., 2022). Planorbids, being air-breathing, tolerate low-oxygen conditions (Koopman et al., 2016), while gill-breathing bithyniids demonstrate metabolic stability across temperature ranges (Hahn, 2005) and effectively exploit nutrient-rich environments through filter-

feeding (Trebitz et al., 2015). These traits make them suited to habitats dominating in Lake Kivu, where ion accumulation and carbonate sedimentation are prevalent (Votava et al., 2017; Hategekimana et al., 2020).

In contrast, the tributaries were dominated by insect orders such as Ephemeroptera and Odonata, taxa typically associated with fast-flowing, well-oxygenated environments. Members of Baetidae and Caenidae, for instance, exhibit strong swimming and clinging traits that help them persist in dynamic flow conditions (Clayton & Westbrook, 2008). Their abundance aligns with their role as indicators of sediment stress and flow regime in lotic systems (Akamagwuna et al., 2019; Principe et al., 2019). Odonates such as Coenagrionidae also showed affinity to lake habitats, likely benefiting from the structure and refuge offered by submerged vegetation (Villalobos-Jimenez et al., 2016; Verdonschot & Peeters, 2012), while others, like Gomphidae and Libellulidae, were more associated with flowing water, utilizing diverse substrates and prey availability (Calvão et al., 2020; Dijkstra et al., 2014).

Indicator taxa further emphasized the ecological distinctions between systems. In lakes, families such as Thiaridae, Bulinidae, Atyidae, Coenagrionidae, and Gomphidae showed strong associations with lentic conditions. This pattern reflects shared adaptations, including reliance on organic-rich sediments and submerged vegetation for feeding, reproduction, and refuge (Page et al., 2008; Villalobos-Jiménez et al., 2016; Remsburg, 2011). For instance, Atyidae and Thiaridae are detritivores that thrive in nutrient-rich benthic zones (Page et al., 2008; Bjelke et al., 2005), while Coenagrionidae and Gomphidae depend on submerged structures for oviposition and predator avoidance (Villalobos-Jiménez et al., 2016; Remsburg, 2011). These shared traits enable them to exploit lacustrine environments effectively. In contrast, tributaries were characterized by families such as Hydropsychidae, Gyrinidae, and Protoneuridae, whose locomotion and respiration traits are well-suited to flowing water and associated prey availability (Wagner, 2000; Raphahlelo et al., 2022; Reis et al., 2023). These taxa not only reflect habitat structure but also provide strong bioassessment potential due to their trait-filtering responses along environmental gradients.

Species-level analysis of molluscs added further depth. Lake-dominant species such as *Melanoides tuberculata* and *Gabbiella humerosa* displayed high tolerance to pollution and hypoxia, with *M. tuberculata* capable of bioaccumulating heavy metals (Orabi & Khalifa, 2020). *G. humerosa*, including the endemic subspecies *G. humerosa kivuensis*, likely benefits from Lake Kivu's

chemical uniqueness, including elevated methane and carbon dioxide levels (Dusabe et al., 2024). In tributaries, *Radix natalensis* and *Pisidium kenianum* were more frequent, their presence associated with vegetated, oxygenated, shallow areas and fine sediment substrates (Soliman, 2008; Loskotová et al., 2019). These molluscs' habitat preferences suggest functional diversity within the malacofauna, offering both bioindicator value and insight into spatial variation in environmental conditions across the basin.

Diversity indices

The significantly higher diversity (Shannon and Dominance indices) observed in the rivers could be attributed to the dynamic flow, varied habitats along the course, and greater connectivity, supporting a wider range of species and ecological interactions. Indeed, the tributaries of Lake Kivu meander through diverse topographical features and landcovers including hills, forests, drylands, farmlands and residential areas (Muvundja et al., 2009; Wronski et al., 2015). This create a rich mosaic of ecological niches that supports a wide range of organisms. Lotic environments have been reported to have higher alpha diversity due to greater ecological heterogeneity and diverse sediment types (Allan & Castillo, 2007; Júnior et al., 2016). Along the river continuum, the flow regime, substrates and vegetation changes, creating a wide range of niches for different species (Vannote et al., 1980; Grubaugh et al., 1997). Moreover, the variation in habitats favors diverse feeding groups since tributaries receive a number of organic inputs such as nutrients and leaf litter (e.g. Masese et al., 2014b; Doong et al., 2021). On the contrary, lakes often exhibit limited colonization rate and less diversity (Johnson et al., 2004). In view of the family-level evenness and richness (Pielou and Margalef indices) between Lake and its tributaries, there was no significant difference. This implies that both lake Kivu and its tributaries exhibit a similar family diversity in relation to the entire population, and both have evenly distributed individuals per family.

Considering the molluscan species, the diversity indicated higher Shannon and Dominance indices in Lake Kivu than in tributaries. As discussed in the first paragraph of the discussion, a number of factors could have contributed to the higher diversity of molluscan species in the lake than rivers. Molluscs could have adapted to relatively stable hydrological conditions and water chemistry, providing efficient medium for growth and local differentiation (Dusabe et al., 2024, Bagalwa et al., 2024). Nonetheless, this varies based on the region and adaptability of the species. For instance, a study focusing on the effects of river–lake disconnection on freshwater molluscs found that

disconnected lakes exhibited significantly lower species richness compared to connected systems (Zheng et al., 2025). This suggests that hydrological connectivity plays a crucial role in maintaining mollusc diversity in an ecosystem. In addition, suspended solids, substrate types and plant presence play an important role in shaping the molluscan communities (Lewin et al., 2023). The factors shaping Molluscan diversity extend beyond the fundamental habitat characteristics of water bodies, as previously mentioned, to encompass a broader range of environmental stressors. For instance, the decline in gastropods diversity in lakes Malawi and Tanganyika, reported by Van Bocxlaer et al., (2012), was attributed to surface water warming, increased runoff and eutrophication. Thus, specific characteristics of Lake Kivu such as desiccation events and volcanic activities (Wood & Scholz, 2017), would have also contributed to the present molluscan diversity status.

Ecological health status of water bodies

The multimetric indices applied for assessment of the lake ecological status, classified Lake Kivu in "moderate" category (Fig. 4). This implies presence of water pollution and habitat degradation, but not yet at the critical levels (Wondmagegn & Mengistou, 2023). However, due to Lake Kivu's large volume and exceptionally long residence time, ranging from 193 years to about 800 years in deep layers (GEF, 2020, Muvundja et al., 2023), the ecological effects of pollution may be delayed. Thus, the current moderate status may underestimate the cumulative and long-term effects of the past and ongoing pressures. Notably, activities such as factory operations, fisheries, and methane gas exploration (Olapade & Omitoyin, 2012; Balagizie et al., 2017), may already be affecting the lake's water quality. This is supported by various forms of water pollution including organic and heavy metal contamination have been reported in different parts of the lake (Olapade & Omitoyin, 2012; Balagizie et al., 2017; Houbraken et al., 2017; Nsabimana et al., 2020; Nyiragatare et al., 2021). Moreover, population in the vicinity of Lake Kivu are engaging numerous potentially pollutant generating activities like farming, and small-scale industries/factories (Balagizie et al., 2017). The increasing pollution of the lake compromises the survival of the inhabiting organisms, especially at the littoral zone (Hyangya et al., 2021), which is an important zone for breeding, refugee and feeding of most aquatic organisms.

Both the northern and Southern sections of Lake Kivu exhibited relatively high Ecological Quality Ratios (EQR=0.64), despite differences in the pollution of their adjacent tributaries. The northern

section, located near Rubavu town, with highly polluted tributaries like Sebeya River, showed unexpectedly good ecological status. This discrepancy may be attributed to hydrodynamic factors such as increased water flow and wind-driven circulation, which could have led to dilution and dispersion of pollutants, thus reducing their impact on benthic communities. In fact, in-lake conditions such as habitat alterations and external pressures (e.g., land-use), have been reported to collectively homogenize macroinvertebrate community in lake (McGoff et al., 2013). In contrast, the southern section's similarly high EQR corresponds more predictably with its relatively less polluted tributaries, indicating a better overall catchment condition. This observation is consistent with findings by Hyangya et al. (2021), who reported that the littoral zones of southern Lake Kivu exhibited water quality ranging from moderate to good. However, the middle section presented the lowest EQR among the other sections. This could plausibly be ascribed to the internal lake processes such as sediment resuspension. This lake internal pollution is recognized as a significant driver of aquatic ecosystem degradation, even in systems where external pollution is reduced (Wang & Jiang, 2016; Wei et al., 2021). It is often driven by wind and bioturbation, which release nutrients and pollutants from sediments (Li et al., 2018). These conditions are unfavorable for sensitive macroinvertebrate taxa and may favor tolerant species ultimately lowering multimetric index values.

The rivers from the northern side of the basin presented lower Ecological Quality Ratio (EQR) compared to the rest, owing to plausibly the structure and anthropogenic activities associated with these rivers. For instance, the River Sebeya flows through various landscapes accumulating pollutants from urban, agricultural and industrial areas. In fact, water quality analysis of Sebeya River revealed high levels of turbidity, total suspended solids, and chemical oxygen demand, particularly in settled areas (Uwacu & Akintande, 2019). Indeed, it experiences high levels of sedimentation, mainly in rainy seasons (Majoro et al., 2023; Hahirwabasenga et al., 2024). The combination of stressors colonized of the habitat dominated by the taxa tolerant to pollution and sedimentation. Moreover, the more polluted or damaged the habitat is, the more tolerant macroinvertebrate species will colonize the area (Adámek et al., 2010; Matlou et al., 2017). However, the Southern part of the basin shows a different trend since rivers run through rural areas with forested catchments. Indeed, rivers in the southern region of the basin are not highly affected by modernized agriculture and landslides, thus having favorable habitats. A study by Mupenzi et al. (2017) reported good quality of southern rivers owing to the forested catchment. In the present

study, the middle side stood as the medium environment, between the northern and southern parts. They are relatively less urbanized than northern but with more human settlements than the Southern. Therefore, the EQR across the basin sides could possibly be attributed to anthropogenic activities, mainly the land-use and land cover of the catchment as the main source of pollution. In fact, EQR in riverine systems depend heavily on the surrounding landscape and associated activities (Wasson et al., 2010).

Tributaries of Lake Kivu were more polluted than the lake water, owing to plausibly their higher proximity to pollution than the lake. Although tributaries contribute much to pollution of the receiving lake (Mooney et al., 2020; Marcarelli et al., 2019), they are more susceptible to pollution than the receiving water bodies (Wieczorek et al., 2024). In fact, tributaries act as protective buffers against pollutants in lake-river systems, through degrading contaminant prior to reaching the lake (Wang et al., 2023). For instance, some rivers flow through urban (e.g: Rivers Sebeya, Koko, Kilimbi, etc), agricultural (e.g: Rivers Sebeya, Ntaruko, Mugonero, etc), and industrial areas (e.g: Sebeya, Koko etc), before reaching the lake. Thus, they accumulate domestic, agricultural runoffs, mining and the industrial wastes (Olapade & Omitoyin, 2012; Bagalwa et al., 2015, Wronski et al., 2015, Hahirwabasenga et al., 2024). Given their limited dilution capacity, the pollutants concentrate more compared to the larger volume in lake allowing dilution of pollutants and reducing the effect on macroinvertebrate community. In fact, dilution significantly reduces the pollutants in lakes (Shinohara et al., 2008; Welch et al., 2020). The dynamic of water pollution plays a crucial role in distribution and diversity of macroinvertebrates, hence perform their role as bioindicators. This takes place through eliminating the sensitive species and dominance of tolerant taxa in highly damaged sites. Some studies reported the same trend in water quality indication using macroinvertebrates in Lake Kivu basin (river/tributaries) (e.g: Wronski et al., 2015; Bagalwa et al., 2019; Baguma et al., 2024), and water quality assessment was done, independently from tributaries (Hyangya et al., 2021, 2022). This research shed light on the linkage between lakes and tributaries, which would be backed up by regular monitoring to confirm this trend. Thus, it could inform effectively of the desired measures for sustainable conservation of this basin.

CONCLUSION

The study investigated macroinvertebrate assemblages in the Lake Kivu basin by comparing the diversity and ecological status of Lake Kivu and its tributaries. The distinct composition of

macroinvertebrate communities highlights the variation between these two interconnected systems. The presence and distribution of indicator taxa/species in these environments are potentially shaped by flow regime, substrate characteristics, and anthropogenic disturbances. As a result, these taxa demonstrate habitat specificity, strong ecological affinity, and bioindicator potential. Furthermore, the incorporation of molluscan species provided additional ecological insights, enhancing the understanding of macroinvertebrate assemblages in a deeper context. The study revealed that, while rivers supported general macroinvertebrate diversity, molluscan diversity was significantly higher in lakes. While the individual indices revealed various ecological status, from "fair" to "good" for lake and from "poor" to "moderate for rivers, the Ecological Quality Ratio (EQR) classified both systems within the moderate range (0.5-0.75). This provided valuable information for sustainable conservation strategies in the basin. Moreover, the investigated river-lake system provided a broader perspective on river-lake interactions.

Although the study assessed community composition and water quality in both Lake Kivu and its tributaries, it could not establish a causal influence of river on lake ecosystem. Thus, targeted research on spatial-temporal dynamics of these systems is needed to better understand the rivers' influence on the lake. Higher taxonomic resolution for other groups such as insects is needed to deepen understanding of macroinvertebrate ecological patterns, relevant in future monitoring of the basin. While assessing the ecological status along the basin, the varied number of sites were considered per section. Although comparison was achieved, future studies would benefit from harmonized site clustering, particularly during sampling. Furthermore, the study recommends increased application of MMIs, and their validation for local efficiency to reduce potential errors associated with individual metrics in biomonitoring approaches. Sustainable land-use planning, riparian zone management, and pollution control practices in the catchment are also recommended to sustain the ecological integrity of this basin.

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REFERENCES

Akamagwuna, F. C., Mensah, P. K., Nnadozie, C. F., & Odume, O. N. (2019). Trait-based responses of Ephemeroptera, Plecoptera, and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *River Research and Applications*, *35*(7), 999-1012. https://doi.org/10.1002/rra.3458

Koopman, K. R., Collas, F. P., van der Velde, G., & Verberk, W. C. (2016). Oxygen can limit heat tolerance in freshwater gastropods: differences between gill and lung breathers. Hydrobiologia, 763(1), 301-312. https://doi.org/10.1007/s10750-015-2386-y

Kaboré, I., Ouéda, A., Moog, O., Meulenbroek, P., Tampo, L., Bancé, V., & Melcher, A. H. (2022). A benthic invertebrates-based biotic index to assess the ecological status of West African Sahel Rivers, Burkina Faso. Journal of Environmental Management, 307, 114503. https://doi.org/10.1016/j.jenvman.2022.114503

Jakubik, B., Koperski, P., & Lewandowski, K. (2014). Diversity of mollusca in lowland river-lake system: lentic versus lotic patches. Polish Journal of Ecology, 62(2), 335-348. https://doi.org/10.3161/104.062.0212

Coelho, P. R., Ker, F. T., Araujo, A. D., Pinto, H. A., Negrão-Corrêa, D. A., Caldeira, R. L., & Geiger, S. M. (2022). Survey on limnic gastropods: relationships between human health and conservation. Pathogens, 11(12), 1533. https://doi.org/10.3390/pathogens11121533

Trebitz, A., Shepard, G., Brady, V., & Schmude, K. (2015). The non-native faucet snail (Bithynia tentaculata) makes the leap to Lake Superior. Journal of Great Lakes Research, 41(4), 1197-1200. https://doi.org/10.1016/j.jglr.2015.09.013

Bækkelie, K. A. E., Schneider, S. C., Hagman, C. H., & Petrin, Z. (2017). Effects of flow events and nutrient addition on stream periphyton and macroinvertebrates: an experimental study using

548 flumes. Knowledge & Management of Aquatic Ecosystems, 418, 47.
549 https://doi.org/10.1051/kmae/2017041

550 Shuman, T. C., Smiley Jr, P. C., Gillespie, R. B., & Gonzalez, J. M. (2020). Influence of physical
551 and chemical characteristics of sediment on macroinvertebrate communities in agricultural
552 headwater streams. Water, 12(11), 2976. https://doi.org/10.3390/w12112976

553 Adámek, Z., Orendt, C., Wolfram, G., & Sychra, J. (2010). Macrozoobenthos response to
554 environmental degradation in a heavily modified stream: Case study the Upper Elbe River,
555 Czech Republic. Biologia, 65(3), 527-536.

556 Akbayeva, L., Yevneyeva, D., Temreshev, I., Abzhalelov, A., Tekebayeva, Z., Temirbekova, A., ...
557 & Mkilima, T. (2025). Potential biodiversity disruptions caused by changes in water body
558 coverage. a case of Lake Taldykol, Kazakhstan. Polish Journal of Environmental Studies, 34(3),
559 3011-3031. 10.15244/pjoes/188834

560 Alemneh, T., Ambelu, A., Zaitchik, B. F., Bahrndorff, S., Mereta, S. T., & Pertoldi, C. (2019). A
561 macroinvertebrate multi-metric index for Ethiopian highland streams. Hydrobiologia, 843, 125-
562 141. https://doi.org/10.1007/s10750-019-04042-x

563 Allan, J. D., & Castillo, M. M. (2007). Stream ecology: structure and function of running waters.
564 Springer, Dordrecht, Netherlands. 436 pp.

565 Anderson N. J. (2014). Landscape disturbance and lake response: temporal and spatial
566 perspectives. Freshwater Reviews, 7(2), 77-120. https://doi.org/10.1608/FRJ-7.2.811

567 Assie, A. F., Arimoro, F. O., Nantege, D., Ndatimana, G., Action, S., Keke, U. N., ... & Edia, E. O.
568 (2025). Exploring macroinvertebrate traits along anthropogenic disturbed gradient in West
569 African Savanna River systems: A case study of Niger state, North central region of Nigeria.
570 Biologia, 80, 1-23. https://doi.org/10.1007/s11756-025-01872-0

571 Aura M., C., Nyamweya, C. S., Njiru, J. M., Omondi, R., Manyala, J., Musa, S., ... & Mwamburi,
572 J. (2021). Using the Multi-metric Index of Biotic Integrity methodological approach to
573 determine the major river catchment that most pollutes a lake. Aquatic Ecosystem Health &
574 Management, 24(1), 12-20. https://doi.org/10.14321/aehm.024.01.04

575 Bagalwa, J. J. M., Batumike, P. C., Ndegeyi, B. K., & Bahizire, J. L. K. (2024). Spatial distribution
576 and abondance of freshwater snails in Lake Kivu, DR Congo side. ESI Preprints, 30, 91-91.
577 https://esipreprints.org/index.php/esipreprints/article/view/1116

578 Bagalwa, M., Majaliwa, J. G. M., Kansiime, F., Bashwira, S., Tenywa, M., & Karume, K. (2015).
579 Sediment and nutrient loads into river Lwiro, in the Lake Kivu basin, Democratic Republic of
580 Congo. International Journal of Biological and Chemical Sciences, 9(3), 1678-1690.
581 10.4314/ijbcs.v9i3.46

582 Bagalwa, M., Mukumba, I., Ndahama, N., Zirirane, N., & Kalala, A. O. (2019). Assessment of
583 river water quality using macroinvertebrate organisms as pollution indicators of Cirhanyobowa
584 River, Lake Kivu, DR Congo. International Journal of Current Microbiology and Applied
585 Sciences, 8(4), 2668-2680. https://doi.org/10.20546/ijcmas.2019.804.310

586 Baguma, G. B., Bisimwa, A. M., Munundu, A. M., Irenge, E. B., Shabani, I. E., & Cubaka, A. K.
587 (2024). Relationships between benthic macroinvertebrates and environmental variables of

Nyamuhinga River, Lake Kivu southwest basin, Sud-Kivu, Democratic Republic of the Congo. Environmental Monitoring and Assessment, 196(10), 917. ttps://doi.org/10.1007/s10661-024-13071-6

Bayumbasire, C., Léonard, M., Andre, N., Isaac, B., Lucien, W., Léon, B., & Jean-Paul, B. (2021). Analyse De La Dynamique Du Paysage Urbain Et Des Mutations Dans L'occupation Du Sol. Cas De La Ville De Bukavu 1986 A 2018, Sud-Kivu, République Démocratique Du Congo. International Journal of Progressive Sciences and Technologies, 27, 2. https://ijpsat.org/index.php/ijpsat/article/view/3299

Benjamin, J., Ayesiga, P., Gomes, M., Dutton, C., Schoelynck, J., & Subalusky, A. (2025). Land-water connections from river source to mouth. In Dalu, T. & Masese, F. O. (Eds). Afrotropical Streams and Rivers: Structure, Ecological Processes and Management. pp. 349-374. Elsevier, Amsterdam, The Netherlands.

Bennett, C., Owen, R., Birk, S., Buffagni, A., Erba, S., Mengin, N., ... & Wasson, J. G. (2011). Bringing European River quality into line: an exercise to intercalibrate macro-invertebrate classification methods. Hydrobiologia, 667, 31-48. https://doi.org/10.1007/s10750-011-0635-2

Bjelke, U., & Herrmann, J. (2005). Processing of two detritus types by lake-dwelling shredders: species-specific impacts and effects of species richness. Journal of Animal Ecology, 74(1), 92-98. https://www.jstor.org/stable/3505383

Brown, D. S. (1994). Freshwater snails of Africa and their medical importance. Taylor and Francis.

Brown, E. D., & Williams, B. K. (2016). Ecological integrity assessment as a metric of biodiversity: are we measuring what we say we are? Biodiversity and Conservation, 25, 1011-1035. https://doi.org/10.1007/s10531-016-1111-0

Brown, K. M., & Lodge, D. M. (1993). Gastropod abundance in vegetated habitats: the importance of specifying null models. Limnology and Oceanography, 38(1), 217-225. https://doi.org/10.4319/lo.1993.38.1.0217

Bundervoet, T., Parby, J.I., Nakamura, S., & Choi, N. (2017). Reshaping urbanization in Rwanda: economic and spatial trends and proposals - note 4: profiling secondary cities in Rwanda - dynamics and opportunities. The World Bank, Washington DC, USA

Cáceres, M. D., & Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. Ecology, 90(12), 3566-3574. https://doi.org/10.1890/08-1823.1

Calvão, L. B., de Paiva, C. K. S., da Silva Brito, J., Fares, A. L., Maia, C., Michelan, T. S., Montag, L. F. & Juen, L. (2020). Influence of biotic and abiotic factors on adult Odonata (Insecta) in Amazon streams. Animal Biology, 71(1), 67-84. https://doi.org/10.1163/15707563-bja10047

Chen, K., Rajper, A. R., Hughes, R. M., Olson, J. R., Wei, H., & Wang, B. (2019). Incorporating functional traits to enhance multimetric index performance and assess land use gradients. Science of the Total Environment, 691, 1005-1015. https://doi.org/10.1016/j.scitotenv.2019.07.047

Clayton, J. A., & Westbrook, C. J. (2008). The effect of the Grand Ditch on the abundance of benthic invertebrates in the Colorado River, Rocky Mountain National Park. River research and applications, 24(7), 975-987. https://doi.org/10.1002/rra.1117

628 Dai, X., Zhou, Y., Ma, W., & Zhou, L. (2017). Influence of spatial variation in land-use patterns
629 and topography on water quality of the rivers inflowing to Fuxian Lake, a large deep lake in the
630 plateau of southwestern China. Ecological Engineering, 99, 417-428.
631 https://doi.org/10.1016/j.ecoleng.2016.11.011

632 Day, J. A., and De Moor, I. J. (2002). Guides to the Freshwater Invertebrates of Southern Africa.
633 Non-arthropods (the protozoans, Porifera, Cnidaria, Platyhelminthes, Nemertea, Rotifera,
634 Nematoda, Nematomorpha, Gastrotrichia, Bryozoa, Tardigrada, Polychaeta, Oligochaeta and
635 Hirudinea). WRC Report No. TT 167/02, 5. Water Research Commission, Pretoria, 304.

636 Day, J. A., de Moor, I. J., Stewart, B. A., & Louw, A. E. (2001a). Guides to the Freshwater
637 Invertebrates of Southern Africa, Volume 3. Crustacea 2: Ostracoda, Copepoda and Brachiura.
638 WRC Report No. TT140/08. Water Research Commission, Pretoria, 177.

639 Day, J. A., Harrison, A. D., & de Moor, I. J. (2002). Guides to the Freshwater Invertebrates of
640 Southern Africa, Volume 3. Diptera. WRC Report TT201/02. Water Research Commission,
641 Pretoria, 200.

642 Day, J. A., Stewart, B. A., De Moor, I. J., & Louw, A. E. (1999). Guides to the Freshwater
643 Invertebrates of Southern Africa: Volume 2, Crustacea I – Notostraca, Anostraca, Conchostraca
644 and Cladocera. WRC Report No. TT 121/00. Water Research Commission, Pretoria, 126.

645 Day, J. A., Stewart, B. A., de Moor, I. J., & Louw, A. E. (2001b). Guides to the Freshwater
646 Invertebrates of Southern Africa, Volume 4. Crustacea 3: Bathynellacea, Amphipoda, Isopoda,
647 Spelaeogripacea, Tanaidacea and Decapoda. WRC Report TT 141/00. Water Research
648 Commission, Pretoria, 141.

649 De Moor, I. J., & Day, J. A. (2002). Guides to the Freshwater Invertebrates of Southern Africa.
650 Arachnida and Mollusca. WRC Report No. TT182/02, 6. Water Research Commission,
651 Pretoria, 141.

652 De Moor, I. J., Day, J. A., & De Moor, F. C. (2003). Guides to the Freshwater Invertebrates of
653 Southern Africa: Volume 7 Insecta I—Ephemeroptera, Odonata and Plecoptera. WRC Report
654 No. TT 207/03. Water Research Commission, Pretoria, 288.

655 Degens, E. T., von Herzen, R. P., Wong, H. K., Deuser, W. G., & Jannasch, H. W. (1973). Lake
656 Kivu: structure, chemistry and biology of an East African rift lake. Geologische Rundschau, 62,
657 245-277. https://doi.org/10.1007/BF01826830

658 Dickens, C. W., & Graham, P. M. (2002). The South African Scoring System (SASS) version 5
659 rapid bioassessment method for rivers. African Journal of Aquatic Science, 27(1), 1-10.
660 https://doi.org/10.2989/16085914.2002.9626569

661 Dijkstra, K. D. B., Kalkman, V. J., Dow, R. A., Stokvis, F. R., & Van Tol, J. A. N. (2014).
662 Redefining the damselfly families: a comprehensive molecular phylogeny of Zygoptera
663 (Odonata). Systematic entomology, 39(1), 68-96. https://doi.org/10.1111/syen.12035

664 Doong, M. K., Anticamara, J. A., & Magbanua, F. S. (2021). Spatial variations in the distribution
665 of benthic macroinvertebrate functional feeding groups in tropical rivers. Indonesian Journal of
666 Limnology, 2(1), 35-52. https://doi.org/10.51264/inajl.v2i1.11

Dusabe, M. C., Kalinda, C., Clewing, C., Hyangya, B. L., Van Bocxlaer, B., & Albrecht, C. (2024). Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu. Journal of Great Lakes Research, 50, 102339. https://doi.org/10.1016/j.jglr.2024.102339

Dusabe, M. C., Wronski, T., Gomes-Silva, G., Plath, M., Albrecht, C., & Apio, A. (2019). Biological water quality assessment in the degraded Mutara rangelands, northeastern Rwanda. Environmental Monitoring and Assessment, 191, 1-13. https://doi.org/10.1007/s10661-019-7226-5

Fierro, P., Valdovinos, C., Vargas-Chacoff, L., Bertrán, C., & Arismendi, I. (2017). Macroinvertebrates and fishes as bioindicators of stream water pollution. Water quality, 2, 23-38. 10.5772/65084

GEF, Global Environment Facility (2020, May). Lake Kivu and Rusizi River Basin Water Quality Management Project. Retrieved June 25, 2025, from https://www.thegef.org/sites/default/files/web-documents/10566_IW_PIF.pdf

Grubaugh, J., Wallace, B., & Houston, E. (1997). Production of benthic macroinvertebrate communities along a southern Appalachian river continuum. Freshwater Biology, 37(3), 581-596. https://doi.org/10.1046/j.1365-2427.1997.d01-578.x

Hahirwabasenga, J., Nilsson, E., Larson, M., Bizimana, H., Wali, U. G., & Persson, M. (2024). Flooding in Sebeya catchment, Rwanda-A review of causes, impacts, and management. International Journal of Disaster Risk Reduction, 114, 105012. https://doi.org/10.1016/j.ijdrr.2024.105012

Hahn, T. (2005). Respiration rates in Bithynia tentaculata (L.) (Gastropoda: Bithyniidae) in response to acclimation temperature and acute temperature change. Journal of molluscan studies, 71(2), 127-131. https://doi.org/10.1093/mollus/eyi015

Hategekimana, F., Ndikuryayo, J. D., Habimana, E., Mugerwa, T., Christian, K., & Digne, R. (2020). Lake Kivu water chemistry variation with depth over time, Northwestern Rwanda. Rwanda Journal of Engineering, Science, Technology and Environment, 3(1). 10.4314/rjeste.v3i1.5

Heino, J. (2008). Influence of taxonomic resolution and data transformation on biotic matrix concordance and assemblage–environment relationships in stream macroinvertebrates. Boreal environment research, 13(4), 359.

Hillbricht-Ilkowska, A., & Węgleńska, T. (2003). River-lake system as mosaic pattern of landscape patches and their transition zones (ecotones). Polish Journal of Ecology, 51(2), 163-174. oai:rcin.org.pl:117552

Hirslund, F., & Morkel, P. (2020). Managing the dangers in Lake Kivu–How and why. Journal of African Earth Sciences, 161, 103672. https://doi.org/10.1016/j.jafrearsci.2019.103672

Houbraken, M., Habimana, V., Senaeve, D., López-Dávila, E., & Spanoghe, P. (2017). Multi-residue determination and ecological risk assessment of pesticides in the lakes of Rwanda. Science of the Total Environment, 576, 888-894. https://doi.org/10.1016/j.scitotenv.2016.10.127

Hyangya, B. L., Kankonda, A. B., Dusabe, M. C., Murhimanya, J. D. K., Kaningini, B. M., & Masilya, P. M. (2022). Choice of benthic macroinvertebrate-based metrics for assessing water quality in the littoral zone under anthropogenic disturbance in southern Lake Kivu (East Africa). Ecohydrology, 15(8), e2468. https://doi.org/10.1002/eco.2468

Hyangya, B. L., Riziki, J. W., Masilya, P. M., Zabene, F. Z., Alunga, G. L., Kaningini, B. M., & Kankonda, A. B. (2021). Physico-chemical characterization of littoral water of Lake Kivu (southern basin, Central Africa) and use of water quality index to assess their anthropogenic disturbances. World Water Policy, 7(2), 166-193. https://doi.org/10.1002/wwp2.12059

Johnson, R. K., Goedkoop, W., & Sandin, L. (2004). Spatial scale and ecological relationships between the macroinvertebrate communities of stony habitats of streams and lakes. Freshwater biology, 49(9), 1179-1194. https://doi.org/10.1111/j.1365-2427.2004.01262.x

Jones, N. E. (2010). Incorporating lakes within the river discontinuum: longitudinal changes in ecological characteristics in stream–lake networks. Canadian Journal of Fisheries and Aquatic Sciences, 67(8), 1350-1362. https://doi.org/10.1139/F10-069

Júnior, S. P., Perbiche-Neves, G., & Takeda, A. M. (2016). The environmental heterogeneity of sediment determines Chironomidae (Insecta: Diptera) distribution in lotic and lentic habitats in a tropical floodplain. Insect Conservation and Diversity, 9(4), 332-341. https://doi.org/10.1111/icad.12172

Kaaya, L. T., Day, J. A., & Dallas, H. F. (2015). Tanzania River Scoring System (TARISS): a macroinvertebrate-based biotic index for rapid bioassessment of rivers. African Journal of Aquatic Science, 40(2), 109-117. https://doi.org/10.2989/16085914.2015.1051941

Karamage, F., Shao, H., Chen, X., Ndayisaba, F., Nahayo, L., Kayiranga, A., & Zhang, C. (2016). Deforestation effects on soil erosion in the Lake Kivu Basin DR Congo-Rwanda. Forests 7(11), 281. https://doi.org/10.3390/f7110281

Kling, G. W., Kipphut, G. W., Miller, M. M., & O'Brien, W. J. (2000). Integration of lakes and streams in a landscape perspective: the importance of material processing on spatial patterns and temporal coherence. Freshwater Biology, 43(3), 477-497. https://doi.org/10.1046/j.1365-2427.2000.00515.x

Kolde, R. (2019). pheatmap: Pretty Heatmaps. R package version 1.0.12, <https://CRAN.R-project.org/package=pheatmap>.

Koperski, P. (2011). Diversity of freshwater macrobenthos and its use in biological assessment: a critical review of current applications. Environmental reviews, 19, 16-31. https://doi.org/10.1139/a10-023

Lei, W. (2010). Review of Agricultural Non-point Pollution in Taihu Lake and Taihu Basin. China Population, Resources and Environment, 20(1), 99-107

Lewin, I., Stępień, E., Szlauer-Łukaszewska, A., Pakulnicka, J., Stryjecki, R., Pešić, V., ... & Zawal, A. (2023). Drivers of the structure of mollusc communities in the natural aquatic habitats along the valley of a lowland river: Implications for their conservation through the buffer zones. Water, 15(11), 2059. https://doi.org/10.3390/w15112059

Li, Y., Wei, J., Gao, X., Chen, D., Weng, S., Du, W., ... & Zhang, S. (2018). Turbulent bursting and sediment resuspension in hyper-eutrophic Lake Taihu, China. Journal of Hydrology, 565, 581-588. https://doi.org/10.1016/j.jhydrol.2018.08.06

Lin, P., & Guo, L. (2016). Dynamic changes in the abundance and chemical speciation of dissolved and particulate phosphorus across the river-lake interface in southwest Lake Michigan. Limnology and Oceanography, 61(2), 771-789. https://doi.org/10.1002/lno.10254

Loskotová, B., Straka, M., & Pařil, P. (2019). Sediment characteristics influence benthic macroinvertebrate vertical migrations and survival under experimental water loss conditions. Fundamental and Applied Limnology, 193(1), 39-49. 10.1127/fal/2019/1138

Majoro, F., Wali, U. G., Munyaneza, O., & Naramabuye, F. X. (2023). Sustainability Analysis of Soil Erosion Control in Rwanda: Case Study of the Sebeya Watershed. Sustainability, 15(3), 1969. https://doi.org/10.3390/su15031969

Marcarelli, A. M., Coble, A. A., Meingast, K. M., Kane, E. S., Brooks, C. N., Buffam, I., ... & Stottlemyer, R. (2019). Of small streams and great lakes: Integrating tributaries to understand the ecology and biogeochemistry of Lake Superior. JAWRA Journal of the American Water Resources Association, 55(2), 442-458. https://doi.org/10.1111/1752-1688.12695

Masese, F. O., Kitaka, N., Kipkemboi, J., Gettel, G. M., Irvine, K., & McClain, M. E. (2014a). Macroinvertebrate functional feeding groups in Kenyan highland streams: evidence for a diverse shredder guild. Freshwater Science, 33(2), 435-450. https://doi.org/10.1086/675681

Masese, F. O., Kitaka, N., Kipkemboi, J., Gettel, G. M., Irvine, K., & McClain, M. E. (2014b). Litter processing and shredder distribution as indicators of riparian and catchment influences on ecological health of tropical streams. Ecological Indicators, 46, 23-37. https://doi.org/10.1016/j.ecolind.2014.05.032

Masese, F. O., Muchiri, M., & Raburu, P. O. (2009). Macroinvertebrate assemblages as biological indicators of water quality in the Moiben River, Kenya. African Journal of Aquatic Science, 34(1), 15-26. https://doi.org/10.2989/AJAS.2009.34.1.2.727

Matlou, K., Addo-Bediako, A., & Jooste, A. (2017). Benthic macroinvertebrate assemblage along a pollution gradient in the Steelpoort River, Olifants River System. African Entomology, 25(2), 445-453. https://hdl.handle.net/10520/EJC-9e3128817

McGoff, E., Solimini, A. G., Pusch, M. T., Jurca, T., & Sandin, L. (2013). Does lake habitat alteration and land-use pressure homogenize E uropean littoral macroinvertebrate communities? Journal of Applied Ecology, 50(4), 1010-1018. https://doi.org/10.1111/1365-2664.12106

Mooney, R. J., Stanley, E. H., Rosenthal, W. C., Esselman, P. C., Kendall, A. D., & McIntyre, P. B. (2020). Outsized nutrient contributions from small tributaries to a Great Lake. Proceedings of the National Academy of Sciences, 117(45), 28175-28182. https://doi.org/10.1073/pnas.2001376117

Mosley, L. M., Zammit, B., Leyden, E., Heneker, T. M., Hipsey, M. R., Skinner, D., & Aldridge, K. T. (2012). The impact of extreme low flows on the water quality of the Lower Murray River

and Lakes (South Australia). Water Resources Management, 26, 3923-3946. https://doi.org/10.1007/s11269-012-0113-2

Mupenzi, C., Li, L., Nahayo, L., Amanambu, A. C., Kayiranga, A., Karamage, F., ... & Nsengiyumva, J. B. (2017). Spatial pattern assessment of Lake Kivu basin rivers water quality using National Sanitation Foundation water quality and rivers pollution indices. Desalination and Water treatment, 95, 118-127. https://doi.org/10.5004/dwt.2017.21566

Muvundja, F. A., Pasche, N., Bugenyi, F. W., Isumbisho, M., Müller, B., Namugize, J. N., ... & Wüest, A. (2009). Balancing nutrient inputs to Lake Kivu. Journal of Great Lakes Research, 35(3), 406-418. https://doi.org/10.1016/j.jglr.2009.06.002

Muvundja, F., A., Mulungula, M. P., Kisse, K. T., Muhigirwa, B. C., Natacha, P., Lwikitcha, H. B., ... & Lawrence, T. (2023). Current status and strategic way forward for long-term management of Lake Kivu (East Africa). Journal of Great Lakes Research, 49(6), 102024. https://doi.org/10.1016/j.jglr.2022.04.004

Navarro, E., Caputo, L., Marcé, R., Carol, J., Benejam, L., García-Berthou, E., & Armengol, J. (2009). Ecological classification of a set of Mediterranean reservoirs applying the EU Water Framework Directive: A reasonable compromise between science and management. Lake and Reservoir Management, 25(4), 364-376. https://doi.org/10.1080/07438140903238567

Ndatimana, G., Arimoro, F. O., Chukwuemeka, V. I., Assie, F. A., Action, S., & Nantege, D. (2023a). Development of lake macroinvertebrate-based multimetric index for monitoring ecological health in North Central Nigeria. Environmental Monitoring and Assessment, 195(12), 1429. https://doi.org/10.1007/s10661-023-12036-5

Ndatimana, G., Nantege, D., & Arimoro, F. O. (2023b). A review of the application of the macroinvertebrate-based multimetric indices (MMIs) for water quality monitoring in lakes. Environmental Science and Pollution Research, 30(29), 73098-73115. https://doi.org/10.1007/s11356-023-27559-0

Nsabimana, A., Habimana, V., & Svetlana, G. (2020). Heavy Metal Concentrations in Water Samples from Lake Kivu, Rwanda. Rwanda Journal of Engineering, Science, Technology and Environment, 3(2). 10.4314/rjeste.v3i2.3

Nsengimana, V., Twagirayezu, E., de Dieu Habiyaremye, J., Rutegwa, M., Nyandwi, V., & Ndatimana, G. (2025). Application of aquatic macroinvertebrates in water quality assessment of the Nyabarongo and Akagera Rivers in Rwanda. Environmental Monitoring and Assessment, 197(4), 370. https://doi.org/10.1007/s10661-025-13817-w

Nyiragatare, A., Habimana, V., Migabo, T., Mutangana, D., Muhizi, T., & Nsabimana, A. (2021). Assessment of Heavy Metals in Water from Lake Kivu, Rwanda. In Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions (2nd Edition) Proceedings of 2nd Euro-Mediterranean Conference for Environmental Integration (EMCEI-2), Tunisia 2019 (pp. 655-660). Springer International Publishing.

Odountan, O. H., Janssens de Bisthoven, L., Abou, Y., & Eggermont, H. (2019). Biomonitoring of lakes using macroinvertebrates: recommended indices and metrics for use in West Africa and developing countries. Hydrobiologia, 826, 1-23. https://doi.org/10.1007/s10750-018-3745-2

825 Oksanen, J., Simpson, G., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Solymos,
826 P., Stevens, M., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D.,
827 Carvalho, G., Chirico, M., De Caceres, M., Durand, S., Evangelista, H., FitzJohn, R., Friendly,
828 M., Furneaux, B., Hannigan, G., Hill, M., Lahti, L., McGlinn, D., Ouellette, M., Ribeiro, Cunha,
829 E., Smith, T., Stier, A., Ter Braak, C., Weedon, J. (2022). vegan: Community Ecology Package.
830 R package version 2.6-4, <https://CRAN.R-project.org/package=vegan>.

831 Olapade, O. J., & Omitoyin, B. O. (2012). Anthropogenic pollution impact on physico-chemical
832 characteristics of Lake Kivu, Rwanda. *African journal of food, agriculture, nutrition and*
833 *development*, *12*(5), 6517-6536. https://doi.org/10.18697/ajfand.53.9840

834 Orabi, O., & Khalifa, M. M. (2020). Biota sediment accumulation and bioconcentration factors of
835 trace metals in the snail Melanoides tuberculata form the agricultural drains of the Manzala
836 Lagoon, Egypt. *Environmental Science and Pollution Research*, *27*(15), 17754-17761.
837 https://doi.org/10.1007/s11356-020-08200-w

838 Ozdemir, S., Celebi, A., Dede, G., Maghrebi, M., & Danandeh Mehr, A. (2025). Impact of Land
839 Use Change on Lake Pollution Dynamics: A Case Study of Sapanca Lake, Turkey. *Water*
840 *(20734441)*, *17*(2). https://doi.org/10.3390/w17020182

841 Page, T. J., Cook, B. D., von Rintelen, T., von Rintelen, K., & Hughes, J. M. (2008). Evolutionary
842 relationships of atyid shrimps imply both ancient Caribbean radiations and common marine
843 dispersals. *Journal of the North American Benthological Society*, *27*(1), 68-83.
844 https://doi.org/10.1899/07-044R.1

845 Panigrahi, J. K. (2007). Water quality, biodiversity and livelihood issues: A case study of Chilika
846 Lake, India. In *2007 Atlanta Conference on Science, Technology and Innovation Policy* (pp. 1-
847 6). IEEE. https://doi.org/10.1109/ACSTIP.2007.4472898

848 Pennock, C. A., Hines, B. A., Elverud, D. S., Francis, T. A., McKinstry, M. C., Schleicher, B. J., &
849 Gido, K. B. (2021). Reservoir fish assemblage structure across an aquatic ecotone: Can river-
850 reservoir interfaces provide conservation and management opportunities? *Fisheries*
851 *Management and Ecology*, *28*(1), 1-13. https://doi.org/10.1111/fme.12444

852 Principe, R. E., Marquez, J. A., & Cibils-Martina, L. (2019). Distribution and habitat preference
853 of Ephemeroptera and Trichoptera in subtropical mountain streams: implications for monitoring
854 and conservation. *Anais da Academia Brasileira de Ciências*, *91*(03), e20180692.
855 https://doi.org/10.1590/0001-3765201920180692

856 R Core Team (2024). R: A language and environment for statistical computing. R Foundation for
857 Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

858 Raphahlelo, M. E., Addo-Bediako, A., & Luus-Powell, W. J. (2022). Distribution and diversity of
859 benthic macroinvertebrates in the Mohlapitsi River, South Africa. *Journal of Freshwater*
860 *Ecology*, *37*(1), 145-160. https://doi.org/10.1080/02705060.2021.2023054

861 Reis, KSS, Braga, CF, & dos Santos, TMT (2023). Predatory Activity of Aquatic Insects (Odonata:
862 Libellulidae and Coleoptera: Hydrophilidae) under Laboratory Conditions. *Acta*
863 *Brasiliensis* , *7* (1), 22-26. 2526-4338, 2526-432X

REMA; Rwanda Environment Management Authority (2009). Chapter 7. Water and wetland management. Report accessed from www.rema.gov.rw

Remsburg, A. (2011). Relative influence of prior life stages and habitat variables on dragonfly (Odonata: Gomphidae) densities among lake sites. *Diversity*, *3*(2), 200-216. https://doi.org/10.3390/d3020200

Ross, K. A., Smets, B., De Batist, M., Hilbe, M., Schmid, M., & Anselmetti, F. S. (2014). Lake-level rise in the late Pleistocene and active subaquatic volcanism since the Holocene in Lake Kivu, East African Rift. *Geomorphology*, *221*, 274-285. https://doi.org/10.1016/j.geomorph.2014.05.010

Sarmento, H., Leitao, M., Stoyneva, M., Compere, P., Coute, A., Isumbisho, M., & Descy, J. P. (2007). Species diversity of pelagic algae in Lake Kivu (East Africa). *Cryptogamie-Algologie*, *28*(3), 245-270.

Shinohara, R., Okunishi, T., Adachi, K., Viet, L. S., Mine, H., Yamashita, T., & Isobe, M. (2008). Evaluation of the impact of water dilution within the hypereutrophic Lake Barato, Japan. Lake and Reservoir Management, *24*(4), 301-312. https://doi.org/10.1080/07438140809354840

Soliman, M. F. (2008). Epidemiological review of human and animal fascioliasis in Egypt. *The Journal of Infection in Developing Countries*, *2*(03), 182-189. https://doi.org/10.3855/jidc.260

Stals, R., and de Moor, I. J. (2007). Guides to the Freshwater Invertebrates of Southern Africa Coleoptera, WRC Report No. TT 320/07, 10.

Tampo, L., Kaboré, I., Alhassan, E. H., Ouéda, A., Bawa, L. M., & Djaneye-Boundjou, G. (2021). Benthic macroinvertebrates as ecological indicators: their sensitivity to the water quality and human disturbances in a tropical river. *Frontiers in Water*, *3*, 662765. https://doi.org/10.3389/frwa.2021.662765

Tian, K., Yang, W., Zhao, Y. W., Yin, X. A., Cui, B. S., & Yang, Z. F. (2020). Development of a hydrological boundary method for the river–lake transition zone based on flow velocity gradients, and case study of Baiyangdian Lake transition zones, China. *Water*, *12*(3), 674. https://doi.org/10.3390/w12030674

Turatsinze, J., Claude, T. J., Ndokoye, P., Yvonne, Y. K., & Bamporineza, D. (2024). Analysing the Impacts of Rusizi Urban Growth on Kivu Coastline: Evidence in Gihundwe and Kamembe Sectors. *Journal of Agriculture & Environmental Sciences*, *8*(1), 98-113. https://doi.org/10.53819/81018102t5334

Unnikrishnan, H., Nagendra, H., & Castán Broto, V. (2021). Water governance and the colonial urban project: the Dharmambudhi lake in Bengaluru, India. *Urban Geography*, *42*(3), 263-288.

Uwacu, R.A. & Akintande, O.J. (2019). Impacts of Land Use on Water Quality in the Sebeya Catchment Area, Rwanda. *Journal of Environment and Health Science* 5(2): 77-89.

Van Bocxlaer, B., Schultheiss, R., PLISNIER, P. D., & Albrecht, C. (2012). Does the decline of gastropods in deep water herald ecosystem change in Lakes Malawi and Tanganyika?. Freshwater Biology, 57(8), 1733-1744. https://doi.org/10.1111/j.1365-2427.2012.02828.x

Vannote, R. L., Minshall, G. W., Cummins, K. W., Sedell, J. R., & Cushing, C. E. (1980). The river continuum concept. *Canadian journal of fisheries and aquatic sciences*, *37*(1), 130-137. https://doi.org/10.1139/f80-017

Vilbaste, S., Pall, P., Haldna, M., Nõges, P., Piirsoo, K., & Nõges, T. (2024). How the catchment-river-lake continuum shapes the downstream water quality. *Journal of Limnology*, *83*:*2167*. doi: 10.4081/jlimnol.2024.2167

Villalobos-Jimenez, G., Dunn, A., & Hassall, C. (2016). Dragonflies and damselflies (Odonata) in urban ecosystems: a review. *European Journal of Entomology*, *113*, 217-232. http://dx.doi.org/10.14411/eje.2016.027

Votava, J. E., Johnson, T. C., & Hecky, R. E. (2017). Holocene carbonate record of Lake Kivu reflects the history of hydrothermal activity. *Proceedings of the National Academy of Sciences*, *114*(2), 251-256. https://doi.org/10.1073/pnas.1609112113

Wagner, F. H. (2000). Spatial drift distributions and their ecological importance. *Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen*, *27*(3), 1631-1634. https://doi.org/10.1080/03680770.1998.11901515

Wang, C., & Jiang, H. L. (2016). Chemicals are used for in situ immobilization to reduce the internal phosphorus loading from lake sediments for eutrophication control. *Critical Reviews in Environmental Science and Technology*, *46*(10), 947-997. https://doi.org/10.1080/10643389.2016.1200330

Wang, D., Tao, T., Zhang, S., Zhao, J., Wang, Z., Zhang, Z., ... & Qian, G. (2023). Tributaries protect the water quality of central lake in an artificial spiderweb-structured water system, from the perspective of runoff pollution control. *Journal of Cleaner Production*, *429*, 139566. https://doi.org/10.1016/j.jclepro.2023.139566

Wasson, J. G., Villeneuve, B., Iital, A., Murray-Bligh, J. O. H. N., Dobiasova, M., Bacikova, S., ... & Chandesris, A. (2010). Large-scale relationships between basin and riparian land cover and the ecological status of European rivers. *Freshwater Biology*, *55*(7), 1465-1482. https://doi.org/10.1111/j.1365-2427.2010.02443.x

Wei, Z., Xu, Y., Zhao, Y., Zhao, Y., Bai, L., Jiang, H., & Wang, C. (2021). The settling of resuspended lake sediment related to physicochemical properties of particles of different sizes: Implication for environmental remediation. International Journal of Sediment Research, *36*(4), 542-554. https://doi.org/10.1016/j.ijsrc.2020.11.005

Welch, E. B., Brattebo, S. K., & Overland, C. (2020). Four decades of diluting phosphorus to maintain lake quality. *Water Environment Research*, *92*(1), 26-34. https://doi.org/10.1002/wer.1207

Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). "Welcome to the tidyverse." *Journal of Open-Source Software* *4*(43), 1686. https://doi.org/10.21105/joss.01686.

941 Wieczorek, K., Turek, A., Szczesio, M., & Wolf, W. M. (2024). A holistic approach to the spatio-
942 temporal variability investigation of the main river water quality–The importance of
943 tributaries. *Science of The Total Environment*, *906*, 167588.
944 https://doi.org/10.1016/j.scitotenv.2023.167588

945 Wondmagegn, T., & Mengistou, S. (2023). Development of macroinvertebrate based multimetric
946 index for ecological health monitoring in Lake Hawassa, Ethiopia. *Environmental and*
947 *sustainability indicators*, *18*, 100242. https://doi.org/10.1016/j.indic.2023.100242

948 Wood, D. A., & Scholz, C. A. (2017). Stratigraphic framework and lake level history of Lake Kivu,
949 East African Rift. Journal of African Earth Sciences, *134*, 904-916.
950 https://doi.org/10.1016/j.jafrearsci.2016.06.014

951 Wronski, T., Dusabe, M. C., Apio, A., Hausdorf, B., & Albrecht, C. (2015). Biological assessment
952 of water quality and biodiversity in Rwandan rivers draining into Lake Kivu. *Aquatic*
953 *Ecology*, *49*, 309-320. https://doi.org/10.1007/s10452-015-9525-4

954 Zerlin, R. A., & Henry, R. (2014). Does water level affect benthic macro-invertebrates of a
955 marginal lake in a tropical river-reservoir transition zone?. *Brazilian Journal of Biology*, *74*(2),
956 408-419. https://doi.org/10.1590/1519-6984.26812

957 Zheng, P., Jiang, X., Shu, F., Zhang, K., Xiang, H., Alahuhta, J., & Heino, J. (2024). Comparative
958 effects of river–lake disconnection on taxonomic and functional composition of molluscan
959 assemblages in floodplain lakes. *Hydrobiologia* 852, 3307. https://doi.org/10.1007/s10750-
960 024-05677-1

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Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu.

