USE OF BIOINDICATORS AND BIOMARKERS TO ASSESS AQUATIC ENVIRONMENTAL CONTAMINATION IN WETLANDS OF LAKE TANA, ETHIOPIA

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ABSTRACT

Anthropogenic pressures on Ethiopian freshwater ecosystems are intensifying due to rapid population growth, industrialization, agriculture, urbanization, and sewage discharge, heightening the need for water quality and biodiversity monitoring. This study employed an integrated ecological approach across six wetlands and four seasons of Lake Tana, combining physicochemical analyses, macroinvertebrate/fish bioindicators, fish histopathology, and health indices to assess ecological health.

Seasonality strongly influenced trophic conditions and physicochemical properties. Spatial pollution gradients emerged: highest at Megech River Mouth, moderate at Avaj and Ras Abbay, and lowest at Wonjeta, Zewdie Girar, and Gumara River Mouth. Macroinvertebrate community structure reflected these gradients, aligning with nutrient and water quality shifts.

Discrepancies were detected between Carlson's Trophic State Index (TSI) and the Water Quality Index (WQI) in wetlands dominated by inorganic turbidity rather than algal biomass. While Carlson's TSI (prioritizing chlorophyll-a, nutrients, and transparency) misclassified some sites as eutrophic, the WQI, integrating broader physicochemical parameters, more accurately reflected ecosystem conditions, highlighting limitations of nutrient-centric indices in sediment-driven turbid systems.

Bioindicator responses were predictable: pollution-sensitive macroinvertebrates thrived in cleaner wetlands (e.g., Zewdie Girar), while tolerant taxa dominated degraded areas. Diversity indices confirmed higher ecological stability at Zewdie Girar, Avaj, Wonjeta, and Ras Abbay.

Fish assemblages similarly reflected water quality: *Oreochromis niloticus* and *Labeobarbus* spp. prevailed in less polluted zones, whereas *Clarias gariepinus* dominated impacted sites. Fishing pressure reduced diversity at Wonjeta, Avaj, and Ras Abbay, while undisturbed Megech, Zewdie Girar, and Gumara River Mouths maintained richer communities.

Histopathology of *Labeobarbus* spp. revealed pollution-linked tissue damage most severe at Megech and Gumara River Mouths, moderate at Ras Abbay, Wonjeta, and Avaj, and minimal at Zewdie Girar. Fish health indices corroborated these stress gradients.

This work underscores the value of incorporating seasonal dynamics and biological indicators particularly macroinvertebrate and fish health assessments for holistic freshwater monitoring. The integrated approach provides critical insights for sustainably managing Ethiopia’s aquatic resources.

DEDICATION

To my father, Mazengia Yimam Ferede

Thank you for giving me a strong educational foundation

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CHAPTER 1

General Introduction

1.1. Overview and rationale

Freshwater ecosystems are among the most vulnerable and critically endangered on Earth, experiencing escalating stress due to anthropogenic activities. The main drivers of ecological degradation, including pollution, overfishing, and habitat destruction, have resulted in declines in water quality, biodiversity loss, and disruptions to aquatic life (Arthington et al., 2016; Wada et al., 2016; Freeman et al., 2019). These pressures are particularly acute in developing nations, where rapid population growth, industrialization, urbanization, and intensified agriculture exacerbate existing environmental challenges (Davis et al., 2015; Hasan et al., 2015; Zhang et al., 2018). Freshwater ecosystems, which harbor approximately 10% of all animal species globally, are increasingly at risk, threatening vital ecosystem services such as water purification, food production, and carbon regulation (Alves et al., 2024). Given the critical role these ecosystems play in global biodiversity and human welfare, monitoring and protecting ecosystems has become an urgent priority (Arthington et al., 2016; Wada et al., 2016; Reid et al., 2019; Bashir et al., 2020; Gatti et al., 2020).

Effective monitoring of freshwater ecosystems involves a variety of methods to assess the health and functionality of the wetlands. Physicochemical assessments, which measure parameters such as temperature, pH, dissolved oxygen, and nutrient concentrations, are commonly used to evaluate water quality and detect pollution (Chapman, 2021). However, these methods may fail to detect subtle ecological changes that can precede noticeable shifts in water quality. In contrast, biomonitoring, which assesses the health of organisms within the ecosystem (such as fish, macroinvertebrates, and phytoplankton), provides deeper insights into ecosystem dynamics and resilience (Barton, 2002; Van Der Putten et al., 2009). By integrating physical, chemical, and biological assessments, researchers gain a more holistic understanding of how human activities impact freshwater ecosystems, which is crucial for developing effective conservation and management strategies (Blazer et al., 2000; Debels et al., 2005; Patil et al., 2012).

The Lake Tana basin, located in the Amhara region of Ethiopia, is the second-largest sub-basin of the Blue Nile River and holds significant ecological, hydrological, and socio-economic importance. The basin provides water for agriculture, industry, and domestic consumption for millions of people (McCartney et al., 2010; Goshu & Aynalem, 2017). Additionally, its waters contribute to the livelihoods of communities in downstream countries, including Sudan, South Sudan, and Egypt, underscoring its transboundary significance (Wale et al., 2013; Mohammed & Mengist, 2018). Despite its importance, the basin faces significant challenges due to the lack of a comprehensive water quality monitoring system, which impedes effective resource management and conservation (Goshu & Aynalem, 2017; Mucheye et al., 2018). Recent studies indicate that shifts in nutrient loads have led to mesotrophic and eutrophic conditions, which have reduced water clarity, altered aquatic habitats, and disrupted biodiversity (Wondie et al., 2007; McCartney et al., 2010; Goshu & Aynalem, 2017; Mucheye et al., 2018; Abegaz et al., 2023).

Human activities, including deforestation, agricultural runoff, and overfishing, have further compounded these environmental stresses. Deforestation in the Lake Tana catchment area accelerates soil erosion, which increases sedimentation and turbidity in the lake, negatively impacting aquatic productivity and water quality (Minale & Rao, 2012; Goshu & Aynalem, 2017). The use of chemical fertilizers and pesticides in agriculture has led to nutrient loading and contamination, disrupting food webs and harming aquatic life (Minale & Rao, 2012; Abebe & Minale, 2017). Overfishing, particularly the depletion of predator species, has destabilized trophic dynamics, diminishing biodiversity, and ecosystem stability (Wana, 2016; Assefa et al., 2019). The spread of invasive species, notably water hyacinth (*Eichhornia crassipes*), has exacerbated these problems. Fueled by nutrient enrichment, the invasive plant has proliferated across the lake, particularly in the northern regions, displacing native vegetation, disrupting fish habitats, and negatively impacting local fisheries (Tewabe, 2015; Gezie et al., 2018). The resulting ecological degradation poses significant risks to the livelihoods of those dependent on the lake for food and income.

A lack of an integrated pollution monitoring system exacerbates these issues, leaving gaps in our understanding of pollution sources and the ecological impacts of pollution sources. Existing methods, such as simple inventory-based approaches, are insufficient for capturing the complex and dynamic nature of pollution in the basin (Goshu & Aynalem, 2017; Tibebe et al., 2019). A more comprehensive monitoring strategy, combining physical, chemical, and biological assessments, is essential for addressing the full spectrum of environmental degradation in the Lake Tana basin. This integrated approach would facilitate the identification of pollution sources, track changes in water quality over time, and provide a more accurate understanding of the impacts of human activities on aquatic ecosystems (Humphrey et al., 1990; Enyew et al., 2020; Gezie et al., 2020; Yoder & Smith, 2020).

The implementation of an integrated monitoring system is vital for tracking long-term ecological changes and detecting emerging environmental threats. Such a system would allow for proactive conservation measures and the timely identification of factors contributing to ecological decline (Humphrey et al., 1990; Martin, 2012; Yoder & Smith, 2020). Biological monitoring, in particular, can offer early warning signals of ecological shifts that may be undetectable through traditional chemical assessments, enabling more effective intervention before irreversible damage occurs (Yoder & Smith, 2020). Moreover, an integrated monitoring system would help inform the sustainable management of the region's natural resources, which are critical to local economies reliant on agriculture, fishing, and tourism (Humphrey et al., 1990; Swain, 1997; Heide, 2012; Yoder & Smith, 2020).

Beyond improving monitoring efforts, addressing the root causes of environmental degradation is paramount. Implementing sustainable agricultural practices that minimize the use of harmful fertilizers and pesticides, alongside soil conservation strategies to prevent erosion, would significantly reduce anthropogenic pressures on the basin (Tilman et al., 2002; Pauly et al., 2002; Pimentel, 2006). Fisheries management should focus on maintaining ecological balance by regulating fishing pressures and protecting key species (Pauly et al., 2002). In addition, urgent efforts are needed to control the spread of invasive species, especially water hyacinth, which has become a major threat to biodiversity and ecosystem services in the lake (Tewabe, 2015; Gezie et al., 2018). By addressing these multifaceted challenges, the ecological health of Lake Tana can be preserved, ensuring it continues to provide essential services to local communities and contribute to the livelihoods of millions (Wondie et al., 2007b; Ayalew et al., 2023).

1.2. Pollution factors in the Lake Tana

Water resources of Ethiopia face significant challenges due to both natural and anthropogenic factors. Natural influences, such as climatic variability, seasonal rainfall fluctuations, and hydrological dynamics, complicate water management and impact both availability and quality across the country (Berhanu et al., 2014; Wassie, 2020; Burnside et al., 2021; Taye et al., 2024). Simultaneously, human-induced pressures, including deforestation, agricultural expansion, urbanization, and land-use changes, exacerbate these challenges, contributing to water scarcity and contamination (Wassie, 2020; Burnside et al., 2021). Additionally, extreme weather events driven by climate change, such as droughts and floods, further complicate resource management, requiring adaptive, integrated strategies to safeguard both environmental and human health (Wassie, 2020; Burnside et al., 2021; Taye et al., 2024).

Lake Tana, Ethiopia's largest freshwater lake, is experiencing severe environmental degradation, primarily due to sedimentation and pollution. Sediment accumulation from soil erosion, caused by deforestation, agricultural expansion, and ineffective land management in the surroundings leads to increased turbidity and nutrient loading, accelerating eutrophication (Abate et al., 2017; Goshu & Aynalem, 2017). This nutrient overload promotes the growth of aquatic plants and phytoplankton, resulting in harmful cyanobacterial blooms, especially in nearshore areas and at river mouths where nutrient inputs are highest (Goshu et al., 2010a; Akale et al., 2017). These blooms, often dominated by toxigenic cyanobacteria, degrade water quality by depleting oxygen levels and producing toxins harmful to aquatic life and human health (Goshuet al., 2010a; Moges et al., 2017).

Pollution from human settlements and agricultural runoff further exacerbates these problems. Fecal contamination from domestic waste and nutrient runoff from fertilizers and pesticides contribute to the eutrophication process, reducing water clarity and harming aquatic habitats and biodiversity (Moges et al., 2017; Wang et al., 2019). The loss of wetlands around Lake Tana, driven by land conversion and encroachment, further degrades water quality by reducing natural filtration and buffering capacity (Moges et al., 2017).

To address these environmental challenges, effective and comprehensive water quality monitoring is essential. Important indicators such as nutrient concentrations (nitrogen, phosphorus), turbidity, dissolved oxygen, pH, and the presence of harmful cyanobacteria should be regularly monitored to identify pollution sources, track changes, and inform adaptive management strategies (Goshu & Aynalem, 2017; Gebremedhin et al., 2018). A combination of physicochemical and biological monitoring methods is necessary, as chemical data alone may not fully capture the impacts of biological contaminants such as cyanobacteria (Blazer et al., 2000; Yoder & Smith, 2020). In addition to monitoring, sustainable land management practices are critical. Erosion control, soil conservation, and reducing agricultural runoff through improved farming techniques are essential to mitigate sedimentation and nutrient pollution (Tilman et al., 2002; Pimentel, 2006).

Addressing the root causes of pollution requires a holistic, multi-stakeholder approach. Integrating local knowledge with scientific research and engaging communities in resource management can help tailor solutions to the region's specific needs. Strategies should prioritize sustainable land use practices such as agroforestry, conservation agriculture, and integrated watershed management to reduce soil erosion and nutrient runoff into the lake (Goshu & Aynalem, 2017; Eneyew & Assefa, 2021). Additionally, controlling urbanization pressures, promoting efficient agricultural practices, and managing invasive species such as water hyacinth are crucial for improving water quality and ecosystem resilience (Tewabe, 2015; Gezie et al., 2018).

Addressing sedimentation and pollution in Lake Tana is crucial for maintaining its ecological health and supporting the socio-economic development of surrounding communities. Reliable monitoring systems targeted pollution control measures, and sustainable resource management practices are essential to preserving the lake's vital ecosystem services, including freshwater supply, agriculture, hydropower generation, and biodiversity conservation. Through implementing integrated management strategies, Ethiopia can enhance the resilience of its water resources, safeguarding Lake Tana for future generations and improving the well-being of local communities.

1.3. Impacts of pollution in the Lake Tana catchments

The escalating consequences of human activities around Lake Tana are severely impacting its ecosystem. The lake, once a relatively pristine body of water, is now suffering from extensive habitat destruction and water pollution, both of which are undermining its ecological integrity (Gebremedhin et al., 2018; Gezie et al., 2020; Getachew et al., 2021). Increased urbanization, agricultural expansion, and industrialization around the lake have introduced significant pressures on its environment, leading to the deterioration of water quality and biodiversity (Moges et al., 2017; Dersseh et al., 2020). The growing human population and associated waste production are overwhelming the lake's natural ability to assimilate pollutants, exacerbating environmental degradation (Gebresllassie et al., 2014; Dejen et al., 2017). The introduction of pollutants, coupled with the loss of natural habitats, is disrupting the delicate balance of the lake's ecosystem, threatening biodiversity and ecosystem services (Gebremedhin et al., 2018; Enyew et al., 2020; Getachew et al., 2021). Historically, the lake's ecosystem could cope with the relatively low levels of pollution generated by a small, scattered population. However, as the region's population continues to grow, alongside the expansion of agricultural and industrial activities, the pollution levels have risen dramatically. These anthropogenic pressures now exceed the lake's natural ability to self-purify, resulting in significant ecological degradation (Vijverberg et al., 2009; Gebresllassie et al., 2014; Dejen et al., 2017).

The primary sources of pollution surrounding Lake Tana are urban runoff and agricultural activities, which collectively contribute a variety of harmful substances to the lake. These include untreated sewage, industrial chemicals, heavy metals, pesticides, and sediment from agricultural runoff (Gebresllassie et al., 2014; Dejen et al., 2017; Goshu et al., 2017). The seasonal nature of the region's rainfall further exacerbates the pollution load. During the rainy season, a large volume of water runoff carries these pollutants from agricultural lands and urban areas directly into the lake. In contrast, the dry season results in reduced water flow, causing the pollutants to become more concentrated, which compounds the ecological impacts on the lake's ecosystem (Wondie, 2010; Khatri & Tyagi, 2015; Engdaw et al., 2022). One of the most critical consequences of this pollution is eutrophication, which occurs when excess nutrients, particularly nitrogen and phosphorus from fertilizers and organic waste, enter the water. These nutrients stimulate the growth of harmful algal blooms that deplete oxygen levels in the water, disrupt aquatic life, and further degrade water quality (Attrill & Depledge, 1997; Ayele & Atlabachew, 2021; Mushi et al., 2021).

Eutrophication has become a significant concern in Lake Tana, as it is directly linked to a host of ecological and economic problems. The enrichment of the lake with nutrients not only reduces water quality but also harms aquatic species. Excessive nutrient levels deplete oxygen, which is essential for the survival of fish and other aquatic organisms, while simultaneously increasing the proliferation of harmful algae, which can be toxic (Dejen et al., 2017; Kassa & Tibebe, 2019). Additionally, the sedimentation resulting from agricultural runoff has created further challenges for the lake's aquatic life. Fine sediments reduce water clarity and suffocate bottom-dwelling organisms, impairing fish respiration and reducing plant productivity, which in turn affects the entire food web (Agumassie, 2019; Damtie et al., 2021). These ecological stresses have led to a marked decline in fish populations, which are critical for local livelihoods, as well as a decrease in biodiversity (Dejen et al., 2017; Kassa & Tibebe, 2019). Furthermore, pollution from nearby urban centers such as Bahir Dar and Gondar, where sanitation systems are often inadequate, has led to the contamination of the lake with harmful bacteria and pathogens. This not only increases the risk of waterborne diseases but also poses a significant public health threat to both humans and animals relying on the lake's water (Wondie et al., 2007b; Wondim et al., 2016; Zelalem & Prokin, 2017).

As pollution continues to accumulate, the quality of water in Lake Tana is deteriorating rapidly, rendering it increasingly unsafe for consumption. The rising levels of nutrients and pathogenic contaminants make the water unsuitable for direct human use, thus requiring costly treatment processes to purify it (Tessema et al., 2021; Dersseh et al., 2022). However, Ethiopia faces significant challenges in mitigating these pollution issues due to a lack of sufficient resources for monitoring water quality and implementing effective pollution control measures (Ademe, 2014; Agumassie, 2019; Ayele & Atlabachew, 2021). The inadequacy of current monitoring systems has hindered the country's ability to track changes in water quality, particularly in less-polluted areas where intervention may still be feasible. Traditional chemical monitoring methods are expensive and often exceed the available budgets for water management programs, making regular water quality assessment a daunting task (Bartell, 2006). As a result, alternative methods, such as biomonitoring, which relies on the use of biological indicators to assess the health of aquatic ecosystems, are gaining traction. These techniques offer a more cost-effective approach to monitoring the state of the lake's water quality and could play a vital role in the long-term management and conservation of Lake Tana's ecosystem (Wepener, 2008; Masese et al., 2013; Plisnier et al., 2022). By using the diversity of living organisms, such as fish, aquatic macroinvertebrates, and algae, researchers can gain valuable insights into the ecological health of the lake, providing a clearer picture of the environmental impacts and the necessary interventions required to restore its health (Wepener, 2008; Masese et al., 2013; Plisnier et al., 2022). Biomonitoring, which relies on the presence and abundance of species, offers an effective and cost-efficient tool for assessing water quality and ecosystem health, particularly in resource-limited settings like Lake Tana (Masese et al., 2013; Plisnier et al., 2022). The rapid expansion of human activities around Lake Tana is having devastating effects on its ecosystem, from pollution and habitat loss to the degradation of water quality. While the challenges are significant, concerted efforts to improve pollution control, enhance monitoring capabilities, and implement sustainable practices in both urban and rural areas are crucial for the long-term preservation of the lake and the well-being of the communities that depend on it.

1.4. Monitoring of water resources in Ethiopia

Ethiopia, like many countries, traditionally monitored water quality by measuring its physical and chemical properties (Wondie, 2010; Lakew & Moog, 2015; Wondim et al., 2016; Goshu et al., 2017; Krylov et al., 2020; Eneyew & Assefa, 2021). However, these methods have limitations, as physical and chemical methods capture only the environmental condition at the time of sampling and fail to account for intermittent human-caused disruptions. Bioaccumulation monitoring, which measures contaminants in organisms, is similarly limited because it only identifies specific pollutants and does not address interactions between chemicals, such as synergistic or antagonistic reactions. Consequently, traditional methods do not provide a comprehensive understanding of complex ecosystems or how multiple stressors affect the health of aquatic systems.

To overcome these limitations, environmental monitoring programs should incorporate a range of chemical, physical, and biological indicators, as each can track different aspects of environmental stress, including xenobiotics (stressors), biomarkers, and bioindicators (Bartell, 2006; Xenopoulos & Lodge, 2006; de Lima Cardoso et al., 2018; Lomartire et al., 2021). Biological assessments are quicker and more cost-effective than chemical analyses, and bioindicators offer the advantage of responding to all toxicants physical and chemical methods encounter, providing insights into chronic effects from intermittent pollution (Xenopoulos & Lodge, 2006; Naigaga, 2012; Shimba & Jonah, 2016).

Biomonitoring uses the biological responses of selected organisms (bioindicators) to detect and track environmental changes, making it a valuable tool for monitoring water quality, stream habitat, and adjacent watershed changes (Wepener, 2008; López-López & Sedeño-Díaz, 2015a; Tanaka et al., 2016; Manzoor et al., 2021). Common bioindicators include diatoms, and benthic macroinvertebrates (Czerniawska-Kusza, 2005; Flores & Zafaralla, 2012; Dar et al., 2021; Getnet et al., 2022).

In Ethiopia, water quality is typically assessed using macroinvertebrate evaluations, fecal coliform tests, and physicochemical parameter analysis (Goshu et al., 2020; Zelalem & Prokin, 2017). However, integrating bioindicators or biomarkers is essential for tracking long-term environmental changes and improving environmental management. The bioindicator-biomarker approach is particularly valuable in emerging nations, where it provides a cost-effective method to monitor water quality and ecosystem health over time (Bartell, 2006; Borisko et al., 2007; Naigaga, 2012; Shimba & Jonah, 2016; Lopes, 2021). By analyzing the responses of organisms, bioindicator-biomarkers offer a reliable and efficient means of assessing environmental conditions and guiding effective management practices (Borisko et al., 2007; Shimba & Jonah, 2016).

1.5. The bioindicator–biomarker approach of aquatic environmental assessment

The bioindicator-biomarker approach offers a powerful and integrated tool for monitoring water quality in aquatic environments, providing a more comprehensive assessment than traditional chemical analysis. Bioindicators, which are organisms sensitive to environmental changes, serve as a reflection of ecosystem health (Ogidi & Akpan, 2022; Pandey & Dhuria, 2023). The presence, or absence of bioindicators, or abundance can indicate pollution levels and broader environmental conditions, offering valuable insights into the health of aquatic ecosystems (Gouda et al., 2024). By using bioindicators, researchers can gain early detection of environmental stressors that may not be immediately visible through chemical analysis alone, allowing for proactive management of water quality (Kassa & Tibebe, 2019; Lomartire et al., 2021). The presence of bioindicators, absence, or abundance can indicate pollution levels and broader environmental conditions (Kadim & Risjani, 2022; Salunke et al., 2024) Biomarkers, on the other hand, refer to measurable biological responses within organisms exposed to pollutants, such as changes in molecular, biochemical, or physiological processes (Kadim & Risjani, 2022). By combining these two approaches, researchers can gain insights into both immediate and long-term environmental stressors. This holistic method goes beyond the static snapshots offered by chemical analyses, revealing the impacts of pollutants on individual organisms and ecosystems (Lomartire et al., 2021; Ndiritu et al., 2021; Calisi, 2023). Furthermore, bioindicators and biomarkers can act as early warning systems, detecting subtle environmental stress before it leads to severe damage, which is particularly crucial for maintaining aquatic biodiversity in the face of complex pollution mixtures (Zelalem & Prokin, 2017; Carere et al., 2021).

Traditional methods for detecting water pollution, such as chemical analysis, often fail to provide a complete picture of environmental health due to the high cost of traditional methods, labor-intensive nature, and limited capacity to track long-term biological impacts (Wondim et al., 2016; Getnet et al., 2022). These methods are especially impractical for routine monitoring in developing regions like Ethiopia, where financial constraints and limited infrastructure hinder consistent data collection (Bartell, 2006; Wepener, 2008). For example, in Lake Tana, water quality data collection is project-based and inconsistent, making it difficult to assess pollution trends or identify areas in need of urgent intervention (Goshu et al., 2010.; Karlberg et al., 2015). As a result, the absence of reliable, continuous monitoring hampers effective policy-making and the implementation of water management strategies aimed at mitigating pollution (Dejen et al., 2017). By integrating bioindicators and biomarkers into monitoring efforts, it becomes possible to gather more real-time, actionable data that can inform environmental policies and management practices, especially in regions with limited resources (Naigaga, 2012; Kassa & Tibebe, 2019).

In the case of Lake Tana, the complexity of pollution, driven by various human activities such as waste disposal and agricultural runoff, further complicates water quality monitoring (Wondim et al., 2016; Gezie et al., 2018). Traditional chemical analyses often fail to account for the cumulative effects of multiple pollutants, especially in environments like Lake Tana, where pollution sources are diverse and not well understood (Zelalem & Prokin, 2017). This makes bioindicators and biomarkers essential for identifying the impacts of poorly characterized or emerging pollutants. Studies have shown that bioindicators, like certain fish species and aquatic macroinvertebrates, can reveal changes in water quality that are not immediately apparent through chemical testing alone (Parmar et al., 2016a; Carere et al., 2021). Biomarkers, including those measuring oxidative stress or genotoxicity, provide further insights into how pollutants accumulate in organisms and affect the physiological functions of organisms (Lomartire et al., 2021; Ogidi & Akpan, 2022). By focusing on both the organismal level and the broader ecological impacts, this integrated approach offers a more dynamic and detailed understanding of aquatic health, which is crucial for sustainable water management in Lake Tana and similar ecosystems (Lomartire et al., 2021).

Due to the escalating threats from human activity, including unsustainable agricultural practices and waste disposal, the ecological health of Lake Tana is under significant pressure (Wondim et al., 2016; Gezie et al., 2018). These anthropogenic stressors lead to water contamination, loss of biodiversity, and the degradation of ecosystem services, which in turn threaten the livelihoods of local communities who depend on the lake's resources (Goshu et al., 2010). Effective management of such a complex ecosystem requires a more thorough understanding of both the direct and indirect effects of pollution. Using bioindicators and biomarkers, researchers can better assess how pollutants impact aquatic life at multiple levels from molecular damage to population declines offering an integrated method for understanding ecosystem resilience (Lomartire et al., 2021; Vaseashta et al., 2021; Calisi, 2023). Moreover, this approach can be adapted for use in areas with limited access to expensive analytical technologies, offering a practical and scalable solution for real-time water quality monitoring in developing countries (Naigaga, 2012; Kassa & Tibebe, 2019).

The bioindicator-biomarker approach represents a promising solution for monitoring and managing water quality in complex aquatic environments like Lake Tana. It not only addresses the shortcomings of traditional methods but also provides valuable early warning signals, making it a vital tool for managing aquatic ecosystems under anthropogenic stress. By incorporating this integrated approach into regular monitoring practices, stakeholders can obtain more accurate and timely data, which will be essential for crafting effective policies aimed at reducing pollution and ensuring the long-term health of aquatic environments (Wepener, 2008; Ogidi & Akpan, 2022; Pandey & Dhuria, 2023; Gouda et al., 2024).

1.6. Aim and objectives of the study

The study aimed to measure the degradation of water quality in certain wetlands in the Lake Tana watershed by integrating fish and macroinvertebrates as bioindicators and fish histology health assessment as a biomarker. To do this, a set of endpoints from several wetlands affected by effluents were compared. (1) Physicochemical parameters; (2) fish bioindicators; (3) water column and benthic macroinvertebrate bioindicators; (4) histopathology biomarkers; and (5) fish health evaluation and inverted parasite indices. Particular goals were to;

(1) characterize wetlands based on physicochemical properties and underlying factors that may have influenced physicochemical variables in the study sites,
(2) assess the appropriateness of macroinvertebrates and fish as biological indicators of water quality deterioration in selected Lake Tana basin wetlands,
(3) investigate fish histopathology as a biomarker of water quality deterioration in selected Lake Tana basin wetlands and,
(4) investigate the fish health assessment index as a bioindicator of water quality deterioration in selected Lake Tana basin wetlands.

1.7. Thesis outline

This thesis explores the use of fish and macroinvertebrate biological indicators (bioindicators) and fish health and histopathology biological markers (biomarkers) for assessing the health of the lake to understand how these methods contribute and compare to describing the environmental conditions in this lake. The context for this study is that efforts towards the prevention of pollution impacts demand efficient and reliable mechanisms of detection, especially for systems contamination.

Chapter One articulates the study concept and rationale, highlights gaps in knowledge regarding the problems associated with the pollution of aquatic resources in Lake Tana, underscores the limitations in the current water quality assessment methods in the country and the need to develop a biological monitoring system of water quality assessment for water bodies in Ethiopia. The chapter gives details on the pollution drivers in the catchments, important types of pollution, and the challenges for pollution management in the Lake Tana wetlands and discusses the need for management intervention. The different impacts of pollution on water quality and aquatic biodiversity are addressed. Key terminologies of biomonitoring, bioindicators, and biomarkers are defined; and the application and advantages of biological monitoring in aquatic environmental assessment are reviewed.

Chapter Two describes the general methods used in data collection and laboratory analysis. Specific details of data processing and interpretation are given in the respective chapters. The chapter describes the six variably impacted wetland ecotones studied along the shoreline of Lake Tana. The chapter also provides a detailed research design. Data for this study were collected at four sampling times, from March 2020 to November 2020. Sampling was conducted in four seasons (dry, early rainy, rainy, and late rainy) in the six wetlands, making a total of 24 samples. This was done to capture changes in temporal variability. Samples included physicochemical variables, fish and macroinvertebrates, and fish tissues for histopathology assessment and fish health assessment. Physicochemical variables were sampled because the physical and chemical variables represent water quality assessment, while fish and macroinvertebrates were sampled because the two faunae are impacted differently by water quality changes. Fish histopathology and fish health assessments were studied because fish cellular structure is sensitive to water quality and represents impacts occurring over time. This chapter also highlights important statistics that were employed in the investigation.

Chapter Three is an account of the physicochemical conditions of the study sites. Physical and chemical conditions gave insight into the aquatic environmental quality of the different study sites and sampling seasons. Based on this information it could be tested to what extent these conditions influenced macroinvertebrate and fish species diversity, fish histopathology, and fish health, all aspects examined in the later chapters. Physicochemical parameters including temperature, oxygen, electrical conductivity, pH, Secchi disk depth a.m., Secchi disk depth p.m., water depth, total dissolved solids, salinity, nitrite, nitrate, soluble reactive phosphorous, ammonia, total nitrogen, total phosphorus, total nitrogen: total phosphorus ratio, and chlorophyll a. These variables were sampled because physical and chemical variables can be used to determine water quality deterioration. Descriptive statistics, including means, standard deviations, and ranges, were calculated for each parameter. The normality of the data for each variable was assessed using the Kolmogorov-Smirnov (K-S) and Lilliefors tests. Since all variables followed a normal distribution ($p > 0.05$), analysis of variance (ANOVA) was used to compare water quality variables across different sampling locations. When a significant interaction between wetland type and season was found, post hoc comparisons of means within the main effects were conducted using Tukey's test. Carlson's Trophic State Index (Carlson, 1977) using total nitrogen, total phosphorus, chlorophyll a, and Secchi disk depth was calculated and used to rank and classify the study sites according to the eutrophic status of study sites. WQI values were calculated for each study location and wetland and were ranked according to Rubio-Arias et al. (2012), Tyagi et al. (2013), and Uddin et al. (2021). Four statistical methods including cluster analysis (CA), principal component analysis (PCA), and factor analysis (FA) were used to analyze water quality data from different locations. These methods helped to understand how water quality varies across the study wetlands and to group similar sites based on water quality conditions of wetlands and level of pollution.

Chapter Four addresses Objective 2 of the study, which focuses on evaluating macroinvertebrates as indicators of water quality deterioration in six wetlands of Lake Tana. A total of 83 macroinvertebrate taxa from 52 families were identified. Community structure was assessed using diversity indices, including Shannon-Wiener, Simpson, Margalef, and Menhinick. To evaluate pollution gradients, biotic indices such as the Hilsenhoff Family Biotic Index (HFBI), Biological Monitoring Working Party (BMWP), and EPT index were applied. Three multivariate statistical techniques that included cluster analysis (CA), principal component analysis (PCA), and factor analysis (FA) were used to show the dimensional distribution of sites based on macroinvertebrate composition. Five different diversity indices including the Shannon-Wiener diversity index, Simpson’s diversity index, Margalef’s richness index, Hill’s index, and Menhinick’s diversity index were used to evaluate the effects of organic contamination on the demographic structure of macroinvertebrates in six wetlands of Lake Tana. To assess the health of the aquatic environment of these wetlands four biological indices including the Hilsenhoff index, biological monitoring working party, the average score per taxon, and the EPT index were used. These indices measure the quality of the water based on the types of aquatic organisms present. Hill-Shannon, Hill-Shannon indices, and coverage were used to estimate species richness. Canonical correspondence analysis (CCA) was employed to evaluate the relationship between macroinvertebrate communities and water quality variables using a paleontological statistics software package for data analysis **(**PAST) software (version 4.14).

Chapter Five examines the health of the chosen wetlands using fish as indicators of water quality. The chapter discusses the importance of fish in water quality assessment, the response of fish species to pollution, and the relationship between environmental physicochemical conditions and fish species diversities. Four multivariate techniques including cluster analysis (CA), principal component analysis (PCA), factor analysis (FA), and canonical correspondence analysis (CCA) were used to show the dimensional distribution of wetlands based on fish species composition. Cluster analysis was conducted using presence-absence data of fish species across the six wetlands. This method was selected to emphasize community similarity based on species occurrence, as abundance data can be disproportionately influenced by dominant taxa. The analysis employed Ward’s method with Euclidean distance to classify wetlands according to their fish community composition. Four diversity indices include the Shannon-Wiener diversity index, Simpson’s diversity index, richness, Margalef’s richness, and Menhinick’s diversity indices used to compare the impacts of contamination on the demographic structure of fish species in six wetlands of Lake. The habitat quality index was used to compare wetlands according to Masese et al. (2013). The Fish Index of Biotic Integrity (FIBI) was applied to compare wetlands based on Karr (1986), Raburu & Masese (2012), Bassa et al. (2020), and Gonino et al. (2020).

Chapter Six investigates fish histopathology related to agriculture and urban effluent exposure in the study wetlands, to explore fish histopathology as a biomarker of water quality deterioration in six wetlands of Lake Tana. Histological alterations in the liver, gills, gonads, and spleen of

Labeobarbus spp. from six variably impacted wetlands were assessed. This chapter presents the histopathological assessment of *Labeobarbus* spp. across six wetlands in the Lake Tana basin, revealing clear spatial and seasonal patterns in tissue-level responses to pollution. The most severe histological alterations were observed in fish from Megech River Mouth (MRM), Gumara River Mouth (GRM), and Avaj (AV), where regressive, inflammatory, and progressive lesions were most pronounced in gill and liver tissues. In contrast, Zewdie Girar (ZG) and Wonjeta (WO) exhibited the lowest organ index scores, indicating minimal tissue damage and better ecological conditions. The dominant reaction types varied by organ and wetland, with gills showing the highest sensitivity to environmental stress. These findings support the use of histopathological biomarkers as reliable indicators of aquatic ecosystem health and provide a foundation for classifying wetland pollution status based on tissue-level responses. The study used statistical methods to analyze how location and season affected the histopathology of organs in six wetlands. As most data were not normally distributed, a non-parametric Kruskal-Wallis test was used to compare medians. The results were categorized based on the histopathology of the organs and lesion severity grades, and the prevalence of fish in each group was calculated for each wetland. The prevalence of lesions was compared among wetlands and seasons. The study wetlands were evaluated and compared in terms of the fish histopathology indices and lesion prevalence according to a modified protocol after Bernet et al. (1999).

Chapter Seven discusses the investigation of fish health and inverted parasite indices for various effluent exposures in the study wetlands, to explore the use of the fish health assessment index as a biomarker of water quality deterioration in six wetlands of Lake Tana. This study followed a structured, seasonally stratified sampling design to assess fish health and parasite indices across six wetlands. Field sampling was conducted during four distinct seasons (dry, early rainy, rainy, and late rainy) between March and November 2020. At each wetland, fish were captured using gillnets of various mesh sizes. Immediately after capture, fish were examined for external anomalies and measured for length and weight. Blood samples were collected for hematocrit analysis, followed by necropsy-based assessments of internal organ anomalies. Parasite loads were evaluated using inverted parasite indices. All data were recorded in the field and later analyzed using statistical methods to compare health indices across wetlands and seasons. This sequential approach ensured consistency and allowed for robust comparisons of fish health in relation to effluent exposure. The study wetlands were evaluated and compared in terms of the fish health assessment and inverted parasite indices and anomaly prevalence, according to a modified protocol after Adams et al. (1993). Additionally, the study used statistical methods to analyze the length and weight of fish in different wetlands across four seasons. Based on the Kolmogorov-Smirnov (K-S) and Lilliefors tests for normality, data on body weight, length, and fish health indices showed non-normal distribution.

Chapter Eight provides a comprehensive synthesis of the ecological implications of pollution by integrating biomarker data specifically fish histopathology and health assessment indices—collected across six wetlands. These biomarkers revealed early warning signs of sub-lethal stress in fish populations that were not detectable through physicochemical or community-level assessments alone. For instance, histopathological alterations such as gill hyperplasia, liver necrosis, and gonadal atrophy were strongly associated with pollution gradients, particularly in Megech River Mouth and Avaj wetlands. Similarly, elevated fish health assessment indices (HAI), including external anomalies and hematological deviations, reflected cumulative environmental stress. These findings underscore the diagnostic value of biomarkers in detecting chronic exposure and distinguishing between wetlands with similar water chemistry but differing biological impacts. Their integration enhances the resolution of ecological assessments and supports their inclusion in routine monitoring frameworks.

CHAPTER 2

General Methods

2.1. Study Sites

Water quality data and biological samples were gathered from six wetlands in four different administrative areas around Lake Tana. These wetlands are located in the same ecology adjacent to Lake Tana. The study wetlands included Wonjeta, Zewdie Girar, Gumara River Mouth, Megech River Mouth, Avaj, and Ras Abbay. Wetlands are exposed to varying degrees of human influence. Two of these wetlands, Avaj and Ras Abbay, are located in Bahir Dar, which is a capital city in the Amhara region. Avaj, Ras Abbay, and Gumara River Mouth are located near Bahir Dar. Avaj is about three kilometers from Bahir Dar, Ras Abbay is about five kilometers northeast, and the Gumara River mouth is in the Dera district. Dera is a rural district about 40 km northeast of Bahir Dar. Megech River Mouth is in the Dembia district, which is about 90 kilometers north of Bahir Dar and 40 kilometers south of Gondar. Wonjeta Wetland is about five kilometers northwest of Bahir Dar. Zewdie Girar is located in the North Achefer district, which is about 50 kilometers west of Bahir Dar. The wetland at the mouth of the Gumara River is polluted by wastewater from nearby agricultural farms (Figure 2.1.).

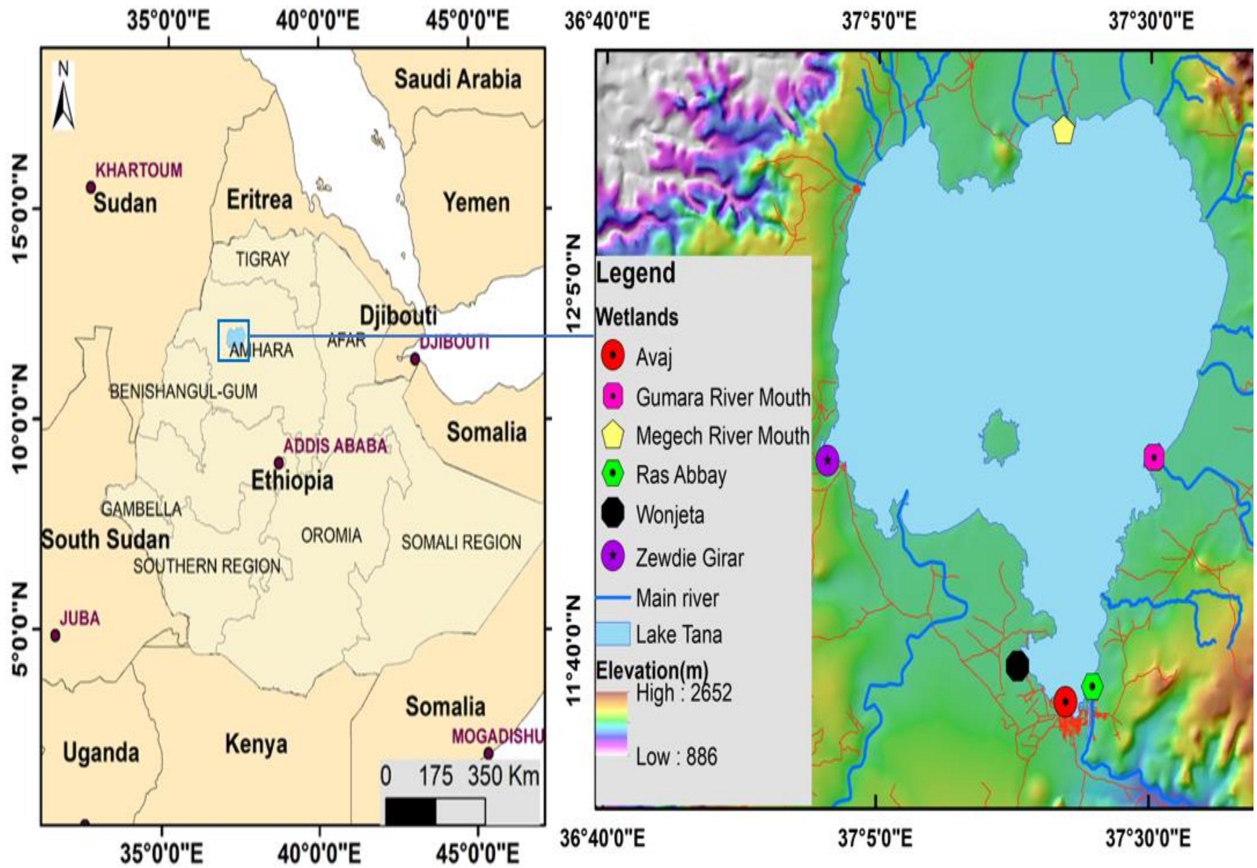


Figure 2. 1. Location map of the study area (left) and positions of the study wetlands (right)

2.2. Research design

The study involved an ecological field experiment; data were collected every three months from March 2020 to November 2020 during a total of 24 field sampling trips. This included sampling for physical and chemical parameters, macroinvertebrates, fish for bioindicators, fish histopathology, and fish health assessment biomarkers. Three sampling locations in the inshore of the lake were used for macroinvertebrate and fish bioindicators while for histopathology and health assessment studies, fish caught at two inshore sites were used. The four seasons were the rainy season with heavy rains during July–September, the late rainy season during October–November, the dry season during December–April, and the early rainy season during May–June (Wondie et al., 2007a; Abebe et al., 2017).

2.3. Sampling and analysis of physicochemical variables

In all study sites, three samples and data on physicochemical variables were measured on the shoreline at three locations of each wetland across four seasons. Samples were taken between 08h00 and 10h00 a.m. Therefore, a total of 72 samples and data were collected for the physicochemical study. To collect the samples surface, middle, and bottom depths in all locations were considered. At each depth, temperature, pH, dissolved oxygen and electrical conductivity, total depth, total dissolved solids, and salinity were measured *in situ* using portable meters (Brand-YSI, Model-556 MPS (multiprobe system) Handheld Multiparameter Water Quality Meter, USA) twice a day. Water transparency at each site was measured using a 25-cm Secchi disk in the morning (10h00) and in the afternoon (15h00), referred to as a.m. and p.m., respectively. Water depth (WD) was measured using a tape measure mounted on a stick. Water samples were collected using a Van Dorn water sampler, for measurement of total phosphorus (TP), total nitrogen (TN), nitrite (NO_2^-), nitrate (NO_3^-), and total ammonia ($NH_4^+ + NH_3$). Chlorophyll-a was measured using the FluoroSense Handheld Fluorometer, to quantify freshwater phytoplankton *in situ* in the morning (10h00) and the afternoon (15h00) (Trent et al., 2017; Shin et al., 2018). Water samples were collected in non-light transparent bottles with tight caps, preserved with hydrochloric acid 37%, and transported on ice for laboratory analysis. Carlson TSI and WQI of wetlands of Lake Tana were calculated to rank wetlands based on the level of contamination of wetlands.

2.4. Sampling and analysis of macroinvertebrate bioindicators

In all study sites, six samples of benthic and water-column macroinvertebrates were measured on the shoreline at three locations of each wetland across four seasons. Samples were taken between 08h00 and 10h00 a.m. Therefore, a total of 144 samples for benthic and water-column macroinvertebrates (72 for each) were collected for the study of the macroinvertebrate community. Sediment samples were collected with the Ekman Grab method (for an area of approximately 0.0254 m^2) for benthic macroinvertebrate collection and a D-frame net (Elliott and Tullet, 1978; Borisko et al., 2007; Phillips, 2008; Malik et al., 2020) to collect water column macroinvertebrates. To sample macroinvertebrates inhabiting the water column, a D-frame sweep net (mesh size = 1.2 mm, mouth area 200.96 cm^2, sweep length 1.5 m) was used to collect macroinvertebrate at the water surface (Meyer et al., 2013; Smith et al., 2019). Three sweeps in an estimated area of 1 m^2 were made for 30 seconds to collect macroinvertebrates in the water column. The samples were taken to a lab, filtered through a fine mesh, and then hand-sorted to separate the living macroinvertebrates from the plants. The collected macroinvertebrates were preserved in 70% alcohol for identification and enumeration. In the laboratory, all organisms were sorted and taxonomically identified to genus level using a dissecting microscope and macroinvertebrate identification manuals (Clifford, 1991; Oscoz et al., 2011; Smith et al., 2019; Feeley et al., 2020). Members of each taxon were counted, and the counts were expressed as individuals/sweep/m^2.

2.1. Sampling for fish bioindicators

Fish for the study were caught using gill nets of different sizes that were left in the water overnight. This involved determining the species diversity and abundance after taking into account all the fish in an overnight catch. In all study sites, two catches to estimate fish bioindicators were obtained on the shoreline of each wetland across each of the four seasons. Samples were taken between 08h00 and 10h00 a.m. Therefore, a total of 48 fish samples were collected for the bioindicator study. All fish caught in each site per season were identified to species level and counted. Different types of nets were used to catch fish in different areas of the lake. In shallow areas near the shore, two nets were used: one with a small mesh and one with a large mesh. In deeper areas, one of each mesh size was used, with one net at the surface and one at the bottom. In areas where the water was deeper than 10 meters, the nets used at the bottom were placed between 7 and 15 meters deep. The nets were left in the water for two hours for the small-meshed net and 15 hours for the large-meshed net. Motorized boats were used to access fish sampling sites that were dominated by water hyacinth, papyrus, and other aquatic plants.

2.3. Sampling for fish histopathology

Fish for histopathology analysis were obtained by the use of gill nets set in the daytime. Because many fish did not stay alive during the overnight gill netting, fish for histopathology assessment were sampled separately. The target was a minimum of 16 fish per site and 24 fish per season. Accordingly, a total of 96 samples from *Labeobarbus* spp. were collected from six wetland ecotones along the shoreline of Lake Tana. Fish were kept alive until euthanasia by continuous exchange of running water from a mobile tap unit in the field. The water used in the mobile tap was collected from the study site. After catching fish in each study wetland, live fish were euthanized immediately in situ by the cervical dislocation technique using a scalpel to incise between the skull and first vertebra, after identification of the location of the junction between the skull and the first cervical vertebra. The fish were dissected to remove the liver, gills, spleen, and gonads for histology processing. The dissected organs were preserved in a 10% neutral-buffered formalin solution and labeled pending laboratory analysis.

2.4. Sampling for health assessment indices

Fish samples were collected from six wetlands around Lake Tana. Because *Labeobarbus* spp. was the most common genus of fish in the lake, this genus was chosen for the study on fish health assessment. Different types of gill nets were used to catch fish in each wetland across four seasons season (Vijverberg et al., 2009). For the fish health assessment index study, hematocrit values, both external and internal organ and tissue anomalies were evaluated as described by Adams et al. (1993), with slight modifications. Additionally, inverted parasite indices were assessed as described by Crafford & Avenant-Oldewage (2009), and the body length and weight relationship was evaluated as described by Sekitar et al. (2015) and used to compare the health of fish of each wetland.

CHAPTER 3

Physical and chemical water quality characteristics in six wetlands of Lake Tana, Ethiopia

3.1. Introduction

The physical and chemical properties of a body of water can have a significant impact on the biological productivity of an aquatic ecosystem (Bhateria & Jain 2016; Elnaggar & El-Alfy, 2016). Hence, healthy aquatic ecosystems require monitoring the physical and chemical properties of the water. Different physicochemical factors in aquatic environments can produce various biochemical and physiological parameters that can be used as biomarkers (Abalaka, 2017; Ha et al., 2020). To effectively use bioindicators and biomarkers for studying ecological biodiversity, a clear understanding is required of physicochemical conditions and the overall health of the ecosystem. Many environmental factors can influence the diversity of life in an ecosystem (Bartell, 2006; Wepener, 2008; Naigaga, 2012; Abalaka, 2017; Lomartire et al., 2021; Marinović et al., 2021).

According to a review on eutrophication and nutrient release in aquatic environments in Sub-Saharan Africa, untreated wastewater from industries and sewage systems is frequently released into the environment as a major source of nutrients that contribute to the eutrophication of surface water bodies (Nyenje et al., 2010; Naigaga, 2012; Ripanda & Miraji, 2022). There are several reports on existing trends of eutrophication in Ethiopian water bodies (Fetahi, 2019; Assefa et al., 2020; Ayele & Atlabachew, 2021; Geletu, 2023). Lake Tana used to be oligotrophic, as reported by Wudneh (1998), Dejen et al. (2004), and Wondie et al. (2007a). However, there has been an increase in the concentrations of nitrate and phosphorus, especially in the areas near the shore and river mouths. This trend could eventually lead to eutrophication, as reported by several researchers (Wondie et al., 2007a; Ligdi et al., 2010; Dersseh et al., 2020; Goshu et al., 2020; Ayele and Atlabachew, 2021).

In-flowing rivers carry heavy loads of suspended silt into Lake Tana, thereby increasing the turbidity of the lake water and reducing primary production (Gebresllassie et al., 2014; Dejen et al., 2017; Gebremedhin et al., 2018; Wondie, 2018; Aragaw, 2021). A significant portion of the lake's shoreline is covered by vast wetlands, which are frequently dominated by thick papyrus stands extending out over the lake waters. These wetlands have an impact on the physical, chemical, and biological conditions of the inshore waters. Subsequently, the quality of the water near the shore is a good indicator of the overall health of the entire lake (Wepener, 2008; Assefa et al., 2020). As water quality deteriorates, ecosystem services may be lost, and organisms will begin to suffer. For example, the fluctuation of the physical and chemical characteristics of a lake has an impact on the diversity and abundance of organisms such as macroinvertebrates, and fish assemblage composition (Czerniawska-Kusza, 2005; Lakew & Moog, 2015; Aragaw, 2021).

It is, therefore, important to gain reliable information on trends in water quality as a prerequisite for planning the prevention and control of lake pollution and the sustainability of an effective water management program (Zelalem & Prokin, 2017; Kassa & Tibebe, 2019). As the human population grows, there is an increasing demand for resources like land and water for housing, farming, and industry. This leads to increased pollution in the lake as more wastewater is released (Gebresllassie et al., 2014; Karlberg et al., 2015; Dejen et al., 2017; Gebremedhin et al., 2018; Wondie, 2018). The use of bioindicators and biomarkers to evaluate environmental quality therefore necessitates that the ecosystem be characterized in terms of physicochemical as well as ecological characteristics. Because Lake Tana and its nearby wetlands are facing pollution from eutrophication, this study measured the levels of nutrients in the water. The wetlands were studied using 16 physicochemical measurements that are commonly used to evaluate the water quality of Lake Tana (Wondie, 2010; Zelalem and Prokin, 2017; Kassa &Tibebe, 2019). The results will be used in the chapters that follow to relate the biological indicators and biomarkers to water quality. This involved comparing the physical and chemical properties of the study wetlands, identifying factors influencing these properties, and analyzing spatial and seasonal variations in water quality and potential pollution sources.