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Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu

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ABSTRACT

Lake Kivu (Rwanda and Democratic Republic of Congo) is known to be unique among the African Great Lakes due to its peculiar history as a volcanic barrier lake and the frequent environmental pertubations caused by limnic eruptions. This lake is a major resource for riparian people but is also characterized by a depauperate fauna. For molluscs, available information is scarce and spatio-temporally restricted. We studied the freshwater molluscs of the lake and its tributaries and their biogeographical affinities, for the first time via genetic characterization. Our study revealed that the malacofauna of Lake Kivu, though admittedly poor compared to other African Great Lakes, is significantly more diverse than previously anticipated. The occurrence of living populations is restricted to a narrow fringe of littoral substrates, but some of the total of eight species occur much deeper than the immediate margins of the lake, i.e. down to a maximum of 15 m. The fauna displays 'Nilotic' biogeographic connections though widespread forms dominate. Differences in diversity occur in the North vs. South of Lake Kivu in species richness and abundance measures. This pattern can be attributed to recent volcanic eruptions and geochemical stressors in the north, but also to current and ongoing anthropogenic effects. A detailed study of schistosomiasis and fasciolosis with high spatial resolution along the local communities and their livestock is recommended since several potential intermediate host snails are present in Lake Kivu and its tributaries.

1. Introduction

Lake Kivu is one of the great lakes in the western branch of the East African Rift System and it is infamous as a dangerous "explosive" lake because of its limnological peculiarities and history of lacustrine volcanic eruptions (Jones, 2021). The lake hosts very substantial fisheries and other natural resources that support the livelihoods of millions of people in the two riparian countries, the Democratic Republic of Congo (DRC) and Republic of Rwanda (Rwanda) (Amisi et al., 2022). Lake Kivu is ancient, as it has existed since the middle Pleistocene, when it formed by uplift of the Virunga Mountains to the north (Degens et al., 1973). Previously, Lake Kivu drained to the north into Lake Edward. However, ~20,000 years ago, volcanic eruptions and resulting lava flows in the Virunga Volcanic Province (VVP) impounded this outlet (Hecky and Degens, 1973; Ross et al., 2014). This barrier led to a lake level rise and a new outlet, the Ruzizi River, was formed in the South which drains to Lake Tanganyika (Degens et al., 1973; Ross et al., 2014). Since its formation, Lake Kivu has been heavily influenced by volcanic activity, particularly that within the VVP (e.g. Smets et al., 2010; d'Oreye et al., 2011; Ross et al., 2014). Lava flows from Nyiragongo and Nyamulagira, the two active volcanoes located north of Lake Kivu in the Virunga Mountains, have repeatedly entered Kabuno Bay and the main basin of Lake Kivu, particularly during the 1938–40 Nyamulagira and 2002 Nyiragongo eruptions (Balagizi et al., 2018). Beyond volcanic eruptions,

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Lake Kivu is exposed to earthquakes and degassing events, which may result in limnic overturns (Balagizi et al., 2018). The methane reservoir in Lake Kivu is a valuable energy resource for neighboring Rwanda and DRC, but also a looming threat to millions of people in the surrounding area if the stability of the lake is disrupted and the gasses are released into the atmosphere. Several researchers have suggested that methane gas exploitation could reduce the risks of dangerous limnetic eruptions due to supersaturation or subaqueous volcanic eruption (Balagizi et al., 2018; Ross et al., 2014; Schmid et al., 2021, 2005).

There are four major cities along Lake Kivu: Goma (DRC) and Rubavu (formerly Gisenyi, Rwanda) straddling the border in the north, Bukavu (DRC) and Rusizi (formerly Cyangugu, Rwanda) in the south. Livelihoods around the lake include hotel operations, fishing, agriculture, boating, methane gas extraction, mineral mining, and local sand mining. Industrial activities are rather limited but include two breweries, mining facilities, as well as tea drying and coffee cleaning factories (Wronski et al., 2015). The waste products of these activities could release organic or mineral contaminants, and their management would need further evaluation. Gas extraction may pose the most risk to destroy aquatic life in the mixolimnion as uncontrolled toxic gas emission may render the lakeshore and waters uninhabitable and, therewith, cause extensive habitat destruction and fragmentation. Methane-producing plants could also cause local pollution with solid waste that may affect water chemistry (Hirslund and Morkel, 2020; Amisi et al., 2022).

The low biodiversity of Lake Kivu likely reflects the unstability of its environments. Compared to other lakes in the East African Rift, Lake Kivu has a relatively low species diversity (Salzburger et al., 2014). Fish diversity in the lake is remarkably low, with only 29 species from the families Cichlidae, Clariidae, Cyprinidae, and Clupeidae (Snoeks et al., 2012). Biodiversity research in Lake Kivu has mainly focused on fish ecology and biology, due to the needs of the fishery (Sarmento et al., 2007). Knowledge of macroinvertebrates in Lake Kivu is still in an early stage of development (Dartevelle and Schwetz, 1947; Verbeke, 1957; Richard et al., 2020), and genetic characterizations have been undertaken for hardly any groups. The combination of genetic assessments in addition to classical morphology-based diversity assessments have considerably improved the knowledge of biodiversity in other tropical lakes including those of the African Great Lakes (e.g. Schultheiß et al., 2009; Van Bocxlaer et al., 2021). Despite their recognized importance to the functioning of aquatic ecosystems and numerous applications to water quality assessment (Doric et al., 2021; Dusabe et al., 2019), macroinvertebrates are generally poorly understood, and little is known about their ecology, diversity, and distribution. This state of affairs is exemplified by the molluscs of Lake Kivu for which very spatially restricted data were presented by Pilsbry and Bequaert (1927), Dartevelle & Schwetz (1947), and Gillet (1960). In a short summary by Brown (1994), Lake Kivu is reported to have seven species of gastropods, including one endemic taxon, *Gabbiella humerosa kivuensis* (Bithyniidae). It should be noted that the available data, collected over the last ~ 100 years, were spatially and temporally very restricted and reflect the lack of a comprehensive mollusc dataset for Lake Kivu. Documenting the diversity and distribution of molluscs is important because of their role in the aquatic food web. They contribute greatly to the processes of nutrient exchange, primarily by controlling algal growth, maintaining water quality, and providing clean substrates for other bottom-dwelling animals such as insects (Johnson, 2009). In addition, some molluscs are intermediate hosts of infectious diseases such as those caused by trematode parasites of the genera *Schistosoma* (blood flukes) and *Fasciola* (liver flukes). The extent of spread of these diseases depends on the availability of snail intermediate hosts in the area of concern and their susceptibility to infection. Understanding the occurrence and distribution of intermediate host snails is an important step towards implementing cost-effective measures to control and monitor schistosomiasis, and complements such efforts by Kagabo et al. (2023) in Rwanda.

Based on extensive field sampling and the use of mitochondrial DNA markers for a genetic assessment of the fauna, we aim to gain new insights into the biodiversity of molluscs in Lake Kivu and its basin. With the present study, we specifically aim to: (a) assess current molluscan species diversity and how this diversity is spatially distributed in Lake Kivu; (b) determine ecological insularity by contrasting tributaries, and lacustrine habitats; (c) evaluate the differences in communities across basins in the lake; and (d) assess effects of anthropogenic disturbances on the diversity and abundances of molluscs. Additionally, we discuss the current status of the mollusc fauna, including their conservation, and their potential role as bioindicators and/or vectors for diseases.

2. Materials and methods

2.1. Study area

Lake Kivu is shared by the DRC and Rwanda (Fig. 1). The lake lies in the centre of the axis of the western branch of the East African Rift and is, with an elevation of 1,463 m above sea level, at highest altitude lake within this branch (Degens et al., 1973). Lake Kivu has a surface area of 2,370 km^2 (Muvundja et al., 2014), a volume of 549 km^3, a catchment area of ~ 7,140 km^2 (Stoffers and Hecky, 1978), and reaches a maximum depth of ~ 485 m in its northern basin (Lahmeyer and OSAE, 1998). The lake consists of four basins (Wong and Herzen, 1974; Schmid and Wuest, 2012): the northern or main basin (off Goma and Gisenyi: max. depths of 485 m), the southern basin (max. depths of ~ 180 m off Ishungu and 100 m off Bukavu Bay and Kamembe), the eastern basin (max. depths 400 m) and the western basin (max depths of ~ 230 m). Kabuno Bay off Minova (max. depths of 150 m) in the northwestern part of the lake is connected to the northern basin by a narrow channel and can be considered to be a pseudo-satellite lake (Fig. 1). The most important water sources of Lake Kivu include direct rainfall (average 1,404 mm/year) and inflows from various streams and rivers. In particular, the Sebeya River and the Koko River, which originate in the Rwandan mountains (Ross, 2014), contribute significantly to the lake's water balance. The only outflow is via the Ruzizi River, which flows south into Lake Tanganyika. The climate is humid with a bimodal precipitation regime (~1,400 mm/$year^{-1}$ over the lake catchment; Muvundja et al., 2009). The long rainy season extends from Febuary to May and short rains from October to December (Bergonzini, 1998). The average water temperature at the surface is about 24 °C (Snoeks, 1994). Lake Kivu has the tenth largest inland island in the world, Idjwi, which is located in the centre of Lake Kivu and is home to ~ 320,000 people (Nyamalyongo et al., 2022).

The catchment of Lake Kivu is relatively small as its shorelines are generally steep, except along the northern shore, where the slope rises more gradually toward the Nyiragongo and Nyamuragira volcanoes. Current land use around Lake Kivu is dominated by subsistence agriculture and cropping, which means that only manure is used for fertilization and comparatively few agrochemicals are used (Muvundja et al., 2009), though there might be significant use of pesticides, too. The main industrial activities in the watershed area are two breweries and a few processing plants for agricultural products such as tea, coffee, and quinine (Wronski et al., 2015; Houbraken et al., 2017). Deforestation in the surroundings of Lake Kivu to meet the increasing demand for fuelwood, given the lack of alternative energy sources for households (Jones, 2003), results in more soil erosion, landslides and sediment influx to the lake (Moeyersons et al., 2004). More than 100 small tributaries, with varying degrees of deforestation along their course, feed the lake (Muvundja et al., 2009) creating a mosaic of impacts.

2.2. Sampling

An extensive field campaign for the collection of mollusc species was carried out from October to November 2022 in northern, central, and southern parts of Lake Kivu by M. C. Dusabe and B. L. Hyangya. Previously sampled material from 2019 (northern basin, Rwanda; C.

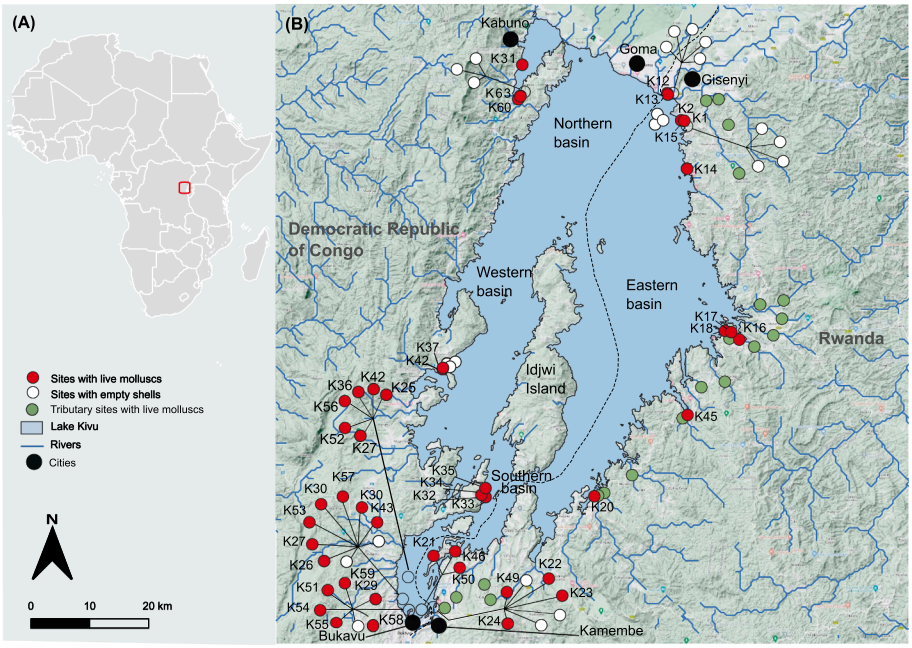

Fig. 1. Map of Lake Kivu with sampling sites and points. A: Overview map of Africa highlighting the central location of Lake Kivu (shown by the red frame). B: Detailed view of sampling sites in Lake Kivu with live molluscs (in red) or empty shells (in white), and in tributaries (in green).

Albrecht, M. C. Dusabe, B. L. Hyangya), 2018 (south-western Lake Kivu, DRC; B. L. Hyangya), and 2010 (south-eastern part of the lake, Rwanda; R. Schultheiß) were also included in this study. In total, we surveyed 34 sites with 64 collecting points (Fig. 1). These points represent different collecting depths (Table 1). In addition, the material collected by M. C. Dusabe in 2014 and used by Wronski et al. (2015) from 19 tributaries on the Rwandan side of Lake Kivu was also included for comparative purposes.

Samples from the shore (0–0.5 m depth) were collected using a scoop net (diameter: 20 cm, mesh size: 1 mm) and a Surber net (250 μm mesh size). At the shoreline, sites were selected to reflect different micro-habitat types: pristine sites, lowly disturbed, moderately disturbed and highly disturbed (Table 1). Site utilization (boating, fishing, swimming, digging etc.) was recorded. The depth, geographical coordinates, and substrate type (vegetation, rocks, stones, sand, mud, detritus, sapropel, artificial substrates) of each site were noted (Table 1).

Lake sampling (0.6–33 m depth) was done using an Ekman's bottom grab from a motorized boat. The depth was measured with an echo sounder (Plastimo Handecholot Echotest II). All sampled materials were sieved in a bucket with a 250 μm Surber sampler and the collecting net was rinsed after each sample. All samples were preserved in 80% ethanol and labelled accordingly. The material was brought to the University of Giessen Systematics and Biodiversity collection (UGSB), Germany or to UMR 8198 - Evo-Eco-Paleo (CNRS, Univ. Lille).

Specimens were identified to species level if possible, but in some cases only to genus level based on shell morphology according to Brown (1994). For each site, two specimens of each species were selected and photographed using a Keyence digital microscope system (KEYENCE VHX-2000; Keyence Deutschland GmbH, Neu-Isenburg, Germany) before processing them in the lab.

2.3. DNA extraction, amplification and sequence analysis

We selected 96 specimens from 9 genera namely *Biomphalaria*, *Bulinus*, *Afrogyrorbis*, *Gabbiella, Sphaerium, Radix, Pisidium, Physella,* and *Melanoides*, for DNA barcoding. Genomic DNA was extracted from a small piece of foot muscle using the CTAB protocol of Wilke et al. (2006) or with a DNeasy Blood and Tissue Kit (Qiagen). Gene fragments of the mitochondrial cytochrome *c* oxidase subunit I (COI) and the large subunit of ribosomal RNA (LSU rRNA or 16S) were amplified with the primers LCO1490 and HCO2198 (Folmer et al., 1994) or COR722b (Wilke and Davis, 2000) for COI and 16Sar and 16Sbr for 16S (Palumbi, 1991) using the PCR conditions decribed in Electronic Supplementary Material (ESM) Appendix S1. Sanger sequencing was performed on an ABI 3730xl DNA analyzer using the BigDye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, Germany). All new sequences were deposited in NCBI GenBank (accession numbers are provided in Table 2).

DNA sequence errors at the beginning and end of the sequences were manually edited and aligned using BioEdit v7.2 (Hall, 1999). Unique haplotypes were calculated using TCS v1.2.1 (Clement et al., 2000; connection limit = 95 %), and were compared to the nucleotide database of NCBI GenBank with BLAST (BLASTn suite: megablast) to find similar sequences (Zhang et al., 2000). BLAST hits were sorted by max *score* (default setting) and the top two hits for each of our specimens were selected. This approach has several potential pitfalls, including that some sequences in GenBank might be wrongly labelled, i.e. based on misidentifications. Additionally, in some cases closely related species may not be present in GenBank yet. In a few of such cases, we used local BLAST searches to compare the sequences of our Kivu specimens with our internal sequence database (unpublished data).

2.4. Phylogenetic analysis

Whereas most recovered genera were monospecific, Biomphalaria was not, so we carried out a phylogenetic analysis of this genus using the COI marker. Additional sequences were downloaded from NCBI GenBank, resulting in a dataset with 48 ingroup specimens, and *Afrogyrorbis natalensis* was used as outgroup. The multi-sequence alignment for the Folmer region of cox1 was conducted using the ClustalW tool implemented in BioEdit. The final alignments comprised 655 bp. The software jModelTest v2.1.4 (Darriba et al., 2012) was used to select the best-fit substitution model for each of the three codon positions. Based on the Akaike information criterion (AIC) and Bayesian information criterion (BIC), the best-fit substitution model for the dataset was HKY + G. We used the software MEGA 11 (Tamura et al., 2021) to construct a phylogenetic tree using maximum likelihood (ML) and bootstrapping with 1,000 replicates. The final tree was visualized and edited in FigTree

Table 1
Information on sampling sites and points (N = 64) including depth, latitude and longitude, and habitat characterization: substrates, sites uzilitazation and degree of anthropogenic disturbance.

Sampling points	Depth/ m	Latitude	Longitude	Substrates	Sites utilization	Level of disturbance
K1	0	−1.73939	29.27028	Detritus, sapropel and artificial (cloth)	Boating, fishing and swimming	high
K2	5	−1.74006	29.27511	Detritus and fine silt	Boating, fishing and swimming	medium
K3	10	−1.74849	29.27708	Detritus and fine silt	Boating and fishing	low
K4	20	−1.74014	29.27461	Detritus and fine silt	Boating and fishing	low
K5	30	−1.74833	29.27650	Detritus and fine silt	Boating and fishing	low
K6	0	−1.70423	29.25916	Stones and sand	Boating, fishing and swimming	medium
K7	5	−1.69867	29.25355	Detritus and fine silt	Boating and fishing	medium
K8	10	−1.70026	29.25012	Detritus and fine silt	Boating and fishing	medium
K9	15	−1.74541	29.23054	Detritus and fine silt	Boating and fishing	medium
K10	20	−1.72982	29.23408	Detritus and fine silt	Boating and fishing	medium
K11	30	−1.73931	29.24282	Detritus and fine silt	Boating and fishing	medium
K12	0	−1.69798	29.24905	Detritus, sand and silt	Swimming, baoting and fishing	medium
K13	0	−1.81300	29.27903	Stones and sand, rocks	Boating, fishing and swimming	medium
K14	0	−1.81336	29.27950	Stones and sand, rocks	Boating, fishing and swimming	medium
K15	0	−1.70000	29.25000	Detritus, sand and silt	Swimming, baoting and fishing	medium
K16	0	−2.07361	29.35914	Detritus, sapropel and artificial (cloth)	Boating and fishing	medium
K17	5	−2.06036	29.33725	Detritus and fine silt	Boating, fishing and cattle swimming	medium
K18	10	−2.06222	29.34678	Detritus and fine silt	Boating, fishing and cattle swimming	medium
K19	0	−2.05329	29.34718	Stones and sand, rocks	Boating, fishing and swimming	medium
K20	0	−2.31239	29.13775	Detritus and sapropel	Boating and fishing	medium
K21	0	−2.47062	28.69626	Detritus and fine silt	Boating and fishing	low
K22	0	−2.47922	28.89847	Detritus and fine silt	Boating and fishing	low
K23	3	−2.48436	28.89408	Detritus and fine silt	Boating and fishing	low
K24	15	−2.48414	28.89387	Detritus and fine silt	Boating and fishing	low
K25	30	−2.48410	28.89351	Detritus and fine silt	Boating and fishing	low
K26	0.5	−2.48599	28.85234	Detritus and fine silt	Boating and fishing	low
K27	5	−2.46946	28.84277	Detritus and fine silt	Boating	low
K28	20	−1.74014	29.27461	Detritus and fine silt	Boating	low
K29	30	−2.46890	28.84514	Detritus and fine silt	Boating	low
K30	0	−2.47167	28.83972	Detritus, sand and silt	Turbid river	low
K31	0	−1.65472	29.02778	Detritus, sand and silt	Boating	medium
K32	0	−2.31361	28.97167	Detritus and fine silt	No activity	pristine
K33	0	−2.31028	28.96556	Detritus, sand and silt	Boating and fishing	medium
K34	0	−2.31028	28.96553	Detritus, sand and silt	Boating and fishing	medium
K35	0	−2.30000	28.97167	Detritus, sand and silt	Boating	medium
K36	10	−2.31056	28.96861	Detritus and fine silt	No activity	pristine
K37	0	−2.11065	28.91298	Detritus and fine silt	No activity	pristine
K38	10	−2.11040	28.91305	Detritus and fine silt	No activity	pristine
K39	20	−2.11051	28.91276	Detritus and fine silt	No activity	pristine
K40	30	−2.10991	28.91293	Detritus and fine silt	No activity	pristine
K41	0	−2.10750	28.92556	Detritus, sand and silt	Movement of people, and a turbid river	high
K42	0	−2.11222	28.91361	Detritus and fine silt	Boating	medium
K43	0	−2.48889	28.84500	Detritus, sand and silt	Market for fish	high
K44	5	−2.46890	28.84514	Detritus, sand and silt	Boating	high
K45	0	−2.18839	29.28014	Detritus and sapropel	Boating, fishing and digging	high
K46	5	−2.49665	28.86323	Detritus and fine silt	Boating and fishing	low
K47	0	−2.11861	28.90611	Stones and gravel	No activity	pristine
K48	0	−2.11667	28.90667	Detritus and fine silt	No activity	pristine
K49	0	−2.48528	28.87411	Detritus, sand and silt	Boating	medium
K50	10.2	−2.50278	28.88556	Detritus and fine silt	Fishing	low
K51	0	−2.48599	28.85234	Detritus, sand and silt	Boating	high
K52	11.2	−2.49889	28.86622	Detritus and fine silt	Fishing	low
K53	30	−2.46890	28.84514	Detritus and fine silt	Fishing	low
K54	32.5	−2.49662	28.86342	Detritus and fine silt	Boating and fishing	low
K55	0	−2.50250	28.85639	Sand and gravel, rocks	Market, boating and big turbid river	high
K56	5	−2.48528	28.85639	Detritus and fine silt	Boating	low
K57	0	−2.48488	28.85669	Detritus and fine silt	Boating	low
K58	33	−2.48519	28.85624	Detritus and fine silt	Boating	low
K59	20.3	−2.48528	28.85621	Detritus and fine silt	Boating	low
K60	0	−1.70722	29.02167	Detritus and fine silt	Boating	low
K61	5	−2.11500	28.91750	Detritus and fine silt	Boating	medium
K62	10	−1.70222	29.02500	Detritus and fine silt	Boating	medium
K63	20	−1.70278	29.02444	Detritus and fine silt	Boating	medium
K64	30	−1.6925	29.03583	Detritus and fine silt	Boating	low

v1.4.2 (Rambaut, 2014). (ESM Appendix S1; Figure S1).

2.5. Ecological analyses

To assess current molluscan species diversity and visualize its spatial distribution in Lake Kivu, we calculated two different diversity indices, Shannon-Wiener index and Pielou's evenness. Heatmaps were used to visualize the spatial distribution of molluscs in Lake Kivu. Descriptive statistics were used to summarize the environmental variables (See above and ESM Appendix S2) that had been collected. To determine the differences in the abundance of molluscs across sites, species, and basins, a non-parametric Kruskal-Wallis test was performed. Furthermore, the effect of environmental variables (See above and ESM Appendix S2) on mollusc abundance was determined using a generalized linear model

Table 2
Results of BLAST searches, indicating species, with voucher code, the associated BLAST hits with % similarity (in squared brackets) and NCBI GenBank accession numbers. UGSB – University of Giessen Systematics and Biodiversity collection. The three letter code indicated for BLAST results indicates the country of origin: CAN = Canada, DRC = DR Congo, KEN = Kenya, RWA = Rwanda, SDN = Sudan, TZA = Tanzania, UGA = Uganda, ZAF = South Africa, ZWE = Zimbabwe, EA = East Africa.

Family	Species (morphological)	Voucher no.	BLAST results (COI)	local BLAST results (COI)	GenBank acc. numbers
GASTROPODA					
Bithyniidae	*Gabbiella humerosa*	UGSB 28063	*Gabbiella* sp. UGA OP339805 [97.2 %] *Gabbiella humerosa* KEN OP339804 [97.2 %]	–	PP510644
		UGSB 28072	*Gabbiella* sp. UGA OP339805 [96.9 %] *Gabbiella humerosa* KEN OP339804 [96.9 %]	–	PP510645
		UGSB 27879 UGSB 27868 UGSB 27901	*Gabbiella* sp. UGA OP339805 [97.2 %] *Gabbiella humerosa* KEN OP339804 [97.2 %]	–	PP510646; PP510647; PP510648
Lymnaeidae	*Radix natalensis*	UGSB 27333	*Radix natalensis* ZWE MZ546834 [98.4 %] *Radix natalensis* ZWE MT888846 [98.4 %]	–	PP510649
Bulinidae	*Bulinus* sp.	UGSB 27317 UGSB 27319	*Bulinus truncatus* RWA MN551581 [99.5 %] *Bulinus truncatus* RWA MN551578 [99.5 %]	–	PP510650; PP510651
		UGSB 27318	*Bulinus truncatus* TZA AM286313 [100.0 %] *Bulinus truncatus* SDN MN853656 [99.8 %]	–	PP510652
		UGSB 27894 UGSB 27898 UGSB 27908 UGSB 28051 UGSB 27905	*Bulinus truncatus* DRC HQ121561 [100.0 %] *Bulinus truncatus* UGA MT707414 [99.7 %]	–	PP510653; PP510654; PP510655; PP510656; PP510657
		UGSB 27906	*Bulinus truncatus* DRC HQ121561 [99.8 %] *Bulinus truncatus* UGA MT707414 [99.5 %]	–	PP510658
		UGSB 27907	*Bulinus truncatus* DRC MT707423 [99.5 %]	–	PP510659
		UGSB 27865	*Bulinus truncatus* UGA GU176748 [99.3 %] *Bulinus truncatus* UGA GU176748 [99.3 %]	–	PP510660
		UGSB 27883	*Bulinus truncatus* UGA MT707423 [99.7 %] *Bulinus truncatus* UGA GU176748 [99.54 %]	–	PP510661
Planorbidae	*Biomphalaria pfeifferi*	UGSB 23723; UGSB23709; UGSB23719; UGSB23720; UGSB23721; UGSB 23722	*Biomphalaria pfeifferi* KEN NC_038059 [99.2 %] *Biomphalaria pfeifferi* KEN OL423116 [99.2 %]	–	PP510662; PP510666; PP510667; PP510668; PP510669; PP510670
	Biomphalaria sp. MT1	UGSB 28055; UGSB 28056; UGSB28880; UGSB28881; UGSB28882; UGSB28883; UGSB23771	*Biomphalaria choanomphala* UGA HM768905 [99.7 %] *Biomphalaria* sp. EA HM769163 [99.5 %]	–	PP510663; PP510664; PP510673; PP510674; PP510675; PP510676; PP510672
	Biomphalaria sp. MT2	UGSB 27886	*Biomphalaria smithi* UGA DQ084836 [99.2 %] *Biomphalaria* sp. EA HM769209 [99.1 %]	–	PP510665
	Biomphalaria cf. *smithi*	UGSB23759	*Biomphalaria smithi UGA DQ084836 [99.5%] Biomphalaria sp. EA HM769209 [99.2%]*	_	PP510671
	Afrogyrorbis kigeziensis	UGSB 27892 UGSB 27892 UGSB 27900	*Gyraulus chinensis* KEN AF199086 [96.6 %] *Gyraulus* sp. UGA OQ849997 [96.3 %]	*Afrogyrorbis* sp. COD UGSB 7770 [96 %] *Afrogyrorbis natalensis* ZAF UGSB 28782 [96 %]	PP510677; PP510678; PP510679

(*continued on next page*)

Table 2 (*continued*)

Family	Species (morphological)	Voucher no.	BLAST results (COI)	local BLAST results (COI)	GenBank acc. numbers
		UGSB 27909	*Gyraulus chinensis* KEN	*Afrogyrorbis* sp. COD	PP510679;
		UGSB 27910	AF199086 [96.6 %]	UGSB 7770 [96 %]	PP510680
			Gyraulus sp. UGA	*Afrogyrorbis natalensis* ZAF	
			OQ849997 [96.3 %]	UGSB 28782 [96 %]	
		UGSB 27895	*Gyraulus* sp. UGA	*Afrogyrorbis natalensis* ZAF	PP510681
			OQ849997 [96.8 %]	UGSB 28784 [96 %]	
			Gyraulus chinensis KEN	*Afrogyrorbis* sp. COD	
			AF199086 [99.6 %]	UGSB 7770 [96 %]	
		UGSB 28067	*Gyraulus chinensis* KEN	*Afrogyrorbis* sp. COD	PP510682
			AF199086 [96.0 %]	UGSB 7770 [95 %]	
			Gyraulus sp. UGA	*Afrogyrorbis natalensis* ZAF	
			OQ849997 [95.8 %]	UGSB 28782 [95 %]	
		UGSB 27897	*Gyraulus* sp. UGA	*Afrogyrorbis natalensis* ZAF	PP510683
			OQ849997 [96.5 %]	UGSB 28786 [96 %]	
			Gyraulus chinensis KEN	*Afrogyrorbis* sp. COD	
			AF199086 [96.2 %]	UGSB 7770 [95 %]	
BIVALVIA					
Sphaeriidae	*Sphaerium* cf. *hartmanni*	UGSB 27314	*Sphaerium* sp. UGA	–	PP510684;
			KF483419 [94.2 %]		PP510685
		UGSB 27315	*Musculium partumeium* CAN		
			MT324076 [93.9 %]		
				–	

with a Poisson link function. However, using this model, we observed that the mean mollusc abundance was greater than the variance indicating over-dispersion. We further followed the poisson model with the *estat gof* function in Stata/SE version 18.0 but used the *glm* to obtain the deviance and Pearson's chi-square values. The deviance was greater than 1, thus making the poisson model unsuitable due to over-dispersion which often leads to underestimated SEs and inflated test statistics (Hilbe, 2014, Hilbe, 2011). Thus, the negative binomial model was used to model the effect of environmental variables (see above and ESM Appendix S2) on mollusc abundance. To test for differences in the diversity of fauna across basins in the lake, the presence or absence of molluscs across the sites was determined. Furthermore, to assess ecological insularity by contrasting tributaries, and lacustrine habitats, the differences in the diversity of tributaries and Lake Kivu (shorelines) were done using the non-parametric Wilcoxon rank-sum (Mann–Whitney).

3. Results

3.1. Sampling and species identification

Living molluscs were found at 40 collection depths from 26 sites, whereas at 24 collection depths from 8 sites we found empty gastropod shells only. For Lake Kivu (excluding the tributaries) we morphologically identified four nominal gastropod species: *Melanoides tuberculata* (Thiaridae), *Gabbiella humerosa* (Bithyniidae), *Radix natalensis* (Lymnaeidae), and *Afrogyrorbis kigeziensis* (Planorbidae), and one bivalve species: *Sphaerium* cf. *hartmanni* (Sphaeriidae). All other taxa were morphologically determined up to the genus level, namely: *Bulinus* sp. (Bulinidae) and *Biomphalaria* spp., for which we distinguished two different morphotypes, MT1, which resembles *Biomphalaria pfeifferi*, and MT2 (Planorbidae). In summary, eight species-level taxa from seven distinct genera were found in Lake Kivu proper. For the tributaries of Lake Kivu six species (representing five distinct genera) could be determined morphologically (ESM Table S1). *Biomphalaria* MT1 (cf. *pfeifferi*) and *Radix natalensis* were found in both the lake and the tributaries, but *Bulinus forskalii* (Planorbidae), *Physella acuta* (Physidae), *Pisidium kenianum* (Sphaeriidae), and *Pisidium* cf. *viridarium* (Sphaeriidae) exclusively occurred in the tributaries (ESM Table S2).

Our analysis of molecular identification focused on 52 specimens from 9 genera of molluscs whose sequences were successfully processed. In most cases, our BLAST searches confirmed at least the genera identified based on shell morphology (Table 2). A seeming exception was for *Afrogyrorbis*, for which the closest BLAST hits related to a different genus, *Gyraulus* (Table 2), but there were no *Afrogyrorbis* sequences in GenBank when we performed these BLAST searches. We thus compared our sequences from Lake Kivu to unpublished sequences of *Afrogyrorbis natalensis* from KwazuluNatal, South Africa and *Afrogyrorbis* sp. from the DRC, which suggested close relationships. Further taxonomic work on *Gyraulus* and *Afrogyrorbis* is required to illucidate relationships, and for now, we retain our assignment of Lake Kivu specimens to *Afrogyrorbis kigeziensis*.

Five sequences of *Gabbiella humerosa* (646 bp length) represented three unique haplotypes. *Gabbiella* sp. (Uganda) and *G. humerosa* (Kenya) with around 97 % identity were the closest hits. For *Radix natalensis* only one COI sequence (468 bp length) could be successfully amplified and this sequence had > 98 % similarity to *R. natalensis* from Zimbabwe. A total of 13 sequences (652 bp length) resulted in 7 unique haplotypes for the *Bulinus* sp. studied. All of these haplotypes had > 99.3 % similarity with *Bulinus truncatus* specimens from various African countries in geographic proximity to Lake Kivu (see Table 5).

Of the five COI sequences of *Biomphalaria* MT1 (cf. *pfeifferi*, length 655 bp), one was an exact match (100 %) to *B. pfeifferi*, and the other four had high similarity (99.24–99.85 %) with *Biomphalaria pfeifferi* from Kenya. For *Biomphalaria* sp. MT2 we generated five sequences with a length of 655 bp, which represented two unique haplotypes. Four sequences could be identified as *Biomphalaria choanomphala* with matches of 99.7 and 99.34 % similarity to *B. choanomphala* from Uganda. The fifth sequence comes out as *Biomphalaria smithi* (99.2 % identity with one sequence of *B. smithi* from Uganda). Maximum Likelihood (ML) phylogenetic analysis of all *Biomphalaria* species revealed that there are only two distinct groups. *B. pfeifferi* clustered with specimens from Kenya, whereas *B. choanomphala* with specimens from Uganda. Branches were extremely short and poorly supported within that clade (ESM Appendix S1; Figure S1), highlighting the unresolved taxonomy within this particular group.

For *Afrogyrorbis kigeziensis,* the eight COI sequences we generated (lengths of 655 bp) represent five unique haplotypes that share appr. 96 % similarity with various *Gyraulus* spp. and *Afrogyrorbis natalensis*. A 16S sequence of *Bulinus forskalii* (16S GenBank accession no.: PP504869) (length of 428 bp) from the Kavungu stream in the Musogoro catchment matched 97.67 % with *Bulinus forskalii* from Tanzania, Tunduma. A sequence of *Physella acuta (16S GenBank accession no.: PP504870)* (length of 505 bp) from the Kiboga stream matched 98.99 % with

Physella acuta from the USA. *Sphaerium* cf. *hartmanni* (397 bp length) was 94 % identical with a *Sphaerium* sp. from DRC. Two *Melanoides tuberculata* samples from Lake Kivu (COI GenBank accession no.: KP774714 and KP774715) have been previously published by Van Bocxclaer et al. (2015). The sequneces of *Pisidium kenianum* (COI GenBank accession no.: OK663625 and OK663626) and *P.* cf. *viridarium* (COI GenBank accession no.: OK663621) from the tributary of Lake Kivu have already been analysed by Clewing et al. (2022).

3.2. Mollusc communities

Our combined morphological and genetic approach to identifying the freshwater molluscs of Lake Kivu revealed eight species (seven gastropod and one bivalve species), which belong to seven genera and six families (Table 3). *Sphaerium* cf. *hartmanni* was recorded for the first time in Lake Kivu from one sampling site (K1) located in Gisenyi region. *Biomphalaria choananomphala*, which was mentioned twice in Lake Kivu (Pilsbry and Bequaert, 1927; Dartevelle and Schwetz, 1947) was found at one sampling site in K22 (South Kivu Rwanda). *Afrogyrorbis kigeziensis* was for the first time mentioned from Lake Kivu by Brown (1994), and occurred at ten sampling sites in our study (Table 3). *Biomphalaria pfeifferi* was previously reported by Gillet et al. (1960) in the Bukavu region and later by Brown (1994) from Lake Kivu, and was obtained here from five sites. *Radix natalensis* was found at 4 sites, whereas *Bulinus truncatus* at 15 sites (Table 3). *Melanoides tuberculata* and *Gabbiella humerosa kivuensis* were found at 5 and 19 sites, respectively (Table 3).

In general, the Shannon-Wiener index was very low, ranging from 0 to 1.1 (ESM Table S3). Species richness at sites ranged from 1 to 5 (Fig. 2) while Pielou evenness ranged from 0 to 1 (Fig. 3). Richness was highest in shallow waters (0–0.5 m) with a mean of 2.45 ± 1.54 and it decreased to 1.33 ± 0.57 on deeper substrates (Fig. 2). Evenness increased with increasing depth (11–15 m: mean 0.94 ± 0.10), suggesting that the number of species obtained was more constant in deeper than in shallow waters (mean 0.80 ± 0.25) (Fig. 3). The northern basin had lower evenness (mean 0.77 ± 0.18) than the southern basin (mean 0.86 ± 0.23) (Fig. 3).

Results from the Wilcoxon rank-sum (Mann–Whitney) test comparing the diversity of the tributaries and Lake Kivu showed no statistically significant difference ($z = 1.593$; $p = 0.071$). The mean diversity for the tributaries was 0.21 (95 % CI: 0.071–0.343) whereas that for Lake Kivu was 0.388 (95 % CI: 0.259–0.515) (see Fig. 4.).

3.3. Mollusc biomass and depth distribution

In the 26 sites where living molluscs were collected, *Gabbiella humerosa,* a species endemic to Lake Kivu recognized as *Gabbiella humerosa kivuensis* (Mandahl-Barth, 1968), accounted for most of the density of gastropod species (31.17 %, 66.41 ± 13.25 indiv. m^2) and was found at 19 sampling sites. *Afrogyrorbis kigeziensis* and *Bulinus truncatus* contributed to 18.18 % (4.93 ± 2.97 indiv. m^2) and 22.08 % (5.49 ± 3.73 indiv. m^2), respectively, and were found at 10 and 15 sampling sites, respectively. *Biomphalaria choanomphala* and *Sphaerium* cf. *hartmanni* were each found at one site only (Table 3). Furthermore, we found no significant differences in the abundance of molluscs across sites (Krukal-Wallis test; $p = 0.4040$). On the other hand, significant differences were observed in the abundance of the various species ($p = 0.0009$).

Results from the nbGLM model suggested that site utilization (nbglm: Coeff: −0.180; $p = 0.001$, 95 % CI: −0.286 − -0.075), substrate types (nbglm: Coeff: −0.2081; $P = 0.041$, 95 % CI: −0.407 − -0.008), and depth (nbglm: Coeff: −0.839; $P = 0.007$, 95 % CI: −1.452 − -0.226) significantly influenced the abundance of molluscs. On another hand microhabitat type (nbglm: Coeff: 0.469; $p = 0.125$; 95 % CI:-0.130–1.068) had no statistically significant effect on snail abundance. In particular, sites with medium disturbance (nbglm: Coeff:-1.477; $p = 0.082$; 95 % CI:-3.141–0.186) and high disturbance (nbglm: Coeff:-1.879; $p = 0.214$; 95 % CI:-4.841–1.082) showed a decline in molluscs compared to sites with low or no disturbance, but these differences were not statistically significant. Compared to the detritus and fine silt, detritus and sapropel and detritus, artificial and sapropel substrates increased the abundance of molluscs by 3.10; $p = 0.001$ (95 % CI:

Table 3
Mollusca species recorded at each site sampled in Lake Kivu. The number of collecting points with live records are given. There are 26 sites and 40 sampling points. For details of the sampling points refer to Table 1.

Site (number of points)	*B. choanomphala*	*B. pfeifferi*	*B. truncatus*	*A. kigeziensis*	*G. humerosa*	*M. tuberculata*	*S.* cf. *hartmanni*	*R. natalensis*
K1(4)		1	1	2	2	1		
K26(2)					2			
K30					1			
K16(2)		1	1	1	1			
K18					1			
K55				1	1	1		
K57					1			
K31				1	1			
K12		1	1				1	
K42				1				1
K37					1			
K43 (2)	1		1	1	1			1
K21(3)		1	1		1			1
K23			1	1	1			
K20		1	1			1		
K16					1			
K13			1					
K14			1					
K33						1		
K34				1				
K35(3)			1		1	1		1
K60					1			
K45 (3)			2	1	1	1		
K48 (3)			2			1		
K51			1					
K15					1			
N points (40)	1	5	15	10	19	7	1	4
Frequency (%)	2.5	12.5	37.5	25	47.5	17.5	2.5	10

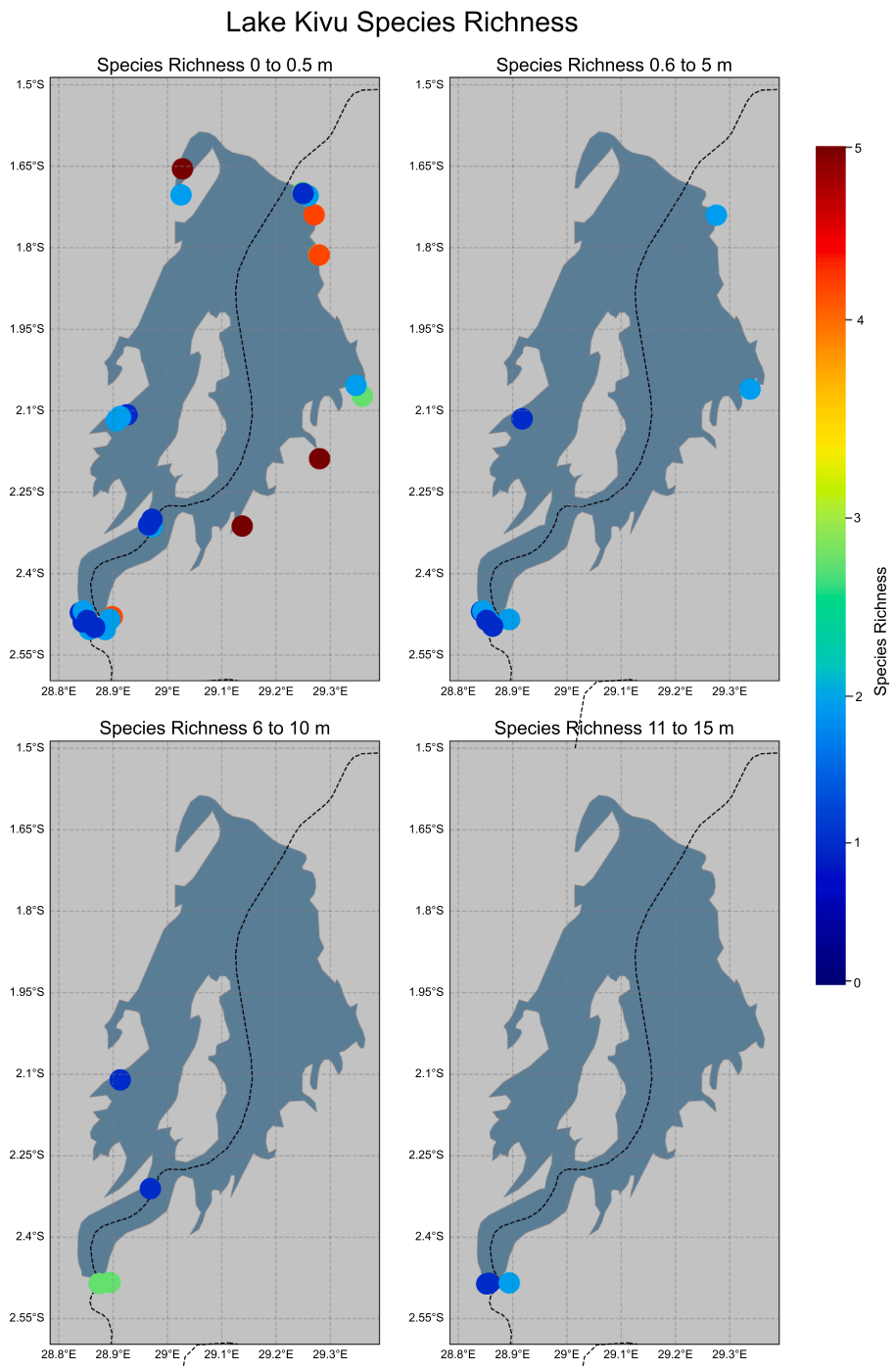

Fig. 2. Heatmap visualization of species richness of molluscs in Lake Kivu with subplots for the different sampling depths, A: 0 to 0.5 m; B: 0.6 to 5 m; C: 5 to 10 m; D: 11 to 15 m. The colour bar indicates the range of species richness in the different sites and depths.

1.202–5.016) and 2.51; $p = 0.003$ (95 % CI: 0.849–4.176) (ESM Appendix S2). Mollusc abundance generally decreased with increasing sampling depth with a notable statistical decrease observed particularly between 11 and 15 m depth (nbglm: Coeff: −2.226; $p = 0.021$, 95 % CI: −1.113 − -0.338).

All species were observed on nearshore substrates (0–0.5 m). *Radix natalensis*, *Sphaerium* cf. *hartmanni*, and *Bulinus truncatus* were found exclusively near the shoreline, whereas *Biomphalaria pfeifferi* and *Biomphalaria choanomphala* were found from the shoreline to a depth of 5 m (Fig. 5), *Afrogyrorbis kigeziensis* up to 11 m, and *Gabbiella humerosa* and *Melanoides tuberculata* up to 15 m. At deeper collecting depths (16–20 m and 26–33 m), we only found empty shells.

In total, we collected 1,983 living mollusc specimens of the various species. Of these, 1,243 individuals were collected from the southern basin, 377 from the eastern basin, 347 from the northern basin and 17 from the western basin. A Kruskal-Wallis test showed no statistically significant difference in the diversity of snails collected from the different basins (Chi 2 (3) = 5.775, $p = 0.138$). Furthermore, the northern basin had lower species richness compared to the southern basin (Fig. 6a). Also, the western basin had one snail species whereas 5 species were found in the eastern basin (Fig. 6b). In addition, there were many sites in the northern basin with empty shells, both near the shore and at all depths compared to other basins (Fig. 1). Of the 23 collecting points examined in the northern basin, only 8 collecting points

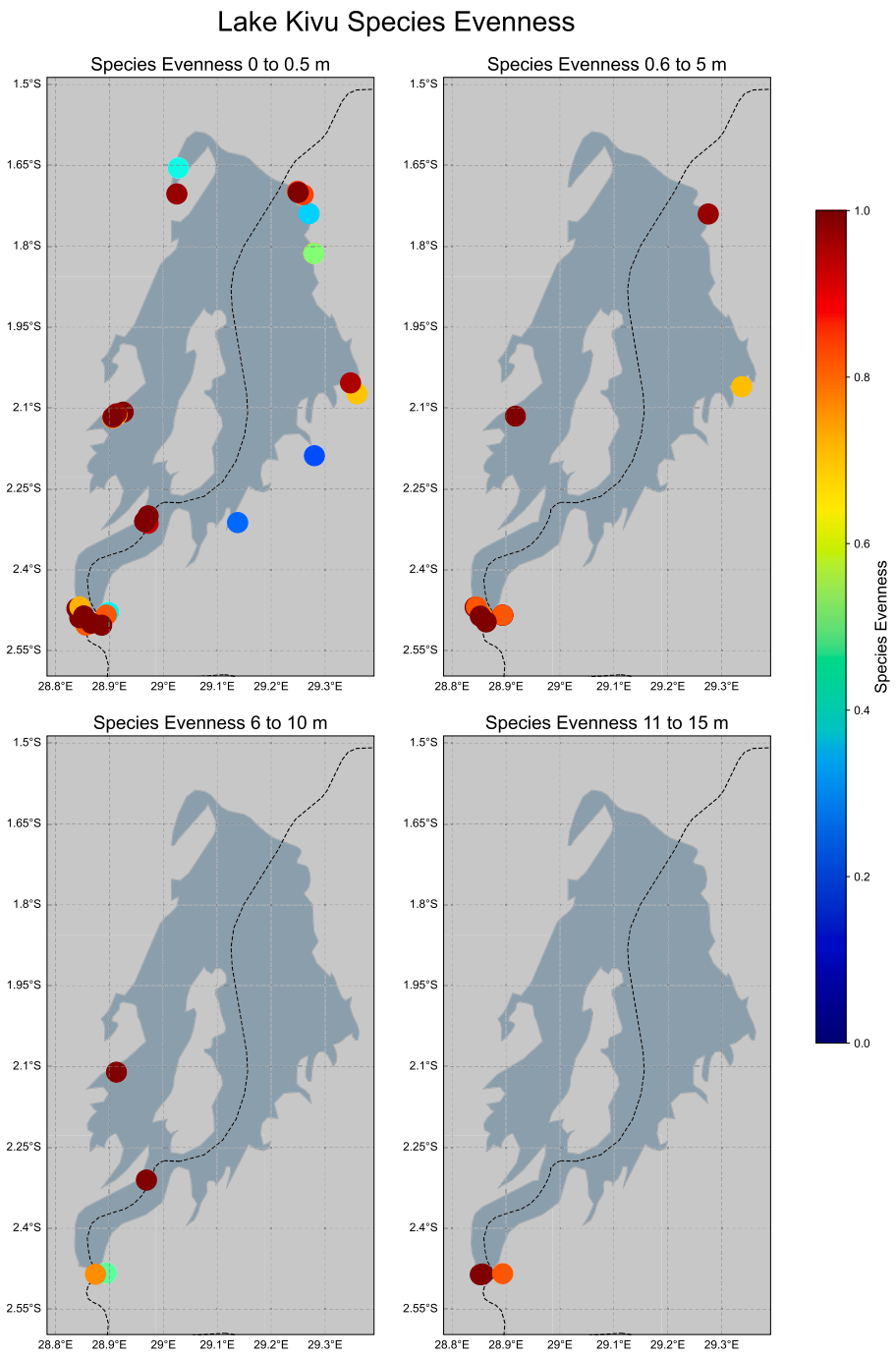

Fig. 3. Heatmap visualisation of evenness of molluscs in Lake Kivu with subplots for the different sampling depths, A: 0 to 0.5 m; B: 0.6 to 5 m; C: 5 to 10 m; D: 11 to 15 m. The colour bar indicate the range of taxa evenness in the different sites and depths.

contained living molluscs, whereas all others had empty shells. In contrast, in the southern basin, we found 30 collecting points with living molluscs and 6 collecting points with only empty shells. In the northern basin, live specimens were found only to a depth of up to 5 m, whereas in the southern basin live species were found up to a depth of 15 m. Although species richness was lower in the northern basin compared to the southern basin, most species are more evenly distributed across sites in the northern basin than in the southern basin (see Figs. 2 & 3).

4. Discussion

4.1. Mollusc diversity and endemism

The mollusc fauna of Lake Kivu has been considered previously to be depauperate (Brown, 1994), reflecting a similar status as for other freshwater organisms (Beadle, 1981; Descy et al., 2012). Here, we present a comprehensive assessment of freshwater molluscs in the Lake Kivu Basin, in fact, one of the most spatially complete surveys of any of the large African lakes. As a result, we present considerable changes to the species list compared to historical records (Table 4). Changes in the list of recognized species relate to new findings during our survey (e.g.

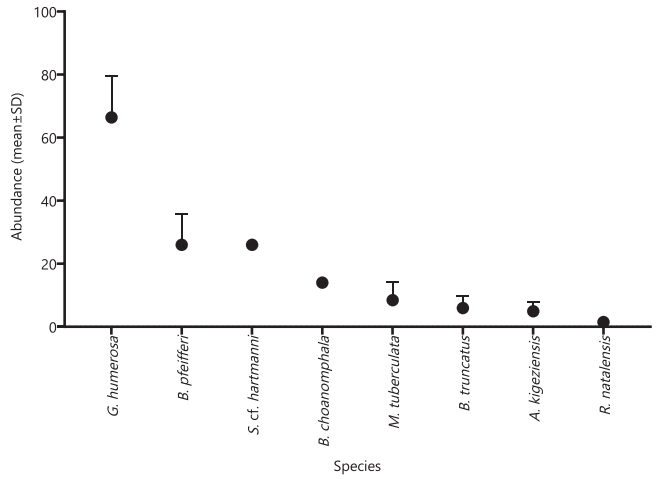

Fig. 4. Average abundances of live molluscs in Lake Kivu. This figure displays the average abundance of live molluscs collected from 26 sites across 40 collection points at varying depths in Lake Kivu. The standard deviation are also presented for reference.

Sphaerium cf. *hartmanni*) but also due to taxonomic changes (e.g. *Afrogyrorbis kigeziensis*). Some taxa that had been previously reported could not be found during our survey (Table 4). Even though our sampling is comparatively dense, it cannot be excluded that a few rare species might have been overlooked, especially small planorbids. As we have complemented morphological examinations with genetic studies, especially for *Biomphalaria*, we consider it unlikely that cryptic but divergent genetic lineages would have been present. Our study casts more doubt on the previous records of *Corbicula* and *Mutela* sp. (Dartevelle and Schwetz, 1947) as we have not been able to obtain any living nor fossil specimens of these taxa.

As in other large rift lakes (Schultheiß et al., 2011; Van Damme et al., 2018; Van Bocxlaer et al., 2012), we found rare and hyperdominant species in Lake Kivu. *Melanoides tuberculata*, *Radix natalensis* and *Gabbiella humerosa* belong to the latter category and are common throughout many places in Africa. Rare species were *Sphaerium* cf. *hartmanni*, and *Biomphalaria choanomphala*, which were each found in only one locality. Such findings outline the possibility of overlooked and/or cryptic species in the tropical regions of Africa as demonstrated for other mollusc genera (e.g. Elderkin et al., 2016). It can also be seen in *Pisidium kenianum* for which at least two different clades have been found in tributaries of Lake Kivu (Clewing et al., 2022) Fig. 7.

Recognizing the presence of cryptic species and illucidating their status requires more extensive surveys and classical taxonomic work. Understanding cryptic diversity is also needed to characterize the level of endemism among local faunas such as that of Lake Kivu, and to characterize patterns and processes of speciation accurately. Currently, the only proposed endemic taxon in Lake Kivu is *Gabbiella humerosa kivuensis* and its relation to other *Gabbiella* species warrants a detailed phylogeographic study covering the diversity and geographic range of the genus.

Our barcoding approach helped to clarify several questions as to species identification that cannot easily be solved with shell-morphological studies alone. Even though not new for studies of poorly known faunas in ancient lakes (Stelbrink et al., 2019; Wiese et al., 2020), a similar genetic characterization has not been used yet for any of the African lakes and should be implemented for other regional faunas of

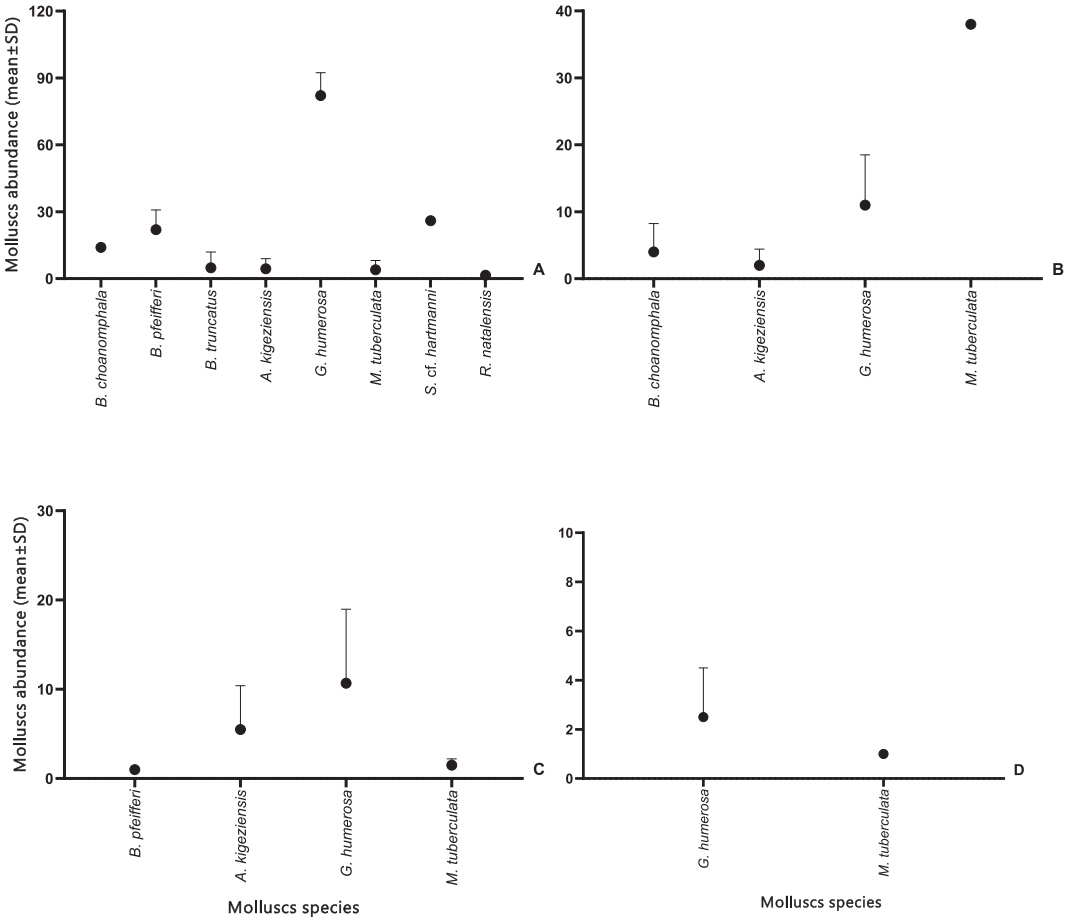

Fig. 5. Mean and standard deviation of snail abundances at different depths. The categories include A: 0 to 0.5 m, B: 0.6 to 5 m, C: 6 to 10 m and D: 11 to 15 m.

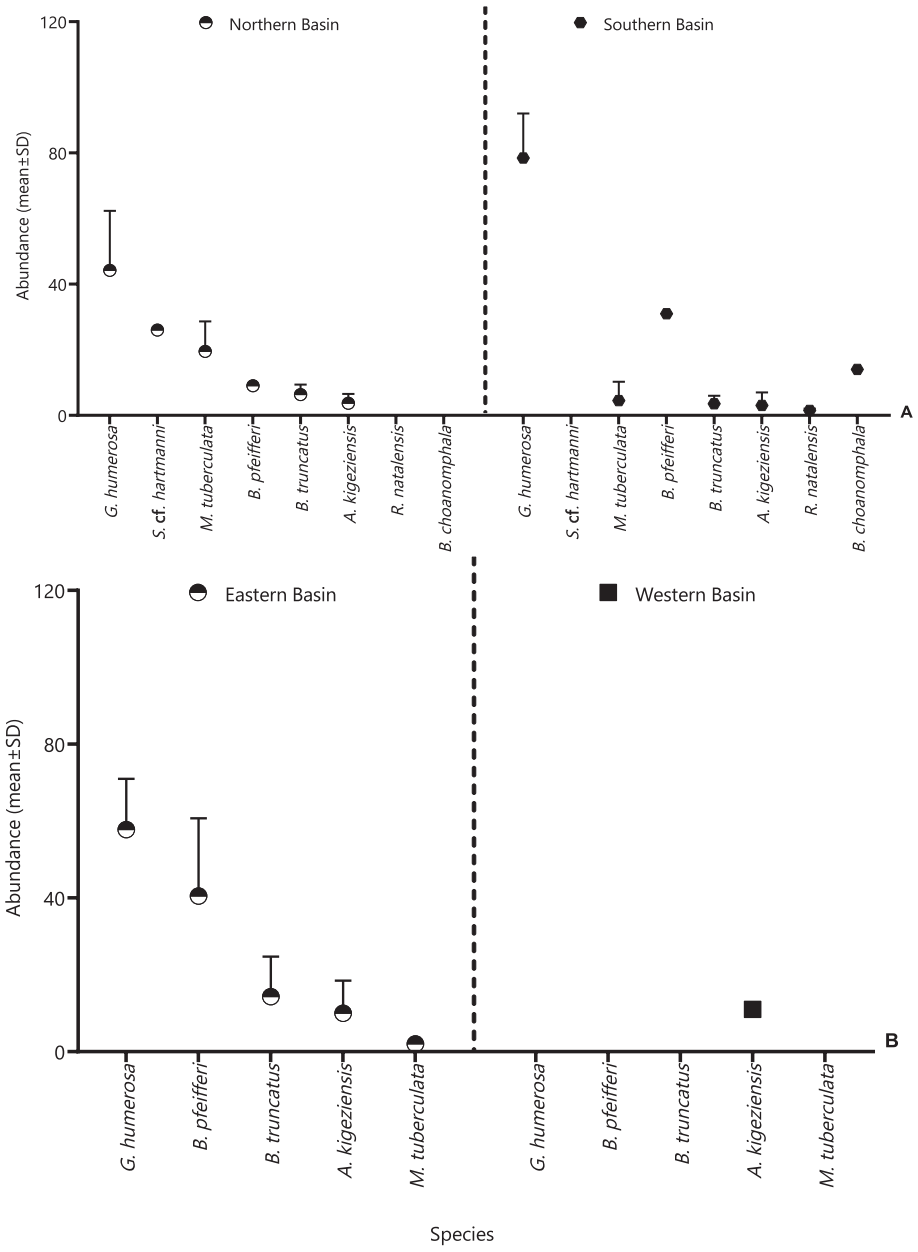

Fig. 6. Abundance and distribution of molluscs species in northern and southern Lake Kivu basin (A) and eastern and western Lake Kivu basin (B). The standard deviation are also presented for reference.

interest. Such approaches will also contribute to a growing reference library of African freshwater molluscs, which is a pre-requisite for studies of whole ecosystems employing technologies such as eDNA metabarcoding. Such studies are becoming more and more common (Schenekar, 2023) although they remain currently rare for Afrotropical lakes (but see Doble et al., 2020). Genetic characterization of species and local populations is also useful to trace faunal connections across scales.

4.2. Biogeography and faunal origin

The mollusc fauna of Lake Kivu resembles that of Lake Edward and as such suggests Nilotic biogeographical relationships. These affinities to Lake Edward likely result from its previous hydrographic connectivity with Lake Kivu (Beadle, 1981) and, as such, they provide evidence of faunal stability over extended time periods despite dramatic changes in the hydrological setting. It would be very interesting to address the faunal origin in a context that involves all Nilotic lakes and the surrounding watersheds (Table 5). It is noteworthy that not a single species of Lake Kivu shows affinities to Congolese taxa or to those of Lake Tanganyika, despite the current hydrographic connectivity of Lake Kivu to Lake Tanganyika and, therefore, to the Congo drainage via the Ruzizi River. These faunal relationships have been observed previously based on morphological similarities (e.g. Pilsbry and Bequaert, 1927; Schultheiß et al., 2011; Van Damme and Van Bocxlaer, 2009).

Beyond geographically restricted species, widespread species may also provide information on faunal origins. For example, *Bulinus truncatus* is more common in northern Africa than elsewhere. Colonization may occur from connected waterbodies or less commonly via long-distance dispersal. For those species found in Lake Kivu which also occur in the studied tributaries, colonization likely took place directly. Interestingly, a significant number of species have not been found in Lake Kivu proper indicating differences in habitat structure or water parameters. *Pisidium* spp., *Physella acuta* and *Bulinus forskalii* were exclusively present in the tributaries whereas some other taxa are

Table 4
Historical account of the malacological exploration and established mollusc species list of Lake Kivu (x = live specimens/F = fossils) for the most comprehensive studies [S1: Pilsbry and Bequaert (1927); S2: Dartevelle and Schwetz (1947); S3: Gillet et al. (1960); S4: Brown (1994)] and our current study (ES). Nomenclature follows MolluscaBase (as of May 2023, except for Pisidium spp.). Previous names refer to names used in the consulted studies (S1–4). [1]Found only in tributaries.

Species	Previous names	S1	S2	S3	S4	ES
GASTROPODA						
Viviparidae						
Bellamya sp.	*Viviparus* sp.		F			
Thiaridae						
Melanoides tuberculata (O.F. Müller, 1774)		x	x/F	x	x	x
Bithyniidae						
Gabbiella humerosa kivuensis Mandahl-Barth, 1968	*Bithynia alberti*	x	x	x	x	x
Lymnaeidae						
Radix natalensis (Krauss, 1848)	*Lymnaea natalensis*		x	x	x	x
Bulinidae						
[1]*Bulinus forskalii* (Ehrenberg, 1831)						x
Bulinus truncatus (Audouin, 1827)	*Bulinus coulboisi*		x		x	x
Physidae						
[1]*Physella acuta* (Draparnaud, 1805)						x
Planorbidae						
Afrogyrorbis kigeziensis (Preston, 1912)	*Ceratophallus kigeziensis*				x	x
Afrogyrorbis natalensis (Kraus, 1848)	*Ceratophallus natalensis, Gyraulus natalensis*			x		
Afrogyrorbis gibbonsi (W. Nelson, 1878)	*Gyraulus gibbonsi, Afrogyrus coretus*		x			
Biomphalaria pfeifferi (Kraus, 1848)				x	x	x
Biomphalaria alexandrina (Ehrenberg, 1831)	*B. boissyi*		F			
Biomphalaria choanomphala (E. v. Martens, 1879)	*B. ruppellii choanomphalus*	x	x/F			x
Biomphalaria smithi Preston, 1910	*B. ruppellii smithi*		F			
Biomphalaria sudanica (E. v. Martens, 1870)	*Planorbis sudanicus*		x/F			
Biomphalaria stanleyi (E. A. Smith, 1888)	*B. ruppellii stanleyi*		x/F			
BIVALVIA						
Cyrenidae						
Corbicula fluminalis (O.F. Müller, 1774)	*Corbicula radiata*		x/F			
Sphaeriidae						
Sphaerium cf. *hartmanni* (Jickeli, 1874)						x
[1]*Pisidium kenianum* Preston, 1911						x
[1]*Pisidium viridarium* Kuiper, 1956						x
Unionidae						
Mutela sp.			F			

exclusively found in the lake, such as *B. choanomphala*, which might not be surprising given that it is a lacustrine species (Gouvras et al., 2017; Standley et al., 2011).

An interesting pattern relates to the presence (or absence) of invasive or non-indigenous species among the molluscs of Lake Kivu. Candidates would have been *Melanoides tuberculata* for which several independent Asian invasions occurred across Africa – including in lakes Malawi and Tanganyika (Van Bocxlaer et al., 2015). However, *Melanoides tuberculata* from Lake Kivu represents a lineage that is native to Africa. A candidate invasive taxon is the globally invasive *Physella acuta*, but so far it was only found in tributaries. This species is known to occur elsewhere in the Lake Kivu region (Lawton et al., 2015; Brown, 1994) and is occasionally reported from sites with some anthropogenic disturbance such as in Lake Naivasha (Brown, 1994) and Lake Victoria (Albrecht et al., unpublished data). Despite historical (geographic) factors, ecological settings may underlie present-day occurrences of molluscs in Lake Kivu.

4.3. Ecology and bioindication

The N-S differences in species richness and the total absence of life molluscs in some northern localities can be linked to the Holocene history of Lake Kivu (Ross et al., 2014) when repeated lava intrusions into the lake occurred from the Virunga volcanoes. These intrusions created (underwater) heatwaves and complete overflow of vast parts of the littoral substrates that would have wiped out entire benthic communities. The latest major lava intrusion dates back to a period from 1938 to 1940 (Beadle, 1981), but similar events would have happened frequently throughout the Holocene (Ross et al., 2014). Additionally, sublacustrine volcanic eruptions also occurred repeatedly during the last 1000 years (Ross et al., 2014), which would have changed the limnological conditions at least temporarily and locally. These events may explain the substantial littoral deposits of subfossil shells, especially along the northern shorelines of the lake. Preservation of such shells (instead of dissolution) is supported furthermore by the high alkalinity of the lake in general. Currently, it is not clear whether ecosystem calamities have been affecting the total benthos of the whole lake or primarily populations in the northern Basin, but concordant patterns in other organisms indicate that effects are more severe in the North. Ash layers from subaerial eruptions sinking in the water column additionally could have caused extinction waves among all Lake Kivu mollusc populations. Such effects have been demonstrated in other lakes worldwide (e.g. Wagner et al., 2014; Jovanovska et al., 2016).

Benthic communities in other African Great Lakes have been shaped by Pleistocene lake level low stands (Schultheiß et al., 2009; Van Bocxlaer, 2017; Van Bocxlaer et al., 2021), and this might have also been the case in Lake Kivu to some extent (e.g. Ross et al., 2014). The more recent volcanic eruptions and the general physico-chemical peculiarities of the lake, including high salinity might have had a greater effect, however, on the composition of the depauperate communities existing today. As a result, macrophytes and soft substrates rich in organic-rich detritus are relatively scarce, so that habitat diversity is low, contributing further to the factors explaining why mollusc communities in Lake Kivu on average only contained 2.5 (1–5) species, which is lower than in the majority of other African rift lakes. Even though most Lake Kivu mollusc species are common, their total biomass remains comparatively small to that in the large Nilotic lakes (Verbeke, 1957; Lange et al., 2013). An interesting pattern relates to the abundance distribution of molluscs along the depth of the lake. Here, a significant extension of live populations has been found for all species compared to the data reported in Gillet et al. (1960) who found live snails (*Melanoides*) at a maximum depth of 7 m only. Here, we found living *Melanoides* down to 15 m and all other species also extend 2 to 3 times deeper than previously known (Fig. 5). The steep decline of mollusc diversity in deeper waters likely relates to oxygen availability and it has been shown that metazoan life is restricted to the upper 40 m of the lake (Richard et al., 2020). The data of Gillet et al. (1960) are from the 1950s and were restricted to the Bukavu region which, however, is one of the more diverse parts of the lake even nowadays. The contrast might be reflecting our sampling effort simply or might hint to the extension of mollusc populations. This has been shown in other African Rift lakes where multiple factors such as overfishing, eutrophication, habitat modification, and invasive plants have led to significant changes in benthic communities and much-extended molluscs abundances for common species and a decline of specialists (e.g. Beeton, 2002). This is significant since molluscs are an important, sometimes dominant component of benthic communities in tropical lakes. Interestingly, both species richness and abundance were

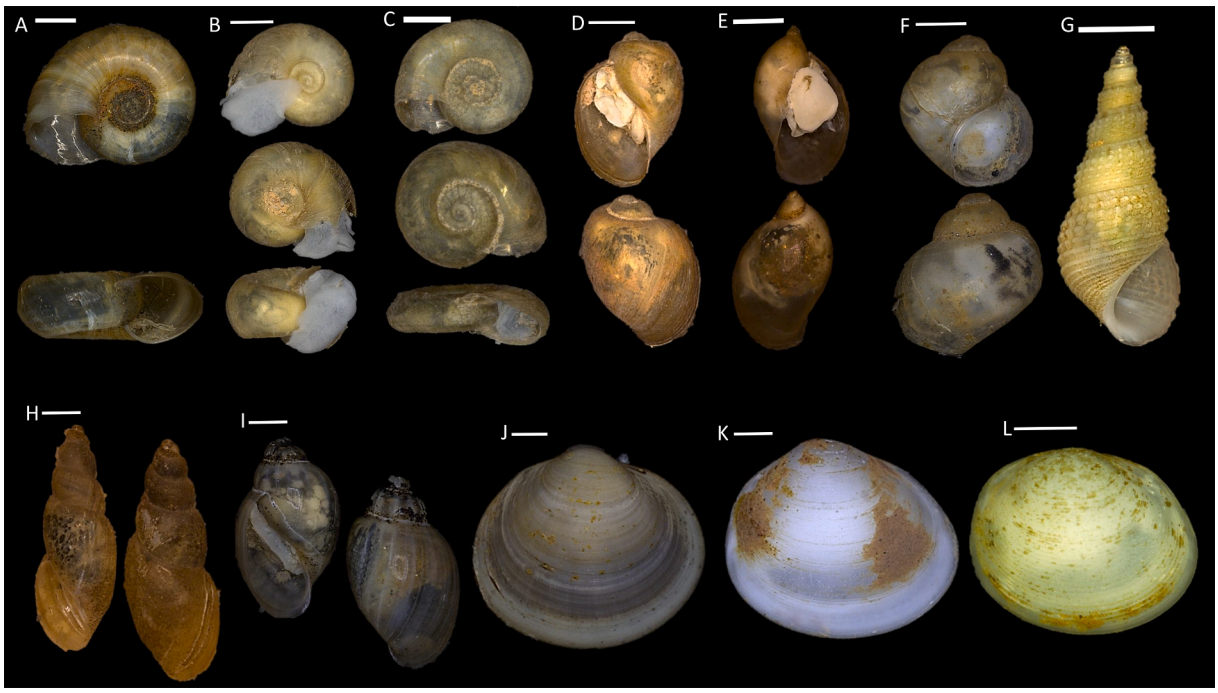

Fig. 7. Mollusc species collected in Lake Kivu and its tributaries (Rwandan side): (A) *Biomphalaria pfeifferi*; (B) *Biomphalaria choanomphala*; (C) *Afrogyrorbis kigeziensis*; (D) *Bulinus truncatus*; (E) *Radix natalensis*; (F) *Gabbiella humerosa*; (G) *Melanoides tuberculata*; (H) *Bulinus forskalii*; (I) *Physella acuta*; (J) *Sphaerium* cf. *hartmanni*; (K) *Pisidium kenianum*; (L) *Pisidium* cf. *viridarium*. Scale bar: 1000 μm.

related to depth (see above) because of a steep decline in oxygen, and changes in salinity but also related to the coarse anthropogenic disturbance measure we employed. This type of disturbance can be expected to even increase in the near future given the ever-growing urban centres at both ends of the lake (Bukavu, Goma-Gisenyi). Given that the anthropogenic impact is observed in even the species-poor mollusc communities of Lake Kivu, supports the potential of lacustrine mollusc species as bio-indicators of littoral conditions. Such indicators are mostly developed at the family level though, and they have been successfully used in lotic systems (Masese et al., 2023; Tumusiime et al., 2019; Dusabe et al., 2019; Wronski et al., 2015). Very recently, biodiversity indicators have been successfully developed for stretches of shoreline along the Congolese side of Lake Kivu (Hyangya et al., 2022), but similar efforts did not work in the Ruzizi River (Dusabe et al., 2022). Enhancing the taxonomic resolution to species level in species-poor communities may offer a solution and should be explored more systematically in other African lakes systems and across taxa. Another interesting factor to which molluscs react sensitively is climate change, specifically to rising temperatures in African lakes (Van Bocxlaer et al., 2012; Cohen et al., 2016). It would be worthwhile to monitor changes in the lake community given that a temperature rise has already been demonstrated in Lake Kivu (Katsev et al., 2014).

4.4. Biomedical implications

Our malacological survey has biomedical implications for both riparian people and their livestock because all genera that typically act as intermediate host species (IHS) for the parasites causing schistosomiasis and fasciolosis are prominently present in the study area. For *Bulinus*, *Bulinus truncatus* only has been identified genetically. This species is a prominent IHS in northern Africa, but it does not seem to be involved in schistosome transmission in equatorial regions, although this condition is to be verified (Brown, 1994). The very few studies available indicate absence of urogenital schistosomiasis in Rwanda (Kagabo et al., 2023; Rujeni et al., 2017), but a more comprehensive study is needed to verify its absence. It is noteworthy that in close geographical proximity, several *Bulinus* species serve as intermediate hosts, namely in Tanzania, Uganda (Babbitt et al., 2023), and the Democratic Republic of Congo (Madinga et al., 2015). *B. truncatus* is also an intermediate host for bovine schistosomiasis (Brown, 1994).

Intestinal schistosomiasis is very common in the Lake Kivu basin and surrounding areas (Clark et al., 2019; Kagabo et al., 2023). We demonstrated the presence of at least two *Biomphalaria* species in the Lake Kivu catchment, namely *B. pfeifferi* and *B. choanomphala* which are often invoked in the transmission of *Schistosoma mansoni*. It has been thought that *B. chonaomphala* is endemic to Lake Victoria (Standley et al., 2011, 2014); however, here we reported it from Lake Kivu too. Identifying which species exactly are involved regionally in transmission is crucial to understand transmission dynamics and to develop potential targeted eradication strategies (Tabo et al., 2024). For example, there is discussion on whether *B. choanomphala* represents a separate species or whether it is synonymous with *B. sudanica* (Standley, 2011, 2014; Zhang et al., 2018). Overall, the findings of our study call for a detailed assessment involving parasitological studies on many of the *Biomphalaria* and *Bulinus* populations in the Kivu basin. Studies on other lakes such as Lake Malawi have shown that transmission patterns can change on short timescales and are often related to human ecosystem alterations (Van Bocxlaer et al., 2014). Our knowledge of IHS transmitting human diseases remains limited in the Lake Kivu catchment, but it is even scarcer for veterinary diseases such as fasciolosis, which is transmitted by lymnaeid snails (e.g. Ngcamphalala et al., 2022; Mahulu et al., 2019; Sun et al., 2020). It can be assumed that transmission occurs along the shoreline of Lake Kivu and its tributaries given the abundance of *Radix natalensis*, but to what extent it is a major burden requires further studies.

5. Conclusions

Our study revealed that the malacofauna of Lake Kivu, though admittedly comparatively poor, is more diverse than previously anticipated. The occurrence of living populations is restricted to a small ring of littoral substrates even though some species penetrate to a maximum

Table 5
Biogeographical relationships of the mollusks of Lake Kivu. The affinities listed a based on molecular studies of phylogenetic relationship of mollusc species wherever available. In cases where no phylogenies have been published or no molecular data are available the standard literature is referred to.

Species	Phylogenetic relationship	Biogeographical affinity	Reference
GASTROPODA			
Thiaridae			
Melanoides tuberculata	African lineage of *M. tuberculata*, i.e. not Asian invasives	Widespread in tropical Africa	Brown (1994), Van Bocxlaer et al. (2015)
Bithyniidae			
Gabbiella humerosa kivuensis	n.a.; endemic subspecies	Large lakes from Albert, Tanganyika to Victoria for *Gabbiella humerosa*	Brown (1994), Mandahl-Barth (1968)
Lymnaeidae			
Radix natalensis	*Radix* s.str.	Wide-spread in African freshwaters, potentially Asian affinities	Aksenova et al. (2018), Albrecht et al. (2023), Brown (1994)
Bulinidae			
Bulinus truncatus	East Africa, Lake Victoria	Widespread throughout Africa but mainly in northern parts of the continent	Brown (1994), Tumwebaze et al. (2019)
Planorbidae			
Ceratophallus kigeziensis	n.a.	Lakes Victoria and Edward	Brown (1994)
Biomphalaria choanomphala	East Africa, Uganda lineage	Lakes Victoria and Albert, Albert Nile	Brown (1994), Joergensen et al., 2007
Biomphalaria pfeifferi	East Africa, Kenya lineage	Tropical Africa	Brown (1994)
BIVALVIA			
Sphaeriidae			
Sphaerium cf. *hartmanni*	n.a.	East Africa	Mandahl-Barth (1988)
[1]*Pisidium kenianum*	*P. viridarium, P. pirothi, P. ethiopicum*	Ethiopia to Zambia	Clewing et al. (2022), Mandahl-Barth (1988)
[1]*Pisidium viridarium*	*P. kenianum, P. pirothi, P. ethiopicum*	Ethiopia to South Africa	Clewing et al. (2022), Mandahl-Barth (1988)

depth of 15 m. The fauna displays 'Nilotic' biogeographic affinities although widespread taxa dominate. Species richness and abundance is more elevated in the south compared to the north, which can be attributed to recent historical ecosystem stresses (volcanic eruptions and associated disturbance) but also to current and ongoing anthropogenic effects. More molecular characterization of African freshwater molluscs is needed for intra- and supraregional comparisons and biodiversity estimates but also to characterize communities for future eDNA studies. A detailed study of schistosomiasis and fasciolosis at high spatial resolution along the shorelines and tributaries of Lake Kivu is recommended.

CRediT authorship contribution statement

MCD collected data, performed laboratory identification, and wrote the draft article; CK performed ecological data analyses and contributed to writing the manuscript; CC contributed to the DNA data analyses and writing the MS; BH contributed to data collection; VB contributed to wiring and revising the MS; CA contributed to the conceptualization of the topic and writing and revising the manuscript. All authors read and approved the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2024.102339.

References

Amisi, M.F., Mulungula, M.P., Kisse, K.T., Muhigirwa, B.C., Natacha, P., Lwikitcha, H.B., Lawrence, T., 2022. Current status and strategic way forward for long-term management of Lake Kivu (East Africa). J. Great Lakes Res. 49, 102024. https://doi.org/10.1016/j.jglr.2022.04.004.

Babbitt, C.R., Laidemitt, M.R., Mutuku, M.W., Oraro, P.O., Brant, S.V., Mkoji, G.M., Loker, E.S., 2023. *Bulinus* snails in the Lake Victoria Basin in Kenya: systematics and their role as hosts for schistosomes. PLoS Negl. Trop. Dis. 17 (2), e0010752. https://doi.org/10.1371/journal.pntd.0010752.

Balagizi, C.M., Kies, A., Kasereka, M.M., Tedesco, D., Yalire, M.M., McCausland, W.A., 2018. Natural hazards in Goma and the surrounding villages. East African Rift System. Nat. Hazards 93, 31–66. https://doi.org/10.1007/s11069-018-3288-x.

Beadle, L.C., 1981. The inland waters of tropical Africa: an introduction to tropical limnology (no.edn 2). Longman Group, London.

Beeton, A.M., 2002. Large freshwater lakes: present state, trends, and future. Environ. Conserv. 29 (1), 21–38. https://doi.org/10.1017/S0376892902000036.

Bergonzini, L. (1998). Bilans hydriques de lacs (Kivu, Tanganyika, Rukwa et Nyassa) du rift est-africain. Musée Royal de l'Afrique Centrale, Tervuren, Belgique: Annales - Serie in 8° - Sciences Géologiques Vol. 103.

Brown, D., 1994. Freshwater snails of Africa and their medical importance, 2nd edition. Taylor & Francis, London, p. 609.

Clark, N.J., Umulisa, I., Ruberanziza, E., Owada, K., Colley, D.G., Ortu, G., Turate, I., 2019. Mapping *Schistosoma mansoni* endemicity in Rwanda: a critical assessment of geographical disparities arising from circulating cathodic antigen versus Kato-Katz diagnostics. PLoS Negl. Trop. Dis. 13 (9), e0007723. https://doi.org/10.1371/journal.pntd.0007723.

Clement, M., Posada, D.C.K.A., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9 (10), 1657–1659. https://doi.org/10.1046/j.1365-294x.2000.01020.x.

Clewing, C., Geertz, T., Rassam, H., Woldekiros, T.H., Albrecht, C., 2022. Freshwater diversity at a biogeographic edge zone: the high-mountain pea-clams of Ethiopia. Syst. Biodivers. 20 (1), 1–15. https://doi.org/10.1080/14772000.2021.2005706.

Cohen, A.S., Gergurich, E.L., Kraemer, B.M., McGlue, M.M., McIntyre, P.B., Russell, J.M., Swarzenski, P.W., 2016. Climate warming reduces fish production and benthic habitat in Lake Tanganyika, one of the most biodiverse freshwater ecosystems. Proc. Natl. Acad. Sci. USA 113 (34), 9563–9568. https://doi.org/10.1073/pnas.1603237113.

d'Oreye, N., González, P.J., Shuler, A., Oth, A., Bagalwa, L., Ekström, G., Fernández, J., 2011. Source parameters of the 2008 Bukavu-Cyangugu earthquake estimated from InSAR and teleseismic data. Geophys. J. Int. 184 (2), 934–948. https://doi.org/10.1111/j.1365-246X.2010.04899.x.

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: More models, new heuristics and parallel computing. Nat. Methods 9 (8), 772. https://doi.org/10.1038/nmeth.2109.
Dartevelle, E., Schwetz, J., 1947. Contribution à l'étude de la faune malacologique des grands lacs africains: les lacs Albert, Édouard et Kivu. Mem. Inst. Royal Colonial Belge. 14 (4), 1–47.
Degens, E.T., von Herzen, R.P., Wong, H.K., Deuser, W.G., Jannasch, H.W., 1973. Lake Kivu: structure, chemistry and biology of an East African rift lake. Geol. Rundsch. 62, 245–277. https://doi.org/10.1007/BF01826830.
Descy, J.P., Darchambeau, F., Schmid, M., 2012. Lake Kivu research: conclusions and perspectives. In: Descy, J.-P., Darchambeau, F., Schmid, M. (Eds.), Lake Kivu: Limnology and biogeochemistry of a tropical great lake, Vol. 15. Springer, Berlin, pp. 181–190. https://doi.org/10.1007/978-94-007-4243-7_11.
Dorić, V., Pozojević, I., Vučković, N., Ivković, M., Mihaljević, Z., 2021. Lentic chironomid performance in species-based bioassessment proving: high-level taxonomy is not a dead end in monitoring. Ecol. Indic. 121, 107041. https://doi.org/10.1016/j.ecolind.2020.107041.
Dusabe, M.C., Wronski, T., Gomes-Silva, G., Plath, M., Albrecht, C., Apio, A., 2019. Biological water quality assessment in the degraded Mutara rangelands, northeastern Rwanda. Environ. Monit. Assess. 191, 139. https://doi.org/10.1007/s10661-019-7226-5.
Dusabe, M.C., Neubauer, T.A., Muvundja, F.A., Hyangya, B.L., Albrecht, C., 2022. Family-level bio-indication does not detect the impacts of dams on macroinvertebrate communities in a low-diversity tropical river. Front. Environ. Sci. 10, 902246. https://doi.org/10.3389/fenvs.2022.902246.
Elderkin, C.L., Clewing, C., Wembo Ndeo, O., Albrecht, C., 2016. Molecular phylogeny and DNA barcoding confirm cryptic species in the African freshwater oyster *Etheria elliptica* Lamarck, 1807 (Bivalvia: Etheriidae). Biol. J. Linn. Soc. 118 (2), 369–381. https://doi.org/10.1111/bij.12734.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3 (5), 294–299.
Gillet, J., Bruaux, P., Wolfs, J., 1960. Study of the depths at which molluscs occur in Lake Kivu and of the survival of *Biomphalaria* in deep water. Ann. Soc. Belg. Med. Trop. 40 (4), 643–649.
Gouvras, A.N., Allan, F., Kinung'hi, S., Rabone, M., Emery, A., Angelo, T., Rollinson, D., 2017. Longitudinal survey on the distribution of *Biomphalaria sudanica* and *B. choanomophala* in Mwanza region, on the shores of Lake Victoria, Tanzania: implications for schistosomiasis transmission and control. Parasit. Vectors 10, 316. https://doi.org/10.1186/s13071-017-2252-z.
Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser. 41, 95–98.
Hecky, R.E., Degens, E.T., 1973. Late Pleistocene-Holocene chemical Stratigraphy and Paleolimnology of the Rift Valley Lakes of Central Africa. Woods Hole Oceanographic Institution. Technical Report (no. 73-28).
Hilbe, J.M., 2011. Negative binomial regression. Cambridge University Press, Cambridge.
Hilbe, J.M., 2014. Modeling count data. Cambridge University Press, Cambridge.
Hirslund, F., Morkel, P., 2020. Managing the dangers in Lake Kivu - how and why. J. Afr. Earth Sci. 161, 103672. https://doi.org/10.1016/j.jafrearsci.2019.103672.
Houbraken, M., Habimana, V., Senaeve, D., López-Dávila, E., Spanoghe, P., 2017. Multi-residue determination and ecological risk assessment of pesticides in the lakes of Rwanda. Sci. Total Environ. 576, 888–894. https://doi.org/10.1016/j.scitotenv.2016.10.127.
Hyangya, B.L., Kankonda, A.B., Dusabe, M.C., Murhimanya, J.D.K., Kaningini, B.M., Masilya, P.M., 2022. Choice of benthic macroinvertebrate-based metrics for assessing water quality in the littoral zone under anthropogenic disturbance in southern Lake Kivu (East Africa). Ecohydrology. 15 (8), e2468.
Joergensen, A., Kristensen, T.K., Stothard, J.R., 2007. Phylogeny and biogeography of African *Biomphalaria* (Gastropoda: Planorbidae), with emphasis on endemic species of the great East African lakes. Zool. J. Linn. Soc. 151 (2), 337–349.
Johnson, P.D., 2009. Freshwater snail biodiversity and conservation. In: Davis, M. (Ed.), Sustaining America's aquatic biodiversity. Virginia Cooperative Extension, Publication, pp. 420–530. https://www.pubs.ext.vt.edu/420/420-530/420-530.html.
Jones, N., 2003. Chock-full of methane, Lake Kivu stores enough energy to power all of Rwanda. New Sci. 177, 2384.
Jones, N., 2021. How dangerous is Afric'a explosive Lake Kivu? Nature 597 (7877), 466–469.
Jovanovska, E., Cvetkoska, A., Hauffe, T., Levkov, Z., Wagner, B., Sulpizio, R., Wilke, T., 2016. Differential resilience of ancient sister lakes Ohrid and Prespa to environmental disturbances during the Late Pleistocene. Biogeosciences 13 (4), 1149–1161. https://doi.org/10.5194/bg-13-1149-2016.
Kagabo, J., Kalinda, C., Nshimiyimana, P., Mbonigaba, J.B., Ruberanziza, E., Nyandwi, E., Rujeni, N., 2023. Malacological survey and spatial distribution of intermediate host snails in schistosomiasis endemic districts of Rwanda. Trop. Med. Infect. Dis. 8 (6), 295. https://doi.org/10.3390/tropicalmed8060295.
Katsev, S., Aaberg, A.A., Crowe, S.A., Hecky, R.E., 2014. Recent warming of Lake Kivu. PLoS ONE 9 (10), e109084.
Lange, C.N., Kristensen, T.K., Madsen, H., 2013. Gastropod diversity, distribution and abundance in habitats with and without anthropogenic disturbances in Lake Victoria, Kenya. Afr. J. Aquat. Sci. 38 (3), 295–304. https://doi.org/10.2989/16085914.2013.797380.
Lawton, S.P., Lim, R.M., Dukes, J.P., Kett, S.M., Cook, R.T., Walker, A.J., Kirk, R.S., 2015. Unravelling the riddle of *Radix*: DNA barcoding for species identification of freshwater snail intermediate hosts of zoonotic digeneans and estimating their inter-population evolutionary relationships. Infect. Genet. Evol. 35, 63–74. https://doi.org/10.1016/j.meegid.2015.07.021.
Madinga, J., Linsuke, S., Mpabanzi, L., Meurs, L., Kanobana, K., Speybroeck, N., Polman, K., 2015. Schistosomiasis in the Democratic Republic of Congo: a literature review. Parasit. Vectors 8, 601. https://doi.org/10.1186/s13071-015-1206-6.
Mahulu, A., Clewing, C., Stelbrink, B., Chibwana, F.D., Tumwebaze, I., Russell Stothard, J., Albrecht, C., 2019. Cryptic intermediate snail host of the liver fluke *Fasciola hepatica* in Africa. Parasite. Vector. 12 (1), 573. https://doi.org/10.1186/s13071-019-3825-9.
Mandahl-Barth, G., 1988. Studies on African freshwater bivalves. Danish Bilharziasis Laboratory, Charlottenlund.
Masese, F.O., Wanderi, E.W., Nyakeya, K., Achieng, A.O., Fouchy, K., McClain, M.E., 2023. Bioassessment of multiple stressors in Afrotropical rivers: Evaluating the performance of a macroinvertebrate-based index of biotic integrity, diversity, and regional biotic indices. Front. Environ. Sci. 11, 1015623. https://doi.org/10.3389/fenvs.2023.1015623.
Moeyersons, J., Tréfois, P., Lavreau, J., Alimasi, D., Badriyo, I., Mitima, B., Nahimana, L., 2004. A geomorphological assessment of landslide origin at Bukavu, Democratic Republic of the Congo. Eng. Geol. 72 (1–2), 73–87. https://doi.org/10.1016/j.enggeo.2003.06.003.
Muvundja, F.A., Pasche, N., Bugenyi, F.W., Isumbisho, M., Müller, B., Namugize, J.N., Wüest, A., 2009. Balancing nutrient inputs to Lake Kivu. J. Great Lakes Res. 35 (3), 406–418. https://doi.org/10.1016/j.jglr.2009.06.002.
Muvundja, F.A., Wüest, A., Isumbisho, M., Kaningini, M.B., Pasche, N., Rinta, P., Schmid, M., 2014. Modelling Lake Kivu water level variations over the last seven decades. Limnologica 47, 21–33. https://doi.org/10.1016/j.limno.2014.02.003.
Ngcamphalala, P.I., Malatji, M.P., Mukaratirwa, S., 2022. Geography and ecology of invasive *Pseudosuccinea columella* (Gastropoda: Lymnaeidae) and implications in the transmission of *Fasciola* species (Digenea: Fasciolodae) - a review. J. Helminthol. 96, e1. https://doi.org/10.1017/S0022149X21000717.
Nyamalyongo, M.M., Makuku, O., Bondekwe, J.C.A., Kabesha, B.T., Tsongo, K.Z., Wembonyama, O.S., 2022. Adolescents' knowledge, attitude and utilization of emergency contraceptive in Idjwi Island in the Democratic Republic of the Congo. Int. J. Fam Commun. Med. 6 (4), 145–151. https://doi.org/10.15406/ijfcm.2022.06.00280.
Palumbi, S., 1991. The simple fool's guide to PCR. University of Hawaii, Honolulu.
Pilsbry, H.A., Bequaert, J., 1927. The aquatic mollusks of the Belgian Congo, with a geographical and ecological account of Congo malacology. Bull. Am. Mus. Nat. Hist. 53, 69–602.
Rambaut, A., 2014. FigTree v1.4.2, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree/.
Richard, A., Caudron, A., Chanez, E., Decourciere, H., Frossard, V., Guillard, J., Descy, J. P., 2020. Conducting fish studies on Lake Kivu and reinforcement of plankton capacities. Research Report. UMR CARRTEL 105p.
Ross, K.A., Smets, B., De Batist, M., Hilbe, M., Schmid, M., Anselmetti, F.S., 2014. Lake-level rise in the late Pleistocene and active subaquatic volcanism since the Holocene in Lake Kivu, East African rift. Geomorphology 221, 274–285. https://doi.org/10.1016/j.geomorph.2014.05.010.
Rujeni, N., Morona, D., Ruberanziza, E., Mazigo, H.D., 2017. Schistosomiasis and soil-transmitted helminthiasis in Rwanda: an update on their epidemiology and control. Infect. Dis. Poverty 6 (1), 8. https://doi.org/10.1186/s40249-016-0212-z.
Salzburger, W., Van Bocxlaer, B., Cohen, A.S., 2014. Ecology and evolution of the African Great Lakes and their faunas. Annu. Rev. Ecol. Evol. Syst. 45, 519–545. https://doi.org/10.1146/annurev-ecolsys-120213-091804.
Sarmento, H., Leitao, M., Stoyneva, M., Compere, P., Coute, A., Isumbisho, M., Descy, J. P., 2007. Species diversity of pelagic algae in Lake Kivu (East Africa). Cryptogam. Algol. 28 (3), 245–270.
Schenekar, T., 2023. The current state of eDNA research in freshwater ecosystems: are we shifting from the developmental phase to standard application in biomonitoring? Hydrobiologia. 850 (6), 1263–1282. https://doi.org/10.1007/s10750-022-04891-z.
Schmid, M., Halbwachs, M., Wehrli, B., Wüest, A., 2005. Weak mixing in Lake Kivu: new insights indicate increasing risk of uncontrolled gas eruption. Geochem. Geophys. Geosyst. 6 (7) https://doi.org/10.1029/2004GC000892.
Schmid, M., Bärenbold, F., Wüest, A.J., 2021. Methane extraction from Lake Kivu - scientific background. Eawag, Kastanienbaum. Available at: https://www.dora.lib4ri.ch/eawag/islandora/object/eawag%3A22449.
Schultheiß, R., Van Bocxlaer, B., Wilke, T., Albrecht, C., 2009. Old fossils–young species: evolutionary history of an endemic gastropod assemblage in Lake Malawi. Proc. R. Soc. B 276, 2837–2846. https://doi.org/10.1098/rspb.2009.0467.
Schultheiß, R., Wembo Ndeo, O., Malikwisha, M., Marek, C., Böß neck, U., Albrecht, C., 2011. Freshwater molluscs of the Eastern Congo: notes on taxonomy, biogeography and conservation. Afr. Invertebr. 52 (2), 265–284. https://doi.org/10.5733/afin.052.0204.
Smets, B., Tedesco, D., Kervyn, F., Kies, A., Vaselli, O., Yalire, M.M., 2010. Dry gas vents ("mazuku") in Goma region (North-Kivu, Democratic Republic of Congo): formation and risk assessment. J. Afr. Earth Sci. 58 (5), 787–789. https://doi.org/10.1016/j.jafrearsci.2010.04.008.
Snoeks, J., 1994. The haplochromines (Teleostei, Cichlidae) of Lake Kivu (East Africa): a taxonomic revision with notes on their ecology. Ann. Mus. R. Afr. Centr. Tervuren Sci. Zool. 270, 1–221.
Snoeks, J., Kaningini, B., Masilya, P., Nyina-Wamwiza, L., Guillard, J., 2012. Fishes in Lake Kivu: diversity and fisheries. In: Descy, J.-P., Darchambeau, F., Schmid, M. (Eds.), Lake Kivu: Limnology and Biogeochemistry of a Tropical Great Lake. Springer, Dordrecht, pp. 127–152.

Standley, C.J., Wade, C.M., Stothard, J.R., 2011. A fresh insight into transmission of schistosomiasis: a misleading tale of *Biomphalaria* in Lake Victoria. PLoS ONE 6 (10), e26563.

Standley, C.J., Goodacre, S.L., Wade, C.M., Stothard, J.R., 2014. The population genetic structure of *Biomphalaria choanomphala* in Lake Victoria, East Africa: implications for schistosomiasis transmission. Parasit. Vectors 7, 524. https://doi.org/10.1186/s13071-014-0524-4.

Stelbrink, B., von Rintelen, T., Albrecht, C., Clewing, C., Naga, P.O., 2019. Forgotten for decades: Lake Lanao and the genetic assessment of its mollusc diversity. Hydrobiologia 843, 31–49. https://doi.org/10.1007/s10750-018-3666-0.

Stoffers, P., Hecky, R.E., 1978. Late Pleistocene - holocene evolution of the Kivu-Tanganyika Basin. In: Matter, A., Tucker, M.E. (Eds.), Modern and Ancient Lake Sediments. Blackwell Scientific Publications, Oxford, pp. 43–55.

Sun, P., Wronski, T., Apio, A., Edwards, L., 2020. A holistic model to assess risk factors of fasciolosis in ankole cattle. Vet. Parasitol. Reg. Stud. Reports 22, 100488. https://doi.org/10.1016/j.vprsr.2020.100488.

Tabo, Z., Breuer, L., Fabia, C., Gorata, S., Albrecht, C., 2024. A machine learning approach for modeling the occurrence of the major intermediate hosts for schistosomiasis in East Africa. Sci. Rep. 14, 4274. https://doi.org/10.1038/s41598-025-54699-1.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7), 3022–3027. https://doi.org/10.1093/molbev/msab120.

Tumusiime, J., Tolo, C.U., Dusabe, M.C., Albrecht, C., 2019. Reliability of the Tanzania river scoring system (TARISS) macroinvertebrate index of water quality: a case study of the river Mpanga system, Uganda. J. Freshw. Ecol. 34 (1), 541–557. https://doi.org/10.1080/02705060.2019.1631895.

Tumwebaze, I., Clewing, C., Dusabe, M.C., Tumusiime, J., Kagoro-Rugunda, G., Hammoud, C., Albrecht, C., 2019. Molecular identification of *Bulinus* spp. intermediate host snails of *Schistosoma* spp. in crater lakes of western Uganda with implications for the transmission of the *Schistosoma haematobium* group parasites. Parasit. Vectors 12, 565. https://doi.org/10.1186/s13071-019-3811-2.

Van Bocxlaer, B., Schultheiss, R., Plisnier, P.D., Albrecht, C., 2012. Does the decline of gastropods in deep water herald ecosystem change in lakes Malawi and Tanganyika? Freshw. Biol. 57 (8), 1733–1744. https://doi.org/10.1111/j.1365-2427.2012.02828.x.

Van Bocxlaer, B., Albrecht, C., Stauffer, J.R., 2014. Growing population and ecosystem change increase human schistosomiasis around Lake Malaŵi. Trends Parasitol. 30 (5), 217–220. https://doi.org/10.1016/j.pt.2014.02.006.

Van Bocxlaer, B., Clewing, C., Mongindo Etimosundja, J.P., Kankonda, A., Wembo Ndeo, O., Albrecht, C., 2015. Recurrent camouflaged invasions and dispersal of an Asian freshwater gastropod in tropical Africa. BMC Evol. Biol. 15 (1), 33. https://doi.org/10.1186/s12862-015-0296-2.

Van Bocxlaer, B., Clewing, C., Duputié, A., Roux, C., Albrecht, C., 2021. Population collapse in viviparid gastropods of the Lake Victoria ecoregion started before the last glacial maximum. Mol. Ecol. 30 (2), 364–378. https://doi.org/10.1111/mec.15599.

Van Bocxlaer, B., 2017. Hierarchical structuring of ecological and non-ecological speciation processes of differentiation shaped ongoing gastropod radiation in the Malawi Basin. Proc. R. Soc. B. 284, 20171494. https://doi.org/10.1098/rspb.2017.1494.

Van Damme, D., Van Bocxlaer, B., 2009. Freshwater molluscs of the Nile Basin, past and present. In: Dumont, H.J. (Ed.), The Nile. Monographiae Biologicae, vol. 89. Springer, Dordrecht, pp. 585–629. https://doi.org/10.1007/978-1-4020-9726-3_29.

Verbeke, J., 1957. Recherche écologique sur la faune des grands lacs de l'Est du Congo belge. Exploration hydrobiologique des lacs Kivu, Edouard et Albert (1952–54). Bull. Inst. R. Sci. Nat. Belg. 3, 1–177.

Wagner, B., Leng, M.J., Wilke, T., Böhm, A., Panagiotopoulos, K., Vogel, H., Sulpizio, R., 2014. Distinct lake level lowstand in lake Prespa (SE Europe) at the time of the 74 (75) ka Toba eruption. Clim. Past 10 (1), 261–267. https://doi.org/10.5194/cp-10-261-2014.

Wiese, R., Clewing, C., Albrecht, C., Rabethge, C., Zhang, H., Iedel, F., 2020. How ancient is Lake Lugu (Yunnan, China)? The gastropods' viewpoint with focus on *Radix* (Lymnaeidae). J. Great Lakes Res. 46 (5), 1099–1112. https://doi.org/10.1016/j.jglr.2020.06.003.

Wilke, T., Davis, G.M., Qiu, D., Spear, R.C., 2006. Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: another case of ancestral polymorphism? Malacologia 48 (1-2), 143.

Wong, H.K., Herzen, R.P., 1974. A geophysical study of Lake Kivu, East Africa. Geophys. J. Int. 37 (3), 371–389.

Wronski, T., Dusabe, M.C., Apio, A., Hausdorf, B., Albrecht, C., 2015. Biological assessment of water quality and biodiversity in Rwandan rivers draining into Lake Kivu. Aquat. Ecol. 49, 309–320. https://doi.org/10.1007/s10452-015-9525-4.

Zhang, S.M., Bu, L., Laidemitt, M.R., Lu, L., Mutuku, M.W., Mkoji, G.M., Loker, E.S., 2018. Complete mitochondrial and rDNA complex sequences of important vector species of *Biomphalaria*, obligatory hosts of the human-infecting blood fluke, *Schistosoma mansoni*. Sci. Rep. 8 (1), 7341.

Zhang, Z., Schwartz, S., Wagner, L., Miller, W., 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. 7 (1–2), 203–214.

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Invaders taking over – mollusc faunal change in volcanic barrier lakes of the Albertine Rift biodiversity hotspot

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Abstract

East African freshwaters are renowned biodiversity hotspots, particularly within the Rift Valley lakes, which exhibit exceptional species richness and endemism. However, research in this region has predominantly focused on large Rift lakes, with smaller volcanic barrier lakes remaining underexplored. This study integrated morphological, DNA barcoding, and ecological analyses to assess mollusc diversity, biogeographical affinities, ecological drivers of distribution, and community changes in lakes Bunyonyi, Mutanda, Mulehe, Ruhondo, and Burera. Results revealed a disproportionately high malacofaunal diversity in these lakes compared to the larger Great Lakes in the region. Assemblages primarily exhibited Nilotic affinities and were dominated by widespread mollusc species. Findings demonstrate ongoing homogenisation of mollusc communities, partially driven by anthropogenic activities and invasive species such as the North American crayfish (*Procambarus clarkii*), *P. acuta*, and the Asian lineage of *Melanoides tuberculata*. This trend, coupled with habitat degradation from agriculture, pollution, and infrastructure development, poses significant threats to the mollusc diversity in these lakes. This research underscores the importance of prioritizing these overlooked fragile ecosystems in the regional biodiversity conservation strategies to safeguard habitats for molluscs and other faunal elements. A comprehensive regional freshwater survey including a metabarcoding initiative is urgently needed to document molluscan diversity patterns amidst contemporary climate change and increasing anthropogenic pressures.
Keywords -Africa, Conservation, Freshwater, Invasive species, Rift valley

Introduction

The freshwaters of Eastern Africa are globally recognized for their high levels of species richness and endemism, especially within the Rift Valley lakes (Darwall et al., 2005). In the East African Rift Valley, comprehensive research efforts in aquatic biodiversity assessment and conservation have focused on the large rift lakes with small volcanic barrier lakes receiving considerably less attention. Similarly, limnology research efforts have disproportionately focused on the large rift lakes (De Crop & Verschuren, 2021).