Accordingly, this chapter addresses Objective 1 of the study, which is to characterize the wetlands based on the physicochemical properties of wetlands and determine the primary factors influencing the wetlands. Six wetlands were evaluated using the selected parameters, with seasonal and spatial variations analyzed. Multivariate statistical techniques, including Principal Component Analysis (PCA) and Factor Analysis (FA), were applied to identify major influencing factors. In addition, the Total Trophic Status Index (TOTTSI) and Water Quality Index (WQI) were computed and visualized to classify the pollution status of each wetland.

3.2. Specific Methods

3.2.1. Measurement of physicochemical variables

Physicochemical variables measured in this study were temperature, dissolved oxygen, conductivity, pH, Secchi disk depth a.m. and p.m., total dissolved solids, salinity, nitrite (NO_2), nitrate (NO_3^-), soluble reactive phosphorous, total ammonia ($NH_4^+ + NH_3$), total phosphorus (TP), total nitrogen (TN), and chlorophyll a (Chl-a). These variables were monitored because physicochemical data define the status and quality of the water and nitrogen can be directly harmful to fish in concentrations beyond the normal ranges (Holmes & Taylor, 2015; Dar et al., 2021; Lopes, 2021; Zhang et al., 2022). This study assessed key physicochemical parameters, including temperature, dissolved oxygen, electrical conductivity, pH, water transparency, total dissolved solids, salinity, and nutrients (nitrate, nitrite, ammonia, total nitrogen, total phosphorus, and chlorophyll-a). These parameters were chosen for the direct relevance of parameters to water quality and ecosystem health. A summary of the measurement methods, units, and regulatory standards for each parameter is provided in Table 3.1.

3.2.2. Assessment of the trophic status of the sampling sites

Based on the value of the Carlson Trophic State Index (TSI), aquatic ecosystems are classified into trophic categories (Carlson, 1977). TSI for the wetlands of Lake Tana was calculated using data on Secchi disk transparency, chlorophyll-a, and the concentration of total phosphorous (TP).

$TSI_{TN} = 54.45 + 14.43 * \ln(TN)$ (mg/L)

$TSI_{TP} = 14.42 * \ln(TP) + 4.15$ (µg/L)

$TSI_{Chla} = 9.81 * \ln(Chl\ a) + 30.6$ (µg/L)

$TSI_{SDT} = 60 - 14.41 * \ln(SD)$ (m)

$$\mathbf{TOT_{TSI}} = \frac{(\mathbf{TSI_{TN}} + \mathbf{TSI_{TP}} + \mathbf{TSI_{Chla}} + \mathbf{TSI_{SDT}})}{\mathbf{4}}$$

Where: TSI_{TN} corresponds to the concentration (mg/L) of total nitrogen, TSI_{SDT} is TSI corresponding to the depth (m) of Secchi disk transparency, TSI_{TP} is TSI corresponding to the concentration (μg/L) of total phosphorus, TSI_{Chla} is TSI corresponding to the concentration (μg /L) of chlorophyll-a, and TOT_{TSI} is total TSI, i.e., the average TSI_{SDT}, TSI_{TP}, $TSI_{TN,}$ and TSI_{Chla}. Generally, TSI values below 40 correspond to an oligotrophic, from 40 - 60 to mesotrophic, from 60 - 80 to eutrophic, and above 80 to a hypertrophic status of the lake (Jarosiewicz et al., 2011).

Table 3. 1. Summary of physicochemical variables, units, and analytical methods used in the assessment of the wetlands of Lake Tana

Parameter	**Abbreviation**	**Units**	**Analytical Tools**
Temperature	Temp	^{o}C	Portable meter
Dissolved Oxygen	DO	mg/L	Portable meter
Electrical conductivity	EC	μS/cm	Portable meter
pH	pH		Portable meter
Secchi Depth a.m.	SD a.m.	M	Secchi disk
Secchi Depth p.m.	SD p.m.	M	Secchi disk
Water depth	WD	M	Tape mounted on the stick
Total dissolved solids	TDS	gL	Portable meters
Salinity	S	g/L	Portable meters
Nitrite nitrogen	NO_2-N	mg/L	Palin test (Photometer)
Nitrate nitrogen	NO_3-N	mg/L	Palin test (Photometer
Soluble reactive phosphorus	SRP	mg/L	Palin test (Photometer)
Total ammonia	NH_4^++NH_3	mg/L	Palin test (Photometer)
Total Nitrogen	TN	mg/L	Ammonium Molybdate (Spectrophotometer)
Total phosphorus	TP	mg/L	Ammonium Molybdate (Spectrophotometer)
Chlorophyll-a	Chl-a	ml/L	Fluorometer

3.2.3. Assessment of the water quality index of the sampling sites

The water quality index (WQI) was calculated using data to understand how water quality changed over time and in different locations (Rubio-Arias et al., 2012; Tyagi et al., 2013; Rocha et al., 2015; Nong et al., 2020; Uddin et al., 2021). WQI is a measure that integrates different factors to assess water quality. If the WQI is high, it means the water is polluted and not suitable for many uses. Different water quality factors were given different importance ratings from 1 to 5. The WQI values were categorized into five groups: excellent (less than 50), good (50-100), poor (100-200), very poor (200-300), and unfit for use (greater than 300) (Yidana & Yidana, 2010). The WQI is computed as;

$$\mathbf{Wi} = \frac{\mathrm{wi}}{\sum \mathrm{wi}}$$

Where ∑Wi is the sum of the weights of all the parameters. In this study, ∑wi was 50.

Table 3.2. presents the wi, Wi, and US Environmental Protection Agency (EPA) standards for each chemical parameter used in this study. A quality rating scale, qi, was computed for each parameter using the equation

$$qi = (Ci/Si) \times 100$$

where Ci and Si respectively refer to the concentration and the US EPA standard for each parameter, in mg/L.

The water quality subindex, SIi was then calculated for each parameter using the equation.

$$WQI = \sum SIi$$

Table 3.2. USEPA standards, weight (wi) and calculated relative weight (Wi) for each parameter. Where US EPA-US Environmental Protection Agency

Physicochemical Parameters	**US EPA**	**Weight (wi)**	**Relative weight (Wi)**
Temperature	25	5	0.1
Dissolved oxygen (mg/L)	5	5	0.1
Electrical conductivity (µs/cm)	1000	4	0.08
pH	7.5	4	0.08
Secchi depth a.m. (m)	NA	1	0.02
Secchi depth p.m. (m)	NA	1	0.02
Water depth (m)	NA	1	0.02
Total dissolved solids (g/L)	0.1	4	0.08
Salinity (g/L)	0.1	4	0.08
Nitrite (mg/L)	0.001	2	0.04
Nitrate (mg/L)	0.1	5	0.1
Soluble reactive phosphorous (mg/L)	0.02	3	0.06
Total ammonia (mg/L)	1.5	3	0.06
Total nitrogen (mg/L)	1.1	3	0.06
Total phosphorous (mg/L)	1.0	3	0.06
Chlorophyll-a (mg/L)	2.0	2	0.04
Sum		**50**	**1**

3.2.4. Statistical analysis

Descriptive statistics comprising the means, standard deviations, and ranges for each parameter were derived. The normality distribution test of the data for each variable under study was checked using Kolmogorov-Smirnov (K-S) and Lilliefors tests. All data followed normal distribution ($P>0.05$) hence analysis of variance (ANOVA) was used to compare the water quality variables between sampling locations. When there is a significant interaction between wetlands by season, a comparison of means within main effects was done using Tukey's post hoc test. To evaluate spatial variation in water quality and to characterize the study sites according to the water quality status of study sites, subsequently defining the degree of contamination of each wetland, the water quality datasets were subjected to four multivariate statistical techniques, namely, ANOVA, cluster analysis (CA), principal component analysis (PCA) and factor analysis (FA). The cut-off value for determining statistical significance was chosen as $p < 0.05$ (Sharma & Sood, 2022). Three sampling sites per offshore side of each wetland were averaged.

Principal component analysis (PCA) was used to decrease the dimensionality of a large number of interrelated variables of a dataset by converting unique, interrelated parameters into a few orthogonal (uncorrelated) parameters known as principal components (PCs). This method is a correlation matrix and reflects stochastic interdependencies between different variables to understand how PCA randomly affect each other. The input water quality parameters of PCA are correlated, whereas the hypothetical parameters (PCs) are uncorrelated and are obtained as a linear combination of the observable water quality parameters (Hatvani et al., 2014).

Cluster analysis was used to group objects (cases) into classes (clusters) based on similarities within a class and dissimilarities between classes. A common method for grouping similar things is called hierarchical agglomerative clustering. This method shows how close each item is to all the others and is often illustrated using a dendrogram (Panda et al., 2006).

FA was examined to further simplify the data structure based on PCA. The purpose of the analysis was to compare the compositional patterns between sites and to identify the various latent factors that influenced each of the study sites. The factors are ranked based on how important PCA is in explaining the differences in the data. The most important factor has the highest eigenvalue (Edori, 2020; Liu et al., 2020). Factor loadings in the factor loadings tables are interpreted as correlation coefficients between the variables and the factors (Panda et al., 2006; Sarmento & Costa, 2017). Thus, only factors with eigenvalues greater than 1 were considered. The study used a common statistical method called R-mode factor analysis. The sampling sites were the grouping (independent) variables, while all the measured variables constituted the independent variables.

The wetlands were ranked based on the Carlson Trophic State Index (TSI) of each wetland and the Water Quality Index (WQI). TSI was calculated using data on Secchi disk transparency, chlorophyll-a, and phosphorus levels. WQI was calculated for each wetland and ranked accordingly.

3.3. Results

3.3.1. Characteristics of study wetlands of Lake Tana

The study was conducted at six different types of wetlands: one riverine, two lacustrine, two river mouths, and one urban wetland. Gumara River Mouth is a wetland that is heavily polluted due to farming activities, livestock grazing, irrigation, and other human activities. This wetland has been invaded by water hyacinths since 2012. Megech River Mouth is dominated by water hyacinths and receives pollution from agricultural lands in the North Gondar administrative zone and untreated wastewater from the city of Gondar. Ras Abbay is a wetland along a river that has many *Cyperus papyrus* and forest trees. It receives pollution from both industries and domestic effluents. The Blue Nile River, which flows through Bahir Dar City, has untreated domestic and industrial wastewater that flows into Ras Abbay. Avaj is a wetland in a city that receives wastewater from hotels, hospitals, and nearby fishing areas. Wonjeta is a wetland that gets water from springs and has many papyrus plants and trees. It is located near rural areas and the spring water is used by people for drinking and small-scale irrigation. Zewdie Girar is a wetland located near a lake that has many reeds and is surrounded by a mountain. It is less affected by pollution from activities like harvesting, sedimentation, water extraction, and the introduction of non-native species. Geographical locations and wetland characteristics of the study wetlands are shown in Table 3.3.

Table 3.3. Summary of the characteristics of study wetlands in Lake Tana. GRM - Gumara River mouth, MRM - Megech River mouth, RA - Ras Abbay, AV - Avaj, WO - Wongeta, and ZG - Zewdie Girar

Wetland	**Location**	**Latitude /Longitude**	**Altitude (masl)**	**Area (ha)**	**Origin of water quality deterioration**	**Type of vegetation**	**Type of wetland**	**Reference**
GRM	Located southeast of the lake, approximately 50 km from Bahir Dar city	37^{0}29’684’’N /11^{0}53’’949’’ E	1850	1500	Catchment agriculture	*Eichhornia*-dominated	River mouth wetland	(Wondie, 2018; Kahsay et al., 2023)
MRM	Located in the northern part of the lake, approximately 90 km from Bahir Dar city and 40 km from Gondar city	37^{0}24’245’’ N /12^{0}16’337’’E	1794	1816	Catchment agriculture and untreated municipal effluent	*Eichhornia*-dominated	River mouth wetland	(Wondie, 2018; Dersseh et al., 2020; Kahsay et al., 2023)
RA	Located in the south of the lake, approximately 5 km from Bahir Dar city	37^{0}24’682’’ N /11^{0}36’140’’ E	1769–1785	1114.5	Domestic wastewater	Grass and trees	Riverine wetland	(Wondie, 2018; Kahsay et al., 2023)
AV	Located south of the lake, approximately 2 km from Bahir Dar city	37^{0}22’464’’ N /11^{0}36’679’’ E	1792	200	Untreated municipal and industrial wastewater	Papyrus and tree natural forests	Urban	(Wondie, 2018; Kahsay et al., 2023)
WO	Located south of the lake, approximately 5 km from Bahir Dar city	37^{0}17’832’’N /11^{0}39’241’’ E	1806	300	Relatively low pressure from farming and wastewater	Papyrus-dominated	Lacustrine	(Wondie, 2018; Kahsay et al., 2023)
ZG	Located southwest of the lake, approximately 4 km from Bahir Dar city	36^{0}59’812’’ N / 11^{0}54’262’’ E	1795	250	Relatively low pressure from farming and wastewater	Papyrus-dominated	Lacustrine	(Wondie, 2018; Kahsay et al., 2023)

3.3.2. Assessment of spatiotemporal variations of selected water physicochemical properties of Lake Tana

Tests of between-subject effects for each dependent variable using univariate analysis (Table 3.4.) showed that the wetlands exhibited a significant difference in electrical conductivity, pH, Secchi depth a.m., Secchi depth p.m., water depth, salinity, nitrate, total ammonia, and chlorophyll-a, while water temperature, dissolved oxygen, total dissolved solids, nitrite, soluble reactive phosphorus, total nitrogen, total phosphorus, and the total nitrogen to total phosphorus ratio did not show significant differences among the six wetlands. On the other hand, season had a significant effect (ANOVA, $p< 0.05$) on water temperature, dissolved oxygen, electrical conductivity, Secchi depth a.m., Secchi depth p.m., water depth, nitrate, soluble reactive phosphorous, total ammonia, total nitrogen, and Chlorophyll-a while pH, total dissolved solids, salinity, nitrite, and total nitrogen did not differ among seasons (ANOVA, $p> 0.05$). There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of dissolved oxygen, electrical conductivity, pH, Secchi depth a.m., Secchi depth p.m., salinity, nitrate, total ammonia, total nitrogen, total phosphorous, total nitrogen to total phosphorous ratio and Chlorophyll-a while water temperature, water depth, total dissolved solids, while nitrate and soluble reactive phosphorous were not affected (ANOVA, $p>0.05$) by the interaction between wetland and season.

Table 3.4. Univariate ANOVA table and test of significance for physicochemical properties in Lake Tana wetlands across four seasons

Effect	Variable	Df	SS	MS	F	P
Wetland	Temperature (^{0}C)	5	11.74	2.35	1.36	0.26
	Dissolved oxygen (mg/L)	5	2.589	0.518	0.96	0.45
	Electrical conductivity (µS/cm)	5	22458	4492	13.05	0.00
	pH	5	5.909	1.182	2.884	0.02
	Secchi depth a.m. (m)	5	24.0054	4.80108	17.84	0.00
	Secchi depth p.m. (m)	5	19.20174	3.84035	15.56	0.00
	Water depth (m)	5	42.6236	8.5247	9.24	0.00
	Total dissolved solids (g/L)	5	0.144555	0.028911	0.75	0.59
	Salinity (g/L)	5	0.004996	0.000999	8.88	0.00
	Nitrite (mg/L)	5	0.054599	0.010920	1.30	0.28
	Nitrate (mg/L)	5	0.31938	0.06388	5.34	0.00
	Soluble reactive phosphorous (mg/L)	5	0.60925	0.12185	1.56	0.18
	Total ammonia (mg/L)	5	0.368185	0.073637	5.23	0.00
	Total nitrogen (mg/L)	5	25.5614	5.1123	0.54	0.74
	Total phosphorous (mg/L)	5	6.60024	1.32005	1.84	0.12
	Total nitrogen: Total phosphorous ratio	5	392.679	78.536	0.82919	0.53
	Chlorophyll-a (mg/L)	5	266.903	53.381	3.0407	0.02
Season	Temperature (^{0}C)	3	29.19	9.73	5.62	0.00
	Dissolved oxygen(mg/L)	3	18.200	6.067	11.240	0.00
	Electrical conductivity µS/cm)	3	3418	1139	3.313	0.03
	pH	3	0.815	0.272	0.663	0.58
	Secchi depth a.m. (m)	3	21.0783	7.02609	26.1061	0.00
	Secchi depth p.m. (m)	3	15.14610	5.04870	20.4560	0.00
	Water depth (m)	3	36.5502	12.1834	13.2047	0.00
	Total dissolved solids (g/L)	3	0.237585	0.079195	2.06663	0.12
	Salinity (g/L)	3	0.000749	0.000250	2.218	0.10
	Nitrite (mg/L)	3	0.030913	0.010304	1.228009	0.31
	Nitrate (mg/L)	3	1.59503	0.53168	44.937	0.00
	Soluble reactive phosphorous (mg/L)	3	1.08765	0.36255	4.7604	0.00
	Total ammonia (mg/L)	3	1.076676	0.358892	25.5014	0.00
	Total nitrogen (mg/L)	3	105.9809	35.3270	3.74764	0.02
	Total phosphorous (mg/L)	3	5.79351	1.93117	2.68797	0.06
	Total nitrogen: Total phosphorous ratio	3	400.579	133.526	1.40978	0.25
	Chlorophyll-a (mg/L)	3	161.819	53.940	3.0725	0.04
Wetland x Season	Temperature (^{0}C)	15	34.73	2.32	1.34	0.22
	Dissolved oxygen(mg/L)	15	18.531	1.235	2.289	0.01
	Electrical conductivity (µS/cm)	15	27947	1863	5.417	0.00
	pH	15	21.137	1.409	3.438	0.00
	Secchi depth a.m.(m)	15	60.7273	4.04849	15.0425	0.00
	Secchi depth p.m. (m)	15	47.22013	3.14801	12.7549	0.00

Continued------

Water depth (m)	15	8.7683	0.5846	0.6336	0.83
Total dissolved solids (g/L)	15	0.446392	0.029759	0.77659	0.69
Salinity (g/L)	15	0.008543	0.000570	5.063	0.00
Nitrite (mg/L)	15	0.176072	0.011738	1.398861	0.19
Nitrate (mg/L)	15	1.94689	0.12979	10.970	0.00
Soluble reactive phosphorous (mg/L)	15	1.43713	0.09581	1.2580	0.26
Total ammonia (mg/L)	15	1.195415	0.079694	5.6627	0.00
Total nitrogen (mg/L)	15	363.3852	24.2257	2.56997	0.01
Total phosphorous (mg/L)	15	20.86332	1.39089	1.93596	0.04
Total nitrogen: Total phosphorous ratio	15	2669.906	177.994	1.87927	0.05
Chlorophyll-a (mg/L)	15	669.931	44.662	2.5440	0.00

The current strategy for managing the quality of surface waters depends on a diverse range of physical and chemical parameters. Figure 3.1 presents the measured values of chemical and physical parameters collected from six wetlands during four different seasons, providing a comprehensive set of data.

Temperature

The overall mean value of water temperature in this study was 24.02 °C ± 1.49. Mean temperature did not differ among wetlands, ranging from 21.02 to 25.00 °C (mean: 23.34 ± 0.34) in GRM and from 22.79 to 26.59°C (mean: 24.63 ± 0.37) in WO (ANOVA, $p > 0.05$). In contrast, the mean value of temperature differed among seasons ranging from 21.02 to 25.69°C (mean: 23.18 ± 1.12) in the rainy season and from 22.82 to 27.92°C (mean: 24.79 ± 1.60) in the early rainy season (ANOVA, $p < 0.05$) (Figure 3.1 a).

Dissolved oxygen (DO)

There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of DO. Oxygen concentration ranged from 4.77 to 5.04 (mean: 4.89 ± 0.14) in WO during the dry season and from 6.86 to 8.64 (mean: 7.62 ± 0.92 mg/L) in GRM during the rainy season. Significant interactions of wetlands and seasons are indicated in Figure 3.1 b.

Electrical conductivity (EC)

There was a significant interaction between wetland and season (ANOVA, $p < 0.05$) for the mean value of EC. Values ranged from 89 to 108 (mean: 96.67 ± 10.02 µS/cm) in GRM during the rainy season and from 196 to 327 (mean: 250 ± 68.47 µS/cm) in MRM during the late rainy season. Figure 3.1 c shows differences between mean values.

pH

The mean value of pH in this study was 6.81 ± 0.82. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of pH. The mean value of pH ranged from 4.67 to 6.15 (mean: 5.62 ± 0.82) in WO during the late rainy season and from 6.63 to 9.41 (mean: 8.41 ± 1.55) in RA during the dry season. Significant differences between combinations of wetlands and seasons are indicated in Figure 3.1d.

Secchi depth (SD) a.m.

The mean value of SD a.m. in this study was 1.02 ± 1.29 m. There was a significant interaction between wetland and season (ANOVA, $p < 0.05$) for the mean value of SD a.m. Values ranged from 0.02 to 0.12 m (mean: 0.077 ± 0.05) in MRM during the early rainy season and from 4.1 to 7.8 m (mean: 6.3 ± 1.96) in GRM during the dry season. Figure 3.1e shows differences between mean values.

Secchi depth (SD) p.m.

The mean value of SD p.m. in this study was 0.94 ± 1.14 m. There was a significant interaction between wetland and season (ANOVA, $p < 0.05$) for the mean value of SD p.m. Values ranged from 0.02 to 0.12 m (mean: 0.07 ± 0.0.05) in MRM during the late rainy season and from 3.38 to 6.90 m (mean: 5.59 ± 1.93) in GRM during the dry season. Figure 3.1f shows differences between mean values.

Water depth (WD)

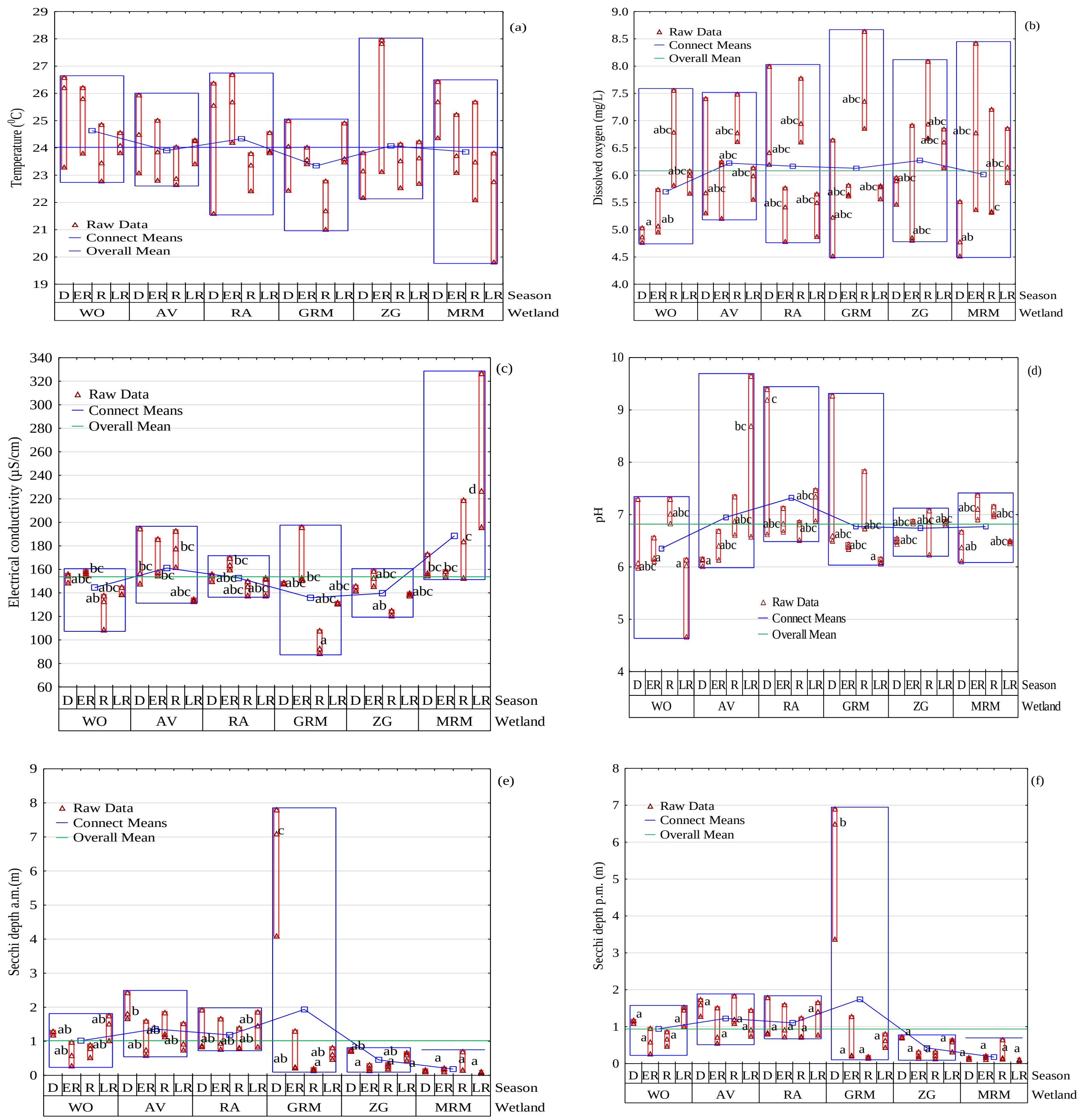
The mean value of WD in this study was 2.22 ±1.36 m. The mean value of WD differed among wetlands, ranging from 0.28 m to 1.87m (mean: 0.78±0.14) in MRM and from 2.05 to 4.10 m (mean: 3.08±0.21) in RA (ANOVA, $p < 0.05$). The season had an effect on the mean value of WD ranging from 0.26 to 2.85 m (mean:1.17 ± 0.86) during the early rainy season and from 0.22 to 6.10 m (mean:2.90 ± 1.34) during the rainy season (ANOVA, $p<0.05$) (Figure 3.1g).

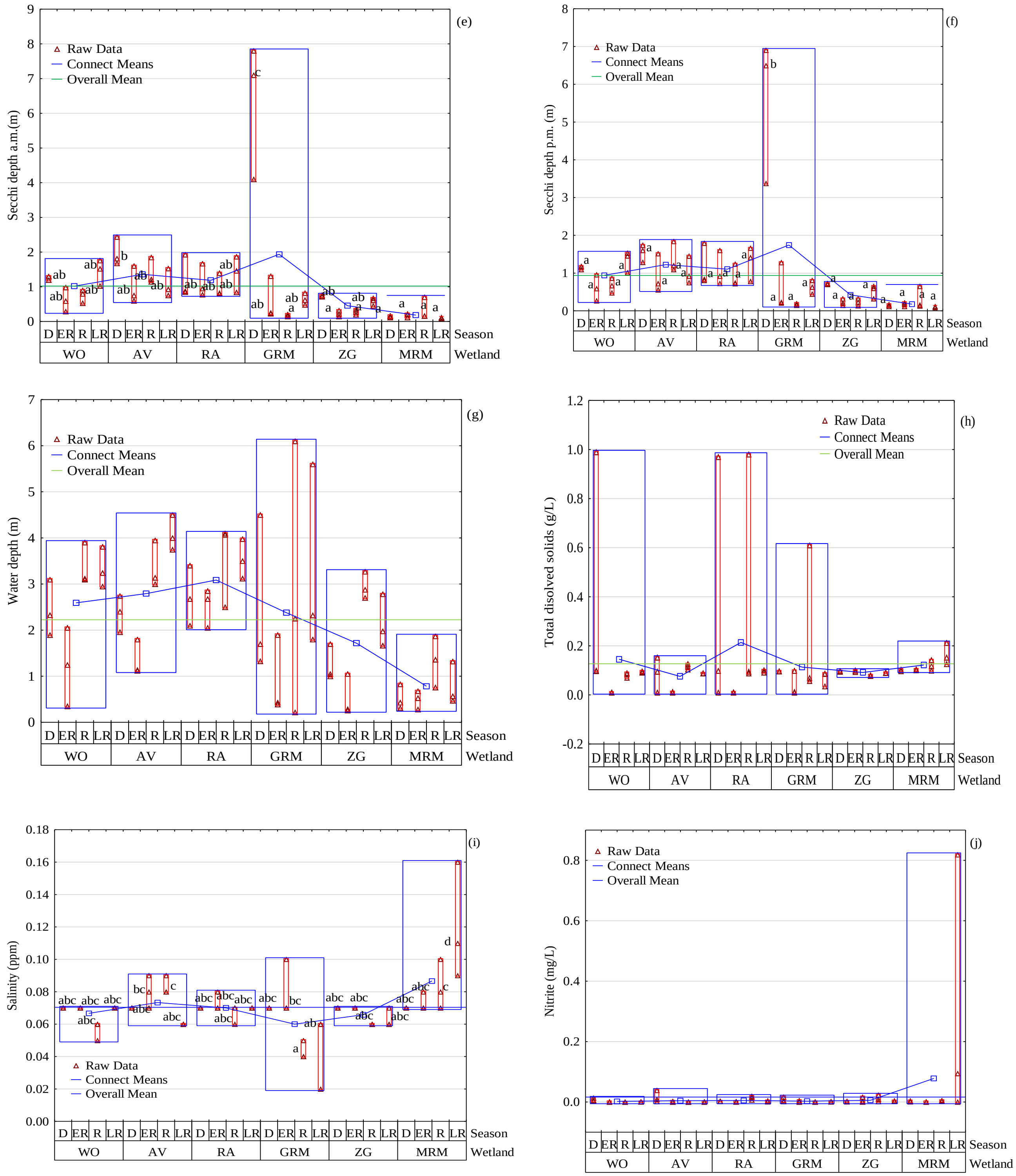
Total dissolved solids (TDS)

The mean value of TDS in this study was 0.13 ± 0.19 g/L. The mean value of TDS did not differ among wetlands, ranging from 0.01 to 0.15 g/L (mean: 0.12±0.04) AV and from 0.01 to 0.98 g/L (mean: 0.21±0.10) in RA (ANOVA, $p > 0.05$). Likewise, the mean value of TDS did not differ among seasons ranging from 0.01 to 0.10 g/L (mean: 0.04) during the early rainy season and from 0.01 to 0.9 g/L (mean: 0.19 ±0.29) during the dry season (ANOVA, $p>0.05$) (Figure 3.1h).

Salinity

The mean value of salinity in this study was 0.07 ± 0.02 ppm. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of Salinity. The mean value of salinity ranged from 0.04 to 0.09 g/L (mean: 0.04 ± 0.0.005) in GRM during the rainy season and from 0.02 to 0.06 g/L (mean: 0.05 ± 0.02) in MRM during the late rainy season. Significant interactions between wetland and season are indicated in Figure 3.1i.


(a)
Temperature (°C)
Raw Data
Connect Means
Overall Mean
Season
Wetland
WO
AV
RA
GRM
ZG
MRM
(b)
Dissolved oxygen (mg/L)
(c)
Electrical conductivity (µS/cm)
(d)
pH
(e)
Secchi depth a.m.(m)
(f)
Secchi depth p.m. (m)


(e)
Raw Data
Connect Means
Overall Mean
Secchi depth a.m.(m)
Season
Wetland
WO AV RA GRM ZG MRM
D ER R LR
(f)
Secchi depth p.m. (m)
(g)
Water depth (m)
(h)
Total disolved solids (g/L)
(i)
Salinity (ppm)
(j)
Nitrite (mg/L)

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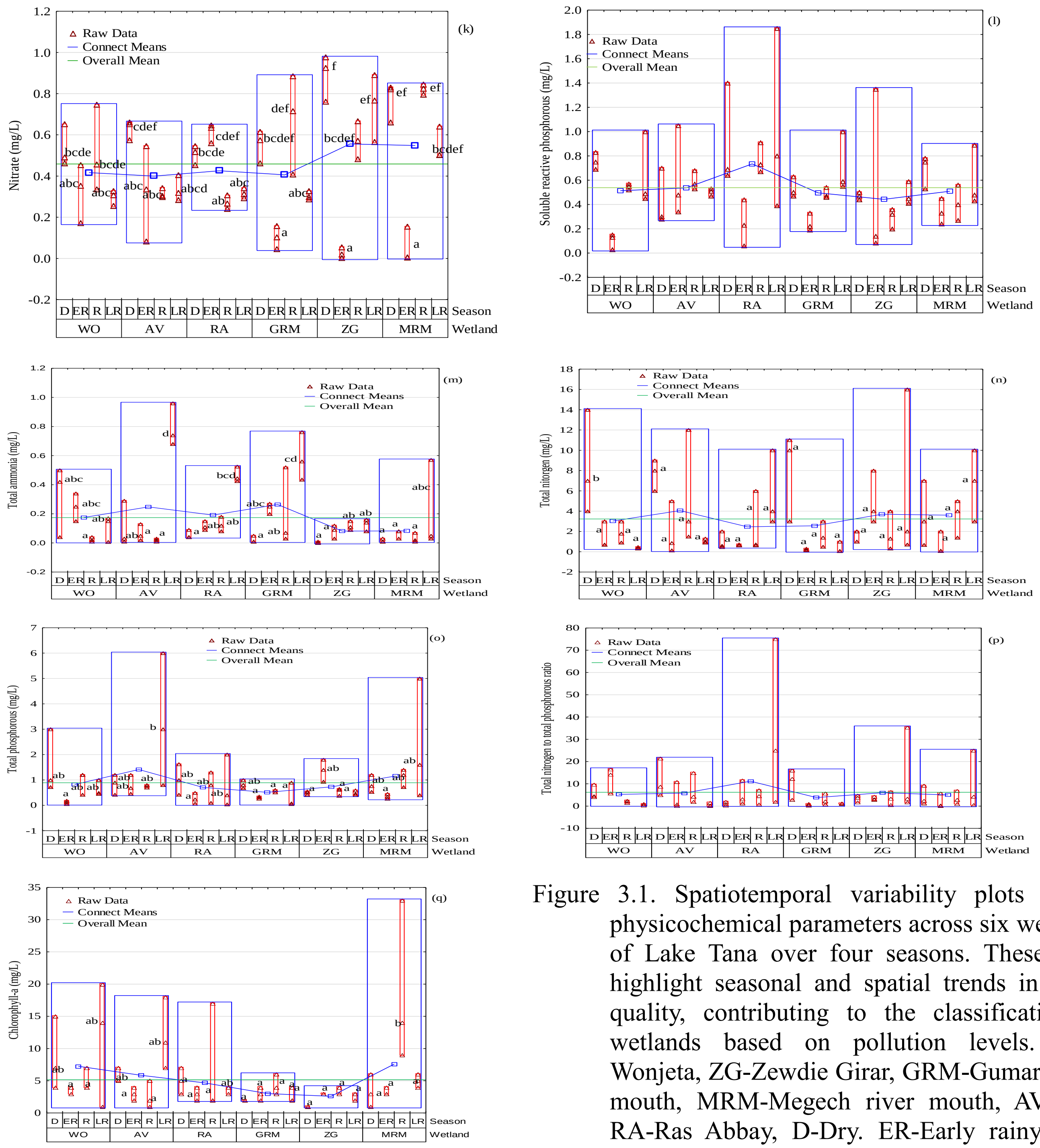


Figure 3.1. Spatiotemporal variability plots of 16 physicochemical parameters across six wetlands of Lake Tana over four seasons. These plots highlight seasonal and spatial trends in water quality, contributing to the classification of wetlands based on pollution levels. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA-Ras Abbay, D-Dry. ER-Early rainy-rainy, LR-Late rainy season.

Nitrite

The mean value of nitrite in this study was 0.02 ± 0.09 mg/L. The mean value of nitrite did not differ among wetlands, ranging from non-detectable to 0.013 mg/L (mean: 0.003 ± 0.001 mg/L) in WO and from non-detectable to 0.819 mg/L (mean: 0.078 ± 0.068) in MRM (ANOVA, $p > 0.05$). Likewise, the mean value of nitrite did not differ among seasons ranging from non-detectable to 0.017 mg/L (0.005 ± 0.007) during the early rainy season and from non-detectable to 0.819 mg/L (mean:0.052 ± 0.193) during the late rainy season (ANOVA, $p > 0.05$) (Figure 3.1j).

Nitrate

The mean concentration of nitrate in this study was 0.46 ± 0.25 mg/L. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of nitrate. Nitrate concentrations ranged from 0.001 to 0.054 mg/L (mean: 0.02 ± 0.0.027) in ZG during the early rainy season and from 0.76 to 0.98 mg/L (mean: 0.89 ± 0.11) in ZG during the dry season. Significant interactions between wetlands and seasons are indicated in Figure 3.1k).

Soluble reactive phosphorus (SRP)

The mean value of SRP in this study was 0.54 ± 0.31 mg/L. The mean value of SRP did not differ among wetlands, ranging from 0.083 to 1.350 mg/L (mean: 0.443±0.093) in ZG and from 0.060to 1.850 mg/L (mean: 0.734±0.142) in RA (ANOVA, $p > 0.05$). In contrast, the mean value of SRP differed among seasons ranging from 0.03 to 1.35 mg/L (mean:0.35 ± 0.34) during the early rainy season and from 0.39 to 1.85 mg/L (mean: 0.66 ± 0.36) during the late rainy season (ANOVA, $p <0.05$) (Figure 3.1l).

Total ammonia

The mean value of ammonia in this study was 0.17 ± 0.21 mg/L. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of total ammonia. Ammonia concentrations ranged from non-detectable to 0.01 mg/L (mean: 0.003 ± 0.006) in ZG during the dry season and from 0.68 to 0.98 mg/L (mean: 0.79 ± 0.15mg/L) in AV during the late rainy season. Significant interactions between wetland and season are indicated in Figure 3.1m.

Total nitrogen (TN)

The mean value of ammonia in this study was 2.23 ± 3.65 mg/L There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of TN. TN concentrations ranged from 0.128 to 0.306 mg/L (mean: .308±0.090) in GRM during the early rainy season and from 4.00 to 14.00 mg/L (mean: 8.333 5.132) in WO during the dry season. Significant interactions between wetland and season are indicated in Figure 3.1n.

Total phosphorous (TP)

The mean value of TP was 0.89 ± 0.98 mg/L. There was a significant interaction between wetland and season (ANOVA, $p <0.05$) for the mean value of TP. The concentration of TP ranged from 0.05 to 0.18 mg/L (mean: 0.12 ± 0.06) in WO during the early rainy season and from 0.80 to 6.00 (mean: 3.27± 2.61) in AV during the late rainy season. Figure 3.10 shows differences between mean values.

Total Nitrogen to Total Phosphorus (TN: TP) Ratio

The mean value of total phosphorous did not differ among wetlands, ranging from 0.37 to 16.2 (mean: 3.8 ± 5.2) in the GRM and from 0.37 to 75.0 (mean: 11 18. ± 21.28 in RA (ANOVA, $p > 0.05$). Likewise, the mean value of TN: TP did not differ among seasons ranging from 0.507 to 15.000 (mean:3.915± 3.257) during the rainy season and from 0.167 to 75.000 (mean: 10.018 ± 19.363) RA (ANOVA, $p > 0.05$) (Figure 3.1p).

Chlorophyll-a (Chl-a)

The mean value of Chl-a in this study was 5.15 ± 5.23 mg/L. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of Chl-a. Chl-a concentrations ranged with a mean value of 1.00 ± 0.00 in ZG during the dry season and from 9.00 to 33.00 mg/L (mean: 718.67±12.67) in MRM during the rainy season. Significant interactions between wetland and season are indicated in Figure 3.1q.

Seasonal variation significantly influenced key physicochemical parameters, including dissolved oxygen, conductivity, pH, water transparency, and nutrient concentrations across the study wetlands. These seasonal changes were consistently observed and aligned with the pollution classifications established for the wetlands. For example, nutrient concentrations such as nitrate, ammonia, and total nitrogen were generally higher during the rainy and late rainy seasons, likely due to increased runoff from agricultural and urban sources, while lower concentrations during the dry season reflected reduced external inputs and dilution effects. These seasonal trends confirmed the ranking of wetlands by pollution level, with Megech River Mouth classified as the most polluted, followed by Avaj and Ras Abbay, while Wonjeta, Zewdie Girar, and Gumara River Mouth exhibited comparatively better water quality. The consistency of these seasonal patterns across wetlands supports the robustness of the pollution classification framework used in the study, without evidence of significant interaction effects.

3.3.3. Spatial Diversity and site grouping based on water quality characteristics

Hierarchical cluster analysis classified the six wetlands into three distinct groups based on similarities in the physicochemical properties of each wetland (Figure 3.2). Cluster 1 included Zewdie Girar and Gumara River Mouth, representing the least polluted wetlands. Cluster 2 contained Wonjeta, Avaj, and Ras Abbay, indicating moderate pollution levels. Cluster 3 consisted solely of Megech River Mouth, which exhibited the highest pollution levels. These groupings were further supported by Canonical Correspondence Analysis (CCA), which replaced the previously used principal component analysis (PCA). The CCA results identified key factors, such as nutrient concentrations, conductivity, and turbidity, as the main drivers of the wetland groupings**,** clearly differentiating the wetlands based on pollution levels.

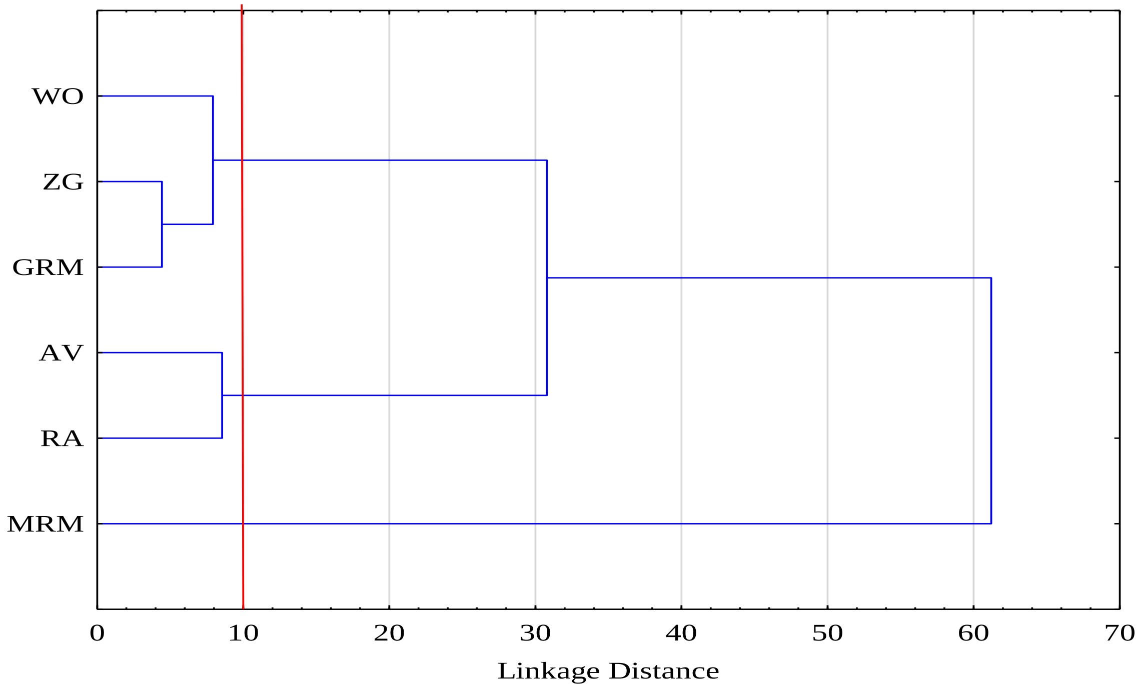


Figure 3.2. Hierarchical Cluster Analysis Dendrogram showing four physicochemical clusters in the wetlands of Lake Tana. Each cluster indicates sites with similar physicochemical characteristics. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was based on Ward's criteria.

3.3.4. Relationship between sampling sites and physicochemical variables

PCA results based on normalized data of the physicochemical components are shown in Table 3.5, and the wetlands are shown in Figure 3.3. Only 12 of the 16 water quality parameters were used for the cluster and PCA analysis because four variables did not explain the cluster and PCA. To ensure the scientific validity and interpretability of the Principal Component Analysis (PCA) and Canonical Correspondence Analysis (CCA), parameters were selected based on a combination of statistical and ecological criteria. Specifically, variables exhibiting multicollinearity, low communalities, or negligible contributions to the explained variance in preliminary exploratory analyses were excluded. This was determined by inspecting correlation matrices and initial factor loadings, where variables with weak loadings (0.5) across principal components were omitted. Furthermore, variables with high multicollinearity were excluded to avoid redundancy and inflation of the variance explained by certain components. Ecologically, the retained parameters are those most directly relevant to wetland water quality dynamics, trophic status, and anthropogenic pollution gradients, as established in previous studies on Lake Tana and similar freshwater ecosystems (Fetahi, 2019; Kassa & Tibebe, 2019; Umer et al., 2020). This combined selection approach ensures that the final set of variables included in PCA and CCA effectively capture the spatial and temporal heterogeneity of water quality and its ecological implications in the study wetlands.

Two components of PCA loaded eigenvalues greater than 1, with Component one (X-axis) explaining 45.3% of the total variance and Component two (Y-axis) explaining 26.4 % of the total variance (Table 3.5). Altogether, the first two components explained 71.7 % of the total variance and 10.2 of the 16 eigenvalues. The PCA plot brought out the four clusters observed in the hierarchical cluster analysis dendrogram (Figure 3.3). Cluster 1, the least polluted cluster 1 (WO, GRM, and ZG), was highly associated with higher values of nitrate and TN: TP ratio. The highly polluted cluster 3 (MRM) was correlated higher with higher values of electrical conductivity, salinity, and nitrite. The moderately polluted cluster 2 (AV and RA), was associated with higher values of SRP, TDS, pH, temperature, water depth, and ammonia (Figure 3.4).

Table 3.5. Eigenvalues, cumulative eigenvalues, percent of total variance, and cumulative percent of the total variance of correlation PCA for physicochemical variable, (n = 6) in the study sites

	Eigenvalues	**Cumulative Total**	**% of Total Variance**	**Cumulative % of Total Variance**
1	5.43	5.43	45.27	**45.27**
2	3.17	8.60	26.44	**71.17**
3	1.75	10.35	14.55	86.26
4	1.16	11.51	9.66	95.92
5	0.49	12.00	4.07	100.00

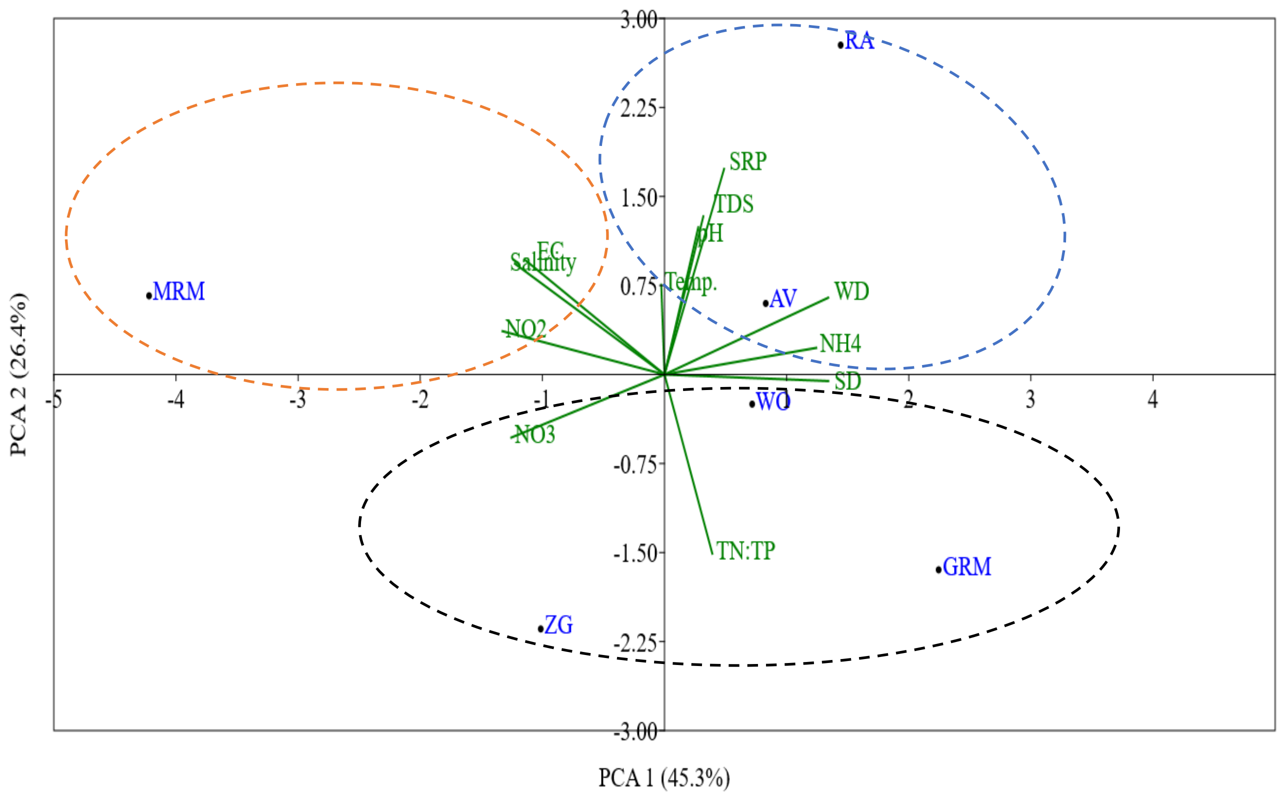


Figure 3.3. PCA plot correlating sampling wetland scores in Lake Tana with water quality vectors of the 12 physicochemical variables for plot component one (X-axis) and plot component two (Y-axis). Note the grouping of the three clusters.

3.3.5. Latent factors influencing water quality in the study sites

Three factors were extracted, explaining 71.71% of the total variance in the water quality data set (Table 3.6). Eigenvalues > 1 were taken as the criterion for the extraction of the factors required for explaining the source of variances in the data set under Kaiser Normalization (Alkarkhi et al., 2008; Panda et al., 2006; Rohe, 2020; Sarmento, 2017; Sayadi et al., 2014; Varol et al., 2012). The parameter loadings for the three identified factors, the factor eigenvalues, the percentage variance, and the cumulative percentage variance are given in Table 3.6. The loading coincided with the correlation coefficients between water quality variables and the factors (Alkarkhi et al., 2008;

Naigaga, 2012; Varol et al., 2012). Factor 1 accounted for 45.27% of the total variance and was positively correlated (loading > 0.70) with Secchi depth, pH, water depth, total dissolved solids, soluble reactive phosphorous, TN: TP ratio, and ammonia concentration while it was negatively correlated with temperature, electrical conductivity, salinity, nitrite, and nitrate (Table 3. 6). Factor 2 explained 26.44% of the total variance and was positively loaded with temperature, Secchi depth, electrical conductivity, pH, water depth, total dissolved solids, salinity, nitrite, soluble reactive phosphorous, and ammonia while it was negatively loaded with nitrate and TN: TP ratio. Factor 1 loading represented the changes and water quality status in the highly polluted Cluster 3 (MRM), while Factor 2 loading explained the changes in the least polluted Cluster 1 (WO, GRM, and ZG), while Cluster 2 (AV and RA) were represented as moderately polluted wetlands.