Lakes Mutanda, Bunyonyi, Mulehe, Ruhondo, and Burera are volcanic barrier lakes in the western arm of the East African Rift Valley (Beadle, 1981). Lakes Mutanda, Bunyonyi, and Mulehe, in Southwestern Uganda along with lakes Ruhondo and Burera in Northern Rwanda were formed as a result of a geological volcanic event that occurred in the western arm of the Rift Valley in the late Pleistocene period ~ 18,000 B.P (Beadle, 1981; Dumont, 2009). This geological event drastically altered the region's hydrology, creating new waterbodies (Beadle, 1981; Stager et al., 2004). Before this, the Lake Edward Basin's southern drainage relied on watersheds near present-day Bukavu, Democratic Republic of Congo, and the western slopes of the Rwanda and Kigezi Highlands. The Virunga volcanoes erupted, releasing lava and ash that blocked streams from these highlands about 100 km south of Lake Edward (Beadle, 1981). This led to a flood which formed the present lakes Ruhondo and Burera, which overflew southward and consistently drained into Lake Victoria via the Nyabarango and Kagera Rivers (Beadle, 1981) joining the Nile Basin. Simultaneously, the eastern branch of Rutshuru River's uppermost valleys was partially blocked by the stream of lava from a small isolated cluster of volcanoes at Muko which led to the formation of Lake Bunyonyi (Beadle, 1981). Furthermore, this lava stream was obstructed at its middle course by the northern edge of the main lava field from the Virunga volcanoes behind which a valley was flooded leading to the formation of Lake Mutanda and consequently Lake Mulehe (Beadle, 1981: 267).

These lakes have for a long time experienced dramatic limnology and ecosystem shifts driven by natural and anthropogenic activities, especially the intentional introduction of alien species, which have caused detrimental effects on organismal community changes, some of which are not fully understood (Fig. 1; Appendix 1).

The introduction of invasive species in the aquatic ecosystem has tremendous disastrous effects on native aquatic biota. A notable example is the intentional introduction of the Nile Perch (*Lates niloticus* (Linnaeus, 1758)) in Lake Victoria to boost commercial fisheries combined with other invaders i.e. water hyacinth, caused a massive ecosystem change consequently resulting in massive endemic cichlid fishes extinction (Van Bocxlaer & Albrecht, 2015). However, invasive mollusc species pose a relatively minor threat to Africa's native molluscan fauna, with only localized impacts reported (Graf, 2011, Albrecht et al., 2025a). In contrast, their effects have been more pronounced in freshwater ecosystems across Eurasia, Europe, and the Americas (Lopes-Lima et al., 2025). However, an agonistic interaction such as inter- and intra-specific competition is a potential threat that has been observed among the native and alien invading molluscs of the Western Rift African Great Lakes (Van Bocxlaer et al., 2015). In 1980, an Asian lineage *Melanoides tuberculata* (O. F. Müller, 1774) invaded Lake Malawi and later dispersed and invaded the ancient Lake Tanganyika, the Congo River, and systematically ascended into the Nile basin. Recently, a negative spatial correlation between the invasive Asian and native *M. tuberculata* morphs in Lake Malawi depicts an agonistic competitive interaction between the two lineages (Van Bocxlaer & Albrecht, 2015; Van Bocxlaer et al., 2015). This has led to the displacement of the native morphs by the more competitive Asian morph in the Southern part of the Lake (Genner et al., 2004), potentially leading to genetic homogenization posing a threat to the conservation of the endemic gastropod diversity. This devastating threat is consistent with anthropogenic stressors that facilitate the invaders' colonisation (Rahel, 2002; Graf, 2011).

Similarly, two other invasive gastropods pulmonates, *Lymaea columella* and *Physella acuta* Draparnaud, 1805, were introduced around 1940 to 1950 in the Republic of South Africa via the aquarium trade (Appleton, 2003), and consequently in the Albertine rift. *Physella acuta* has recently been encountered only in tributaries of an Albertine volcanic Lake Kivu by Dusabe et al. (2024) but never in the lake proper. Nonetheless, *P. acuta* is predicted to continue its global invasion including in Africa (Albrecht et al., 2025b).

An important detrimental invasion in the volcanic barrier lakes was the introduction of the North American invasive crayfish (*Procambarus clarkii* (Girard, 1852)) (Madzivanzira et al., 2020). The species is reported to negatively impact the native freshwater biodiversity, especially the macroinvertebrates, due to its intensive predatory behavior and high fecundity (Twardochleb et al., 2013). Furthermore, their tolerance to a wide range of environmental conditions, enormous size, and omnivorous feeding behaviour have facilitated their rapid establishment (Lodge et al., 2012; Twardochleb et al., 2013; Madzivanzira et al., 2020) thereby interspecifically outcompeting the native fauna. The snail-feeding preference of *P. clarkii* has been evidenced in Kenya and Egypt based on laboratory experiments and field observations (Hofkin et al., 1991; Mkoji et al., 1999; Khalil & Sleem, 2011). This non-native invasive crayfish was introduced in Lake Bunyonyi and was reported well-established and abundant as early as 2007 (Foster & Harper, 2007). The evident widespread dispersion of the species within the Bunyonyi system indicates potential colonization of lakes Mutanda and Mulehe, adjacent to Lake Bunyonyi. In Rwanda, little is reported about the spread and establishment of *P. clarkii* except for its first introduction in Mukungwa Valley as a biological control agent of water hyacinth (Madzivanzira et al., 2020). Exploring the threat could provide substantial insights into combating the issue, potentially mitigating expected mollusc diversity loss by guiding control efforts.

The establishment of *P. clarkii* in Lake Bunyonyi and its potential spread to adjacent lakes pose a significant threat to native mollusc populations, including endemic and conservation-priority species such as *Bulinus mutandaensis* (Preston, 1913) and *Gyraulus costulatus exilis*. These conservation-priority species are thought to be endemic to the lakes Mutanda and Bunyonyi system. *B. mutandaensis* has been treated as a distinct species by Mandahl-Barth (1962) though considered to be a lacustrine form of *Bulinus truncatus* (Audouin, 1827) (Brown & Wright, 1972; Brown, 1994). Furthermore, *G. costulatus exilis* has been considered a subspecies of *Gyraulus costulatus* (Krauss, 1848) by Mandahl-Barth (1962) while considered morphologically indistinguishable from *G. costulatus* by Brown and Wright (1972). The taxonomic discrepancy on these species remained unresolved for a long time based on morphological, anatomical, conchological, and ploidy level (*B. mutandaensis*) characteristics. It is possible that the currently observed shell variations in *B. mutandaensis* and *G. costulatus* might represent ecophenotypic plasticity. However, DNA barcoding is a tool that might offer an opportunity to resolve this long-standing taxonomic question.

Currently, there have been very few surveys of macrobenthos, including molluscs, in these satellite volcanic barrier lakes. Most of what we know about the malacofauna in these lakes comes from the early expeditions published in Thiele (1911) and Worthington (1932), conducted in 1907 and 1931, respectively. Given this knowledge vacuum for almost 100 years, this study seeks to update our knowledge of the malacofaunal assemblage of these lakes.

Based on an integrated approach using morphological, DNA barcoding, and ecological analyses of mollusc communities, this study 1) determines the diversity and distribution, 2) quantifies ecological factors driving the mollusc distribution, 3) tests faunal change, and 4) discusses the biogeographical affinity of molluscs in lakes Bunyonyi, Mutanda, Mulehe, Ruhondo and Burera in the Western Rift valley region.

We also document and discuss threats to the mollusc diversity, and suggest conservation and management interventions.

Materials and Methods

Study area

The sampled volcanic barrier lakes are located in southwestern Uganda and northwestern Rwanda. In Uganda, these include Lake Mutanda (1,179° S, 29,698° E, 1,792 m) with a total surface area of 29km^2 and a maximum depth of 56 m. Lake Bunyonyi (1,283° S, 29,927° E, 1,973 m) covers 56 km^2 and a maximum depth of 40 m. Lake Mulehe (1°13′5" S, 29°43′17" E, 1,806m) has a surface area of 5km^2 and a maximum depth of 6 m (Dumont, 2009). In northwestern Rwanda, the lakes include Lake Ruhondo (1,534° S, 29.711° E, 1,765 m) with a surface area of 26.6 km^2 and a maximum depth of 68 m. Lake Burera (1.45° S, 29.78° E, 1,860 m) spans 51.8 km^2 and a reaches depth of 179 m (Habimana & Nsabimana, 2020). These lakes were formed by a volcanic event that happened in the late Pleistocene (~18,000 BP) in the western arm of the Rift Valley during the late Pleistocene (~18,000 BP)(Dumont, 2009). They are all located near the foot of the Virunga mountain range (Dumont, 2009).

The Ugandan volcanic barrier lakes are connected by several adjoining streams and rivers i.e. lakes Mutanda and Mulehe are connected by approximately 2 km long, River Mucha. Lake Bunyonyi is connected to Lake Mutanda through River Ruhezamyenda and Lake Mutanda drains into River Mkarara which drains into Lake Edward (Magumba, 2000; Papius et al., 2016). The lakes' catchments are dominated by cultivated hills with the lakes' buffer zones encroached on by agricultural activities and several hotel constructions (Papius et al., 2016). Lakes Ruhondo and Burera are hydrologically interconnected through short streams and underground watercourses due to their proximity and difference in elevation. Both lakes drain into the Nile Basin, with their outflow directed through the Mukungwa River, which flows southward via Lake Ruhondo. The Mukungwa River subsequently joins the Nyabarongo River, which, after converging with the Ruvubu River from Burundi, forms the Kagera River, ultimately discharging into Lake Victoria (Habiyakare & Zhou, 2015; Habimana & Nsabimana, 2020).

The study area is situated in a predominantly agricultural region, characterized by intensive farming practices (Nzabuheraheza & Nyiramugwera, 2017). The surrounding landscape features extensive human settlement, infrastructure development, and hydropower activities, including the Ntaruka Power Station (Habimana & Nsabimana, 2020). These anthropogenic activities pose significant threats to the ecological integrity of lakes Ruhondo and Burera, including sedimentation, agrochemical runoff, and sewage pollution.

Sampling

Extensive malacological surveys were conducted in 2023 and 2024, at lakes Bunyonyi, Mutanda, Mulehe, Ruhondo and Burera. A total of 44 sites were comprehensively sampled (Fig. 1). Snails were collected using a scoop net (20 cm diameter, 1 mm mesh size) and a Surber net (250 µm mesh size). Samples from stones and rocky substrates were collected by direct picking using feather-light forceps. An Ekman grab was used to collect samples from the bottom of the lake at deeper sites (up to 20 m) in the littoral zone. Crayfish traps were set with bait (cat food and bacon) to catch *P. clarkii* in the littoral zone. Selected physicochemical parameters such as dissolved oxygen (DO), water temperature, total dissolved solids, electrical conductivity (EC), and pH, and geographical information such as GPS coordinates and altitude at each site were measured *in situ* using a calibrated handheld multiparameter probe meter (HI 9829, HANNA, Romania) and a portable GPS (Garmin GPSMAP 64) respectively following the manufacturer's guidelines (Table 1). Furthermore, we documented site use and substrate types (vegetation, rock, sand, mud, etc.) All collected samples were preserved in 80% ethanol and labeled accordingly. Specimen vouchers are stored in the Systematics and Biodiversity collection of the Justus Liebig University, Giessen (UGSB). Specimens were identified to genus and species level based on shell morphology (Mandahl-Barth, 1954; Danish Bilharziasis Laboratory, 1987; Mandahl-Barth, 1988; Brown, 1994). Two specimens per species and locality were photographed with a Keyence digital microscope (KEYENCE VHX-2000) before processing in the molecular laboratory.

DNA extraction, amplification and sequence analysis

A total of 80 specimens of genera: *Biomphalaria*, *Bulinus*, *Afrogyrorbis*, *Sphaerium*, *Radix*, *Pisidium*, *Physella*, *Melanoides*, *Bellamya*, *Segmentorbis*, *Gyraulus*, and *Procambarus* were selected for molecular barcoding. Genomic DNA was extracted from a small piece of foot muscle for molluscs and from the abdomen for *P. clarkii* using the CTAB protocol (Wilke et al., 2006). The mitochondrial cytochrome *c* oxidase subunit I (COI) and large subunit ribosomal RNA (16S rRNA) gene fragments were amplified using primers COF14 and COR722b (Wilke & Davis, 2000) for COI and 16Sar and 16Sbr for 16S (Palumbi et al., 1991). PCR conditions are described in the supplementary materials (Table S?). Sequencing was performed using the BigDye Terminator Kit on an ABI 3730xl DNA analyzer (Life Technologies, LGC Genomics GmbH). The new sequences will be deposited in NCBI GenBank, and accession numbers will be provided upon acceptance (Appendix 5). Sequence editing was performed manually and aligned using BioEdit v7.2 (Hall, 1999).

Edited sequences were analysed using BLASTn (megablast) against the NCBI GenBank nucleotide database. BLAST results were ranked by maximum score, and the top two hits for each specimen were selected for further comparison. For Sphaeriidae, local BLAST searches were conducted against an internal sequence database (unpublished data), as closely related sequences were not available in NCBI GenBank. This approach ensured more accurate taxonomic identification and minimized potential misannotations associated with incomplete public reference databases.

Phylogenetic and phylogeographical analyses

A phylogenetic analysis was performed for only *Bulinus*, *Biomphalaria*, and *Melanoides* species using the COI marker to test the taxonomic status of *Bulinus mutandaensis*, to adress the *Biomphalaria pfeifferi* (Krauss, 1848) and *B. choanomphala* species delimitation problem, and to ascertain whether the recorded *M. tuberculata* is of the invasive Asian or the native lineage (Appendix. 3). For *Bulinus* sp., a total of 47 in-group specimens were included and *B. pfeifferi* was used as outgroup. For *Biomphalaria* sp., a total of 19 in-group specimens were included and *Biomphalaria glabrata* was used as an outgroup (Appendix 4). For *Melanoides* sp. a total of 16 in-group specimens were included and *Thiara scabra* served as outgroup. Alignments were performed using ClustalW in BioEdit, with final alignments (COI) consisting of 600 base pairs for *Bulinus* sp., 588 base pairs for *Biomphalaria* sp., and 512 base pairs for *Melanoides* sp. The most appropriate substitution model for each codon position was selected based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) in MEGA v11. Based on the AIC and the BIC criterion, the Maximum Likelihood method and Hasegawa-Kishino-Yano model were used (Hasegawa et al., 1985). Phylogenetic trees were constructed using maximum likelihood (ML) with bootstrapping (1,000 replicates) in MEGA v11, and the final tree was visualised and edited in Interactive Tree of Life (ITOL) online software v6.9.1. Statistical parsimony haplotype networks within PopArt v1.7 (Leigh et al., 2015) were created to assess the haplotype diversity of *Bulinus* sp. in these lakes in order to ascertain the taxonomic status of *Bulinus mutandaensis* (Appendix 2).

Mollusc diversity and ecological data analyses

The mollusc diversity was computed using the Shannon Wiener (H) index in PAST (Version 3.15) for each sampled site. The H' index values along with their coordinates were imported in QGIS software (Version 3.36.1) and thematically mapped using a graduated colour method to display the spatial distribution of mollusc species diversity in the studied lakes.

Non-metric Multidimensional Scaling (NMDS) was used to explore the variations in mollusc community composition across sampling sites among the sampled lakes. A Bray-Curtis dissimilarity matrix was calculated from mollusc species abundance data. The NMDS ordination (k = 2 dimensions) was performed using the *metaMDS()* function in the vegan package of R studio (version 4.3.3), resulting in a stress value of 0.0001, indicating a reliable representation of the data.

Environmental variables, including altitude, physicochemical parameters; DO, pH, TDS, EC, temperature, human activities (fishing, agriculture, boating, tourism, settlement, water sporting), substrate type (sand, Sp-Sapropel, detritus, vegetation, and stones), and the system type (lake or stream) were fitted onto the ordination using the *envfit()* function. The categorical variables were analyzed as presence/absence data (1/0). Vector significance was determined via permutation testing (999 permutations, $p < 0.05$). Significant group differences were confirmed using PERMANOVA in R studio (version 4.3.3).

Results

Diversity and distribution of molluscs

We identified 20 mollusc species belonging to eight families, comprising 13 gastropod species and 7 bivalve species (Table 2). The highest mollusc diversities (H index = 0.99-1.24) were found in lakes Mulehe, Bunyonyi and Ruhondo (Fig. 3). In contrast, lakes Mutanda and Burera exhibited the lowest mollusc diversity ranging between 0 and 0.25 (Fig. 3).

Pila ovata (Olivier, 1804) was exclusively recorded from Lake Mulehe, while *Bulinus* cf. *mutandaensis*, was found only in Lake Mulehe. *Bulinus tropicus* (Krauss, 1848) was restricted to Lake Ruhondo. The invasive American species *P. acuta* was detected exclusively in lakes Burera and Ruhondo. *Afrogyrorbis natalensis* (Krauss, 1848) and *Segmentorbis* cf. *angustus* (Jickeli, 1874) were recorded solely from Lake Bunyonyi, whereas *Lentorbis* sp. was found only in Lake Ruhondo. *Bellamya* cf. *unicolor* (Olivier, 1804) was also confined to Lake Ruhondo. *Biomphalaria* cf. *choanomphala* (E. von Martens, 1879) was recorded in all three lakes in Uganda, i.e. lakes Bunyonyi, Mulehe, and Mutanda. *Radix natalensis* (Krauss, 1848) and *M. tuberculata* were recorded in all five lakes (Table 2). However, two distinct lineages of *M. tuberculata* i.e. the indigenous and the invasive Asian morphs were identified in the region (Appendix 3). Notably, the invasive Asian morph was detected exclusively in Lake Mulehe.

Sphaeriid bivalves were encountered exclusively in lakes Bunyonyi and Ruhondo (Table 2). However, due to an 'ambiguous taxonomic assignment as a result of the sequence multi-species match during sequence blasting in the GenBank, the encountered species were assigned working names (see Appendix 5 for details). Notably, five out of the seven recorded sphaeriid species were found in Lake Bunyonyi. All *Euglesa* spp. were collected from tributaries supplying Lake Bunyonyi, whereas both *Sphaerium* sp. was recorded from the lakes Bunyonyi Ruhondo.

Ecological factors driving the mollusc distribution

The NMDS ordination biplot shows the variation in malacofaunal community composition among the sampling sites in the five lakes. The stress value of 0.0001 suggests that the ordination adequately represents the data. Samples from the Lake Mulehe-Mutanda system, lakes Ruhondo and Bunyonyi cluster together, indicating mollusc species composition similarity. Lake Bunyonyi and Lake Burera sites are widely spread apart with no overlap, indicating a significant dissimilarity among the mollusc fauna communities of these lakes from the rest of the four lake systems. The environmental parameters, i.e. altitude ($p=0.033$), pH ($p=0.014$), TDS ($p=0.003$), EC ($p=0.003$), and detritus ($p=0.004$) significantly determine mollusc community composition.

The current study reports an extensive distribution of the invasive crayfish *P. clarkii*, with well-established populations in lakes Bunyonyi, Ruhondo, and Burera (Fig. 3). The invasion is ongoing, with early-stage establishment observed in lakes Mulehe and Mutanda. The invasive *M. tuberculata* was encountered from only one locality in Lake Mulehe (Fig. 3). On the other hand, *P. acuta* was encountered from two lakes, i.e. lakes Ruhondo, and Burera (Fig. 3).

Shifts in molluscan community composition in volcanic barrier lakes

A comparative analysis of historical records and our recent surveys reveals significant shifts in the molluscan communities of the sampled volcanic barrier lakes over the past 91 years. Worthington (1932) previously documented *B. tropicus*, *Bulinus ugandae*, *B. pfeifferi*, and *Burnupia* sp. in lakes Bunyonyi, Mutanda, and Mulehe. However, our current survey found no evidence of these species in these lakes, suggesting potential local extirpation or population decline. Notably, we report the first record of a live *Ferrissia* sp. from Lake Bunyonyi, based on a single specimen collected at one site (C. Albrecht, pers. obs. 2013). Additionally, we document the first occurrences of *B. truncatus*, *M. tuberculata*, *Euglesa* cf. *keniana* (Preston, 1911), *P. ovata*, and *B.* cf. *choanomphala* in these lakes (Table 3), indicating a notable shift in species composition.
On the other hand, Thiele (1911) previously encountered *Bulinus trigonus*, *Bulinus zanzibaricus*, *Pettancylus (=Ferrissia) ruandensis*, and *Sphaerium nyanzae* in Lake Ruhondo and in the waterfalls between lakes Ruhondo and Burera (Table 3). It needs to be discussed whether these species conform to the *Bulinus*, *Ferrissia* and *Sphaerium* taxa encountered during our survey.

Phylogenetic and phylogeographical analysis results

Our phylogenetic analysis revealed the presence of four well-supported *Bulinus* clades within the sampled volcanic barrier lakes, all clustering within the *B. truncatus/tropicus* complex (Bayesian posterior probability [BPP] 0.81; Appendix 2B). Notably, *Bulinus* cf. *mutandaensis* formed a supported clade (BPP 0.92) within this complex, clustering specifically with *B. truncatus* (Appendix 2B). Within our study area, we identified three haplotypes of *B. truncatus*, among which the *B.* cf. *mutandaensis* haplotype (UGSB 29948) was exclusively detected in Lake Mulehe and its primary inflow, River Mucha (Appendix 2A).

Additionally, we identified two highly supported *M. tuberculata* lineages: an invasive Asian lineage and a native African lineage (Appendix 3). The two *M. tuberculata* specimens from Lake Mulehe clustered within the invasive lineage from China, forming a strongly supported branch (BPP 1.00). In contrast, the specimens from Lake Bunyonyi grouped within the native *M. tuberculata* lineage, also forming a well-supported branch (BPP 0.97; Appendix 3).

Discussion

Diversity and distribution of molluscs

In general, the montane freshwater malacofauna of the East African Rift Valley system, including its crater and volcanic barrier lakes, has historically been considered species-poor (Brown, 1994; Van Damme & Van Bocxlaer, 2009). From the Western Ugandan crater lakes, only eight gastropod species were reported, with *B. tropicus toroensis* and *Gabbiella kichwambae* considered endemic (Brown, 1994). This low species richness has been attributed to unfavorable environmental conditions, including high salinity, low dissolved oxygen levels due to prolonged water residence time, and permanent stratification, all of which severely constrain mollusc survival (Brown, 1994).

The “volcanic barrier lakes cluster” hosts a relativelly rich malacofaunal assemblage for high-altitude lakes. The cluster surpasses the mollusc species richness of several Western Rift Great Lakes of comparatively larger size. For instance, Lake Kivu, shows a total of eight mollusc species following recent studies (Dusabe et al., 2024). The lake has a low mollusc species diversity (Shannon H index of 0-1.1). Similarly, lakes George and Edward also record a lower mollusc species richness of about 10 molluscs in the lake proper, and 15 mollusc species with the Kazinga channel species inclusive respectively (Mandahl-Barth, 1954; Brown, 1994; Holmberg et al., 2011).

The volcanic barrier lake cluster harbors a high gastropod species richness, with 14 recorded species, two fewer than Lake Albert, which supports 16 gastropod species, including eight endemics (Mandahl-Barth, 1954; Brown, 1994). On the other hand, Lake Victoria’s gastropods species richness surpasses that of the volcanic barrier lakes cluster by fourteen mollusc species since it harbours a total of 28 mollusc species of which 13 species are endemic to the lake (Brown, 1994). Considering Lake Victoria’s significantly larger surface area (approximately 68,460 km² (Dumont, 2009)) compared to the combined surface area of 168.4 km² for the five volcanic barrier lakes, this higher species richness is not surprising. .

The high bivalve diversity disproportionate to the number of the sampled high-altitude volcanic barrier lakes in this study is not a surprise (see Clewing et al., 2022). All *Euglesa* spp. (formerly under *Pisidium* spp.) were exclusively collected from tributaries supplying Lake Bunyonyi, whereas *Sphaerium* sp. was recorded in the lake proper of lakes Bunyonyi and Ruhondo (Table 2). This distribution pattern aligns with observations from Lake Kivu, where *Pisidium kenianum* and *Pisidium* cf. *viridarium* were restricted to streams, while *S.* cf. *hartmanni* was found in the lake proper (Dusabe et al., 2024). Furthermore, we record no Unionid species in the sampled volcanic barrier lakes which aligns with the previous observations in these lakes specifically lakes Bunyonyi, Mutanda, and Ruhondo (Thiele, 1911; Mandahl-Barth, 1954). It is worth noting that in the current study, the taxonomic sampling of certain groups, particularly bivalves, may have underestimated mollusc species richness in the volcanic barrier lakes region. Further rigorous taxonomic studies are recommended to refine these estimates.

Ecological factors driving the snail distribution

Abiotic factors also play a crucial role in shaping aquatic macroinvertebrates, including molluscs' community composition and dynamics (Hämäläinen et al., 2003; Hauffe et al., 2016; Chi et al., 2024). For instance, lake morphology, particularly surface area, strongly correlates with macroinvertebrate richness (Zenker & Baier, 2009). This influences the habitat availability and heterogeneity, which indirectly influence mollusc species composition and distribution. Lake Bunyonyi, with a surface area of approximately 56 km² and its amoeboid shape featuring

numerous islands, provides diverse microhabitats and ecological niches, partially explaining the presence of 12 mollusc species-higher than in other volcanic barrier lakes considered in this study.

Physico-chemical parameters significantly influence snail distribution patterns on both regional and local scales in Africa (Brown, 1994). Total dissolved solids (TDS), a surrogate for salinity and electrical conductivity (EC), strongly affect snail distributions in inland waterbodies (Brown, 1994: 489; Marie et al., 2015). We demonstrate a significant influence of the TDS and EC on the mollusc community composition in the selected five volcanic barrier lakes (P=0.003, fig. 4), consistent with observations from other African regions, such as Niger and South Africa (Meier-Brook et al., 1987; Brown, 1994; Marie et al., 2015).

Similarly, extreme pH conditions have been shown to affect mollusc assemblages in freshwater systems (Tchakonté et al., 2014; Spyra, 2017). The current study reports pH as a significant factor influencing mollusc community dynamics ($p = 0.014$, fig. 4), likely due to its impact on shell formation physiology via calcium ion leaching under acidic conditions (Tchakonté et al., 2014).Additionally, the presence of detritus significantly influenced mollusc species composition in these lakes ($p = 0.004$, fig. 4). Detritus serves as a critical dietary component for snails, alongside periphytic algae, other algae, and microbes (Civitello et al., 2022), corroborating our observations. These findings highlight the need for integrated approaches that consider both biotic and abiotic factors to manage and conserve mollusc biodiversity in these ecosystems.

The Albertine Rift volcanic barrier lakes experienced the intentional introduction of the North American crayfish (*P. clarkii*) in the 1960s, aimed at economic benefits without adequate consideration of ecological consequences (Madzivanzira et al., 2020). *Procambarus clarkii* is known to drastically reduce populations of invertebrates (particularly molluscs), macrophytes, fish, and amphibians in aquatic systems. The ongoing invasion of *P. clarkii* in the sampled volcanic barrier lakes is evident (Fig. 3) and has significantly influenced mollusc community composition (Fig. 4). However, current assessments are based solely on presence/absence data. We emphasize the need for comprehensive regional studies to quantify the ecological impacts of *P. clarkii*, particularly its effects on mollusc assemblages and broader aquatic biota.

In addition to *P. clarkii*, emerging invaders such as the gastropods *P. acuta* and the invasive Asian *M. tuberculata* lineage were detected (Fig. 3). Invasive freshwater molluscs exert both direct and indirect pressures on native species by outcompeting and displacing them through resource monopolization, habitat alteration, and modification of abiotic conditions (Preston et al., 2022). These changes can facilitate the spread of novel diseases and contribute to ecosystem destabilization (Geist et al., 2025; Lopes-Lima et al., 2025). The ecological consequences of such invasions can be severe, leading to declines in native biodiversity and disruptions in ecosystem functioning (Lopes-Lima et al., 2025). However, their impact varies depending on abiotic factors, biotic resistance, genetic diversity, and stochastic events (Sousa et al., 2024). These invasive species pose potential threats to mollusc diversity through native species replacement and community homogenisation, a phenomenon previously observed in Lake Malawi due to *M. tuberculata* (Van Bocxlaer & Albrecht, 2015; Van Bocxlaer et al., 2015). The arrival of new global invaders in the region such as *Pseudosuccinea columella* (Say, 1817) and *Pomacea canaliculata* (Lamarck, 1822) in East African waters such as Lake Naivasha adds another layer of complexity to the problem (Albrecht et al., 2025). Although in a broader context, currently the mollusc invasion is not perceived as a serious threat to the African malacofauna, this might be a transient as similar invasions have caused significant impacts in other regions, such as the Caribbean Islands (Graf, 2011). Continuous monitoring is required to quantify the impact of these invasives on the malacological fauna of this region to better inform conservation efforts.

Tests historical mollusc's faunal change

Most ecosystems are currently undergoing community homogenisation driven by species and subspecies loss, making ecosystems more uniform (Brodersen & Seehausen, 2014). This phenomenon has become a focal point in evolutionary and biodiversity conservation studies.

Our findings confirm the ongoing ecosystem homogenization in the region, as several molluscan species and subspecies previously described by Mandahl-Barth (1954) are becoming increasingly rare. Notable examples include *Bulinus ugandae*, *Lentorbis sp.*, *Gyraulus costulatus exilis*, and *Burnupia kempi* (Table 3). Conversely, *Bulinus* cf. *mutandaensis* was found to be extremely rare in the lake proper, with very few specimens collected from the lake and more specimens collected from only one site in River Mucha, which drains Lake Mulehe into a swamp connected to Lake Mutanda. Phylogenetic analysis (Appendix 2) supports Brown (1994)'s assertion that *B. mutandaensis* is a lacustrine form of *B. truncatus*, rather than a subspecies of *B. tropicus* as proposed by Mandahl-Barth (1954).

Other interesting species previously described from the region are limpet-like species such as *Ferrissia toroensis* Mandahl-Barth, 1954, a critically endangered species and endemic to this region (Brown, 1994; Albrecht & Clewing, 2022) and *Pettancylus ruandensis* Thiele, 1911. It remains unknown which species was found in Lake Bunyonyi (single specimen (C. Albrecht, pers. obs. 2013).
We also observed new records of species not previously encountered in this lake system, including *M. tuberculata* and several Sphaeriidae species (Table 3). These species may have been overlooked in earlier less exhaustive surveys, which primarily focused on the littoral zone. Furthermore, some might be 'newcomers' such as the invasive *M. tuberculata* (Asian lineage) which we recorded in L. Mulehe.

Overall, the observed shifts in molluscan community composition can be attributed to dramatic ecosystem and limnological changes in the region. These include macroinvertebrate invasions (e.g., crayfish, *P. acuta*, and *M. tuberculata*), water pollution from hotel construction and extensive agriculture in the lake buffer zones, habitat loss exacerbated by buffer zone encroachment, and fish introductions all driven by anthropogenic activities (reviewed in Appendix 1; fig. 1). While some of these patterns undoubtedly reflect true ecological changes, the possibility of discrepancies in species records must also be considered. For example, certain taxa reported in earlier surveys (such as *Sphaerium nyanzae* in 1931) may correspond to the species that is now identified by DNA as *Sphaerium* sp. This highlights the limitations of relying solely on shell morphology for species identification, and emphasises the recognised taxonomic challenges surrounding *Sphaerium* in East Africa.

Biogeographical affinities

The molluscan fauna encountered in the volcanic barrier lakes exhibits a predominantly Nilotic affinity, dominated by species with broad Afrotropical distributions (Table 4). Notable among these species are *B. truncatus*, which is widespread across Africa, including North Africa and the Congo Basin (Brown, 1994; Tumwebaze et al., 2019; Dusabe et al., 2024), and *R. natalensis*, a species common throughout the Nile Basin and other parts of Africa, including the Zaire Basin (Brown, 1994). Similarly, *B. pfeifferi* is extensively distributed in tropical Africa (Danish Bilharziasis Laboratory, 1987; Brown, 1994), while *Sphaerium* spp. and *E. keniana* are widely found in East Africa (Danish Bilharziasis Laboratory, 1987; Mandahl-Barth, 1988; Clewing et al., 2022), with the latter extending its range from Ethiopia to Zambia (Mandahl-Barth, 1988; Clewing et al., 2022). Another widely distributed species is *B. tropicus*, ranging across East Africa from Ethiopia to South Africa (Danish Bilharziasis Laboratory, 1987; Brown, 1994). *Pila ovata* is another species of interest, distributed from Egypt across much of Africa, including regions with Palearctic affinities and the Zaire Basin (Brown, 1994). Additionally, *M. tuberculata* is commonly found in African waterbodies, including the Zaire Basin (Brown, 1994); however, an invasive Asian lineage of this species was notably encountered in Lake Mulehe. *Segmentorbis angustus*, which is distributed from Sudan to Natal and from Ethiopia to Lower Zaire (Danish Bilharziasis Laboratory, 1987), is found in major African great lakes such as Lake Malawi, Lake Tanganyika, Lake Turkana, Lake Victoria, and Lake Albert.

The introduction of alien and invasive species is also evident in these systems. *Physella acuta*, a widely invasive species, is particularly encountered in the Akagera-Victoria Nile system i.e Ruhondo and Burera system, where it has been recorded in lakes such as Naivasha and Kivu tributaries (Albrecht et al., 2025; Dusabe et al., 2024), but it remains absent from the Edward Nile Basin i.e Bunyonyi, Mutanda and Mulehe system currently. Similarly, *Bulinus* cf. *mutandaensis* a vulnerable species, initially thought to be restricted to Lake Mutanda (Brown, 1994), was for the first time recorded in Lake Mulehe. However, resolving whether it is as species level entity or a subspecies of local form of *Bulinus truncatus*, it is beyond the scope of the current study.

Geographically, there are limited differences in species composition between the Edward Nile Basin, which includes lakes Mutanda, Bunyonyi, and Mulehe in Uganda, and the Kagera-Victoria Nile system, represented by lakes Ruhondo and Burera on the Rwanda side (Fig. 5). However, *Sphaeriuum* sp. and *B.* cf. *unicolor* were found exclusively in the Akagera-Victoria Nile system and were absent from the Edward Nile Basin. Additionally, the invasive *P. acuta* was recorded in the Akagera-Victoria Nile system but was not observed in the Edward Nile system.

These findings underscore the complex biogeographic patterns in the volcanic barrier lakes, highlighting the interplay between few endemic species, the majority with broad Afrotropical distributions, and invasive species. This biogeographic situation reflects the ecological and evolutionary processes shaping the molluscan communities in these unique aquatic ecosystems.

Conclusions

The significant changes observed both in mollusc community composition and the ecosystem limnology might be responses to a nexus of multiple natural and anthropogenic factors leading to decline and potential loss of endemic diversity. Better reference databases and new tools such as environmental DNA approaches are crucial for documenting the conservation status of molluscs. Another critical observation in this context is the ongoing mollusc community homogenization, largely driven by invasive species such as the North American crayfish (*P. clarkii*), *P. acuta*, and the Asian lineage of *M. tuberculata*. These invasions, coupled with habitat degradation caused by agricultural activities, pollution, and infrastructure development within lake buffer zones, pose significant threats to mollusc biodiversity. There is an urgent need for the protection and sustainable management of these fragile ecosystems by international, national, and local authorities to mitigate further biodiversity loss.

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Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Francis Ssenkuba and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

All the data supporting the findings of this study would be made available by the corresponding author when genuinely requested. NCBI GenBank (accession numbers).

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Declarations

The authors declare no conflicts of interest.

Tables

Table 1. Characteristics of the sampled lakes and streams including altitude, physical and chemical characteristics of the water, substrate type, utilization and the degree of anthropogenic disturbance rated qualitatively using a comparative approach. Abbreviations: DO-Dissolved oxygen, TDS-Total Dissolved Solids, Temp-Temperature, EC-Electrical conductivity

Parameters	Rwanda		Uganda				
	Burera	Ruhondo	Bunyonyi	Tributaries to Bunyonyi	Mutanda	Mulehe	P-value
Altitude (m)	1862	1759	1948	1944.50±6.50	1786	1795	_
DO (mg/l)	6.81±0.87	6.16±1.25	1.74±0.65	1.49±0.03	2.28±0.92	1.93±0.25	<0.01
pH	7.93±0.41	8.26±0.31	8.72±0.15	8.84±0.30	8.93±0.09	8.50±0.23	0.01

TDS (ppm)	116.53±50.08	205.74±5.20	118.75±1.48	66.00±8.00	137.00±40.31	114.00±1.63	<0.01
Temp (°C)	23.26±1.52	22.99±0.49	24.54±2.11	18.37±1.23	22.82±1.16	21.57±1.40	>0.01
EC (µs)	232.86±100.92	411.59±10.58	231.75±9.28	115.00±11.00	263.00±67.18	217.67±0.47	<0.01
Substrates type	Detritus, stones, sapropel	Sand, sapropel, detritus	Vegetation, detritus, sapropel	Stones, Soil, Silt	Vegetation, Stones, Detritus, sapropel	Stones, Stand, Detritus, Vegetation	
Utilisation	Fishing, boating, tourism, Agriculture	Fishing, boating, tourism, Agriculture	Settlement, Tourism, Agriculture	Agriculture	Settlement, Tourism, Agriculture	Agriculture, Tourism	
Level of disturbance	Medium	High	Medium	High	Low	Medium	

Table 2. Historical malacofauna versus species found in the current study. Current mollusc species found in lakes Bunyonyi (BN), Mutanda (MT), Mulehe (MH), Ruhondo (RH), and Burera (BR) are compared with the past encounters by Worthington (1932) in 1931, and Thiele (1911) in 1907-1908 respectively. Synonyms adopted here refer to the names used in the original papers i.e. Worthington (1932) and Thiele (1911). Nomenclature follows MolluscaBase (https://www.molluscabase.org).

Species	**Past** (1907/1931)			**Recent** (2023/2024)				
	Synonym	**MT-BN** (1931) (Worthington, 1932)	**RH** (1907) (Thiele, 1911)	**BN**	**MT**	**MH**	**RH**	**BR**
GASTROPODA								
Ampullariidae								
Pila ovata (Olivier, 1804)						**X**		
Bulinidae								
Bulinus truncatus (Audouin, 1827)	*Bulinus tropicus mutandaensis*	**X**				**X**		
Bulinus trigonus (E. Von Martens, 1892)	*Isidora strigosa*		**X**					
Bulinus tropicus (Krauss, 1848)		**X**					**X**	
Bulinus truncatus (Audouin, 1827)				**X**		**X**	**X**	
Bulinus ugandae Mandahl-Barth, 1954	*Bulinus globosus ugandae*	**X**						
Bulinus zanzibaricus (Clessin, 1886)*	*Isidora zanzibarica*		**X**					
Lymnaeidae								
Radix natalensis (Krauss, 1848)				**X**	**X**	**X**	**X**	**X**
Physidae								
Physella acuta Draparnaud, 1805							**X**	**X**
Planorbidae								
Afrogyrorbis natalensis (Krauss, 1848)	*Anisus natalensis*	**X**		**X**				
Biomphalaria cf. *choanomphala* (E. von Martens, 1879)				**X**	**X**	**X**		
Biomphalaria pfeifferi (Krauss, 1848)	*Biomphalaria ruppelli* *Planorbis nairobiensis*	**X**	**X**				**X**	
Burnupia sp.		**X**						
Ferrissia sp.				**X**				
Gyraulus costulatus (Krauss, 1848)	*Gyraulus costulatus exilis*	**X**		**X**				

Pettancylus ruandensis (Thiele, 1911)	*Ancylus ruandensis*		**X**					
Segmentorbis cf. *angustus* (Jickeli, 1874)	*Segmentorbis kempi*	**X**		**X**			**X**	
Thiaridae								
Melanoides tuberculata (O.F. Müller, 1774)				**X**		**X**	**X**	**X**
Viviparidae								
Bellamya cf. *unicolor* (Olivier, 1804)							**X**	
BIVALVIA								
Sphaeriidae								
Euglesa cf. *keniana* (Preston, 1911)				**X**				
Euglesa sp. I				**X**				
Euglesa sp. II				**X**				
Euglesa sp. III				**X**				
Sphaerium nyanzae E.A. Smith, 1892			**X**					
Sphaerium sp.		**X**		**X**			**X**	

Table 3. Biogeographical affinity of molluscs of the volcanic barrier lakes. The molluscs biogeographical affinities listed here are based on the phylogenetic analyses where the sequences are available and the respective standard literature.

Species	Phylogenetic relationship	Biogeographical affinity	Reference
GASTROPODA			
Ampullariidae			
Pila ovata (Olivier, 1804)	East Africa, Lake Victoria, Uganda lineage	Widespread throughout Africa	Brown (1994), Jorgensen et al., 2008
Bulinidae			
Bulinus cf. *mutandaensis* (Preston, 1913)	East Africa, Uganda lineage: in the group of *Bulinus truncatus*	Potentially endemic to Western Uganda crater lakes	Brown (1994), Tumwebaze et al. (2019), Dusabe et al., 2024
Bulinus tropicus (Krauss, 1848)	East Africa, Uganda Crater lakes lineage	Widespread throughout Africa but mainly in northern parts of the continent	Tumwebaze et al. (2019), Brown (1994)
Bulinus truncatus (Audouin, 1827)	East Africa, Rwanda lineage	Widespread throughout Africa but mainly in northern parts of the continent	Tumwebaze et al. (2019), Brown (1994), Dusabe et al. (2024)
Lymnaeidae			
Radix natalensis (Krauss, 1848)	East Africa, Kenya and Rwanda lineage	Wide-spread in African freshwaters, potentially Asian affinities	Brown (1994), Mahulu et al., 2019, Dusabe et al., 2024
Physidae			
Physella acuta Draparnaud, 1805	Invasive lineage, USA	Wide spread in Africa, Asia, USA affinities	Brown (1994), Albrecht et al. (2025)
Planorbidae			
Afrogyrorbis natalensis (Krauss, 1848)	n.a	East Africa, from Ethiopia to South Africa	Brown (1994)
Biomphalaria cf. *choanomphala* (E. von Martens, 1879)	East Africa, Rwanda lineage	Lake Kivu, lakes Victoria and Albert, Albert Nile	Brown (1994), Dusabe et al. (2024)
Biomphalaria pfeifferi (Krauss, 1848)	East Africa, Uganda, Kenya lineage	Tropical Africa	Brown (1994), Dusabe et al. (2024)

Ferrissia sp.	n.a	Wide spread throughout Africa	Brown (1994)
Lentorbis sp.	n.a	Widespread in eastern and central Africa	Brown (1994) Mandahl-Barth (1954)
Segmentorbis cf. *angustus* (Jickeli, 1874)	n.a	Tropical Africa	Brown (1994) Mandahl-Barth (1954)
Thiaridae			
Melanoides tuberculata (O. F. Müller, 1774)	East Africa, Uganda, Rwanda native lineage	Widespread in tropical Africa	Brown (1994), Van Bocxlaer & Albrecht (2015)
Melanoides tuberculata (O. F. Müller, 1774)	Asian invasive lineage, China	Widespread in tropical Africa	Van Bocxlaer & Albrecht (2015)
Viviparidae			
Bellamya cf. *unicolor* (Olivier, 1804)	East Africa, Lake Victoria, Uganda lineage	Widespread throughout Africa	Brown (1994), Schultheiß et al., 2014, Gu et al., 2019
BIVALVIA			
Sphaeriidae			
Euglesa sp. I	n.a	n.a	Mandahl-Barth (1988)
Euglesa sp. II	n.a	n.a	Mandahl-Barth (1988), Clewing et al. (2022)
Euglesa sp. III	n.a	n.a	Mandahl-Barth (1988), Clewing et al. (2022)
Euglesa cf. *keniana* (Preston, 1911)	n.a	Ethiopia to Zambia East African affinities	Mandahl-Barth (1988), Clewing et al. (2022)
Sphaerium sp.	East Africa, Rwanda	Egypt, Ethiopia to Northern Sudan, East Africa	Mandahl-Barth (1988), Dusabe et al., 2024

Figures

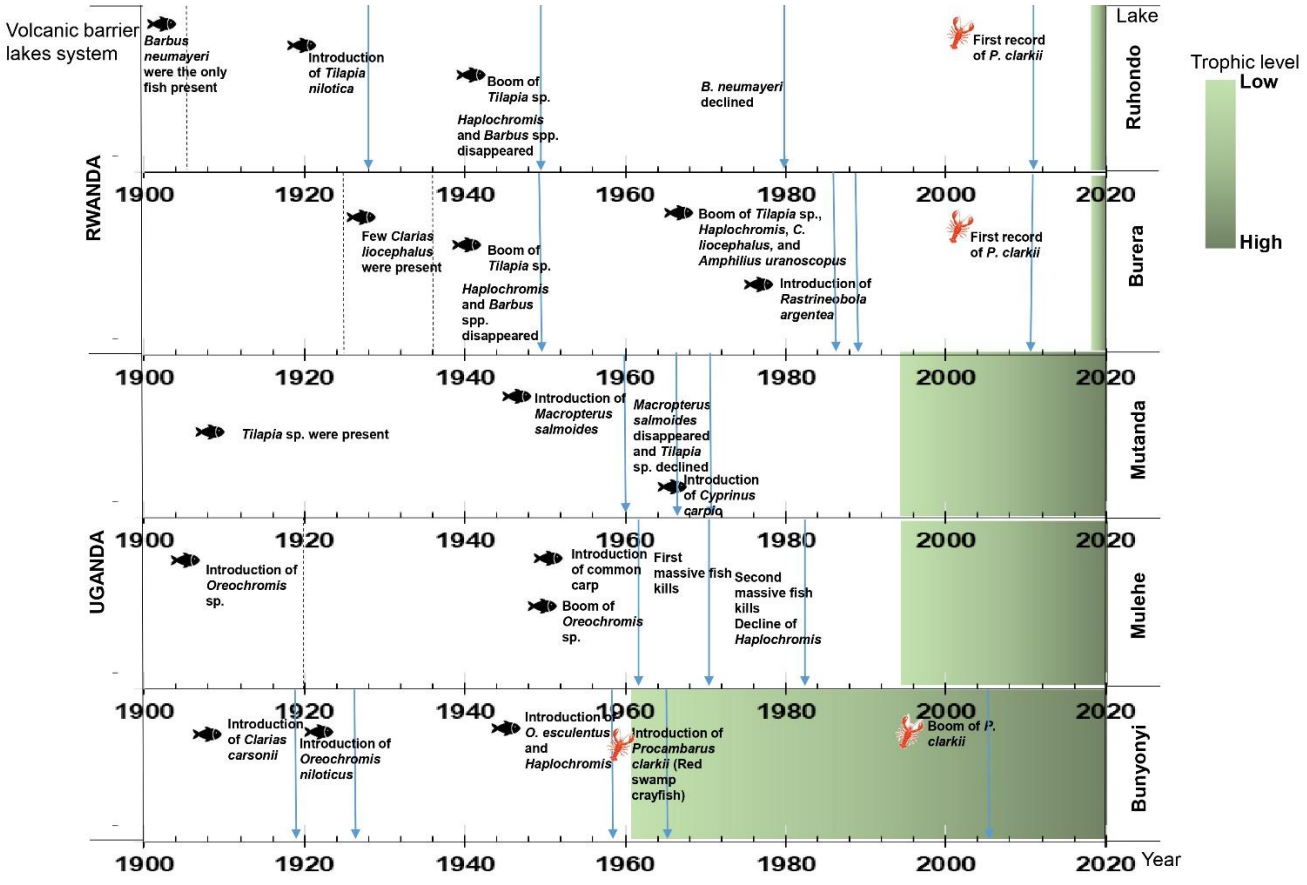
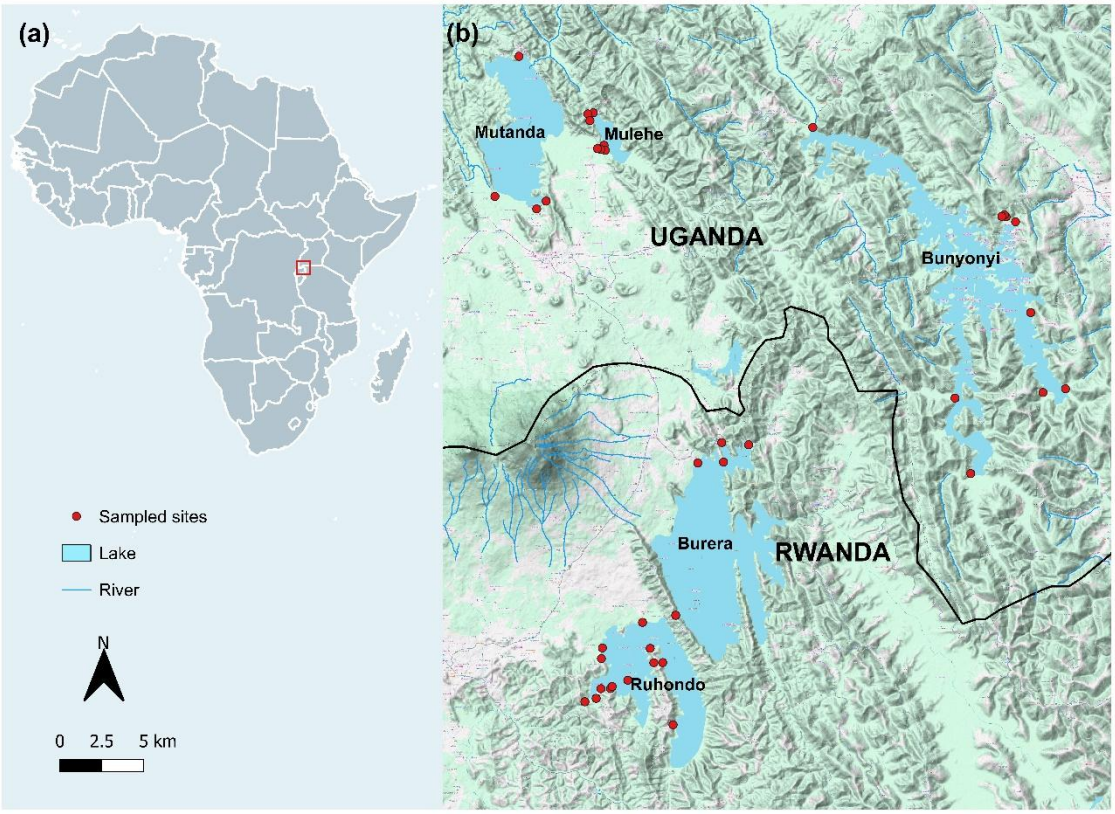


Fig. 1 Historical summary of the major ecological events documenting the major ecosystem changes in the volcanic-barrier lakes. (data from a review in Appendix 1).



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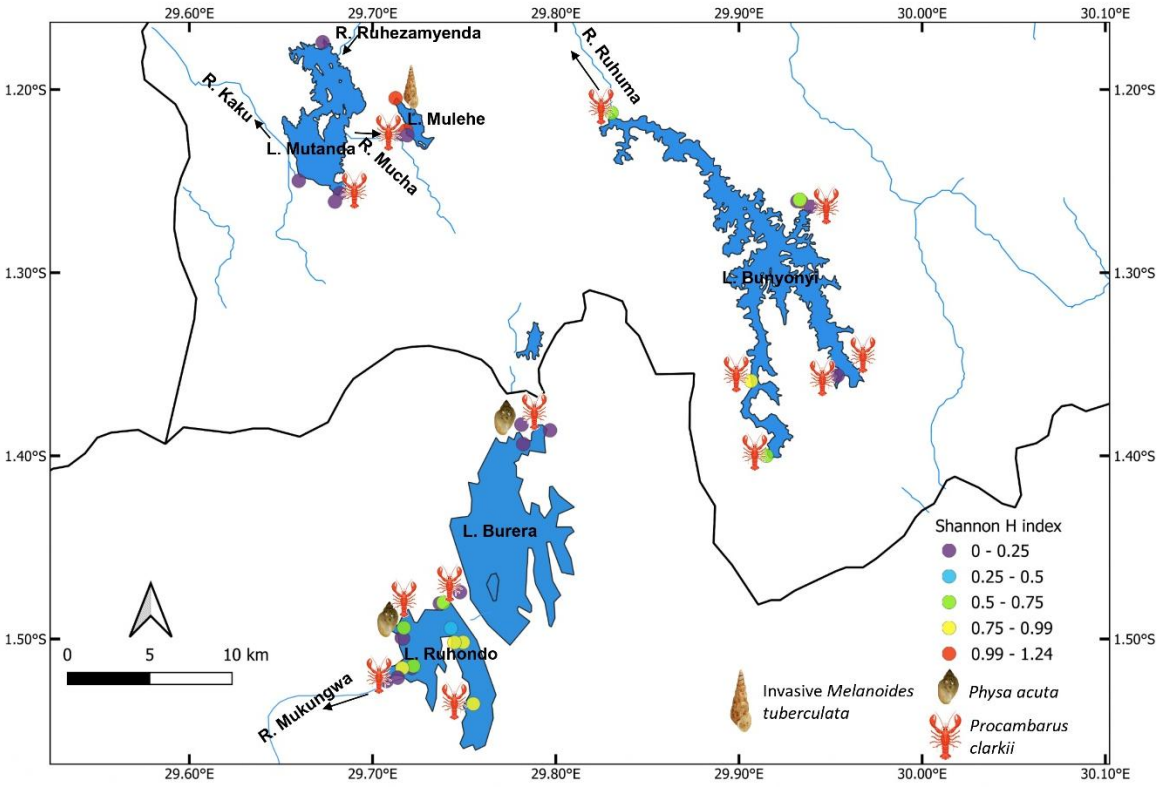
463

464 **Fig. 2** (**a**) Map of Africa highlighting the region of the sampled volcanic barrier lakes (in a red frame) (**b**) Map of the sampled
465 volcanic barrier lakes showing the location of the sampled sites (red points)



466

467 **Fig. 3** Mollusc diversity distribution (heatmap) and occurrence of the invasive *Melanoides tuberculata* lineage, *Physella acuta*,
468 and *Procambarus clarkii*. Shannon H index represents mollusc diversity based on the Shannon-Wiener diversity index. Where
469 Purple - very low mollusc diversity site, Pale-blue - low mollusc diversity site, Pale-green - medium mollusc diversity site,
470 Yellow - high mollusc diversity site, Red - very high mollusc diversity site.

471

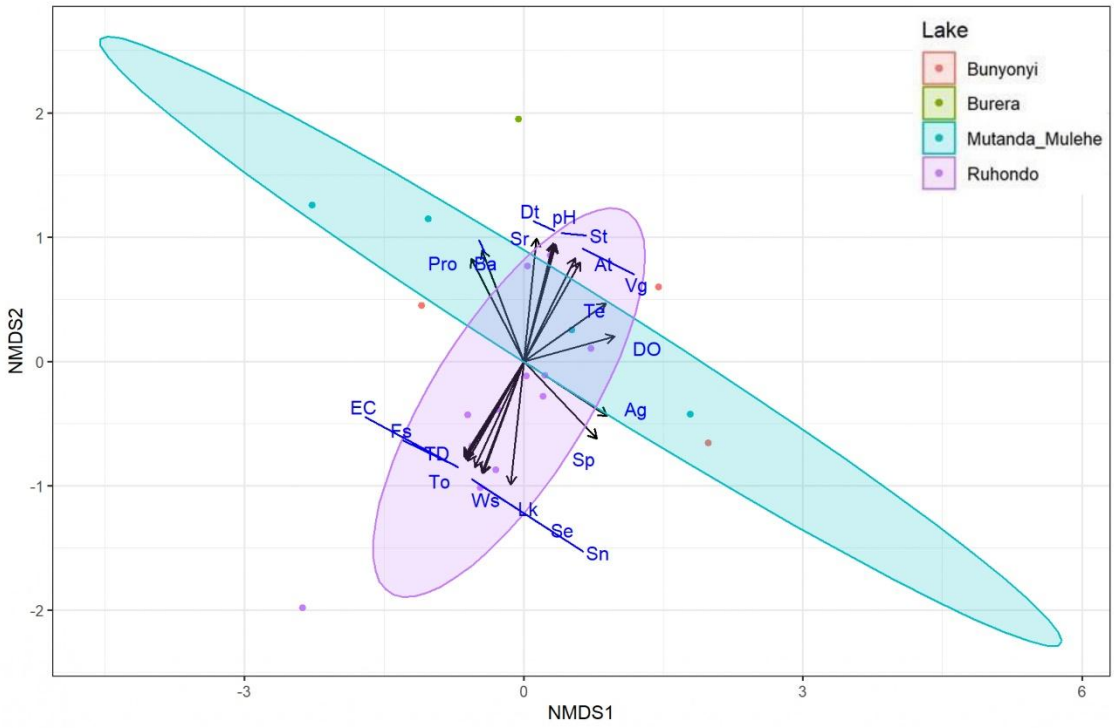


Fig. 3 NMDS ordination of mollusc community composition explained by environmental, *Procambarus* presence and human impact parameters. Where: Ag-Agriculture, At-Altitude, Ba-Boating, Dt-Detritus, DO-Dissolved oxygen, EC-Electrical conductivity, Fs-Fishing, Lk-Lake, **Pro-** *Procambarus* presence, Sn-Sandn, Sp-Sapropel, Sl-Settlement, St-Stones, Sr-Stream, TD-Total Dissolved Solids, Te-Temperature, To-Tourism, Vg-Vegetation, Ws-Water sport.

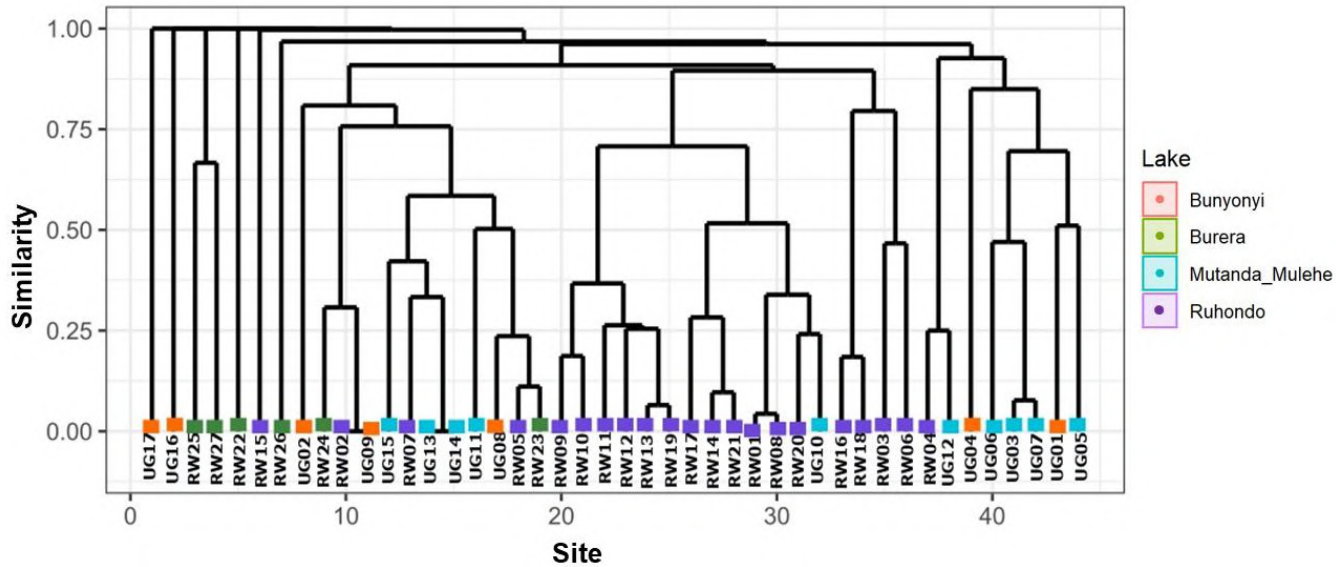


Fig. 4 Bray-Curtis similarity cluster analysis for mollusc faunal similarity. Where: Lake Ruhondo sites; RW01, RW02, RW03, RW04, RW05, RW06, RW07, RW08, RW09, RW10, RW11, RW12, RW13, RW14, RW15, RW16, RW17, RW18, RW19, RWA20, RW21. Lake Burera sites; RW22, RW23, RW24, RW25, RW26, RW27. Lake Bunyonyi; UG01, UG04, UG08, UG09, UG16. Stream to Lake Bunyonyi; UG02, UG17. Lake Mutanda; UG03, UG05, UG14, UG15. R. Mucha drains L. Mulehe; UG06. L. Mulehe; UG07, UG10, UG11, UG12, UG13.

References

Albrecht, C., & Clewing, C. (2022). *Pettancylus toroensis. The IUCN Red List of Threatened Species 2022: e.T184630A187684642. 8235*. https://doi.org/https://dx.doi.org/10.2305/IUCN.UK.2022-2.RLTS.T184630A187684642.en

Albrecht, C., Clewing, C., Seebens, H., Chibwana, F. D., Da Silva, E. L., Leal, M. F., Lingofo Bolaya, R., Marwoto, R. M., Odaibo, A., & Pinheiro, T. G. (2025). When One Global Invasion Hides Another—Cryptic Interspecific Invasion in Freshwater Gastropods. *Diversity and Distributions*, *31*(1), e13958.

Albrecht, C., Kipyegon, J. K., & Junginger, A. (2025). *Returners and New Arrivals After the Crash : Intermediate Hosts and Global Invaders Dominate Gastropod Fauna of Lake*. 1–12. https://doi.org/https://doi.org/10.3390/d17040265

Albrecht, C., Kipyegon, J. K., Junginger, A., & Clewing, C. (2025). Returners and New Arrivals After the Crash: Intermediate Hosts and Global Invaders Dominate Gastropod Fauna of Lake Naivasha, Kenya. *Diversity*, *17*(4), 265. https://doi.org/https://doi.org/10.3390/d17040265

Albrecht, C., Wilke, T., Kuhn, K., & Streit, B. (2004). Convergent evolution of shell shape in freshwater limpets: the African genus Burnupia. *Zoological Journal of the Linnean Society*, *140*(4), 577–586. https://doi.org/10.1111/j.1096-3642.2003.00108.x

Appleton, C. C. (2003). Alien and invasive fresh water Gastropoda in South Africa. *African Journal of Aquatic Science*, *28*(1), 69–81. https://doi.org/10.2989/16085914.2003.9626602

Beadle, L. C. (1981). *The inland waters of tropical Africa: an introduction to tropical limnology.* (Second edi, Issue Edn 2). Longman Group Limited.

Brodersen, J., & Seehausen, O. (2014). Why evolutionary biologists should get seriously involved in ecological monitoring and applied biodiversity assessment programs. *Evolutionary Applications*, *7*(9), 968–983. https://doi.org/10.1111/eva.12215

Brown, D. (1994). *Freshwater Snails of Africa and their Medical Importance* (2nd ed.). Taylor & Francis. https://doi.org/10.1201/9781482295184

Brown, D. S. (1994). *Freshwater snails of Africa and their medical importance* (2nd ed.). Taylor & Francis. https://doi.org/10.1201/9781482295184

Brown, D. S., & Wright, C. A. (1972). On a polyploid complex of freshwater snails (Planorbidae: Bulinus) in Ethiopia. *Journal of Zoology*, *167*(1), 97–132. https://doi.org/10.1111/j.1469-7998.1972.tb01723.x

C. Marlene Fiol and Marjorie A. Lyles. (2006). Linked references are available on JSTOR for this article. *Academy of Management Review*, *31*(2), 386–408.

Chi, S., Hu, J., Zheng, J., Li, S., Li, M., & Hu, J. (2024). The role of abiotic and biotic factors within influencing macroinvertebrate communities in a subtropical eutrophic reservoir with thermal stratification. *Chemistry and Ecology*, *40*(5), 535–552. https://doi.org/https://doi.org/10.1080/02757540.2024.2329076

Civitello, D. J., Angelo, T., Nguyen, K. H., Hartman, R. B., & Starkloff, N. C. (2022). *Transmission potential of human schistosomes can be driven by resource competition among snail intermediate hosts*. *119*(6), 1–9. https://doi.org/10.1073/pnas.2116512119

Clewing, C., Geertz, T., Rassam, H., Woldekiros, T. H., & Albrecht, C. (2022). Freshwater diversity at a biogeographic edge zone: the high-mountain pea-clams of Ethiopia. *Systematics and Biodiversity*, *20*(1), 1–15. https://doi.org/10.1080/14772000.2021.2005706

Danish Bilharziasis Laboratory. (1987). *A Field guide to African freshwater snails* (2nd ed.). World Health Organization.

Darwall, W., Smith, K., Lowe, T., & Vié, J.-C. (2005). The status and distribution of freshwater biodiversity in Eastern Africa. *IUCN SSC Freshwater Biodiversity Assessment Programme. IUCN, Gland, Switzerland and Cambridge, UK. Viii*.

De Crop, W., & Verschuren, D. (2021). Mixing regimes in the equatorial crater lakes of western Uganda. *Limnologica*, *90*, 125891. https://doi.org/10.1016/j.limno.2021.125891

De Vos, L., Snoeks, J., & van den Audenaerde, D. T. (1990). The effects of tilapia introductions in Lake Luhondo,

534 Rwanda. *Environmental Biology of Fishes*, *27*, 303–308.
535 https://doi.org/https://doi.org/10.1007/BF00002748

536 Dumont, H. J. (2009). *The Nile: origin, environments, limnology and human use* (Vol. 89). Springer Science &
537 Business Media.

538 Dusabe, M. C., Kalinda, C., Clewing, C., Hyangya, B. L., Van Bocxlaer, B., & Albrecht, C. (2024). Environmental
539 perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake
540 Kivu. *Journal of Great Lakes Research*, 102339. https://doi.org/10.1016/j.jglr.2024.102339

541 Foster, J., & Harper, D. (2007). Status and ecosystem interactions of the invasive Louisianan red swamp crayfish
542 Procambarus clarkii in East Africa. In *Biological invaders in inland waters: Profiles, distribution, and*
543 *threats* (pp. 91–101). Springer.

544 Geist, J., Benedict, A., Dobler, A. H., Hoess, R., & Hoos, P. (2025). Functional interactions of non-native aquatic
545 fauna with European freshwater bivalves: implications for management. *Hydrobiologia*, *852*(5), 1397–1419.
546 https://doi.org/https://doi.org/10.1007/s10750-022-05121-2

547 Genner, M. J., Michel, E., Erpenbeck, D., De Voogd, N., Witte, F., & POINTIER, J. (2004). Camouflaged invasion
548 of Lake Malawi by an Oriental gastropod. *Molecular Ecology*, *13*(8), 2135–2141.
549 https://doi.org/10.1111/j.1365-294X.2004.02222.x

550 Graf, D. (2011). Freshwater molluscs of Africa: diversity, distribution, and conservation. *Gland: IUCN*, 92–125.

551 Green, J. (1965). Zooplankton of Lakes Mutanda, Bunyonyi and Mulehe. *Proceedings of the Zoological Society*
552 *of London*, *144*(3), 383–400. https://doi.org/https://doi.org/10.1111/j.1469-7998.1965.tb05189.x

553 Green, J. (2009). Nilotic lakes of the Western Rift. In *The Nile: Origin, Environments, Limnology and Human Use*
554 (pp. 263–286). Springer. https://doi.org/https://doi.org/10.1007/978-1-4020-9726-3_14

555 Gu, Q. H., Husemann, M., Wu, H. H., Dong, J., Zhou, C. J., Wang, X. F., ... & Nie, G. X. (2019). Phylogeography
556 of Bellamya (Mollusca: Gastropoda: Viviparidae) snails on different continents: contrasting patterns of
557 diversification in China and East Africa. *BMC Evolutionary Biology*, *19*(1), 82.
558 https://doi.org/10.1186/s12862-019-1397-0

559 Habimana, V., & Nsabimana, A. (2020). Water Physico-Chemical Characteristics of the Lakes Burera and
560 Ruhondo, Rwanda. *Rwanda Journal of Engineering, Science, Technology and Environment*, *3*(2).
561 https://doi.org/10.4314/rjeste.v3i2.5

562 Habiyakare, T., & Zhou, N. (2015). Water resources conflict management of Nyabarongo river and Kagera river
563 watershed in Africa. *Journal of Water Resource and Protection*, *7*(12), 889.
564 https://doi.org/10.4236/jwarp.2015.712073

565 Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for
566 Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*(41), 95–98.

567 Hämäläinen, H., Luotonen, H., Koskenniemi, E., & Liljaniemi, P. (2003). Inter-annual variation in
568 macroinvertebrate communities in a shallow forest lake in eastern Finland during 1990–2001.
569 *Hydrobiologia*, *506*, 389–397. https://doi.org/101023/B:HYDR.0000008581.86095.0b

570 Hasegawa, M., Kishino, H., & Yano, T. (1985). Dating of the human-ape splitting by a molecular clock of
571 mitochondrial DNA. *Journal of Molecular Evolution*, *22*, 160–174.
572 https://doi.org/https://doi.org/10.1007/BF02101694

573 Hofkin, B. V, Koech, D. K., Oumaj, J., & Loker, E. S. (1991). The North American crayfish Procambarus clarkii
574 and the biological control of schistosome-transmitting snails in Kenya: laboratory and field investigations.
575 *Biological Control*, *1*(3), 183–187. https://doi.org/10.1016/1049-9644(91)90065-8

576 Holmberg, R. D., Madsen, H., Kristensen, T. K., & Jørgensen, A. (2011). Gastropod distribution in Lakes George
577 and Edward, Uganda, relative to copper and cobalt levels. *African Journal of Aquatic Science*, *36*(2), 191–
578 196. https://doi.org/https://doi.org/10.2989/16085914.2011.589117

579 Isumbisho, M., Petit, P., Gashagaza, J. B., & Moreau, J. (2011). The feeding habit of the Cyprinidae Rastrineobola
580 argentea in its new habitat, lakes Bulera and Ruhondo, two Rwandan lakes (Eastern Africa). *Knowledge and*
581 *Management of Aquatic Ecosystems*, *403*, 4. https://doi.org/https://doi.org/10.1051/kmae/2011044

582 Jørgensen, A., Kristensen, T. K., & Madsen, H. (2008). A molecular phylogeny of apple snails (Gastropoda,

Caenogastropoda, Ampullariidae) with an emphasis on African species. *Zoologica Scripta*, *37*(3), 245–252. https://doi.org/10.1111/j.1463-6409.2007.00322.x

Kamanyi, J. R., Ndawula, L. M., & Magumba, M. (2000). *The history and fishery potential of Kisoro Minor Lakes: part two: Preliminary survey of Lakes Mutanda and Mulehe fisheries*.

Khalil, M. T., & Sleem, S. H. (2011). Can the freshwater crayfish eradicate schistosomiasis in Egypt and Africa. *J. Am. Sci*, *7*(7), 457–462.

Leigh, J. W., Bryant, D., & Nakagawa, S. (2015). POPART: full-feature software for haplotype network construction. *Methods in Ecology & Evolution*, *6*(9).

Lodge, D. M., Deines, A., Gherardi, F., Yeo, D. C. J., Arcella, T., Baldridge, A. K., Barnes, M. A., Chadderton, W. L., Feder, J. L., & Gantz, C. A. (2012). Global introductions of crayfishes: evaluating the impact of species invasions on ecosystem services. *Annual Review of Ecology, Evolution, and Systematics*, *43*, 449–472.

Lopes-Lima, M., Lopes-Lima, A., Burlakova, L., Douda, K., Alonso, Á., Karatayev, A., Ng, T. H., Vinarski, M., Zieritz, A., & Sousa, R. (2025). Non-native freshwater molluscs: a brief global review of species, pathways, impacts and management strategies. *Hydrobiologia*, 1–24. https://doi.org/https://doi.org/10.1007/s10750-024-05780-3

Madzivanzira, T. C., South, J., Wood, L. E., Nunes, A. L., & Weyl, O. L. F. (2020). A review of freshwater crayfish introductions in Africa. *Reviews in Fisheries Science & Aquaculture*, *29*(2), 218–241.

Mahulu, Anna, Catharina Clewing, Björn Stelbrink, Fred D. Chibwana, Immaculate Tumwebaze, J. Russell Stothard, and Christian Albrecht. "Cryptic intermediate snail host of the liver fluke Fasciola hepatica in Africa." *Parasites & vectors* 12, 573. https://doi.org/10.1186/s13071-019-3825-9

Magumba, M. K. (2000). *Physical, chemical, algal composition and primary production in the four Kisoro minor lakes.*

Mandahl-Barth, G. (1954). *The freshwater mollusks of Uganda and adjacent territories*. Koninklijk Museum van Belgisch-Congo.

Mandahl-Barth, G. (1962). Key to the identification of East and Central African freshwater snails of medical and veterinary importance. *Bulletin of the World Health Organization*, *27*(1), 135.

Mandahl-Barth, G. (1988). *Studies on African freshwater bivalves*. Danish bilharziasis laboratory.

Marie, M.-A. S., El-Deeb, F. A. A., Hasheesh, W. S., Mohamed, R. A., & Sayed, S. S. M. (2015). Impact of seasonal water quality and trophic levels on the distribution of various freshwater snails in four Egyptian governorates. *Applied Ecology and Environmental Sciences*, *3*(4), 117–126. https://doi.org/10.12691/aees-3-4-4

Meier-Brook, C., Haas, D., Winter, G., & Zeller, T. (1987). Hydrochemical factors limiting the distribution of Bulinus truncatus(Pulmonata: Planorbidae). *American Malacological Bulletin. 1987.*

Mkoji, G. M., Hofkin, B. V, Kuris, A. M., Stewart-Oaten, A., Mungai, B. N., Kihara, J. H., Mungai, F., Yundu, J., Mbui, J., & Rashid, J. R. (1999). Impact of the crayfish Procambarus clarkii on Schistosoma haematobium transmission in Kenya. *The American Journal of Tropical Medicine and Hygiene*, *61*(5), 751–759.

Nzabuheraheza, F. D., & Nyiramugwera, A. N. (2017). Food security status in developing countries: a case study of Burera and Musanze districts of Rwanda. *African Journal of Food, Agriculture, Nutrition and Development*, *17*(3), 12413–12426.

Palumbi, S. R., Martin, A., Romano, S., McMillan, W. O., Stice, L., & Grabowski, G. (1991). *The Simple Fool, s Guide to PCR.,(University of Hawaii: Honolulu, Hawaii.)*.

Papius, D. M. T., William, O., Alex, B., Dismas, M., Jimmy, O., & Vincent, K. (2016). Status of Kigezi minor Lakes: A limnological survey in the Lakes of Kisoro, Kabale and Rukungiri Districts. *International Journal of Water Resources and Environmental Engineering*, *8*(5), 60–73. https://doi.org/10.5897/ijwree2016.0645

Petkevičiūtė, R., Stunžėnas, V., & Stanevičiūtė, G. (2018). Comments on species divergence in the genus Sphaerium (Bivalvia) and phylogenetic affinities of Sphaerium nucleus and S. corneum var. mamillanum based on karyotypes and sequences of 16S and ITS1 rDNA. *PLoS One*, *13*(1), e0191427. https://doi.org/https://doi.org/10.1371/journal.pone.0191427

Preston, D. L., Crone, E. R., Miller-ter Kuile, A., Lewis, C. D., Sauer, E. L., & Trovillion, D. C. (2022). Non-native freshwater snails: a global synthesis of invasion status, mechanisms of introduction, and interactions with natural enemies. *Freshwater Biology*, *67*(2), 227–239. https://doi.org/https://doi.org/10.1111/fwb.13848

Rahel, F. J. (2002). Homogenization of freshwater faunas. *Annual Review of Ecology and Systematics*, *33*(1), 291–315. https://doi.org/10.1146/annurev.ecolsys.33.010802.150429

Saturday, A., Kangume, S., & Bamwerinde, W. (2023). Content and dynamics of nutrients in the surface water of shallow Lake Mulehe in Kisoro District, South–western Uganda. *Applied Water Science*, *13*(7), 150. https://doi.org/https://doi.org/10.1007/s13201-023-01953-y

Saturday, A., Lyimo, T. J., Machiwa, J., & Pamba, S. (2023). Spatial and temporal variations of trophic state conditions of Lake Bunyonyi, south-western Uganda. *Applied Water Science*, *13*(1), 7. https://doi.org/https://doi.org/10.1007/s13201-022-01816-y

Schultheiß, R., Van Boxlaer, B., Riedel, F., von Rintelen, T., & Albrecht, C. (2014). Disjunct distributions of freshwater snails testify to a central role of the Congo system in shaping biogeographic patterns in Africa. *BMC Evolutionary Biology*, *14*, 1471–2148. https://doi.org/10.1186/1471-2148-14-42

Sousa, R., Nogueira, J. G., & Padilha, J. (2024). Moving from the species to the population level in biological invasions. *Global Change Biology*, *30*(7), e17396. https://doi.org/https://doi.org/10.1111/gcb.17396

Stager, J. C., Day, J. J., & Santini, S. (2004). Comment on "Origin of the superflock of cichlid fishes from Lake Victoria, East Africa". *Science*, *304*(5673), 325–329. https://doi.org/10.1126/science.1091978

Tabo, Z., Neubauer, T. A., Tumwebaze, I., Stelbrink, B., Breuer, L., Hammoud, C., & Albrecht, C. (2022). Factors controlling the distribution of intermediate host snails of schistosoma in crater lakes in Uganda: A machine learning approach. *Frontiers in Environmental Science*, *10*, 871735. https://doi.org/https://doi.org/10.3389/fenvs.2022.871735

Tchakonté, S., Ajeagah, G. A., Diomandé, D., Camara, A. I., & Ngassam, P. (2014). Diversity, dynamic and ecology of freshwater snails related to environmental factors in urban and suburban streams in Douala–Cameroon (Central Africa). *Aquatic Ecology*, *48*, 379–395. https://doi.org/10.1007/s10452-014-9491-2

Thiele, J. (1911). *Mollusken der deutschen Zentral-Afrika-Expedition. Wissenschaftliche Ergebnisse der deutschen Zentral-Afrika-Expedition, 1907–1908.* Klinkhardt & Biermann.

Tumwebaze, I., Clewing, C., Dusabe, M. C., Tumusiime, J., Kagoro-Rugunda, G., Hammoud, C., & Albrecht, C. (2019). Molecular identification of Bulinus spp. intermediate host snails of Schistosoma spp. in crater lakes of western Uganda with implications for the transmission of the Schistosoma haematobium group parasites. *Parasites & Vectors*, *12*, 1–23. https://doi.org/10.1186/s13071-019-3811-2

Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*(4), 1367–1382.

Van Bocxlaer, B., & Albrecht, C. (2015). Ecosystem change and establishment of an invasive snail alter gastropod communities in long-lived Lake Malawi. *Hydrobiologia*, *744*, 307–316. https://doi.org/10.1007/s10750-014-2093-0

Van Bocxlaer, B., Clewing, C., Mongindo Etimosundja, J.-P., Kankonda, A., Wembo Ndeo, O., & Albrecht, C. (2015). Recurrent camouflaged invasions and dispersal of an Asian freshwater gastropod in tropical Africa. *BMC Evolutionary Biology*, *15*, 1–18. https://doi.org/10.1186/s12862-015-0296-2

Van Damme, D., & Gautier, A. (2013). Lacustrine mollusc radiations in the Lake Malawi Basin: experiments in a natural laboratory for evolution. *Biogeosciences*, *10*(9), 5767–5778. https://doi.org/https://doi.org/10.5194/bg-10-5767-2013

Van Damme, D., & Van Bocxlaer, B. (2009). *Freshwater molluscs of the Nile Basin, past and present. In: Dumont, H.J. (eds) The Nile. Monographiae Biologicae, vol 89. Springer, Dordrecht.* 585–629. https://doi.org/https://doi.org/10.1007/978-1-4020-9726-3_29

Wilke, T., & Davis, G. M. (2000). Infraspecific mitochondrial sequence diversity in Hydrobia ulvae and Hydrobia ventrosa (Hydrobiidae: Rissooidea: Gastropoda): do their different life histories affect biogeographic patterns and gene flow? *Biological Journal of the Linnean Society*, *70*(1), 89–105. https://doi.org/10.1111/j.1095-8312.2000.tb00202.x

682 Wilke, T., Davis, G. M., Qiu, D., & Spear, R. C. (2006). Extreme mitochondrial sequence diversity in the
683 intermediate schistosomiasis host Oncomelania hupensis robertsoni: another case of ancestral
684 polymorphism? *MALACOLOGIA-PHILADELPHIA-*, *48*(1/2), 143.

685 Worthington, E. B. (1932). *A Report on the Fisheries of Uganda Investigated by the Cambridge Expedition to the*
686 *East African Lakes, 1930-31 with 3 Appendices, 5 Maps and 21 Other Illustrations*.
687 https://doi.org/10.2307/1784052

688 Zenker, A., & Baier, B. (2009). Relevance of abiotic criteria used in German lake typology for macroinvertebrate
689 fauna. *Hydrobiologia*, *636*, 379–392. https://doi.org/10.1007/s10750-009-9967-6

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Paper 6: Dusabe et al., (in revision)

Historical drainage evolution and water level fluctuations in the African Great Lakes shaped phylogeny and biogeography of *Gabbiella* gastropods.

Dusabe, M. C., Clewing, C., Kagoro, G. R., Rwibutso, M., Tumusiime, T., Ndeo, W. O., & Albrecht, C. (2025). *Journal of Biogeography*. (in revision).

Historical drainage evolution and water level fluctuations in the African Great Lakes shaped phylogeny and biogeography of freshwater gastropods

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Abstract

Aim: The role of historical drainage connectivity and the influence of water level fluctuations in African lakes on the evolution and distribution of macroinvertebrates remains poorly understood. This is partly because evolutionary biology research has largely focused on mobile vertebrates, such as cichlids. Here, we studied an African freshwater snail genus and assessed its phylogenetic relationships and phylogeographic patterns, and determined its colonization history in African waters.

Location: African Great Lakes (AGL)

Taxon*: Gabbiella* (Gastropoda: Bithyniidae)

Methods: We applied a multi-locus molecular phylogenetic approach combining two mitochondrial DNA markers (COI and 16S) and two nuclear DNA markers (H3 and 28S) based on samples covering six AGL, their major connected rivers, and two crater lakes in western Uganda. Using maximum likelihood (ML) and Bayesian inference (BI) approaches, we inferred a dated phylogeny of *Gabbiella* and estimated the ancestral areas of the *Gabbiella humerosa*

subspecies using BioGeoBEARS. We used statistical parsimony haplotype networks and genetic *p*-distances to investigate phylogeography and quantify genetic divergence within and between *Gabbiella* clades.

Results: The ML and BI analyses yielded nearly identical topologies. Our BEAST analysis suggests that *Gabbiella* probably originally diversified in the Miocene and later in the Pliocene and Pleistocene, too. We found high genetic distance among *Gabbiella* species and typical intraspecific genetic distance among *G. humerosa* subspecies. Phylogenetic inferences suggest that the most recent common ancestor (MRCA) of all *G. humerosa* subspecies originated in the Kivu-Tanganyika region during the Late Pleistocene. The MRCA of the Nilotic *G. humerosa* subspecies was most likely distributed in the Lake Kivu and Lake Albert regions.

Main conclusions: The timing of diversification of *Gabbiella* coincides with significant geological events, such as rifting, historical drainage changes and lakes-level fluctuations. These events suggest that the tectonic and climatic history of the region plays an integral role in diversification. Following each episode of vicariance, dispersal rather than extinction seems to determine the distribution patterns, underlining the high ecological tolerance of *G. humerosa*. The spatial diversification patterns of *Gabbiella* show similarities with those of other African molluscs and several fish taxa, suggesting that diversification is influenced by common underlying factors.

Keywords: Ancient lakes, Bithyniidae, dispersal, *Gabbiella*, phylogeography, rifting

1. Introduction

Formed by tectonic activity that split the earth's crust, creating deep depressions that were later filled with water, the African Great Lakes (AGL) are located in the rift valleys between East and Central Africa (Cohen et al., 1997; Danley et al., 2012; Ogutu-Ohwayo et al., 2020). Together they cover a catchment area of over 850,000 km² in seven large lake basins: lakes Tanganyika, Kivu, Edward, Albert, Victoria, Turkana, and Malawi (also known as Nyasa or Niassa) (Salzburger et al., 2014; Cowx & Ogutu-Owhayo et al, 2019). The AGL represent the largest freshwater lake system in the tropics (Plisnier et al., 2023), an invaluable resource at local, regional and global scales. They provide water, support biodiversity, enable food security, drive economic activities, and regulate the climate (Daley, 2003; Salzburger et al., 2014). The AGL are known as biodiversity

hotspots due to their exceptional species diversity, making them of great interest to evolutionary biologists (e.g. Brooks, 1950; Martens et al., 1994; Martens, 1997; Rossiter & Kawanabe, 2000). The lakes' structure, age and habitat diversity have given rise to unique and endemic organisms (Salzburger et al., 2014). Their drainage history, shaped by tectonic activities and climatic changes, has had a lasting impact on the speciation and dispersal patterns of aquatic organisms (Salzburger et al., 2014; Daniels et al., 2015; Ogutu-Ohwayo et al., 2020). For example, genetic evidence suggests that tectonically induced drainage reversals have played a crucial role in the evolution and dispersal of cichlids and other endemic AGL species (Verheyen et al., 2003). The formation of a cichlid superflock following the drying events in Lake Victoria indicates historical drainage connectivity, which is defined here as former surface water linkages between basins, among lakes Albert, Edward and Kivu (Elmer et al., 2009; Vranken et al., 2022; Meier et al., 2023). These connections are now mainly restricted to the Nile River (Schultheiß et al., 2014). Lake Malawi, which is part of the Zambezi River system, has remained isolated from the other AGL (Salzburger & Meyer, 2004). However, several authors have suggested a connection between the Congo River system and Lake Malawi (Van Bocxlaer et al., 2015; Schultheiß et al., 2011; Ortiz-Sepulveda et al., 2020).

While much of the research in evolutionary biology has focused on fish such as the famous cichlids, catfish and killifish (Verheyen et al., 2003; Seehausen, 2006; Friedman et al., 2013; Day et al., 2013; Van der Merwe et al., 2021; Meier et al., 2023), less is known about macroinvertebrates. Most fish are highly mobile and can disperse over long distances. They can move against the current, cross barriers, and use temporary water connections in wet conditions (Hohausová et al., 2010). This mobility leads to dispersal patterns that are often very different from those of other invertebrates (Tonkin et al., 2018). In addition, human-induced relocations of fish can blur natural biogeographical patterns (e.g. Van Steenberge et al., 2020). Biogeographical studies of less mobile invertebrates could enable more accurate reconstructions of drainage connectivity through time. Their restricted movement subjects them to localized evolutionary pressures, making them valuable for biogeographic studies and the reconstruction of drainage connections (Mahulu et al., 2021). Recent studies found some freshwater molluscs particularly valuable for studying ancient evolutionary processes (Ortiz-Sepulveda et al., 2020; Van Bocxlaer et al., 2021; Mahulu et al., 2021). However, none of these studies covered the AGL entirely or their connected rivers.

Gabbiella, is a genus of freshwater gastropod belonging to the family Bithyniidae. Here it serves as a model for reconstructing phylogeographic patterns because it is widely distributed in African lakes and rivers, less commonly in permanent small water bodies, and rarely in habitats that dry out frequently (Mandahl-Barth, 1968; Brown, 1994). Each AGL harbors an endemic *Gabbiella* species or subspecies (Mandahl-Barth, 1968; Brown, 1994). This strong pattern of local endemism is likely due to limited dispersal ability and strict habitat specificity. *Gabbiella* speciesare freshwater specialists with narrow ecological tolerances (Mandahl-Barth, 1968; Brown, 1994). They dependent on stable, localized habitats such as fine substrates or rocky littoral zones (Brown, 1994), making them highly sensitive to historical changes in drainage connectivity. The oldest fossil record of *Gabbiella* dates to the Miocene (12.5–10.5 Ma) from the pre-Obweruka or Kisegi stage (Van Damme & Pickford, 2003) and other fossils are found in African paleo-lakes (see Table S3). The *Gabbiella* genus is divided into three subgenera of which two subgenera, *Gabbiella* sensu stricto and *Conogabbia*, are distributed across the AGL and surrounding waters (Mandahl-Barth, 1968). *Gabbiella humerosa* is very diverse in the AGL of East Africa and comprises six subspecies: *G. h. tanganyicensis* (Lake Tanganyika), *G. h. kivuensis* (Lake Kivu), *G. h. edwardi* (Lake Edward), *G. h. alberti* (Lake Albert), *G. h. kyogae* (Lake Kyoga & Victoria Nile River), and *G. h. humerosa* (Lake Victoria) (Mandahl-Barth, 1968). *Gabbiella kichwambae* occurs in the crater lakes of western Uganda and is morphologically similar to *G. humerosa*, but larger (Brown, 1994). Lake Albert harbors not only *G. h. alberti*, but also two other endemic *Gabbiella* species: *G. candida* and *G. walleri* (Mandahl-Barth, 1968). Lake Malawi is home to the endemic *G. stanleyi*, while Lake Turkana hosts the endemic *G. rosea* (Mandahl-Barth, 1968).

Knowledge of the Bithyniidae in sub-Saharan Africa remains limited as populations are not extensively surveyed and species richness and taxonomy are still primarily based on shell and radula morphology and distribution patterns (Mandahl-Barth, 1968; Brown, 1994). To date, no comprehensive large-scale phylogenetic analysis of the *Gabbiella* genus has been conducted.

In this context, we sampled *Gabbiella* species in the AGL (Figure 1 and Figure S3). We (1) determined the phylogenetic relationships, genetic variability, and phylogeographic patterns of *Gabbiella* species in the AGL. We (2) determined the geographical origin, and colonization history of the subspecies of *G. humerosa* in the AGL of East Africa, and we (3) assessed the impact of drainage changes and water level fluctuations on the dispersal and adaptive radiation of *Gabbiella* species. Although most *Gabbiella* species are restricted to large lakes, the evolutionary links

between these lakes remain unresolved, particularly the question of whether lacustrine species evolved from widespread riverine ancestors or by dispersal between lakes.

To address these objectives, we used a multi-locus molecular phylogenetic approach, constructed haplotype parsimony networks, and reconstructed ancestral ranges. We discuss the diversification patterns of *Gabbiella* in the context of AGL basin evolution, water level fluctuations, and large-scale biogeographical patterns in Africa. Finally, we compare our results with patterns in cichlid fishes and other freshwater molluscs.

2. Material and methods

2.1 Data collection and morphological identification

A targeted sampling of *Gabbiella* was carried out, specifically focusing on type localities summarized by Mandahl-Barth (1968). Fieldwork was conducted at Lake Tanganyika (LT), Lake Kivu (LK), Lake Edward (LE), Lake Albert (LA), Lake Kyoga (LKY), Lake Victoria (LV), the Victoria Nile River (VNR), the Semliki River (SR), Lake Malawi (LM), Lake Turkana (LTK), Lake Bunyonyi (LB), several crater lakes in western Uganda (CL), and a stream in the Kano Plain near Awasi (SKA) in western Kenya. The samples were taken in a survey carried out in 2022 and 2023. Additional *Gabbiella* spp. material from multiple previous collections spanning from 2006 to 2018, stored in the UGSB (University Giessen Systematic & Biodiversity) collection at the Justus Liebig University Giessen (Germany), complemented this study. Specimens were hand-picked from hard substrates in shallow waters on stones and rocks, while scoop nets and dredges were used on soft substrates. Sampling was extended to deeper parts of the littoral zone (down to depths of 40 m) using an Ekman grab and a dredge. All specimens were preserved in 80% ethanol. We then used the relevant literature of Mandahl-Barth (1962) and Brown (1994) for morphological taxonomic identification. Prior to DNA extraction, high-resolution images were taken using a digital microscope (Keyence VHX-2000).

2.2 DNA isolation, amplification and sequencing

DNA was extracted from two specimens per species per locality, resulting in a total of 120 *Gabbiella* specimens, using either an entire specimen or a foot tissue sample (for larger specimens)

using the CTAB protocol described by Wilke et al. (2006). Fragments of two mitochondrial markers, cytochrome *c* oxidase subunit I (COI) and large subunit ribosomal RNA (16S), were amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994), and 16Sar and 16Sbr (Palumbi et al., 1991), respectively. In addition, two nuclear markers, histone 3 (H3) and large subunit ribosomal RNA (28S), were amplified with the primers H3F and H3R (Colgan et al., 2000), and D23F and D6R (Park & Foighil, 2000), respectively. PCR conditions are described in the supplementary materials (Table S4). DNA vouchers and, where available, shells and tissue remains are stored in the UGSB collection. Sanger sequencing was performed on an ABI 3730xl DNA analyzer using the BigDye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, Germany).

2.3 Dataset Compilation and Alignment

Additional Bithyniidae sequences for all four genetic markers were downloaded from the NCBI GenBank database (see Table S1). We used *Bythinella pannonica* (COI: FJ029077; 16S: FJ028877; 28S: FJ028895) and *Lithoglyphus naticoides* (COI: AF4445332; 16S: FJ160287; 28S: FJ160290) as outgroup taxa. Alignments for the protein coding 66 sequences (49 newly generated) of COI (658 bp) and 25 sequences (15 newly generated) H3 (328 bp) were performed using ClustalW implemented in BioEdit version 7.2.5 (Hall, 1999). The non-coding gene fragments with 37 sequences (22 newly generated) 16S (516 bp) and 22 sequences (10 newly generated) 28S (820 bp) were aligned using the online program MAFFT version 7.0 (Katoh & Standley, 2013; default settings). Ambiguous sites within the 28S alignment were removed using Gblocks version 0.91b (Castresana, 2000) with the settings for a less stringent selection (i.e., allowing smaller final blocks, gap positions within the final blocks and less strict flanking positions). The final alignment of 28S was 677 bp long, representing 82.5% of the original 820 positions. All new sequences are deposited in GenBank (accession numbers are given in Table 1). A concatenated dataset was created using SequenceMatrix version 1.7.8 (Vaidya et al., 2011), resulting in a dataset composed of 66 taxa with a total length of 2,179 bp.

2.4 Phylogenetic and molecular-clock analyses

The phylogenetic relationships were reconstructed using both maximum likelihood (ML) and Bayesian inference (BI). Prior to the analyses, we used jModelTest version 2.1.10 (Darriba et al., 2012) to select the best-fit substitution model for each gene partition. The models were selected based on the corrected Akaike information criterion (AICc) and Bayesian information criterion (BIC). For the 28S and 16S partitions, the AICc and BIC selected the GTR + I + Γ model, while for the COI and H3 partitions, the HKY + I + Γ and GTR + I models were selected, respectively. The ML analysis was performed for the concatenated dataset (N = 66) using IQ-TREE version 2.2.2.6 (Minh et al., 2020). The node supports were evaluated using 10,000 ultra-fast bootstrap iterations.

The estimation of divergence times was performed with BEAST version 1.8.4 (Drummond et al., 2012) using a concatenated dataset reduced to unique haplotypes (N = 49). The following settings were used : speciation = birth–death process; number of MCMC generations = 40,000,000 and a sample frequency = 2,000. Due to uncertainty about the age and identification of the fossils (see Table S3), fossil calibrations were not used. Instead, we applied a mean molecular-clock rate ranging from 0.0124 to 0.0157 (substitutions per site and My) proposed for the COI gene for different Protostomia groups referring to the substitution models HKY and HKY + I + Γ, respectively (Wilke et al. 2009). A uniform prior was applied to this range. Two different clock models, the strict-clock model and the uncorrelated lognormal relaxed-clock model, were used. These clock-models were compared in BEAST using the marginal likelihood estimation based on path sampling (PS) and stepping-stone (SS) sampling (Baele et al., 2012). Based on marginal likelihood comparison, the relaxed clock-model was favored (see Table S5) and a maximum clade credibility (MCC) tree was generated with TreeAnnotator version 1.8.4 (BEAST package; node heights = mean heights) with a 50% burn-in. To address convergence issues with more complex substitution models (major ESS values < 25), the less complex HKY substitution model was applied to the 16S, 28S and H3 partitions (28S and 16S = HKY + I + Γ; H3 = HKY + I), reaching stationarity and increasing the effective sample size (ESS) values for all parameters. The ESS values were visualized on Tracer version 1.5 (Rambaut & Drummond, 2007) revealing for both clock models values >300.

Species delimitation was based on congruence between shell morphological identification and phylogenetic structure inferred from the phylogenetic analyses.

2.5 Phylogeographical analyses

To explore the phylogeographic patterns of *Gabbiella* spp., we performed a statistical parsimony network analysis based on COI newly generated sequences using TCS version 1.21 (Clement et al., 2000; connection limit = 95%). In addition, uncorrected genetic *p*-distances between groups of different *Gabbiella* (sub)species were calculated using the COI gene fragment in MEGA 11 (Tamura et al., 2021).

2.6 Ancestral area estimation

To infer the biogeographical history of the *G. humerosa* group (see Figure 2) we used the R package BioGeoBEARS version 1.1.3 (Matzke 2013a,b) for the R statistical environment 4.4.2 (R Core Team, 2024). Six biogeographical models were tested and compared using the AICc: DEC, DEC+J, DIVALIKE, DIVALIKE+J, BayAreaLIKE and BayAreaLIKE+J (Landis et al., 2013; Ree et al., 2005; Ree and Smith, 2008; Ronquist, 1997; Matzke, 2014). The geographic distribution of each subspecies was coded based on occurrences of the sampled populations resulting in seven regions (see 2.1.): LT (A), LK (B), LE (C), LA (D), and LV (E), as well as SR (F) and VNR (G). The maximum number of ancestral areas possibly inhabited was set to two. Prior to the analyses, the BEAST maximum clade credibility (MCC) tree was pruned to a *G. humerosa* subspecies (OTU-like) tree in BioGeoBEARS.

3 Results

3.1 Species richness and communities

Of the 13 type localities surveyed, five *Gabbiella* spp. were found at 9 localities (Figure 1). No *Gabbiella* spp. were found in LTK, which harbored only other snail shells during sampling. Similarly, no *Gabbiella* spp. were found in LB, LKY and in the SKA, although other living

molluscs were present. Six subspecies of *G. humerosa* were found one per lake in LT, LK, LE, LA and LV as well as in the VNR (see about *G. humerosa kyogae* in 3.3) (Figure 1). Two endemic species, *G. candida* and *G. walleri*, occurred exclusively in LA, with 90% of them often co-occurring with *G. humerosa alberti* (Figure 1). Lake Albert is thus the only lake with more than a single *Gabbiella* species. *Gabbiella kichwambae* was found in the CL (lakes Nyinambuga and Lyantonde), and *G. stanleyi* was recorded from various locations across LM, but was not found in the tributaries.

3.2 Phylogenetic relationships and estimation of divergence times

The ML and BI trees yielded nearly identical topologies, except for the position of *G. kichwambae* from the crater lakes of western Uganda and *Hydrobioides* sp. from Myanmar, the latter position rendering the genus *Gabbiella* non-monophyletic (see taxonomic implications in supplementary materials: Appendix 1). Overall, strongly supported phylogenies were obtained (Figure 2A and Figure S1). The *Gabbiella* samples from the AGL yielded three major clades with Bayesian posterior probabilities (BPP) above 0.95 and bootstrap values (BS) of 1.00 for clade 1 and clade 2, and BPP of 0.98 and BS of 0.74 for clade 3 (Figure 2A and Figure S1). Clade 1 consists of *G. humerosa* (incl. all six subspecies) and *G. kichwambae*, and is the sister of clade 2, which consists of *G. candida* (BPP = 1.00 and BS = 100). Clade 3, comprising *G. stanleyi* and *G. walleri* (BPP = 1.00 and BS = 100), is sister to *Hydrobioides* sp. from Myanmar. Clade 3 and *Hydrobioides* sp. form a sister group to clades 1 and 2, and all three clades form a sister group to the remaining bithyniid.

According to the Bayesian divergence time estimates, the split between *Gabbiella* (including *Hydrobioides* from Myanmar) and the Eurasian bithyniids occurred in the Late Oligocene 25.03 Ma [95% highest posterior density (HPD) = 18.16–32.22 Ma] (Figure 2A). The most recent common ancestor (MRCA) of *G. stanleyi* and *G. walleri* (lakes Malawi and Albert, respectively) and *Hydrobioides* sp. (Myanmar) split in the Middle Miocene 17.31 Ma (95% HPD = 11.88–23.62 Ma). The MRCA of *G. candida*, *G. kichwambae*, and *G. humerosa* was estimated to be 14.35 million years old (95% HPD = 9.43–19.91 Ma). The split between *G. stanleyi* and its sister species (*G. walleri*) is estimated to 3.36 Ma (95% HPD = 1.99–4.80 Ma) and thus dates back to the

Pliocene. The *G. humerosa* subspecies originated in the Early Pleistocene 1.50 Ma (95% HPD = 0.94–2.26 Ma).

3.3 Phylogeographical patterns

The statistical parsimony network analysis resulted in 32 unique haplotypes out of 49 COI sequences organized in seven single networks (Figure 3 and Table 1). *Gabbiella humerosa alberti* (LA) clustered together with *G. humerosa humerosa* (LV) and *G. humerosa kyogae* (VNR) in group A, which comprised eleven specimens separated by a maximum of four mutational steps (Figure 3A). Two specimens from LV and the VNR share the same haplotype (H17) showing that *G. humerosa humerosa* (LV) and *G. humerosa kyogae* (VNR) are genetically identical. *Gabbiella humerosa kivuensis* (LK) clustered together with *G. humerosa edwardi* (LE) in group B which comprised 11 specimens separated by a maximum of seven mutational steps (Figure 3B). The *G. humerosa edwardi* haplotype (H11) from the SR was separated by one mutational step from specimens from LE. Four specimens of *G. humerosa tanganyicensis* (LT) formed a single haplotype (group C; Figure 3C) and did not cluster with any other *G. humerosa* subspecies. *Gabbiella kichwambae* formed an independent cluster comprising two specimens from the crater lakes Nyinambuga and Lyantonde (group D; Figure 3D). *Gabbiella candida* and *G. walleri* from Lake Albert (N = 6 and N = 5, respectively) formed the independent networks (see Figures 3E and F). The haplotypes from LM were clustered in group G (see Figure 3G), which comprised 10 specimens of *G. stanleyi* with populations from the southern basin (H24, H27–H29) connected to those from the northern basin (H22, H23) by a maximum of 18 mutational steps.

The *p*-distance between the group A (*G. h. alberti*, *G. h. humerosa* and *G. h. kyogae*) and the group B (*G. h. kivuensis* and *G. h. edwardi*) was 2.8%, while the *p*-distance to group C (*G. h. tanganyicensis*) was 2.5% and 3.3%, respectively (Table 2). The *p*-distance between *G. humerosa* (groups A, B, C) and *G. kichwambae* (group D) ranged between 3.6% and 4.7%. The genetic *p*-distance between *G. candida* (group E) and *G. walleri* (group F) and *G. humerosa* subspecies ranged between 16.6% and 18.2% (Table 2), while the *p*-distance between the two sister taxa *G. walleri* (group F) and *G. stanleyi* (group G) was 7.7%.

3.4 Ancestral area estimation

According to the AICc as implemented in BioGeoBEARS, the best-fit biogeographical model was DIVALIKE (d = 0.5781; e = 0.4158; j = 0; LnL = -14.66; Figure S2). The MRCA of *G. humerosa* probably inhabited the area of LT and LK (Figures 2B, 4 and Figure S2). A vicariant event around 1.17 Ma (95% HPD = 0.76–1.59 Ma) gave rise to the ancestor of all Nilotic subspecies (*G. h. alberti, G. h. humerosa, G. h. kyogae, G. h. kivuensis,* and *G. h. edwardi*) and the LT subspecies (*G. h. tanganyicensis*; see Figures 2B, 4). Subsequently, the ancestral area of all Nilotic subspecies was expanded via anagenetic dispersal from the LK region into the LA area. Around 1.00 Ma (95% HPD = 0.64–1.4 Ma) another vicariant event separated the ancestral populations inhabiting LK and LA, which both expanded their range to LK+LE and LA+LV, respectively, via anagenetic dispersal. Both ancestral areas (LK+LE and LA+LV) were further subdivided via vicariance c. 400 ka (95% HPD = 0. 20–0.61 Ma) and c. 300 ka (95% HPD = 0.12–0.4 Ma), respectively, giving rise to all currently known Nilotic subspecies.

4 Discussion

4.1 Species diversity, phylogenetic relationships and divergence times

The present study provides the first molecular-based approach to *Gabbiella* species diversity in the AGL and major connected rivers. Our analyses identified the presence of five *Gabbiella* species and five of the six *G. humerosa* subspecies at their type localities (Figure 1, note that *G. h. kyogae* is with *G. h. humerosa*). Our results also confirm the occurrence of *G. humerosa edwardi* in the SR, which is consistent with earlier findings by Schultheiß et al. (2011). Our phylogenies reveal the presence of three highly divergent *Gabbiella* clades aligned with taxonomic classifications rather than geographic distribution (i.e. samples from LA do not form a cluster). Importantly, the phylogenetic relationships within the clades (except clade 1) and between the clades are not reflected in the classification of *Gabbiella* into different subgenera based on morphological, anatomical and radula characters from previous studies (see taxonomic implications in the supplementary materials: Appendix 1; Mandahl-Barth, 1968; Brown, 1994). Our multi-gene phylogenies strongly support a close relationship within the subspecies of *G.*

humerosa found in the AGL, as recognized by Mandahl-Barth (1968), and support a close relationship of *G. humerosa* and *G. kichwambae* from the western Uganda crater lakes (Figure 2A and Table 2). The uncorrected genetic *p*-distance between subspecies of *G. humerosa* of 2.5% to 3.3% corresponds to the intraspecific *p*-distance of 1.5% to 5.0% in *Bythinella pannonica*, which is thought to be comparatively high due to a spatial distribution characterized by habitat fragmentation and dispersal over long distances (Fehér et al., 2013). The *p*-distance between *G. kichwambae* and *G. humerosa* ranging between 3.6% and 4.7% corresponds to interspecific *p*-distances from the family Hydrobiidae (Beran et al., 2022) and thus supports a close relationship between *G. humerosa* and *G. kichwambae* as recognized by Mandahl-Barth (1968).