Table 3.6. R-mode varimax rotated factor analysis of water quality variables (number of variables = 12) factor loadings > 0.70 in bold, Extraction Method: Principal Axis Factoring. Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 12 iterations.

Variable	**Factor 1**	**Factor 2**
Temperature	-0.06	0.39
Electrical conductivity	-0.82	0.42
pH	0.12	0.66
Water depth	0.87	0.42
Total dissolved solids	0.14	0.71
Salinity	-0.87	0.39
Nitrite	-0.91	0.09
Nitrate	-0.82	-0.36
Soluble reactive phosphorous	0.24	0.92
Total nitrogen-total phosphorous ratio	0.34	-0.75
Ammonia	0.82	0.19
Eigenvalues	5.43	3.17
% Variance	45.27	26.44
Cumulative Variance	**45.27**	**71.71**

3.3.6. Spatiotemporal variations of trophic status indices using multivariate analysis

Tests between-subject effects for each dependent variable using univariate analysis are shown in Table 3.7. Wetland had a significant effect (ANOVA, $p< 0.05$) on TOT_{TSI}, TSI_{TP}, TSI_{Chla}, and TSI_{STD} while TSI_{TN} did not differ (ANOVA, $p> 0.05$) among the six wetlands. On the other hand, the season had a significant effect (ANOVA, $p < 0.05$) on TOT_{TSI}, TSI_{TP}, and TSI_{SDT} while TSI_{Chla} did not differ (univariate ANOVA, $p > 0.05$) across the four seasons. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of TOT_{TSI}, TSI_{TN}, TSI_{TP}, TSI_{Chla}, and TSI_{STD}.

Table 3.7. Univariate ANOVA table and test of significance of Carlson Trophic Status Indices in Lake Tana wetlands

Effect	**Variable**	**Df**	**SS**	**MS**	**F**	**P**
Wetland	Total nitrogen trophic state index	5	2069.3	413.9	2.146	0.08
	Total phosphorous trophic state index	5	1847.23	369.45	2.8345	0.02
	Chlorophyll-a trophic state index	5	670.4	134.1	4.207	0.00
	Secchi disk transparency trophic state index	5	8369.7	1673.9	38.611	0.00
	Total trophic state index	5	1338.9	267.8	8.025	0.00
Season	Total nitrogen trophic state index	3	4404.8	1468.3	7.613	0.00
	Total phosphorous trophic state index	3	1409.24	469.75	3.6040	0.02
	Chlorophyll-a trophic state index	3	256.0	85.3	2.678	0.06
	Secchi disk transparency trophic state index	3	1824.1	608.0	14.025	0.00
	Total trophic state index	3	442.5	147.5	4.420	0.01
Wetland x Season	Total nitrogen trophic state index	15	11629.9	775.3	4.020	0.00
	Total phosphorous trophic state index	15	4519.97	301.33	2.3119	0.01
	Chlorophyll-a trophic state index	15	1267.7	84.5	2.652	0.00
	Secchi disk transparency trophic state index	15	4201.4	280.1	6.461	0.00
	Total trophic state index	15	1832.0	122.1	3.660	0.00

Total trophic state index (TOT_{TSI})

The mean TOT_{TSI} was 64.4 ± 8.7. There was a significant interaction between wetland and season (ANOVA, $p < 0.05$) for TOT_{TSI}. Values ranged from 43.74 to 62.53 (mean: 50.69 ± 10.30) in GRM during the late rainy season and from 77.18 to 86.81 (mean: 81.43 ± 4.91) in MRM during the late rainy season (Figure 3.4a).

Total nitrogen trophic state index (TSI_{TN})

The mean TSI_{TN} was 94.2 ± 19.6. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of TSI_{TN}. The mean value ranged from 48.67 to 87.68 (mean: 63.15 ± 21.36) in GRM during the late rainy season and from 107.68 to 125.76 (mean: 116.86 ±

9.06) in WO during the dry season. Significant differences between combinations of wetlands and seasons are indicated in Figure 3.4b.

Total phosphorous trophic state index (TSI_{TP})

The overall mean of TSI_{TN} in this study was 29.7 ± 14.0. There was a significant interaction between wetland and season (ANOVA, $p < 0.05$). Values ranged from 8.45 to 12.63 (mean: 4.52 ± 9.44) in WO during the early rainy season and from 34.14 to 63.19 (mean: 50.17 ± 14.76) in AV during the late rainy season (Figure 3.4c).

Chlorophyll-a trophic state index (TSI_{Chla})

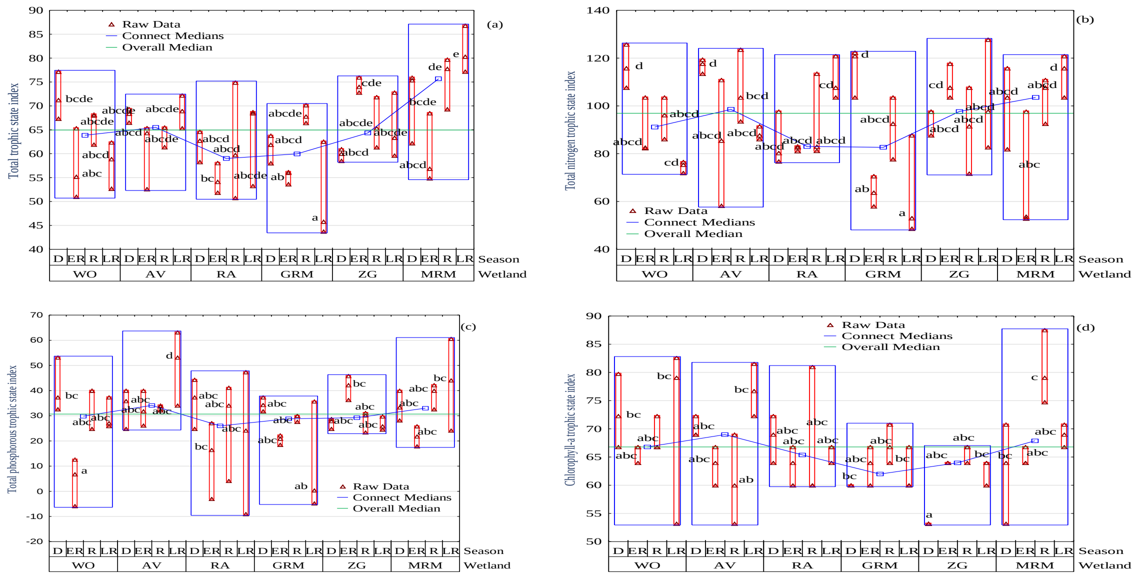
The mean TSI_{TN} was 66.3 ± 7.2. There was a significant interaction between wetland and season (ANOVA, $p<0.05$) for the mean value of TSI_{Chla}. The mean value of TSI_{Chla} was 52.19 ± 0.001 in GRM during the late rainy season and from 53.19 to 70.76 (mean: 80.44 ± 80.86) in MRM during the rainy season (Figure 3.4d.)

Secchi disk transparency trophic state index (TSI_{STD})

TSI_{TN} averaged 67.6 ± 15.2. There was a significant interaction between wetland and season (ANOVA, $p < 0.05$). Values ranged from 34.26 to 40.99 (mean: .34.87 ± 5.33) in GRM during the dry season and from 90.33 to 116.37 (mean: 100.54 ±13.86) in MRM during the late rainy season (Figure 3.3 e).

3.3.7. Spatiotemporal variations in water quality indices (WQI) of Lake Tana wetlands

The mean WQI value in this study was 56.48 ± 101.14. There was no significant interaction between the wetland and the season (ANOVA, $p > 0.05$). Likewise, the mean value of WQI did not differ (ANOVA, $p>0.05$) among wetlands ranging from 14.76 to 61.69 (mean: 39.76 ± 14.53) in GRM and from 17.43 to 880.19 (mean:117.23 ± 242.27) in MRM. The season had no effect (ANOVA, $p>0.05$) on the mean value of WQI ranging from 10.63 to 81.31 (mean: 27.68 ± 18.15) in the early rainy season and from 30.72 to 880.19 (mean:97.94 ± 197.21) in the late rainy season. WO, GRM, AV, and ZG were under the category of excellent water while RA is considered good water. In contrast, MRM falls under the category of poor water (Figure 3.5.



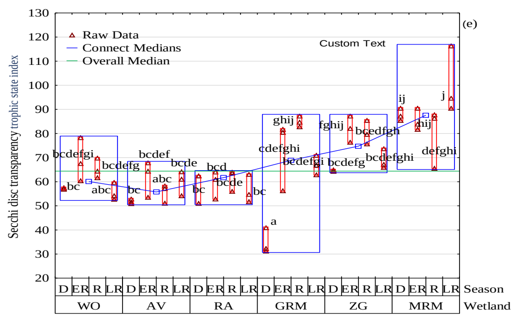


Figure 3.4. Spatiotemporal variability plot of Trophic State Indices in the wetlands of Lake Tana. WO-Wonjeta, AV-Avai, RA-Ras Abbay, GRM-Gumara River mouth, MRM-Megech River mouth, ZG-Zedie Girar, D-Dry, ER-Early rainy, R-Rainy, LR-Late rainy. Standard TSI criteria: < 40 = Oligotrophic, 40 – 50 = Mesotrophic, 50 – 70 = Eutrophic, > 70 = Hypereutrophic. N = 24.

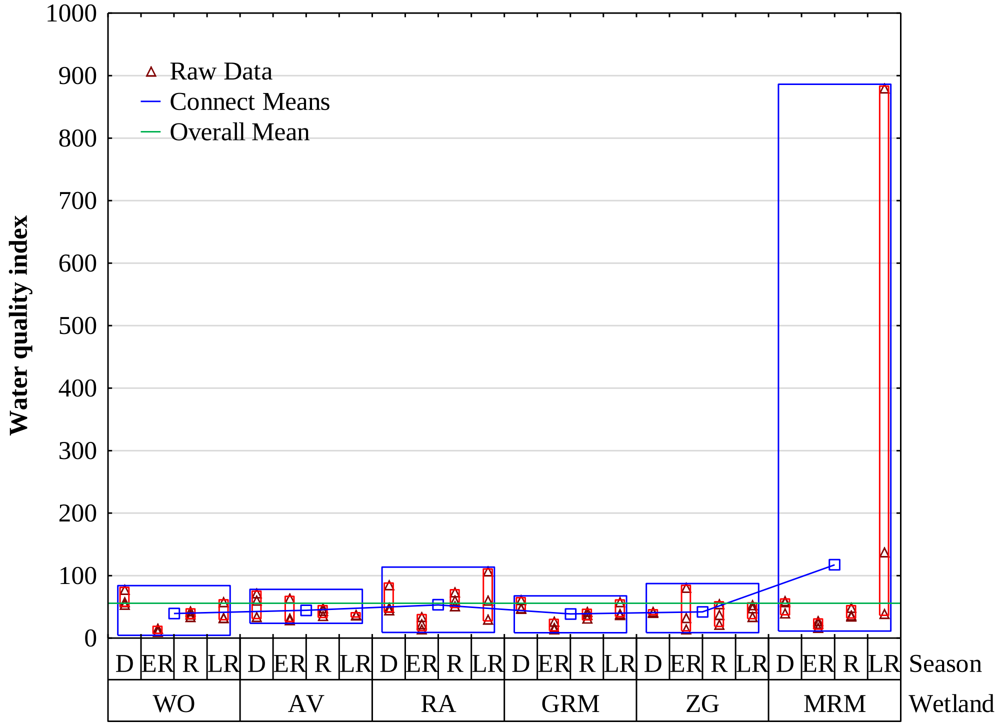


Figure 3.5. Spatiotemporal variability plot of Water Quality Index (WQI) in the wetlands of Lake Tana. WO-Wonjeta, AV-Avai, RA-Ras Abbay, GRM-Gumara river mouth, MRM-Megech river mouth, ZG-Zedie Girar, D-Dry, ER-Early rainy, R-Rainy, LR-Late rainy

3.4. Discussion

3.4.1. Evaluation of physicochemical variables using multivariate analysis

Physicochemical characteristics of the study wetlands showed distinct spatiotemporal trends. Wetland and season had a significant interaction for dissolved oxygen, pH, Secchi depth a.m., Secchi depth p.m., salinity, nitrate, ammonia, total nitrogen, total phosphorous, and chlorophyll-a, based on seventeen physicochemical variables. Previous studies on Ethiopian water bodies have shown similar results (Fetahi, 2019; Kassa & Tibebe, 2019; Tibebe et al., 2019; Umer et al., 2020; Dersseh et al., 2020; Getnet et al., 2020; Dagne et al., 2021; Wagaw et al., 2021; Tibebe et al., 2022). Many of these studies have highlighted seasonal and spatial variations in nutrient and physicochemical levels during dry and wet seasons (Wondim et al., 2016; Umer et al., 2020;

Tibebe et al., 2019; Getnet et al., 2020; Dagne et al., 2021; Wagaw et al., 2021; Tibebe et al., 2022). Several studies on Ethiopian water quality have focused on assessing water quality variables and trophic state indices (Adimasu, 2015; Fetahi, 2019; Teshome, 2020; Lencha et al., 2021), as well as some aspects of comprehensive pollution indices (Ghebremedhin & Gupta, 2023). However, these studies often overlooked seasonal variations, relied on limited water quality indicators and trophic state indices, and lacked integrated approaches for aquatic environmental quality assessment (Tibebe et al., 2019; Dersseh et al., 2020; Lencha et al., 2021; Aragaw et al., 2022; Mohammed et al., 2023). Although multivariate techniques have been recommended for national water quality monitoring programs (Begum et al., 2023), no previous study in Ethiopian water bodies has applied these techniques to analyze and interpret multidimensional water quality data in Ethiopian aquatic systems. This includes identifying pollution sources, understanding temporal and spatial variations across four seasons, and informing effective water quality management strategies. Unlike previous research, this study collected water quality data across four seasons: dry, early rainy, rainy, and late rainy. It employed various methods to assess aquatic environmental conditions in the wetlands and to compare the effectiveness of each method. By utilizing multivariate techniques, this study aimed to advance scientific knowledge of the spatial extent of water quality problems in nine districts along Lake Tana's shoreline in Ethiopia.

3.4.2. Assessment of spatiotemporal variations of physical parameters of Lake Tana

Temperature is an important factor that regulates the biogeochemical activities in the aquatic environment. Although the mean value of surface water temperature did not differ among wetlands, the mean temperature observed in this study was higher than the water temperature recorded in Lake Tana (Wondie et al., 2007b; Vijverberg et al., 2009; Wondim et al., 2016; Tibebe et al., 2019b), Lake Navaishia (Ndungu, 2014), and Soda lakes (Melese & Debella, 2023), Lake Ziway (Abnet & Seyoum, 2020), and Eleyele Lake (Ayoade & Ikulala, 2007).

The mean water temperature in this study showed significant variation across seasons with maximum mean values in the early rainy season. The higher water temperature during the early rainy season could be attributed to high air temperatures (Atobatele & Ugwumba, 2008; Vajravelu et al., 2018) Seasonal variability of African lake temperatures has been reported by many studies

(Damo & Icka, 2013). Umer et al. (2020) reported maximum temperatures in the rainy and dry seasons of 35.5 °C and 28.4 °C., respectively in Beseka in the Rift Valley of Ethiopia. Therefore, higher water temperature is detected in WO and RA wetlands. The variations in water temperature among these studies might be explained by the differences in atmospheric temperature of the regions, sampling seasons, and heat absorption potential of the lakes. On the other hand, the significant effect of season on RA, GRM, and MRM might be associated with the presence of the floating macrophytes and the water hyacinth mats which restrained the increase of the water temperature (Van de Moortel et al., 2010). The variability of temperature in different seasons in African lakes has been reported by many studies (Damo & Icka, 2013).

Table 3.8. Comparison of the physical and chemical parameters and nutrients of Lake Tana with other tropical lakes for nutrients

Lake	Temp	DO	EC	pH	SD	WD	TDS	SAL	NO_2	NO_3	SRP	NH_4	TN	TP	Chl-a	Reference
Ziway	23	5	404	8.1	0.2	-	-	-	-	0.21	0.006	-	-	0.311	-	(Tibebe et al., 2022)
Hawasa	23.5	5–7	846	8.66	0.8-5	-	-	-	-	0.025	0.015	-	-	0.034	-	(Tilahun & Ahlgren, 2010)
Chamo	26.3	5–9	1910	8.84	0.1-8	-	-	-	-	0.033	0.118	-	-	0.182	-	(Tilahun & Ahlgren, 2010)
Hayq	18.2	1-8.4	910	9	2.7					0.042	0.022			0.058	-	(Fetahi, 2010)
Abaya	-	-	623	8.9	-			-	-	-	0.04	-		-	-	(Wondie & Mengistou, 2006)
Langano	-	-	1810	9.4	-	-	-	--	-	-	0.09	-	-	-	-	(Wood & Talling, 1988)
Bishoftu	-	-	1830	9.2	-	-	-	-	-	-	0.005-0.1	-	-	-	-	(Wood & Talling, 1988)
Abijata	-	-	15,800	10.2	-	-	-	-	-	-	0.005	-	-	-	-	(Wood & Talling, 1988)
Shala	-	-	19,200	9.9	0 -20	-	-	-	-	-	0.76	-	-	-	-	(Melese & Debella, 2023; Wood & Talling, 1988)
Beseka	28.4-35.5		1407 - 3321	7.5-10.9	-	-	-	--	-	-	-	-	-	-	-	(Umer et al., 2020)
Chitu	-	-	28,600	9.8	-	-	-	-	--	-	1.7	-	-	-	-	(Wood & Talling, 1988)
Tana	23.2	6.7	132.8	7.7	0.05-0.8	2.0 2.5	0.02-0.5	0.01	0-3.4	0.003 -4.7	0.326-1.6	0.0-6.6	1.1	0.5	6.4-9.9	(Melaku & Yalew, 2022; Wondim et al., 2016; Vijverberg et al., 2009; Wondie et al., 2007b)
Tana	24.02	6.1	153.8	6.8	0.9	2.2	1.3	0.07	0.02	0.5	0.5	0.2	2.2	0.9	5.1	Present study

The overall mean DO concentration in this study (6.08 ± 0.9 mg/L) was similar to the range of value (1-8.4) reported in Ethiopian lakes (Fetahi, 2010; Tilahun & Ahlgren, 2010; Vijverberg et al., 2009; Wondie et al., 2007a). In contrast, the overall mean DO concentration in this study was lower than the value reported in Hawassa by (Abate et al., 2015a) (11.2-21.42 mg/L) which may be due to a result of hyper-eutrophication combined with measuring in the afternoon, and Soda lakes by Melese & Debella, (2023) while Tibebe et al. (2022) reported a lower mean (5 mg/L) DO value in Ziway. The lowest DO values in the dry season at WO were attributed to human impacts like fishing and laundry washing while the lowest DO values in MRM were attributed to its muddy water from agricultural and urban waste runoff (Tibebe et al., 2022). The highest values of DO in AV, RA, GRM, and ZG wetlands in the rainy season may be due to high dilution. The mean concentration of DO in the present study was greater than the minimum requirement for the survival of aquatic life (i.e. >5 mg/L; (Johansen et al., 2006)). Concentrations below 4.0 mg/L adversely affect aquatic life (EEPA, 2003). The value of DO in this study is within the EU Directive (1998) and USEPA (2000) permissible limits. According to the EU Directive (1998) and EPA (2015), the standard for dissolved oxygen for fisheries and aquatic life is between 5.0 to 9.0 mg/L.

Mean EC at the six wetlands ranged from 89 µS/cm to 327 µS/cm with the highest values in MRM in the late rainy season. This range is lower than the values from previous reports on Ethiopian water bodies (Tibebe et al., 2022; Umer et al., 2020; Fetahi, 2010; Tilahun & Ahlgren, 2010; Wood & Talling, 1988). The mean value of EC in the present study was lower than that reported by Melese & Debella, (2023) in Soda Lakes. Significant seasonal variation was noted during the study with maximum EC values during the rainy season of 327 µs/cm. The seasonal variation of EC of Lake Tana may be due to different anthropogenic and naturally induced pollutants such as inorganic and organic pollutants from run-off. According to EEPA (2003), EC levels above 1000 µs/cm limit the use of water for drinking. The mean value of EC in this study was within the normal ranges mentioned in EU and WHO guidelines. The conductivity of most freshwater bodies ranges from 10–1000 µS /cm (Rice et al., 2012) but may exceed 1000 µS /cm, especially in polluted waters, or those receiving large quantities of land run-off (Eaton et al., 1995).

The mean pH of the lake water was 6.81 ± 0.82 which is lower than reported by Girma and Ahlgren (2010) (8.65), (8.44), by Tibebe et al. (2022) (8.1) and by Melese and Debella (2023) (>8.5). The maximum and lower values of pH in WO and RA can be attributed to the high alkalinity of the lake due to different ions for example, K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+} (Umer et al., 2020). However, no significant seasonal variation was noted in the present study. This is not in agreement with the report by Umer et al. (2020) for Lake Beseka with maximum pH values for the rainy and dry seasons. Exudates from freshwater swamps can primarily adjust the pH level by controlling the acidity of water bodies. It is usual to record a pH between 6 and 8.5 (Rice et al., 2012). According to EPA (2015), Lake Tana's pH is within allowable limits.

The mean SD value (a.m. and p.m.) of 0.94 ± 1.29 m is in agreement with other studies in Ethiopia (Wondie et al., 2007a; Vijverberg et al., 2009; Tilahun & Ahlgren, 2010; Wondim et al., 2016; Melese & Debella, 2023). However, the mean value of SD in this study is higher than values of 0.21 m in previous studies in Lake Ziway by Tibebe et al. (2022) while it is lower than the mean SD in Hayq reported by Fetahi (2010) (2.7 m). The minimum SD in MRM may be attributed to the accumulation of sediments from agriculture and urban effluents drained by the Megech River. The seasonal variation in SD at WO, ZG, and GRM can be primarily attributed to lower levels of catchment degradation and siltation in these wetlands. One of the signs that point to an increase in the lake's turbidity is the decreasing trend in SD, which is mostly caused by siltation and catchment degradation. In general, the SD was found to be at its lowest level during the rainy season, and highest during the dry season at all sampling sites, due to the large amount of run-off silt that enters the lake in the wet season. Ndungu, (2014) reported that SD increased during the dry season in tropical freshwater water bodies. This may be due to the undisturbed watershed, which keeps the soil system intact during the dry season.

The average WD value was 2.22 ± 1.36 m, which is consistent with other research conducted in Lake Tana (Wondie et al., 2007b; Vijverberg et al., 2009; Wondim et al., 2016). However, the mean value of WD in this study is lower than the values in previous studies in Shala by Melese & Debella (2023) of 0 – 20 m and higher than the depth in Ziway by Tibebe et al. (2022) (0.21m). The highest mean value of water depth in RA may be attributed to the lower load of sediment drained into this wetland. The significant effect of season on the mean value of WD in WO, AV, RA, and ZG may be associated with the complex pattern of water losses and inputs that can cause large daily and seasonal water level fluctuations. Water levels are highest at the end of the main rainy season and during the post-rainy period, slowly decreasing to a minimum around the end of the dry season (Vijverberg et al., 2009). The lower mean water depth in MRM is likely associated with a high amount of sediment load from agricultural and urban effluents.

Total dissolved solids (TDS) is one of the most important water quality parameters. The mean TDS was 0.13 g ± 0.19 g/l. This was higher than the values reported for Lake Tana by Wondim et al. (2016) (0.02 - 0.5 g/l). The mean TDS value in this study was lower than the mean TDS value reported by (Zelalem & Prokin, 2017) in Lake Tana, which ranged from 0.065 g/L to 0.77 g/L. However, it was higher than the mean TDS values of 0.74 to 1.598 g/L found in Lake Beseka by Umer et al. (2020). When compared with the TDS values of Lake Naivasha in Kenya (values ranged from 1.24 to 2.05 mg/L, with a mean of 1.52) by Ndungu (2014) and the mean of TDS value of Lake Hawassa in the southern part of Ethiopia (with the highest value of 4.56 mg/L) by Adimasu (2015), the TDS value of Lake Tana was low.

The mean value of salinity in this study was 0.07 ± 0.02 ppm which is in line with the results in Lake Tana (Kassa et al., 2021; Kassa & Tibebe, 2019; Tibebe et al., 2022) with ranges of 0.07 ppm - 0.16 ppm). However, the mean value of salinity in this study is lower than in previous reports for Lake Tana by (Wondie et al., 2007a) (0.1ppm). The high level of salinity in MRM in the rainy season is in agreement with previous reports in Soda lakes of Ethiopia by Melese & Debella, (2023), in Lake Shala, and by Kihwele et al. (2015) in Manyara Lake. Alkalinity levels were high during the post-rainy season in Lakes Beseka and Chittu and during the dry season in Lake Shala (Melese & Debella, 2023). Overall, the salinity levels were still low and within normal ranges of EEPA (2003) and WHO guidelines (500 mg/L).

3.4.3. Spatiotemporal variations of nutrients in Lake Tana

The mean nitrite value of 0.02 ± 0.09 mg/L was higher than values reported in previous studies. For instance, (Beneberu & Mengistou, 2009) and (Tamire & Mengistou, 2013) reported 0.06 and 0.01 mg/L nitrite, respectively. However, the mean value of nitrite in the present study was lower than the reports for Lake Tana by Wondim, (2016) (0.2mg/L), Shitaw et al. (2018), (0.418), and in Lake Adele of eastern Ethiopia (30.67 mg/L), and by Tibebe et al (2022) (0.5 mg/L). Relatively higher nitrite concentrations were measured in MRM in the late rainy season which could be due to the application of the high amount of fertilizer for crop production in the adjacent farming lands of Megech River catchment. The presence of nitrite in water may mainly result from excessive application of fertilizers. The mean nitrite levels were comparatively low but above the normal ranges given by EEPA (2003) and USEPA (2000) (0.001 mg/L).

The mean nitrate in this study (0.46 ± 0.25 mg/L) was higher than values of 0.21 0.17, 0.003, and 0.06 mg/L reported by (Tamire & Mengistou, 2013; Tibebe et al., 2022; Tilahun, 1988; Tilahun & Ahlgren, 2010), respectively. The high concentration of nitrate in ZG and MRM in the rainy and late rainy seasons is probably because of nutrient enrichment of the littoral zone of the lake from agricultural effluent sources from the catchment area. The observed nitrate concentration in this study is within normal ranges of EEPA (2003) and USEPA (2000) (50 mg/L).

The mean SRP concentration (0.54 ± 0.31 mg/L) was higher than in previous reports in freshwater lakes of Ethiopia (Tibebe et al. 2022; Tibebe et al. 2019; Tamire and Mengistou, 2013; Wondie and Mengistou, 2006; Gebre-Mariam and Desta, 2002; Jeppesen et al., 2000; Tilahun, 1988) was higher than that of the previous reported which was 0.016, 0.01, 0.059 0.029, 0.06, and 0.326 mg/L respectively. The high value of SRP in RA wetlands during the early rainy season may be attributed to organic and non-organic discharge of water from domestic sources around the wetland vicinity. The measured concentration is also beyond the range of its threshold (0.05 to 0.1mg/L) as a nutrient for natural waters (Jeppesen et al., 2000; Wondie & Mengistou, 2006).

The mean SRP concentration (0.660 ± 0.084 mg/L) in the late rainy season was higher than the values reported by (Gebre-Mariam & Desta, 2002; Kebede et al., 1994; Tamire & Mengistou, 2013; Tilahun & Ahlgren, 2010) for other Ethiopian Lakes (0.016, 0.035, 0.01 and 0.029 mg/L). However, this value is lower than that reported by Melaku & Yalew (2022) (1.6 mg/L). The maximum allowable concentration of phosphorous that is permissible in drinking water is 1 mg/L (USEPA, 2000; WHO, 2009). The elevated phosphate levels in RA during the late rainy season may result from the heavy use of chemicals such as detergents and runoff from car washes, which introduce both organic and inorganic pollutants from domestic sources into the lake.

The mean concentration of ammonia (0.17 ± 0.21 mg/L) (table 3.11) was similar to reports by (Tibebe et al., 2022) (0.121 mg/L) (Tilahun 1988) (0.111 mg/L), and (Tamire & Mengistou, 2013) (0.143 mg/L) but higher than values reported by (Kebede et al., 1994) (0.036 mg/L). However, the mean value of ammonia in this study was lower than that reported for Lake Tana by Wondim (2016) (0.0-6.6 mg/L) and (Melese & Debella, 2023) ammonium nitrogen had the highest value (56.39-161.93) in Lake Arenguade and the lowest in Lake Beseka. When compared with the mean total ammonia values of Lake Naivasha in Kenya (the mean varied between 0.045 to 0.085 mg/L with a mean of 0.063 mg/L by Ndungu (2014), the total ammonia value of Lake Tana was higher. The high value of total ammonia in AV and GRM during the late rainy season may be attributed to hospital effluent discharged into AV, and to agricultural effluent discharged into GRM, respectively.

The mean TN concentration of 2.23 mg/L was lower than the values (5.6–21 mg/L) reported in Lake Ziway of Ethiopia by Tibebe et al. (2022). The relatively high concentration of TN in AV and MRM in the late rainy season could be due to chemicals from the hospital in AV the application of fertilizers on cropland and the decomposition of organic matter washed off into MRM. Season did not influence TN values, which is not in line with reports by (Tibebe et al., 2018; Tibebe et al., 2022). The agricultural office report indicates that the application of diammonium phosphate (DAP) and urea fertilizer for rain-fed and irrigation agriculture is increasing in farmlands adjacent to Lake Tana. The observed TN concentration value in this study is within normal ranges of EEPA (2003) and USEPA (2000) (1.1 mg/L).

The mean TP value of the lake water was 0.89 ± 0.98 mg/L which is higher than in reports in freshwater lakes of Ethiopia (Tibebe et al., 2022), by Melaku and Yalew (2022), Kebede et al. (1994), and Tilahun (1988), which were 0.311, 0.48, 0.069 and 0.219 mg/L, respectively. A higher TP concentration was also measured in this study as compared to that reported for other Ethiopian rift valley lakes like Lake Awasa and Chamo (Tilahun, 1988). In contrast, the mean value of TP in this study was lower than the values in Soda Lakes of Ethiopia (Melese & Debella, 2023) (0.75-2.41 mg/L). However, the mean value of TP in this study was lower than in the report by (Dersseh et al., 2020) (0.01-1.8 mg/L) in Lake Tana. The increasing trend in TP is probably due to nutrient enrichment of the lake from agricultural activities around the lake watershed (Goshu & Aynalem, 2017; Wondie, 2018; Ayele & Atlabachew, 2021). The observed TP concentration value in this study was outside the normal ranges of EEPA (2003) and USEPA (2000) (0.05 to 0.1 mg/L).

The mean value of Chl-a in this study was 5.15 ± 5.23 mg/L which was lower than the report in Lake Tana by Melaku and Yalew (2022), (0.99 mg/L), by Mucheye et al. (2022) (2.52 mg/L), by Kahsay et al. (2022) (1.0-4.0 mg/L), by Tibebe et al. (2019) (8.0 mg/L) and by Wondie & Mengistu (2017) (0.03-13 mg/L) by Vijverberg et al. (2009), (0.64 mg/L), by Wondie et al. (2007) (0.61 mg/L) and by Dejen et al. (2004) (0.64 mg/L). The highest value of Chl-a concentration in MRM in the late rainy may be attributed to the influx of sediment and nutrient load from the upper catchment. Lake Tana water Chlorophyll–levels were above the permissible level (0.3 mg/L) (Trodden & O'Boyle, 2020).

The ratio of total nitrogen to total phosphorus (TN: TP) in lakes is important because it helps us understand whether nitrogen or phosphorus is limiting the growth of algae. According to the report by Smith, (1962) a TN: TP ratio less than 29 can lead to an overgrowth of blue-green algae, while a ratio greater than 29 is less likely to have this problem. The mean value of the TN: TP ratio was 6.1 ± 10.6, which was lower than reported for Lake Ziway by (Tibebe et al., 2018) (48:1), and Lake Hawass by (Lencha et al., 2021) (31:1). There was no significant difference in the TN: TP ratio among the six wetlands and the four seasons, thus, Lake Tana wetlands are hypereutrophic (Downing & McCauley, 1992).

The underlying reasons for such spatial and temporal variations in the water quality parameters are likely unsustainable anthropogenic activities such as agricultural activities, urbanization, and the resultant discharge of waste into the lake. Seasonal variation significantly influenced key physicochemical parameters, including dissolved oxygen, conductivity, pH, water transparency, and nutrient concentrations. These seasonal changes were consistently observed across all wetlands and aligned with the classification of pollution levels. For instance, nutrient concentrations such as nitrate, ammonia, and total nitrogen were generally higher during the rainy and late rainy seasons, likely due to increased runoff from agricultural and urban sources. In contrast, lower concentrations during the dry season reflected reduced external inputs and dilution effects. These patterns support the classification of Megech River Mouth as the most polluted, followed by Avaj and Ras Abbay, with Wonjeta, Zewdie Girar, and Gumara River Mouth showing comparatively better water quality. The consistency of seasonal trends across parameters reinforces the robustness of the wetland pollution classifications. Most of the water parameters in the disturbed wetlands revealed lower qualities in the rainy and late rainy season, which can be associated with a high influx of effluents from agricultural lands. Significant differences were recorded in the concentration of nutrients (nitrate, ammonia, and total nitrogen) between seasons. The higher level of nitrate and total nitrogen in the dry season may be attributed to a lower dilution effect in the dry season. Similar reports on the seasonal distribution of nitrate and nitrite levels in the wetlands of Nigeria were reported by Nwankwoala et al. (2010) and Udom et al. (2018). The higher phosphate and ammonia levels recorded during the late rainy season could be attributed to additional discharge from the catchment areas, such as sewage discharge from Bahir Dar and Gondar towns, as well as runoff from the surrounding farmlands due to heavy rainfall. The seasonal influx of allochthonous organic and inorganic materials during the rainy and late rainy seasons is characteristic in most tropical wetlands (Angello et al., 2020; Bagalwa et al., 2021; Nwankwoala et al., 2010; Saturday et al., 2021; Soro et al., 2020).

Overall, the average concentrations of SRP, ammonia, nitrite, nitrate, TN, and TP were low during the dry season, while higher average concentrations were noted during the rainy and late rainy seasons. This indicates point source pollution for these parameters, which might be associated with industrial effluents, human interference, and agricultural and urban effluents (Tibebe et al., 2022). During the dry season, both decreased precipitation and increased agricultural croplands contributed to lower flows of those nutrients, however, SRP, nitrite, nitrate, TN, and TP all had higher concentrations during rainy and late rainy seasons. Wondie & Mengistou, (2006) reported that nutrients are higher in the dry season than in the rainy season when nutrients come from specific sources that have a constant supply. Nutrients from other sources, like runoff during heavy rains, tend to be higher in the rainy season. The analysis of water quality data in Lake Tana revealed that there has been a progressive increase in the concentration of various parameters like EC, TDS, nitrate, ammonia, total nitrogen, and total phosphorous when compared to earlier records (Dersseh et al., 2022; Vijverberg et al., 2009; Wondie et al., 2007a; Wondim, 2016) as temperature increased from 23.2°C to 24.02 °C, EC increased from 132.8 µS/l to 153.8 µS/cm, TDS increased from 0.3 g/l to 1.3 g/l, TN increased from 1.1 mg/L to 2.2 mg/L and TP increased from 0.5 mg/L to 0.9

mg/L between 2009 and 2020 (Setegn et al., 2009; Wondim et al., 2016; Zimale et al., 2018; Mucheye et al., 2018; Goshu et al., 2020; Kebedew et al., 2020; Ayele & Atlabachew, 2021; Engdaw et al., 2022)

3.4.4. Spatial Diversity and site grouping based on water quality characteristics

The six study wetlands/clusters of least polluted (WO, GRM, and ZG RA), moderately polluted (AV and RA), and highly polluted (MRM) were investigated for based on water quality physicochemical characteristics following multivariate analyses. The groups were first ranked using hierarchical clustering based on the similarity of the physicochemical characteristics, which was then confirmed by PCA and FA. The moderately polluted cluster comprised two wetlands, AV and RA, and correlated highly with electrical conductivity, salinity, and nitrite on the PCA plot. This cluster was confirmed following the varimax rotation factor analysis and was linked to the positive loading of Secchi depth, pH, water depth, TDS, SRP, TN: TP ratio, and ammonia on Factor One. This registered higher Secchi depth, pH, water depth, TDS, SRP, TN: TP ratio, and ammonia, implying that there was potentially higher photosynthetic activity and algal growth. Moreover, the maximum values of these values in MRM point to the fact that the nutrients in the highly polluted site could be mainly attributed to non-point-sources effluents from urban waste from hospitals in Bahir Dar city. This concurs with the fact that municipal and industrial discharges can contribute ions to receiving waters, increasing the conductivity and nutrients of the receiving waters (Moges et al., 2017; Wondim et al., 2016; Zelalem & Prokin, 2017; Kassa & Tibebe, 2019; Engdaw et al., 2022). These studies also reported that specific physical, chemical, and biological parameters were used to detect pollution sources (Goshu et al., 2010a; Aragaw, 2021; Mushi et al., 2021). Overall, these results reflect the high dissolved nutrients and organic pollution originating from the different catchment activities, implying that Factor One originates from industrial and municipal anthropogenic activities. This concurs with other research that traced the causes of pollution during different study periods in Lake Tana (Mucheye et al., 2018; Zimale et al., 2018; Kebedew et al., 2020; Ayele & Atlabachew, 2021; Dersseh et al., 2022).

The highly polluted cluster, Cluster 3, comprised one wetland, MRM, with electrical conductivity, salinity, and nitrite on the PCA plot. This cluster was confirmed following the varimax rotation factor analysis and was linked to the positive loading of electrical conductivity, salinity, and nitrite Factor Two. The high-value water conductivity, salinity, and nitrite point to the fact that the nutrients in the highly polluted site could be mainly attributed to non-point sources from agriculture and urban effluent from Gondar City. The catchment in MRM is dominated by subsistence agriculture and urban effluent from Gondar town, and this may contribute to the organic matter in the lake. This concurs with many studies in which it was found that catchment agriculture and urban effluent contributed more to water quality deterioration than municipal and industrial effluent (Setegn et al., 2009; Taffese et al., 2014; Assefa et al., 2020; Kebedew et al., 2020; Engdaw et al., 2022). As with the highly polluted wetland findings, high nutrient levels have also been observed in studies on other lakes in the tropics (Namugize & Nsengimana, 2010; Naigaga, 2012; Samanta et al., 2015; Assefa et al., 2020; Obubu et al., 2022).

The Secchi depth, total depth, and ammonia levels in MRM were relatively low compared with other wetlands studies. This could be attributed to the fact that the wetland contains high amounts of particles, which could be from algae or eroded sediment from agricultural farmlands in the catchment (Setegn et al., 2009; Gebremedhin et al., 2018; Wondie, 2018; Zimale et al., 2018; Kebedew et al., 2020; Engdaw et al., 2022). These authors observed relatively high sediments in bays that received effluent from catchments. However, this study was limited to shallow coastline bays (coastal wetland areas) and could not confirm how nutrient levels compare with those in open waters.

The moderately polluted cluster, Cluster 2, i.e., AV and RA, was associated with high temperature, soluble reactive phosphorous, total dissolved solids, pH, water depth, and ammonia. These were confirmed by the positive loadings of these variables following the varimax rotation factor analysis under Factor Two. This explains and confirms the moderate pollution in these sampling locations. This difference was attributed to the dilution effect of the sewage effluent as it moves off the shoreline. This finding is in agreement with studies that pointed to a stronger eutrophication effect in the inshore areas of Lake Tana wetlands by Goshu et al. (2010b), Ademe, (2014), and Wondim et al. (2016). For other highland lakes of Ethiopia it has been shown that sewage discharge reduces water quality, depending on the degree of dilution, the degree of treatment of the original material, the composition, and the response of the ecosystem (Assefa et al., 2020; Dersseh et al., 2022).

In the least polluted cluster, Cluster One, the sampling locations under this group included WO, GRM, and ZG. This cluster correlated highly with nitrate and TN: TP ratio values on the PCA plot, and these were confirmed by the negative loadings of these variables following the varimax rotation factor analysis under Factor Two. The higher values of nitrate and TN: TP ratio in WO, GRM, and ZG.

The higher and stable values of temperature and the higher values of Secchi depth in WO, GRM, and ZG were higher than the values for the rest of the wetlands studied. The higher temperature could be attributed to the fact that these wetlands did not experience wastewater cooling effects as there is no wastewater inlet, and the higher Secchi depth a.m. and p.m. may be attributed to low amounts of sediment particles. The higher temperature in WO, GRM, and ZG is in line with previous findings by various researchers (Namugize & Nsengimana, 2010; Moges et al., 2017; Dallas, 2018; Mucheye et al., 2018).

Overall, the study showed that the MRM wetland, which receives urban and domestic wastewater discharges, was more polluted than the rest of the sites, emphasizing the impact of discharges on water quality. This is in line with findings by many researchers who point to urban effluents as an important underlying factor responsible for surface water quality deterioration in Lake Tana (Setegn et al., 2009; Wondim et al., 2016; Mucheye et al., 2018; Zimale et al., 2018; Goshu et al., 2020; Kebedew et al., 2020; Ayele & Atlabachew, 2021; Engdaw et al., 2022).

Results from the cluster analysis, principal component analysis, and factor analysis complemented each other and led to the establishment of the six clusters. This synchronization in results concurs with previous studies on water quality, which have all recommended the application of different multivariate statistical techniques when dealing with environmental data (Panda et al., 2006; Landau & Chis Ster, 2010; Varol et al., 2012; Liu et al., 2020).

3.4.5. Spatiotemporal status in trophic status of Lake Tana using Carlson trophic state index model

Wetland, season and the interaction of wetland by season had effects on the TSI of Lake Tana. This may be attributed to each wetland receiving different loads of effluents from agriculture and urban. The average TOT_{TSI}, TSI_{TN}, TSI_{TP}, $TSI_{STD,}$ and $TSI_{Chl\text{-}a,}$ values were 64.4± 8.7, 94.2 ± 19.6, 29.7 ± 14.0, 66.3 ± 7.2 and 67.6 ± 15.2, respectively. TOT_{TSI} ranked WO, ZG, GRM, AV, and RA under the category of the eutrophic level while it ranked MRM as hypereutrophic. The findings of this study are not in agreement with several reports on the assessment of aquatic environmental quality using water quality indices in different water bodies of Ethiopia (Lencha et al., 2021; Menberu et al., 2021). Eutrophication causes the impairment of activities, discomfort, and visual unpleasantness that hamper the recreational use of water severely (Breen et al., 2018). Melaku & Yalew, (2022) reported that the trophic state index value of Lake Tana according to the three parameters of the trophic state (TSIC) was eutrophic.

The overall average value of the Trophic State Index (TSI) of Lake Tana was 69.77. This TSI value, based on Carlson's trophic state classification criteria (Kratzer & Brezonik, 1981; Jarosiewicz et al., 2011), suggests that Lake Tana is eutrophic during the early rainy, rainy, and late rainy seasons. When comparing this TSI value to the OECD's standard (Vollenweider & Kerekes, 1982), Lake Tana is in a hypereutrophic state. Similarly, Tibebe et al. (2019), and Wondie et al. (2007a), reported that the lake is above the eutrophic threshold values, placing it in mesotrophic and oligotrophic states, respectively. This could be due to the current anthropomorphic activities around the lake, as well as seasonal effects. The trophic state index of SD exhibited a higher trophic state, probably due to water transparency being the variable most affected by rainfall variations (Klippel et al., 2020).

MRM, which receives municipal, industrial, and agricultural effluents, may be considered hypertrophic. This concurs with (Wondim et al., 2016; Dersseh et al., 2020; Enyew et al., 2020; Ayele & Atlabachew, 2021; Damtie et al., 2021; Dersseh et al., 2022), who studied three shallow bays along Lake Tana shoreline and reported the highest eutrophication in the agriculture-impacted bays due to the invasion of the wetlands and inshore lake by water hyacinth. The findings also agree with studies carried out in other African Lakes, for example, in the Lake Kyoga basin of Uganda by Obubu et al. (2022), Lake Victoria of Kenya by Otieno et al. (2022), in Lake Victoria of Uganda by Wanda et al. (2015), and in the African Great Lakes (Plisnier et al., 2022).

The presence of water hyacinth (*Eichhornia crassipes*) in the agricultural-impacted wetlands (GRM and MRM) may be the cause of water quality changes, with higher levels of EC, pH, SRP, TP, NH_4^{+}, and Chl-a in the impacted wetlands, compared to the least impacted wetlands. Similarly, the water quality values across seasons showed lower values of water transparency and higher values of NO_3, SRP, NH_4^{+}, TP, and Chl-a in the early rainy, rainy, and late rainy seasons compared to the dry seasons in the agricultural-impacted wetlands (GRM and MRM), which are infested with water hyacinth. This finding is in line with the report by (Mucheye et al., 2022), who found seasonal variation in the invasive water hyacinth as well as changes in water quality values (Chl-a and TDS) at the end of the main rainy season. Several reports have indicated that the plausible reason for the infestation of the lake by water hyacinth could be changes in the physicochemical characteristics of the lake (Wubie et al., 2016; Gebremedhin et al., 2018; Damtie & Mengistu, 2022). In addition, Dersseh et al. (2022) demonstrated that water hyacinths appeared in Lake Tana around 2010 after the nitrogen assimilation capacity of the lake was exceeded. This trend was seen mainly in the northeastern part of Lake Tana during rainy seasons, although nutrient concentrations are suitable for growing water hyacinths throughout the lake. The area covered by water hyacinth has increased significantly and positively correlates with the seasonal lake level fluctuation (Asmare, 2017; Asmare et al., 2020; Dersseh et al., 2019, 2020). Similarly, Kipng'eno (2019) reported the spread of water hyacinth in Lake Victoria using satellite imagery and demonstrated that growth in urban areas with high effluent was proportional to the degree of water hyacinth spread.

3.4.6. Spatiotemporal variations in water quality indices (WQI) of Lake Tana wetlands

Wetland, season and the interaction of wetland by season had effects on the WQI of Lake Tana. The overall mean WQI value of 57.7 ± 101.1 was lower than the mean WQI in Lake Tinishu Abaya (Enawgaw & Lemma, 2018)(188-222), In Lake Hawassa by (Meberu et al., 2021) (120.06–228.29), by (Ghebremedhin & Gupta, 2023) in Lake Chamo (102.9-359.5). However, a report for the Ribb reservoir by Mekonnen et al. (2023) (65.42 -101.96) is comparable with this finding. The high mean value WQI in WO, RA, GRM, and MRM during rainy and late rainy seasons is similar to the report by (Teshome et al., 2015) in Hawssa. Therefore, the cumulative result of WQI for drinking, aquatic life, and recreational uses showed that the environmental situation has become worse in the last few decades.

3.5. Conclusions and recommendations

In conclusion, the physicochemical analysis grouped WO, GRM, and ZG as the least polluted, AV and RA as moderately polluted, and MRM as highly polluted wetlands. Elevated concentrations of nitrite, nitrate, ammonia, and soluble reactive phosphorus were consistently recorded in MRM and to a lesser extent in GRM, correlating with higher electrical conductivity, total dissolved solids, and salinity values. Seasonal patterns showed that the rainy season had comparatively improved water quality, while the dry and late rainy seasons exhibited higher pollutant loads. Multivariate analyses (PCA and CA) confirmed these patterns by consistently clustering wetlands according to pollution levels. The findings underscore the strong influence of agricultural runoff, urban effluents, and natural wetland buffering capacity on spatial and temporal water quality variations. These results reinforce the need for continuous monitoring, targeted pollution management, and conservation interventions in the Lake Tana wetlands.

CHAPTER 4

Macroinvertebrates as biological indicators of aquatic environmental quality in wetlands of Lake Tana, Ethiopia

4.1. Introduction

Even with water scarcity and misuse being common, many regions rely solely on surface water for essential needs like drinking, farming, and industry. This makes assessing water quality especially critical (Karr 1998; Sharma and Chowdhary 2011; Shokri et al., 2014). Though traditional methods using physical and chemical measurements provide a snapshot of water quality at a specific time, these methods fall short of revealing the overall health of the water (Ashegh-Malla et al., 2016).

Bioassessment methods, particularly those using macroinvertebrates, offer a more comprehensive and cost-effective alternative. These methods are quick, require minimal laboratory equipment, and provide valuable insights compared to traditional techniques (Azimi et al., 2015; MacDonald et al., 2017). Different species of macroinvertebrates can withstand different levels of pollution. By analyzing the presence, absence, and sensitivity of macroinvertebrates to contaminants, it is possible to evaluate the health of the aquatic environment. This allows us to identify areas with poor water quality and to take steps for improvement (Cooper and Knight 1991; Bere et al., 2016; Raburu et al., 2017). Therefore, macroinvertebrates are excellent bioindicators for monitoring water quality due to several factors. Different macroinvertebrate groups have varying tolerances to oxygen concentrations (Czerniawska-Kusza, 2005; López-López and Sedeño-Díaz 2015). By studying the biodiversity of aquatic macroinvertebrates, scientists can estimate the amount of oxygen in the water. Macroinvertebrates are commonly found in most freshwater environments, including small streams with limited fauna (Clarke et al., 2008; López-López & Sedeño-Díaz, 2015). Macroinvertebrates are easy to collect and analyze, making macroinvertebrates a practical choice for biomonitoring. Most macroinvertebrates have restricted movement or live in one place (Bass, 2007; Muralidharan et al., 2010). This makes macroinvertebrates good indicators of local water quality, reflecting conditions at the specific location where macroinvertebrates are collected. Many macroinvertebrate species have lifespans of a year or more (Muralidharan et al., 2010). This allows macroinvertebrate studies to integrate the effects of long-term environmental conditions, including exposure to pollutants.

Ethiopia's current water quality monitoring relies heavily on measuring physical and chemical properties. However, this approach has several drawbacks (Zelalem and Prokin, 2017; Awoke et al., 2016; Wondim, 2016; Getnet et al., 2022). While many studies have examined how human activities impact Ethiopian wetlands, (Dixion, 2002; Mereta et al., 2012; Gezie et al., 2017; Chawaka et al., 2018), the need for ongoing assessments of these aquatic ecosystems remains critical (Dixion 2002; Mehari et al. 2014; Gezie et al. 2017; Getnetet al., 2022).