Interestingly, unlike other AGL, where each lake harbors a single endemic *Gabbiella* species, LA contains three highly distinct species. The uncorrected *p*-distance between these species exceeds the *p*-distance observed in hydrobiids (Delicado et al., 2019). It is similar to the family or subfamily divergence observed in the Truncatelloidea (Wilke et al., 2001, 2023). These species are not closely related and thus do not represent a species flock, but rather a species scatter resulting from multiple colonization events (Hauswald et al., 2008). Similar situations for Bithyniidae have so far only been found in temperate, potentially ancient lakes Trichonis and Prespa (Albrecht et al., 2009, 2012). While formal taxonomic changes are beyond the scope of this study, our results emphasize the need for a comprehensive phylogenetic re-evaluation of the genus *Gabbiella* (see taxonomic implications in supplementary materials: Appendix 1). Compared to other smaller AGL such as LE and LK, LA hosts a relatively high molluscan diversity. Similarly, the western Uganda crater lakes exhibit a comparatively high molluscan diversity, including a potentially endemic *Gabbiella* species, raising questions about the origins and colonization mechanisms of species in these isolated water bodies. The inferred sister relationship between *G. walleri* (LA) and *G. stanleyi* (LM) should be interpreted with caution due to the incomplete sampling.

The fossil record of *Gabbiella* is summarized (Table S3), but the actual dating, taxonomic classification, and age of most the records are uncertain, limiting their applicability in determining their precise placement on the phylogenetic tree. Such uncertainties reduce the trustworthiness of using the fossil record as a reference for dating evolutionary relationships. Therefore, we used the mean molecular-clock rate for the COI gene according to Wilke et al. (2009), which closely matches clock rates determined by fossil calibrations in freshwater molluscs (Schultheiß et al., 2014; Tumwebaze et al., 2022). As such, the suggested divergence time of *Gabbiella* from the

Eurasian bithyniids (Figure 2A) thus predates the geological evidence for the closure of the Tethys Seaway (18–20 Ma; Okay et al., 2010) and it aligns with the earliest evidence for mammalian exchange between Africa and Eurasia (Rögl, 1999; Harzhauser et al., 2007; Harzhauser & Piller, 2007). Moreover, a significant portion of the eastern branch of the East African Rift System developed somewhat later, approximately 20 Ma (Pickford, 1982; Chorowicz, 2005). The colonization of *Gabbiella* in the African region began later than that of co-occurring freshwater molluscs like *Lanistes* (Mahulu et al., 2021) and freshwater crabs of the Potamonautidae, which originated during the Eocene (Daniels et al., 2015). However, it aligns with that of *Melanoides* in the Early Miocene (Genner et al., 2007) and fish genera, such as *Distichodus* in the Late Oligocene (Arroyave et al., 2020). The split of *G. stanleyi* and *G. walleri* (clade 3) from the Asian *Hydrobioides* (Myanmar) coincides with a period of significant climatic and geological changes in Africa during the Middle Miocene, with the climatic optimum around 15–17 Ma representing a warmer phase with a peak in precipitation (Zachos et al., 2001). This may have increased the connectivity of African rivers, facilitating divergence and diversification. Other fish genera have also been identified as having diversified around the same period, including spiny eels (*Mastacembelus*) and killifish (*Nothobranchius*) during the Miocene (Day et al., 2017; Van der Merwe et al., 2021) as well as the Early Miocene onset of diversification within Haplochromini cichlids (Schedel et al., 2019). It is noteworthy that, *Gabbiella*'s diversification occurred earlier than that of *Bellamya* snails (Schultheiß et al., 2011, 2014) and *Coelatura* bivalves (Ortiz-Sepulveda et al., 2020). The *G. candida* from LA diverged from the *G. humerosa* subspecies and *G. kichwambae* in the Middle Miocene, 14.35 Ma (95% HPD = 9.43–19.91). The relatively long branch, likely indicates prolonged isolation and an absence of further diversification. Alternatively, we could also miss taxa because they are either extinct or have not been sampled. The initial colonization of the paleo-lake Obweruka occurred during the Kisegi stage (12.0 and 10.5 Ma), where the oldest fossils of *Gabbiella* were discovered (Van Damme & Pickford, 2003) overlaps the timing of the split of *G. candida*. By 2.0 Ma, however, all Albertine endemics had gone extinct, leaving only the widespread thiarids remaining in the Kaiso and Lusso paleo-lakes (Van Damme & Pickford, 2003). Identifying and classifying older fossils within a modern DNA-based phylogenetic framework is challenging (Wilke et al., 2009). Using such older fossils with uncertain phylogenetic placement as calibration points can lead to significant errors (Nguyen et al., 2020). The SR, which formed a connection between LE and LA about 10 ka ago (Beadle,

1981), adds to the difficulty of accurately placing the associated fossil record. Similarly, the estimated age of the *G. stanleyi* fossils from paleo-lake Chiwondo in the Malawi Basin is unclear (Van Damme & Gautier, 2013). Our estimate for the initial start of diversification within *G. stanleyi* in Middle Pleistocene, 930 ka (95% HPD = 0.48–1.46 Ma) coincides with *Lanistes* (Schultheiß et al., 2009; Mahulu et al., 2021), *Bellamya* (Schultheiß et al., 2011, 2014), and thus supports the founder flush pattern recognized by Schultheiß et al. (2009). Lake Malawi was most likely dry during long periods between 1.6 Ma and 800 ka (see Figure 5; Delvaux, 1995; Lyons et al., 2015; Ivory et al., 2016), and it is likely that *G. stanleyi*, among other taxa, recolonized the lake after the crisis (Van Damme & Gautier, 2013).

4.3 Biogeography and phylogeographic patterns

The phylogeny of *G. humerosa* from the AGL suggests a strong connection between the region's historical drainages, and underscores the impact of vicariance and dispersal on biogeographical patterns. Each evolutionary divergence in *G. humerosa* is associated with a discrete episode of drainage evolution, which either separated ancestral populations into new habitats or facilitated their dispersal. This was likely driven by rifting or climatic change. Our phylogenetic framework suggests similar patterns of colonization of the AGL by *G. humerosa* and the cichlid fish of the LV Region Superflock (LVRS; cichlids of LV and the nearby LA, LKY, LE and LK; Verheyen et al, 2003; Meier et al., 2023). A slight difference with other molluscs such as *Bellamya* and *Coelatura* exists (Schultheiß et al., 2011, 2014; Ortiz-Sepulveda et al., 2020; Van Bocxlaer et al., 2021). Notably, *Bellamya* and *Coelatura* are absent in LK (Brown, 1994; Mandahl-Barth, 1988; Dusabe et al., 2024), and *Bellamya* is absent in LE and Lake George (Brown, 1994; Schultheiß et al., 2011). Apart from these absences, the phylogeographic patterns of *G. humerosa* closely match those of the molluscs mentioned above.

The MRCA of *G. humerosa* inhabited LK and LT in the Early Pleistocene 1.50 Ma (95% HPD = 0.94–2.26 Ma; Figure 2A). With the progression of rifting, a vicariance likely disrupted the drainage connection between LK and LT around 1.17 Ma (95% HPD = 0.76–1.59 Ma). This period coincides with increasing aridity and climate variability across Africa, as documented by Trauth et al. (2021) for the period from 1.9 to 1.4 Ma. Faunal fluctuation must have occurred on a larger African scale around 2.0 to 1.75 Ma (Bibi & Kissling, 2015) and 0.75 to 0.50 Ma (Cohen et

al., 2022b). The vicariance also matches the time when the water level of LT dropped significantly, reaching up to 600 meters in Plio-Pleistocene (Russell et al., 2020) or even 650–700 meters below present-day levels by 1.1 Ma (Figure 5; Lezzar et al., 1996). In addition, the final uplift of the northern LT boundary fault, which occurred around 1.0 Ma, further corroborates the vicariance (Lezzar et al., 2002; Wright et al., 2020). This vicariance led to the initial allopatric speciation of *G. humerosa* (Figure 2B) giving rise to the ancestor of all Nilotic subspecies (*G. h. kivuensis*, *G. h. alberti*, *G. h. humerosa*, *G. h. kyogae*, and *G. h. edwardi*) and the LT subspecies (*G. h. tanganyicensis*; see Figures 2B, 4). The ancestral area of all Nilotic subspecies expanded via anagenetic dispersal from the LK region into the LA area within a timeframe of ~1.17–1 Ma (Figure 4). Around 1.00 Ma (95% HPD = 0.64–1.4 Ma), a vicariance event occurred, separating the ancestral populations inhabiting the LK and LA regions. This separation likely resulted from the cooling and drying of climate in East Africa between 1.7 and 1.0 Ma (Peter, 2004; Hoag et al., 2017), which likely triggered ecological fragmentation and genetic isolation. The time of the vicariance also corresponds with the estimated age of the MRCA of cichlids in the LVRS (913 ka; Meyer et al., 2017). However, the MRCA of viviparid snails in the LV ecoregion is older, at approximately 2.50 Ma (Schultheiß et al., 2014; Van Bocxlaer et al., 2020). Following this divergence, the descendant populations appear to have expanded their range to the LK+LE region (timeframe: ~1 Ma–300 ka) and to the LA+LV region (timeframe: ~1 Ma–400 ka).

Notably, the LK's inclusion in the MRCA of other Nilotic AGL *Gabbiella* species aligns with prior research highlighting the pivotal role of LK in the cichlids distribution within the Nilotic AGL (Verheyen et al., 2003; Meier et al., 2023). This also highlights the historical drainage connections between the LK and other Nilotic lakes (Beadle, 1981; Ross et al., 2014). However, the dispersal pattern of the catfish *Clarias gariepinus* tells a different story. The ancestors of the LK catfish were distributed throughout the Congo Province, reflecting the current drainage system through which the LK flows into the Congo River via the LT (Van Steenberge et al., 2020). Both ancestral areas (LA+LV and LK+LE) were further subdivided by vicariance around 400 ka (95% HPD = 0.20–0.61 Ma) and 300 ka (95% HPD = 0.12–0.4 Ma), respectively, giving rise to all the Nilotic subspecies known today. The vicariance event that separated LA+LV at 400 ka corresponds to the estimated origin of LV (Johnson et al., 1996; Talbot & Williams, 2009; Danley et al., 2012; Salzburger et al., 2014) and corroborates the mitochondrial divergence time of the cichlid LVRS (Schedel et al., 2019). The LA and LV subspecies cluster in the same haplotype

network, separated by a maximum of four mutational steps (Figure 3A), suggesting a recent common ancestor that nevertheless experienced some divergence with persistent gene flow. Lake Victoria may have completely desiccated during the megadroughts (Beverly et al., 2022; Renaut & Owen, 2023) or experienced widespread desiccation with localized seasonal wetlands during the Last Glacial Maximum (Wienhues et al., 2024; Tryon et al., 2016). Survival of organisms would potentially require that a source area existed in the vicinity of LV during arid phases and that recolonization happened when the lake refilled. Lake Victoria is currently connected to the LA via the VNR and LKY. However, the VNR is estimated to be only about 11.2 ka (Beuning et al., 1997; Johnson et al., 2000; Table S2C), which corroborates the timeframe of species dispersal between the LV and VNR. This dispersal likely began with the formation of the VNR (see Figure 2B). Mandahl-Barth (1968) and Brown (1994) recorded the occurrence of *G. humerosa kyogae* in the VNR. Our phylogeographic analysis shows that *G. humerosa kyogae* shares the same haplotype with *G. humerosa humerosa* (Figure 3A). Due to the absence of clear genetic, morphological, and ecological differences (see Figure S3B and C), we hypothesize that these populations represent a single, contiguous lineage and are not independently evolving subspecies.

The vicariance event that separated LK+LE at 300 ka coincides with the Middle Pleistocene lava flows (300–350 ka), which caused a flow reversal in the paleo-Nyabarongo River system. This diversion of water from the Kivu Rift into the Nile system occurred as a result of the lava flows (Holzförster & Schmidt, 2007). A significant drop in the LK water level around 475 ka preceded this event and may have set the stage for genetic and ecological divergence (Figure 5; Wood & Scholz, 2017). The clustering of the LK and LE subspecies within the same haplotype network, separated by a maximum of seven mutational steps (Figure 3B), emphasizes their close phylogenetic relationship and historical connectivity of their drainage systems. According to geological evidence, the SR did not flow out of the LE until the Late Pleistocene (Beadle, 1981), which supports the timeframe of population dispersal between these areas (Figure 2B). Recent immigration is evident (Figure 3B), and slight genetic differentiation is likely due to local adaptation to the riverine habitat.

Gabbiella stanleyi (LM) harbors distantly related haplotypes from the north separated by more than 18 mutational steps to the south of the lake (Figure 3G). The LM has experienced several prolonged lake level fluctuations, which may have led to habitat fragmentation (Figure 5; Delvaux, 1995; Lyons et al., 2015; Ivory et al., 2016). Thus, for several thousand years, the lake was

confined to a single paleo-lake in the northern half of the depression, where the current water depth reaches more than 700 meters (Delvaux, 1995; Johnson et al., 2016; Ivory et al., 2016). The distantly related haplotypes observed in *G. stanleyi* may therefore indicate prolonged isolation, reflecting cryptic speciation or independently evolving species that may have been shaped by historical isolation from refugia during low lake levels.

The interpretations of historical drainage changes and lake-level fluctuations referenced here are drawn from published geological literature (see Table S2). An increasing number of deep-drilling campaigns across Africa will likely provide a higher temporal resolution of patterns in paleolimnological configurations (Cohen et al., 2022 a,b). This information will be invaluable for interpreting future biogeographical patterns of aquatic taxa across the continent. Although reticulate evolution, resulting from mechanisms such as hybridization or introgression, could theoretically explain the patterns of shared genetic variation, the resolution of our multilocus dataset does not allow rigorous inference of admixture. The strong spatial structure and consistency with historical drainage patterns also make passive dispersal unlikely. Many species have low tolerance for desiccation, low survival rates during overland transport, and require specific habitats for successful colonization. Thus, the observed endemism reflects the isolation of catchments after the termination of hydrological connectivity. Our results are consistent with those of other studies on the historical drainage changes in the AGL. These studies demonstrate that historical drainage changes and water-level fluctuations impact the evolution of organisms in the AGL (Salzburger et al., 2014; Seehausen, 2015; Cohen et al., 2013). The biogeography of *G. humerosa*, in particular, serves as one of the most informative models for drainage evolution in the AGL during the Pleistocene.

5. Conclusion

This study provides the first molecular phylogeny for freshwater bithyniids of the genus *Gabbiella* inhabiting the AGL. Accordingly, ancestors of *Gabbiella* from the AGL date back to the early Miocene. The spatial patterns of diversification of *Gabbiella* show similarities with those of other molluscs and various fish taxa. This suggests that diversification is influenced by common underlying factors and corroborates various events such as rifting, historical drainage changes, and climate fluctuations. Our analysis shows that the current diversity and distribution of the AGL

Gabbiella humerosa subspecies result from vicariance and dispersal patterns caused by rifting and associated historical drainage changes during the Pleistocene. A taxonomic revision of *Gabbiella* spp. is necessary, as the previous morphological classification is not reflected in the phylogenetic classification. This study provides new insights into the patterns and process of diversification of *Gabbiella* in the African Great Lakes and contributes to phylogeographic studies of freshwater biodiversity in Africa.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author contributions

MCD carried out fieldwork, performed laboratory work and analyses, created figures and wrote the first draft of the manuscript. CC checked the quality of the sequences, helped with data

compilation and analyzed data. GK helped with fieldwork and permits. MR assisted with field and lab work, OWN and JT collected materials. CA conceived the study, collected material, advised in the data analyses and revised the manuscript. All authors contributed to drafting and reviewing the manuscript.

Data availability statement

All sequences are deposited in GenBank of the manuscript under the accession numbers given in Table 1.

References

Albrecht, C., Hauffe, T., Schreiber, K., & Wilke, T. (2012). Mollusc biodiversity in a European ancient lake system: lakes Prespa and Mikri Prespa in the Balkans. *Hydrobiologia, 682*, 47–59. https://doi.org/10.1007/s10750-011-0830-1

Albrecht, C., Hauffe, T., Schreiber, K., Trajanovski, S., & Wilke, T. (2009). Mollusc biodiversity and endemism in the potential ancient lake Trichonis, Greece. *Malacologia, 51*, 357–375. https://doi.org/10.4002/040.051.0209

Arroyave, J., Denton, J. S., & Stiassny, M. L. (2020). Pattern and timing of diversification in the African freshwater fish genus *Distichodus* (Characiformes: Distichodontidae). *BMC Evolutionary Biology, 20*, 48. https://doi.org/10.1186/s12862-020-01615-6

Baele, G., Lemey, P., Bedford, T., Rambaut, A., Suchard, M. A., & Alekseyenko, A. V. (2012). Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Molecular Biology and Evolution, 29*, 2157–2167. https://doi.org/10.1093/molbev/mss084

Beadle, L. C. (1981). *The inland waters of tropical Africa: An introduction to tropical limnology* (2nd. ed.). Longman Group Limited, London, UK.

584 Beran, L., Jaszczyńska, A., Hofman, S. (2022). Notes on the distribution of
585 *Dalmatinella simonae* (Gastropoda: Hydrobiidae) in Croatia and the utility of barcoding in its
586 determination. *Folia Malacologica*, *30*, 128–134. https://doi.org/10.12657/folmal.030.016

587 Beuning, K. R., Kelts, K., Ito, E., & Johnson, T. C. (1997). Paleohydrology of Lake Victoria,
588 East Africa, inferred from $^{18}O/^{16}O$ ratios in sediment cellulose. *Geology*, *25*, 1083–1086.
589 https://doi.org/10.1016/S0031-0182(02)00709-5

590 Beverly, E. J., White, J. D., Peppe, D. J., Faith, J. T., Blegen, N., & Tryon, C. A. (2020). Rapid
591 Pleistocene desiccation and the future of Africa's Lake Victoria. *Earth and Planetary Science*
592 *Letters*, *530*, 115883. https://doi.org/10.1016/j.epsl.2019.115883

593 Bibi, F., & Kiessling, W. (2015). Continuous evolutionary change in Plio-Pleistocene mammals
594 of eastern Africa. *Proceedings of the National Academy of Sciences*, *112*, 10623–10628.
595 https://doi.org/10.1073/pnas.1504538112

596 Brooks, J. L. (1950). Speciation in ancient lakes (concluded). *The Quarterly Review of Biology*,
597 *25*, 131–176. https://doi.org/10.1086/397539

598 Brown, D. S. (1994). *Freshwater Snails of Africa and their Medical Importance (2nd. ed.)*.
599 Taylor and Francis, London, UK.

600 Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in
601 phylogenetic analysis. *Molecular Biology and Evolution*, *17*, 540–552.
602 https://doi.org/10.1093/oxfordjournals.molbev.a026334

603 Chibwana, F. D., Tumwebaze, I., Mahulu, A., Sands, A. F., & Albrecht, C. (2020). Assessing the
604 diversity and distribution of potential intermediate hosts snails for urogenital schistosomiasis:
605 *Bulinus* spp. (Gastropoda: Planorbidae) of Lake Victoria. *Parasites & Vectors*, *13*, 418.
606 https://doi.org/10.1186/s13071-020-04281-1

607 Chorowicz, J. (2005). The East African rift system. *Journal of African Earth Sciences*, *43*, 379–
608 410. https://doi.org/10.1016/j.jafrearsci.2005.07.019

609 Clement, M., Posada, D. C. K. A., & Crandall, K. A. (2000). TCS: A computer program to
610 estimate gene genealogies. *Molecular Ecology*, *9*, 1657–1659. https://doi.org/10.1046/j.1365-
611 294x.2000.01020.x

Cohen, A. S., Campisano, C. J., Arrowsmith, J. R., Asrat, A., Beck, C. C., Behrensmeyer, A. K., Deino, A. L., Feibel, S. C., Foerster, V., Kinston, J.D., Lamb, H. F., Lowenstein, T. K., Lupien, R. L, Muiruri, V., Olago, D. O., Owen, R. B., Potts, R., Russell, J. M., Schaebitz, F., Stone, J. R., & Yost, C. L. (2022a). Reconstructing the environmental context of human origins in Eastern Africa through scientific drilling. *Annual Review of Earth and Planetary Sciences, 50*, 451–476. https://doi.org/10.1146/annurev-earth-031920-081947

Cohen, A. S., Du, A., Rowan, J., Yost, C. L., Billingsley, A. L., Campisano, C. J., Brown, E. T., Deino, A. L., Feibel, C. S., Grant, K., Kingston, J. D, Lupien, R. L., Muiruri, V., Owen, R. B., Reed, K. E, Russell, J., & Stockhecke, M. (2022b). Plio-Pleistocene environmental variability in Africa and its implications for mammalian evolution. *Proceedings of the National Academy of Sciences*, *119*, e2107393119. https://doi.org/10.1073/pnas.2107393119

Cohen, A. S., Lezzar, K. E., Tiercelin, J. J., & Soreghan, M. (1997). New palaeogeographic and lake-level reconstructions of Lake Tanganyika: Implications for tectonic, climatic and biological evolution in a rift lake. *Basin Research*, *9*, 107–132. https://doi.org/10.1046/j.1365-2117.1997.00038.x

Cohen, A. S., Van Bocxlaer, B., Todd, J. A., McGlue, M., Michel, E., Nkotagu, H. H., Grove, A. T., & Delvaux, D. (2013). Quaternary ostracodes and molluscs from the Rukwa Basin (Tanzania) and their evolutionary and paleobiogeographic implications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *392*, 79–97. https://doi.org/10.1016/j.palaeo.2013.09.007

Colgan, D. J., Ponder, W. F., & Eggler, P. E. (2000). Gastropod evolutionary rates and phylogenetic relationships assessed using partial 28S rDNA and histone H3 sequences. *Zoologica Scripta*, *29*, 29–63. https://doi.org/10.1046/j.1463-6409.2000.00021.x

Cowx, I. G., & Ogutu-Owhayo, R. (2019). Towards sustainable fisheries and aquaculture management in the African Great Lakes. *Fisheries Management and Ecology*, *26*, 397–405. https://doi.org/10.1111/fme.12391

Daley, R. (2003). The knowledge imperative for the African Great Lakes. *Journal of Great Lakes Research*, *29*, 1–2. https://doi.org/10.1016/S0380-1330(03)70534-6

639 Daniels, S. R., Busschau, T., & Cumberlidge, N. (2019). Two new species of freshwater crabs of
640 the genus *Potamonautes* MacLeay, 1838 (Decapoda: Brachyura: Potamonautidae) from the
641 forests of KwaZulu-Natal, South Africa. *Journal of Crustacean Biology, 39,* 426–435.
642 https://doi.org/10.1093/jcbiol/ruz024

643 Daniels, S. R., Busschau, T., Gullacksen, G., Marais, H., Gouws, G., & Barnes, A. (2023).
644 Cryptic and widespread: a recipe for taxonomic misidentification in a freshwater crab species
645 (Decapoda: Potamonautidae: *Potamonautes sidneyi*) as evident from species delimitation
646 methods. *Zoological Journal of the Linnean Society, 197*, 1005–1033.
647 https://doi.org/10.1093/zoolinnean/zlac068

648 Daniels, S. R., Phiri, E. E., Klaus, S., Albrecht, C., & Cumberlidge, N. (2015). Multilocus
649 phylogeny of the Afrotropical freshwater crab fauna reveals historical drainage connectivity and
650 transoceanic dispersal since the Eocene. *Systematic Biology, 64*, 549–567.
651 https://doi.org/10.1093/sysbio/syv011

652 Danley, P. D., Husemann, M., Ding, B., DiPietro, L. M., Beverly, E. J., & Peppe, D. J. (2012).
653 The impact of the geologic history and paleoclimate on the diversification of East African
654 cichlids. *International Journal of Evolutionary Biology, 2012*, 574851.
655 https://doi.org/10.1155/2012/574851

656 Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new
657 heuristics and parallel computing. *Nature Methods, 9*, 772–772.
658 https://doi.org/10.1038/nmeth.2109

659 Day, J. J., Fages, A., Brown, K. J., Vreven, E. J., Stiassny, M. L., Bills, R., Friel, J. P., & Rüber,
660 L. (2017). Multiple independent colonizations into the Congo Basin during the continental
661 radiation of African *Mastacembelus* spiny eels. *Journal of Biogeography, 44*, 2308–2318.
662 https://doi.org/10.1111/jbi.13037

663 Day, J. J., Peart, C. R., Brown, K. J., Friel, J. P., Bills, R., & Moritz, T. (2013). Continental
664 diversification of an African catfish radiation (Mochokidae: *Synodontis*). *Systematic Biology, 62*,
665 351–365. https://doi.org/10.1093/sysbi o/syt001

Delicado, D., Arconada, B., Aguado, A., & Ramos, M. A. (2019). Multilocus phylogeny, species delimitation and biogeography of Iberian valvatiform springsnails (Caenogastropoda: Hydrobiidae), with the description of a new genus. *Zoological Journal of the Linnean Society*, *186*, 892–914. https://doi.org/10.1093/zoolinnean/zly093

Delvaux, D. (1995). Age of Lake Malawi (Nyasa) and water level fluctuations. *Musée Royal de l'Afrique Centrale Tervuren (Belgium), Département de Géologie et de Minéralogie, 1995–1996*, 99–108.

Drummond, A. J., Suchard, M. A., Xie, D., & Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, *29*, 1969–1973. https://doi.org/10.1093/molbev/mss075

Dusabe, M. C., Kalinda, C., Clewing, C., Hyangya, B. L., Van Bocxlaer, B., & Albrecht, C. (2024). Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu. *Journal of Great Lakes Research*, *50*, 102339. https://doi.org/10.1016/j.jglr.2024.102339

Elmer, K. R., Reggio, C., Wirth, T., Verheyen, E., Salzburger, W., & Meyer, A. (2009). Pleistocene desiccation in East Africa bottlenecked but did not extirpate the adaptive radiation of Lake Victoria haplochromine cichlid fishes. *Proceedings of the National Academy of Sciences*, *106*, 13404–13409. https://doi.org/10.1073/pnas.0902299106

Fehér, Z., Major, Á., & Krízsik, V. (2013). Spatial pattern of intraspecific mitochondrial diversity in the Northern Carpathian endemic spring snail, *Bythinella pannonica* (Frauenfeld, 1865) (Gastropoda: Hydrobiidae). *Organisms Diversity & Evolution*, *13*, 569–581. https://doi.org/10.1007/s13127-013-0141-7

Folmer, O., Black, M., Hoeh, W., Lutz, R., & Vreijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biological Biotechnology*, *3*, 294–299.

Friedman, M., Keck, B. P., Dornburg, A., Eytan, R. I., Martin, C. H., Hulsey, C. D., Wainwright, P. C, & Near, T. J. (2013). Molecular and fossil evidence place the origin of cichlid fishes long

693 after Gondwanan rifting. *Proceedings of the Royal Society B: Biological Sciences*, *280*,
694 20131733. https://doi.org/10.1098/rspb.2013.1733

695 Genner, M. J., Seehausen, O., Lunt, D. H., Joyce, D. A., Shaw, P. W., Carvalho, G. R. & Turner,
696 G. F. (2007). Age of cichlids: New dates for ancient lake fish radiations. *Molecular Biology and*
697 *Evolution*, *24*, 1269–1282. https://doi.org/10.1093/molbev/msm050

698 Hall, T. A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis
699 program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, *41*, 95–98.

700 Harzhauser, M., & Piller, W. E. (2007). Benchmark data of a changing sea—palaeogeography,
701 palaeobiogeography and events in the Central Paratethys during the Miocene. *Palaeogeography,*
702 *Palaeoclimatology, Palaeoecology, 253*, 8–31. https://doi.org/10.1016/j.palaeo.2007.03.031

703 Harzhauser, M., Kroh, A., Mandic, O., Piller, W. E., Göhlich, U., Reuter, M., & Berning, B.
704 (2007). Biogeographic responses to geodynamics: A key study all around the Oligo–Miocene
705 Tethyan Seaway. *Zoologischer Anzeiger - A Journal of Comparative Zoology*, *246*, 241–256.
706 https://doi.org/10.1016/j.jcz.2007.05.001

707 Hauswald, A. K., Albrecht, C., & Wilke, T. (2008). Testing two contrasting evolutionary
708 patterns in ancient lakes: Species flock versus species scatter in valvatid gastropods of Lake
709 Ohrid. *Hydrobiologia*, 615, 169–179. https://doi.org/10.1007/s10750-008-9556-0

710 Hoag, C., & Svenning, J. C. (2017). African environmental change from the Pleistocene to the
711 Anthropocene. *Annual Review of Environment and Resources*, *42*, 27–54.
712 https://doi.org/10.1146/annurev-environ-102016-060653

713 Hohausová, E., Lavoy, R. J., & Allen, M. S. (2010). Fish dispersal in a seasonal wetland:
714 influence of anthropogenic structures. *Marine and Freshwater Research*, *61*, 682–694.
715 https://doi.org/10.1071/MF09140

716 Holzförster, F., & Schmidt, U. (2007). Anatomy of a river drainage reversal in the Neogene
717 Kivu–Nile Rift. *Quaternary Science Reviews*, *26*, 1771–1789.
718 https://doi.org/10.1016/j.quascirev.2007.04.018

719 Ivory, S. J., Blome, M. W., King, J. W., McGlue, M. M., Cole, J. E., & Cohen, A. S. (2016).
720 Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2

721 million years. *Proceedings of the National Academy of Sciences, 113*, 11895–11900.
722 https://doi.org/10.1073/pnas.1611028113

723 Johnson, T. C., Kelts, K., & Odada, E. (2000). The Holocene history of Lake Victoria. *Ambio,*
724 *29*, 2–11. https://doi.org/10.1579/0044-7447-29.1.2

725 Johnson, T. C., Scholz, C. A., Talbot, M. R., Kelts, K., Ricketts, R. D., Ngobi, G., Beuning, K.,
726 Ssemmanda, I., & McGill, J. W. (1996). Late Pleistocene desiccation of Lake Victoria and rapid
727 evolution of cichlid fishes. *Science, 273*, 1091–1093. https://doi.org/10.1126/scien
728 ce.273.5278.1091

729 Johnson, T. C., Werne, J. P., Brown, E. T., Abbott, A., Berke, M., Steinman, B. A., Halbur, J.,
730 Contreras, S., Grosshuesch, S., Deino, A., Lyons, R. P., Scholz, C. A., Schouten, S., & Damsté,
731 J. S. (2016). A progressively wetter climate in southern East Africa over the past 1.3 million
732 years. *Nature, 537*, 220–224. https://doi.org/10.1038/nature19065

733 Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7:
734 Improvements in performance and usability. *Molecular Biology and Evolution*, *30*, 772–780.
735 https://doi.org/10.1093/molbev/mst010

736 Landis, M. J., Matzke, N. J., Moore, B. R., & Huelsenbeck, J. P. (2013). Bayesian analysis of
737 biogeography when the number of areas is large. *Systematic Biology*, *62*, 789–804.
738 https://doi.org/10.1093/sysbio/syt040

739 Lezzar, K. E., Tiercelin, J. J., Batist, M. D., Cohen, A. S., Bandora, T., Rensbergen, P. V., Turdu,
740 C. L., Mifundu., & Klerkx, J. (1996). New seismic stratigraphy and late Tertiary history of the
741 North Tanganyika Basin, East African Rift system, deduced from multichannel and high-
742 resolution reflection seismic data and piston core evidence. *Basin Research*, *8*, 1–28.
743 https://doi.org/10.1111/j.1365-2117.1996.tb00112.x

744 Lezzar, K. E., Tiercelin, J. J., Le Turdu, C., Cohen, A. S., Reynolds, D. J., Le Gall, B., & Scholz,
745 C. A. (2002). Control of normal fault interaction on the distribution of major Neogene
746 sedimentary depocenters, Lake Tanganyika, East African rift. *AAPG Bulletin*, *86*, 1027–1059.
747 https://doi.org/10.1306/61EEDC1A-173E-11D7-8645000102C1865D

748 Lyons, R. P., Scholz, C. A., Cohen, A. S., King, J. W., Brown, E. T., Ivory, S. J., Johnson, T. C.,
749 Deino, A. L., Reinthal, P. N., McGlue, M. M. & Blome, M. W. (2015). Continuous 1.3-million-
750 year record of East African hydroclimate, and implications for patterns of evolution and
751 biodiversity. *Proceedings of the National Academy of Sciences of the United States of America*,
752 *112*, 15568–15573. https://doi.org/10.1073/pnas.1512864112

753 Mahulu, A., Stelbrink, B., Van Bocxlaer, B., Riedel, F., & Albrecht, C. (2021). Going with the
754 flow? Diversification of gastropods reflects drainage evolution in Africa. *Journal of*
755 *Biogeography*, *48*, 1579–1593. https://doi.org/10.1111/jbi.14096

756 Mandahl-Barth, G. (1962). Key to the identification of east and central African freshwater snails
757 of medical and veterinary importance. *Bulletin of the World Health Organization*, *27*, 135–150.

758 Mandahl-Barth, G. (1968). Revision of the African Bithyniidae (Gastropoda Prosobranchia).
759 *Revue de Zoologie et de Botanique Africaines*, *78*, 129–159.

760 Mandahl-Barth, G. (1988). *Studies on African freshwater bivalves*. Danish Bilharziasis
761 Laboratory, Charlottenlund.

762 Martens, K. (1997). Speciation in ancient lakes. *Trends in Ecology & Evolution*, *12*, 177–182.
763 https://doi.org/10.1016/s0169-5347(97)01039-2

764 Martens, K., Coulter, G., & Goddeeris, B. (1994). Speciation in ancient lakes – 40 years after
765 Brooks. *Ergebnisse der Limnologie*, *44*, 75–75.

766 Matzke, N. J. (2013a). *BioGeoBEARS: BioGeography with Bayesian (and Likelihood)*
767 *evolutionary analysis in R scripts*. University of California, Berkeley, CA.

768 Matzke, N. J. (2013b). Probabilistic historical biogeography: new models for founder-event
769 speciation, imperfect detection, and fossils allow improved accuracy and model-testing.
770 *Frontiers of Biogeography*, *5*, 242–248. https://doi.org/10.21425/F55419694

771 Matzke, N. J. (2014). Model selection in historical biogeography reveals that founder-event
772 speciation is a crucial process in island clades. *Systematic Biology*, *63*, 951–970.
773 https://doi.org/10.1093/sysbio/syu056

Meier, J. I., McGee, M. D., Marques, D. A., Mwaiko, S., Kishe, M., Wandera, S., Neumann, D., Mrosso, H., Chapman, L. J., Chapman, C. A., Kaufman, L., Taabu-Munyaho, A., Wagner, C. E., Bruggmann, R., Excoffier, L., & Seehausen, O. (2023). Cycles of fusion and fission enabled rapid parallel adaptive radiations in African cichlids. *Science, 381*, eade2833. https://doi.org/10.1126/science.ade2833

Meyer, B. S., Matschiner, M., & Salzburger, W. (2017). Disentangling incomplete lineage sorting and introgression to refine species-tree estimates for Lake Tanganyika cichlid fishes. *Systematic Biology*, *66*, 531–550. https://doi.org/10.1093/sysbio/syw069

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*, 1530–1534. https://doi.org/10.1093/molbev/msaa015

Nguyen, J. M., & Ho, S. Y. (2020). Calibrations from the fossil record. In S. Y. Ho (ed), *The Molecular Evolutionary Clock: Theory and Practice* (pp. 117–133). Springer. https://doi.org/10.1007/978-3-030-60181-2_8

Ogutu-Ohwayo, R., Natugonza, V., Olokotum, M., Rwezawula, P., Lugya, J., & Musinguzi, L. (2020). Biogeography: Lakes—African great lakes. In M. I. Goldstein & D. A. DellaSala (Eds.), Encyclopedia of the world's biomes (pp. 243–260). Elsevier. https://doi.org/10.1016/B978-0-12-409548-9.12090-1

Okay, A. I., Zattin, M., & Cavazza, W. (2010). Apatite fission-track data for the Miocene Arabia-Eurasia collision. *Geology*, *38*, 35–38. https://doi.org/10.1130/G30234.1

Ortiz-Sepulveda, C. M., Stelbrink, B., Vekemans, X., Albrecht, C., Riedel, F., Todd, J. A., & Van Bocxlaer, B. (2020). Diversification dynamics of freshwater bivalves (Unionidae: Parreysiinae: Coelaturini) indicate historic hydrographic connections throughout the East African Rift System. *Molecular Phylogenetics and Evolution*, *148*, 106816. https://doi.org/10.1016/j.ympev.2020.106816

Palumbi, S., Martin, A., Romano, S., McMillan, W. O., & Grabowski, G. (1991). The simple fool's guide to PCR. In *Special publication of the University of Hawaii, Department of Zoology and Kewalo Marine Laboratory* (pp. 1–23).

Park, J. K., & Foighil, D. Ó. (2000). Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Molecular Phylogenetics and Evolution, 14,* 75–88. https://doi.org/10.1006/mpev.1999.0691

Peer, N., Gouws, G., Maliwa, L., Barker, N., Juby, P., & Perissinotto, R. (2023). Description of a new montane freshwater crab (Arthropoda, Malacostraca, Decapoda, Potamonautidae) from the Eastern Cape, South Africa. *ZooKeys, 1160,* 89–108. https://doi.org/10.3897/zookeys.1160.100844

Peter, B. D. (2004). African climate change and faunal evolution during the Pliocene–Pleistocene. *Earth and Planetary Science Letters, 220,* 3–24. https://doi.org/10.1016/S0012-821X(04)00003-2

Pickford, M. (1982). The tectonics, volcanics and sediments of the Nyanza rift valley, Kenya. *Zeitschrift für Geomorphologie. Supplementband Stuttgart*, 42, 1–33.

Plisnier, P. D., Kayanda, R., MacIntyre, S., Obiero, K., Okello, W., Vodacek, A., Cocquyt, C., Abegaz, H., Achieng, A., Akonkwa, B., Albrecht, C., Balagizi, C., Barasa, J., Bashongwa, R. A., Bishobibiri, A. B., Bootsma, H., Borges, A. V., Chavula, G., Dadi, T., & Lawrence, T. (2023). Need for harmonized long-term multi-lake monitoring of African Great Lakes. *Journal of Great Lakes Research, 49,* 101988. https://doi.org/10.1016/j.jglr.2022.01.016

R Core Team. 2024. R: A language and environment for statistical computing. https://www.R-project.org. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut, A., & Drummond, A. J. (2007). Tracer v. 1.5. Available from http://tree.bio.ed.ac.uk/softw are/tracer.

Ree, R. H., & Smith, S. A. (2008). Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology, 57,* 4–14. https://doi.org/10.1080/10635150701883881

Ree, R. H., Moore, B. R., Webb, C. O., & Donoghue, M. J. (2005). A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution*, *59*, 2299–2311. https://doi.org/10.1111/j.0014-3820.2005.tb00940.x

Renaut, R.W., & Owen, R.B. (2023). Lake Victoria. In: R. B. Owen & R. W. Renaut (Eds.), The Kenya Rift Lakes: Modern and Ancient (pp 527–576). Syntheses in Limnogeology. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-25055-2_13

Rögl, F. (1999). Mediterranean and Paratethys: facts and hypotheses of an Oligocene to Miocene paleogeography (short overview). *Geologica Carpathica*, *50*, 339–349.

Ronquist, F. (1997). Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology*, *46*, 195–203. https://doi.org/10.1093/sysbio/46.1.195

Ross, K. A., Smets, B., De Batist, M., Hilbe, M., Schmid, M., & Anselmetti, F. S. (2014). Lake-level rise in the late Pleistocene and active subaquatic volcanism since the Holocene in Lake Kivu, East African Rift. *Geomorphology*, *221*, 274–285. https://doi.org/10.1016/j.geomorph.2014.05.010

Rossiter, A., & Kawanabe, H. (2000). *Ancient Lakes: Biodiversity, Ecology and Evolution* (Vol. 31).

Russell, J. M., Barker, P., Cohen, A., Ivory, S., Kimirei, I., Lane, C.,Leng, M., Maganza, N., McGlue, M., Msaky, E., Noren, A., Boush, L. P., Salzburger, W., Scholz, C., Nuru, S., & Lake Tanganyika Scientific Drilling Project (TSDP) Consortium (2020). ICDP workshop on the Lake Tanganyika Scientific Drilling Project: A late Miocene–present record of climate, rifting, and ecosystem evolution from the world's oldest tropical lake. *Scientific Drilling*, *27*, 53–60. https://doi.org/10.5194/sd-27-53-2020

Salzburger, W. (2018). Understanding explosive diversification through cichlid fish genomics. *Nature Reviews Genetics*, *19*, 705–717. https://doi.org/10.1038/s41576-018-0043-9

Salzburger, W., & Meyer, A. (2004). The species flocks of East African cichlid fishes: Recent advances in molecular phylogenetics and population genetics. *Naturwissenschaften*, *91*, 277–290. https://doi.org/10.1007/s00114-004-0528-6

Salzburger, W., Van Bocxlaer, B., & Cohen, A. S. (2014). Ecology and evolution of the African Great Lakes and their faunas. *Annual Review of Ecology, Evolution, and Systematics*, *45*, 519–545. https://doi.org/10.1146/annurev-ecolsys-120213-091804

Schedel, F. D. B., Musilova, Z., & Schliewen, U. K. (2019). East African cichlid lineages (Teleostei: Cichlidae) might be older than their ancient host lakes: New divergence estimates for the east African cichlid radiation. *BMC Evolutionary Biology*, *19*, 94. https://doi.org/10.1186/s12862-019-1417-0

Schultheiß, R., Ndeo, O. W., Malikwisha, M., Marek, C., Bößneck, U., & Albrecht, C. (2011). Freshwater molluscs of the Eastern Congo: Notes on taxonomy, biogeography and conservation. *African Invertebrates*, *52*, 265–284. https://doi.org/10.5733/afin.052.0204

Schultheiß, R., Van Bocxlaer, B., Wilke, T., & Albrecht, C. (2009). Old fossils–young species: Evolutionary history of an endemic gastropod assemblage in Lake Malawi. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 2837–2846. https://doi.org/10.1098/rspb.2009.0467

Schultheiß, R., Van Boxlaer, B., Riedel, F., von Rintelen, T., & Albrecht, C. (2014). Disjunct distributions of freshwater snails testify to a central role of the Congo system in shaping biogeographic patterns in Africa. *BMC Evolutionary Biology*, *14*, 1471–2148. https://doi.org/10.1186/1471-2148-14-42

Schultheiß, R., Wilke, T., Jørgensen, A., & Albrecht, C. (2011). The birth of an endemic species flock: demographic history of the *Bellamya* group (Gastropoda, Viviparidae) in Lake Malawi. *Biological Journal of the Linnean Society*, *102*, 130–143. https://doi.org/10.1111/j.1095-8312.2010.01574.x

Seehausen, O. (2006). African cichlid fish: a model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 1987–1998. https://doi.org/10.1098/rspb.2006.3539

Seehausen, O. (2015). Process and pattern in cichlid radiations–inferences for understanding unusually high rates of evolutionary diversification. *New Phytologist*, *207*, 304–312. https://doi.org/10.1111/nph.13450

Talbot, M.R., & Williams, M.A. (2009). Cenozoic Evolution of the Nile Basin. In: Dumont, H.J. (eds) The Nile. Monographiae Biologicae, vol 89. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-9726-3_3

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, *38*, 3022–3027. https://doi.org/10.1093/molbev/msab120

Tonkin, J. D., Altermatt, F., Finn, D. S., Heino, J., Olden, J. D., Pauls, S. U., & Lytle, D. A. (2018). The role of dispersal in river network metacommunities: Patterns, processes, and pathways. *Freshwater Biology*, *63*, 141–163. https://doi.org/10.1111/fwb.13037

Trauth, M. H., Asrat, A., Berner, N., Bibi, F., Foerster, V., Grove, M., Kaboth-Bahr, S., Maslin, M. A., Mudelsee, M., & Schäbitz, F. (2021). Northern Hemisphere Glaciation, African climate and human evolution. *Quaternary Science Reviews*, *268*, 107095. https://doi.org/10.1016/j.quascirev.2021.107095

Tryon, C. A., Faith, J. T., Peppe, D. J., Beverly, E. J., Blegen, N., Blumenthal, S., Chritz, K., Driese, S., Patterson, D., & Sharp, W. (2015). The Pleistocene prehistory of the Lake Victoria basin. *Quaternary International*, *404*, 100–114. https://doi.org/10.1016/j.quaint.2015.11.073

Tumwebaze, I., Clewing, C., Chibwana, F. D., Kipyegon, J. K., & Albrecht, C. (2022). Evolution and biogeography of freshwater snails of the genus *Bulinus* (Gastropoda) in afromontane extreme environments. *Frontiers in Environmental Science*, *10*, 902900. https://doi.org/10.3389/fenvs.2022.902900

Vaidya, G., Lohman, D. J., & Meier, R. (2011). SequenceMatrix: Concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, *27*, 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Van Bocxlaer, B., Clewing, C., Duputié, A., Roux, C., & Albrecht, C. (2021). Population collapse in viviparid gastropods of the Lake Victoria ecoregion started before the Last Glacial Maximum. *Molecular Ecology*, *30*, 364–378. https://doi.org/10.1111/mec.15599

Van Bocxlaer, B., Clewing, C., Mongindo Etimosundja, J. P., Kankonda, A., Wembo Ndeo, O., & Albrecht, C. (2015). Recurrent camouflaged invasions and dispersal of an Asian freshwater

910 gastropod in tropical Africa. *BMC Evolutionary Biology, 15*, 33. https://doi.org/10.1186/s12862-
911 015-0296-2

912 Van Bocxlaer, B., Ortiz-Sepulveda, C. M., Gurdebeke, P. R., & Vekemans, X. (2020). Adaptive
913 divergence in shell morphology in an ongoing gastropod radiation from Lake Malawi. *BMC*
914 *Evolutionary Biology*, *20*, 5. https://doi.org/10.1186/s12862-019-1570-5

915 Van Damme, D., & Gautier, A. (2013). Lacustrine mollusc radiations in the Lake Malawi Basin:
916 Experiments in a natural laboratory for evolution. *Biogeosciences*, *10*, 5767–5778.
917 https://doi.org/10.5194/bg-10-5767-2013

918 Van Damme, D., & Pickford, M. (2003). The late Cenozoic Thiaridae (Mollusca, Gastropoda,
919 Cerithioidea) of the Albertine Rift Valley (Uganda-Congo) and their bearing on the origin and
920 evolution of the Tanganyikan thalassoid malacofauna. *Hydrobiologia*, *498*, 1–83.
921 https://doi.org/10.1023/A:1026298512117

922 Van der Merwe, P. D. W., Cotterill, F. P., Kandziora, M., Watters, B. R., Nagy, B., Genade, T.,
923 Flügel, T. J, Svendsen, D., & Bellstedt, D. U. (2021). Genomic fingerprints of palaeogeographic
924 history: The tempo and mode of rift tectonics across tropical Africa has shaped the
925 diversification of the killifish genus *Nothobranchius* (Teleostei: Cyprinodontiformes). *Molecular*
926 *Phylogenetics and Evolution*, *158*, 106988. https://doi.org/10.1016/j.ympev.2020.106988

927 Van Steenberge, M. W., Vanhove, M. P. M., Chocha Manda, A., Larmuseau, M. H. D., Swart, B.
928 L., Khang'Mate, F., Arndt, A., Hellemans, B., Van Houdt, J., Micha, J. C., Koblmüller, S.,
929 RoodtWilding, R., & Volckaert, F. A. M. (2020). Unravelling the evolution of Africa's drainage
930 basins through a widespread freshwater fish, the African sharptooth catfish *Clarias gariepinus*.
931 *Journal of Biogeography*, *47*, 1739–1754. https://doi.org/10.1111/jbi.13858

932 Verheyen, E., Salzburger, W., Snoeks, J., & Meyer, A. (2003). Origin of the superflock of
933 cichlid fishes from Lake Victoria. *East Africa. Science*, *300*, 325. https://doi.org/10.1126/scien
934 ce.1080699

935 Vranken, N., Van Steenberge, M., Heylen, A., Decru, E., & Snoeks, J. (2022). From a pair to a
936 dozen: The piscivorous species of *Haplochromis* (Cichlidae) from the Lake Edward system.
937 *European Journal of Taxonomy*, *815*, 1–94. https://doi.org/10.5852/ejt.2022.815.1749

Wienhues, G., Temoltzin-Loranca, Y., Vogel, H., Morlock, M. A., Cohen, A. S., Anselmetti, F. S., Bernasconi, S. M., Jaggi, M., Tylmann, W., Kishe, M. A., King, L., Ngoepe, N., Courtney-Mustaphi, C. J., Muschick, M., Matthews, B., Mwaiko, S., Seehausen, O., Tinner, W., & Grosjean, M. (2024). From desiccation to wetlands and outflow: Rapid re-filling of Lake Victoria during the latest Pleistocene 14–13 ka. *Journal of Great Lakes Research, 50*, 102246. https://doi.org/10.1016/j.jglr.2023.102246

Wilke, T., Davis, G. M., Falniowski, A., Giusti, F., Bodon, M., & Szarowska, M. (2001). Molecular systematics of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): Testing monophyly and phylogenetic relationships. *Proceedings of the Academy of Natural Sciences of Philadelphia, 151*, 1–21. https://doi.org/10.1635/0097-3157(2001)151[0001:MSOHMG]2.0.CO;2

Wilke, T., Davis, G. M., Qiu, D., & Spear, R. C. (2006). Extreme mitochondrial sequence diversity in the intermediate schistosomiasis host *Oncomelania hupensis robertsoni*: Another case of ancestral polymorphism? *Malacologia, 48*, 143–157.

Wilke, T., Kehlmaier, C., Stelbrink, B., Albrecht, C., & Bouchet, P. (2023). Historical DNA solves century-old mystery on sessility in freshwater gastropods. *Molecular Phylogenetics and Evolution, 185*, 107813. https://doi.org/10.1016/j.ympev.2023.107813

Wilke, T., Schultheiß, R., & Albrecht, C. (2009). As time goes by: A simple fool's guide to molecular clock approaches in invertebrates. *American Malacological Bulletin, 27*, 25–45. https://doi.org/10.4003/006.027.0203

Wood, D. A., & Scholz, C. A. (2017). Stratigraphic framework and lake level history of Lake Kivu, East African Rift. *Journal of African Earth Sciences, 134*, 904–916. https://doi.org/10.1016/j.jafrearsci.2016.06.014

Wright, L. J., Muirhead, J. D., & Scholz, C. A. (2020). Spatiotemporal variations in upper crustal extension across the different basement terranes of the Lake Tanganyika Rift, East Africa. *Tectonics, 39*, e2019TC006019. https://doi.org/10.1029/2019TC006019

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001). Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science, 292*, 686–693. https://doi.org/10.1126/science.1059412

TABLE CAPTIONS

Table 1. Sampling sites for the studied *Gabbiella* species, including specimen codes, location information (country, region, name of waterbody, coordinates), haplotype number, and GenBank accession numbers. Abbreviations for lakes are as follows: LA – Lake Albert, LE – Lake Edward, LK – Lake Kivu, LT – Lake Tanganyika, LV – Lake Victoria, CL – Lake Nyinambuga and Lake Lyantonde (crater lakes), LM – Lake Malawi. GenBank accession numbers will be provided upon acceptance.

Table 2. Uncorrected genetic *p*-distances between *Gabbiella* species groups, based on COI dataset (658bp).

FIGURE CAPTIONS

[Double column] Figure 1. Map of Africa highlighting the African Great Lakes and rivers (in red frame) within their respective drainage basins where *Gabbiella* spp. were sampled (A). The sampled *Gabbiella* spp. are shown at their locations in the map (B) and (C) and the color codes represent their lakes of origin. White dots in the map (C) and (D) indicate locations where no *Gabbiella* spp. were found.

[Double column] Figure 2. Maximum clade credibility (MCC) tree derived from the BEAST analyses including 95% HPD. The Bayesian posterior probabilities (PP) are indicated at the nodes. For detailed information on the specimens see Table 1; Figure 1 (A). Historical biogeography of *Gabbiella humerosa* subspecies, ancestral area reconstruction from BioGeoBEARS, derived from the Bayesian chronogram, the best-fit model was DIVALIKE. Pie charts represent the probabilities of ancestral states at each node (color codes represent different sub/species) (B).

[Double column] Figure 3. Phylogeography of *Gabbiella* spp. using statistical parsimony haplotype networks implemented in TCS (95% connection limit). 32 unique haplotypes of the COI gene fragment are color-coded according to lakes and rivers (Figure 1). Seven clusters were

identified. The size of the circles is proportional to the number of specimens sharing the same haplotype.