Lake Tana basin's shoreline wetlands are abundant with diverse aquatic life (Vijverberg et al., 2012; Dejen et al., 2017; Gebremedhin et al., 2018; Ayele and Atlabachew, 2021). The wetlands also support a wide range of macroinvertebrate species, with varying tolerance levels to pollution (Czerniawska-Kusza, 2005; Flores-Lopes & Thomaz, 2011). By analyzing the types of macroinvertebrates present, scientists can gauge the overall health of the aquatic environment. Within a specific region, macroinvertebrate communities tend to respond predictably to changes in water quality (Muralidharan et al., 2010; Lakew and Moog 2015; López-López and Sedeño-Díaz 2015; Bekele et al., 2021; Getnet et al., 2022). This predictability allows researchers to establish baseline data and identify changes over time. With growing pollution in urban waterways around Lake Tana and limited resources dedicated to environmental monitoring, there is a critical need for regular environmental quality assessments (Goshu et al., 2010; Ewnetu et al., 2014; Goshu et al., 2017; Goshu et al., 2020).

Over the past 15 years, numerous studies have been conducted on the diversity of macroinvertebrates in Lake Tana (Vijverberg et al., 2009; Negash et al., 2011; Mehari et al., 2014; Zelalem & Prokin, 2017; Bekele et al., 2021; Getnet et al., 2022). Previous studies have examined macroinvertebrates and physicochemical characteristics in the southern Gulf of Lake Tana; however, macroinvertebrates did not cover wetlands across the entire lake. This chapter expands on that by investigating the role of macroinvertebrates as biological indicators of wetland health in Lake Tana over four seasons and explores the potential application of biological indicators in the environmental quality assessment of wetlands in Ethiopia. Additionally, it aims to contribute to the development of effective monitoring methods for wetland ecosystems.

4.2. Specific Methods

4.2.1. Field sampling

The study involved collecting macroinvertebrate data in the dry, early rainy, rainy, and late rainy seasons over 24 sampling trips between 8 a.m. and 10 a.m. The distance from the dry land to the first point in the wetland was approximately one hundred meters, while the distance of the sample point from the end of the wetland to the lake was approximately sixty meters. The distance from subsequent sample points along the transect was fifty meters. The above-described strategy was used across all wetlands to ensure an unbiased comparison of wetlands. Water-column macroinvertebrates were collected at surface, middle, and bottom depths. Samples for benthic macroinvertebrates were collected using the Ekman Grab method (for an area of approximately 0.0254 m^2) for benthic collection and a D-frame net (Elliott and Tullet, 1978; Borisko et al., 2007; Phillips, 2008) for collecting water column macroinvertebrates. The hauls of benthic macroinvertebrates were pooled and preserved with 5% buffered formalin in polythene bags for transport to the laboratory. To sample macroinvertebrates inhabiting the water column, a D-frame sweep net (mesh size = 1.2 mm, mouth area = 200.96 cm^2, sweep length = 1.5 m) was used to collect macroinvertebrates at the water surface (Meyer et al., 2013; Smith et al., 2019). Three sweeps in an estimated area of 1 m^2 were made for 30 seconds to collect macroinvertebrates in the water column. Samples were taken to the laboratory, washed through a 0.5-mm sieve, and placed in a white enamel pan where live animals were manually separated from plant material. Macroinvertebrate specimens were then preserved in 70% ethanol for identification and enumeration. In the laboratory, all organisms were sorted and taxonomically identified to genus level using a dissecting microscope and macroinvertebrate identification manuals (Clifford, 1991; Feeley et al., 2020; Oscoz et al., 2011; Smith et al., 2019). Members of each taxon were counted, and results were expressed as individuals/sweeps/m^2.

Diversity indices

This study employed multiple diversity indices because no single index can comprehensively capture all facets of biodiversity within an ecosystem or community (Arya, 2021; Gatti et al., 2020; Rajamani & Iyer, 2023). Biodiversity is a complex concept encompassing not only species richness but also species evenness, functional diversity, and genetic diversity (Ogidi & Akpan, 2022). Different indices are designed to highlight specific aspects of this multifaceted concept.

Shannon-Wiener diversity index (H')

The Shannon-Wiener diversity index, often simply called the Shannon Index or Shannon Entropy, is a widely used measure of species diversity. It considers both species richness (the number of different species) and evenness (how evenly individuals are distributed among species). This system uses a number called the Shannon-Wiener diversity index (Wilhm & Dorris 1968). A low index (below 1) indicates high pollution, a medium index (1 to 3) suggests moderate pollution and a high index (above 3) means the ecosystem is not polluted. The Shannon-Wiener Diversity Index (H) was calculated using the following formula:

$$H=\sum_{i\text{-}1}^{S}\frac{Ni}{N}\,\mathrm{Ln}\,\frac{NI}{N}$$

Where N is the total population size of all species, Ni is the proportion of individuals found in species i, and S is the total number of species.

Simpson's diversity index (D)

The Simpson Diversity Index (D) is a measure of biodiversity developed by Simpson in 1949. In 1972, the formula used the following information: "ni" is the number of individuals of a specific species, "N" is the total number of individuals of all species, and "S" is the total number of different macroinvertebrates.

$$D= 1\text{-}\frac{\sum_{i\text{-}1}^{S} n(n\text{-}1)}{N(N\text{-}1)}$$

Margalef's richness index (R)

Margalef's index, developed by Spanish ecologist Ramon Margalef Lopez in the 1950s, assesses species richness, a key aspect of biodiversity. Unlike other richness indices, it adjusts for sampling effort by dividing the number of species by the natural logarithm of the number of individuals. This helps to avoid misleading comparisons between samples with different sampling sizes.

Margalef's Richness Index (R) is derived from the following formula:

$$R = \frac{S-1}{\mathrm{LnN}}$$

Where S is the number of species of macroinvertebrate and N is the total number of individuals in the sample.

Menhinick's index (DMn)

The Menhinick index is an ecological tool used to compare species diversity between different groups of organisms or to track changes in diversity over time. It accounts for sample size by adjusting species richness relative to community size, reducing bias. While it provides a simple, relative measure of species richness, it doesn't consider how evenly distributed species are within a community. Therefore, it's often used alongside other indices to get a more complete picture of community composition and structure. Menhinick's index (DMn) is the number of taxa (S) divided by the square root of the total number of individuals (N).

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

Hill's index (inverted Simpson's index) (H)

Hill's Index, a measure developed by British ecologist Michael O. Hill in the 1970s (Hill, 1973), quantifies community diversity by considering both species richness and evenness. It's a family of indices with different values depending on the parameter "q", which controls sensitivity to species abundance. The index can be mathematically represented as:

$$H' = \frac{1}{\sum_{i-1}^{S} p2\ i}$$

S – species richness, pi – Relative abundance of i^{th} taxon in the sample.

Biotic indices

Hilsenhoff index (HFBI)

The Hilsenhoff Biotic Index measures how tolerant different types of macroinvertebrates are to pollution. A score of 0 means the macroinvertebrates are very sensitive to pollution, while a score of 10 means macroinvertebrates are very resistant to contamination (Hilsenhoff, 1988a). The index is calculated using a specific formula:

$$HFBI=\sum_{i=0}^{n}(TVi)(ni)/N$$

Where "TVi" is the score for each group of macroinvertebrates, "ni" is the number of individuals in each group, and "N" is the total number of individuals in the sample. After calculating the Hilsenhoff index scores, the water quality class was determined based on the interpretation in Table 4.1.

Table 4. 1. A water classification system based on the values of the HFBI Index (Hilsenhoff, 1988b)

HFBI	Water quality	Degree of organic pollution
0.00–3.75	Excellent	Organic pollution unlikely
3.76–4.25	Very good	Possible slight organic pollution
4.26–5.00	Good	Some organic pollution is probable
5.01–5.75	Fair	Fairly substantial pollution is likely
5.76–6.50	Fairly poor	Substantial pollution likely
6.51–7.25	Poor	Very substantial pollution is likely
7.26–10.00	Very poor	Severe organic pollution is likely

Biological Monitoring Working Party (BMWP)

The scoring in this system is based on the resistance of each genus to organic contamination. The highest and lowest scores are assigned to genera with the highest and lowest resistance to contamination, respectively. The scores for each group of macroinvertebrates are added together to get the BMWP score for each location. The BMWP index is calculated using a specific formula:

$$BMWP=\sum N*B$$

Where, "N" is the number of individuals of different groups of macroinvertebrates found in each location, and "B" is the score for the BMWP index (Hawkes, 1998). The water quality is classified based on the BMWP index using Table 4.2.

Average score per taxon (ASPT)

The ASPT index is calculated by dividing the BMWP score by the total number of different groups of macroinvertebrates found in the sample. The water quality is then classified based on the ASPT index using Table 4.2.

ASPT=BMWP/N

EPT index

The EPT index is based on three types of macroinvertebrates, Ephemeroptera, Plecoptera, and Trichoptera because macroinvertebrates are very sensitive to pollution. The higher the EPT index, the less polluted the water is. The index is calculated by adding up the number of families of these three types of macroinvertebrates in the sample (Mandaville, 2002). The water quality is then classified based on the EPT index using Table 4.2.

Table 4.2. Water quality classification based on the values of the BMWP Index (Mandaville, 2002), ASPT Index (McCafferty, 1983), and EPT Index (Hamzaraj et al., 2014; Zakaria & Mohamed, 2018)

Index							
BMWP Score	Category	Interpretation	ASPT Score	Category	Interpretation	EPT Score	Interpretation
0–15	Very poor	Heavily polluted	<4	Poor	Severely polluted	<2	Polluted
16–50	Poor	Polluted or impacted	4–5	Moderate	Moderately polluted	2–5	Clean
51–100	Moderate	Moderately impacted	5–6	Doubtful	Probable polluted	6–10	Good
101–150	Good	Clean but slightly impacted	6<	Good	Unpolluted	>10	Very good
150 <	Very good	Unpolluted, unaffected					

4.2.2. Statistical analysis

Descriptive statistics comprising the means and standard deviation for each parameter were derived. The Simpson diversity index, Margalef's richness index, and Hill's index were not normally distributed based on the Kolmogorov-Smirnov and Shapiro-Wilk tests ($p<0.05$), hence the Kruskal-Wallis nonparametric ANOVA was used to compare sampling locations. However, Shannon-Wiener diversity and Menhinick's diversity indices were normally distributed ($P > 0.05$), hence one-way ANOVA was used for comparison among the study locations. Hilsenhoff index, biological monitoring working party, the average score per taxon, and the EPT index were not normally distributed based on Kolmogorov-Smirnov and Shapiro-Wilk normality tests ($p<0.05$), hence the Kruskal-Wallis nonparametric ANOVA was used to compare sampling locations. Three multivariate statistical methods including cluster analysis (CA), principal component analysis (PCA), and fraction analysis (FA) were used to group the wetlands based on the types of macroinvertebrates found in each. This helped to understand how the wetlands are similar or different in terms of the macroinvertebrate communities in each wetland. The data was analyzed using Statistica software (14.0). Canonical correspondence analysis (CCA) was employed to evaluate the relationship between macroinvertebrate communities and environmental variables using a paleontological statistics software package for data analysis **(**PAST) software (4.14). CCA is a powerful tool for simplifying complex data sets and being a direct gradient analysis, it allows integrated analysis of both taxa and environmental data (Palmer, 1993; Chahouki, 2011; Arimoro & Keke, 2017).

Macroinvertebrate density per square meter was calculated by dividing the total number of individuals collected from each sampling unit by the area sampled. For benthic macroinvertebrates, the Ekman Grab sampler covered an area of approximately 0.0254 m^2 per grab. The total number of individuals from replicate grabs was summed and divided by the total sampled area to obtain density in individuals/m^2. For water-column macroinvertebrates, the D-frame net sweep covered an estimated area of 1 m^2 per sweep. To address potential dominance effects, where a few taxa may skew density estimates, density values were interpreted alongside diversity and biotic indices (e.g., Shannon-Wiener, Simpson's, HFBI) to provide a more balanced view of community structure and ecological condition.

Interpretation of Diversity and Biotic Indices

To ensure clarity and consistency in ecological assessment, the following interpretation ranges were applied: Simpson's Diversity Index (D): Values range from 0 to 1, where values closer to 1 indicate higher diversity and ecosystem stability. Values below 0.5 suggest dominance by a few taxa and potential ecological stress. Margalef's Richness Index (R): Typically ranges from 0 to 5 in freshwater systems. Values <2 indicate low richness (potential pollution), 2–4 moderate richness, and >4 high richness. Menhinick's Index (DMn): Values <1 suggest low diversity, 1–2 moderate, and >2 high diversity. Sensitive to sample size. Hill's Index (H): Values closer to 1 indicate high evenness and diversity; values <0.5 suggest dominance by few taxa. Hilsenhoff Family Biotic Index (HFBI): Interpreted as follows: 0–3.75 (excellent), 3.76–4.25 (very good), 4.26–5.00 (good), 5.01–5.75 (fair), 5.76–6.50 (fairly poor), 6.51–7.25 (poor), 7.26–10.00 (very poor). BMWP (Biological Monitoring Working Party): 0–15 (very poor), 16–50 (poor), 51–100 (moderate), 101–150 (good), >150 (very good). ASPT (Average Score Per Taxon): <4 (poor), 4–5 (moderate), 5–6 (doubtful), >6 (good). EPT Index: <2 (polluted), 2–5 (clean), >10 (very good). Higher values indicate better water quality due to the sensitivity of Ephemeroptera, Plecoptera, and Trichoptera.

The relative abundance of each genus was calculated using the following formula.

$$\text{Relative abundance} = \frac{\text{Abundance of Genus}}{\text{Total abundance of all Genera}}\ x100$$

In the laboratory, all organisms were sorted and identified to the genus level using a dissecting microscope and field guides for freshwater macroinvertebrates from South Africa (Arimoro, 2009). Identification of the genus level was further aided by identification keys from Clifford, (1991), Feeley et al. (2020), and Smith et al. (2019). The number of individuals of each taxon was counted and expressed as individuals/sweep / m^2.

Interpretation of Percent Relative Abundance

Percent relative abundance values were interpreted to assess the dominance and ecological role of macroinvertebrate taxa in each wetland. The following ranges were used for interpretation: >50%: Indicates dominance by a single taxon, suggesting potential ecological imbalance or pollution tolerance. 20–50%: Suggests sub-dominance, where a few taxa are more prevalent but not overwhelmingly dominant. 5–20%: Reflects moderate representation, indicating a more balanced community structure; <5%: Represents rare or less abundant taxa, which may include pollution-sensitive species or those with specialized niches.

4.3. Results

4.3.1. Composition and occurrence of macroinvertebrate taxa in wetlands of Lake Tana

A total of 83 macroinvertebrate taxa, representing 52 families across 19 orders, were recorded, as detailed in Table 4.3. The relative abundance percentages varied significantly between wetlands (H test, $p<0.05$), ranging from 0.11 to 61.36 (median: 1.54) in WO and from undetected to 80.05 (median: 4.60) in MRM. Multiple comparisons revealed significantly higher relative abundance in MRM, GRM, AV, RA, and ZG compared to WO while MRM and GRM had higher relative abundance than AV. There were no seasonal differences in relative abundance (H test, $p > 0.05$) (Figure 2 A).

Macroinvertebrate density per m^2 differed significantly among wetlands (H test, $p<0.05$), ranging from undetectable to 118.20 (median: 4.0) in AV and from undetectable to 12,332 (mean: 39.40) in MRM. MRM had significantly higher densities compared to RA, AV, WO, and ZG. There were no seasonal differences in macroinvertebrate density (H test, $p > 0.05$) (Figure 2 B).

Taxa richness showed no significant differences across wetlands (H test, $p > 0.05$), with a range from undetectable to 29 (median: 2.50) in AV and 6 to 34 (median: 19.50) in WO. Mean richness did not vary by season (ANOVA, $p > 0.05$) (Figure 2 C).

The highest relative abundance of Odonata (e.g., *Anax*, *Enallagma*, *Epitheca*) and Ephemeroptera (e.g., *Baetis*, *Centropitium*) was found at WO, AV, RA, and ZG wetlands, while Oligochaeta (e.g., *Branchiura*, *Chaetogaster*) and Diptera larvae (e.g., *Chironomus*) dominated at ZG, GRM, and MRM.

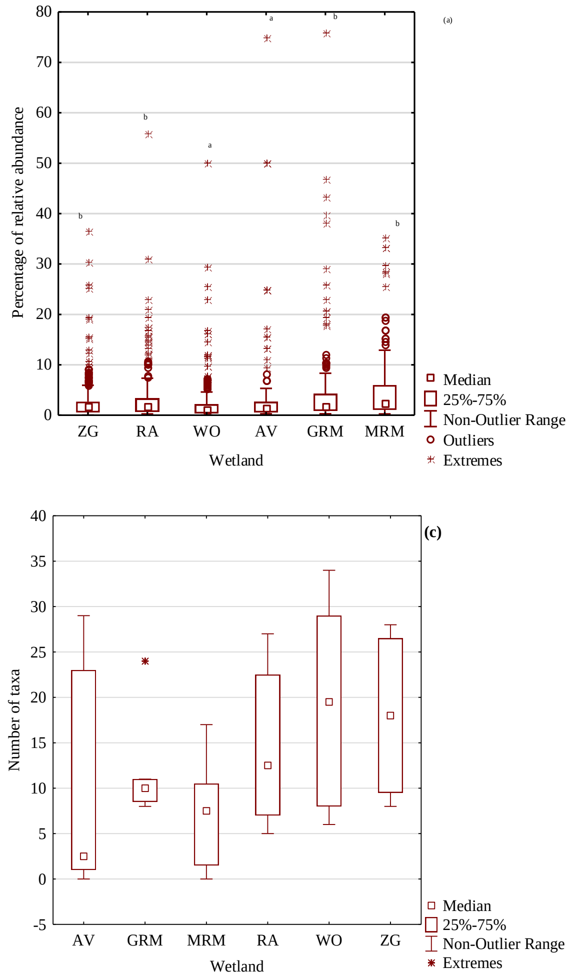


Figure 4.1. Box plots showing (a) relative abundance (%), (b) number of taxa, and (c) density (individuals/m²) of macroinvertebrates across six wetlands. These metrics reflect biodiversity patterns and pollution gradients. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, WO-Wonjeta, RA-Ras Abbay, ZG-Zewdie Girar

Table 4.3. Relative abundance (%) of macroinvertebrate taxa (by order, family, and genus) across six wetlands of Lake Tana. This table offers a detailed taxonomic breakdown used in the calculation of diversity and biotic indices., WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara River mouth, MRM-Megech River mouth, AV-Avaj, and RA-Ras Abbay. A summary row showing the number of taxa per wetland is included for clarity. Note: To improve clarity, a new row titled "Number of Taxa per Wetland" has been added at the bottom of Table 4.3. This row summarizes the total number of macroinvertebrate taxa identified in each wetland, facilitating easier comparison of biodiversity across sites.

Order	**Family**	**Genus**	**AV**	**GRM**	**MRM**	**RA**	**WO**	**ZG**
Odonata	Aeshnidae	*Aeshna*	0.00	0.00	0.00	66.67	0.00	33.33
		Anax	15.90	2.27	4.40	11.36	44.31	22.72
	Coenagrionidae	*Amphiagrion*	0.00	0.00	0.00	0.00	0.00	100.00
		Enallagma	27.27	0.77	0.39	27.66	32.49	11.41
		Ischnura	11.93	1.70	5.11	19.89	31.81	29.54
	Corduliidae	*Epitheca*	0.00	7.69	0.00	0.00	69.23	23.08
		Somatochlora	15.93	1.77	2.65	28.32	23.01	28.32
		Cordulia	0.00	0.00	0.00	100.00	0.00	0.00
		Macromia	0.00	0.00	0.00	0.00	100.00	0.00
	Gomphidae	*Gomphus*	33.33	0.00	0.00	0.00	100.00	0.00
	Libellulidae	*Leucorrhinia*	66.67	0.00	0.00	0.00	0.00	33.33
		Pachydiplax	0.00	0.00	0.00	0.00	50.00	50.00
		Sympetrum	21.48	0.37	0.37	18.15	44.44	15.18
		Libellula	0.00	0.00	0.00	0.00	100.00	0.00
	Lestidae	*Lestes*	12.78	0.34	0.00	29.81	43.61	13.46
Ephemeroptea	Baetidae	*Baetis*	13.80	3.68	0.31	21.77	43.56	16.87
		Centroptilum	18.48	0.54	1.63	21.19	31.52	26.63
		Pseudocloeon	2.00	0.00	0.00	30.00	48.00	20.00
	Caenidae	*Caenis*	19.67	0.30	2.53	20.72	45.16	11.62
	Ephemeridae	*Hexagenia*	100.00	0.00	0.00	0.00	0.00	0.00
	Heptageniidae	*Pseudiron*	0.00	0.00	0.00	0.00	100.00	0.00
	Leptophlebiidae	*Choroterpes*	100.00	0.00	0.00	0.00	0.00	0.00

Continued---

Order	Family	Genus	AV	GRM	MRM	RA	WO	ZG
		Paraleptophlebia	0.00	0.00	0.00	0.00	100.00	0.00
	Polymitarcyidae	*Ephoron*	25.00	0.00	0.00	25.00	50.00	0.00
	Acanthametropodidae	*Analetris*	0.00	0.00	0.00	0.00	100.00	0.00
		Parameletus	17.98	0.74	0.00	9.74	52.06	19.48
	Tricorythidae	*Ranorythus*	100.00	0.00	0.00	0.00	0.00	0.00
Trichoptera	Philopotamidae	*Chimera*	0.00	0.00	0.00	0.00	0.00	100.00
		Wormaldia	0.00	20.00	40.00	0.00	0.00	40.00
	Polycentropodidae	*Polycentropus*	0.00	100.00	0.00	0.00	0.00	0.00
Diptera	Chaoboridae	*Chaoborus*	0.00	0.00	0.00	100.00	0.00	0.00
	Chironomidae	*Chironomus*	1.90	35.00	16.90	12.81	3.41	29.10
		Tanypodinae[*]	0.00	66.67	0.00	0.00	33.33	0.00
		Tanytarsini[**]	7.86	25.00	20.00	15.71	7.86	23.57
	Ceratopogonidae	*Dasyhelea*	0.00	0.00	0.00	0.00	100.00	0.00
	Culicidae	*Mansonia*	100.00	0.00	0.00	0.00	0.00	0.00
	Dixidae	*Dixella*	0.00	0.00	100.00	0.00	0.00	0.00
Coleoptera	Dytiscidae	*Dytiscus*	0.00	25.00	0.00	12.5	62.5	0.00
		Laccophilus	9.10	27.27	0.00	36.36	27.27	0.00
	Gyrinidae	*Gyrinus*	4.08	10.20	10.20	4.08	14.29	57.14
	Haliplidae	*Haliplus*	0.00	93.75	0.00	0.00	6.25	0.00
		Peltodytes	15.62	21.90	0.00	18.75	0.00	43.75
	Hydraenidae	*Hydraena*	0.00	0.00	0.00	0.00	0.00	100.00
	Hydrophilidae	*Berosus*	0.00	0.00	0.00	0.00	0.00	100.00

Continued---

Order	Family	Genus	AV	GRM	MRM	RA	WO	ZG
		Hydrochara	14.29	0.00	0.00	0.00	85.71	0.00
		Hydrochus	0.00	63.64	0.00	4.55	15.91	15.91
		Hydrophilus	100.00	0.00	0.00	0.00	0.00	0.00
	Scirtidae	*Cyphon*	50.00	0.00	0.00	0.00	50.00	0.00
Basommatophora	Lymnaeidae	*Lymnaea*	3.23	6.45	6.45	9.68	25.81	48.39
		Radix	9.09	0.00	0.00	9.09	72.73	9.09
		Stagnicola	6.67	0.00	0.00	20.00	60.00	13.33
	Physidae	*Physa*	13.49	2.38	5.56	21.43	30.95	26.19
	Planorbidae	*Planorbula*	3.23	30.97	2.58	10.97	40.65	11.61
Littorinimorpha	Valvatidae	*Valvata*	5.88	0.00	0.00	1.47	85.29	7.35
Haplotaxida	Tubificidae	*Branchiura*	0.00	25.72	40.52	8.17	3.88	21.70
	Naididae	*Chaetogaster*	0.41	14.40	54.73	5.35	4.94	20.16
	Haplotaxidae	*Haplotaxis*	0.00	13.27	38.78	14.29	9.18	24.49
	Lumbriculiade	*Lumbriculus*	0.00	0.00	100.00	0.00	0.00	0.00
Hemiptera	Belostomatidae	*Lethocerus*	11.43	12.86	1.43	41.43	10.00	22.86
	Corixidae	*Cymatia*	14.39	9.35	7.91	15.83	43.17	9.35
		Micronecta	0.00	0,00	0.00	18.89	53.33	27.78
		Sigara	0.00	0.00	0.00	0.00	100.00	0.00
	Gerridae	*Gerris*	27.27	0.00	0.00	27.27	18.18	27.27
		Limnoporus	0.00	0.00	0.00	0.00	100.00	0.00
	Mesoveliidae	*Mesovelia*	0.00	0.00	0.00	100.00	0.00	0.00
	Notonectidae	*Notonecta*	20.00	0.00	0.00	0.00	0.00	80.00
		Buenoa	4.55	22.73	0.00	13.64	9.09	50.00
	Pelidae	*Paraplea*	35.00	10.00	0.00	0.00	0.00	55.00

Continued---

Order	Family	Genus	AV	GRM	MRM	RA	WO	ZG
Veneroida	Corbiculidae	*Corbicula*	0.00	41.42	27.99	1.12	0.37	29.10
Sphaeriida	Sphaeriidae	*Pisidium*	3.03	12.12	3.03	3.03	3.03	75.76
		Sphaerium	10.00	0.00	10.00	3.33	53.33	23.33
Arhynchobdellida	Erpobdellidae	*Dina*	0.00	9.09	0.00	63.64	9.09	18.18
Plecoptera	Perlodidae	*Kogotus*	100.00	0.00	0.00	0.00	0.00	0.00
Araneae	Pisauridae	*Dolomedes*	4.35	13.04	13.04	4.35	39.13	26.09
Amphipoda	Hyalellidae	*Hyalella*	100.00	0.00	0.00	0.00	0.00	0.00
Oribatida	Hydrozetidae	*Hydrozetes*	0.00	0.00	0.00	0.00	100.00	0.00
Architaenioglossa	Thiaridae	*Melanoides*	0.87	31.30	1.22	18.43	4.52	43.65
Rhynchobdellida	Piscicolidae	*Acanthobdella*	31.58	2.63	7.89	5.26	42.11	10.53
		Piscicola	0.00	0.00	0.00	0.00	66.67	33.33
	Glossiphoniidae	*Placobdella*	0.00	100.00	0.00	0.00	0.00	0.00
		Theromyzon	0.00	0.00	0.00	0.00	100.00	0.00
Lepidoptera	Crambidae	*Paraponynx*	14.29	0.00	0.00	0.00	85.71	0.00
		Petrophila	0.00	0.00	0.00	0.00	0.00	100.00
Number of Taxa			82	91	50	116	153	144

A single asterisk (*) in the Genus column indicates the subfamily, while a double asterisk (**) denotes the tribe.

4.3.2. Relationship between wetlands and macroinvertebrate communities

Hierarchical cluster analysis grouped the six sampling locations into four clusters based on the similarity of the macroinvertebrate community structures (Figure 4.2). The four clusters, as displayed in the dendrogram (Figure 4.2), included WO (Cluster 1), RA and AV (Cluster 2), ZG and GRM (Cluster 3), and MRM (Cluster 4).

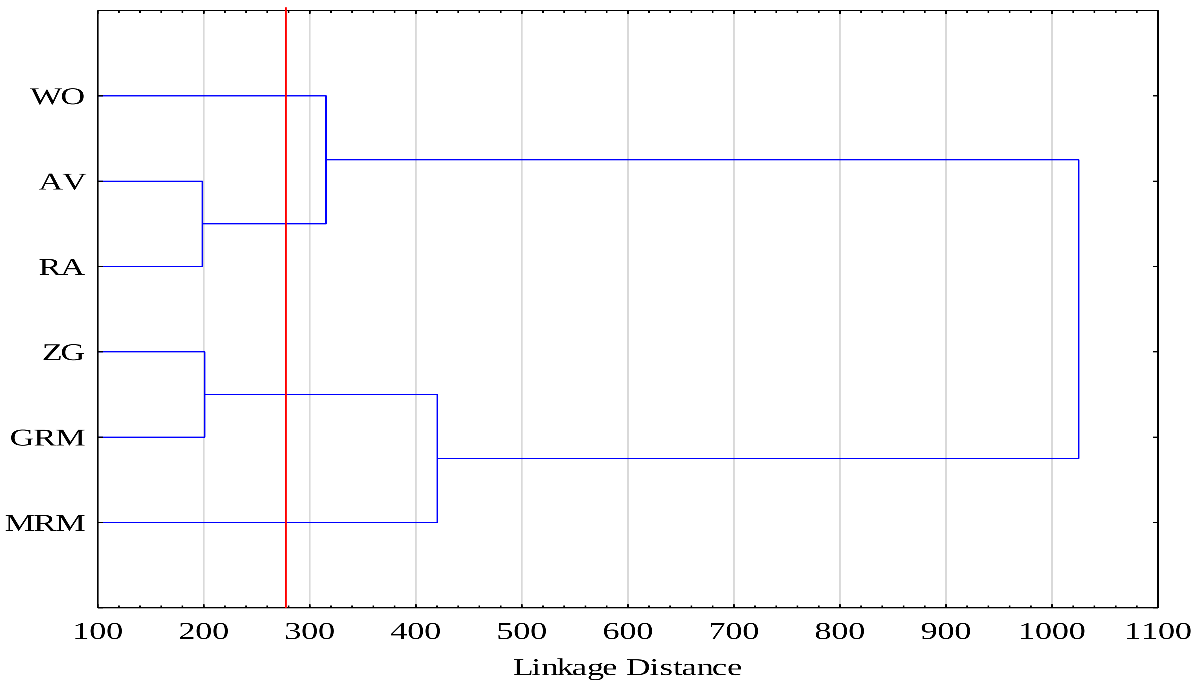


Figure 4.2. Hierarchical Cluster Analysis dendrogram showing four clusters of macroinvertebrates for six wetlands of Lake Tana. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was based on Ward's method WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara River mouth, MRM-Megech River mouth, AV-Avaj, RA-Ras Abbay. Note: Hierarchical Cluster Analysis (HCA) was applied using a subset of 16 macroinvertebrate families selected based on their ecological relevance and contribution to variance in the dataset. These families were chosen after preliminary screening showed that 38 of the 54 families had negligible influence on clustering outcomes. The analysis used Euclidean distance and Ward's method to group wetlands based on macroinvertebrate community similarity.

The PCA plots (Figure 4.3 and Table 4.5) produced a four-dimensional distribution of wetlands similar to the grouping formed by the hierarchical cluster analysis dendrogram. Only 16 of the 54 macroinvertebrate families were used for the cluster and PCA analysis as thirty-eight of the macroinvertebrates did not explain the cluster and PCA. Hence, Aeshnidae, Baetidae, Caenidae, Chironomidae, Coenagrionidae, Corbiculidae, Corduliidae, Haplotaxidae, Lestidae, Libellulidae, Naididae, Physidae, Planorbidae, Siphlonuridae, Thiaridae, and Tubificidae were the macroinvertebrate included in the analysis. The least polluted cluster 1, comprising WO, was highly associated with the eight families Aeshnidae, Baetidae, Corduliidae, Corixidae, Lestidae, Libellulidae, Physidae, and Planorbidae. The least sensitive cluster 1 (WO) was associated with a high number of nine pollution-sensitive families such as Aeshnidae, Coenagrionidae, Cordulidae, Gamphidae, Libelluliidae, Lestidae, Baetidae, Caenidae, and Heptageniidae. The slightly polluted cluster 2 (RA and AV) was associated with a low number of pollution-sensitive groups and comprised a high number of one family, Coenagrionidae. The moderately polluted cluster 3 (ZG and GRM) was mainly associated with a high number of three families, such as Chironomidae, Corbiculidae, and Thiaridae. The highly polluted cluster 4, comprising MRM, was highly associated with pollution-tolerant groups that included three families such as Haplotaxidae, Naididae, and Tubificidae.

Table 4. 4. Eigenvalues, cumulative eigenvalues, percent of total variance, and cumulative percent of total variance of correlation PCA for the macroinvertebrate families (n = 16) in wetlands of Lake Tana

	Eigenvalues	**Cumulative Total**	**% of Total Variance**	**Cumulative % of Total Variance**
1	10.42	10.42	65.15	65.15
2	2.37	12.79	14.83	79.98
3	1.79	14.58	11.21	91.19
4	1.11	15.69	6.94	98.13
5	0.30	15.99	1.87	100.00

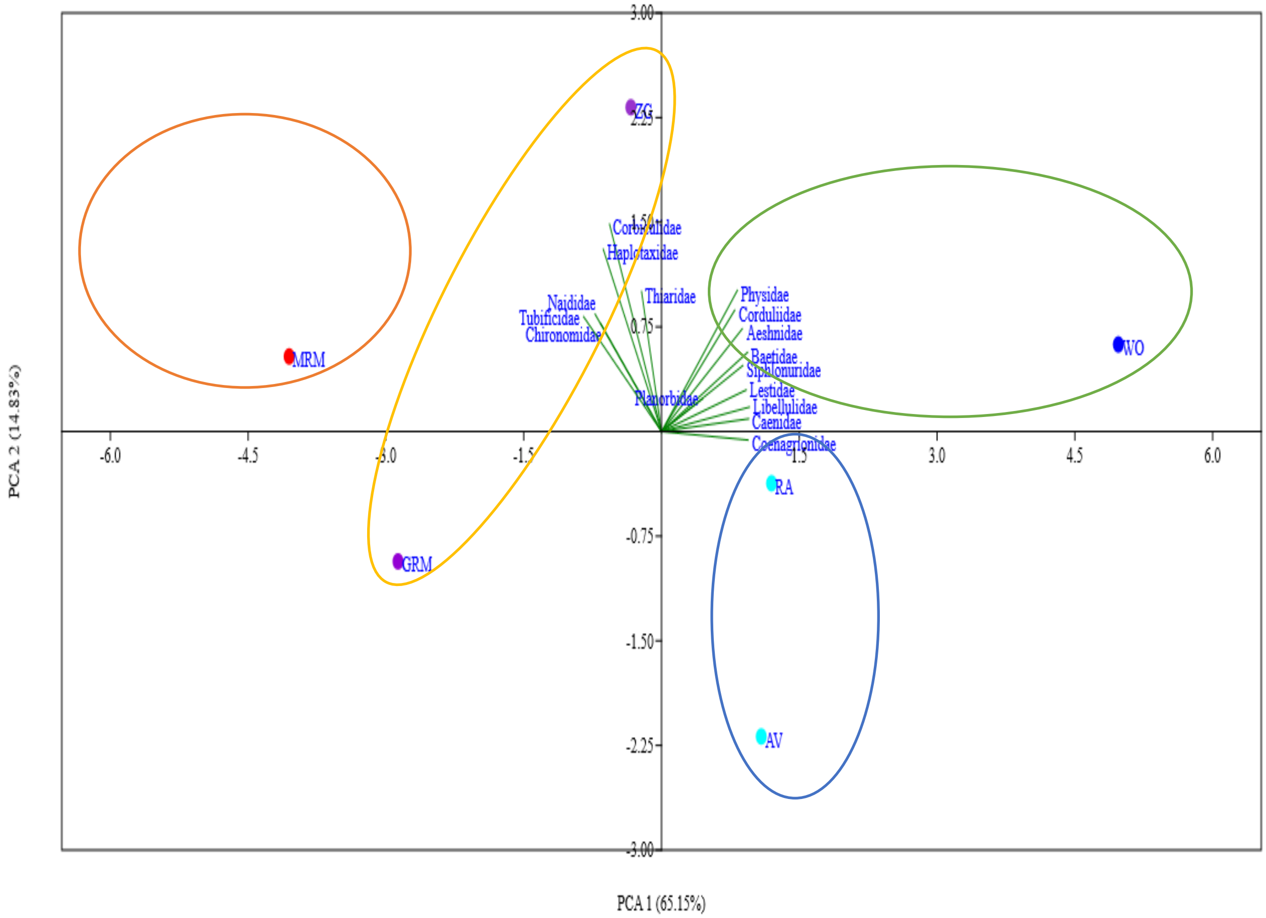


Figure 4.3. PCA plot correlating sampling wetland scores of the six study wetlands with macroinvertebrate vectors of the 16 macroinvertebrate families for plot Component one (X-axis) and plot Component two (Y-axis). Note the grouping of the four clusters. Where AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar

4.3.3. Relationship between macroinvertebrate taxa and environmental variables

The high family-environmental correlation coefficient associated with each axis in the Canonical Correspondence Analysis (CCA) ordination demonstrated a strong association between the measured environmental variables in the six wetlands and the distribution of macroinvertebrate families. This means that the environment greatly influences the distribution of macroinvertebrates (Table 4.6). The strongest explanatory factors were nitrite, water depth, Secchi depth, ammonia, salinity, temperature, total nitrogen: total phosphorous ratio, and total dissolved solids. pH was excluded from the CCA plot due to a high multicollinearity with oxygen. The pollution-sensitive families (Aeshnidae, Baetidae, Corduliidae, Corixidae, Lestidae, Libellulidae, and Siphonariidae) were more abundant in the WO and were mainly associated with the high temperatures in this wetland. MRM was associated with a high total nitrogen-total phosphorous ratio, total dissolved solids, and high conductivity favored the pollution-resistant Oligochaetes such as Haplotaxidae, Naididae, and Tubificidae. GRM and ZG were associated with high salinity, soluble reactive phosphorous, and nitrate that favored Thiaridae, Chironomidae, and Planoribidae. AV and RA were associated with high ammonia, Secchi depth, and water depth which may have favored Corbiculidae. The first three axes accounted for 98.06% of the total taxa variance (Table 4.6) explained in the observed patterns in the CCA plot (Figure 4.4).

Table 4.5. Summary of CCA axis length showing axis eigenvalues, correlation between family and the environmental gradients, and variance of family, following canonical correspondence analysis of macroinvertebrate taxa abundance data in six wetlands of Lake Tana

	CCA Axis 1	**CCA Axis 2**	**CCA Axis 3**
Canonical eigenvalue	0.288	0.104	0.048
Cumulative percentage variance	63.86	22.99	10.61
Family-environmental correlation	7.19		
Number of family (response variable)	16		
Number of environmental variables	12		
Total variance in family data	97.46		

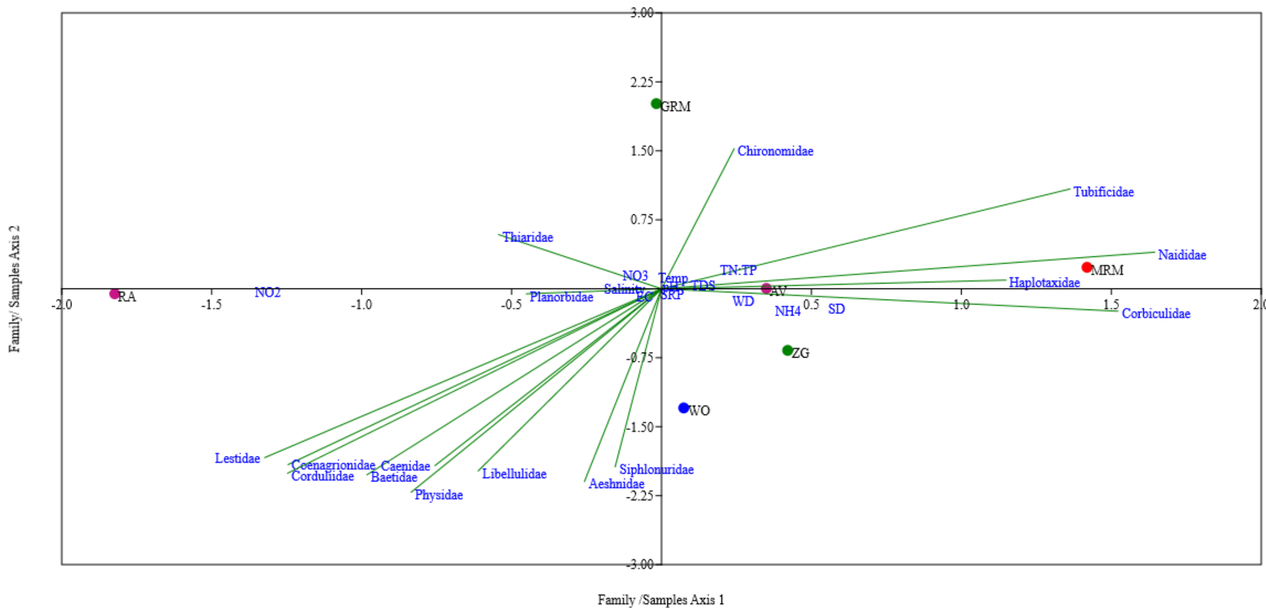


Figure 4.4. CCA plot of first and second CCA axes of macroinvertebrate taxa, environmental variables, and the corresponding wetlands.`Eigenvalues: axis 1, 0.006, axis 2,0.002. The first 2 axes account for 98.06% of the variance. Where Wetlands AV-Avaj, GRM-Gumara River Mouth, MRM- Megech River Mouth, Ras Abbay, WO -Wonjeta, ZG- Zewdie Girar. Note: The orientation of environmental vectors in this CCA plot reflects the specific ecological responses of macroinvertebrate taxa to environmental gradients. These orientations may differ from those of other biological groups, such as fish, due to variations in ecological tolerances, habitat preferences, and environmental sensitivities.

4.3.4. Evaluation of aquatic environmental quality of Lake Tana wetlands using macroinvertebrate diversity indices

The Shannon-Wiener Diversity Index (H’)

The Shannon-Wiener Diversity Index of macroinvertebrate species differed across the studied wetlands (ANOVA, $p < 0.05$). Wetlands in ZG had significantly more species diversity than those in GRM, AV, and MRM. RA and WO had intermediate levels of diversity. While the Shannon-Wiener Diversity Index varied among wetlands, it did not differ between seasons (ANOVA, $p > 0.05$) (Figure 4.4 a).

Simpson's diversity index (D)

Simpson's diversity index did not differ among wetlands, ranging from non-detectable to 0.99 (median: 0.70) in AV and from 0.94 to 1.00 (median: 0.99) in ZG (K-W ANOVA, $p > 0.05$). The season did not have an effect on Simpson's diversity index (K-W-ANOVA, $p > 0.05$) (Figure 4.4b).

Margalef's richness index (R)

The mean value of Margalef's richness index did not differ among wetlands, ranging from non-detectable to 4.76 (median: 1.44) in AV and from 1.32 to 5.17 (median: 3.57) in WO (K-W-ANOVA, $p > 0.05$). Likewise, the mean value of Margalef's richness index did not differ (ANOVA, $p > 0.05$) among seasons (Figure 4.4c).

Menhinick's index (DMn)

The mean DMn did not differ among wetlands (ANOVA, $p > 0.05$), ranging from non-detectable to 1.82 (mean ± SD: 0.78 ± 0.71) in MRM and from 0.90 to 1.93 (mean ± SD: 1.43 ± 0.38) in WO. Likewise, the mean value of DMn did not differ (ANOVA, $p > 0.05$) among seasons, ranging from non-detectable to 1.84 (mean± SD: 1.14 ± 0.53) during the rainy season and from non-detectable to 2.63 (mean± SD: 1.20 ± 0.71) during the dry season (ANOVA, $p > 0.05$) (Figure 4.4d).

Hill's index (H)

The mean Hill's index did not differ among wetlands, ranging from non-detectable to 1.04 (median: 1.00) in MRM and from 1.00 to 1.08 (median: 1.01) in WO (K-W-ANOVA, $p > 0.05$). Likewise, the mean Hill's index differed among wetlands, ranging from non-detectable to 1.08 (median: 1.00) during the rainy season and from non-detectable to 1.10 (median: 1.01) during the dry season (Figure 4.4e).

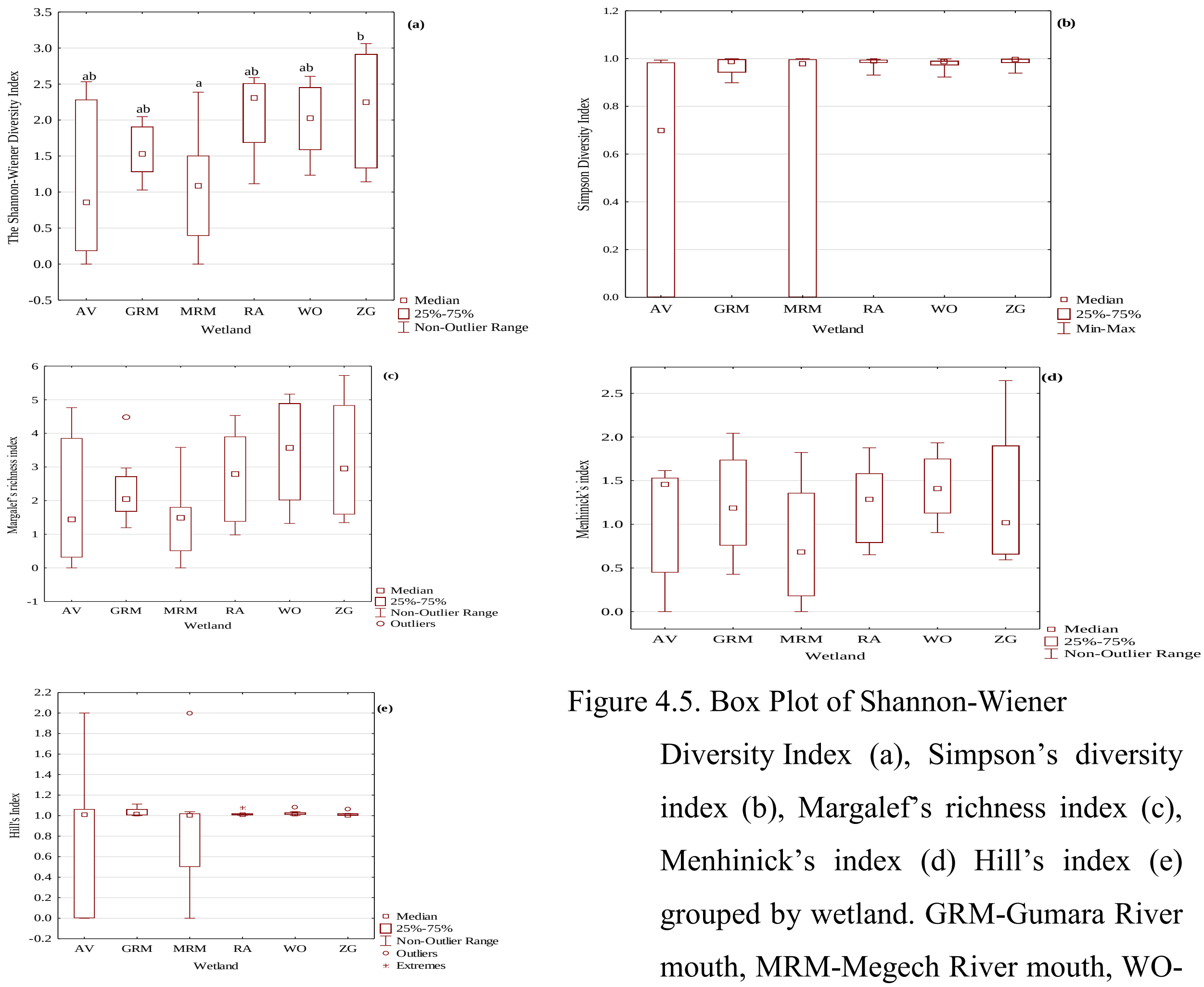


Figure 4.5. Box Plot of Shannon-Wiener Diversity Index (a), Simpson's diversity index (b), Margalef's richness index (c), Menhinick's index (d) Hill's index (e) grouped by wetland. GRM-Gumara River mouth, MRM-Megech River mouth, WO-Wonjeta, RA-Ras Abbay, ZG-Zewdie Girar

4.3.5. Evaluation of aquatic environmental quality using biotic indices

Hilsenhoff index (HFBI)

Hilsenhoff's index differed among wetlands, ranging from non-detectable to 1.38 (median: non-detected) in MRM and from non-detectable to 4.0 (median: 0.40) in RA (K-W, $p < 0.05$). Multiple comparison tests showed significantly higher Hilsenhoff indexes of macroinvertebrates in AV, RA, and WO than GRM, MRM, and ZG. HFBI ranked wetlands in decreasing order: AV > RA > WO > ZG> GRM> MRM (Figure 4.5a). The HFBI index did not differ among seasons, ranging from non-detected to 3.86 (median: non-detected) in the late rainy season and from non-detected to 3.87 (median: 0.02) in the dry season (ANOVA, $p > 0.05$).

Biological Monitoring Working Party (BMWP)

The biological monitoring working party differed among wetlands, ranging from non-detectable to 450.0 (median: 9.0) in GRM and from non-detectable to 1430.0 (median: 30.0) in AV (K-S-ANOVA, $p<0.05$). Multiple comparisons of mean ranks for all groups showed significantly higher BMWP in macroinvertebrates recorded in AV, ZG, and RA than GRM, MRM, and WO. The BWMP index ranked wetlands in decreasing order: AV > ZG > RA > WO> MRM> GRM, and there was a significant difference in BWMP between AV and GRM and AV and MRM. Using the BMWP biotic index, AV was classified as having “very good water,” indicating that it was unpolluted, while WO was classified in the “moderate" category, indicating that it was a moderately polluted wetland. GRM, MRM, RA, and ZG were classified as “polluted water,” indicating that the wetlands were impacted wetlands. BWMP did not differ among seasons (Figure 4.5 b).

Average score per taxon (ASPT)

The value of ASPT did not differ (K-W-ANOVA) among wetlands, ranging from non-detectable to 3.02 (median: 0.09) in WO and from non-detectable to 17.50 (median: 0.18) in AV. Likewise, ASPT did not differ (K-W-ANOVA) among seasons. Using the ASPT index, all wetlands were within the “poor water" quality class, indicating that the wetlands were severely polluted (Figure 4.5 c).

EPT index

The mean EPT index differed among wetlands, ranging from non-detectable to 17.0 (mean: non-detectable) in MRM and from non-detectable to 110.0 (median: non-detectable) in AV (K-W-ANOVA, $p <0.05$). Multiple comparisons of mean ranks for all groups showed significantly higher EPT indexes of macroinvertebrates in AV, WO, and RA than ZG, GRM, and MRM, while there was no significant difference in EPT index between AV and WO. However, the mean value of the EPT index did not differ among seasons (K-W ANOVA, $p < 0.05$). Using the EPT index wetlands, MRM, GRM, and ZG were classified as "polluted water," while AV, WO, and RA belonged to the "clean" category (Figure 4.5 d).