[Single column] Figure 4. Biogeographical history scenario of *Gabbiella humerosa* subspecies from the Early Pleistocene to the present. Map of the African Great Lakes and the rivers in their respective drainage basins in which samples of *Gabbiella humerosa* subspecies were found. The dotted ellipse shows the distribution area of the ancestor (red star), while the red arrow indicates the origin of the subspecies' dispersal and the time frame indicating the range of dispersal is shown (see Figure 2B).

[Double column] Figure 5. The water level fluctuations of the African Great Lakes from the Early Pleistocene to the present, dominated by three types of droughts (glacial drought, megadrought and last glacial maximum) (This figure was created based on the detailed information in Table S2).

List of tables

Table 1

Specimen code	Country	Location	Coordinates		Species	Haplo-type no.	Voucher no.	GenBank accession no.			
			Latitude (°N)	Longitude (°E)				COI	16S	28S	H3
LA01/1	Uganda	Butiaba, Lake Albert	1.81914	31.32879	*G. candida*	H01	UGSB 28863	PX149791		PX169358	PX204858
LA02/1	Uganda	Ntoroko, Lake Albert	1.03089	30.51656	*G. candida*	H01	UGSB 28834	PX149792			PX204859
LA03/1	Uganda	Butiaba, Lake Albert	1.81475	31.31992	*G. candida*	H01	UGSB 28861	PX149793			
LA04/1	Uganda	Ntoroko, Lake Albert	1.03166	30.51738	*G. candida*	H01	UGSB 28868	PX149794			
LA05/1	Uganda	Hoima, Lake Albert	1.58376	31.10286	*G. candida*	H02	UGSB 28839	PX149795			
LA05/2	Uganda	Hoima, Lake Albert	1.58376	31.10286	*G. candida*	H03	UGSB 28840	PX149796			
LA05/3	Uganda	Hoima, Lake Albert	1.58376	31.10286	*G. humerosa alberti*	H04	UGSB 28837	PX149797	PX169336		

LA05/4	Uganda	Hoima, Lake Albert	1.58376	31.10286	*G. humerosa alberti*	H04	UGSB 28842	PX149 798				
LA06/1	Uganda	Hoima, Lake Albert	1.58120	31.12238	*G. humerosa alberti*	H04	UGSB 28847	PX149 799				
LA07/1	Uganda	Ntoroko, Lake Albert	1.05228	30.53475	*G. humerosa alberti*	H05	UGSB 28843	PX149 800	PX169 337			
LA04/2	Uganda	Ntoroko, Lake Albert	1.03166	30.51738	*G. humerosa alberti*	H06	UGSB 28852	PX149 801	PX169 338			
LA03/2	Uganda	Butiaba, Lake Albert	1.81475	31.31993	*G. humerosa alberti*	H07	UGSB 28858	PX149 802				
LE01/1	DR Congo	Lunyasenge, Lake Edward	-0.30085	29.45000	*G. humerosa edwardi*	H08	UGSB 28885	PX149 803				
LE02/1	DR Congo	Lunyasenge, Lake Edward	-0.49245	29.33960	*G. humerosa edwardi*	H09	UGSB 28887	PX149 804				
LE02/2	DR Congo	Lunyasenge, Lake Edward	-0.49245	29.33960	*G. humerosa edwardi*	H10	UGSB 28888	PX149 805	PX169 339			
SR01/1	DR Congo	Kyavinyonge, Semliki River	-0.11697	29.57148	*G. humerosa edwardi*	H11	UGSB 28892	PX149 806	PX169 340			PX204 860
LE03/1	Uganda	Rwenshana, Lake Edward	-0.38809	29.75127	*G. humerosa edwardi*	H12	UGSB 28894	PX149 807	PX169 341			
LK01/1	Rwanda	Nyamasheke, Lake Kivu	-2.31244	29.13772	*G. humerosa kivuensis*	H13	UGSB 27868	PX149 808	PX169 342	PX169 359		PX204 861
LK02/1	Rwanda	Nyamasheke, Lake Kivu	-2.29037	29.15549	*G. humerosa kivuensis*	H14	UGSB 27869	PX149 809	PX169 343			PX204 862
LK03/1	Rwanda	Kigufi, Lake Kivu	-1.73938	29.27026	*G. humerosa kivuensis*	H15	UGSB 27871	PX149 810				
LK04/1	Rwanda	Gitesi, Lake Kivu	-2.07361	29.35914	*G. humerosa kivuensis*	H15	UGSB 27873	PX149 811				PX204 863
LK05/1	Rwanda	Cyangugu, Lake Kivu	-2.47920	28.89837	*G. humerosa kivuensis*	H15	UGSB 27877	PX149 812	PX169 344			
LK06/1	Rwanda	Cyangugu, Lake Kivu	-2.47921	28.89836	*G. humerosa kivuensis*	H15	UGSB 28071	PX149 813				
LT01/1	Congo	Kasanga, Lake Tanganyika	-5.73446	29.36347	*G. humerosa tanganyicaensis*	H16	UGSB 17452	PX149 814	PX169 345	PX169 361		PX204 864
LT01/2	Congo	Kasanga, Lake Tanganyika	-5.73446	29.36347	*G. humerosa tanganyicaensis*	H16	UGSB 17453	PX149 815	PX169 346	PX169 362		PX204 865

LT01/3	Congo	Kasanga, Lake Tanganyika	-5.73446	29.36347	*G. humerosa tanganyicaensis*	H16	UGSB 17454	PX149 816	PX169 347			
LT01/4	Congo	Kasanga, Lake Tanganyika	-5.73446	29.36347	*G. humerosa tanganyicaensis*	H16	UGSB 17455	PX149 817	PX169 348			
VN01/1	Uganda	Atura, Victoria Nile River	2.12852	32.32919	*G. humerosa kyogae*	H17	UGSB 28870	PX149 818	PX169 349			PX204 866
LV01/1	Uganda	Ssese Archipelago, Lake Victoria	0.00735	32.35314	*G. humerosa humerosa*	H17	UGSB 28897	PX149 819	PX169 351			
LV02/1	Uganda	Dagusi Island, Lake Victoria	0.15662	33.56414	*G. humerosa humerosa*	H18	UGSB 28939	PX149 820				
LV03/1	Uganda	Ssese Archipelago, Lake Victoria	0.05037	32.39346	*G. humerosa humerosa*	H19	UGSB 28896	PX149 821	PX169 350	PX169 360	PX204 868	
LV04/1	Uganda	Dagusi Island, Lake Victoria	0.13707	33.60149	*G. humerosa humerosa*	H20	UGSB 28898	PX149 822				
CL01/1	Uganda	Isunga, Lake Nyinambuga (crater lake)	0.48092	30.28825	*G. kichwambae*	H21	UGSB 28974	PX149 823	PX169 352			
CL02/1	Uganda	Nyakasunsa, Lake Lyantonde (crater lake)	0.48716	30.28048	*G. kichwambae*	H21	UGSB 29660	PX149 824				
LM01/1	Malawi	Chilumba, Lake Malawi	-10.43677	34.26531	*G. stanleyi*	H22	UGSB 29376	PX149 825	PX169 353	PX169 363	PX204 868	
LM02/1	Malawi	Chilumba, Lake Malawi	-10.43593	34.26424	*G. stanleyi*	H22	UGSB 31033	PX149 826				
LM03/1	Malawi	Chilumba, Lake Malawi	-10.43675	34.26533	*G. stanleyi*	H23	UGSB 29377	PX149 827	PX169 354	PX169 364	PX204 869	
LM04/1	Malawi	Pemba, Lake Malawi	-13.90833	34.62748	*G. stanleyi*	H24	UGSB 0096	PX149 828				
LM05/1	Malawi	Kalonga, Lake Malawi	-10.43593	34.26425	*G. stanleyi*	H25	UGSB 0111	PX149 829				
LM05/2	Malawi	Kalonga, Lake Malawi	-10.43593	34.26425	*G. stanleyi*	H26	UGSB 31032	PX149 830				
LM06/1	Malawi	Pemba, Lake Malawi	-13.90055	34.63010	*G. stanleyi*	H27	UGSB 0058	PX149 831				

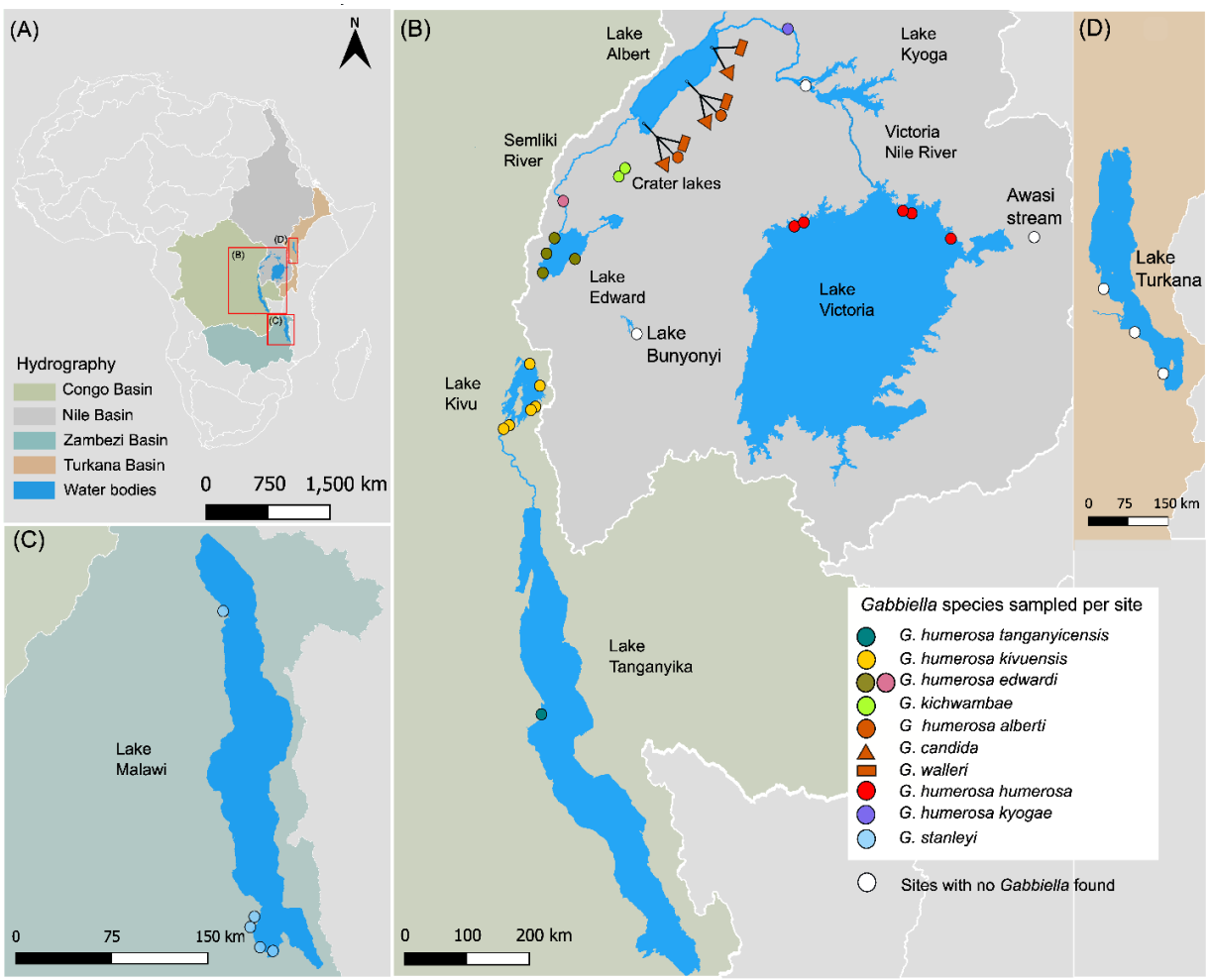
LM06/2	Malawi	Pemba, Lake Malawi	-13.90055	34.63010	*G. stanleyi*	H28	UGSB 31034	PX149 832			
LM07/1	Malawi	Mangochi, Lake Malawi	-14.25548	34.80618	*G. stanleyi*	H29	UGSB 0176	PX149 833			
LM08/1	Malawi	Kalonga, Lake Malawi	-10.42556	34.25018	*G. stanleyi*	H30	UGSB 0216	PX149 834			
LA08/1	Uganda	Hoima, Lake Albert	1.69745	31.06365	*G. walleri*	H31	UGSB 28849	PX149 835			
LA09/1	Uganda	Ntoroko, Lake Albert	1.06135	30.53561	*G. walleri*	H31	UGSB 28862	PX149 836	PX169 355	PX169 365	PX204 870
LA09/2	Uganda	Hoima, Lake Albert	1.69745	31.06365	*G. walleri*	H31	UGSB 28850	PX149 837			
LA09/3	Uganda	Hoima, Lake Albert	1.69745	31.06365	*G. walleri*	H31	UGSB 28865	PX149 838	PX169 356	PX169 366	PX204 871
LA09/4	Uganda	Hoima, Lake Albert	1.69745	31.06365	*G. walleri*	H32	UGSB 28864	PX149 839	PX169 357	PX169 367	PX204 872

Table 2

Group	***G. candida***	***G. h. alberti*, humerosa& kyogae**	***G. h. kivuensis* & edwardiensis**	***G. kichwambae***	***G. h. tanganyicensis***	***G. walleri***
G. candida						
G. h. alberti, humerosa & kyogae	16.6					
G. h. kivuensis & edwardiensis	16.7	2.8				
G. kichwambae	15.9	3.7	4.7			
G. h. tanganyicensis	16.2	2.5	3.3	3.6		
G. walleri	18.6	18.2	16.9	18.5	17.8	
G. stanleyi	17.3	18.1	17.2	17.8	17.7	7.7

List of Figures

Figure 1



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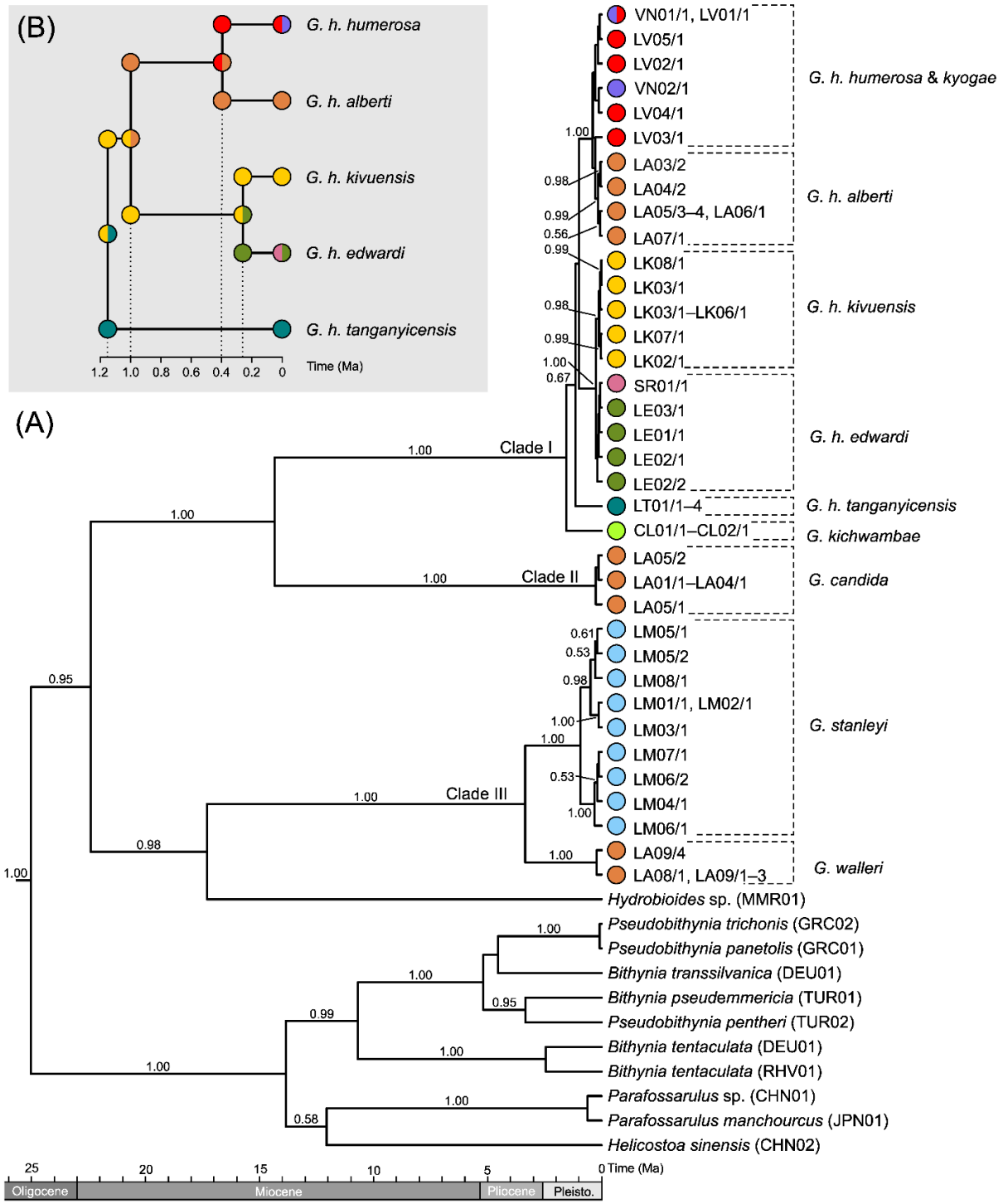

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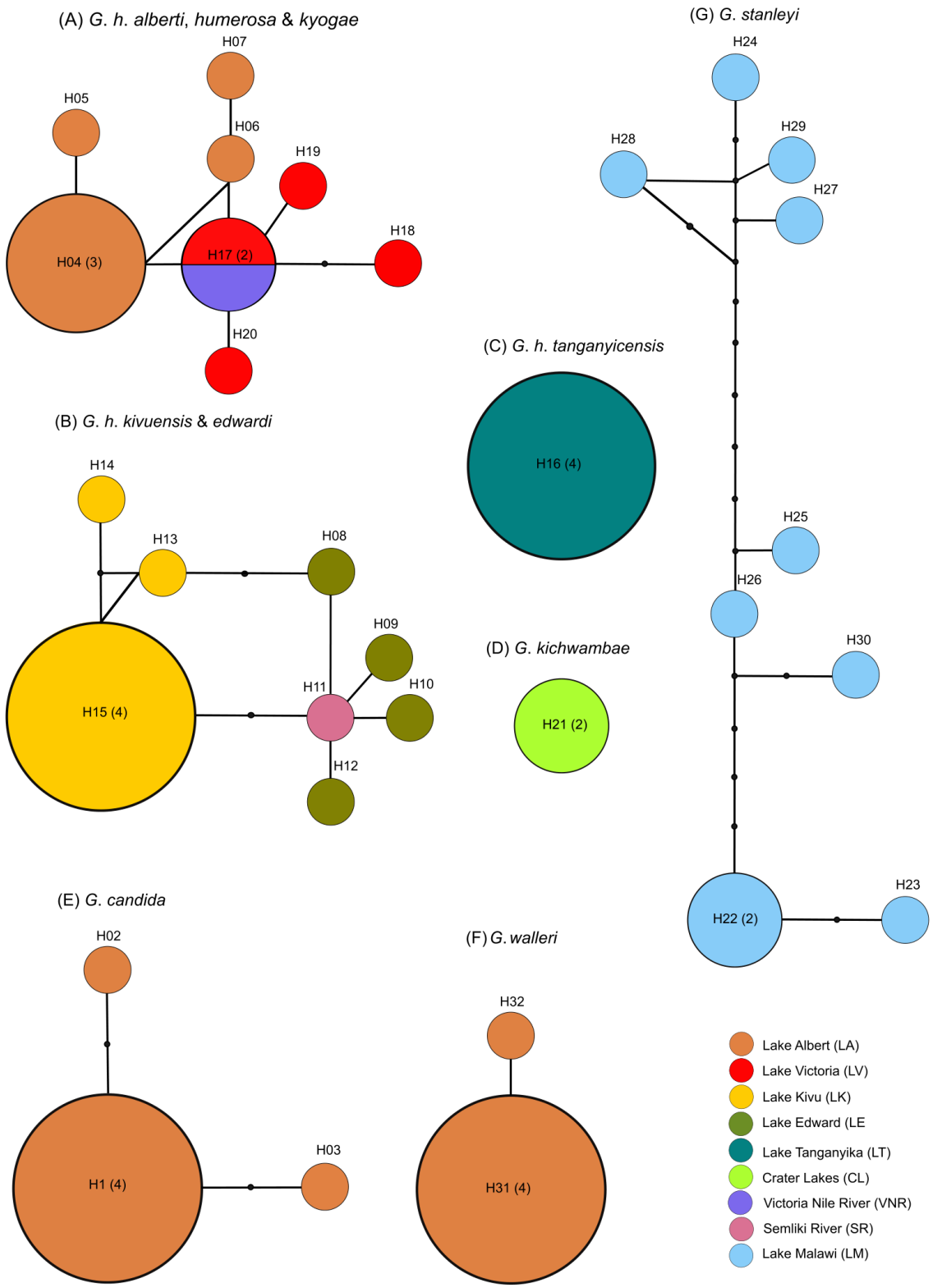

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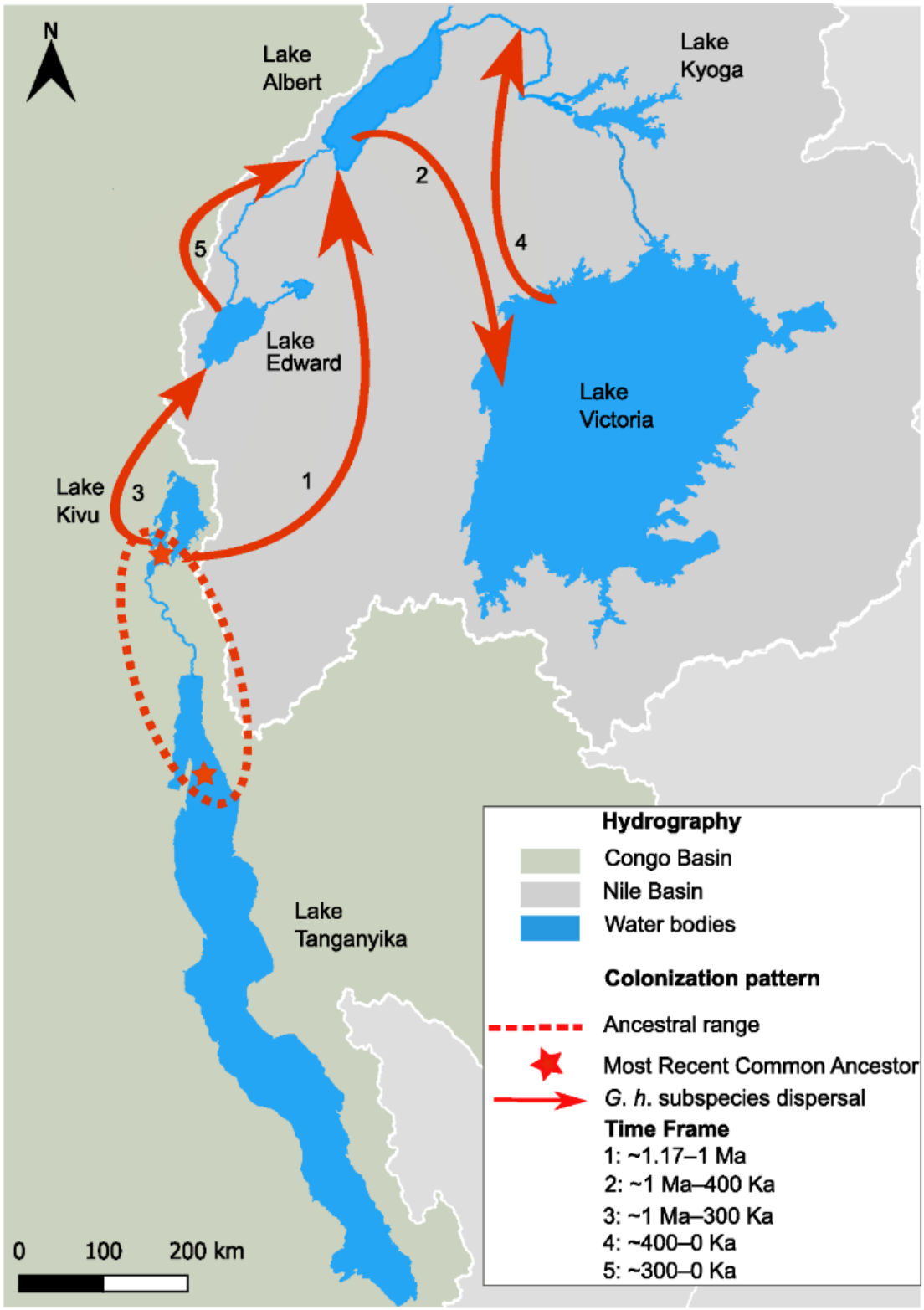

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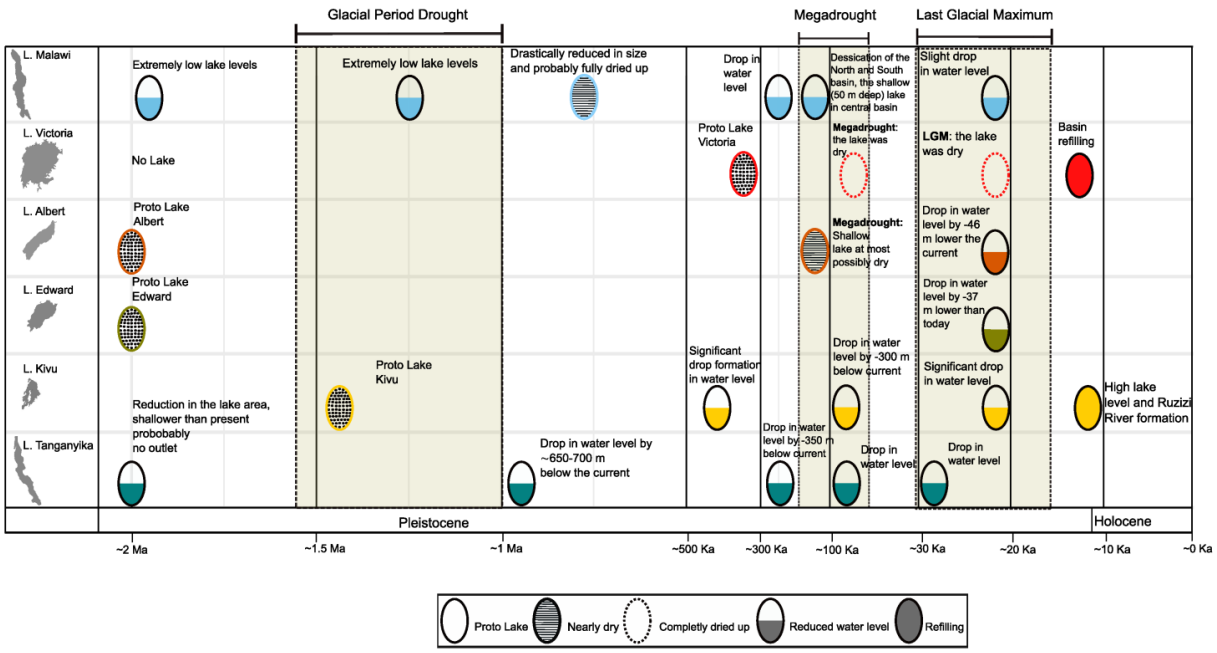

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Paper 7: Bálint et al., 2024

Environmental DNA barcoding reveals general biodiversity patterns in the large tropical rift Lake Albert.

Bálint, M., Tumusiime, J., Nakintu, J., Baranski, D., Schardt, L., Romahn, J., Dusabe, M.C., Tolo, C.U., Kagoro, G.R., Ssenkuba, F. and Junginger, A., & Albrecht, C. (2024). *Science of the Total Environment, 957, 177308. https://doi.org/10.1016/j.scitotenv.2024.177308*

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Environmental DNA barcoding reveals general biodiversity patterns in the large tropical rift Lake Albert

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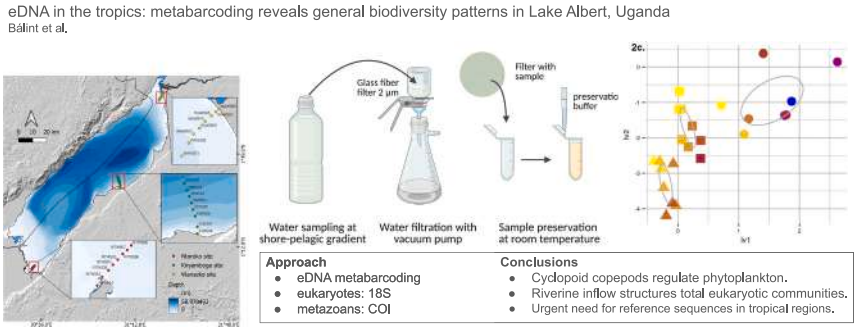
HIGHLIGHTS

- eDNA is well preserved in large, warm tropical freshwaters.
- eDNA metabarcoding confirms patterns in tropical plankton distribution.
- Comprehensive reference sequences are urgently needed.
- eDNA already allows efficient taxonomy-free biomonitoring in the tropics.

GRAPHICAL ABSTRACT



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ABSTRACT

Lake Albert, Africa's seventh-largest lake and a biodiversity hotspot, faces significant environmental challenges, including unregulated anthropogenic pressure and a lack of comprehensive biological studies. To address the scarcity of biodiversity data, we utilized environmental DNA (eDNA) metabarcoding to assess the lake's eukaryotic and metazoan communities. Surface water samples were collected at three distinct locations: close to the southern inflow of the Semliki River, the central part of the lake, and close to the northern inflow of the Victoria Nile and outflow of the Albert Nile. We aimed to study ecological patterns across the lake, focusing on sequence variant richness and community composition, testing for differences among locations and between shoreline and pelagic zones. Consistent with previous morphology-based observations, our results revealed differences in community composition among the three sites, with cyclopoid copepods dominating the communities. Distance from shore was a significant factor influencing community composition, confirming expectations about the effects of nutrient and oxygen availability gradients. However, the lack of comprehensive reference sequence data limited accurate taxonomic assignments. Despite these limitations, our study

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demonstrates that eDNA metabarcoding is highly useful for assessing biodiversity in underexplored tropical freshwater ecosystems. We advocate for urgent efforts to generate reference sequences from tropical regions to enhance the utility of eDNA for biodiversity monitoring and conservation. Our findings underscore the potential of eDNA in providing insights into ecological patterns of entire communities and emphasize the need for comprehensive studies addressing the full taxonomic spectrum in tropical freshwater ecosystems.

1. Introduction

Africa's freshwater systems are widely acknowledged as biodiversity hotspots, yet they face significant challenges. Despite the high levels of biodiversity present, there is increasing unregulated anthropogenic pressure on these ecosystems, leading to reported declines in species populations and even the likelihood of many species facing a high threat of extinction (IPBES, 2019). Another major challenge is the taxonomic crisis, with many taxa in African freshwaters remaining unstudied or unknown. In order to address these issues, one approach that has gained traction in studying entire aquatic organism communities is environmental DNA (eDNA) analysis (Schenekar, 2023). While this method has been somewhat established in extratropical regions for monitoring and assessing biodiversity, Africa significantly lags behind in adopting this approach (Takahashi et al., 2023).

Tropical freshwaters are dramatically underrepresented in eDNA studies (von der Heyden, 2023). This is particularly true for Africa, with only six eDNA studies reported until 2023 from all of the continent's hyperdiverse freshwater ecosystems (Schenekar, 2023). The focus of the handful of existing studies is either on disease vectors and human pathogens like parasitic flatworms of the genus *Schistosoma* (Sengupta et al., 2022) in Lake Tanganyika (Doble et al., 2020) and River Okavango (von der Heyden et al., 2023). There is a notable lack of comprehensive studies covering the entire taxonomic spectrum in large lakes or rivers (Takahashi et al., 2023), including all of the African Great Lakes, well-known centers of tropical freshwater biodiversity (Odada and Olago, 2006; Salzburger et al., 2014). At the same time, human action, including anthropogenic climate change is severely threatening these delicate systems (Salzburger et al., 2014). Considering that rift lakes are characterized by high levels of endemism, researching these systems is crucial. Given these research gaps, there is a pressing need for comprehensive eDNA studies which address the full taxonomic spectrum of African freshwater ecosystems. Further, establishing present-day biodiversity baselines using eDNA in these areas can help to track changes over time, offering critical insights for conservation and sustainable management, given that historical biodiversity data is largely lacking from these regions.

One such ecosystem that exemplifies the need for detailed study is Lake Albert. The lake is Africa's seventh largest lake (Ali and Abd Ellah, 2023), located in the western arm of the African Rift System at 619 m asl (Fig. 1). It is sitting on major sediment deposits of Miocene origin, but it is relatively young in geological terms (i.e. Pleistocene). The lake experiences major lake level fluctuations, and it was likely even completely dried out during the Pleistocene (Berke et al., 2014; Beuning et al., 1997). It is a typical rift lake, ca. 150 km long and 35 km wide on average with a maximum depth of only 56 m and covering 5600 km^2

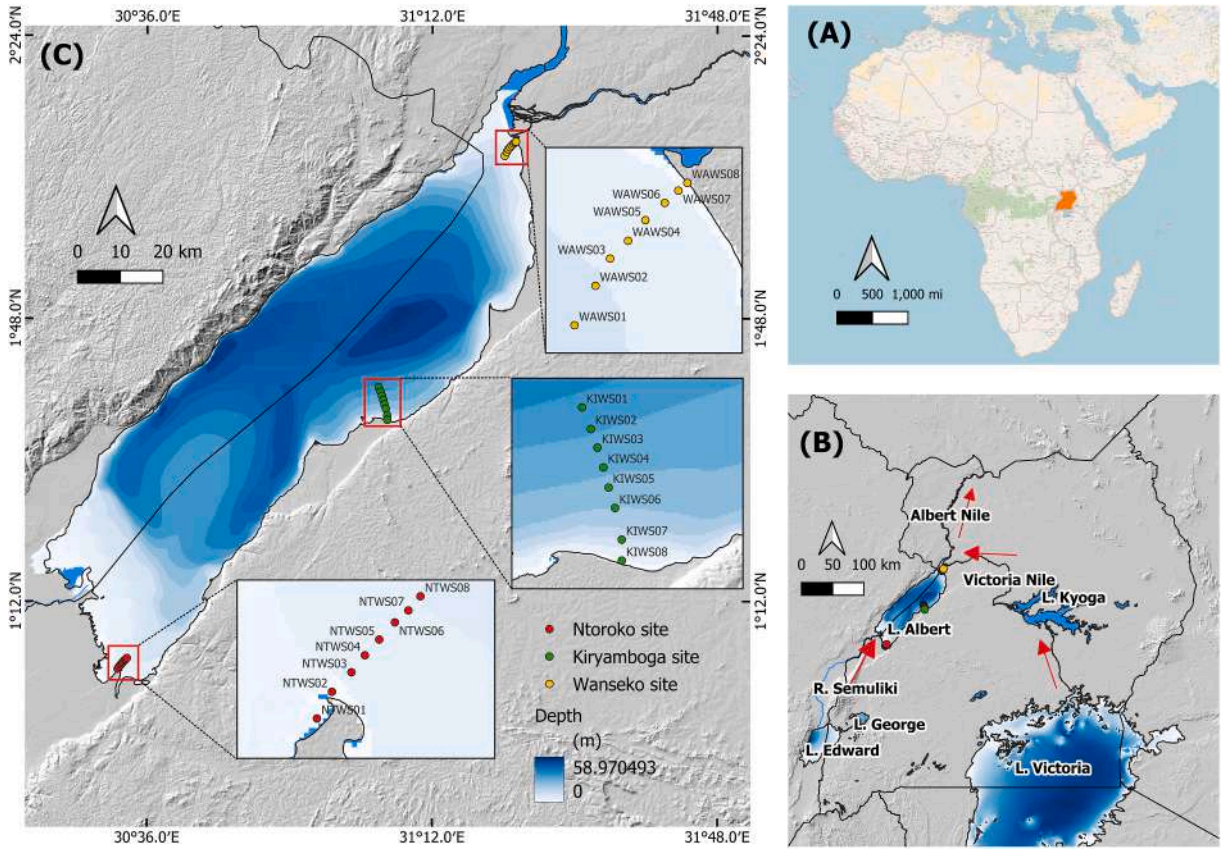

Fig. 1. A) Location of Uganda in Africa, B) sampling locations in Uganda, C) detailed sampling points on Lake Albert. Samples were collected from the southern (Ntoroko), central (Kiryamboga) and northern (Wanseko) parts of the lake along distance gradients from the shore. Red arrows mark the flow directions of the Semliki River, Victoria Nile and Albert Nile.

(Green, 2009). Mixing of its water column is pronounced during several months (Green, 2009). This is reflected in its mesotrophic state (Morana et al., 2023) and relatively high oxygenation down to the profundal (Beadle, 1981). Lake Albert's water level is mostly balanced by the Nile River which flows in and out of the lake at its northern tip (Beadle, 1981). Another major tributary is the Semliki River in the south, connecting from the Lake Edward-George system. These two rivers largely determine the current limnological conditions in Lake Albert (Beadle, 1981; Nakiyende et al., 2023), although there are some minor tributaries from the Rwenzori Mountains to the west of the lake. The lake is mesotrophic, dominated by non-humic primary production (Morana et al., 2023).

Lake Albert supports an immense range of aquatic biodiversity, including macro- and micro-invertebrates, fish, zooplankton, reptiles, birds, aquatic macrophytes, and mammals (Wandera and Balirwa, 2010). The lake is notable for endemisms, particularly with fish species such as *Lates macropthalmus* and *Engraulicypris bredoi (*Froese and Pauly, 2024*)*, and molluscs like *Bellamya rubicunda*, *Biomphalaria stanleyi*, and several species of *Gabbiella* (Brown, 1994). This highlights the urgent need for a comprehensive biodiversity inventory of the lake to inform sustainable management strategies and protect its diverse ecosystems. However, knowledge of the lake's fauna and flora is still scant, as information collection started around the middle of last century (Verbeke, 1957). As part of the Upper Nile freshwater ecoregion, nilotic elements dominate the fauna and flora in these reports. The few biodiversity studies concentrated on fish as Lake Albert supports the most diverse commercial fisheries in Uganda with at least 55 species (Wandera and Balirwa, 2010), although with comparatively low degree of fish species endemism (Nakiyende et al., 2023). Another prominent group of organisms that has been studied in this lake are molluscs. Some gastropod groups have received more attention due to their role as intermediate hosts for schistosomiasis (Rowel et al., 2015; Tumusiime et al., 2024). Knowledge about the lake's phytoplankton comes from a one-year sampling campaign (1961–1962), published in 1997 by Evans. The data shows that nutrient conditions in the lake are largely influenced by hydrological inflows from the two main rivers. The influence of the Victoria Nile in the north is limited, as it flows out of the lake almost immediately after its inflow. Nonetheless, the Nile has a considerable impact on the lake at its northernmost end (Beauchamp, 1956). The Semliki River in the south is a major source of nutrients, especially in the southern parts of the lake. This results in an end to end compositional polarization of the phytoplankton, with the riverine inflows of Semliki and Nile creating more eutrophic conditions, compared to the more oligotrophic free pelagic zone in the mid-lake center (Evans, 1997). Interestingly, phytoplankton biomass decreased between the 1960s and the 1990s, likely as a result of changes in wind conditions (Lehman et al., 1998). There is little information available about the zooplankton and macro-invertebrate communities which are key contributors to the food web and ecosystem functioning of aquatic systems. Green (1967) focused on fish predation of a *Daphnia* species and reported a reduction of fish predation on zooplankton in the mid sections of the lake. Lehman et al. (1998) report an overall dominance of cyclopoid copepods in Lake Albert's zooplankton, with a much smaller contribution from calanoids and cladocerans. Rotifers were found to be rare in the lake (Lehman et al., 1998), in comparison with other African lakes (Green, 2009).

Gradients from shoreline to pelagic conditions have been detected in Lake Albert, where shoreline waters experience more temporal oscillations, likely affecting water movement and stratification (Talling, 1963). This variability is reflected in distinct phytoplankton biomasses between inshore and offshore areas (Lehman et al., 1998). Similarly, the composition of zooplankton differs between pelagic and shoreline zones in Lake Albert (Green, 1971). (Green, 1967) even observed a morphological shift of *Daphnia lumholtzi* along the shore-pelagic gradient, attributed to feeding pressure from planktivorous fish. Compositional differences of plankton between shoreline and pelagic areas likely result from variations in nutrient concentrations and other biological or environmental factors (Chomicki et al., 2022; Dusabe et al., 2024; Green, 1967).

However, records of all organism groups are sporadic at best (and inexistent at worst) in Lake Albert, with many organism groups completely unstudied. This highlights a significant gap in understanding its ecosystem dynamics. Consequently, we likely face a massive underestimation of the actual degree of biodiversity and endemism. At the same time, Lake Albert faces serious biodiversity threats with anticipated extinctions, especially now that the lake ecosystem is exposed to oil exploration risks (Verheyen et al., 2016). The oil exploration activities came with infrastructural developments i.e. improved road networks which have accelerated rural-urban migration into this region exacerbating the problem (Ericson, 2014). A biodiversity crisis has been identified as the lake's most important recent challenge (Nakiyende et al., 2023), threatening its fisheries. Other important stressors include a destruction of shoreline vegetation, and increasing water pollution (Nakiyende et al., 2023). Considering that the lake is part of the Nile basin, its anthropogenic degradation can impact ecosystem health far downstream. However, the methodological and general knowledge gaps hamper conservation efforts and sustainable management ((Nakiyende et al., 2023)).

Environmental DNA is a highly promising approach for large scale biodiversity surveys in neglected tropical freshwaters, and to complement the so far sparse records of biodiversity. Environmental DNA might also become an important tool for monitoring the lake's biodiversity, contributing to a sustainable use and management of its ecological resources. However, the use of eDNA in large tropical lakes might encounter several issues. Conditions in the water column of tropical lakes are very distinct compared to temperate lakes, with high temperatures throughout the water column resulting in frequent mixing (Talling, 1969). This mixing causes nutrient redistribution and deep oxygen penetration (De Crop and Verschuren, 2021). Hydrological differences might translate into potential preservation issues for eDNA. DNA might decompose faster at high temperatures (Lamb et al., 2022), and available oxygen supports microbial activity, which might result in rapid DNA removal from the water column (Bairoliya et al., 2022). Finally, even if DNA is preserved and suitable for community assessments, identification problems arise due to the lack of comprehensive sequence databases. Very few sequence data exist for tropical regions (Lopes-Lima et al., 2024), posing a key problem for African eDNA studies (von der Heyden, 2023). An explicit evaluation of this problem is still lacking. Altogether, the suitability of eDNA must be evaluated for tropical lakes.

Our specific objectives were to evaluate the suitability of eDNA for describing general biodiversity patterns in tropical large lakes. Specifically, our study aimed to achieve three primary objectives. First, we sought to test environmental DNA (eDNA) methodologies by examining both previously observed and expected patterns within plankton communities of Lake Albert. Second, we aimed to evaluate the feasibility of making accurate taxonomic assignments using existing sequence references, thereby assessing the reliability of current genetic databases for identifying tropical freshwater biodiversity. Third, we wanted to generate the first comprehensive, community-wide eDNA baseline dataset of plankton diversity from any of the African Rift Lakes. This baseline is intended to serve as a crucial reference point for future biodiversity monitoring and conservation efforts in these unique and ecologically significant regions.

Along these aims we hypothesize that 1) eDNA reflects previous findings on plankton composition, 2) eDNA is sensitive enough to detect expected shoreline - pelagic gradients, and 3) currently available sequence data is insufficient for a reasonable taxonomic assignment of African lake plankton biodiversity.

2. Materials and methods

2.1. Sampling

Permission to collect and transfer samples was granted by Uganda National Council of Science and Technology (UNCST) under permission number NS148ES. Sampling was conducted in March 2023 at three distinct locations within the lake: the southern region near the inflow of the Semliki River (Ntoroko District), which carries water from Lake Edward; the central part of the lake (Kiryamboga) in the Tonya area (Hoima District); and the northern area, Wanseko (Buliisa District), close to the inflow and outflow of the White Nile (Victoria Nile and Albert Nile, respectively). Eight surface water samples were collected at each location along a transect from a boat, spaced approximately 500 m apart, starting from the deepest end of the transect (Fig. 1, Supp. Table 1). Samples were collected into plastic bottles and filtered using glass fiber filter membranes (2 μm pore size, thickness 380 μm, Ø 47 mm, Sigma-Aldrich, Darmstadt, Germany) with a reusable bottle-top filter, fitting 45 mm bottle threads (Fisher Scientific, Schwerte, Germany). The filter was operated manually with a vacuum pump. After filtration, the glass fiber membranes were folded and stored in 2 ml test tubes, preserved with Zymo DNA/RNA Shield buffer (Zymo Research, Freiburg, Germany). These tubes were kept at ambient temperature in the field (up to two weeks) and subsequently frozen at −20 °C in the laboratory prior to DNA extraction.

2.2. DNA extraction

2.2.1. Laboratory preparations before DNA/RNA extractions

PCR workstations (VWR International GmbH, Darmstadt, Germany) were cleaned with a 10 % dilution of a bleach-based cleaner (DanKlorix, CP GABA GmbH, Hamburg, Germany), followed by distilled water before every use. Pipettes in the workstations were cleaned with DNA/RNA-ExitusPlus IF (ITW Reagents, S.R.L., Monza, Italy) before every use. The designated RNA workstation and pipettes were additionally cleaned with RNAse AWAY (Molecular BioProducts, Thermo Fisher Scientific, Massachusetts, USA) before every use. These cleaning steps were repeated once the lab work in the boxes was done for the day. Prior to the lab work starting and after finishing, both boxes had the internal UV lights running for 30 min. Forceps were coated in 70 % denatured ethanol and then sterilized with fire. Pipette tip boxes were put into a shortwave UV crosslinker with open lids for 10 min each.

2.2.2. Filter preparations and lysis

Samples were randomly assigned to four different extraction batches (Supp. Table 1) to avoid batch effects (Bálint et al., 2018). These batches were processed on four separate days in June 2023. Filters were removed from storage solution tubes using sterilized forceps (changed between every sample tube) and unfolded on a stainless steel surface in the UV workstation. One-third of the filter membranes was cut into small pieces with scissors and placed in a bashing bead lysis tube of the ZymoBIOMICS DNA/RNA Miniprep kit (Cat. nr. R2002, Zymo Research Europe GmbH, Freiburg, Germany). Additionally, 400 μl of the storage buffer from the same sample was transferred to the lysis tube. The unused portion of the filter membranes was placed back in the original sample tube (still containing leftover Shield buffer) and kept at −20 °C as a backup. A negative extraction control was run alongside each extraction batch (two controls for the last extraction batch), consisting of 400 μl of DNA/RNA Shield buffer transferred to a lysis tube. Lysis tubes were shaken for 40 min on a Vortex Genie 2 using a horizontal tube adapter, set to maximum speed (3200 rpm). The stainless steel surface was cleaned between processing every sample with 10 % bleach solution, and rinsed with distilled water. Scissors were submerged into a 10 % bleach solution for a few minutes and rinsed with distilled water prior to processing the next filter.

2.2.3. DNA/RNA extraction

DNA and RNA were extracted in parallel from the cut and lysed filter membranes using the ZymoBIOMICS DNA/RNA Miniprep kit (Cat. nr. R2002, Zymo Research Europe GmbH, Freiburg, Germany), following the manufacturer's instructions. DNA was eluted in 80 μl of AccuGENE molecular biology grade water (Lonza, Verviers, Belgium); RNA was eluted in 50 μl of molecular biology grade water. DNA extracts were subsequently stored at −20 °C until further processing; RNA extracts were subsequently stored at −80 °C.

2.3. PCR reactions and sequencing

Metabarcoding PCR reactions were automated using a Biomek i7 workstation (Beckman Coulter GmbH, Germany) in a molecular prePCR laboratory. To avoid contamination, several measures were taken to sterilize the laboratory equipment and all surfaces before and after pipetting. All surfaces were treated with DNA Exitus (Kisker, Steinfurt, Germany), bleach and UV light, and plastic equipment was sterilized under UV light. In addition, protective equipment (full body suits, face masks, shoe covers, face masks, etc.) was used. DNA extracts were amplified with a) the Euka02 primers (F: 5'-TTTGTCTGSTTAATTSCG-3′, 5'-CACAGACCTGTTATTGC-3′, (Guardiola et al., 2015)) and b) the miCOIint/jgHCO2198 primers (F: 5′-GGWACWGGWTGAACWGTWTAYCCYCC, R: 5′-TAAACTTCAGGGTGACCAAARAAYCA), (Geller et al., 2013; Leray et al., 2013) using AmpliTaq Gold™ Mastermix (Thermo Fisher, Waltham MA, USA). Individually labeled PCR replicates of the Lake Albert samples were pooled with reactions from three other unrelated projects. We followed a one-step-PCR approach in which every sample had a unique nucleotide index combination of both primer pairs of 11 base pairs. This allowed three base pair mismatches for index identification (Taberlet et al., 2018). PCR was performed using 10 μl AmpliTaq GOLD Reaction buffer, 2 μl GC Enhancer, 3 μl water, 2 primer pair mixture (5 μM), 3 μl sample. The PCR positive control for Euka02 was DNA extract of a single Nematoda species (*Rhomborhabditis regina*, 800 individuals); the PCR positive control for the metazoan primer pair was a mock community containing DNA extracts of 24 species. The metabarcoding PCRs were run with eight PCR negative controls, 24 multiplexing negative controls and four PCR positive controls. The multiplexing negative controls were no-template no-mastermix control samples used to control for index jumps during the library preparation process (Taberlet et al., 2018). PCR reactions were performed in four individually indexed replicates for all samples and controls. The cycling program for Euka02 included an initial denaturation step at 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 45 °C for 30 s, 72 °C for 60 s, and a final elongation step at 72 °C for 7 min. The cycling program for miCOIint/jgHCO2198 included an initial denaturation step at 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 57 °C for 90 s, 72 °C for 60 s, and a final elongation step at 72 °C for 7 min. After amplification, PCR products were pooled and purified using the MinElute PCR Purification Kit (Qiagen). Library preparation and paired-end sequencing (NovaSeq 6000, 2 × 150 bp for Euka02 and Miseq 2 × 250 bp) was performed with the MetaFast protocol at Fasteris SA (Geneva, Switzerland).

2.4. Bioinformatics and taxonomic assignment

Bioinformatic analysis was conducted using the Obitools pipeline v4.0.5 (Boyer et al., 2016). The paired-end reads were assembled with a minimum overlap of ten bases and then demultiplexed into PCR replicates. These replicates were de-replicated into amplified sequence variants (ASVs). Sequences were discarded which had variants of other sequences with a count >5 % of their own count. We kept ASVs only if they had lengths between 70 and 350 bp, and if they were represented by at least 10 reads.

The ASV x PCR replicate matrix underwent further processing with a custom R script, following the recommendations of (Taberlet et al., 2018). To account for potential PCR or sequencing errors, ASVs with low

read abundance were removed if their total reads fell below a threshold (18S: 100 reads; COI: 75 reads) based on frequency criteria (Drake et al., 2022). Additionally, replicates with spurious richness were filtered out based on their richness - read profiles, specifically those with fewer than 7000 reads for 18S and fewer than 75 ASVs for COI. For quality control, if an ASV was present in any of the negative controls (including extraction, PCR, and multiplexing negatives), the maximum number of reads for that ASV in any negative control was calculated and subtracted from each replicate. Nonmetric multidimensional scaling (NMDS) ordination plots of the replicates were generated using the "vegan" package in R (v4.4.0) with Bray-Curtis dissimilarity. Replicates highly dissimilar to others from the same sample were removed. Subsequently, PCR replicates for each sample were summed into a community matrix, and altered observation depths due to replicate removal were corrected.

Taxonomic assignment of 18S ASVs was performed using local BLAST searches against the NCBI database, while COI ASVs were matched against all COI sequences from both NCBI and the Barcode of Life database (Meglécz, 2023). Only the best 100 BLAST hits with an e-value <0.001 were retained. The BLAST results were parsed with MEGAN v6 (Huson et al., 2016), and each ASV was assigned to its lowest common ancestor based on the top 10 % of bit scores of BLAST results.

2.5. Statistical analyses

All statistical analyses were performed in R v4.4.0 (R Core Team, 2024). Unequal water sample volumes and unequal sequencing depths of replicates were explicitly accounted for in all analyses by including them into models as predictors. This has three benefits in comparison to simple rarefactions: 1) avoids data loss, 2) allows remove their effects when using model-based analysis tools, and 3) explicitly quantifies the effect of biases linked to observation depth, in comparison to the effect of predictors of interest (reviewed in (Bálint et al., 2016)). We considered only the taxonomic groups specifically targeted by the primers in the analyses of community properties. This means that all eukaryotic ASVs were considered in the analyses which were amplified with the primers euka02, but all other ASVs (e.g. a few ASVs assigned to prokaryotes, or not assigned to anything known) were omitted. We considered only metazoan ASVs amplified with the COI primers, but all other ASVs, e.g. algae, were omitted.