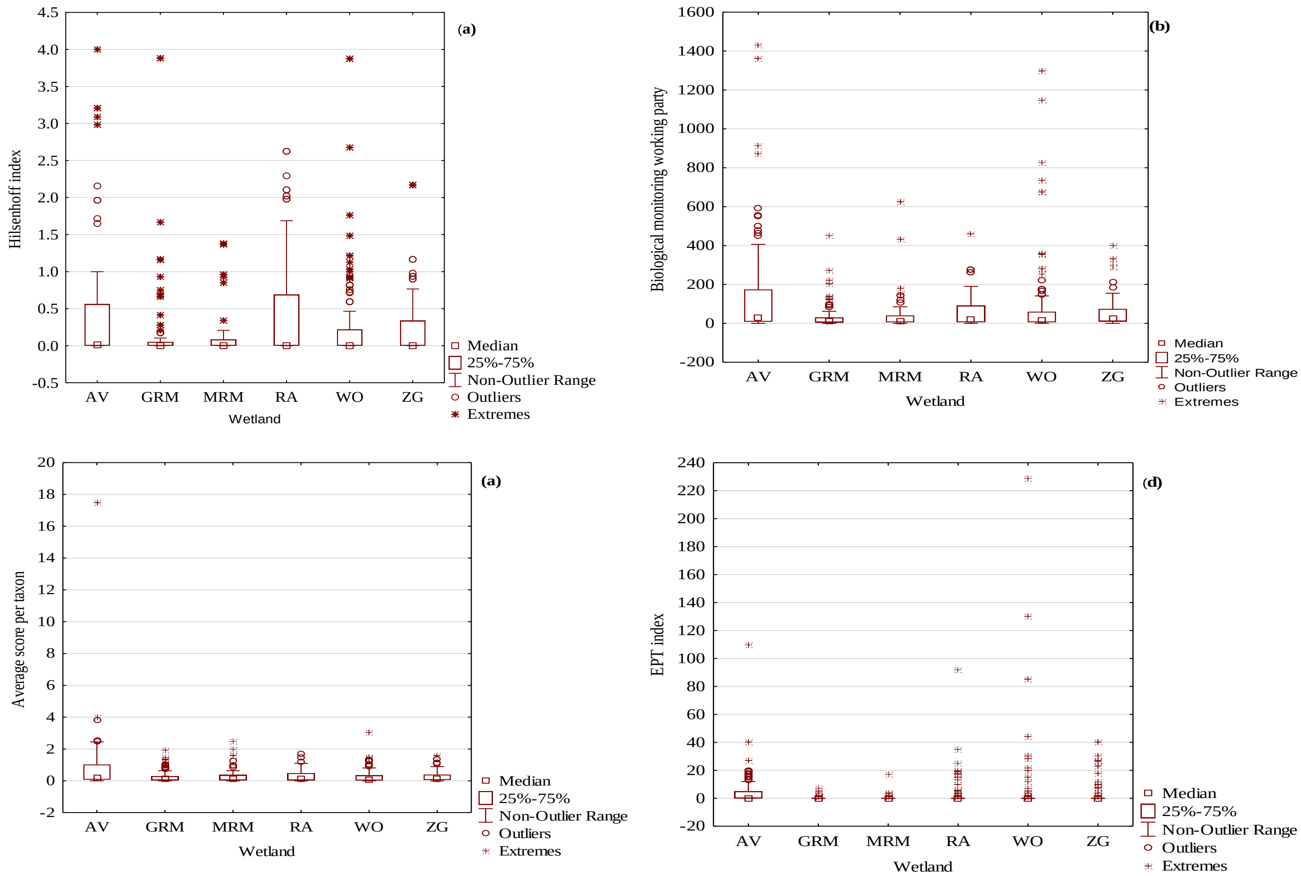


Figure 4.6. Box Plot of Biotic Indices, Hilsenhoff index (a), Biological monitoring working party (b), Average score per taxon (C), EPT index (d) grouped by Wetland. AV-Avaj GRM-Gumara River mouth, MRM-Megech River mouth, WO-Wonjeta, RA-Ras Abbay, ZG-Zewdie Girar

4.4. Discussion

The macroinvertebrate taxonomic composition in this chapter grouped the wetlands into four clusters, i.e., least, slightly, moderately, and highly polluted. This provided a distinction between wetlands, which included grouping WO in the least impacted cluster, RA and AV in the slightly polluted cluster, ZG and GRM in the moderately polluted cluster, and MRM in the highly polluted cluster. In this chapter, multivariate techniques that included CA, PCA, CCA, biodiversity, and biotic indices were used to explore variations in macroinvertebrate taxa and community structure about deterioration in environmental water quality. This was done with the view of exploring water column and benthic macroinvertebrates as biological indicators of water quality in selected wetlands in Lake Tana and to provide a basis for further comparisons for other sites in the equatorial region. The findings of this study are consistent with those of (Agboola et al., 2020), who reported that macroinvertebrate assemblages respond predictably to pollution gradients in tropical freshwater systems. (Agboola et al., 2020) also observed that pollution-sensitive taxa such as Ephemeroptera and Odonata were more abundant in less disturbed sites, while pollution-tolerant groups like Oligochaeta and Chironomidae dominated in more impacted areas. This alignment reinforces the reliability of macroinvertebrate-based indices in assessing ecological integrity and highlights the broader applicability of these bioindicators across African aquatic ecosystems. In addition to describing spatial patterns, this study critically assessed the appropriateness of macroinvertebrate diversity and biotic indices for wetland classification. While diversity indices such as Shannon-Wiener and Simpson's provided general insights into community structure, they were less effective in distinguishing pollution gradients due to their sensitivity to sample size and dominance effects. In contrast, biotic indices like the BMWP, EPT, and Hilsenhoff Index proved more robust for classifying wetlands by pollution level, as they incorporate taxa-specific tolerance values. These indices aligned well with multivariate clustering and environmental gradients, supporting their appropriateness for ecological classification in Lake Tana wetlands.

The macroinvertebrate taxa identified in this study were categorized based on their tolerance to pollution, allowing for the classification of wetlands by water quality. Pollution-sensitive taxa such as Aeshnidae, Baetidae, Corduliidae, Heptageniidae, Caenidae, and Ephemeridae were most abundant in WO and ZG, indicating good water quality. Moderately tolerant taxa, including Coenagrionidae, Planorbidae, and Physidae, were more common in RA and AV, suggesting moderate pollution levels. Pollution-tolerant taxa such as Naididae, Tubificidae, Chironomidae, and Thiaridae dominated in MRM and GRM, reflecting poor water quality and high organic pollution. These distribution patterns align with the physicochemical and trophic classifications, reinforcing the role of macroinvertebrate assemblages as reliable indicators of aquatic ecosystem health. Composition, occurrence, and density of macroinvertebrate taxa in wetlands of Lake Tana

The number of different families (54) of macroinvertebrates found in this study was high compared to previous studies in Ethiopia (Sitotaw, 2006; Masese et al., 2009; Kasangaki et al., 2008; Kibichii et al. 2007; Mehari et al., 2014; Gezie et al., 2017). This can be explained by the survey's approach, which involved collecting both water-column and benthic macroinvertebrates throughout all four seasons. In contrast to this study, some studies on the composition and occurrence of macroinvertebrates in Lake Tana were conducted only during wet and dry seasons on benthic macroinvertebrates (Gezie et al., 2017; Zelalem and Prokin 2017; Gezie et al., 2020; Mohammed et al., 2022).

The number and genus of macroinvertebrates differed among the wetlands. GRM and MRM had more tolerant macroinvertebrates, while others had fewer sensitive ones. Previous studies have shown that a high number of oligochaetes and chironomids in freshwater ecosystems can be an indicator of ecological problems (Odume 2020). Camargo et al. (2004) and Czerniawska-Kusza, (2005) have shown that pollution caused by human activities and increased nutrients can significantly impact the number of gatherers and collectors in river mouths. In contrast, in WO, ZG, AV, and RA, the most sensitive macroinvertebrate taxa were abundant, while tolerant macroinvertebrate taxa were absent. The high percentage of certain macroinvertebrates in MRM and GRM may be due to the large number of benthic organisms, such as oligochaetes and Diptera fly larvae. These organisms often indicate that the aquatic ecosystem is polluted by agricultural and urban wastewater. In contrast, the lower percentage of relative abundance of macroinvertebrates in WO, ZG, AV, and RA may be attributed to the lower abundance of benthic macroinvertebrates. The high vegetation cover and lower water turbidity compared to other study wetlands may have favored the macroinvertebrates in WO, ZG, RA, and AV (Muluye et al., 2024). The lower percentage of certain macroinvertebrates in WO, ZG, RS, and AV compared to GRM and MRM might be due to problems in the ecosystem caused by changes in factors like water quality, the type of substrate, food availability, and high turbidity. These factors could have negatively impacted the abundance of macroinvertebrates (Nkwoji & Edokpayi, 2013).

The highest density of macroinvertebrates in MRM, ZG, and GRM compared to WO, AV, and RA may be associated with a higher occurrence of benthic macroinvertebrates such as Chironomidae, Naididae, and Gastropods, which may be related to the amount of detritus that has accumulated in these wetlands. MRM and GRM are dominated by water hyacinths, and these macroinvertebrates were found to be more abundant and diverse in the water hyacinth-dominated habitat. The high number of macroinvertebrates in areas with water hyacinths might be because of the plant's roots, stems, and leaves, which provide a good habitat. This has been observed in other studies in Lake Tana (Zelalem & Prokin, 2017; Gezie et al., 2018; Tamiru, 2019; Getnet et al., 2021; I. Mohammed et al., 2022), Lake Victoria (Mailu, 2001; Masifwaet al., 2001; Muli et al., 2000; Jones, 2005; Owalo et al., 2021; Nyamweya et al., 2023) and other water bodies worldwide (Villamagna and Murphy 2010; Uwadiae et al., 2011; Barker et al., 2014; Coetzee et al., 2014; Rocha-Ramírez et al., 2014; Nguyen et al., 2015; Asmath et al., 2023; Basaula, 2023). At the open water/macrophyte interface, the beds of water hyacinth supported higher populations of macroinvertebrates than the consolidated areas with the *Cyperus papyrus*, Indigenous tree-dominated areas, and open water fringe. In Lake Tana, the surface-floating fringe vegetation at the open-water interface supports a richer abundance and diversity of macroinvertebrates. The lowest density of macroinvertebrates in AV might be due to substrate variation and pollution from untreated municipal and industrial wastewater from Felege-Hiwot Hospital.

Diversity indices in the assessment of water bodies have been proven to be useful tools for describing the structure of communities, but diversity indices do not indicate the pollution level of aquatic bodies. Diversity indices are good for assessing organic pollution and eutrophication but poor for assessing toxicity and physical changes (Malvandi et al., 2021). Hence, the various indices, such as Simpson's, Menhinick's, Margalef's richness, and Hill's indices, and the average score per taxa have been less successful in ranking the wetlands. This might be associated with these indices being influenced by sample size, biased towards abundant species, not suitable for highly diverse and rare communities, and not providing detailed information on species evenness (Keylock, 2005; Magurran, 2021; Rajamani & Iyer, 2023).

4.4.1. Spatial diversity and site grouping based on macroinvertebrate taxa characteristic

The CA plot and the PCA ordination confirmed that the highly polluted MRM was associated with a high total nitrogen-total phosphorous ratio, total dissolved solids, and high conductivity favored the pollution-resistant Oligochaetes such as Haplotaxidae, Naididae, and Tubificidae. This may be due to the impact of various anthropogenic activities such as catchment agriculture and urban waste discharge without treatment into the Megech River that might influence the macroinvertebrate communities, as the diversity and abundance of MRM are known to predictably change in response to aquatic environmental degradation. The highly polluted MRM comprises macroinvertebrate species tolerant to high organic matter and trace metals due to the ability of macroinvertebrates to thrive in a low-oxygen environment. The opportunistic pollution-tolerant macroinvertebrate taxa recorded in this study are known to thrive in organically polluted areas of the aquatic environment. On the other hand, the absence of some species, such as the taxa of Ephemeroptera and Odonata in MRM, may be due to the sensitivity of Ephemeroptera and Odonata to pollutants associated with this wetland and most probably linked to the severity of ecological degradation. This finding supports what was found in several reports in Lake Tana (Mohammed et al., 2022; Tamiru, 2021; Gezie et al., 2020) that observed that Gastropoda, Oligochaeta and Hirudinidae, Bivalvia, Odonata, Trichoptera, and Chironomidae were the dominant macroinvertebrate taxa in the Gulf of Lake Tana. There are several reports on taxa-specific indicators for very poor water quality in other water bodies that found that macroinvertebrate groups shifted from pollution-sensitive in urban impacted wetlands to pollution-resistant macroinvertebrates at high-activity agricultural and urban sites (Barber-James et al., 2008; Lenat & Crawford, 1994; Odume, 2020; Pelletier et al., 2010).

The slightly polluted cluster 2, RA and AV, was associated with high levels of ammonia, Secchi depth, and water depth which favored Corbiculidae, and with a low number of pollution-sensitive groups comprising taxa of Odonata and Ephemeroptera. This may be explained by the fact that the groups are known to respond to degradation in water quality. The moderately polluted clusters 3, ZG, and GRM were mainly associated with increased salinity, soluble reactive phosphorous, and nitrate that favored Thiaridae, Chironomidae, and Planoribidae. The relatively low taxa macroinvertebrate assemblage in ZG and GRM comprising Thiaridae, Chironomidae, and Planorbidae could be attributed to the high tolerance of these taxa to anoxic and eutrophic conditions. The pollution-sensitive families such as Aeshnidae, Baetidae, Corduliidae, Corixidae, Lestidae, Libellulidae, and Siphonariidae, were more abundant in the WO and were mainly associated with the high temperatures in this wetland. This showed the presence of a relatively clean water environment in this wetland. The high abundance of the sensitive macroinvertebrate taxa of Ephemeroptera and Odonata in less impacted areas has been similarly reported for middle Awash (Muluye et al., 2024), upper Awash (Lakew and Moog, 2015; Kebede et al., 2020; Dabessa et al., 2021), and other Ethiopian waterbodies (Mezgebu et al., 2019; Tamiru, 2019; Wondmagegn and Mengistou, 2020). The higher abundance of Ephemeroptera in clean water is a globally accepted indicator. This is because these orders of insects reside only in good water (Rife and Moody, 2004). These groups of insects require clean water with high levels of dissolved oxygen for insects to survive. As such, Ephemeroptera are usually not found in polluted water bodies (Begum et al., 2023; Lim and Do, 2023; Mohammed et al., 2023; Sharma et al., 2023). Ephemeroptera families are often used to assess the health of wetland ecosystems (Couceiro et al., 2007). Ephemeroptera are typically found in areas with submerged vegetation (Mereta et al., 2012). Odonates are also closely linked to wetland vegetation because Ephemeroptera are herbivores that feed on plants (Shelly et al., 2011).

4.4.2. Diversity indices for biomonitoring water quality in Lake Tana wetlands

The highest values of Shannon-Wiener indices were obtained in ZG, RA, and WO, whereas the lowest values were recorded for AV, MRM, and GRM. The highest Shannon-Wiener indices in ZG, RA, and WO from multiple comparison tests may be associated with the decline of aquatic environments from point and non-point source pollutants into these wetlands without treatment. This indicated that MRM, GRM, and AV were heavily impacted by human activities. Moreover, these wetlands are under high pressure due to catchment agriculture in harvesting, livestock grazing, irrigation developments, sedimentation, water extraction, and the introduction of alien species. The Shannon-Wiener diversity index is among the most commonly used indices in ecological studies, and it accounts for abundance and evenness (Magurran, 2021). The Shannon-Wiener index discriminated between the composition of taxa in reference and impaired wetlands. A high Shannon index indicates a good macroinvertebrate habitat and non-impacted water quality (Damanik-Ambarita et al., 2016). ZG, RA, and WO had greater Shannon-Wiener diversity indices than GRM, MRM, and AV. This might be due to the elimination of sensitive taxa in GRM, MRM, and AV. The results concur with Sponseller et al. (2001). Vinson (2006) also revealed that taxonomic diversity decreases with decreasing water quality. The median Shannon-Wiener index was lower than in the reports by Negash et al. (2011), Welela (mean: 2.28), and Shesher (mean: 1.92) in wetlands. However, the results are similar to the results in several studies in Lake Tana (Mohammed et al., 2023; Tela and Masayi, 2023; Getnet et al., 2022; Zewudu et al., 2022; Mehari et al., 2014; Negash et al., 2011), as the Shannon-Wiener diversity index generally decreased with increasing degradation of habitat quality. In contrast, this result is lower than in other reports (Custodio et al. 2018; Patrick et al. 2013; Brraich and Kaur 2017; Sharma and Rawat 2009) and higher than in the report of Shabani et al. (2019) in other water bodies worldwide.

The median of Simpson's diversity index did not differ among wetlands, ranging from minimum values in MRM to maximum values in ZG. The overall median values of Simpson's index were less than one, indicating severe pollution of aquatic bodies, as reported by Azimi et al. (2015) and Kratzer and Batzer (2007). The Simpson index measures the dominance of certain species. It is often used to determine how evenly distributed species are. Results showed that the distribution of individuals between species was very uneven in all wetlands, from ZG to MRM. The Simpson diversity index is a suitable measure for research that focuses on the relative abundance of common species, requires minimal sampling, and is interested in the likelihood of randomly choosing two individuals of the same species (Hurlbert, 1971).

The Margalef Diversity Index is a measure of biodiversity in biological populations. It is particularly useful for comparing macroinvertebrate communities and can differentiate between ecosystems with high and low species richness. The greater its numerical value, the better the river's quality (McCafferty, 1983). During the present investigation, the range of this index varied from 0 to 5.72. A low index value that was obtained during the present study indicates extremely low species richness and low abundance in physically disturbed areas (Bhandarkar, 2013).

The mean value of Menhinick's index (DMn) did not differ between wetlands or across seasons. However, the median value for the DMn index ranged from 0 to 2.0, indicating heavy pollution. Based on Menhinick's index diversity indices, the water quality was classified into four grades:

no pollution (Dmg > 5.0), light pollution (5.0 > Dmg > 4.0), moderate pollution (4.0 > Dmg > 3.0), and heavy pollution (3.0 > Dmg> 0.0) (Mechanic, 1964). The DMn index is restricted to categorical data, it can only be used with data that can be divided into a specific number of groups. (Menhinick, 1964). Moreover, the DMn index considers the influence on the diversity of the analysis scale; therefore, the index value does not take into account class frequencies and it remains strongly influenced by sample size (Gatti et al., 2020).

The mean value of Hill's index did not differ among wetlands, with a maximum value in ZG and the lowest value in AV. ZG, RA, and WO (Hill's index values from 5.33 to 9.26) were the least polluted, and AV, GRM, and MRM (Hill index values from 2.80 to 3.79) were highly polluted. The macroinvertebrate assemblage, distribution, and diversity across the lake indicate the level of disturbance and water quality variation. Karr (1998) described that the health of a water body can be best assessed by examining the condition of its biological communities.

All diversity indices, except Hill's index, have shown the highest values in ZG and WO, whereas the lowest values were registered in MRM and GRM. The species diversity showed improvement in wetland WO, ZG, AV, and RA, probably as a result of better environmental conditions. Human activities like dumping waste from hospitals, hotels, and resorts, untreated stormwater, and altering the shoreline, which includes destroying habitats and adding artificial structures, probably contributed to the decrease in biodiversity. This pattern was also seen in other Ethiopian water bodies, including the Lake Tana wetlands (Gezie et al., 2017), the source of the Blue Nile River (Wosnie and Wondie, 2014), the Kebena and Akaki Rivers (Beyene et al., 2009), the Upper Awash River (Degefu et al., 2013), and the Tikur Wuha River (Abraha, 2007).

4.4.3. Biotic indices for biomonitoring water quality in Lake Tana wetlands

The median value of Hilsenhoff's index differed among wetlands, and the mean index values ranged from non-detectable to 1.38 in MRM, and from non-detectable to 4.00 in AV. The Hilsenhoff Biotic Index suggests that Lake Tana has excellent water quality. Multiple comparisons of mean ranks for all groups indicated the mean value of Hilsenhoff's index decreased in the order of AV > RA > WO >ZG > GRM > MRM. This index measures the ability of different families of benthic macroinvertebrates to tolerate pollution. Scores range from 0 to 10, with 0 meaning no tolerance to pollution and 10 indicating high tolerance (Hilsenhoff, 1988). There are several reports in support of this in Ethiopia and worldwide on the assessment of the pollution status of aquatic environmental quality using Hilsenhoff's index. For example, research on the Enfranz River in Ethiopia using macrobenthos data and the HFBI index revealed that water quality was worse in downstream areas, heavily impacted by anthropogenic activities (Mehari et al., 2014). The HFBI classified stations in the Haraz River as ranging from very good to very poor (Banagar et al., 2018). In Taiwan, the Beishi, Tonghou, Nanshi, and Xindian streams were also categorized as excellent to poor (Narangarvuu et al., 2014). In the Shahroud River in Iran, the HFBI indicated that the highest index values were found at stations downstream of fish farms (Dadgar et al., 2014). In Mashhad, Iran, the HFBI showed that stations in the Dehbar River fell into four categories: good, fair, poor, and very poor (Malvandiet al., 2021).

The higher value of BWMP in AV, ZG, RA, and WO compared to GRM and MRM indicated by the multiple comparison tests may be due to a decline in aquatic environmental quality in MRM and GRM due to the discharge of agricultural and urban effluents from point and non-point sources. Based on the BMWP index, the wetlands of Lake Tana were categorized into three water quality classes: poor, moderate, and very good. AV was deemed "very poor water," suggesting it was unaffected by pollution. RA, WO, and ZG were classified as "moderate," indicating those wetlands were moderately polluted. In contrast, GRM and MRM were classified as "poor water," indicating polluted or impacted wetlands. Other studies on lentic ecosystems (Rashid & Pandit, 2014; Yazdian et al., 2014), including those in Ethiopia (Gezie et al., 2017; Wondmagegn and Mengistou, 2020), have found similar results (Rashid & Pandit, 2014; Yazdian et al., 2014; Gezie et al., 2017; Wondmagegn & Mengistou, 2020). The BMWP index rates each family of organisms according to the ability of the BMWP index to withstand pollution (Mohammed et al., 2023).

The index was higher in less impaired wetlands (AV, ZG, RA, and WO) compared with disturbed wetlands (GRM and MRM); the higher the BMWP score, the clearer the water. In support of the argument, Varnosfaderany et al. (2010) concluded that the index showed a greater correlation with water quality parameters than that of the richness and diversity indices. Due to its simplicity and affordability, the BMWP index has been adopted in numerous countries across Africa, Asia, Oceania, and Latin America (Chang et al., 2014). Its effectiveness in distinguishing between polluted and less affected wetlands has led to its inclusion in water quality assessments of wetlands.

The maximum value of ASPT was recorded in AV, RA, and ZG, while the minimum values were recorded in GRM, MRM, and WO. The ASPT Index analysis revealed that the wetlands of Lake Tana are heavily polluted. The lowest value of the ASPT index was recorded in AV during the rainy season and the highest in AV during the late rainy season. The ASPT Index scores, analyzed using a post-hoc test, showed a decline from MRM to ZG. This decrease might be attributed to increased pollution from fish farming, urban and industrial wastewater, and agricultural waste in areas near the river (Wondie 2018; Engdaw 2023; Mohammed et al. 2023). Using a benthic macroinvertebrate-based score for assessing the ecological conditions of highland streams and rivers in Ethiopia, the overall ASPT values in this study (<2.4) suggest bad water, indicating heavily degraded conditions.

The mean value of the EPT index differed among wetlands with maximum GRM, MRM, and ZG. Based on the EPT index wetlands, MRM, GRM, and ZG were classified as “polluted water,” while AV, WO, and RA were in the “clean” category. The EPT index classified dry, early rainy, and late rainy seasons under the “polluted’’ class, while the rainy season was categorized under the “good” class. The low score of EPT in MRM, GRM, and ZG wetlands indicated that these wetlands are being subjected to pollution. Taxa belonging to the groups within the EPT indices are recognized to be very sensitive to perturbations. Overall, in MRM, GRM, and ZG, pollution-sensitive organisms decreased, while pollution-resistant ones increased. This finding is in line with several reports that indicated pollution-resistant macroinvertebrates increased in polluted water (Narangarvuu et al., 2014; Onana et al., 2019). Changes in the types and numbers of macroinvertebrates reflect environmental factors and stresses and help to preserve ecological balance (Fore et al.,1996).

4.5. Conclusion and recommendations

The water quality environmental data in Chapter 3 grouped WO, ZG, and GRM as the least polluted, AV and RA as moderately polluted, and MRM as highly polluted clusters. On the other hand, the macroinvertebrate taxonomic composition in Chapter 4 grouped WO as the least polluted, AV and RA as slightly polluted, GRM and ZG as moderately polluted, and MRM as highly polluted. CCA analysis revealed that pollution-sensitive families (Aeshnidae, Baetidae, Corduliidae, Corixidae, Lestidae, Libellulidae, and Siphonariidae) were more prevalent in WO and may be primarily associated with higher temperatures in this wetland. MRM had higher total nitrogen-total phosphorus ratios, total dissolved solids, and conductivity, favoring pollution-resistant Oligochaetes like Haplotaxidae, Naididae, and Tubificidae. The GRM and ZG had elevated salinity, soluble reactive phosphorus, and nitrate levels, which favored Thiaridae, Chironomidae, and Planorbidae. AV and RA were associated with higher ammonia levels, Secchi depth, and water depth, favoring Corbiculidae. This was further supported by diversity indices like Shannon-Wiener Diversity and Margalef's richness indices and biotic indices, like the BMWP, EPT, and Hilsenhoff's richness index. This chapter gives an account of the potential of using macroinvertebrates as biological indicators of water quality in the wetlands of Lake Tana. This study is one of the few studies in the region to investigate the quantification of changes in macroinvertebrate diversity and biotic indices about environmental condition assessment in Ethiopia. The results suggest that the relative abundance, Shannon-Wiener diversity index, Margalef diversity index, density per square meter, and biotic indices of the macroinvertebrate taxa were influenced by the wetlands' human disturbance of the aquatic ecosystem in Ethiopia. The high mean values of diversity and biotic indices recorded in ZG, AV, WO, and RA compared to GRM and MRM may not be associated with pollution of water; rather, this may be linked with the predominance of indigenous tree forests in these wetlands and the presence of *Cyperus papyrus*. This study, therefore, recommends that, in addition to the physicochemical variables, further investigations on the habitat and biological effects should be considered, and a wider spatial coverage should be considered, including the findings from this study. The predominance of the oligochaetes, Gastropoda, Veneroida, and dipteran taxa associated with eutrophication in MRM and GRM and the absence of some of the pollution-sensitive taxa such as Ephemeroptera, Odonata, Trichoptera, and Plecoptera in these wetlands point to the fact that effluent-induced environmental changes may have led to the ecological transformation and environmental degradation of the lake, rendering the environment unfavorable to some macroinvertebrates. Another important finding was that of the endpoints used to describe the macroinvertebrate community structure and variation across study wetlands, CA, PCA, and CCA separated the wetlands into four clusters according to the levels of environmental degradation of the wetlands: highly polluted, moderately polluted, slightly polluted, and least polluted. The clusters were consistent with the four clusters discussed in Chapter 3, regarding the classification of the wetlands according to water quality and physicochemical variables. The classification of each wetland as CA, PCA, CCA, Shannon-wiener diversity index, and biotic indices showed the differences in the pollution status of each wetland as good, poor, and very poor. These findings suggest that macroinvertebrates are good candidates for assessing ecosystem integrity, and the investigation of macroinvertebrates should be considered in the development of biomonitoring networks and programs to assess water quality in wetlands. This warrants further research on the spatial and temporal distribution of macroinvertebrates in the lake

CHAPTER 5

Fish biodiversity as a biological indicator of aquatic environmental quality in wetlands of Lake Tana, Ethiopia

5.1. Introduction

Estimating the number of species occurring in a particular area is central to biodiversity studies and remains the fundamental theme of ecology (Dudgeon et al., 2006; Biggs et al., 2017). Measures of fish diversity and biotic integrity are frequently seen as indicators of the well-being of ecological systems and are important in understanding the mechanisms and effects of certain ecological phenomena, such as environmental disturbances (Simon, 1999; Jha, 2006; Naigaga, 2012; Parmar et al., 2016a; Pelletier et al., 2020). Fish are commonly used as indicators of water quality because the sensitivity of fish to pollution varies between species (Authman et al., 2015; Dunier, 1996; Reid et al., 2016). Fish community structure, diversity, and biotic indices are widely used to evaluate changes in aquatic ecosystems (Fausch et al., 1990; Chakona & Swartz, 2012; Zelalem et al., 2022; Pinna et al., 2023).

The decline and extinction of many fish species in the Lake Tana wetlands have been reported, and the degradation of critical aquatic habitats have been addressed (Goshu et al., 2010a; Gezie et al., 2017; Goshu & Aynalem, 2017; Dersseh et al., 2020, 2019; Ayele & Atlabachew, 2021; Kahsay et al., 2022). The deterioration seems to be largely due to overfishing, deforestation, dam construction, poor fisheries management, and ineffective legislation (Wondie, 2018; Enyew et al., 2020). Traditionally, farming was practiced in the high-altitude regions of the Blue Nile-Lake Tana basin. However, due to growing population pressure and limited land and water resources in the past two decades, farming has shifted to wetlands, including lake shorelines and riverbanks (Atnafu et al., 2011; Dejen et al., 2017; Wondie, 2018). Farming practices such as drainage and water diversions for small-scale irrigation have resulted in soil erosion and loss of soil fertility (Hurni et al., 2010; Wondie, 2010; Goshu & Aynalem, 2017; Mucheye et al., 2018; Zimale et al., 2018). Socio-economic research has revealed that landowners near the wetlands in Fogera, a district in northwestern Ethiopia, follow the receding waterline and cultivate the land until it dries out (Wondie, 2010; Atnafu et al., 2011; Dejen et al., 2017).

The destruction of macrophytes, which are breeding habitat for fish, due to deforestation along the lake's shoreline, has negatively impacted fish populations (Wondie, 2010). This led to a decline in spawning areas. The withdrawal of water from pools for irrigation has resulted in many tributary rivers of Lake Tana drying up during April and May. This has had a significant negative impact on the downstream migration of juvenile *Labeobarbus* spp. fish to the lake (Anteneh et al., 2012a; Mingist & Gebremedhin, 2016; Teshome et al., 2021; Mequanentet al., 2022). The building of large hydropower and irrigation dams on the tributary rivers of Lake Tana is anticipated to interfere with the migratory spawning of *Labeobarbus* spp. Moreover, these dams may reduce water flow to downstream floodplains, resulting in insufficient inundation of spawning areas for *C. gariepinus* and *O. niloticus* (de Graaf et al., 2008; Anteneh et al., 2012a). The shore macro habitats of Lake Tana have become infested with water hyacinth (*Eichhornia crassipes*) since 2011 (Enyew et al., 2020; Dersseh et al., 2022; Cai et al., 2023). Water hyacinth has spread to cover about 34,500 hectares, exceeding one-third of the lake's shoreline (approximately 128 kilometers) (Asmare, 2017; Damtie et al., 2021). Early research findings show that juvenile fish are more likely to be found in areas with native plants on the shoreline and less likely in areas infested with water hyacinths (Gezie et al., 2018; Dersseh et al., 2019).

The fish family with the greatest number of species in the lake is the Cyprinidae, represented by four genera found in the Lake Tana basin: *Barbus*, *Garra*, *Labeobarbus*, and *Varicorhinus* (de Graaf et al., 2008; Dejen et al., 2017; Gebremedhin et al., 2018). There have been several studies on the species composition and temporal changes of endemic *Labeobarbus* spp. in the Lake Tana basin (de Graaf et al., 2008; Vijverberg et al., 2009; Goshu et al., 2010b; Anteneh et al., 2012a; Gebremedhi et al., 2013; Mequanent et al., 2014; Shitaw et al., 2018; Mequanent et al., 2022). Previous studies have demonstrated that Lake Tana and its catchment are suitable areas for using fish communities as indicators of water quality deterioration. Employing biological indicators is vital not only for the conservation of key fish families such as Cichlidae and Cyprinidae but also for informing regulatory measures to prevent further biodiversity loss. In this context, this chapter investigates suitable biological indicators for assessing water quality decline in the Lake Tana basin wetlands.

Specifically, the chapter contributes to Objectives 2 and 4 by analyzing fish community structure and applying fish-based indices of biotic integrity. A total of 18 fish species were recorded across the study wetlands. Ecological health was evaluated using diversity indices and the Fish Index of Biotic Integrity (FIBI). Multivariate analyses were used to examine the relationship between fish distribution patterns and environmental gradients. Additionally, the chapter highlights the influence of fishing pressure and habitat quality on fish diversity and biotic integrity.

5.1. Specific Methods

5.1.1. Field sampling

Fish for bioindicator analysis were collected at all study locations using gillnets of various mesh sizes during four seasons and 24 field trips (de Graaf et al., 2008; Vijverberg et al., 2009; Dejen et al., 2017). Different-sized gillnets were used to catch fish in two areas of each wetland: near the shore and 60 meters off-shore. Every fish caught was identified and counted. Motorized boats were used to reach fish sampling spots that were mostly covered by water hyacinth, papyrus, and other aquatic plants. The captured fish were identified and counted using identification keys from various authors (Nagelkerke et al., 1994; Nagelkerke & Sibbing, 2000; de Graaf et al., 2008). The species diversity and abundance were assessed by analyzing all the fish caught overnight.

Diversity indices

Shannon-Wiener diversity index (H’)

The Shannon-Wiener Diversity Index (H’) was calculated using the following formula:

$$H' = \sum_{i=1}^{s} \frac{Ni}{N} Ln \frac{Ni}{N}$$

Where N is the total population size of all species, Ni is the proportion of individuals found for species i, and S is the total number of species.

To assess how organic pollution affects the population structure of aquatic ecosystem organisms, the classification system suggested by Wilhm & Dorris (1968) was employed. According to this classification, a Shannon-Wiener diversity index below 1 indicates a severely contaminated ecosystem, values between 1 and 3 suggest a moderately contaminated ecosystem and an index above 3 indicates a non-contaminated ecosystem.

Simpson's diversity index (D)

The Simpson Diversity Index (D) was introduced by Simpson in 1949 (Keylock, 2005). In 1972, Krebs, (2008) presented a formula for calculating it. Here, ni represents the total number of individuals of a specific species, N is the total number of individuals of all species, and S is the total number of species.

$$D = 1 - \frac{\sum_{i=1}^{S} n(n-1)}{N(N-1)}$$

Margalef's richness index (R)

Margalef's Richness Index (R) is derived from the following formula:

$$R = \frac{S\text{-}1}{\mathrm{LnN}}$$

Where S is the number of species and N is the total number of individuals in the sample.

Margalef's Richness Index (R): Typically ranges from 0 to 5 in freshwater systems. Values <2 indicate low richness (potential pollution), 2–4 moderate richness, and >4 high richness.

Menhinick's index (DMn) is the number of taxa (S) divided by the square root of the total number of individuals (N)

$$D_{Mn} = \frac{S}{\sqrt{N}}$$

Menhinick's Index (DMn): Values <1 suggest low diversity, 1–2 moderate, and >2 high diversity. This index is sensitive to sample size.

Hill's index (inverted Simpson's index) (H)

$$H = \frac{1}{\sum_{i=1}^{n} p^{2i}}$$

S – species richness, pi – Relative abundance of i^{th} taxon in the sample.

Hill's Index (H): Values closer to 1 indicate high evenness and diversity; values <0.5 suggest dominance by few species.

Fish-Based Index of Biotic Integrity

To determine FIBI a total of 12 metrics, namely, native, intolerant, rheophilic, benthic, tolerant, Cyprinidae, detritivorous, herbivorous, carnivorous, omnivorous, exotic species, the number of individual fish species per 50 m^2 of sampling and modified fish index of wellbeing were determined (Table 5.1). The feeding guild status of herbivores, omnivores, and carnivores was determined (de Graaf et al., 2008; Gebremedhin et al., 2018). FIBI was estimated using the metrics in Table 5.2.

Habitat Quality Index (HQI)

The Habitat Quality Index (HQI) was used to evaluate ecological conditions of the wetlands based on key habitat features such as in-stream cover, substrate composition, water level, bank stability, buffer vegetation, invasive species presence, aesthetic quality, and channel morphology. Each attribute was scored on a scale of 0–4 or 0–5, with total HQI values ranging from 0 to 25. Wetlands were categorized as good (≥20), moderate (10–19), or poor (<10) (Smith, 2023).

Table 5. 1. Various aspects of Lake Tana fishes such as habitat and feed preferences, reproductive behavior, spawning grounds, and IUCN status by (de Graaf et al., 2004), (Gebremedhin et al., 2018), and (Abdissa et al., 2022)

Species	**Preferred Habitat (Water Depth)**	**Feeding Strategy**	**Reproductive Strategy**	**Spawning Grounds**	**IUCN Status since 2000**
Clarias gariepinus	Shore areas and sublittoral	Omnivore	Lacustrine	Flood plain	Least concern
Garra dembecha	Benthic	Phytoplanktivore	Lacustrine	Shore area	Least concern
Labeobarbus acutirostris	All habitat	Piscivore	Migratory	Stream	Vulnerable
Labeobarbus beso	Not known	Phytoplanktivore	Lacustrine	Shore area	Least concern
Labeobarbus brevicephalus	Offshore pelagic	Zooplanktivore	Migratory	Stream	Not evaluated
Labeobarbus crassibarbis	Offshore benthic	Benthivore	Migratory	Stream	Not evaluated
Labeobarbus gorgorensis	Muddy, sandy, and rocky substrates	Molluscivore	Migratory	Stream	Not evaluated
Labeobarbus gorguari	Rocky littoral	Piscivore	Lacustrine	Unknown	Vulnerable
Labeobarbus intermedius	All habitat	Wide range of prey	Migratory	Stream	Not evaluated
Labeobarbus longissimus	Rocky littoral	Piscivore	Lacustrine	Unknown	Not evaluated
Labeobarbus macrophthalmus	Offshore pelagic	Piscivore	Migratory	Stream	Endangered
Labeobarbus megastoma	Offshore pelagic	Piscivore	Migratory	Stream	Least concern
Labeobarbus nedgia	Rocky littoral	Macrobenthivore	Migratory	Stream	Least concern
Labeobarbus platydorsus	Offshore benthic	Piscivore	Migratory	Stream	Vulnerable
Labeobarbus truttiformis	Offshore pelagic	Piscivore	Lacustrine	Unknown	Least concern
Labeobarbus tsanensis	Offshore benthic	Benthivore	Migratory	Stream	Least concern
Labeobarbus surkis	Muddy, sandy, and rocky substrates	Macrophytivore	Lacustrine	Unknown	Not evaluated
Oreochromis niloticus		Phytoplanktivore	Lacustrine	Shore area	Not evaluated

Table 5.2. Fish-Index of Biotic Integrity (FIBI) Metrix and its scoring criteria for Lake Tana, (Karr, 1986; Raburu & Masese, 2012; Bassa et al., 2020; Gonino et al., 2020)

Category	**Metric**	**Scoring criteria**		
		1 (worst)	3	5 (best)
Species richness and Composition	No. of native species	<3	3–5	≥6
	No. of intolerant species	<3	3–5	≥6
	No. of rheophilic species	0	1	>1
	% of benthic species	<7.5	7.5–15	>15
	% of tolerant individuals	>20	10–20	<10
	% of Cyprinidae individuals	<40	40–80	>80
Trophic metric	% of detritivorous individuals	<7.5	7.5–15	≥15
	% of carnivorous individuals	<1	1–4.4	≥4.5
	% of omnivorous individuals	>45	20–45	<20
Abundance and condition	No. of individuals per 50 m^2 of sampling	<25	25–50	>50
	No. of exotic species	>2	1	0
	Modified index of well being	<1.25	1.25–2.50	≥2.50

Each metric was scored, and a sum of all scores was obtained. The stations were then translated as excellent, good, fair, poor, or very poor depending on whether the value was within the range of 50–60, 40–49, 30–39, 20–29, or < 20 (Bassa et al., 2020).

A final range of FIBI was extracted from the individual ranges. Biodiversity indices used as metrics in the estimation of fish indices of biological integrity were calculated using the following formulae. The percentage proportion of individual species (P) was estimated using the formula:

$$\mathrm{P} = \frac{\text{Population size}}{\text{Total number of fish community}} \times 100$$

The modified index of wellbeing (MIWB) was estimated using the formula (Raburu & Masese, 2012):

MIWB = 0.5 1nN + 0.5 lnB + (H)N + H(B)

Where,

Ln- Natural log, N- Number of fish individuals caught per unit sampled,

B- Biomass of fish individuals caught per unit distance excluding tolerant and exotic species,

HN, **HB** - Shannon-Wiener diversity index based on fish numbers and biomass, respectively.

MIWB- the modified index of wellbeing.

5.2.1. Statistical analysis

Descriptive statistics comprising the means and standard deviation for each parameter were derived. Catch Per Unit Effort (CPUE), Shannon-Wiener diversity index, Simpson diversity index, percentage of relative abundance, and Habitat Quality Index were not normally distributed based on the Kolmogorov-Smirnov and Shapiro-Wilk test ($p<0.05$) hence the Kruskal-Wallis nonparametric ANOVA was used to compare sampling locations. Likewise, richness, Margalef's richness, and Menhinick's diversity indices were normally distributed based on the Kolmogorov-Smirnov test ($p>0.05$) while not normally distributed ($p<0.05$) based on the Shapiro-Wilk test ($p<0.05$). Since the Shapiro-Wilk test ($p<0.05$) is more powerful for the normality test and hence the Kruskal-Wallis nonparametric ANOVA was used to compare sampling locations. As Hill's diversity index was normally distributed ($p>0.05$), one-way ANOVA was used for comparison among the study locations. Cluster analysis (CA), principal component analysis (PCA), and factor analysis (FA) multivariate techniques were used. The modified index of biotic integrity (FIBI) was used to analyze field data on fish (Karr, 1986). Tolerant species were considered *C. gariepinus* due to their capacity to breathe atmospheric oxygen (Cala, 1987; Pinto & Araújo, 2007; Masson et al., 2017). Each metric can take the value 1, 3, or 5 (table 5.2), and the FIBI value is the result of the summation of the metric values (Karr, 1986). The data were analyzed using Statistica software (14.0).

5.3. Results

5.4.1. Composition and abundance of fish taxa in wetlands of Lake Tana

One-thousand and six-hundred sixteen fish were collected, and eighteen species, 4 genera, and 3 families were identified.

In AV, 224 fish, 8 species, and 3 genera were found and *L. beso* and *L. megastoma* were the most abundant species while *O. niloticus* was the second most abundant species. *O. niloticus* and *L. beso* were the main representatives of the phytoplanktivores and *O. niloticus* and *L. beso* represented 14.28%. Piscivores were represented by *L. megastoma* (6.25%) and *L. beso* was only present in this wetland (Table 5.3).

In RA, 117 fish, 10 species, 4 genera, and 3 families were found. These fish included an omnivore, i.e., *C. gariepinus* (11.96%), phytoplankitovores, *O. niloticus* and *G. dembecha* (9.40%), A zooplanktivore represented by *L. brevicephalus* (23.93%), fish with wide range of prey represented by *L. intermedius* (32.48%), and a macrobenthivore represented by *L. nedgia* (13.67%). *G. dembecha* was only present in this wetland (Table 5.3).

In WO, 166 organisms, 12 species, 3 genera, and 3 families were found. The most abundant fish in this wetland was *O. niloticus* while other abundant fish species included *C. gariepinus*, *L. brevicephalus, L. platydorsus, L. intermedius*, and *L. nedgia*. Phytoplanktivores were represented by *O. niloticus* (17.47%). Omnivores were represented by *C. gariepinus* (9.64%), fish with wide ranges of prey were represented by *L. intermedius* (31.92%), Zooplanktivores were represented by *L. brevicephalus* (13.86%), piscivores by *L. platydorsus* and *L. macrophthalmus* (10.84%), and benthivores were represented by *L. nedgia*. *L. macrophthalmus* was only present in this wetland (Table 5.3).

In GRM, 294 specimens belonging to 12 species, 3 genera, and 3 families were found. This wetland was dominated by C. *gariepinus, O. niloticus, L. brevicephalus, L. intermedius, L. nedgia*, and *L. tsanesis.* Fish with a wide range of prey were represented by *L. intermedius* (47.96%). Zooplanktivores were represented by *L. brevicephalus* (19.73%), macrobenthivores by *L. nedgia* (7.82%), phytoktonvore by *O. niloticus* (6.46%), omnivores by *C. gariepinus* (4.08%), and *L. acutirostris* was only present in this wetland (Table 5.3).

In ZG, 358 fish specimens in 12 species, 3 families, and 3 genera were found. *L. platydorsus* was the most dominant species while *L. brevicephalus, L. crassibarbus, L. intermedius*, and *L. tsanesis* were the second most dominant species. Omnivores were represented by *C. gariepinus* (7.26%), benthivores by *L. crassibarbus* and *L. tsanesis* (7.26%), zooplanktivores by *L. brevicephalus* (19.83%), and piscivores by *L. platydorsus* and *L. truttiformis* (7.82%). *L. truttiformis* was only present in this wetland (Table 5.3).

In MRM, 457 fish in 3 families, 3 genera, and 12 species were found. *C. gariepinus, L. brevicephalus, L. crassibarbis, L. gorgorensis, L. platydorsus, L. intermedius, L. nedgia,* L. *sukris*, and *L. tsanesis* were the most abundant species. *L. intermedius* was the main representative of fish with a wide range of prey representing 36.98%. Meanwhile, the zooplanktivores were represented by *L. brevicephalus* (19.04%), the omnivores by *C. gariepinus* (8.75%), benthivores by *L. crassibarbus, L. nedgia, L. tsanesis* (17.94%), the piscivores (4.81%) by *L. platydorsus* (4.81%), and molluscivores (2.62%) by *L. gorgorensis* (Table 5.3). MRM and ZG were dominated by *C. gariepinus* while WO and AV were dominated by *O. niloticus*. *L. brevicephalus* was abundant in AV, GRM, and MRM while *L. intermedius* and *L. nedgia* were abundant in GRM, MRM, and ZG (Table 5.3).

Among the *Labeobarbus* spp, *L. intermedius* and *L. brevicephalus* were the most abundant in the study wetlands. In contrast, *L. beso*, *L. gorguari*, *L. truttiformis*, *L. acutirostris*, *L. longissimus*, and *L. macrophthalmus* were less prevalent. Four *Labeobarbus* spp. (*L. brevicephalus*, *L. intermedius*, *L. nedgia*, and *L. surkis*), along with *C. gariepinus* and *O. niloticus*, were found in all wetlands studied (100% frequency). *L. megastoma* and *L. tsanensis* were also frequently observed, appearing in 75% of the wetlands (Figure 5.1).

Catch Per Unit Effort (CPUE)

The mean value of CPUE differed among wetlands ranging from non-detectable to 39.0 (median:13.0) in WO and from 44.00 to 110.00 (median: 53.00) in MRM. Post-hoc pairwise comparison using Tukey's HSD test showed the maximum CPUE was recorded in MRM and the lowest CPUE in RA and WO. On the other hand, the season had no effect on the median value of CPUE (K-W ANOVA, P>0.05) (Figure 5.2 a).

Number of taxa

The mean value of taxa did not differ among wetlands ranging from non-detectable to 13.0 (median: 3.0) in WO and from 1.0 to 18.00 (median: 6.0) in MRM (K-W ANOVA, P>0.05). Likewise, the season had no effect on the median value of the number of taxa (K-W ANOVA, P>0.05) (Table 5.4 and Figure 5.2 b).

Table 5. 3. Abundance of fish communities in wetlands of Lake Tana. Where, WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA-Ras Abbay

Family	**Genus**	**Species**	**AV**	**GRM**	**MRM**	**RA**	**WO**	**ZG**
Clariidae	*Clarias*	*C. gariepinus*	10	12	40	14	16	26
Cichlidae	*Oreochromis*	*O. niloticus*	26	19	12	10	29	12
Cyprinidae	*Garra*	*G. dembecha*	0	0	0	1	0	0
	Labeobarbus	*L. beso*	6	0	0	0	0	0
		L. brevicephalus	50	58	87	28	23	71
		L. crassibarbis	6	6	11	2	2	10
		L. gorgorensis	2	4	12	0	0	0
		L. gorguari	0	0	4	0	4	0
		L. megastoma	14	2	4	4	4	4
		L. platydorsus	10	6	22	0	16	24
		L. truttiformis	0	0	0	0	0	4
		L. acutirostris	0	2	0	0	0	0
		L. intermedius	69	141	169	38	53	161
		L. nedgia	20	23	36	16	10	22
		L. surkis	3	6	25	2	4	6
		L. tsanensis	8	15	35	2	3	16
		L. longissimus	0	0	0	0	0	2
		L. macrophthalmus	0	0	0	0	2	0

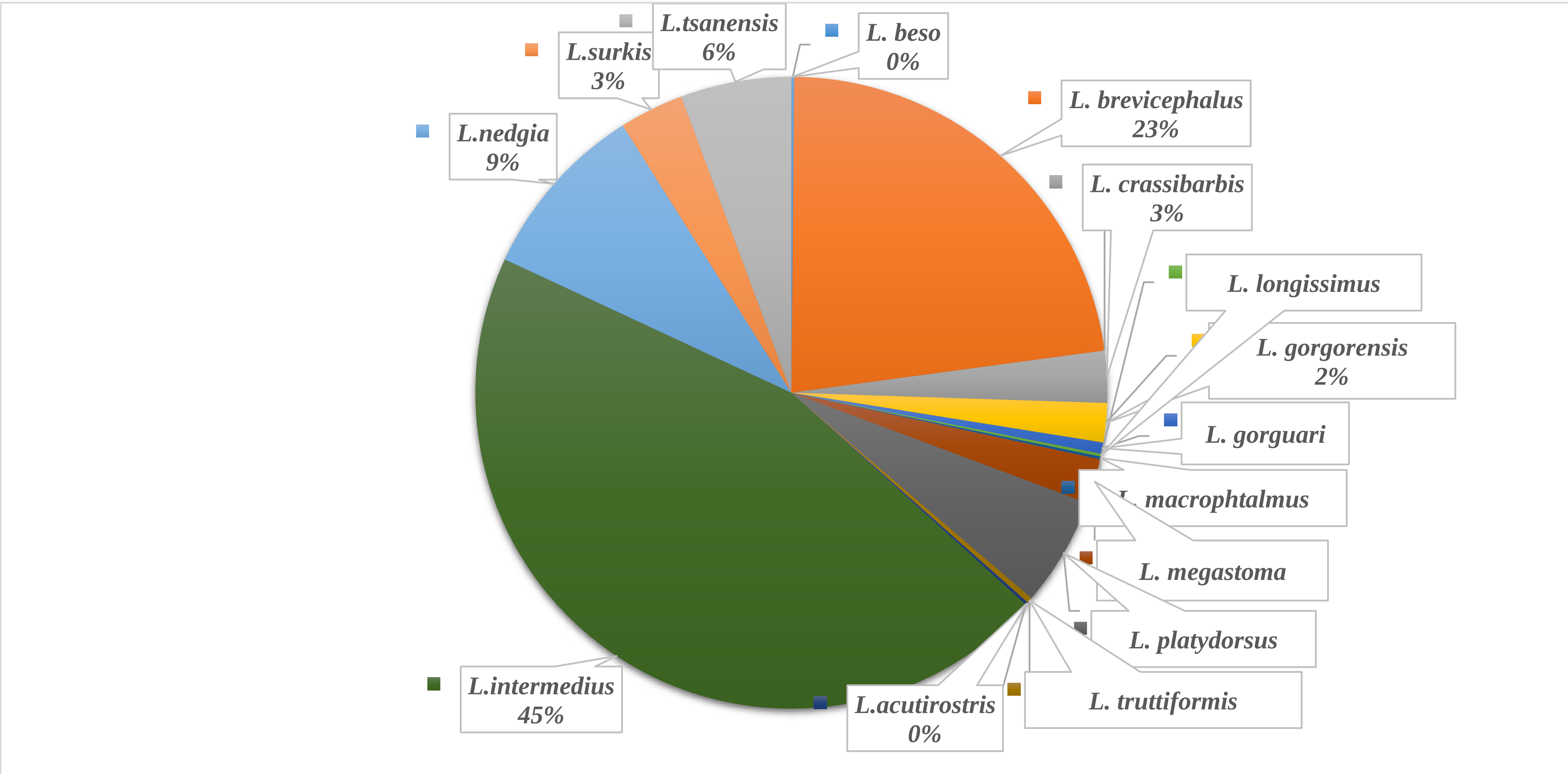


Figure 5.1. Relative abundance of the *Labeobarbus* spp. captured during the four-season gillnet catches in the wetlands of Lake Tana

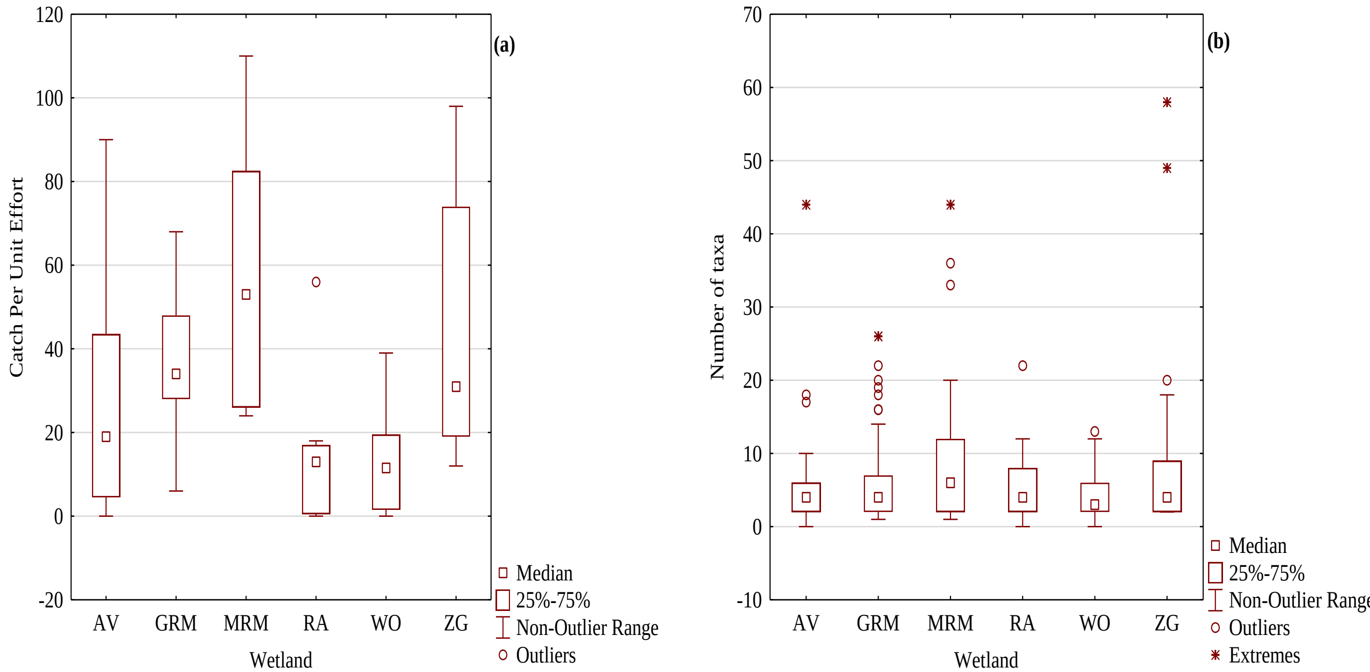


Figure 5.2. Box plot for median value of Catch Per Unit Effort (a) and number of taxa (b) by wetlands of Lake Tana.

5.4.2. Relationship between sampling wetlands and fish communities

Hierarchical cluster analysis grouped the six sites into three clusters, based on the similarity of fish community structures (Figure 5.3). The three clusters displayed in the dendrogram included WO, RA, and AV (Cluster 1), ZG and GRM (Cluster 2), and MRM (Cluster 3).

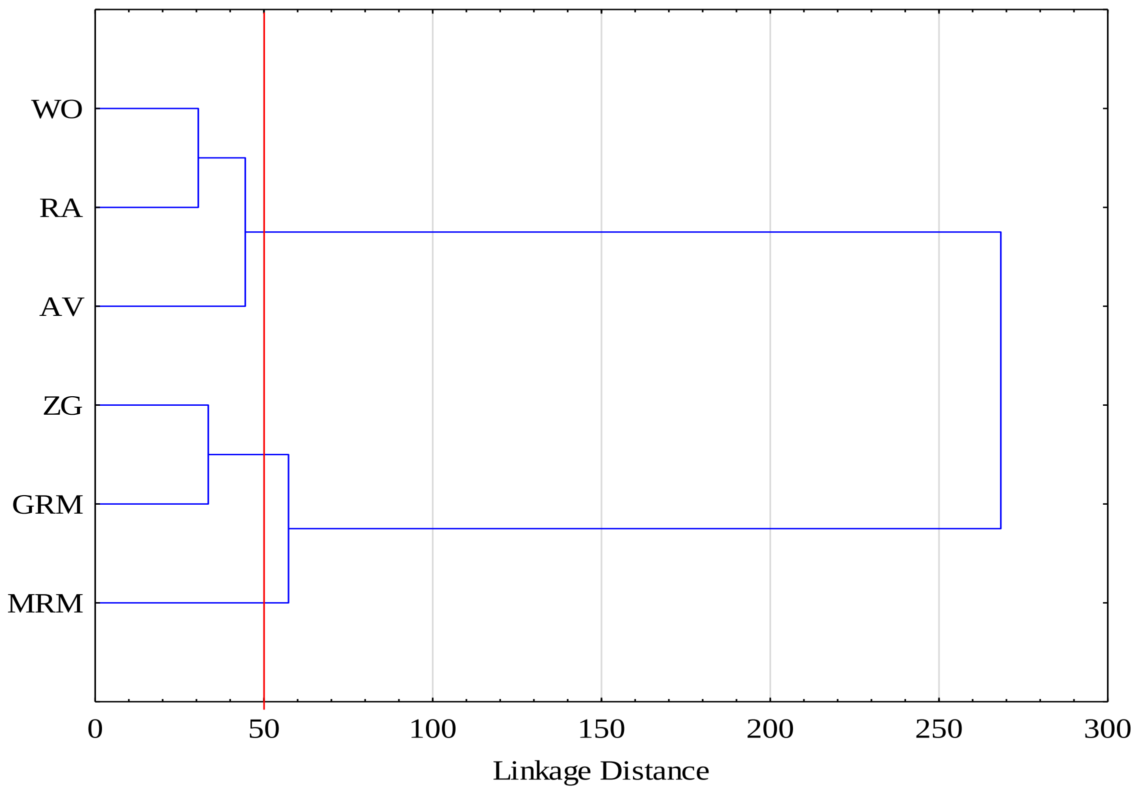


Figure 5.3. Hierarchical Cluster Analysis dendrogram showing three clusters of wetland composition, each cluster indicates wetlands with fish communities. Homogeneity within clusters was based on Euclidean distance and the heterogeneity between clusters was based on Ward's method. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA -Ras Abbay

The results of the PCA, based on normalized data of fish components, are expressed in Table 5.5, and the sampling stations are shown in Figure 5.4. Only 11 of the 18 fish species were used for the cluster and PCA analysis because six of the fish species did not explain the cluster and PCA. Only 11 of the 18 fish species were used for the cluster and PCA analysis because seven of the fish species did not explain the cluster and PCA.

The PCA analysis identified two significant components, with Component One (X-axis) accounting for 68.38% of the total variation and Component Two (Y-axis) explaining 12.20%.

The first two components accounted for 80.58% of the five eigenvalues. The PCA plot clearly shows the three clusters observed in the hierarchical cluster analysis dendrogram (Figure 5.4). Cluster 3 depicts MRM, associated with *L. platydorsus*, *L. crassibarbus*, *L. brevicephalus,* and *L. nedgia*. Cluster 2 (GRM and ZG) was associated with *L. intermedius, C. gariepinus,* and *L. surkis*. Cluster 1, (WO, RA, and AV) was associated with *O. niloticus* and *L. megastoma*.

Table 5. 4. Eigenvalues, cumulative eigenvalues, percent of the total variance, and cumulative percent of the total variance of correlation PCA for fish communities in wetlands of Lake Tana

	Eigenvalues	Cumulative Total	% of Total Variance	Cumulative % of Total Variance
1	7.52	7.52	68.38	68.38
2	1.34	8.86	12.20	80.58
3	0.93	9.79	8.47	89.05
4	0.76	10.55	6.88	95.93
5	0.45	11.00	4.07	100.00

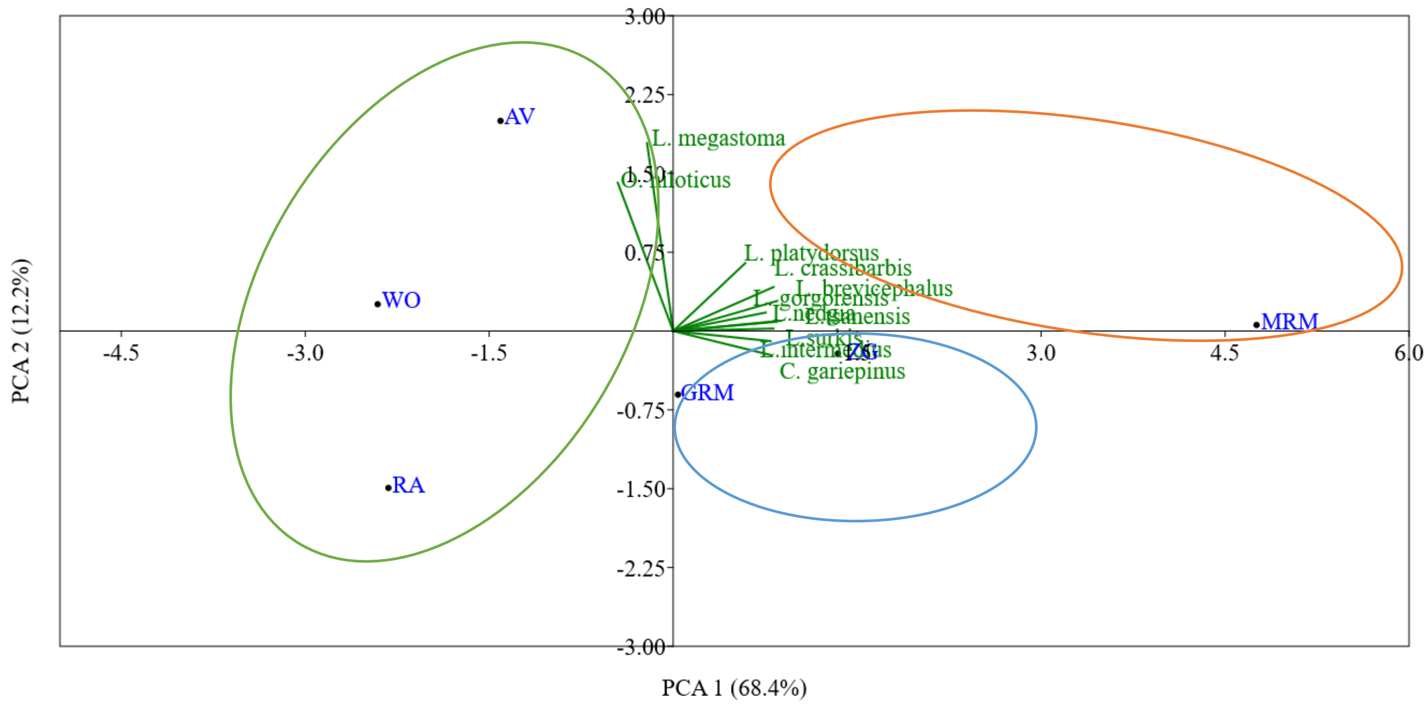


Figure 5.4. PCA plot correlating sampling wetland scores of the six study locations with water quality vectors of the fish community structure for plot Component one (X-axis) and plot Component two (Y-axis). Note the grouping of the six clusters.

5.4.3. Relationship between fish species and environmental variables

The high species-environmental correlation coefficient associated with each axis in the Canonical Correspondence Analysis (CCA) ordination demonstrated a strong association between the measured environmental variables in the six wetlands and the distribution of fish species (Table 5.6). The strongest explanatory factors were water depth, Secchi depth, ammonia, soluble reactive phosphorous, total nitrogen to total phosphorous ratio, and total dissolved substance. pH was excluded from the CCA plot due to a high multicollinearity with oxygen. *O. niloticus* and *L. megastoma* were more abundant in the RA, AV, and WO and were mainly associated with the high water depth, Secchi depth, soluble reactive phosphorous, ammonia, and total dissolved substances in these wetlands. MRM was associated with a high nitrite and nitrate-favored C. *gariepinus*, *L. intermedius*, *L. platydorsus*, *L. crassibarbus*, *L. brevicephalus*, *L. tsanensis*, *L. nedgia*, and *L. surkis*. GRM and ZG were associated with high salinity, soluble reactive phosphorous, TN: TP ratio, and nitrate that favored *L. intermedius*, and *L. nedgia*. The first three axes accounted for 98.45% of the total taxa variance (Table 4.6) explained in the observed patterns in the CCA plot (Figure 5.5).

Table 5.5. Summary of CCA axis length showing axis eigenvalues, correlation between family and the environmental gradients, and variance of family, following canonical correspondence analysis of invertebrate taxa abundance data in six wetlands of Lake Tana

	CCA Axis 1	CCA Axis 2	CCA Axis 3
Canonical eigenvalue	0.006	0.002	0.0007
Cumulative percentage variance	69.15	21.50	7.79
Species-environmental correlation	1.43		
Number of species (response variable)	11		
Number of environmental variables	12		
Total variance in family data	98.45		

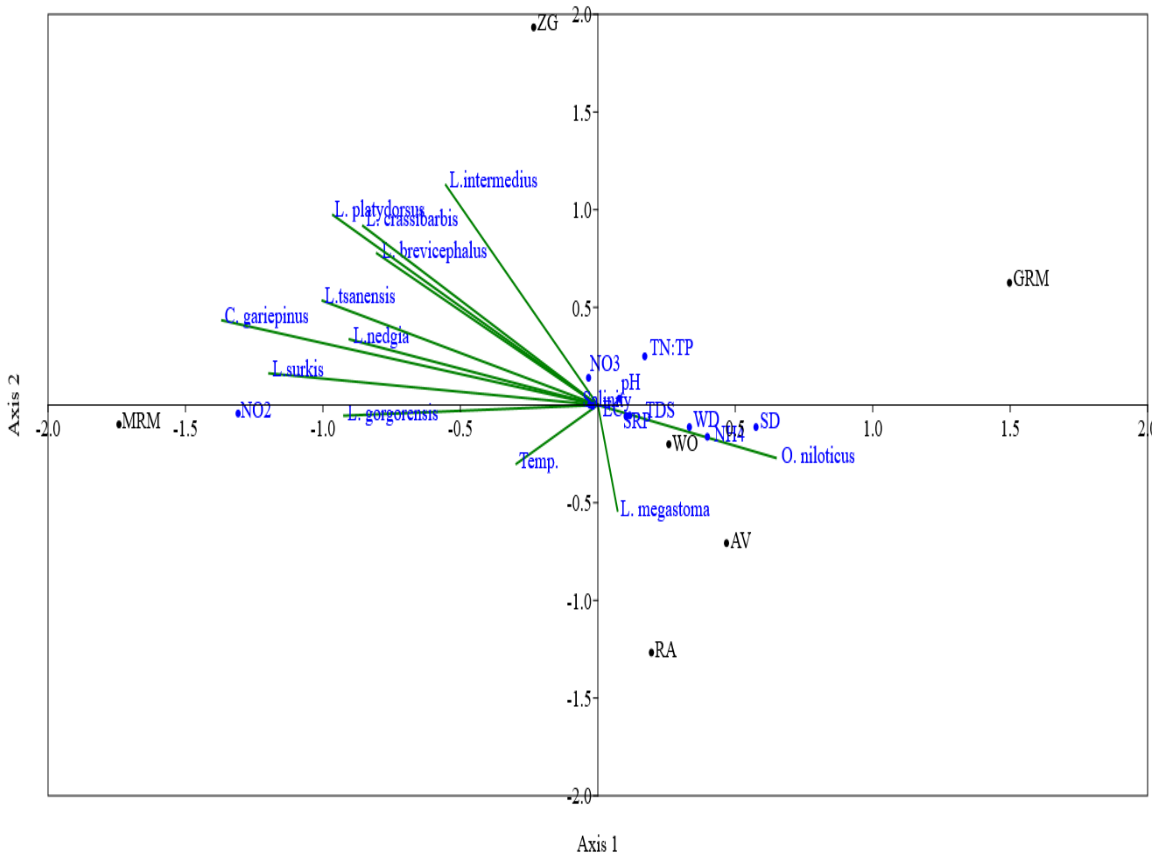


Figure 5 4. CCA plot of first and second CCA axes of fish species, environmental variables, and their corresponding wetlands. Eigenvalues: axis 1, 0.006, axis 2,0.002. The first 2 axes account for 98.06% of the variance. Where Wetlands AV-Avaj, GRM-Gumara River Mouth, MRM- Megech River Mouth, Ras Abbay, WO -Wonjeta, ZG- Zewdie Girar.

5.4.2. Habitat quality index (HQI)

The mean estimated HQI of the differed (K-W ANOVA, $p < 0.05$) among wetlands, ranging from 12.86 to 13.00 (median:13.0) in AV and from 18.75 to 19.00 (median: 18.88) in ZG. Post-hoc pairwise comparison using Tukey's HSD test indicated the highest HQIs were recorded ZG and WO while the lowest HQI was observed in RA. In contrast, the season had an effect on the median value of HQI (K-W ANOVA, $p > 0.05$) (Figure 5.6).

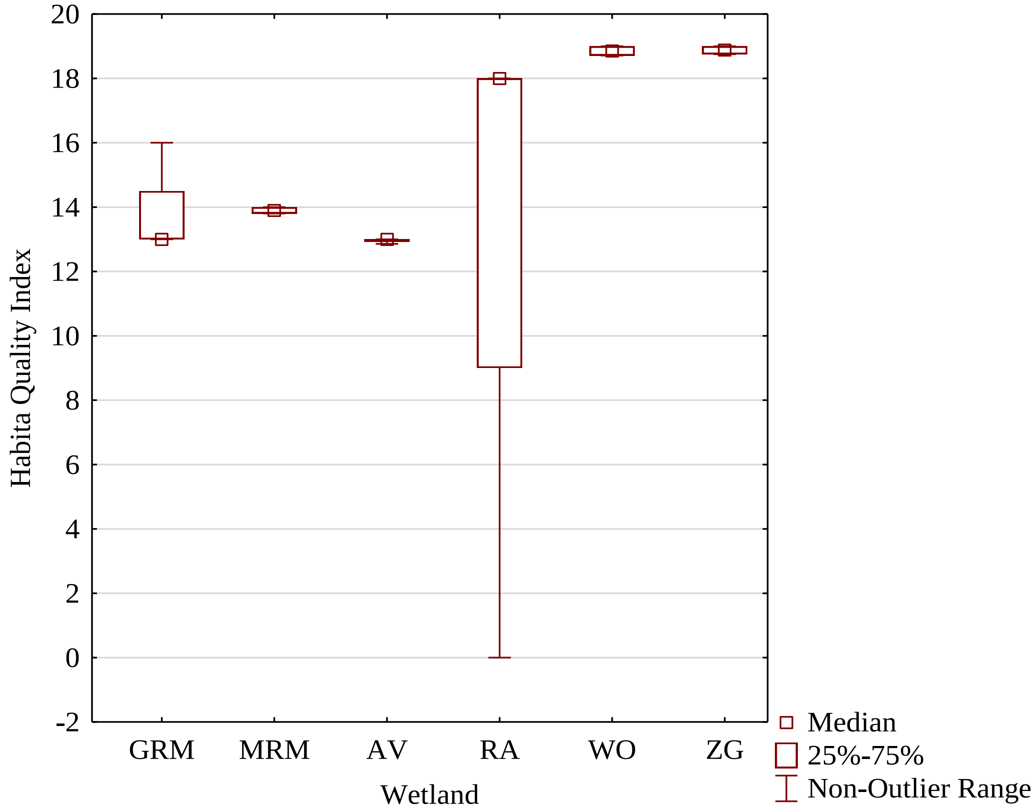


Figure 5. 5. Box plot for median value of HQI by wetlands of Lake Tana, AV- Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay and WO-Wonjeta, ZG-Zewdie Girar

5.4.3. Relationship among wetlands and fish diversity indices

Shannon-Wiener Diversity Index (H')

The median Shannon-Wiener diversity index did not differ among wetlands, ranging from non-detectable to 2.16 (median: 0.82) in WO and from 0.69 to 48.00 (median:1.58) in MRM (K-W ANOVA, P>0.05). Likewise, the median Shannon-Wiener diversity index did not differ among seasons ranging from non-detectable to 16 (median: 1.15) during the dry season and from 0.56 to 2.02 (median: 1.61) during the rainy season (K-W ANOVA, p >0.05) (Figure 5.7a).

Simpson's diversity index (D)

The median Simpson's diversity index did not differ among wetlands, ranging from non-detected to 1.0 (median: 0.09) in RA and from 0.05 to 0.27 (median: 0.13) in ZG (K-W ANOVA, $p > 0.05$). The median Simpson's diversity index did not differ among seasons. (K-W ANOVA, $p >0.05$) (Figure 5.7 b).

Margalef's richness index (R)

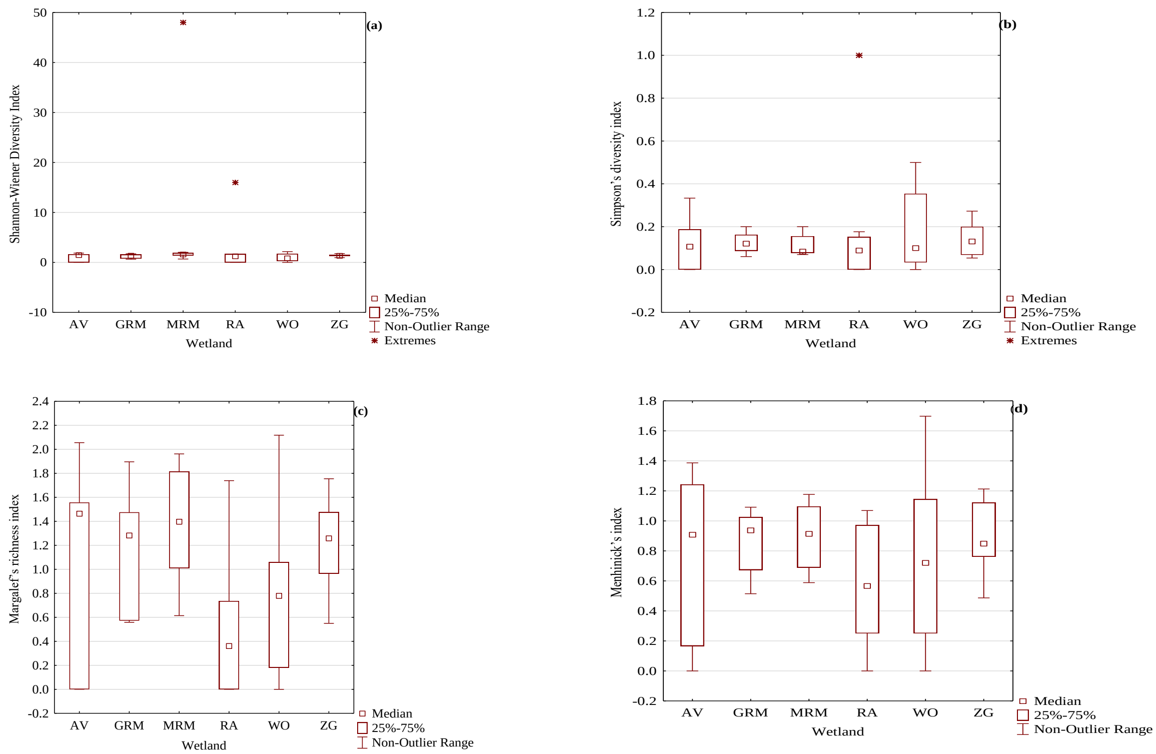
The median Margalef's richness index differed among wetlands, ranging from non-detectable to 1.74 (median: 0.36) in RA and from 0.55 to 1.75 (median: 1.26) in AV (K-W ANOVA, $p > 0.05$). In contrast, the median value of Margalef's richness index did not differ among seasons ranging from non-detectable to 1.53 (mean: 0.60) during the dry season and from non-detectable to 2.1 (median:1.5) (K-W ANOVA, $P>0.05$) (Figure 5.7c).

Menhinick's index (DMn)

The median Menhinick's index did not differ ranging from non-detectable to 1.06 (median:0.57) in RA and from 0.49 to 1.21 (median: 0.85) in ZG (K-S-ANOVA, $p>0.05$). However, the median value of Menhinick's index differed among seasons ranging from non-detectable to 1.18 (median:0.65) during the dry season and from 0.50 to 0 1.69 (median:1.06) in the late rainy season (K-W ANOVA, $p<0.05$) (Figure 5.7d).

Hill's index (H)

The median value of Hill's index differed among wetlands ranging from non-detectable to 4.13 (1.74 ± 1.41) in RA and from 2.43 to 4.58 (mean:3.41 ±0,79) in ZG (ANOVA, $p<0.05$). Likewise, the median value of Hill's index did not differ among seasons (Figure 5.7 e).



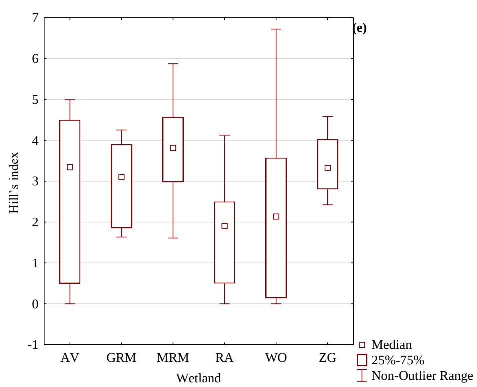


Figure 5.6. Box plot for median value of fish diversity indices in Lake Tana wetlands, The Shannon-Wiener Diversity Index (a), Simpson's diversity index (b), Margalef's richness index (c), Menhinick's index (DMn) (d), Hill's index (H)

5.4.4. Relationship among wetlands and fish biotic integrity indices

Fish-Index of Biotic Integrity (FIBI)

The median FIBI differed among wetlands ranging from 18.0 to 29.0 (median: 8.0) in RA and from 30.0 to 48.0 (median: 8.0) in MRM (K-W ANOVA, $p<0.05$). Post-hoc pairwise comparison using Tukey's HSD test indicated the highest median values of FIBI in GRM, MRM, and ZG while the lowest median of FIBI in RA and WO. However, the median value of FIBI did not differ among seasons (K-W ANOVA, p<0.05) (Figure 5.8a).

Percentage of benthic individuals

The median value of the percentage of benthic individuals did not differ among wetlands ranging from non-detectable to 15.6 (median: 1.94) in AV and from non-detectable to 58.8 (median:19.01) in ZG (K-W ANOVA, p>0.05). In contrast, the median value of the percentage of benthic individuals differed among seasons ranging from non-detectable in the dry season and from non-detectable to 100.0 (mean: 9.82) in the late rainy season (K-W ANOVA, p<0.05) (Figure 5.8b).

Percentage of tolerant individuals

The median value of the percentage of tolerant individuals did not differ among wetlands ranging from non-detectable to 100.0 (median:1.79) in RA and from non-detectable to 100.0 (median: 27.21) in WO (K-W ANOVA, p>0.05). However, the median value of the percentage of tolerant individuals differed among seasons ranging from non-detectable to 33.33 (median: non-detectable) during the rainy season and from 2.04 to 100.00 (median: 20.80) during the early rainy season (K-W ANOVA, p<0.05) (Figure 5.8c).

Percentage of Cyprinidae individuals

The median value of the percentage of Cyprinidae individuals differed among wetlands ranging from non-detectable to 60.00 (median:14.0) in RA and from 54.17 to 100.0 (mediaan:91.24) in GRM (K-W ANOVA, p<0.05). Post-hoc pairwise comparison using Tukey’s HSD test indicated the highest median for the percentage of Cyprinidae individuals was recorded in GRM and MRM while the lowest median for the percentage of Cyprinidae individuals was observed in WO and RA. The season had no effect on the median value of the percentage of Cyprinidae individuals (K-W ANOVA, p>0.05) (Figure 5.8d).

Percentage of detritivorous individuals

The median value of the percentage of detritivorous individuals did not differ among wetlands ranging from non-detectable to 3.57 (median: non-detected) in RA and from non-detectable to 16.67 (median: non-detectable) in ZG (K-W ANOVA, p>0.05). However, the median value of the percentage of detritivorous individuals differed among seasons ranging from non-detectable during the dry season and from non-detectable to 11.76 during the late rainy season (K-W ANOVA, $p<0.05$) (Figure 5.8e).

Percentage of carnivorous individual

The median percentage of carnivorous individuals did not differ among wetlands ranging from non-detectable to 7.14 (median: non-detectable) in RA and from non-detectable to 33.33 (median: 7.23) in ZG (K-W ANOVA, $p>0.05$). However, the median percentage of carnivorous individuals differed among seasons ranging from non-detectable to 13.79 (median: non-detectable) during the dry season and from non-detected to 33.33 (median:5.97) during the rainy season (K-W ANOVA, $p>0.05$) (Figure 5.8f).

Percentage of omnivorous individuals

The percentage of omnivorous individuals did not differ among wetlands ranging from non-detectable to 5.26 (median: non-detectable) in AV and from non-detectable to 16.67 (median:9.27) in MRM (K-W ANOVA, $p>0.05$). Likewise, the mean percentage of omnivorous individuals did not differ among seasons ranging from non-detectable to 33.33 (median: non-detectable) in the rainy season and from non-detectable to 40.00 (median: 7.73) in the early rainy season (K-W ANOVA, $p>0.05$) (Figure 5.8g).

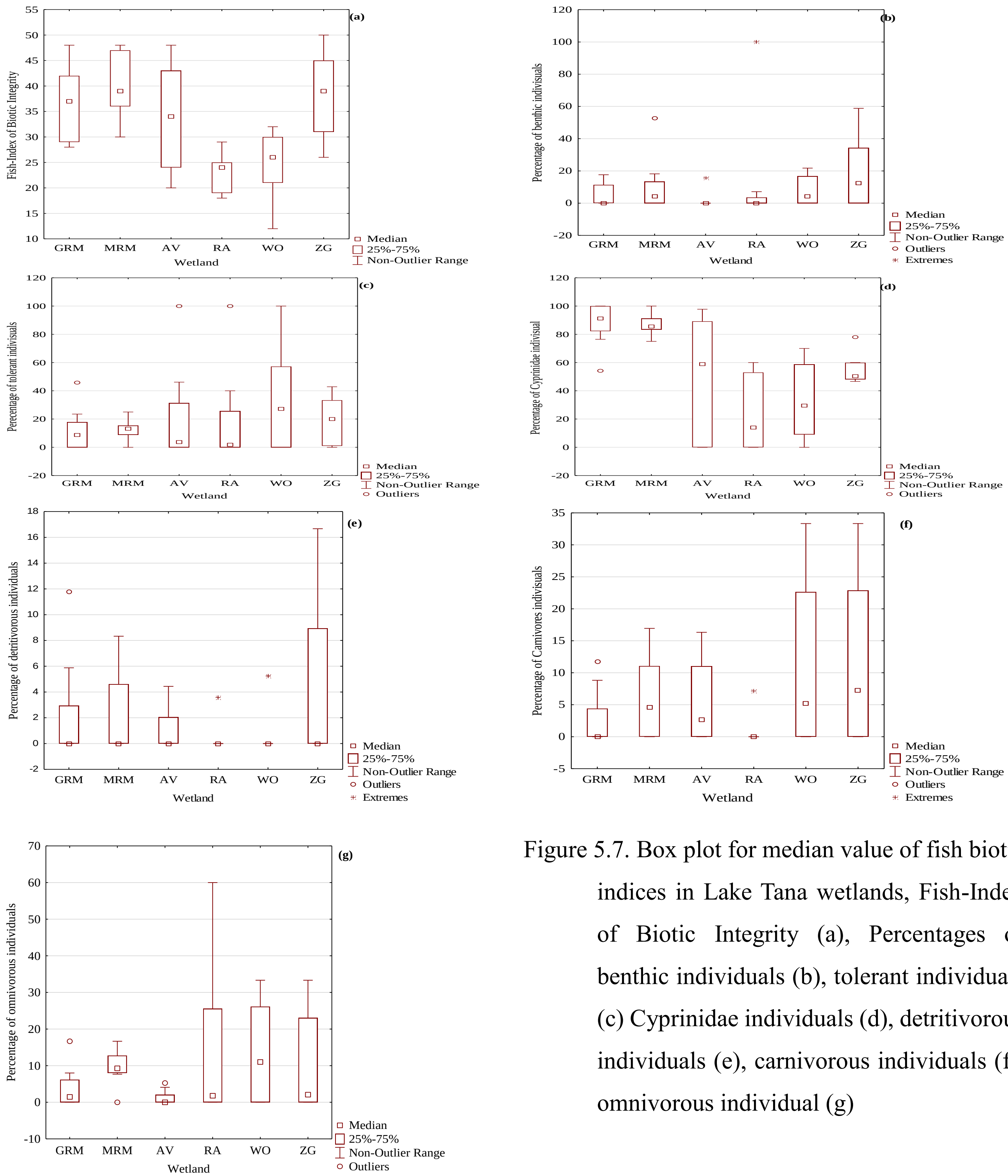


Figure 5.7. Box plot for median value of fish biotic indices in Lake Tana wetlands, Fish-Index of Biotic Integrity (a), Percentages of benthic individuals (b), tolerant individuals (c) Cyprinidae individuals (d), detritivorous individuals (e), carnivorous individuals (f), omnivorous individual (g)

5.4. Discussion

5.4.1. Composition and abundance of fish species in the study wetlands

In this study 18 fish species, 3 families, and 4 genera were identified from catches in six wetlands of Lake Tana. These numbers are lower than the total of 28 fish species categorized into four families (Cyprinidae, Balitoridae, Clariidae, and Cichlidae) and seven genera (*Labeobarbus*, *Barbus*, *Garra*, *Varicorhinus*, *Afronemacheilus*, *Clarias*, and *Oreochromis*) found in Lake Tana (Getahun & Dejen, 2012; Habteselassie, 2012).

Eighty-six percent of the fish species identified in this study belonged to the Cyprinidae family. This aligns with previous research that found Cyprinidae to be the dominant group in Lake Tana. Goshu et al. (2010b) identified *Labeobarbus* spp., *Oreochromis niloticus*, *Clarias gariepinus*, and *Varicorhinus beso* as commercially important fish species, accounting for 77%, 13%, 9%, and 1% of the total experimental fish catch, respectively. There are several reports in Lake Tana and other lacustrine environments in the Eastern African Great Lakes that reported *Labeobarbus* spp., *Oreochromis niloticus,* and *Clarias gariepinus* as the most dominant species (Wudneh, 1998; de Graaf et al., 2006; Vijverberg et al., 2009; Anteneh et al., 2012a; Gebremedhi et al., 2013; Dejen et al., 2017; Gebremedhin et al., 2018; Tefera et al., 2019). The higher abundance of piscivores represented by *L. beso* and *L. megastoma* and phytoplanktivores represented by *O. niloticus* in AV may be associated with a high density of papyrus and forest trees (de Graaf et al., 2008; Gebremedhin et al., 2018; Abdissa et al., 2022). The lower abundance of the less tolerant *Labeobarbus* spp. in AV may be attributed to the discharge of untreated wastewater from urban effluents.

The higher abundance of fish with a wide range of prey represented by *L. intermedius* and zooplanktivores represented by *L. brevicephalus*, the omnivore represented by *C. gariepinus,* and macrobenthivore represented by *L. nedgia* in AV may be associated with a higher density of grass and trees (de Graaf et al., 2008; Gebremedhin et al., 2018; Abdissa et al., 2022). The lower abundance of *Labeobarbus* spp. in RA may be due to overfishing by motorized commercial fishers (Getahun and Dejen, 2012; Dejen et al., 2017).

The higher abundance of phytoplanktivore represented by fish with wide ranges of prey was represented by *L. intermedius* and zooplanktivore was represented by *L. brevicephalus* in WO. This may be associated with higher papyrus in this wetland. This wetland is characterized by relatively low pressure from farming and wastewater (Wondie, 2018; Kahsay et al., 2023). Although the initial assumption was that the ranking of wetlands would be primarily determined by pollution levels, vegetation cover, and human activity, the actual results suggest that other factors may have played a significant role. WO wetlands are lacustrine wetlands, papyrus-dominated with relatively low pressure from human activities.

GRM was dominated by fish with a wide range of prey, represented by *L. intermedia,* and zooplanktivores represented by *L. brevicephalus*. This may be associated with domination by *Eichhornia crassipes* which provide shelter from predators (Gezie et al., 2018; Erarto et al., 2020).

ZG was dominated by zooplanktivores represented by *L. brevicephalus*, and piscivores represented by *L. platydorsus* and *L. truttiformis.* This may be associated with the high dominance of papyrus and indigenous trees (Anteneh et al., 2012a).

The higher abundance of fish species in MRM includes fish with a diverse diet, such as the zooplanktivore *L. brevicephalus,* the omnivore *C. gariepinus*, and the benthivore *L. crassibarbus*, *L. nedgia*, and *L. tsanensis*. The greater abundance of fish communities in this wetland might be connected to the presence of water hyacinth, which has been documented as a refuge for fish (Gebremedhin et al., 2012; Gezie et al., 2018; Mequanent et al.,2022).

MRM, GRM, and ZG had the highest species richness and CPUE, compared to AV, RA, and WO. This might be associated with the lower access of these wetlands to fishermen with motorized boats. The lowest species richness, and individuals per catch in the AV, RA, and WO wetlands may be linked to a higher number of motorized fisheries in the southern gulf of the lake near Bahir Dar City. Before the mid-1980s, the fisheries in Lake Tana were primarily subsistence-based, carried out by the Negada-Woito people using traditional reed boats (de Graaf et al., 2006; Dejen et al., 2017). Due to the lack of motorized boats, fishermen were limited to the lake's shoreline.

Fishermen used locally made fishing gear, such as traps, hooks, and small gillnets, mainly targeting *O. niloticus*. In 1986, the Lake Tana Fisheries Resources Development Program introduced modern fishing equipment like motorized boats and nylon gillnets. This allowed fishermen to access offshore wetlands near Bahir Dar and distant river mouths in the northeast, leading to increased fish catches, overfishing of young fish, and a decline in fish populations.

5.4.2. Relationship among wetlands and fish diversity indices

The six study wetlands of cluster 1 (AV, RA, and WO), cluster 2, (GRM and ZG), and cluster 3 (MRM) were investigated for the fish community structure of fish following multivariate analyses. The groups were first ranked using hierarchical clustering based on the similarity of the fish community of groups, a finding that was then confirmed by PCA and FA. Cluster 3, MRM was mainly associated with the highly adaptive and resilient *C. gariepinus* and Cyprinidae species such as *L. gorguari*, *L. gorgorensis*, *L. surkis*, and *L. nedgia*. *L. tsanensis*, while cluster 1 (WO, RA, and AV) was mainly associated with *Cyprinidae* species, which included, *G. mecha, L. macrophthalmus*, *L. acutirostris,* and *L. megastoma.* The higher abundance of *C. gariepinus* in MRM is in agreement with previous sightings of the high abundance of gonadally mature *C. gariepinus* at MRM (Anteneh et al., 2012a; Vijverberg et al., 2012; Teshome et al., 2015; Gebremedhin et al., 2018; Assefa et al., 2019). Gebremedhin et al. (2018) reported that *C. gariepinus* migrates to the floodplains to spawn. Chemical and physical factors may influence migratory fish to gather at the river mouths (Anteneh et al., 2012a). Flooding events, which raise the lake level and increase turbidity, can also trigger the migration of fish (de Graaf et al., 2006; Mingist & Gebremedhin, 2016). It has been proposed that the abundant *L. intermedius* and *L. platydorsus* in the heavily polluted MRM wetland have developed a new reproductive strategy, preferring muddy and sandy habitats for spawning in streams near Lake Tana (Mingist & Gebremedhin, 2016; Gebremedhin et al., 2018).

The most abundant fish were Cyprinidae species, including *L. brevicephalus*, *L. megastoma*, and *L. nedgia*, as well as the adaptable cichlid *O. niloticus*, which were predominantly found in WO, RA, and AV. These species favored sandy and rocky habitats with dense vegetation in the inshore wetlands. The abundant vegetation in AV, RA, and WO supported the production of zooplankton and phytoplankton, which are preferred by the adaptable *O. niloticus* (Paugy and Levêque 1999; de Graaf et al., 2006; Dejenet al., 2017; Gebremedhin et al., 2018; Assefa et al., 2019).

5.4.3. Habitat quality index and fish index of biotic integrity in the study of wetlands

The highest habitat quality indices in WO and ZG are associated with a high density of indigenous macrophytes and trees (Gebresllassie et al., 2014; Mehari et al., 2014; Kahsay et al., 2022). WO is known for its papyrus and indigenous tree forests, and ZG is a lacustrine wetland rich in reed swamps (over 75%) and surrounded by mountain forests. RA is a riverine wetland covered with vegetation and forest trees, crossed by the Blue Nile River. Additionally, the diverse wetland habitats of RA receive untreated and industrial wastewater. AV is an urban wetland that receives stormwater, drugs, and waste from the hospital, and domestic wastewater generated by hotels and fish landing sites from surrounding communities (Atnafu et al., 2011; Gezie et al. 2017; Mucheye et al., 2018; Wondie, 2018; Kahsay et al., 2022). GRM and MRM are dominated by sediment from agriculture catchments as well as water hyacinth (Gezie et al., 2018; Dersseh et al., 2019; Enyew et al., 2020; Damtie et al., 2021; Dersseh et al., 2022). The low HQI in the MRM and GRM wetlands is in line with recent research findings, which have indicated that the biotic indices in Lake Tana significantly decreased with increasing human disturbance (Wudneh, 1998; Vijverberg et al., 2009; Wondie, 2018; Erarto et al., 2020). Fish-based indices of biotic integrity have been widely adopted and applied in riverine ecosystems, where the indices have been useful in providing information on levels and sources of degradation and developing biological criteria for aquatic ecosystem protection and restoration (Dudgeon et al., 2006; Masese et al., 2013; Naigaga et al., 2011; Reynolds, 2013).

5.4.4. Spatiotemporal diversity and site grouping based on fish species characteristics

The Shannon Wiener diversity index was highest at MRM and RA while the Margalef species richness index was highest at MRM and ZG. Shannon-Wiener diversity and Margalef species richness indices were lowest at AV and WO. Thus, the AV and WO which are located closer to Bahir Dar city registered the lowest diversity and richness, while the highly impacted effluentrecipient MRM recorded the highest indices. This difference may be due to the existence of motorized commercial fishers in locations closer to Bahir Dar while MRM and ZG were situated far from Gondar City and are less accessible for commercial fishers (Gebremedhin et al., 2018; Dejenet al., 2017; Getahun and Dejen, 2012; de Graaf et al., 2006). The higher Shannon diversity and Margalef species richness indices in rainy and late rainy seasons may be associated with the spawning migration of *Labeobarbus* spp. in the rainy season (Anteneh et al., 2012a; Gebremedhi et al., 2013; Teshome et al., 2015; Abdissa et al., 2022; Mequanent et al., 2022).

Simpson's diversity index was higher in RA and WO while the lowest values were recorded in AV and MRM. This implies that although there was a higher diversity at the RA and WO, these sites had low species richness, while at AV and MRM, diversity was low while species richness was high. The maximum Simpson's diversity index was in the late rainy and early rainy seasons while the lowest was recorded in the rainy and dry seasons. This could be a result of the poor water quality during the late and early rainy season making the environment less habitable for many fish species.

The highest Menhinick's and Hill's diversity indices in AV and MRM may be attributed to the spawning migration of *Labeobarbus* spp. in WO and MRM during the late rainy season. The maximum Menhinick's and Hill's diversity indices in AV and MRM may be associated with these wetlands that are characterized by fast-flowing and highly oxygenated water (Teshome, 2020; Wondie, 2018). Gebremedhin et al. (2012) found that the peak spawning season for *Labeobarbus* species occurred from August to October (late rainy season), based on the gonado-somatic index of *Labeobarbus* spp. Similarly, Anteneh et al. (2012b) reported that six species of *Labeobarbus*, including *L. brevicephalus*, *L. intermedius*, *L. megastoma*, *L. nedgia*, *L. truttiformis*, and *L. tsanensis*, migrated more than 60 kilometers upstream in the Ribb River to spawn.

5.5.5. Relationship among wetlands and fish biotic indices

The higher FIBI values in MRM, GRM, and ZG contradict the anticipated reduction in species richness, which can be expected along a degradation gradient. The weak relationship between the FIBI and HQI can be explained by the fact that the lower access of motorized fisheries to MRM, GRM, and ZG influenced fish assemblage characteristics which were not captured by habitat assessment. However, other reports have indicated that habitat quality significantly influences FIBI which indicates that the fish index can identify near-aquatic and aquatic habitats impacted by human activities like sand mining and watering animals in rivers (Raburu et al., 2017; Masese & Raburu, 2017; Bassa et al., 2020).

The predominance and migration of Cyprinidae in the river mouth of Gumara and Megech and the decline of Cyprinidae in lacustrine wetlands such as WO and ZG during the rainy season suggest that Cyprinidae are sensitive to poor water quality and habitat degradation. Although the percentages of functional groups (e.g., benthic, tolerant, omnivorous species) are components of the Fish Index of Biotic Integrity (FIBI), they are presented here for transparency and to demonstrate how individual metrics contribute to the overall FIBI score. These metrics are not interpreted in isolation but are integrated within the composite index to provide a comprehensive assessment of ecological integrity. In contrast, the significant difference in seasonal/spatial variation for the mean percentage of carnivorous and omnivorous individuals in this study is in line with the findings of de Graaf et al. (2004). In contrast, Shitaw et al. (2018) and Goshu et al. (2010) found that *Labeobarbus* spp. of Lake Tana showed significant variation in catches at four sampling sites and that these species showed significant variation in dry and rainy seasons. In this study, habitat quality and fish biotic indices were used in six wetlands of Lake Tana and the mean HQI, % of detritivorous and carnivorous individuals varied among wetlands. Likewise, the percentage of benthic individuals, % of tolerant individuals, detritivorous individuals, % of carnivorous individuals differed among seasons. This finding is in line with observations in different tropical freshwater assessments using a fish-based index of biotic integrity for monitoring (Desmond et al., 2002; Bhagat and Ruetz, 2011; Raburu and Masese, 2012; Sheaves et al., 2012; Chea et al., 2020; Masese et al., 2020).

The HQI and FIBI used in this study have revealed environmental degradation and the potential of ecosystems to support healthy fish populations. The indices suggest that pollution from poor agricultural practices and urban waste were major factors in the significant decline of environmental conditions, as evidenced by the proliferation of invasive water hyacinths. This demonstrates the impact of disturbances on fish assemblages in Lake Tana. The higher biotic integrity assessment tool offers water resource managers a scientifically sound basis for reducing freshwater habitat degradation and non-point source pollution. FIBI integrated data from individuals, populations, groups, fish distribution, and ecosystems into a single water quality index, thereby offering a more complete understanding of lake health than physical and chemical indicators (Bozzetti & Schulz, 2004; Miller et al., 2018).

WO and ZG had low FIBI while HQI indices of these wetlands were high. In contrast, the more disturbed wetlands MRM and GRM had high FIBI scores and low HQIs, which might encourage conservation or restoration (Masese & Raburu, 2017). As using FIBI could not delineate different wetlands according to the level of degradation, its use with HQI is encouraged, to identify wetlands with potential problems in Lake Tana where interventions are needed to avoid further degradation to ecosystem integrity. For example, human activity and water hyacinth invasion in GRM and MRM influenced fish diversity and FIBI.

In addition to summarizing spatial and seasonal patterns, this study also considered the ecological roles of fish species in relation to their indicator potential. Species such as *Clarias gariepinus* and *Oreochromis niloticus*, which are ecological generalists, were found across all wetlands and exhibited tolerance to a wide range of environmental conditions. While their ubiquity makes them less suitable as sensitive indicators of pollution, their dominance in disturbed wetlands highlights their value in signaling degraded conditions. In contrast, certain *Labeobarbus* spp., particularly those with narrow habitat preferences and specialized feeding strategies, were more restricted in distribution and showed stronger associations with specific environmental gradients. These species demonstrate higher indicator potential for assessing ecological integrity and wetland classification.