2.5.1. ASV richness

With respect to ASV richness and community composition, we tested 1) the impact of inflowing rivers (three locations: Ntoroko - Semliki River, Kyriamboga - no major effluent, Wanseko - Victoria Nile, Fig. 1), and 2) the effects of a shoreline - pelagic gradient. ASV richness was modeled with four predictors (water sample volume, sequencing depth, sampling locality and distance from shore) in linear regressions.

2.5.2. Community composition

To select abundant and frequent ASVs, we modeled the log-normalized average read abundance of ASVs against their incidence with generalized additive models (mgcv package, (Wood, 2017)). We used GAMs primarily for exploring patterns, rather than for hypothesis testing (Simpson, 2018). GAMs are particularly suitable for this purpose because they do not impose a strict parametric relationship between variables, allowing for flexible pattern discovery. By doing so, we selected taxa with a high signal-to-noise ratio, enabling us to focus on taxa with meaningful biological variation rather than noise (Bálint et al., 2016). For analyses of 18S community composition, we retained abundant and frequent ASVs: these were represented by more than the average number of reads, and were present in at least six samples. For analysis of COI community composition, we retained frequent OTUs: these were present in at least eight samples. This resulted in sample x ASV matrices with 278 ASVs (18S), and 241 ASVs (COI).

For testing hypotheses about community composition, we employed generalized linear models (GLMs), which are well-suited for handling non-normally distributed data. Specifically, we modeled taxon read abundances using a negative binomial distribution to account for overdispersion in the data, as recommended for ecological counts (Lindén and Mäntyniemi, 2011). Although distance-based methods like PERMANOVA or NMDS are commonly used for community composition data, they rely on relative abundances, which may be biased due to technical factors like primer bias (Krehenwinkel et al., 2017). As such, we opted for GLMs within a joint species distribution model framework (Ovaskainen et al., 2017; Warton et al., 2015) to explicitly model the read counts per taxon, considering the underlying data generation process. The individual GLM results are summarized into community-level statistics at the end, providing an overview of driver importance of community-level differences in a format similar to results of distance-based approaches such as PERMANOVA (Wang et al., 2012). We consider this approach is better suited for metabarcoding data and allows for community-wide statistics to be calculated more robustly (Bálint et al., 2016).

We predicted community composition of frequent and/or highly incident ASVs with a model-based analysis of multivariate abundance data as implemented in the mvabund package (Wang et al., 2012). mvabund models the abundances of single taxa in a community against a set of predictors in a generalized linear modeling (GLM) framework, and summarizes model results into a community-level statistics. As ecological count data including sequencing reads are generally overdispersed, model-based community analyses are particularly helpful as they allow to include parameters about data properties (Warton et al., 2015). We fitted community models by specifying the distribution family as negative binomial using a log link, with an unknown overdispersion parameter. We created an analysis of deviance (ANOVA) table for the GLM, using a likelihood ratio test with Monte Carlo resampling (1000 bootstrap iterations).

We visualized compositional differences among the ASV communities with Bayesian ordination and regression as implemented in the boral package (Hui, 2016), by fitting the models with a negative binomial distribution. We parameterized the models with sample volume and sequencing depth as covariates, and accounted for variation unexplained by these covariates on two latent variables. Similarities and differences in ASV composition among samples were visualized along these latent variables in two dimensions, similar to a conventional ordination (Warton et al., 2015). We ran the MCMC sample for 40,000 iterations, discarded 10,000 iterations as burn-in with a thinning rate of 30.

3. Results

After the Obitools processing and subsequent R cleanup, the Lake Albert dataset contained 3,841,955 reads assigned to 4527 ASVs. The COI dataset contained 2,756,941 reads assigned to 1544 ASVs. Of the 4527 18S ASVs, 707 were assigned to algae, 330 to fungi, 839 to metazoans, 997 to non-algae protists, 36 to oomycetes, 65 to streptophytes, and 1553 could not be assigned to any taxonomic levels below the superkingdom of eukaryotes (Fig. 2, Supp. Table 2). Of the 1524 COI ASVs, 473 were assigned to algae, 16 to fungi, 87 to non-algae protists, 439 to other eukaryotes, and 509 to metazoans (Fig. 2, Supp. Table 2). Of the 4527 18S ASVs, 2874 (63.5 %) were shared across at least two locations, and 1653 (36.5 %) were observed at only a single location. Of the 509 metazoan COI ASVs, 321 (63 %) were shared across at least two locations, and 188 (37 %) were observed at only a single location.

3.1. Ecological patterns

3.1.1. ASV richness

The linear regression of 18S ASV richness had an adjusted $R^2 = 0.826$ ($p < 0.001$, $F = 22.89$, $df = 18$). The volume of water had no statistically significant effect. ASV richness statistically significantly increased with ~7 ASVs for every 10,000 reads ($p < 0.001$), and it was different

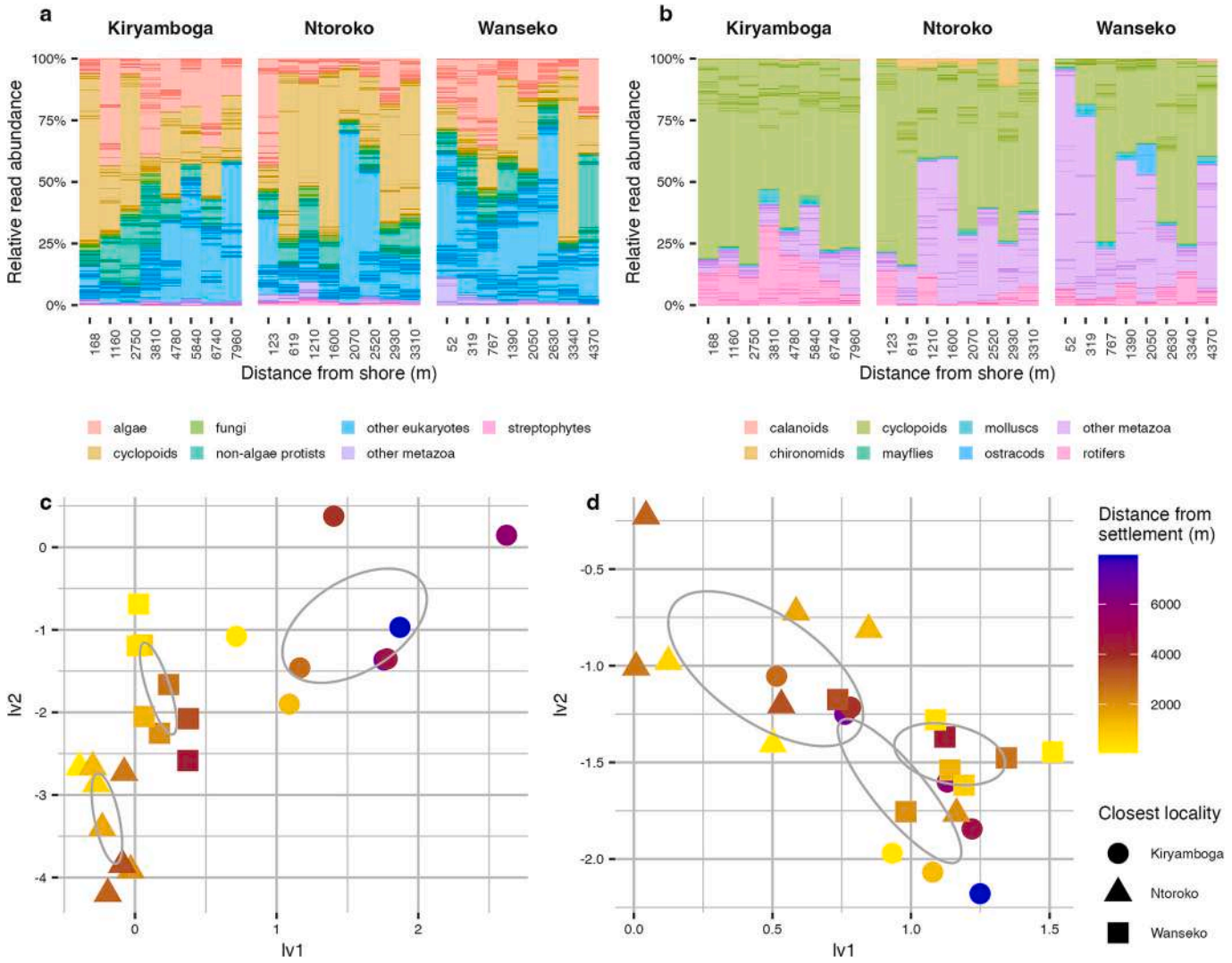

Fig. 2. Community composition recorded by eDNA. a) Dominant eukaryotic groups recorded by the 18S marker gene. b) Dominant metazoan groups recorded by COI. c–d) Model-based ordinations of eukaryotic (c) and metazoan (d) community composition.

between Kiryamboga and Wanseko ($p = 0.001$), but not between Kiryamboga and Ntoroko ($p = 0.169$). ASV richness statistically significantly decreased with ~6 ASVs for every 100 distance increase from the shore ($p = 0.002$).

The linear regression of COI ASV richness had an adjusted $R^2 = 0.705$ ($p < 0.001$, F = 12, df = 18). The volume of water had no statistically significant effect. ASV richness statistically significantly increased with ~5 ASVs for every 10,000 reads ($p < 0.001$), and it was different between Kiryamboga and Wanseko ($p = 0.007$), but not between Kiryamboga and Ntoroko ($p = 0.447$). ASV richness had no statistically significant relationship with the distance from the shore ($p = 0.445$).

3.1.2. Community composition

Composition of both 18S and COI ASV communities differed statistically significantly among locations (Table 1, Fig. 2). Of the 278 abundant and frequent 18S ASVs, 85 (31 %) had statistically significantly different read abundances among locations (Fig. 3, Supp. Fig. 1). These ASVs belonged to algae (20 ASVs), fungi (6) and protists (14). Many ASVs could not be identified beyond eukaryotes (45). Of the 241 frequent COI ASVs, 15 (6 %) had statistically significantly different read abundances among locations (Fig. 3, Supp. Fig. 2). These ASVs belonged to an annelid (likely an aquatic enchytraeid with 78 % sequence similarity to a *Limnodrilus* species), eight arthropods (two cyclopid ASVs, an amphipod, two insects, two branchipods and an ASV which could not be identified beyond arthropods), a chordate (likely a rodent, with ~79 % sequence similarity to a *Chinchilla* species), three snails and two rotifers (Supp. Fig. 2).

Table 1
ANOVA table of compositional differences of the model-based analysis of multivariate ASV abundances.

		Residual df	Df difference	Deviance	*p*
18S	(Intercept)	23			
	Sample volume	22	1	2101	0.001
	Sequencing depth	21	1	415	0.001
	Locality	19	2	3585	0.001
	Distance from shore	18	1	1701	0.001
COI	(Intercept)	23			
	Volume_filtered_ml	22	1	818	0.001
	seq_depth	21	1	360	0.001
	Closest_locality	19	2	1178	0.002
	dist_settlement_m	18	1	680	0.001

Composition of both 18S and COI ASV communities was statistically significantly affected by the distance from shore (Table 1, Fig. 2). Read abundances of statistically significantly affected 18S ASVs ($n = 42$) generally decreased with distance from the shore with the exception of a

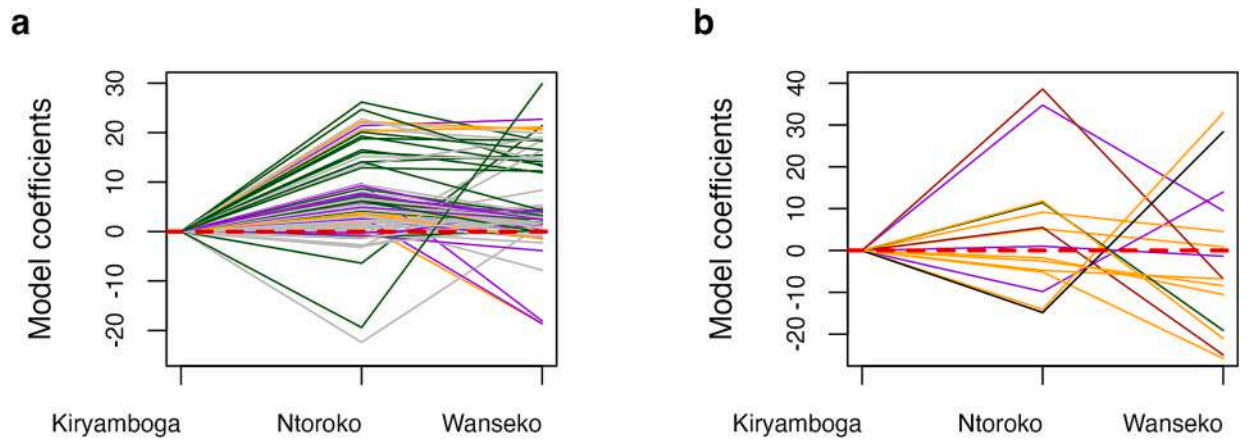

Fig. 3. Mean-centered model coefficients show the extent of differences in read abundance of ASVs at river-affected locations (Ntoroko: Semliki river, Wanseko: Victoria Nile), compared to a baseline (Kiryamboga, no major inflow). Most ASVs show higher read abundances at the river-affected locations. Black continuous lines represent ASV coefficients from the multivariate community GLM with statistically significantly different read abundances among the localities. Dashed red line marks the base of comparison. Colors mark major organism groups: a) 18S: green - algae; orange - fungi; purple - non-algae protists; gray - other eukaryotes. b) COI: green - annelids; orange - arthropods; chordates - black; molluscs - purple; rotifers - brown.

diatom ASV belonging to Coscinodiscophyceae (Supp. Fig. 3). The individual COI ASVs related to the distance from the shore belonged to arthropods, molluscs and rotifers. Read abundances of all statistically affected COI ASVs (n = 10) decreased with distance from the shore (Supp. Fig. 4).

3.2. Limitations of taxonomic identification

Average identity among 18S query and database sequences was 96 % (Fig. 4). Most assignments had low bit scores, even if sequence identity between query and database sequences was high (Supp. Fig. 5). Average identity among COI query and database sequences was 91 % (Fig. 4). The relationship between bit score and sequence identity was almost linear (Supp. Fig. 5).

4. Discussion

4.1. Environmental DNA confirms known and expected biodiversity patterns

4.1.1. Detected taxonomic composition and north-south differences

Most organisms recorded with the two marker fragments belonged to plankton (Fig. 2). This is not surprising given that we sampled only surface water. We found large read abundances of cyclopoid copepods in both 18S and COI data (Fig. 2). This confirms that the zooplankton communities are dominated by cyclopoid copepods, whereas calanoids and Cladocera represent <10 % of the total biomass (Lehman et al., 1998). Rotifers were also well represented as planktonic consumers in the eDNA data, especially at the central location of Kiryamboga (Fig. 2). Interestingly, previous accounts listed only a few rotifers, low compared to other lakes on the continent (Green, 2009). Rotifer abundance was reported to be highly localized with most species in the South of Lake Albert (Green, 2009). These differences may result from a reduction in phytoplankton biomass over recent decades, compared to the 1960s, likely due to diminished nutrient availability, which is itself a consequence of changes in wind mixing (Lehman et al., 1998). Additionally, reduced and/or selective predation pressure by planktivorous fish on zooplankton, attributable to the introduction of the Nile perch (Green, 1967; Lehman et al., 1998), might also play a role. Observed differences might originate from alterations in nutrient and temperature conditions since the 1990s, driven by increasing human impact and climate warming, as trophic conditions in the lake can shift within a matter of years or decades (Lehman et al., 1998).

Producers were mostly represented by diverse strains of algae, likely components of the phytoplankton, suggested by existing information on chlorophyll concentrations, which indicate algal photosynthesis to at least 25 m depth (Lehman et al., 1998). Interestingly, most of the ASVs with strongly different read numbers among the locations belonged to algae (Supp. Fig. 1). Read numbers of these algae were significantly higher at the river-influenced locations of Ntoroko and Wanseko, in comparison with the centrally located Kiryamboga. This is consistent with the only comprehensive data available (to our best knowledge) on the composition of phytoplankton communities from the lake, which dates back >60 years (Evans, 1997). This phytoplankton dataset reports an end to end polarization in the composition of phytoplankton, with higher concentrations at the two extremes (south and north) influenced by the Semliki and Nile rivers. Here the lake is more eutrophic, compared to the more oligotrophic free pelagic zone in the mid-lake center (Evans, 1997). Lake Albert is considered a rare case among tropical lakes as its planktonic communities are largely controlled by its hydrographic and hydrological character (Evans, 1997). The eDNA results are in line with this observation that the two large rivers determine the limnological conditions in the lake (Beadle, 1981; Nakiyende et al., 2023). Rivers carry nutrients into lakes and strongly influence primary productivity at the river-lake transition zones (Cotte et al., 2023). This selects for species which can rapidly utilize nutrients for growth (Nhu et al., 2019). Localities with less nutrients often support specialized, highly efficient nutrient utiliser phytoplankton species which are competitive at low trophicity (Atkins et al., 2021).

eDNA recorded only a few clearly benthic taxa, represented by relatively few reads. This is expected as we sampled surface water. Consequently, most DNA fragments should originate from pelagic organisms. The macrobenthic fauna of Lake Albert is not well studied, but general records hint at a comparatively rich fauna (Guignot, 1957; Verbeke, 1957), representing groups such as Chironomidae, Ephemeroptera, and Ostracoda. Insects alone are estimated to number at least 200 species (Green, 2009). Green (2009) attributed the relative species richness of Lake Albert to the presence of sandpits along the Ugandan shores, which creates habitat heterogeneity. Chironomids were relatively abundant at Ntoroko in the eDNA data, but Ephemeroptera and ostracods were detected as well. eDNA supported the absence of other benthic taxa, such as decapod crustaceans which were so far not observed in the lake (Cumberlidge and Clark, 2018). A prominent group of benthic organisms are the molluscs, with the lake's fauna consisting of approximately 13 bivalve and 18 gastropod taxa (Brown, 1994; Mandahl Barth, 1954). eDNA recorded several mollusc ASVs such as

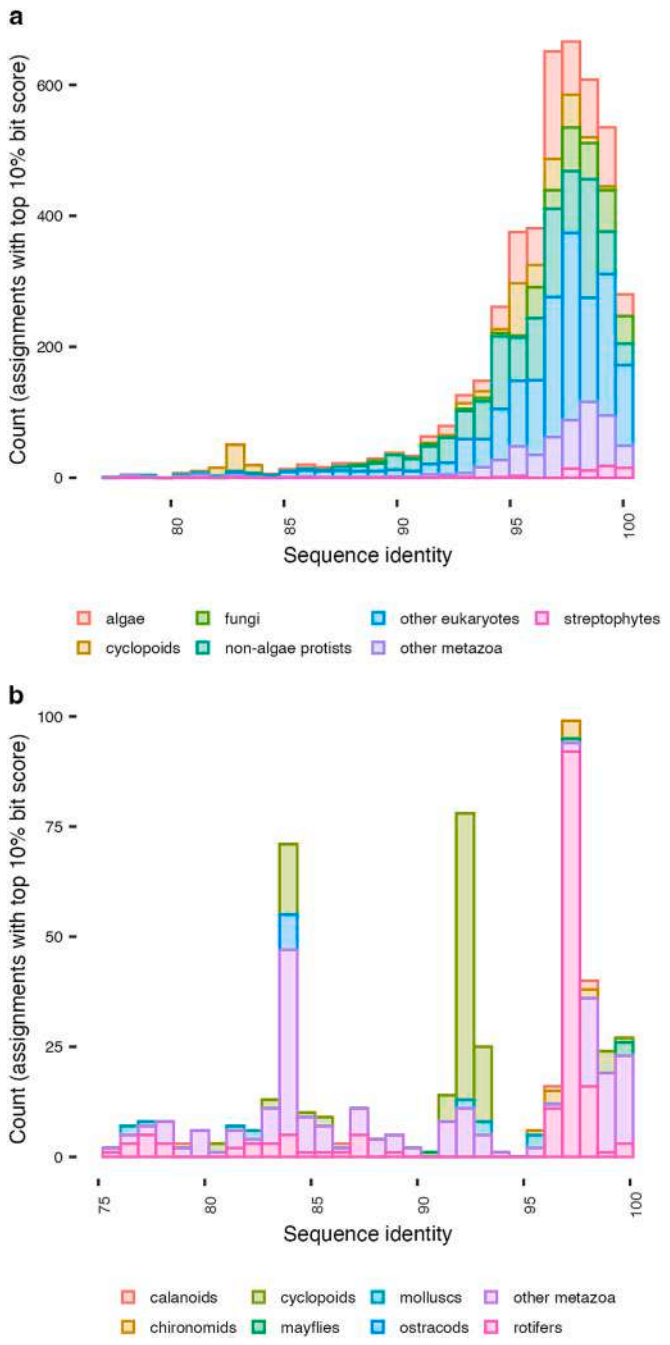

Fig. 4. Sequence identity of BLAST query and database sequences. a) Eukaryotes recorded with 18S. b) Metazoans recorded with COI.

sphaeriid bivalves (*Pisidium* sp.). Members of that genus are known to occur in Lake Albert (Mandahl Barth, 1954).

4.1.2. Differences in shore vs open water

Both markers showed statistically significant differences in community composition along a distance gradient from the shore toward the more central areas of the lake. The 18S dataset also showed a decrease in ASV richness from the shoreline toward the lake center. Read abundance of individual ASVs of both markers mostly decreased from the shore toward pelagic environments (Supp. Figs. 3–4) except one ASV assigned to diatoms. This is in agreement with expectations about differences between shoreline and central parts of large lakes, as nutrient concentrations generally decline from the shoreline to offshore areas. For example, in Lake Ontario, mean nutrient concentrations rapidly decline with increasing distance from the shore to background levels (Chomicki et al., 2022). Shoreline erosion can be an important source of nutrients, influencing lake productivity and trophic status (Hewlett et al., 2015). Water mixing can also affect nutrient availability, with more dynamic mixing of shallow shoreline waters promoting higher nutrient concentrations due to sediment resuspension (MacIntyre et al., 1999). Using traditional sampling, a steep decline in oxygen levels was observed with increasing depth, which has an impact on species diversity and richness (Dusabe et al., 2024). We collected our samples at a time of the year that is known for pronounced circulation of nutrients (Talling, 1965). Highly heterogeneous conditions at the shoreline, e.g. supported by macrophytes, also promote more taxonomically rich and compositionally distinct communities (Wang et al., 2018). Finally, reduction in oxygen in the pelagic zones, as observed in other African Great Lakes (Dusabe et al., 2024), might also play a role. The observed compositional gradient might also be a result of nutrient enrichment resulting from human activity, as all transects started at settlements. Many more locations will be necessary to disentangle the causes of the observed compositional gradients. A new sampling designed to disentangle these causes should contrast gradients starting at human settlements with gradients starting at shores without human impact. Vegetation conditions and the presence/absence of water inflows should be also considered when designing such a study in the future. While a designated sampling will be necessary to distinguish among the relative contribution of these effects to the observed patterns, eDNA records confirmed expectations about compositional differences between shoreline and pelagic communities.

4.2. Taxonomic identification of tropical eDNA sequences

The 18S sequences had an average identity of 96 % with sequences deposited in GenBank (Fig. 4). An average high identity with GenBank sequences is expected since 18S is a relatively conserved taxonomic marker, suitable for studying higher-level taxonomic units (Bininda-Emonds, 2021). However, many of the 18S ASVs (34 %) could not be assigned to taxonomic levels beyond eukaryotes. This might be caused by three processes. First, the query sequence might belong to an unknown eukaryotic group not present in the database. Second, the sequence in the database is misidentified, e.g., it is assigned to an organism but actually originates from a symbiont of this organism or contamination (Valkiūnas et al., 2008). Third, some of the assignments considered by the LCA algorithm are very low level, e.g. eukaryotes, which frequently happens when environmental sequences are deposited in NCBI. It is difficult to clearly distinguish among these three processes. Issues with reference sequence databases are common (summarized e.g. by (Chorlton, 2024). Issues with the identification quality of sequences deposited in NCBI certainly play a role (Valkiūnas et al., 2008). Nonetheless, taxonomic assignments in GenBank are fairly reliable (Leray et al., 2019), often comparable in quality to metazoan assignments made with BOLD (Baena-Bejarano et al., 2023). For the 18S marker gene we found a mismatch between the bit score (reflecting alignment length, sequence identity and database size) and sequence identity (Supp. Fig. 5). The vast majority of 18S ASV assignments were based on fairly low bit scores, regardless of the sequence identity, although all high bit score assignments also had high identity. This results from a short alignment length between query and database sequences, consistent with the level of conservation of the 18S: the ~150 bp V7 region amplified by us has a 20–25 bp highly variable core, flanked by conserved regions (Hadziavdic et al., 2014). Consequently, the most likely explanation for the large number of unidentified 18S eukaryotic ASVs is a general lack of eukaryotic sequences from African Great Lakes in GenBank.

Of the 1524 COI ASVs, 509 (33 %) could be assigned to metazoans. On average, these ASVs showed 91 % sequence identity with sequences deposited in GenBank and BOLD. Many other eukaryotes, including algae, fungi, and protists, were also amplified. This is not surprising, as

degenerate metazoan COI primers frequently amplify various groups beyond metazoans. Similarly to what we found here, metazoans might not even be the group with the highest read count (Collins et al., 2019). We analyzed only metazoans because the primers were optimized to specifically address primer biases for metazoans (Leray et al., 2013). We found a clear, almost linear relationship between identity and bit score (Supp. Fig. 5), which results from the highly conserved length of COI across the tree of life (Guo et al., 2022), as variable sites in COI are generally the second and third codon positions. Sequence identity of COI query and database sequences ranged between 75 and 100 %, with several distinct peaks (Fig. 4). Two prominent groups in the COI data are arthropods and rotifers. Arthropod query sequences had three distinct identity peaks: ~84 % (diverse metazoans), ~93 % (mostly cyclopoid copepods), and ~ 97–98 % (mostly rotifers). Rotifer COI queries had a distinct peak with most ASVs showing 97 % sequence identity with GenBank sequences. These ASVs were assigned to two cosmopolitan *Polyarthra* species (*P. dolichoptera* and *P. vulgaris*), both known for high levels of cryptic diversity even within the same water bodies (Liang et al., 2022; Obertegger et al., 2014). The two species accounted for >50 % of all rotifer counts in the COI dataset and >10 % of all metazoan counts. Both species were already reported from Lake Albert (Kiggundu et al., 2022; Lopez et al., 2023). Altogether, COI assignments results mirror those based on 18S: most sequenced COI fragments originate from species, genera, and families which do not have reference COI sequences available in GenBank. This is not surprising considering that many genera in well-researched regions such as North America also lack publicly available COI barcodes (Curry et al., 2018).

4.3. Management suggestions

Understanding of aquatic resources is crucial to inform policy formulation and decision-making. Currently, institutions such as NAFIRRI (National Fisheries Resources Research Institute), which hold the mandate for monitoring and conducting aquatic biodiversity research in Uganda, often omit important microbiota, such as zooplankton and phytoplankton, in their monitoring efforts due to limited resources. Most existing biodiversity data from Lake Albert are focused on fish, neglecting other critical organisms like zooplankton and algal communities, vital components of the food web and essential indicators of ecosystem health. Our study helps fill this gap by providing baseline data especially on the planktonic community composition of Lake Albert. Such baseline data is essential to support monitoring and management efforts, particularly in light of ongoing oil exploration activities.

Although our results show that the precise taxonomic assignment of eDNA sequences is problematic due to lack of reference sequence data, the results also highlight that aquatic eDNA is already suitable for taxonomy-free biomonitoring in the tropics. We recommend the urgent setup of an Africa-wide taxonomy-free eDNA-based monitoring scheme across freshwaters of the continent. Taxonomy-free biomonitoring directly links sequence data to environmental conditions and/or human impact, and allows large-scale DNA-based biomonitoring efforts (Cordier et al., 2019; Mächler et al., 2021). An eDNA-based, taxonomy-free biomonitoring scheme could efficiently track biodiversity dynamics by collecting aquatic eDNA samples at strategically placed locations at regular intervals. Such a scheme would be based on variation in α-, β- and γ-diversity of amplified sequence variants. The scheme would also allow the identification of bioindicator sequence variants, useful to authorities to quickly detect ecosystem disturbances. Further, a taxonomy-free eDNA monitoring scheme could be easily extended and refined to include economically important species and pathogens, highly relevant for societies across the continent. Systematic biomonitoring will be particularly important for managing local and large-scale threats, such as pollution, resource overuse, species invasions, climate change, allowing for interventions to ensure resilience.

5. Conclusions and outlooks

In summary, our study confirmed that aquatic eDNA is suitable to record biodiversity patterns in large, warm tropical freshwaters. Environmental DNA reflected both previous and expected findings about the structure and composition of planktonic communities in Lake Albert, in support of the first and second study hypotheses. Environmental DNA revealed significant differences in community composition in distinct localities, confirming previous, morphology-based findings about processes that shape planktonic communities. The eDNA data, consistent with limited morphology-based observations, indicated that cyclopoid copepods dominate pelagic communities, controlling planktonic producers. The data also confirmed previous findings about the importance of riverine nutrient input as the primary factor influencing planktonic microorganisms in the lake, confirming earlier observations. Environmental DNA was sufficiently sensitive to reveal gradients in plankton community composition. These gradients were expected both from historic observations, and on the basis of limnological differences between shoreline and pelagic regions.

However, the current dataset only included pelagic zones, neglecting the benthic regions and the entire water column. This limitation, along with the restricted spatial and temporal scope of the data, highlights the need for more comprehensive spatial experimental designs. These should include replicated sampling at both riverine and non-riverine sites, and transects starting from both settlements and natural areas. When designing these studies, we recommend considering further limnological factors specific to tropical lakes. Several of these factors, such as wind mixing together with a weak thermal stratification of the water column (Lewis, 1987) may strongly influence community composition of the plankton, as oxygen and nutrient conditions might be homogenized vertically and horizontally. This may result in more homogeneous planktonic communities, requiring longer gradients or more samples to disentangle compositional patterns. Furthermore, seasonal aspects, such as dry and wet seasons, and temporal variations in planktonic composition and abundance should be considered.

The results of the present study clearly confirmed expected strong patterns in the distribution of the most common taxa, even with a relatively limited sample set from three transects at strategically selected locations. However, investigating rarer taxa, particularly those with restricted distributions within the lake, would benefit from sampling at a much higher spatial resolution. Future studies should consider the shoreline of Lake Albert in the Democratic Republic of Congo. Ideally, such efforts would involve international teams from both countries. Sampling along the entire shoreline will undoubtedly increase the number of identified taxa and significantly contribute to our understanding of high-resolution patterns in planktonic community composition. The results also confirmed the third hypothesis: although eDNA data reveals strong biodiversity patterns, a major challenge to its application in tropical regions like Africa is the urgent need for generating comprehensive reference sequence data. The lack of reference sequences significantly hampers the ability to accurately interpret eDNA results from the most biodiverse regions of Earth, as many taxa remain unidentified or misidentified due to insufficient database coverage. Without reference sequences, eDNA can record ecological patterns but struggles with accurate interpretation. This is because eDNA results cannot be effectively linked to existing knowledge about taxa, their ecology, habitat preferences, and traits. This gap underscores the necessity for coordinated efforts to expand genetic databases with sequences from diverse taxa present in tropical freshwater ecosystems. We recommend prioritizing potentially high biomass species when generating reference sequence data, as these species likely have a large impact on ecosystem functioning.

Despite these challenges, we advocate for eDNA as a highly suitable monitoring tool in tropical regions, even in the current absence of reliable marker gene data. Our approach underscores the importance of targeting entire ecosystems, including both producers and consumers,

rather than focusing on specific groups. It is crucial to exploit the capabilities of molecular tools for comprehensive surveys in highly diverse tropical ecosystems. Only by doing this can we achieve a holistic understanding of ecosystems and the complex interaction networks among microorganisms, plants, animals, fungi, and protists, and perform efficient interventions to ensure their resilience.

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CRediT authorship contribution statement

Miklós Bálint: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Julius Tumusiime:** Writing – review & editing, Investigation. **Justine Nakintu:** Writing – review & editing, Investigation. **Damian Baranski:** Writing – review & editing, Methodology. **Leonie Schardt:** Writing – review & editing, Methodology. **Juliane Romahn:** Writing – review & editing, Methodology, Data curation. **Marie-Claire Dusabe:** Writing – review & editing, Investigation. **Casim Umba Tolo:** Writing – review & editing, Investigation. **Grace Rugunda Kagoro:** Writing – review & editing, Investigation. **Francis Ssenkuba:** Writing – review & editing, Investigation. **Annett Junginger:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Christian Albrecht:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT v4 in order to improve the readability and language of the manuscript and to optimize data analysis scripts. The authors also used Perplexity for literature research. After using these tools, the authors reviewed and edited the content as needed. They take full responsibility for the content of the published article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

High throughput sequence data will be accessible in NCBI or ENA upon acceptance. Analyses and results can be reproduced with R scripts and data deposited on FigShare (doi: https://doi.org/10.6084/m9.figshare.26506030).

References

Ali, M.M., Abd Ellah, R.G., 2023. Chapter 1 - history and formation of African Lakes. In: El-Sheekh, M., Elsaied, H.E. (Eds.), Lakes of Africa. Elsevier, pp. 1–31.
Atkins, K.S., Hackley, S.H., Allen, B.C., Watanabe, S., Reuter, J.E., Schladow, S.G., 2021. Variability in periphyton community and biomass over 37 years in Lake Tahoe (CA-NV). Hydrobiologia 848, 1755–1772.
Baena-Bejarano, N., Reina, C., Martínez-Revelo, D.E., Medina, C.A., Tovar, E., Uribe-Soto, S., Neita-Moreno, J.C., Gonzalez, M.A., 2023. Taxonomic identification accuracy from BOLD and GenBank databases using over a thousand insect DNA barcodes from Colombia. PloS One 18, e0277379.
Bairoliya, S., Koh Zhi Xiang, J., Cao, B., 2022. Extracellular DNA in environmental samples: occurrence, extraction, quantification, and impact on microbial biodiversity assessment. Appl. Environ. Microbiol. 88, e01845-21. https://doi.org/10.1128/aem.01845-21.
Bálint, M., Bahram, M., Eren, A.M., Faust, K., Fuhrman, J.A., Lindahl, B., O'Hara, R.B., Öpik, M., Sogin, M.L., Unterseher, M., Tedersoo, L., 2016. Millions of reads, thousands of taxa: microbial community structure and associations analyzed via marker genes. FEMS Microbiol. Rev. 40, 686–700.
Bálint, M., Márton, O., Schatz, M., Düring, R.-A., Grossart, H.-P., 2018. Proper experimental design requires randomization/balancing of molecular ecology experiments. Ecol. Evol. 8, 1786–1793.
Beadle, L.C., 1981. The Inland Waters of Tropical Africa: An Introduction to Tropical Limnology. Longman.
Beauchamp, R.S.A., 1956. The electrical conductivity of the head-waters of the white Nile. Nature 178, 616–619.
Berke, M.A., Johnson, T.C., Werne, J.P., Livingstone, D.A., Grice, K., Schouten, S., Sinninghe Damsté, J.S., 2014. Characterization of the last deglacial transition in tropical East Africa: insights from Lake Albert. Palaeogeogr. Palaeoclimatol. Palaeoecol. 409, 1–8.
Beuning, K.R.M., Talbot, M.R., Kelts, K., 1997. A revised 30,000-year paleoclimatic and paleohydrologic history of Lake Albert, East Africa. Palaeogeogr. Palaeoclimatol. Palaeoecol. 136, 259–279.
Bininda-Emonds, O.R.P., 2021. 18S rRNA variability maps reveal three highly divergent, conserved motifs within Rotifera. BMC Ecol Evol 21, 118.
Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., Coissac, E., 2016. obitools: a unix-inspired software package for DNA metabarcoding. Mol. Ecol. Resour. 16, 176–182.
Brown, D.S., 1994. Freshwater Snails of Africa and Their Medical Importance. CRC Press.
Chomicki, K.M., Taylor, W.D., Brown, C.J.M., Dove, A., Bowen, G.S., Mohamed, M.N., 2022. Seasonal variation in the influence of environmental drivers on nearshore water quality along an urban northern Lake Ontario shoreline. J. Great Lakes Res. 48, 914–926.
Chorlton, S.D., 2024. Ten common issues with reference sequence databases and how to mitigate them. Front Bioinform 4, 1278228.
Collins, R.A., Bakker, J., Wangensteen, O.S., Soto, A.Z., Corrigan, L., Sims, D.W., Genner, M.J., Mariani, S., 2019. Non-specific amplification compromises environmental DNA metabarcoding with COI. Methods Ecol. Evol. 10, 1985–2001.
Cordier, T., Lanzén, A., Apothéloz-Perret-Gentil, L., Stoeck, T., Pawlowski, J., 2019. Embracing environmental genomics and machine learning for routine biomonitoring. Trends Microbiol. 27, 387–397.
Cotte, G., Soulignac, F., dos Santos Correia, F., Fallet, M., Ibelings, B.W., Barry, D.A., Vennemann, T.W., 2023. Controlling factors of phytoplankton distribution in the river–lake transition zone of a large lake. Aquat. Sci. 85, 37.
Cumberlidge, N., Clark, P.F., 2018. Albertine Rift Valley endemics: three new species of freshwater crabs (Brachyura: Potamoidea: Potamonautidae) from Uganda, the Democratic Republic of the Congo, and Rwanda. J. Nat. Hist. 52, 1637–1656.
Curry, C.J., Gibson, J.F., Shokralla, S., Hajibabaei, M., Baird, D.J., 2018. Identifying North American freshwater invertebrates using DNA barcodes: are existing COI sequence libraries fit for purpose? Freshw. Sci. 37, 178–189.
De Crop, W., Verschuren, D., 2021. Mixing regimes in the equatorial crater lakes of western Uganda. Limnologica 90, 125891. https://doi.org/10.1016/j.limno.2021.125891.
Doble, C.J., Hipperson, H., Salzburger, W., Horsburgh, G.J., Mwita, C., Murrell, D.J., Day, J.J., 2020. Testing the performance of environmental DNA metabarcoding for surveying highly diverse tropical fish communities: a case study from Lake Tanganyika. Environ. DNA 2, 24–41.
Drake, L.E., Cuff, J.P., Young, R.E., Marchbank, A., Chadwick, E.A., Symondson, W.O.C., 2022. An assessment of minimum sequence copy thresholds for identifying and reducing the prevalence of artefacts in dietary metabarcoding data. Methods Ecol. Evol. 13, 694–710.
Dusabe, M.C., Kalinda, C., Clewing, C., Hyangya, B.L., Van Bocxlaer, B., Albrecht, C., 2024. Environmental perturbations and anthropogenic disturbances determine mollusc biodiversity of Africa's explosive Lake Kivu. J. Great Lakes Res. 50, 102339.
Ericson, K., 2014. A Crude Awakening: The Relationship between Petroleum Exploration and Environmental Conservation in Western Uganda. Collection, Independent Study Project (ISP).
Evans, J.H., 1997. Spatial and seasonal distribution of phytoplankton in an African Rift Valley lake (L. Albert, Uganda, Zaire). Hydrobiologia 354, 1–16.
Froese, R., Pauly, D., 2024. FishBase. World Wide Web electronic publication [WWW Document]. https://fishbase.de/ (accessed 10.11.24).

Geller, J., Meyer, C., Parker, M., Hawk, H., 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Mol. Ecol. Resour. 13, 851–861.
Green, J., 1967. The distribution and variation of *Daphnia lumholtzi* (Crustacea: Cladocera) in relation to fish predation in Lake Albert. East Africa. J. Zool. 1987 (151), 181–197.
Green, J., 1971. Associations of Cladocera in the zooplankton of the lake sources of the White Nile. J. Zool. 1987 (165), 373–414.
Green, J., 2009. Nilotic Lakes of the Western rift. In: Dumont, H.J. (Ed.), The Nile: Origin, Environments, Limnology and Human Use. Springer, Netherlands, Dordrecht, pp. 263–286.
Guardiola, M., Uriz, M.J., Taberlet, P., Coissac, E., Wangensteen, O.S., Turon, X., 2015. Deep-Sea, deep-sequencing: Metabarcoding extracellular DNA from sediments of marine canyons. PloS One 10, e0139633.
Guignot, F., 1957. Haliplidae et Dytiscidae. Exploration hydrobiologique des lacs Kivu, Edouard et Albert 3, 131–169.
Guo, M., Yuan, C., Tao, L., Cai, Y., Zhang, W., 2022. Life barcoded by DNA barcodes. Conserv. Genet. Resour. 14, 351–365.
Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E.M., Troedsson, C., 2014. Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. PloS One 9, e87624.
Hewlett, C., North, R.L., Johansson, J., Vandergucht, D.M., Hudson, J.J., 2015. Contribution of shoreline erosion to nutrient loading of the Lake Diefenbaker reservoir, Saskatchewan. Canada. J. Great Lakes Res. 41, 110–117.
Hui, F.K.C., 2016. Boral - Bayesian ordination and regression analysis of multivariate abundance data in r. Methods Ecol. Evol. 7, 744–750.
Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.-J., Tappu, R., 2016. MEGAN Community edition - interactive exploration and analysis of large-scale microbiome sequencing data. PLoS Comput. Biol. 12, e1004957.
IPBES, 2019. Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES secretariat, Bonn, Germany.
Kiggundu, V., Egessa, R., Mwebaza-Ndawula, L., 2022. Zooplankton Occurrences and Abundance in the Lake Albert System, Uganda. https://doi.org/10.15468/WQ98M3.
Krehenwinkel, H., Wolf, M., Lim, J.Y., Rominger, A.J., Simison, W.B., Gillespie, R.G., 2017. Estimating and mitigating amplification bias in qualitative and quantitative arthropod metabarcoding. Sci. Rep. 7, 17668.
Lamb, P.D., Fonseca, V.G., Maxwell, D.L., Nnanatu, C.C., 2022. Systematic review and meta-analysis: Water type and temperature affect environmental DNA decay. Mol. Ecol. Resour. 22, 2494–2505. https://doi.org/10.1111/1755-0998.13627.
Lehman, J.T., Litt, A.H., Mugidde, R., Lehman, D.A., 1998. Nutrients and plankton biomass in the rift Lake sources of the White Nile: Lakes Albert and Edward. In: Lehman, J.T. (Ed.), Environmental Change and Response in East African Lakes. Springer, Netherlands, Dordrecht, pp. 157–172.
Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., Machida, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10, 34.
Leray, M., Knowlton, N., Ho, S.-L., Nguyen, B.N., Machida, R.J., 2019. GenBank is a reliable resource for 21st century biodiversity research. Proc. Natl. Acad. Sci. U. S. A. 116, 22651–22656.
Lewis, W.M., 1987. Tropical Limnology. Annu. Rev. Ecol. Syst. 18, 159–184.
Liang, D., McManus, G.B., Wang, Q., Sun, X., Liu, Z., Lin, S., Yang, Y., 2022. Genetic differentiation and phylogeography of rotifer Polyarthra dolichoptera and P. vulgaris populations between Southeastern China and eastern North America: high intercontinental differences. Ecol. Evol. 12, e8912.
Lindén, A., Mäntyniemi, S., 2011. Using the negative binomial distribution to model overdispersion in ecological count data. Ecology 92, 1414–1421.
Lopes-Lima, M., Prié, V., Camará, M., Ceríaco, L.M.P., Fernandes, V., Ferreira, S., Gonçalves, D.V., Lecoq, M., Martins, F.M.S., Parrinha, D., de Barros, A.R., Valentini, A., Veríssimo, J., Palma, L., Beja, P., 2024. Rapid eDNA survey reveals a unique biodiversity hotspot: the Corubal River, West Africa. BioScience 74, 405–412. https://doi.org/10.1093/biosci/biae036.
Lopez, Z.F., Cancellario, T., Fontaneto, D., Kamburska, L., Karimullah, K., Wallace, R.L., Walsh, E.J., Smolak, R., 2023. African Rotifer Records. https://doi.org/10.15468/VE5YGW.
Mächler, E., Walser, J.-C., Altermatt, F., 2021. Decision-making and best practices for taxonomy-free environmental DNA metabarcoding in biomonitoring using Hill numbers. Mol. Ecol. 30, 3326–3339.
MacIntyre, S., Flynn, K.M., Jellison, R., Romero, J.R., 1999. Boundary mixing and nutrient fluxes in Mono Lake. California. Limnol. Oceanogr. 44, 512–529.
Mandahl Barth, G., 1954. The freshwater mollusks of Uganda and adjacent territories. In: Annales du Musée Royal du Congo Belge, Sciences Zoologiques, 32, pp. 1–206.
Meglécz, E., 2023. COInr and mkCOInr: building and customizing a nonredundant barcoding reference database from BOLD and NCBI using a semi-automated pipeline. Mol. Ecol. Resour. 23, 933–945.
Morana, C., Borges, A.V., Deirmendjian, L., Okello, W., Sarmento, H., Descy, J.-P., Kimirei, I.A., Bouillon, S., 2023. Prevalence of autotrophy in non-humic African Lakes. Ecosystems 26, 627–642.
Nakiyende, H., Basooma, A., Ikwaput Nyeko, J., Okello, W., Rugadya, R., Albrecht, C., Lawrence, T., Van Steenberge, M., Smith, S., Muderhwa, N., Matunguru, J., Mulongaibalu, M., Ajode, M.Z., 2023. Limitations for informed decision making and better management of the transboundary Lake Albert fisheries resources. J. Great Lakes Res. 49, 102165.
Nhu, Y.D.T., Hoang, N.T., Lieu, P.K., Harada, H., Brion, N., Van Hieu, D., Van Hop, N., Olde Venterink, H., 2019. Effects of nutrient supply and nutrient ratio on diversity-productivity relationships of phytoplankton in the Cau Hai lagoon. Vietnam. Ecol. Evol. 9, 5950–5962.
Obertegger, U., Flaim, G., Fontaneto, D., 2014. Cryptic diversity within the rotifer *Polyarthra dolichoptera* along an altitudinal gradient. Freshw. Biol. 59, 2413–2427.
Odada, E.O., Olago, D.O., 2006. The East African Great Lakes: Limnology. Springer Science & Business Media, Palaeolimnology and Biodiversity.
Ovaskainen, O., Tikhonov, G., Norberg, A., Guillaume Blanchet, F., Duan, L., Dunson, D., Roslin, T., Abrego, N., 2017. How to make more out of community data? A conceptual framework and its implementation as models and software. Ecol. Lett. 20, 561–576.
R Core Team, 2024. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Rowel, C., Fred, B., Betson, M., Sousa-Figueiredo, J.C., Kabatereine, N.B., Stothard, J.R., 2015. Environmental epidemiology of intestinal schistosomiasis in Uganda: population dynamics of biomphalaria (gastropoda: planorbidae) in Lake Albert and Lake Victoria with observations on natural infections with digenetic trematodes. Biomed. Res. Int. 2015, 717261.
Salzburger, W., Van Bocxlaer, B., Cohen, A.S., 2014. Ecology and evolution of the African Great Lakes and their faunas. Annu. Rev. Ecol. Evol. Syst. 45, 519–545.
Schenekar, T., 2023. The current state of eDNA research in freshwater ecosystems: are we shifting from the developmental phase to standard application in biomonitoring? Hydrobiologia 850, 1263–1282.
Sengupta, M.E., Lynggaard, C., Mukaratirwa, S., Vennervald, B.J., Stensgaard, A.S., 2022. Environmental DNA in human and veterinary parasitology - current applications and future prospects for monitoring and control. Food Waterborne Parasitol 29, e00183.
Simpson, G.L., 2018. Modelling palaeoecological time series using generalised additive models. Front. Ecol. Evol. 6. https://doi.org/10.3389/fevo.2018.00149.
Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: For Biodiversity Research and Monitoring, Illustrated Edition. ed. Oxford Univ Pr.
Takahashi, M., Saccò, M., Kestel, J.H., Nester, G., Campbell, M.A., van der Heyde, M., Heydenrych, M.J., Juszkiewicz, D.J., Nevill, P., Dawkins, K.L., Bessey, C., Fernandes, K., Miller, H., Power, M., Mousavi-Derazmahalleh, M., Newton, J.P., White, N.E., Richards, Z.T., Allentoft, M.E., 2023. Aquatic environmental DNA: a review of the macro-organismal biomonitoring revolution. Sci. Total Environ. 873, 162322.
Talling, J.F., 1963. Origin of stratification in an African rift lake. Limnol. Oceanogr. 8, 68–78.
Talling, J., 1965. The photosynthetic activity of phytoplankton in East African lakes. Int. Rev. Hydrobiol. 50, 1–32.
Talling, J.F., 1969. The incidence of vertical mixing, and some biological and chemical consequences, in tropical African lakes. SIL Proc. 17, 998–1012. https://doi.org/10.1080/03680770.1968.11895946.
Tumusiime, J., Bhraunxs, N.V., Kagoro-Rugunda, G., Namirembe, D., Albrecht, C., Twongyirwe, R., Tolo, C.U., Jacobs, L., Huyse, T., 2024. Citizens can help to map putative transmission sites for snail-borne diseases. PLoS Negl. Trop. Dis. 18, e0012062.
Valkiūnas, G., Atkinson, C.T., Bensch, S., Sehgal, R.N.M., Ricklefs, R.E., 2008. Parasite misidentifications in GenBank: how to minimize their number? Trends Parasitol. 24, 247–248.
Verbeke, J., 1957. Recherches écologiques sur la faune des grands lacs de l'est du Congo belge. Bulletin de l'Institut royal des Sciences naturelles de Belgique: Résultats scientifiques de l'exploration hydrobiologique (1952-1954) des lacs Kivu, Edouard et Albert. - Bulletin van het Koninklijk Belgisch Instituut voor Natuurwetenschappen: Wetenschappelijke resultaten van exploratie hydrobiologische (1952-1954) meren van Kivu. Edward en Albert. III, 3–177.
Verheyen, E., Abila, R., Akoll, P., Albertson, C., Antunes, D., Banda, T., Bills, R., Bulirani, A., Manda, A.C., Cohen, A.S., Cunha-Saraiva, F., Derycke, S., Donohue, I., Du, M., Dudu, A.M., Egger, B., Fritzsche, K., Frommen, J.G., Gante, H.F., Genner, M. J., Härer, A., Hata, H., Irvine, K., Mwapu, P.I., de Bisthoven, L.J., Jungwirth, A., Kaleme, P., Katongo, C., Kéver, L., Koblmüller, S., Konings, A., Lamboj, A., Lemmel-Schaedelin, F., Schiaffino, G.M., Martens, K., Mulungula, P.M., Meyer, A., More, H. L., Musilova, Z., Bukinga, F.M., Muzumani, R., Ntakimazi, G., Okello, W., Phiri, H., Pialek, L., Plisnier, P.D., Raeymaekers, J.A.M., Rajkov, J., Rican, O., Roberts, R., Salzburger, W., Schoen, I., Sefc, K.M., Singh, P., Skelton, P., Snoeks, J., Schneider, K., Sturmbauer, C., Svardal, H., Svensson, O., Dowdall, J.T., Turner, G.F., Tyers, A., van Rijssel, J.C., Van Steenberge, M., Vanhove, M.P.M., Weber, A.-T., Weyl, O., Ziegelbecker, A., Zimmermann, H., 2016. Oil extraction imperils Africa's Great Lakes. Science 354, 561–562.
von der Heyden, S., 2023. Environmental DNA surveys of African biodiversity: state of knowledge, challenges, and opportunities. Environ. DNA 5, 12–17.
von der Heyden, S., Neef, G., Grevesse, T., Cwecwe, Y., Sado, T., Miya, M., Mosie, I., Creer, S., Skelton, P., von Brandis, R., 2023. Environmental DNA biomonitoring in biodiversity hotspots: a case study of fishes of the Okavango Delta. Environ. DNA 5, 1720–1731.
Wandera, S.B., Balirwa, J.S., 2010. Fish species diversity and relative abundance in Lake Albert—Uganda. Aquat. Ecosyst. Health Manag. 13, 284–293.
Wang, S.C., Liu, X., Liu, Y., Wang, H., 2018. Contrasting patterns of macroinvertebrates inshore vs. offshore in a plateau eutrophic lake: implications for lake management. Limnologica 70, 10–19.

Wang, Y., Naumann, U., Wright, S.T., Warton, D.I., 2012. mvabund– an R package for model-based analysis of multivariate abundance data. Methods Ecol. Evol. 3, 471–474.

Warton, D.I., Blanchet, F.G., O'Hara, R.B., Ovaskainen, O., Taskinen, S., Walker, S.C., Hui, F.K.C., 2015. So many variables: joint modeling in community ecology. Trends Ecol. Evol. 30, 766–779.

Wood, S.N., 2017. Generalized Additive Models: An Introduction with R, Second edition. CRC Press.

IV. APPENDIX

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