5.5. Conclusions and recommendation

The physicochemical analysis in Chapter 3 grouped WO, GRM, and ZG in the least polluted, AV and RA in moderately polluted, and MRM in highly polluted clusters while the macroinvertebrate taxonomic composition in Chapter 4 grouped WO in the least polluted, AV and RA in the slightly polluted, GRM and ZG in the moderately polluted, and MRM in the highly polluted cluster. However, the fish community taxonomic composition in this chapter grouped WO, RA, and AV in cluster 1, GRM and ZG in cluster 2, and MRM in cluster 3. *O. niloticus* and *L. megastoma* were more prevalent in RA, AV, and WO, and were often found in areas with deeper water, higher Secchi depth, elevated soluble reactive phosphorus, ammonia, and total dissolved solids. MRM was associated with higher nitrite and nitrate levels, which favored *C. gariepinus*, *L. intermedius*, *L. platydorsus*, *L. crassibarbus*, *L. brevicephalus*, *L. tsanensis*, *L. nedgia*, and *L. surkis*. GRM and ZG had higher salinity, soluble reactive phosphorus, TN: TP ratios, and nitrate levels, which may have favored *L. intermedius* and *L. nedgia*. While the evidence in this chapter suggests that commercial fishing in the Southern Gulf of Lake Tana wetlands, such as WO, AV, and RA, has led to lower fish diversity and biotic indices, the opposite was observed in MRM, ZG, and GRM. These wetlands, being less accessible and remote, were less impacted by overfishing, resulting in higher catch per unit effort, species richness, fish diversity, and biotic integrity. The quality of the water in Lake Tana's wetlands significantly impacted the fish populations, as measured by various ecological indices. These indices included the Habitat Quality Index, Margalef's richness index, Hill's index, Fish-Biotic Integrity, and the percentage of Cyprinid individuals. However, this was not further supported by diversity indices like Shannon-Wiener Diversity, Simpson's diversity, Menhinick's indices, and biotic indices like percentage of benthic, percentage of tolerant, percentage of detritivorous, percentage of carnivorous, and percentage of omnivorous individuals. Fish diversity and biotic indices could be valuable biological indicators for water quality assessments in Lake Tana, as these indices appear to be unaffected by overfishing. The findings recommend using fish community structure for biomonitoring aquatic environments in Ethiopia. Historical data on fishing activities should be collected before conducting assessments. This research also advocates for the sustainable use and conservation of fish stocks, biodiversity, and aquatic environmental quality. Conservation efforts should consider the impact of societal changes in the surrounding areas on water quality.

CHAPTER 6

Fish histopathology as a biomarker of aquatic environmental quality in wetlands of Lake Tana, Ethiopia

6.1. Introduction

Developing suitable biological indices for environmental quality assessments is becoming more important as the evaluation and observation of the aquatic environment are critical for aquatic ecosystem health management. The use of biomarkers is advantageous in the study of intricate geographical and temporal variations in pollutant exposure or biological impacts in the field (McCarthy et al., 1990; Lewis et al., 1999; Abdel Moneim et al., 2012; Liebel et al., 2013; Yancheva et al., 2016) (see Chapter 1). Even though fish pathological changes caused by environmental stressors can look similar regardless of a specific cause, pathological changes can still be valuable indicators of environmental stress (van der Oost et al., 2003; Bernet et al., 2004; Dalzochio et al., 2016a).

Histopathological changes in various fish tissues have been used in evaluating aquatic pollution and as a source of additional information to supplement physicochemical analyses (Bernet et al., 1999; van der Oost et al., 2003; Dalzochio et al., 2016). Furthermore, histopathological changes in different fish organs and tissues have been used as biomarkers for the assessment of the ecological quality of aquatic ecosystems and biomonitoring (Bernet et al., 1999; Abdel-Moneim et al., 2012; Abalaka, 2017; Lebepe et al., 2020). Schwaiger et al. (1997), Reddy (2012), and Yancheva et al. (2016) stated that histopathological alterations can be used as indicators of the effects of various human-caused pollutants and that pathological changes reflect the overall health of the entire population in the studied ecosystem. Histopathology in animal tissues has long been applied as a diagnostic tool in human and animal medicine (Nikolić et al., 2021). In the past two decades, however, histopathological characteristics have become potent indicators of prior exposure to environmental stressors in fish (Bernet et al., 1999; van der Oost et al., 2003; Zimmerli et al., 2007; Yancheva et al., 2016).

Histopathological biomarkers provide a view of pathogenic and biotic factors, in addition to water quality, thus enabling a more holistic view of environmental deterioration and its effects on organisms such as fish (van der Oost et al., 2003; Bartell, 2006; Hook et al., 2014b; Dalzochio et al., 2016a; de Lima Cardoso et al., 2018). Therefore, fish histopathology biomarkers are considered to be an excellent method for assessing environmental quality (de Lima Cardoso et al., 2018). Several reports suggest that changes in fish tissues can be a helpful tool to evaluate the level of pollution, especially for sub-lethal and long-term effects (Bernet et al., 1999; Reddy, 2012). Histopathological analysis of fish organs and tissues exposed to environmental pollutants is a valuable biomarker that can help identify the harmful environmental factors an organism has been exposed to over a long period (Shuman et al., 2019). One of the key strengths of histopathological biomarkers is the ability of histopathological indices to target specific organs for examination in environmental pollution monitoring (Parikh et al., 2010; Naeemi et al., 2013). Histopathological changes in fish tissues and organs are indicators of the impact of exposure to environmental stressors (Liebel et al., 2013). Moreover, for field assessments, histopathology is the quickest method of detecting harmful short-term and long-term effects of exposure in the different tissues and organs of a fish or shellfish (Hinton, 2021).

The objective of this study was to investigate fish histopathology in relation to effluent exposure and evaluate its potential as a biomarker for water quality in the wetlands of Lake Tana. *Labeobarbus* spp., consistently present in all six study wetlands, was selected as the bioindicator species. Histological alterations in the liver, gills, gonads, and spleen were examined to detect seasonal and spatial variations in environmental conditions across wetlands with varying levels of impact. The study sites were compared using fish histopathology indices and lesion prevalence to assess pollution effects.

Accordingly, this chapter addresses Objective 3 by examining histopathological changes in *Labeobarbus* spp. as indicators of water quality deterioration. Organ-specific indices were calculated based on reaction patterns such as circulatory, regressive, progressive, inflammatory, and neoplastic changes. The severity of tissue damage was then used to classify wetlands and evaluate the ecological impacts of pollution on fish health.

6.2. Specific Methods

6.2.1. Sampling protocol and sample preparation

Ninety-six samples were collected from six wetland ecotones along the shoreline of Lake Tana. *Labeobarbus* spp. specimens were kept alive by the continuous exchange of running water from a mobile tap unit in the field until euthanasia. The water used in the mobile tap was sourced from the study location. After catching fish in each study wetland, live fish were euthanized immediately *in situ* by the cervical dislocation technique using a scalpel to incise between the skull and the first vertebra. The fish were dissected to remove the liver, gills, spleen, and gonads for histopathological processing. The dissected organs were preserved in a 10% neutral-buffered formalin solution and labeled for subsequent tissue processing. In the laboratory, samples were dehydrated in alcohol, cleared in xylene, embedded in paraffin, sectioned at 3–5 μm thickness, stained with Hematoxylin-Eosin stain, and observed under a light microscope (Leica) and described according to Liebel et al. (2013).

6.2.2. Histological assessment

Light microscopy was used for the examination of the tissues. Histological alterations were qualitatively assessed in all organs. To reduce error due to observer bias during histopathological analysis, a 'blind' procedure was employed. Histopathology slides with codes were interpreted, results were recorded, and an expert interpreted the coded slides independently for comparison. Human error discrepancy was expected due to an unfamiliar task or a routine, and fatigue may increase the chances of error. To minimize this problem repeated observations with a minimum of three readings and interpretation per slide were made to obtain an average value. The averaged results were semi-quantitatively evaluated according to a protocol proposed by Bernet et al. (1999), which was slightly modified in nomenclature and included gonadal and splenic histopathology, which were not included in the Bernet protocol. For each organ, the pathological changes were classified into five "reaction patterns": "circulatory disturbances" (C), "regressive changes" (R), "progressive changes" (P), "inflammation" (I), and "neoplasms" (N), and the lesions under each reaction pattern were identified and scored. Circulatory disturbances were the result of a pathological condition of blood and tissue fluid flow, such as congestion, hyperemia, edema, thrombosis, and embolism. Regressive changes were defined as processes that could terminate in a functional reduction or loss of an organ, such as atrophy, degenerative changes, and necrosis. "Progressive changes" were defined as processes that lead to increased activity of cells or tissues, such as hypertrophy and hyperplasia. Inflammatory changes were often associated with processes belonging to other reaction patterns, such as cellular infiltration and exudates. Lastly, a tumor or neoplasm depicts uncontrolled cell and tissue proliferation (autonomous proliferation).

Each alteration (lesion) was assigned a factor of 1 to 3 depending on its "pathological importance" in affecting the organ function and ability of the fish to survive, with 1 assigned to a lesion that was considered easily reversible, such as hemorrhage and plasma alterations, and 3 to a lesion that may lead to partial or total loss of the organ, for example, necrosis (Bernet et al., 1999). Every lesion was then assessed using a "score" ranging from 0 to 6, depending on the degree of alteration, whereby (0) was unchanged; (2) "mild occurrence"; (4)" moderate occurrence"; and (6) "severe occurrence" (diffuse lesion)." Intermediate" values were also considered (Bernet et al., 1999).

For each lesion, a lesion index was calculated by multiplying the importance factor by the score value. The reaction pattern for each organ was determined by summing the lesion indices associated with that pattern. A circulatory index (IC), regressive index (IR), progressive index (IP), inflammatory index (II), and neoplastic index (IN) were calculated. The sum of the reaction indices for each organ resulted in the total organ index: IL for the liver, Gl for the gill, GdI for the gonads, and IS for the spleen. These indices indicate the severity and extent of histological changes in the respective tissues, as pathological changes convert qualitative observations into quantitative values (Bernet et al. 1999; van der Oost et al., 2003; Zimmerli et al. 2007).

To classify the study sites according to the severity of the histological response, the organ index results were categorized into four grades. These classifications were adapted from a scoring system designed for trout in Swiss rivers by (Zimmerli et al., 2007) and a system used in studies of *Clarias gariepinus* and *Oreochromis mossambicus* in reconstructed and polluted waters by van Dyk et al. (2009). In this study, *Labeobarbus* spp. was consistently present in all six study wetlands, making it the preferred species for histopathology biomarker analysis. Organ histological indices were divided into four categories: Classes 1 and 2 were deemed normal and recoverable, while Classes 3 and 4 were considered pathological, with Class 4 indicating severe changes.

The description of each class is provided below.

- Class 1 (index < 15) displayed normal tissue structures with minimal histological changes.
- Class 2 (index 16-30) showed normal tissue structure with moderate histological alterations.
- Class 3 (index 31-40) exhibited significant changes in organ tissue.
- Class 4 (index > 41) showed severe alterations in organ tissue.

6.2.3. Statistical analysis

Descriptive statistics were used to derive the range and median of the different reaction pattern indices in each study organ, and significant differences among wetlands and the four seasons were established. Most data reaction patterns showed a non-normal distribution based on the Kolmogorov-Smirnov and Shapiro-Wilk tests ($p<0.05$); hence, the Kruskal-Wallis nonparametric ANOVA by ranks was used to compare sampling locations at an alpha error of $p < 0.05$. The multiple comparison was used for p-values < 0.05. The four classes of organ index results, i.e., index < 15, 16–30, 31–40, and > 41, for each organ, were derived and graphically presented to show the proportion (percentage) of each class among the studied fish population for each wetland. The Type 1 error level of all statistical tests was set at 5% ($p < 0.05$). The number of fish affected by a specific histological alteration in each wetland was presented as a percentage prevalence. Contingency analysis was conducted to test the significance levels of prevalence between wetland levels and across the four seasons.

6.3.Results

6.3.1. The sample size for histopathology-biomarker in the study wetlands of Lake Tana

A total of 96 fish were examined in this study. The sample size for each wetland and season varied slightly. Most wetlands (AV, GRM, MRM, and ZG) included 16 fish, with four fish collected in each season. Wetland RA had 15 fish, while wetland WO had 17 fish.

6.3.2. Histopathological indices and index grades in the wetlands of Lake Tana

The changes observed in the organs were condensed into numerical values based on the five reaction patterns: circulatory (C), regressive (R), progressive (P), inflammatory (I), and neoplastic (N). The sensitivity of the organs decreased in the following order: gills > liver > spleen > gonad. When combining the gonad and spleen, the combined organ index values of the gonad and spleen were lower than those of either the gill or liver individually (Table 6.1).

To enhance the interpretability of histopathological findings, the prevalence and severity of reaction indices across organs and wetlands were synthesized into a summary table (Table 6.2). This table identifies the dominant organ affected, the most prevalent reaction types (e.g., regressive, inflammatory), and the highest index values observed in each wetland. These patterns provide insight into the physiological stress experienced by *Labeobarbus* spp. and serve as indicators of ecological degradation. Wetland status was inferred based on the cumulative severity of organ-level responses, with higher index values and multiple dominant reactions indicating greater pollution pressure. This integrative approach supports the use of histopathological biomarkers as reliable tools for wetland health classification.

The gills and liver exhibited significantly higher levels of circulatory disturbance, along with increased regressive and inflammatory indices compared to the gonad and spleen ($p<0.05$). Conversely, the gills and spleen showed significantly higher progressive indices than the liver and gonad ($p<0.05$). Additionally, the gills and gonad displayed significantly elevated tumor indices in comparison to the liver and spleen ($p<0.05$).The fish species from AV had the highest gill and gonad indices, while ZG had the lowest indices for all organs (gill, liver, gonad, and spleen). GRM had the highest liver index, and MRM had the highest spleen index.

Of the four categories of pathological grades only Grades 1, 2, and 3 were present in the gill and liver. In contrast, the spleen and gonads showed only Grades 1 and 2 pathological changes. None of the organs (gill, liver, spleen, or gonads) showed any severe (Grade 4) pathological changes. The organ index results, which indicate the severity of histological alterations for each organ, are depicted in Figures 6.1 to 6.4. Among the organs examined, the gills and liver were identified as the most sensitive and diagnostically useful for rapid environmental monitoring. Gills responded consistently to pollutant exposure with early regressive changes such as epithelial lifting and progressive changes like lamellar hyperplasia, which are well-established biomarkers of waterborne contaminants. The liver, as the primary organ for detoxification, exhibited inflammatory responses including granuloma formation and vascular degeneration, indicating chronic exposure to pollutants. These two organs showed the highest histopathological indices across wetlands and seasons, making them reliable indicators of ecological stress. In contrast, the spleen and gonads showed fewer and less severe alterations, suggesting lower diagnostic sensitivity for early detection.

The severity of gill lesions varied across wetlands and seasons. Grade I lesions were most prevalent (100%) in MRM during the dry and early rainy seasons and in WO throughout the year. Grade II lesions were most prevalent in MRM during the dry season, while Grade III lesions were primarily observed in AV during the rainy season. In some wetlands and seasons, no gill lesions of a particular grade were observed (Figure 6.1).

The percentage of fish with liver lesion grades varied across wetlands and seasons. Grade 1 liver lesions were most prevalent in MRM, RA, and WO, while Grade 2 lesions were most common in MRM and RA. The most severe liver lesions (Grade 3) were exclusively observed in AV during the rainy season (Figure 6.2).

The prevalence of Grade 1 spleen lesions was high across all wetlands and seasons, except for AV during the dry season. Grade 2 spleen lesions were only observed in AV during the dry season. (Figure 6.3). The prevalence of gonad Grade 1 lesions varied across wetlands and seasons. The highest prevalence (100%) was observed in AV during the early and rainy seasons, as well as in GRM, MRM, RA, and WO throughout the year. Lower prevalence rates (50%) were found in AV during the dry and late rainy seasons in GRM during the late rainy season, and in MRM during the dry season (Figure 6.4).

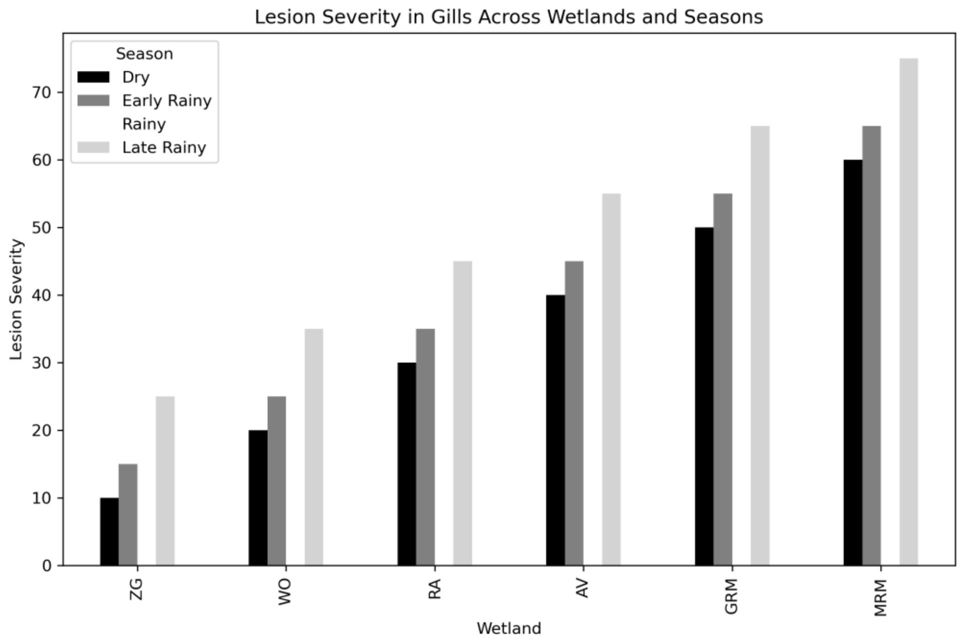


Figure 6. 1. Grades of lesion severity in the study wetlands show the percentage of fish in different classes of histological response based on the gill index. Index < 15 = normal tissue structure with slight histological alterations; Index 16–30 = normal tissue structure with moderate histological alterations; Index 31–40 = pronounced alterations of organ tissue. GRM-Gumara River Mouth, Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, LR-Late rainy season

Table 6. 1. Organ-specific histopathological indices (IC, IR, IP, II) in *Labeobarbus* spp. from six Lake Tana wetlands. IC = circulatory disturbances, IR = regressive changes, IP = progressive changes, II = inflammatory responses. Indices quantify tissue reactions to pollution in gill, liver, gonad, and spleen tissues for biomarker-based aquatic health assessment. AV = Avaj, GRM = Gumara River Mouth, MR = Megech River Mouth, RA = Ras Abbay, WO = Wonjeta, ZG = Zewdie Girar; Seasons: D = Dry, ER = Early Rainy, R = Rainy, LR = Late Rainy.

Wetland	**Index**	**Gill**	**Liver**	**Gonad (Male, Female)**	**Spleen**
AV	*I*C	64	56	4, 12	16
	*I*R	158	72	91, 0	20
	*I*P	66	48	0,0	26
	*I*I	20	64	8, 10	16
	*I*T	36	0	0,0	0
	Organ Index	**344**	**240**	**103, 22**	**78**
GRM	*I*C	58	52	4, 6	7
	*I*R	76	109	36, 0	30
	*I*P	92	20	0,0	51
	*I*I	12	92	16, 0	0
	*I*T	20	0	54, 0	0
	Organ Index	**262**	**273**	**110, 6**	**88**
MRM	*I*C	44	45	4, 0	14
	*I*R	98	81	60, 42	22
	*I*P	100	0	0,0	60
	*I*I	20	76	0, 16	0
	*I*T	0	0	0,0	4
	Organ Index	**262**	**202**	**64, 58**	**100**
RA	*I*C	60	12	0, 2	14
	*I*R	77	72	24, 12	12
	*I*P	50	41	0	86
	*I*I	38	79	0, 8	4
	*I*T	0	0	0,0	0
	Organ Index	**225**	**204**	**24, 22**	**116**
WO	*I*C	48	52	4, 6	24
	*I*R	54	48	18, 12	10
	*I*P	56	27	12, 0	60
	*I*I	0	62	16, 0	0
	*I*T	0	0	0,0	0
	Organ Index	**158**	**189**	**50, 18**	**94**
ZG	*I*C	36	20	0, 4	2
	*I*R	80	40	0,0	0
	*I*P	60	20	0,0	58
	*I*I	40	32	4, 0	4
	*I*T	0	8	24, 0	0
	Organ Index	**216**	**120**	**28, 4**	**64**

Table 6.2. Summary of Histopathological Reaction Index Prevalence and Indicative Wetland Status in *Labeobarbus* spp., and Corresponding Wetland Status Classification. AV = Avaj, GRM = Gumara River Mouth, MR = Megech River Mouth, RA = Ras Abbay, WO = Wonjeta, ZG == Zewdie Girar.

Wetland	**Dominant Organ(s)**	**Dominant Reaction Type(s)**	**Highest Index Value(s)**	**Indicative StatuS**
AV	Gill, Liver	Regressive, Inflammatory	IR (158), II (64)	Highly Polluted
GRM	Liver, Gill	Regressive, Progressive	IR (109), IP (92)	Highly Polluted
MRM	Spleen, Gill	Progressive, Regressive	IP (100), IR (98)	Highly Polluted
RA	Liver, Gill	Regressive, Inflammatory	IR (77), II (79)	Moderately Polluted
WO	Gill, Liver	Progressive, Inflammatory	IP (56), II (62)	Slightly Polluted
ZG	Gill, Liver	Regressive, Progressive	IR (80), IP (60)	Least Polluted

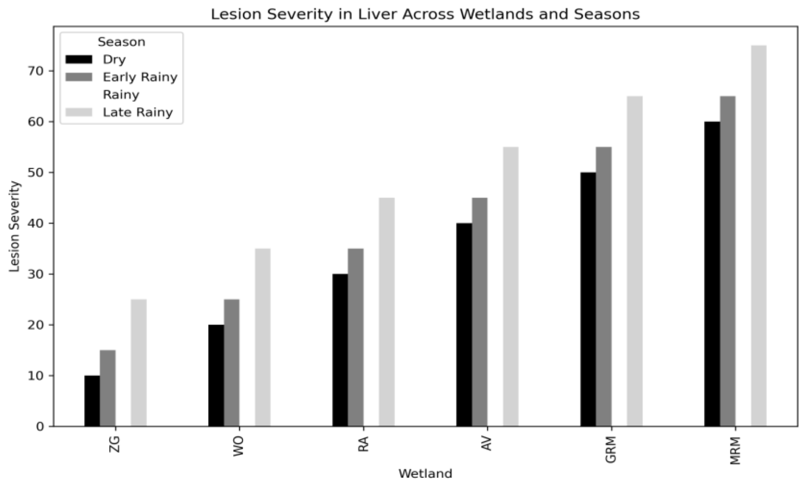


Figure 6.2. Grades of lesion severity in the study wetlands show the percentage of fish in different classes of histological response based on a liver index. Index < 15 = normal tissue structure with slight histological alterations; Index 16–30 = normal tissue structure with moderate histological alterations; Index 31–40 = pronounced alterations of organ tissue. GRM-Gumara River Mouth, Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, LR-Late rainy season

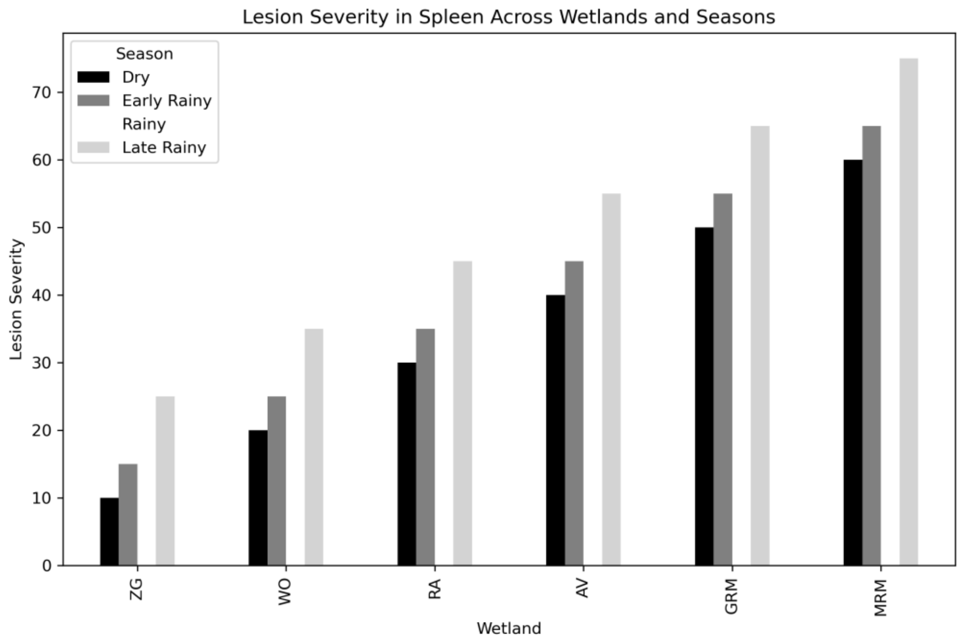


Figure 6.2. Grades of lesion severity in the study wetlands show the percentage of fish in different classes of histopathological responses based on spleen index. Index < 15 = normal tissue structure with slight histological alterations; index 16–30 = normal tissue structure with moderate histological alterations. GRM-Gumara River Mouth, Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, LR-Late rainy season.

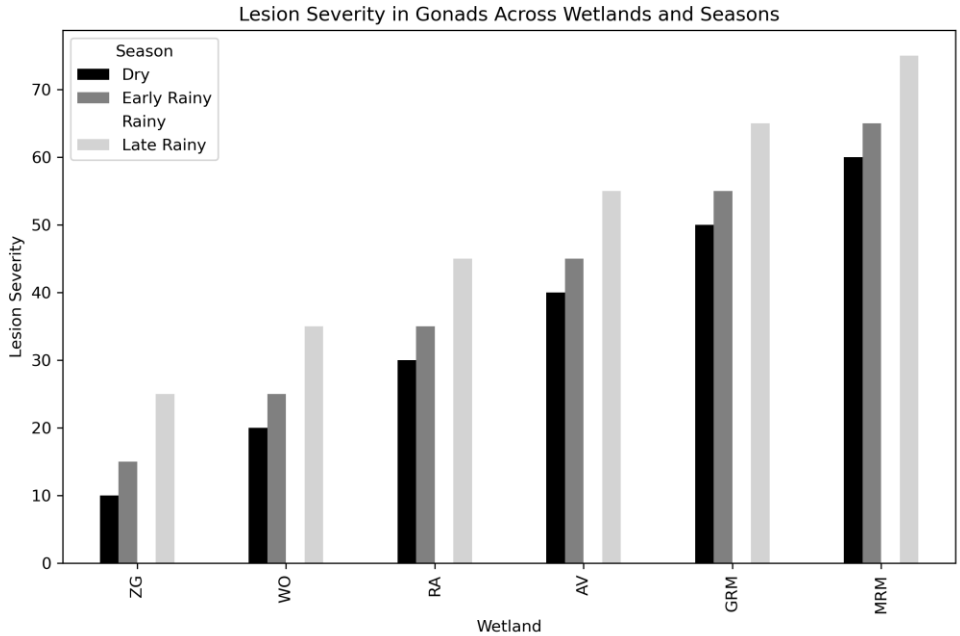


Figure 6.3. Grades of lesion severity in the study wetlands show the percentage of fish in different classes of histopathological responses based on the gonad index. Index < 15 = normal tissue structure with slight histological alterations; index 16–30 = normal tissue structure with moderate histological alterations. GRM-Gumara River Mouth, Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, LR-Late rainy season.

To enhance clarity and address reviewer feedback, the six study wetlands were classified according to the dominant histopathological grades observed in *Labeobarbus* spp. tissues. This classification integrates the severity of organ-level alterations (Grades 1–3) with the ecological context and known pollution sources to infer each wetland's environmental status. The summary is presented in Table 6.3.

Table 6.3. Classification of Study Wetlands Based on Dominant Histopathological Grades in *Labeobarbus* spp. and Corresponding Pollution Status. AV = Avaj, GRM = Gumara River Mouth, MR = Megech River Mouth, RA = Ras Abbay, WO = Wonjeta, ZG = Zewdie Girar.

Wetland	**Dominant Pathology Grade**	**Interpretation of Grade**	**Wetland Status**
ZG	Grade 1 (Index < 15)	Normal tissue structure with minimal histological changes	Least Polluted
WO	Grade 1–2 (Index 15–30)	Normal to moderate histological alterations	Slightly Polluted
RA	Grade 2 (Index 16–30)	Moderate histological alterations	Moderately Polluted
AV	Grade 2–3 (Index 16–40)	Moderate to significant histological alterations	Moderately
GRM	Grade 2–3 (Index 16–40)	Moderate to significant histological alterations	Highly Polluted
MRM	Grade 2 (Index 16–30)	Moderate histological alterations	Highly Polluted

Note: Wetland status was determined by integrating histopathological index values with ecological context and identifying pollution sources.

6.4.2. Reaction indices for each organ

Gill

Medians for gill circulatory disturbance index, regressive index, progressive index, inflammatory, and tumor index did not differ between wetlands and seasons (K-W ANOVA, $p > 0.05$; Figures 6.5a-e), while gill index was influenced by wetland ($p < 0.05$, figure 6.5f). There was a significantly higher gill index in AV than in WO. The median gill index value differed among seasons ranging from non-detectable to 12 (median = 4) in the dry and early rainy seasons and from 1.0 – 18.0 in the rainy season (K-W ANOVA, $p < 0.05$; figure 6.5f).

Liver

The median for liver circulatory disturbance index, regressive index, and inflammatory index did not differ among wetlands (K-W ANOVA, $p > 0.05$; figures 6.6 a, b, and d), while the progressive index, tumor index, and liver index were influenced by wetland (K-W ANOVA, $p < 0.05$; figures

6.6 c, e, and f). There were significantly higher liver indices in GRM and MRM than in ZG. The season had an effect on either of the liver pathological indices (K-W ANOVA, $p > 0.05$).

Spleen

The median of spleen circulatory disturbance index, regressive index, progressive index, inflammatory index, tumor index, and spleen index did not differ among wetlands (K-W ANOVA, $p > 0.05$; figures 6.7 a-f). Spleen circulatory index, progressive index, and tumor index differed (K-W ANOVA, $p < 0.05$) among seasons. However, the median spleen regressive index differed among seasons ranging from non-detectable in the late rainy season and from non-detectable to 15.0 (median = 1.5) in the dry season (K-W ANOVA, $p<0.05$). Likewise, the median of the spleen index differed among seasons ranging from non-detectable to 6.0 (median = 6) during the late rainy season and from non-detectable to 15.0 (median = 15.0) during the dry season (K-W ANOVA, $p<0.05$).

Gonad

The median of the gonad circulatory index and the inflammatory index did not differ among wetlands (K-W ANOVA, $p > 0.05$; figures 6.8 a and d), while the regressive index, progressive index, tumor index, and gonad index were influenced by wetland (K-W ANOVA, $p < 0.05$, figure 6.8 b, c, e, and f). There was a significantly higher gonad index in MRM than in ZG. The season had no effect on the circulatory index, regressive index, and tumor index. However, the median of the gonad index differed among seasons ranging from non-detectable to 18 (median = 4) in the rainy season and from non-detectable to 18 (median = 6) in the late rainy season.

Fish histopathology index

The median fish histopathology index differed among wetlands and ranged from 14.0 to 50.0 (median = 25.5) in ZG and from 24.0 to 62 (median = 46.0) in MRM (K-W ANOVA, $p < 0.05$). Multiple comparisons of mean ranks for all groups revealed that MRM, AV, and GRM exhibited the highest fish histopathology indices. Moderate indices were observed in RA and WO, while the lowest fish histopathology index was found in ZG. However, the median value of the fish histopathology index did not differ among seasons (K-W ANOVA, $p > 0.05$) (Figure 6.9).

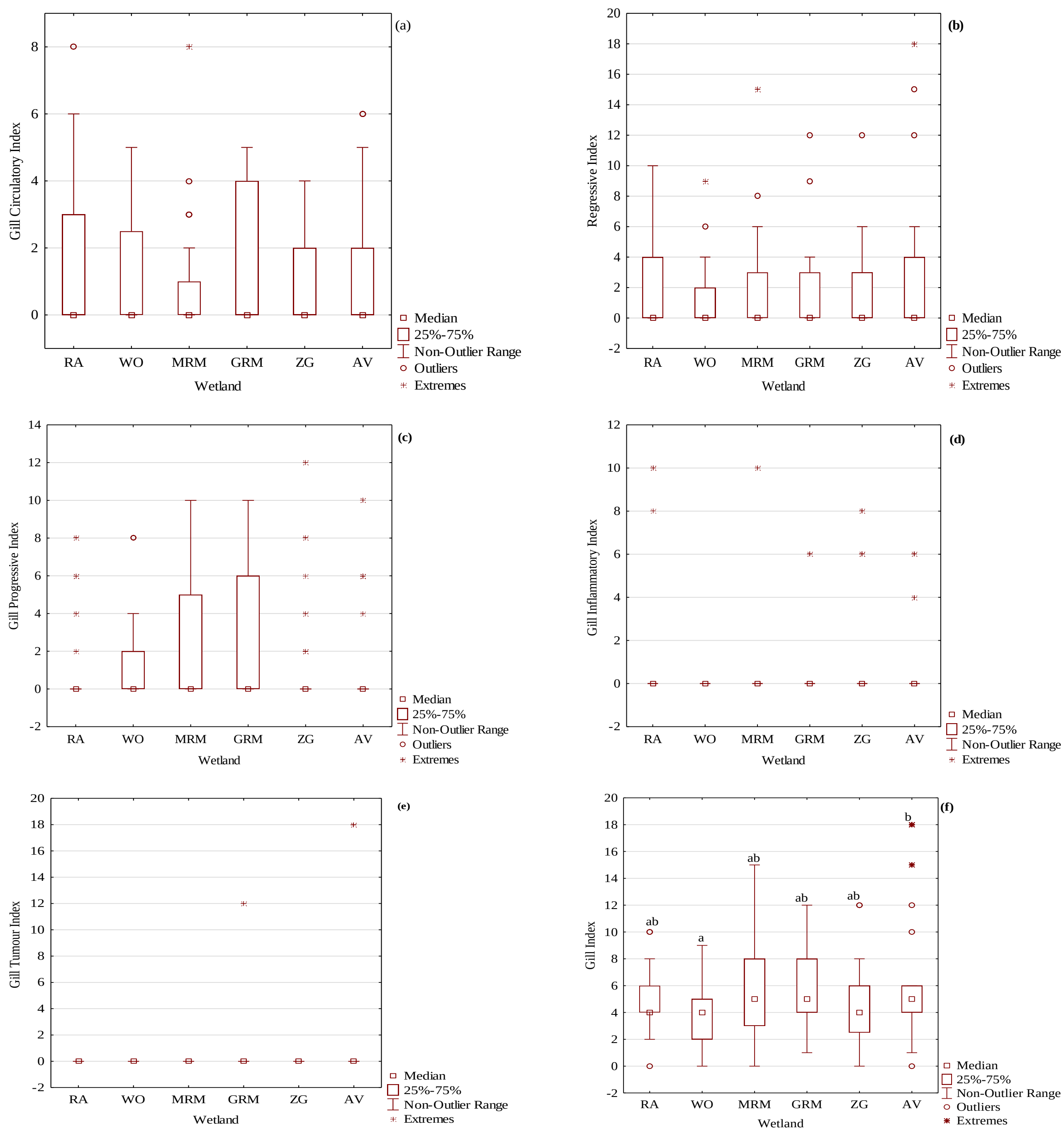


Figure 6.4. Box plot for median value of gill reaction pattern index by wetlands of Lake Tana, Gill circulatory index (a), regressive index (b), progressive index (c), inflammatory index (d), tumor index (e) Gill index (f) AV-Avaj, GRM-Megech River mouth, MRM-Megech River mouth, RA-Ras Abbay, WO-Wonjeta, and ZG-Zewdie Girar

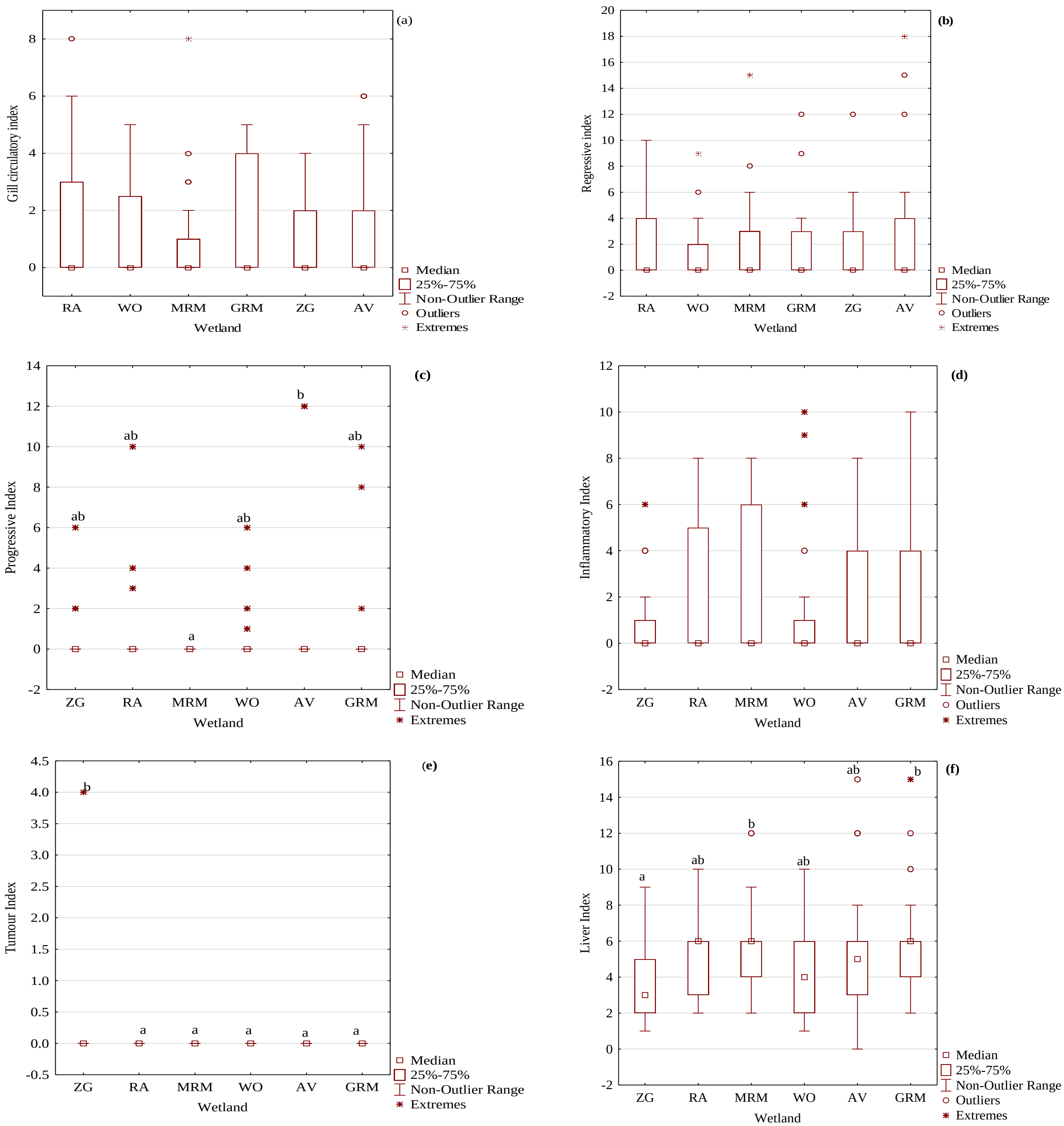


Figure 6.5. Box plot for medians of liver reaction pattern by wetlands of Lake Tana: liver circulatory index (a), regression index (b), progressive index (c), inflammatory index (d), tumor index (e), and liver index (f). AV-Avaj, GRM-Megech River mouth, MRM-Megech River mouth, RA-Ras Abbay, WO-Wonjeta, and ZG-Zewdie Girar.

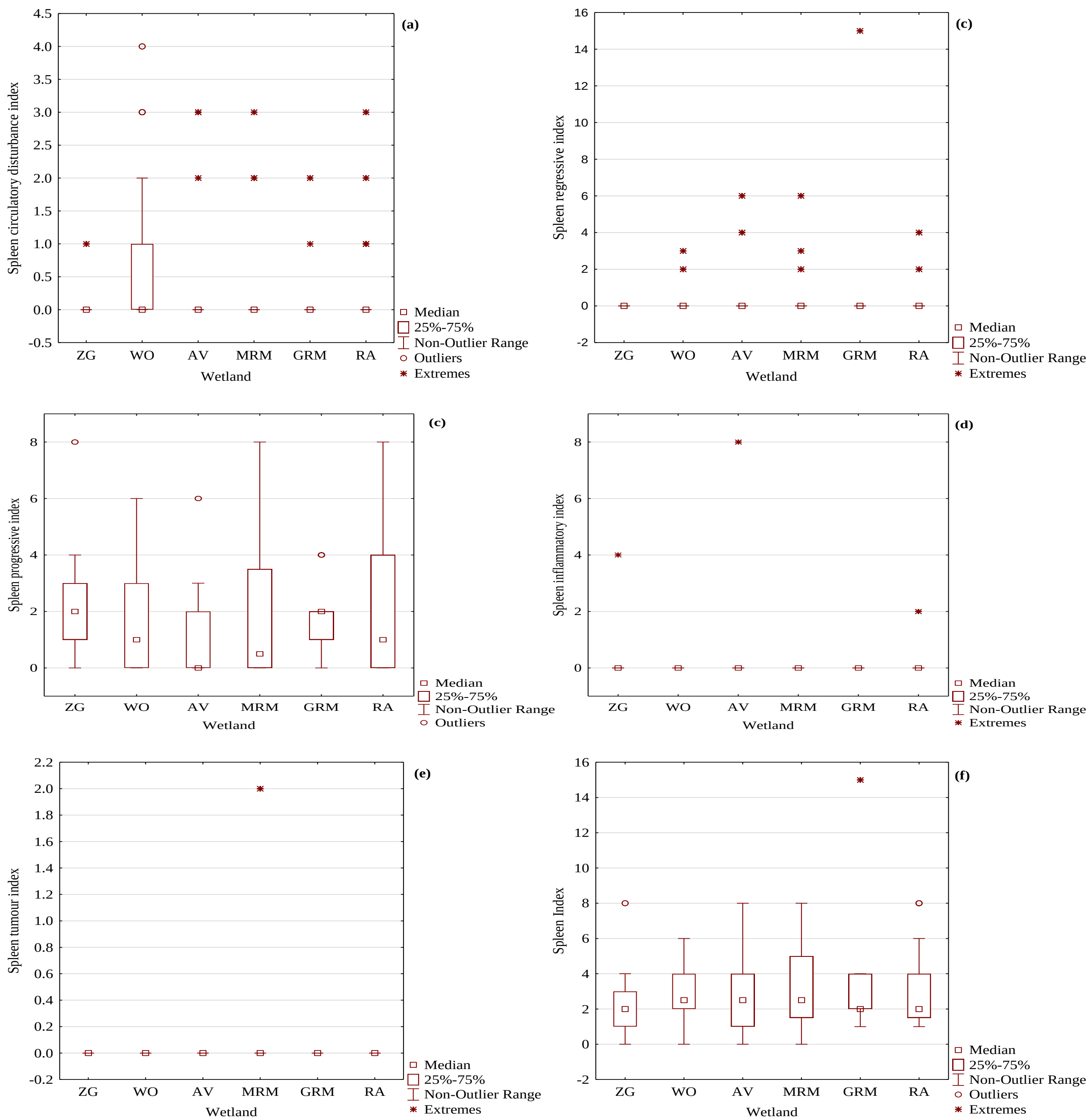


Figure 6.6. Box plot for a median of spleen reaction pattern by wetlands of Lake Tana: spleen circulatory index (a), regression index (b), progressive index (c), inflammatory index (d), tumor index (e), and spleen index (f). AV-Avaj, GRM-Megech River mouth, MRM-Megech River mouth, RA-Ras Abbay, WO-Wonjeta, and ZG-Zewdie Girar

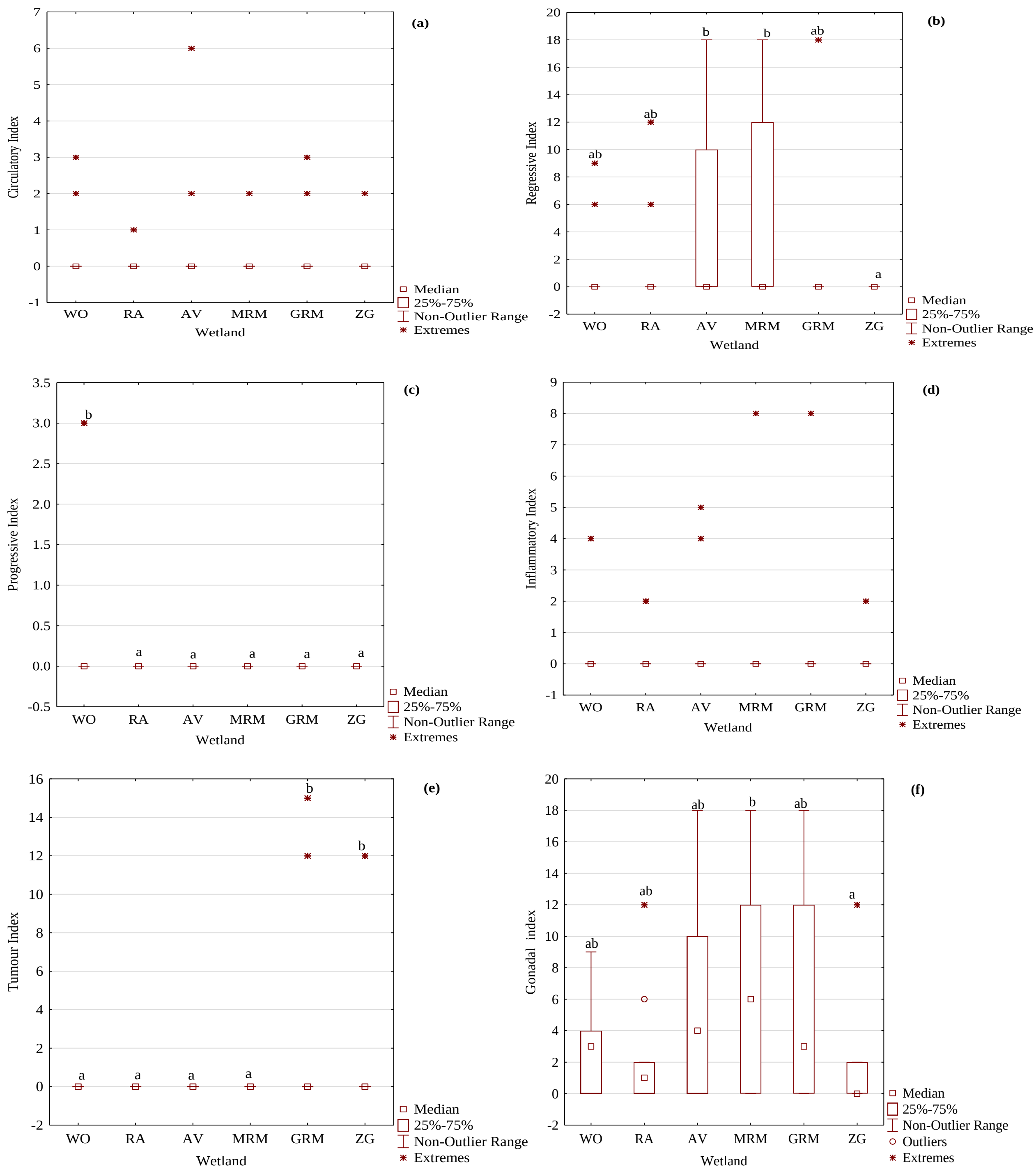


Figure 6.7. Box plot for median of gonad reaction pattern by wetlands of Lake Tana, Gonad circulatory index (a), regression index (b), progressive index (c), inflammatory index (d), tumor index (e), and Gonad index (f). AV-Avaj, GRM-Megech River mouth, MRM-Megech River mouth, RA-Ras Abbay, WO-Wonjeta, and ZG-Zewdie Girar.

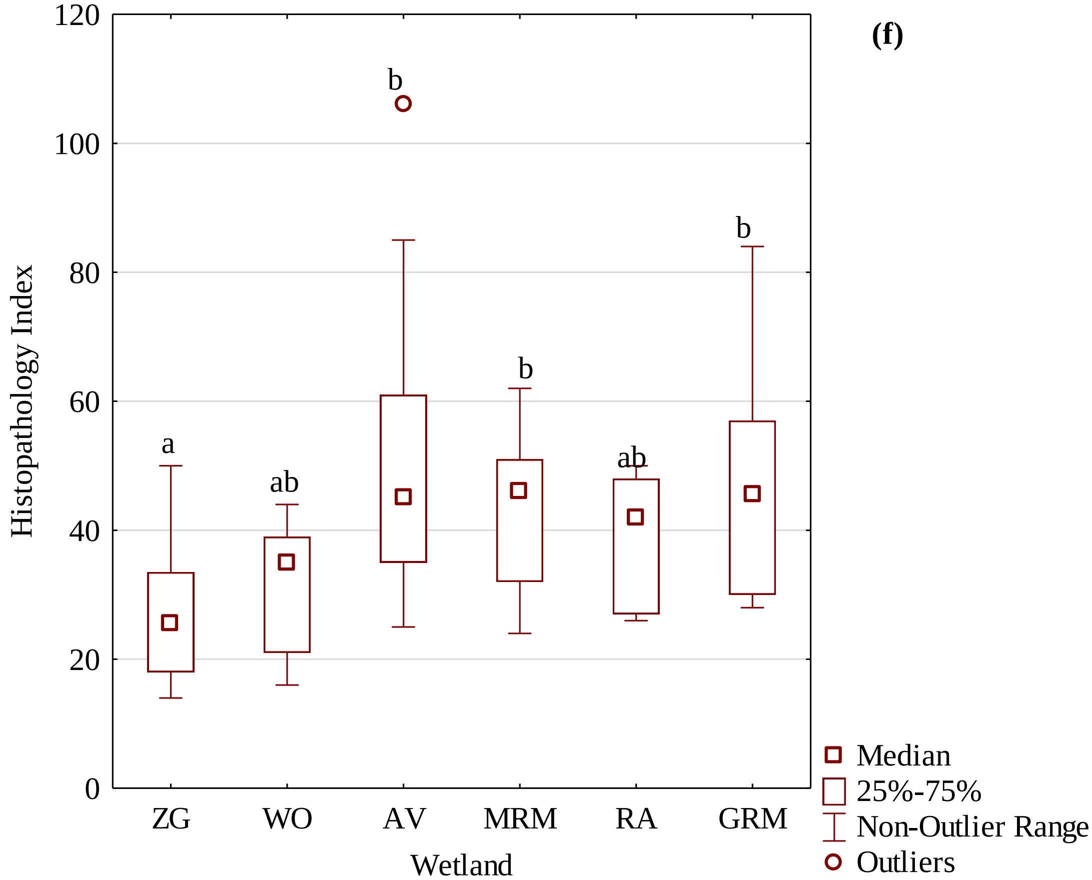


Figure 6.8. Box plot for a median of histopathology index by wetlands of Lake Tana, AV-Avaj, GRM-Megech River mouth, MRM-Megech River mouth, RA-Ras Abbay, WO-Wonjeta, and ZG-Zewdie.Girar.

6.4.3. Prevalence of specific histopathological alterations

Gill

Regressive gill lesions, including structural alterations, necrosis, and atrophy of secondary lamellae, were the most prevalent gill abnormalities, occurring in approximately 35% (range: 16.42-65.69%) of cases. Progressive changes, such as epithelial hyperplasia, circulatory disturbances, and inflammatory changes, were less frequent, affecting approximately 25% (range: 0-52.94%), 20% (11.76-41.18%), and 15% (range: 0-43.75%) of fish, respectively. Tumorous changes (malignant tumors) were the least common, occurring in only 5% of cases (range: 0-25%).

Normal gill architectural structure showed the gill arch with primary lamellae that had a series of secondary lamellae perpendicular to the primary lamellae (Figure 6.11, Plate A). Mild epithelial lifting (Figure 6.11, Plate B), atrophy of secondary lamellae, and congestion and epithelial lifting of primary lamellae (Figure 6.11, Plate E) indicate regressive changes. Hyperplasia of secondary lamellae (Figure 6.11, Plate C) and its advancement to fusion of secondary lamellae (Figure 6.11, Plate D) indicate progressive changes. Severe aneurism or telangiectasia of gills infested with gill parasites (Figure 6.11, Plate F) indicate circulatory disturbances. Micrographs of gill histological sections are shown in Figure 6.11.

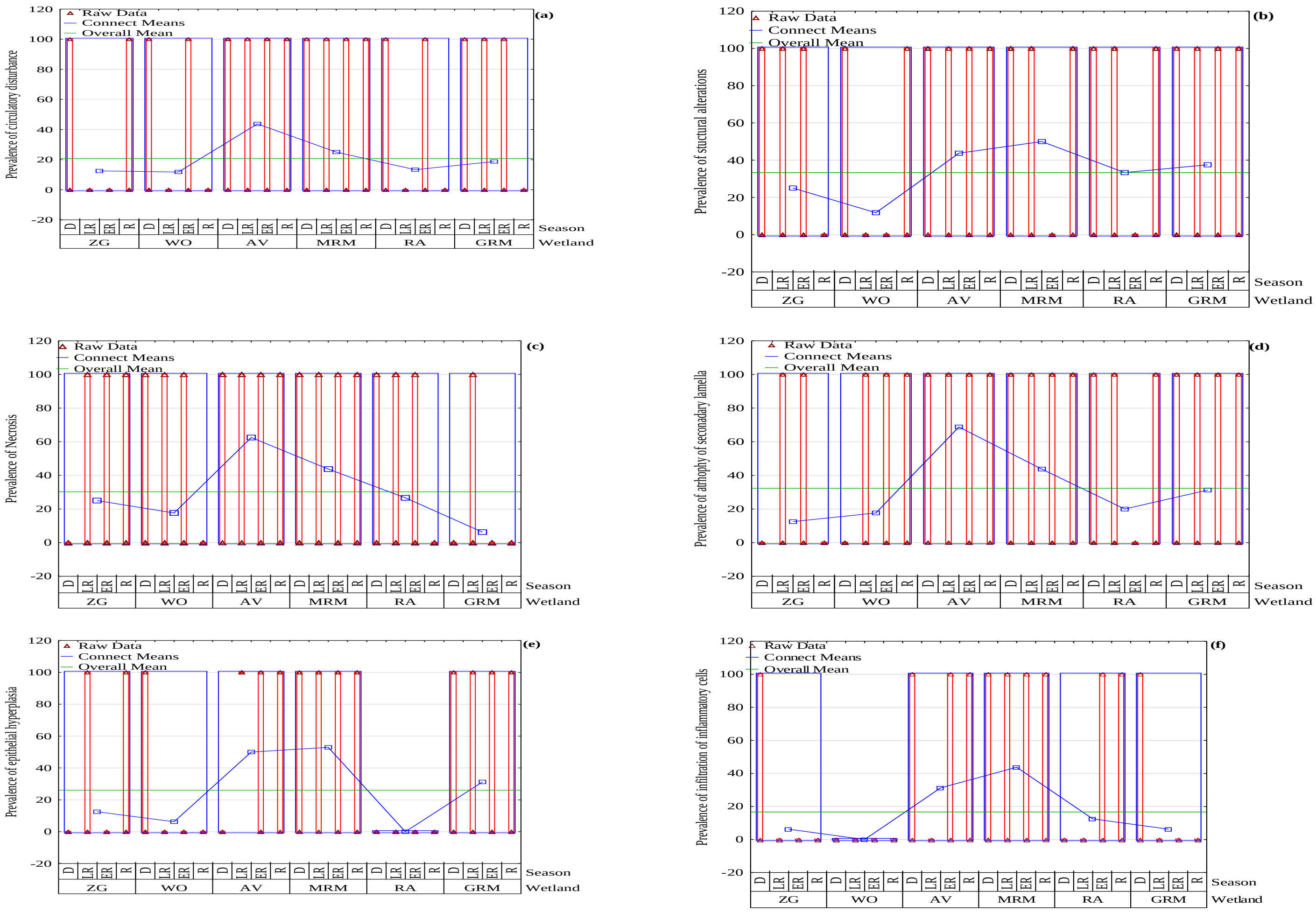
The prevalence of several gill pathologies varied significantly across wetlands. Atrophy of secondary lamellae (χ^2=1 6.27, df=5, p <0.05), necrosis (χ^2=15.23, df=5, p <0.05), infiltration of inflammatory cells (χ^2=16.81, df=5, p <0.05), and epithelial hyperplasia (χ^2=21.44, df=5, p <0.05) were more prevalent in some wetlands compared to others. Wetland AV exhibited the highest prevalence for most of these pathologies. In contrast, wetland GRM showed the lowest prevalence. However, the prevalence of circulatory disturbances (χ^2=7.34, df =5, p>0.05) and structural alterations (χ^2= 6.97, df=5, p >0.05) in the gills did not differ significantly between wetlands.

The prevalence of malignant tumors (χ2=9.07, df=3, p<0.05) in the gills varied significantly across seasons, with the highest prevalence observed during the early rainy season. In contrast, atrophy of secondary lamella (χ2= 0.14, df=3, p >.05), necrosis (χ2= 2.12, df=3, p >0.05), structural alteration (χ2= 3.25, df=3, p >0.05), infiltration of inflammatory cells (χ2= 3.00, df =3, p >0.05), and epithelial hyperplasia in the gills (χ2= 4.49, df=3, p >.05) did not differ among seasons. The prevalence of gill histopathological alterations in the wetlands of Lake Tana is shown in Figure 6.10.

Liver

The most common liver lesions were regressive changes, including necrosis, affecting 32% (range:18.75-81.25%) of the fish. Inflammatory changes, including infiltration and tissue damage, were also prevalent at 31% (range: 20.83-60.42%). Circulatory disturbances, such as hemorrhage and vascular degeneration, were observed in 25% (range: 12.5-43.75%) of cases. Progressive changes and tumors were less frequent, occurring in 10% (range: 2.08-39.18%) and 2% (range: 0-

6.5%) of the fish, respectively. The normal liver architectural structure showed a network of hepatic cell cords, with hepatocytes located among the sinusoids with a centrally located nucleus (Figure 6.13, Plate A). Hyperplasia of the bile duct (Figure 6.13, Plate B) indicates progressive changes, and congestion of the liver parenchyma (Figure 6.13, Plate C) and hemorrhages (Figure 6.13, Plate E) indicate circulatory disturbance. Aggregations of melano-macrophages (Figure 6.13, Plate D) indicate progressive changes. Vacuolar degeneration and hepatic necrosis in the hepatic parenchyma (Figure 6.13, Plate G) indicate regressive changes, and granuloma formation with bacterial colonies at the center (Figure 6.13, Plate F) indicates inflammatory changes. Micrographs of liver histological sections are shown in Figure 6.13.



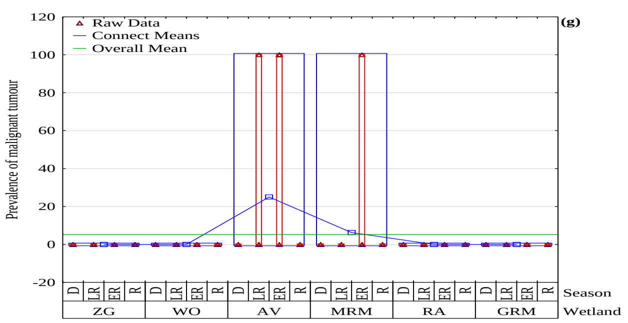


Figure 6.9. Variability plot on the prevalence of gill histopathological alterations in the wetlands of Lake Tana (a) Circulator disturbance, (b) Structural alteration, (c) Necrosis, (d) Atrophy of the secondary lamella, (e) Epithelial hyperplasia, (f) Infiltration of cells (g) Malignant tumor. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA-Ras Abbay, D-dry, ER-Early rainy, R-rainy, LR-late rainy season.

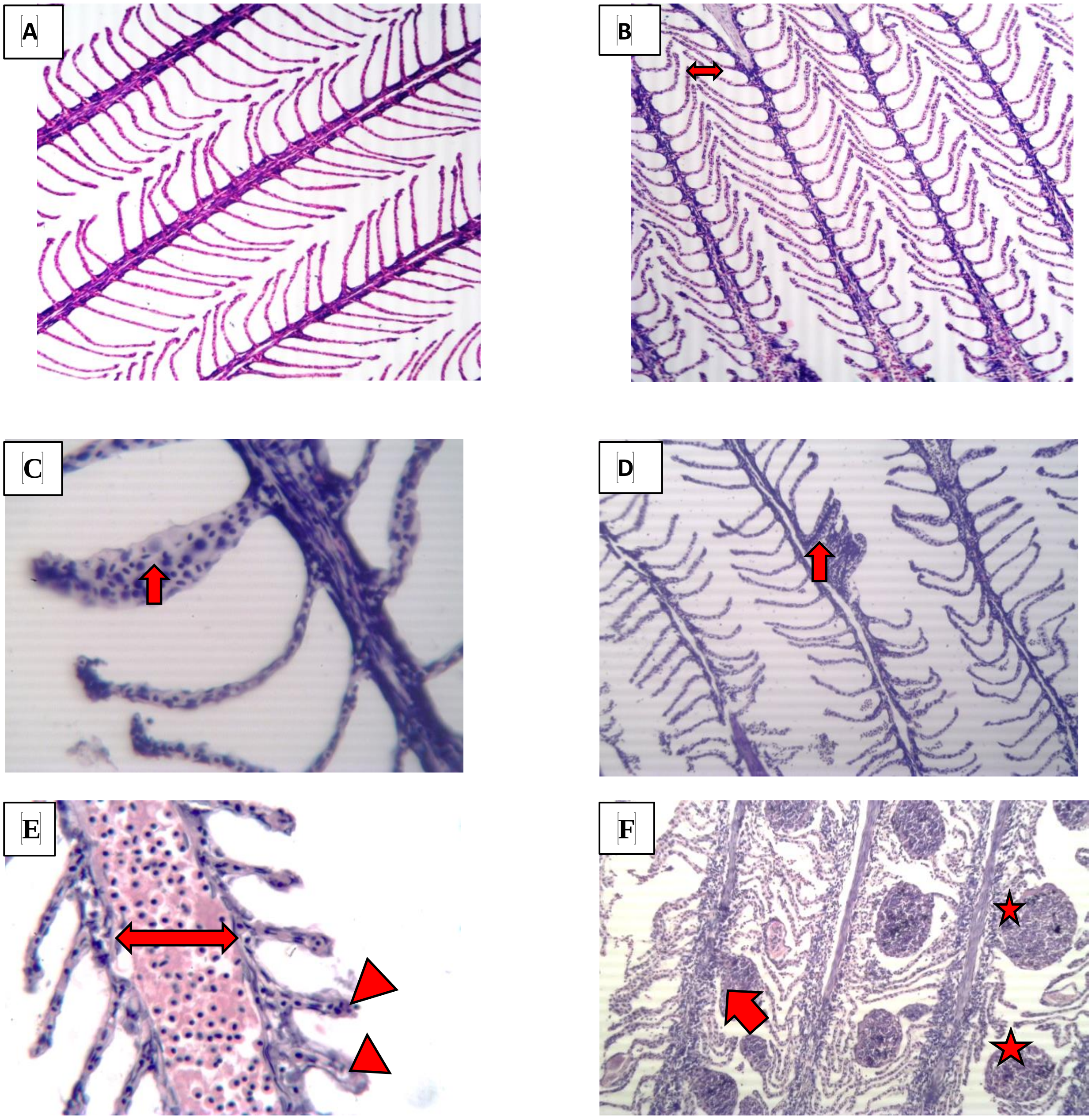
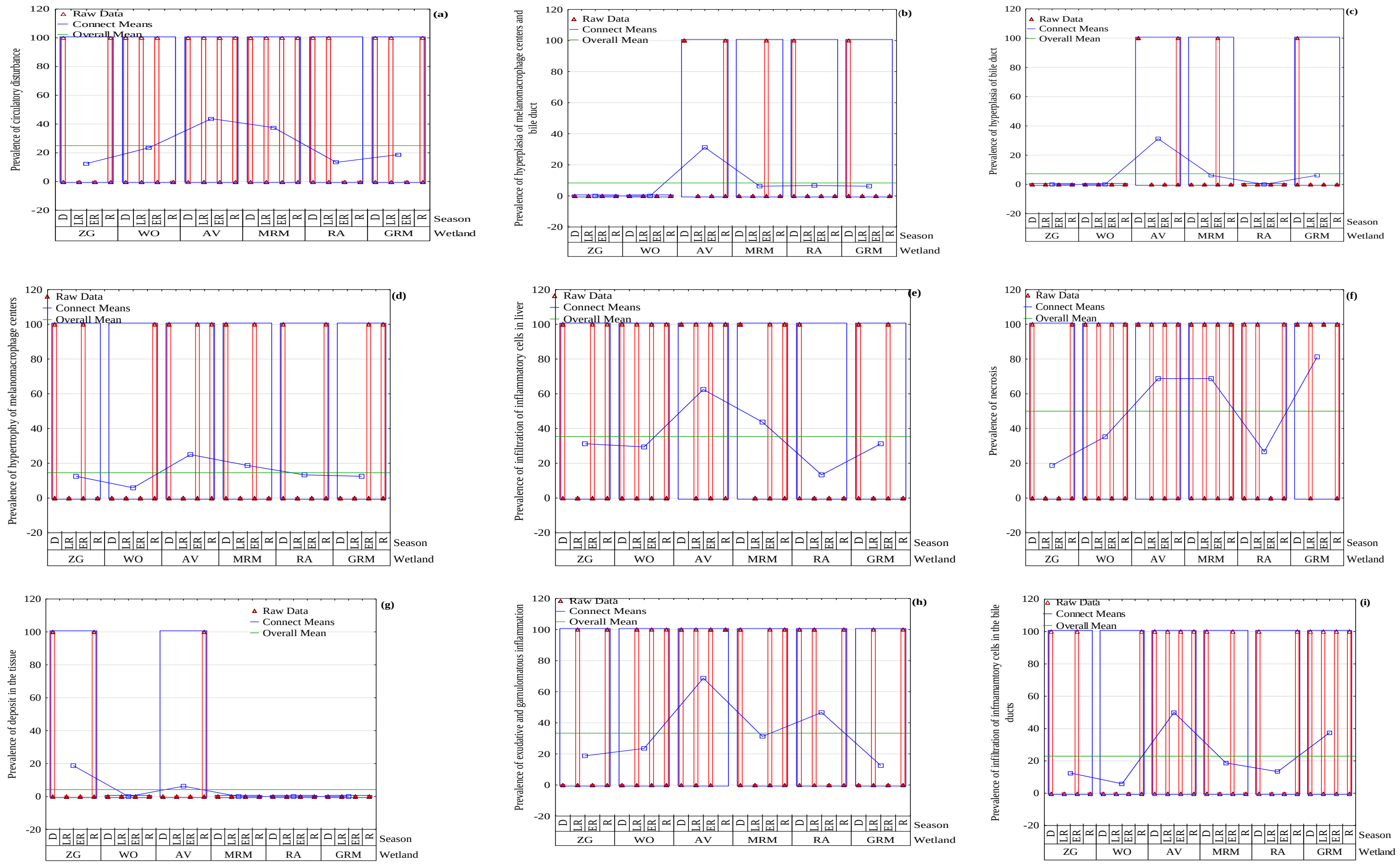


Plate 6.1. Micrographs of gill histological sections: (A) normal gill histology; (B) almost normal gill histology with mild epithelial lifting (double-pointed arrow); (C) hyperplasia of secondary lamella (bold arrow) and its advancement to (D) fusion of secondary lamellae (bold arrow); (E) atrophy of secondary lamellae (arrowheads) and congestion and epithelial lifting (double-pointed arrow) of primary lamella; and (F) severe aneurysm or telangiectasias (stars) characteristic of gills infested with gill parasites (bold arrow).

The prevalence of liver pathologies related to circulatory problems ($\chi 2=8.97$, df=5, $p >0.05$), hypertrophy of melano-macrophage centers ($\chi 2=2.78$, df=5, $p >0.05$), infiltration in hepatic tissue ($\chi 2=9.33$, df=5, $p >0.05$), and malignant tumors in the bile duct ($\chi 2=4.09$, df=5, $p >0.05$) did not differ across wetlands. However, other liver pathologies, such as hyperplasia of melano-macrophage centers and bile ducts ($\chi 2=14.24$, df=5, $p <0.05$), hyperplasia of the bile duct ($\chi 2=17.41$, df=5, $p >0.05$), necrosis ($\chi 2=23.55$, df=5, $p >0.05$), hepatic tissue deposits ($\chi 2=11.42$, df=5, $p >0.05$), exudative and granulomatous tissue ($\chi 2=11.40$, df=5, $p >0.05$), and infiltration of inflammatory cells in the bile duct ($\chi 2=13.28$, df=5, $p >0.05$), exhibited significant differences among the wetlands. Wetland AV had the highest frequency of these pathologies, while wetlands WO and ZG had the lowest.

The prevalence of hyperplasia of melano-macrophage centers ($\chi 2 = 12$, df = 3, $p <0.05$) and cell infiltration of inflammatory cells in the liver ($\chi 2 = 9.33$, df = 3, $p <0.05$) varied depending on the season. However, other liver problems, such as the prevalence of circulatory disturbance ($\chi 2 = 5.66$, df = 3, $p >0.05$), hyperplasia of the bile duct ($\chi 2 = 7.43$, df = 3, $p >0.05$), hypertrophy of melano-macrophage centers ($\chi 2 = 2.78$, df = 3, $p >0.05$), necrosis ($\chi 2 = 2.33$, df = 3, $p >0.05$), deposit on hepatic tissue ($\chi 2 = 4.17$, df = 3, $p >0.05$), exudative and granulomatous inflammation ($\chi 2 = 4.88$, df = 3, $p >0.05$), infiltration of inflammatory cells ($\chi 2 = 4.48$, df = 3, $p >0.05$) in the bile duct, and malignant tumors ($\chi 2 = 2.04$), df = 3, $p >0.05$) did not differ among seasons. A variability plot on the prevalence of liver histopathological alterations in the wetlands of Lake Tana is shown in Figure 6.12.



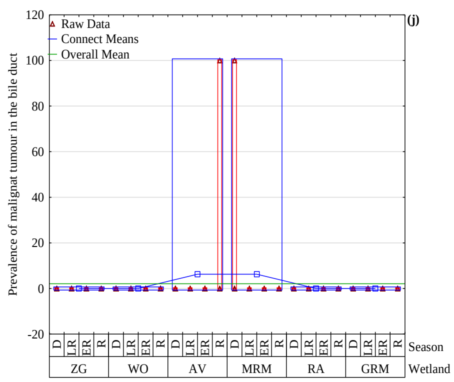
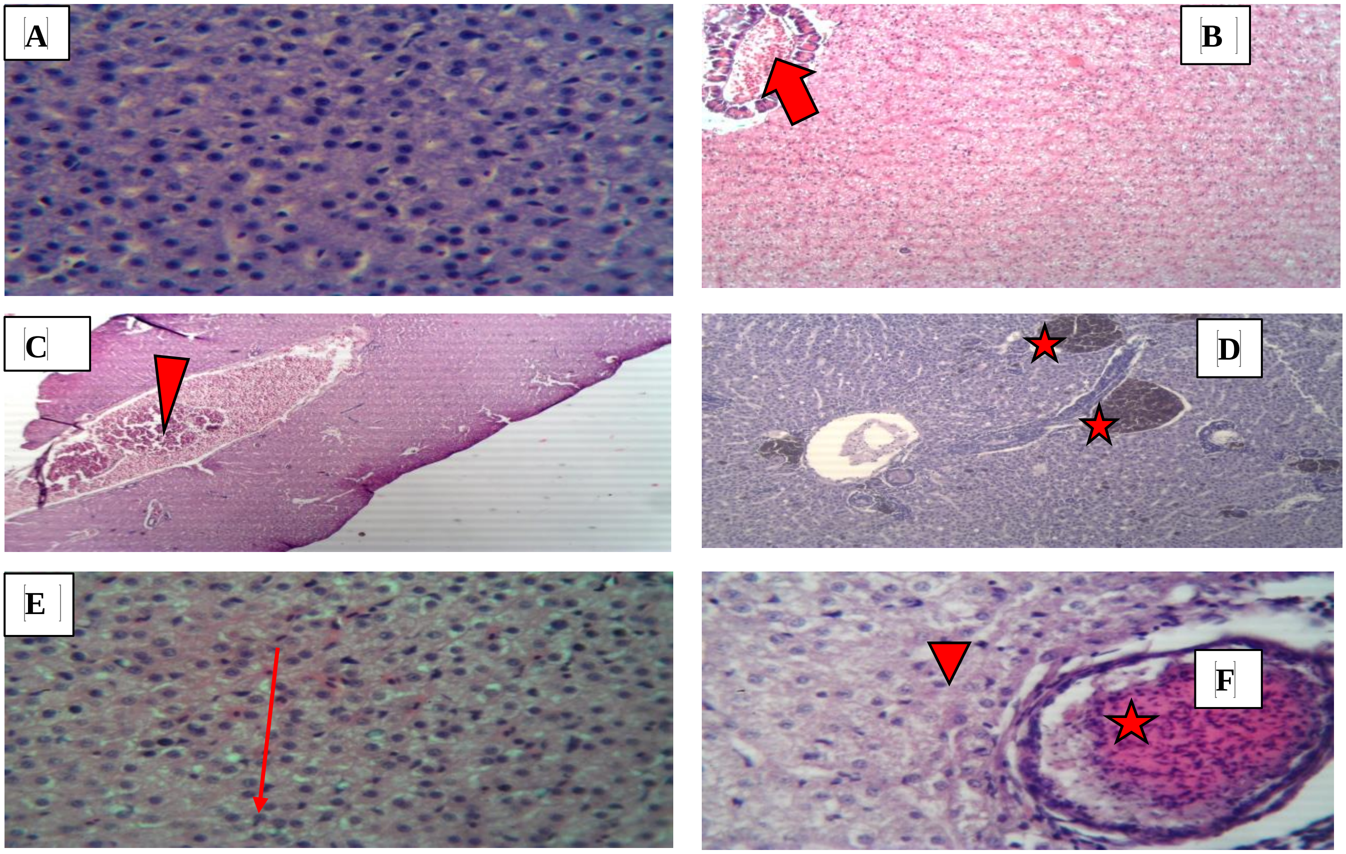


Figure 6.10.Variability plot on the prevalence of liver histopathological alterations in the wetlands of Lake Tana (a) Circulatory disturbance (b) Hyperplasia of MMCs and bile duct (c) Hyperplasia of the bile duct (d) Hypertrophy of MMCs (e) Infiltration of inflammatory cells in liver tissue (f) Necrosis (g) Deposits (h) Exudative and granulomatous inflammation (i) Infiltration of inflammatory cells in the bile duct (j) Malignant tumor in the bile duct. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA-Ras Abbay, D-dry, ER-early rainy, R-rainy, LR-late rainy season



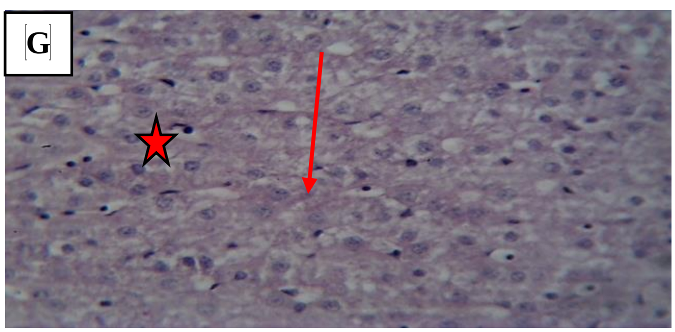


Plate 6.2. Micrographs of liver histological sections: (A) liver with relatively normal histology; (B) hyperplasia of the bile duct (bold arrow) and hemorrhages (reddened areas) (C) Circulatory disturbance or congestion (arrowhead) (D) Aggregations of melano-macrophage centers (stars) (E) hemorrhages (arrows) (F) Granuloma formation (star) with bacterial colonies at the center, probably as a result of *Edwardsiella tarda* or a similar infection, and necrotic areas (arrowhead) (G) vacuolar degeneration (arrow) and hepatic necrosis (star)

Spleen

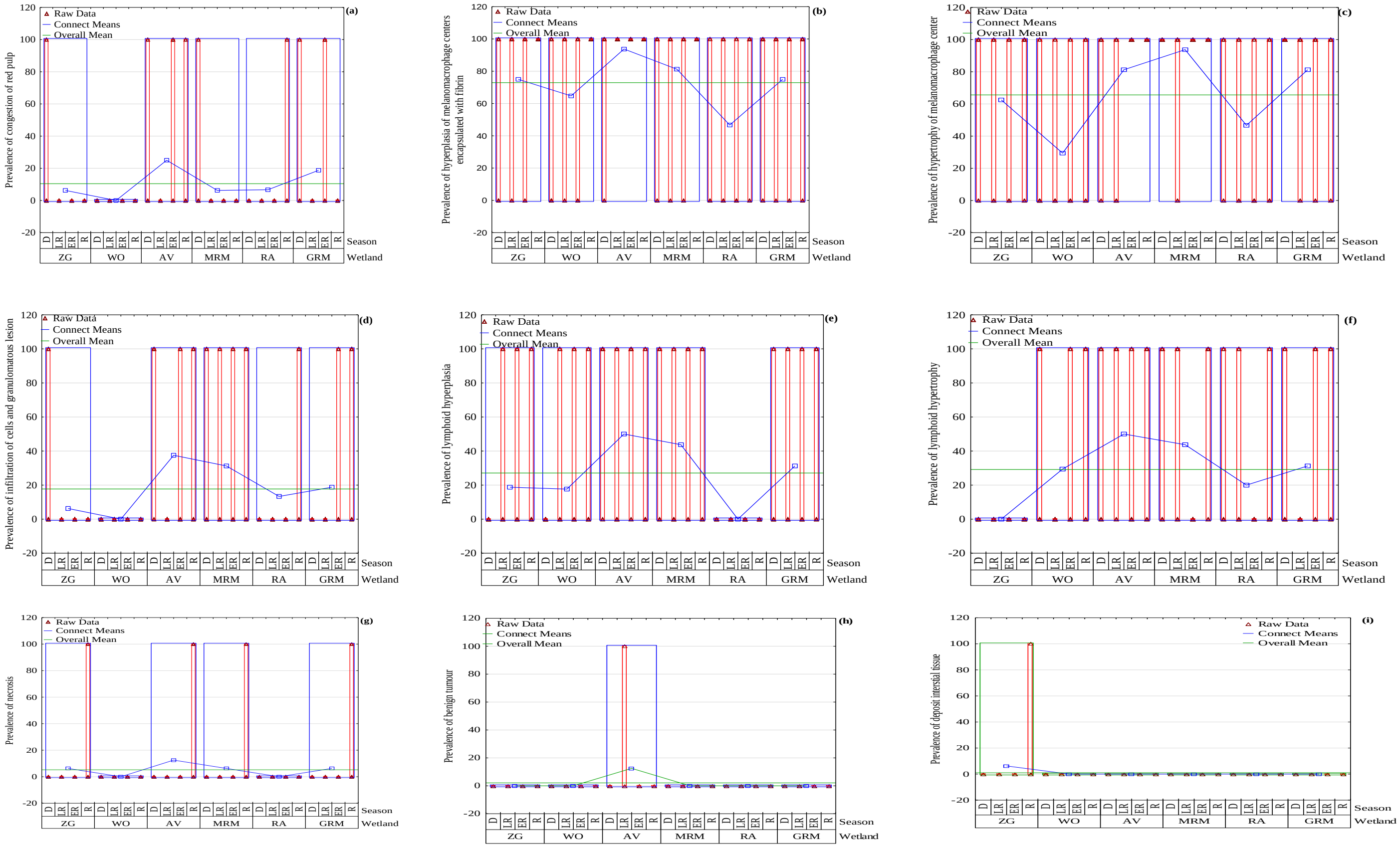
Progressive changes, particularly hyperplasia and hypertrophy of melano-macrophage centers and lymphoid hyperplasia and lymphoid hypertrophy were registered in 50% (range: 26.83-68.75%) of cases. Regressive changes, such as tissue damage and inflammation, were observed in 17% (range: 0-34.06%) of the spleen samples. Circulatory disturbances were present in approximately 10% (range: 6.25-37.5%) of cases, while tumors were relatively rare, occurring in only 2% (range;0-12.5%) of the spleen samples. The normal spleen architectural structure showed spleen tissue, with MMCs (Figure 6.15 Plate A), MMC encapsulated by the fibrin layer (Figure 6.15 Plate B), melano-macrophages and splenic congestion (Figure 6.15 Plate C), and granulomatous inflammatory responses (Figure 6.15 Plate D) all indicate progressive changes. Figure 6.15 shows some of the histopathological changes observed in the spleen. Micrographs of spleen histological sections are shown in Figure 6.15.

The prevalence of congestion of red pulp ($\chi2=7.64$, df=5, $p>0.05$), benign tumors ($\chi2=10.21$, df=5, $p>0.05$), exudative inflammation ($\chi2=7.36$, df=5, $p>0.05$), and hyperplasia of melano-macrophage centers encapsulated with fibrin ($\chi2=9.96$, df=5, $p>0.05$) in the spleen did not differ significantly between wetlands. However, there were significant differences in the prevalence of infiltration of cells ($\chi2=11.62$, df=5, $p<0.05$), hypertrophy of melano-macrophage centers ($\chi2=10.21$, df=5, $p<0.05$), lymphoid hyperplasia ($\chi2=13.55$, df=5, $p<0.05$), and lymphoid hypertrophy ($\chi2=12.24$, df=5, $p<0.05$) among wetlands. The highest prevalence for infiltration of cells, hyperplasia of melano-macrophage centers, lymphoid hyperplasia, and lymphoid hypertrophy was observed in wetland AV, while the lowest values were found in wetlands WO and RA.

The prevalence of congestion of red pulp ($\chi2=4.91$, df=3, $p>0.05$), benign tumors ($\chi2=6.12$, df=3, $p>0.05$), exudative inflammation ($\chi2=2.13$, df=3, $p>0.05$),, infiltration of inflammatory cells ($\chi2=7.65$, df=3, $p>0.05$), hyperplasia of melano-macrophage centers encapsulated with fibrin ($\chi2=7.38$, df=3, $p>0.05$), hypertrophy of melano-macrophage centers ($\chi2=2.72$, df=3, $p>0.05$), lymphoid hyperplasia ($\chi2=1.05$, df=3, $p>0.05$), and lymphoid hypertrophy ($\chi2=4.03$, df=3, $p>0.05$) did not differ among seasons. A variability plot on the prevalence of spleen histopathological alterations in the wetlands of Lake Tana is shown in Figure 6.14.

Gonads

Regressive changes, including structural alterations, necrosis, and atrophy, were the most common abnormalities observed in the gonads, affecting approximately 60% (range: 31.25-64.20%) of the examined samples. Congestion and edema were less prevalent, occurring in about 16% (range: 6.25-31.25%) of cases. Inflammatory changes, such as the infiltration of leukocytes and fibrotic tissue, were also observed in 16% (range: 0-20%) of the samples. Progressive changes and tumors were the least common, affecting only 4% and 2% (range: 0-6.25%) of the gonads, respectively.



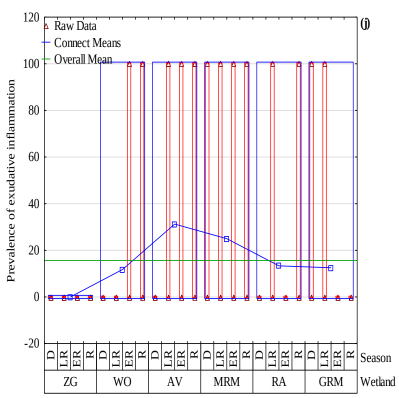


Figure 6.11. Variability plot on prevalence of spleen histopathological alterations in the wetlands of Lake Tana (a) Congestion of red pulp (b) Hyperplasia of MMCs encapsulated with fibrin (c) Hypertrophy of MMCs (d) Infiltration of cells and granulomatous lesions (e) Lymphoid hyperplasia (f) and lymphoid hyperplasia (g) Necrosis (h) Benign tumor (i) Deposits on inertial tissue (j) Exudative inflammation. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara river mouth, MRM-Megech river mouth, AV-Avaj, RA-Ras Abbay, D-dry, ER-early rainy, R-rainy, LR-late rainy season

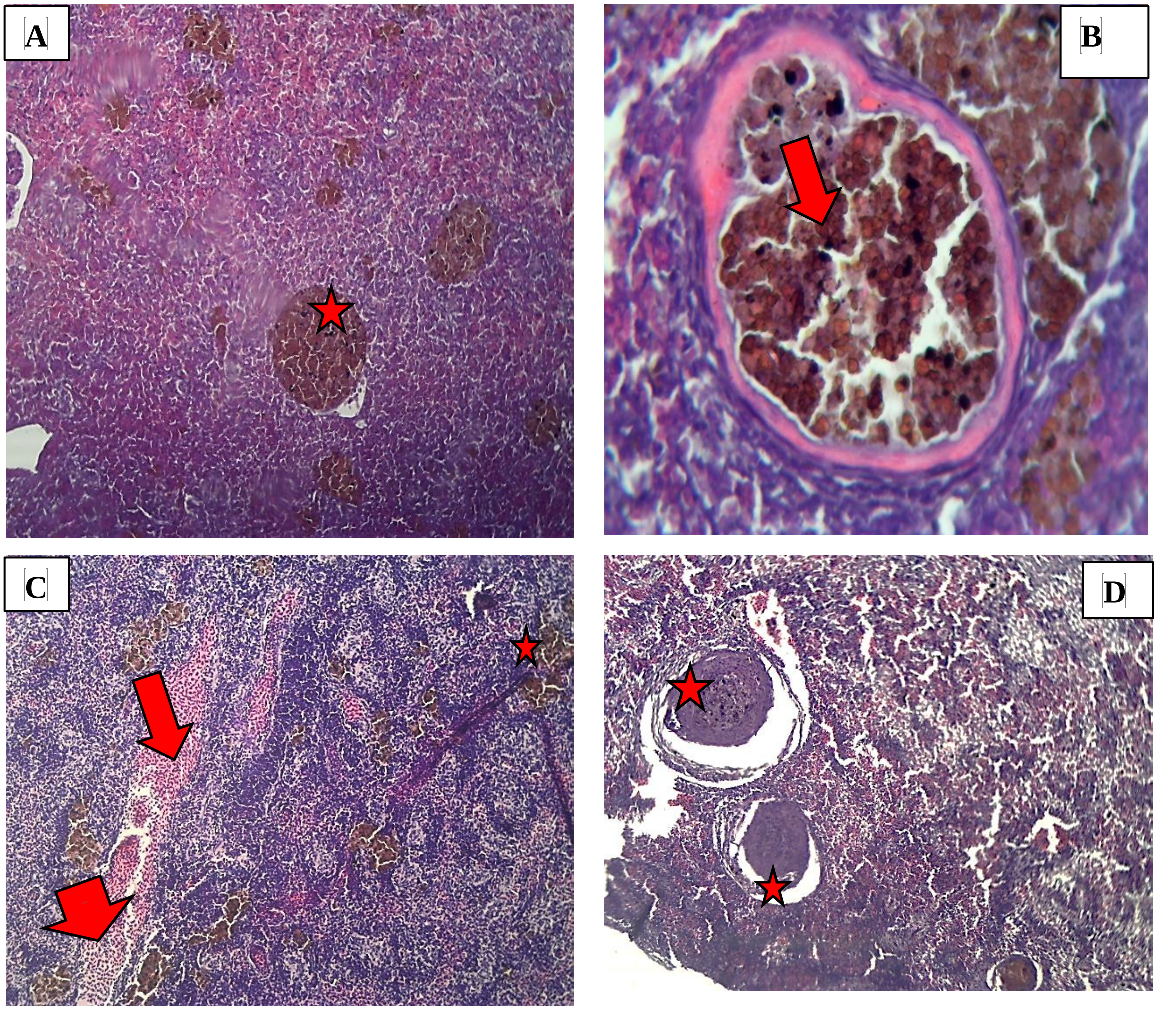


Plate 6.3. Micrographs of spleen histological sections. (A) Melano-macrophage centers (stars): hypertrophied (an artifact of the cutting and staining) (B) MMC (arrow) encapsulated by a fibrin layer (arrowhead) (C) Melano-macrophage (arrows) and splenic congestion (star) and (D) Granulomatous (stars) inflammatory response, probably due to *Edwardsiella tarda* or another bacterial infection

The normal histology of mature testes was characterized by the presence of spermatozoa (eosinophilic) in the seminiferous tubules shown (Figure 6.17, Plate A). Cysts of spermatogonia (Figure 6.17, Plate B) in the seminiferous tissue of male fish indicate inflammatory changes. Slight congestion (Figure 6.17, Plate C) in the interstitial cells of Leydig indicates a circulatory disturbance. Spermatocytic seminoma (Figure 6.17, Plate D) represents a distinct testicular neoplasm and testes with an aggregation of melano-macrophages (Figure 6.17, Plate E), indicating an inflammatory change in the interstitial Leydig cells. Micrographs of gonad histological sections are shown in Figure 6.17.

The prevalence of fibrosis ($\chi2$=15.20, df=5, p >0.05), spermatic seminoma ($\chi2$=7.23, df=5, p >0.05), hyperplasia of melano-macrophage centers ($\chi2$=7.10, df=5, p >0.05), congestion and edema ($\chi2$=9.44, df=5, p >0.05), and infiltration of inflammatory cells ($\chi2$=13.29, df=5, p >0.05) in the gonads did not vary significantly across different wetlands. There were statistically significant differences in the frequency of structural alteration ($\chi2$=21.61, df=5, p <0.05), hypertrophy of melano-macrophage center enlargement ($\chi2$=14.68, df=5, p <0.05), necrosis ($\chi2$=21.18, df=5, p <0.05), and atrophy ($\chi2$=12.36, df=5, p <0.05) across wetlands.

Most of the examined gonad pathologies, including congestion and edema ($\chi2$=6.13, df=3, p >0.05), necrosis ($\chi2$=2.88, df=3, p >0.05), structural alterations ($\chi2$=3.11, df=3, p >0.05), fibrosis ($\chi2$=2.13, df=3, p >0.05), spermatic seminoma ($\chi2$=3.78, df=3, p >0.05), hyperplasia of melano-macrophage center ($\chi2$=3.75, df=3, p >0.05), and hypertrophy of melano-macrophage center ($\chi2$=3.56, df=3, p >0.05) did not show significant variations across different seasons. However, there were significant differences in the prevalence of infiltration ($\chi2$=10.18, df=3, p <0.05) and atrophy ($\chi2$=8.47, df=3, p <0.05) of the gonadal tissue among seasons. A variable plot on the prevalence of gonad histopathological alterations in wetlands of Lake Tana is in Figure 6.16.

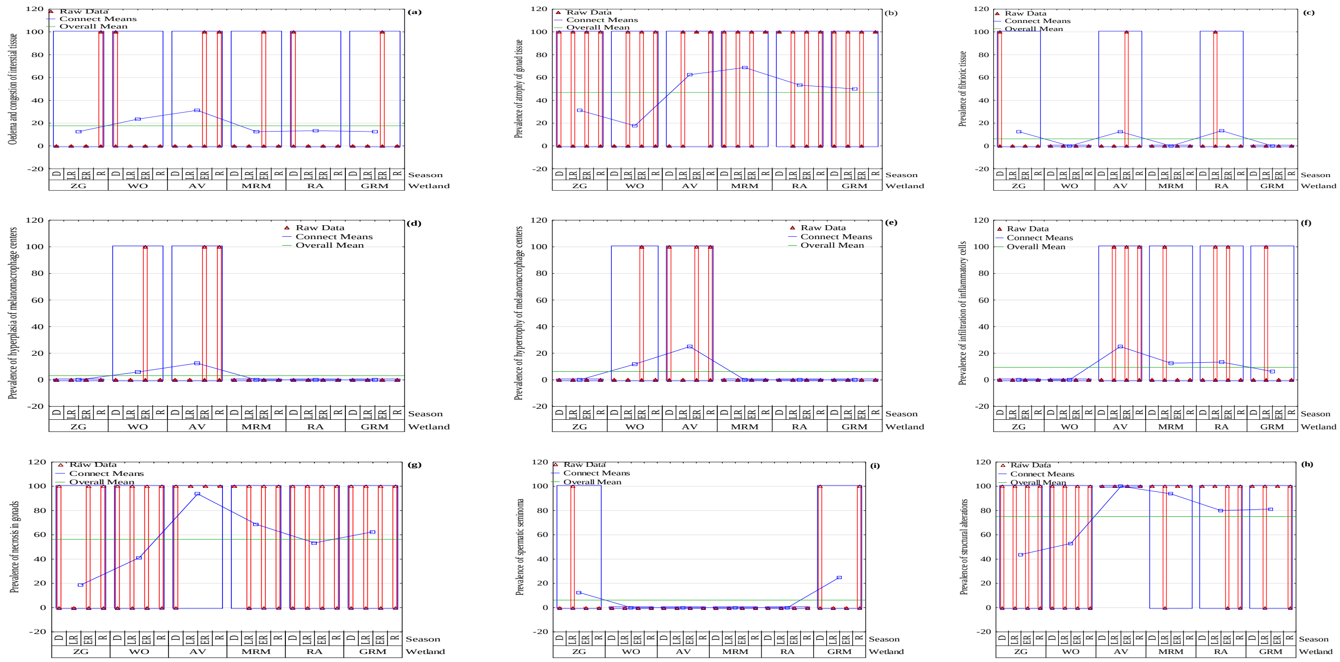


Figure 6.12. Variability plot on the prevalence of spleen histopathological alterations in the wetlands of Lake Tana (a) Edema of interstitial tissue (b) Atrophy (c) Fibrotic tissue (d) Hyperplasia of melano-mamacrophage centers (e) Hypertrophy of melano-mamacrophage centers (f) Inflammatory cells (g) Necrosis (h) Spermatic Seminoma (i) Structural alteration. WO-Wonjeta, ZG-Zewdie Girar, GRM-Gumara River mouth, MRM-Megech River Mouth, WO-Wonjeta, ZG-Zewdie Girar, AV-Avaj, RA-Ras Abbay, D-dry, ER-early rainy, R-rainy, LR-late rainy season.

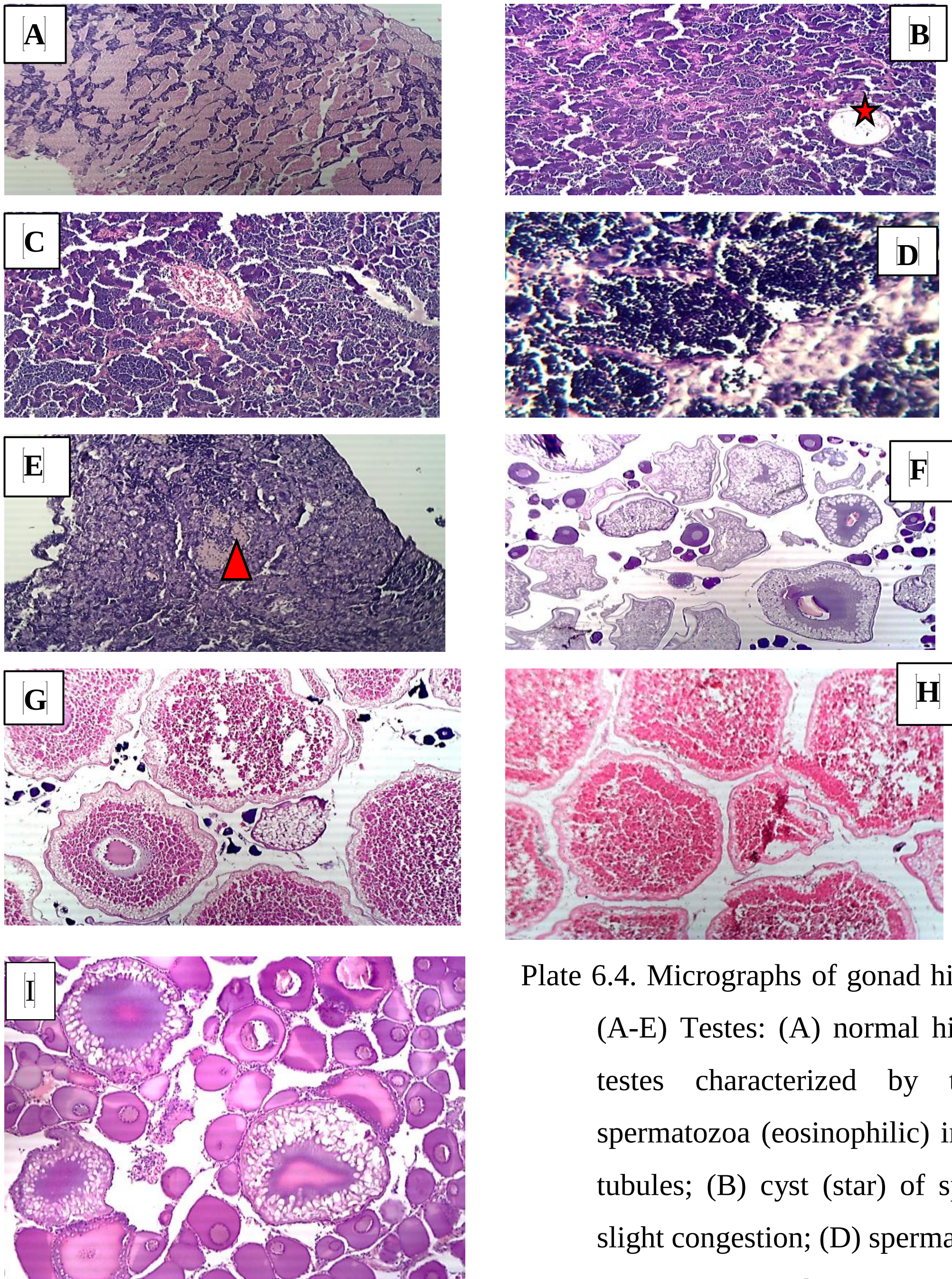


Plate 6.4. Micrographs of gonad histological sections (A-E) Testes: (A) normal histology of mature testes characterized by the presence of spermatozoa (eosinophilic) in the seminiferous tubules; (B) cyst (star) of spermatogonia; (C) slight congestion; (D) spermatocytic seminoma; (E) testes with aggregation of melano-macrophages (arrowhead); and (F-I) ovaries (F) normal histology of the ovary after spawn; (G and H) during spawning; and (I) postovulatory regression period.

6.4. Discussion

This chapter investigated fish histopathology as a biomarker of water quality deterioration in six wetlands receiving runoff across four seasons in Lake Tana, Ethiopia. A linkage between wetlands in four seasons and some of the histopathological responses of *Labeobarbus* spp. was established. Organ sensitivity and spatial and temporal diversity in histopathological alterations were recorded. The quantitative differences in spatiotemporal histopathology index grades, the severity of reaction indices, and lesion prevalence were established in six wetlands of Lake Tana.

The study wetlands were evaluated and compared in terms of the fish histopathology indices and lesion prevalence according to a modified protocol (Bernet et al., 1999). While comparisons with previous studies provide valuable context, the primary aim of this study was to assess spatial and seasonal variations in the histopathological responses of *Labeobarbus* spp. in relation to water quality across Lake Tana wetlands. The observed predominance of regressive and inflammatory lesions in gill and liver tissues from MRM, GRM, and AV directly addresses this objective by highlighting pollution-induced tissue damage in the most ecologically stressed sites. Although similar lesion types have been documented in fish populations from other regions, including Uganda, Portugal, and South Africa, the relevance of these comparisons lies chiefly in confirming that the histopathological responses observed here are consistent with those reported in other polluted freshwater ecosystems. Crucially, this study extends beyond many previous investigations by integrating multi-organ, multi-seasonal data and correlating histopathological trends with measured physicochemical parameters. This comprehensive approach reinforces the conclusion that gill and liver tissues serve as reliable biomarkers of chronic pollutant exposure in Lake Tana wetlands and underscores their suitability for future ecological monitoring and management programs.

There have been several reports on the wider application of histopathology for the assessment of aquatic environments. For example, Van Dyket al. (2012) found higher levels of damage likely caused by toxins, along with pre-neoplastic changes, in the livers of *C. gariepinus* from polluted sites compared to those from unpolluted areas. Liebel et al. (2013) used histopathology to examine gill and liver damage like aneurysms, excessive cell growth, and tumors, in *Astyanax aff. fasciatus* and *Oreochromis niloticus* exposed to pollution. More recent research findings demonstrate the effectiveness of histopathology alongside other biomarkers in identifying effects caused by environmental contamination (de Lima Cardoso et al., 2018; Doherty et al., 2019). Moore and Simpson (1992) identified potential biomarkers that could detect early changes in organisms at the molecular and cellular level. These authors determined the underlying causes of these changes. Bernet et al. (1999) and Yancheva et al. (2016) suggested that changes in fish anatomy caused by water pollution could be used to measure exposure to contaminants, especially for low-level and long-term effects. Teh (1997) examined changes in the gills, liver, and spleen of wild fish from three freshwater environments with varying levels of pollution. These studies, among others, demonstrate the increasing popularity of using histopathology to assess the health of aquatic ecosystems.

The study aimed to identify variations in histopathological alterations within *Labeobarbus* spp. in six wetlands throughout four seasons, based on a sample size of 96 fish. The sample size is contingent upon the variability observed across the different locations. A smaller distinction necessitates a larger sample size for statistically confirming such differences. As indicated by Bernet et al. (1999), Van Dyk et al. (2012), and Hinton et al. (2018) sample size plays a crucial role in any histopathological monitoring initiative. The number of samples needed for histopathological studies should be determined by the research question and the desired level of statistical confidence. As exemplified by Bernet et al. (1999) the selection of an appropriate sample size is crucial for the success of histopathological monitoring programs. However, a standardized, uniform approach is not universally applicable. The ideal number of samples depends on the objectives of the study. If the objective of histopathological monitoring is to detect the presence of disease to statistically confirm the presence of a specific disease in a population with 95% confidence, an appropriate sample size is required (Bernet et al., 1999; Hinton et al., 2018). The emphasis of these studies would be to collect a single individual having the pathological condition of interest. However, if the objective is to compare fish from polluted and unpolluted sites, the sample size needs to be large enough to detect even subtle differences in histopathological changes between the groups (Bernet et al., 1999; Van Dyk et al., 2012). Hence, the smaller the expected difference, the more samples are needed for a statistically valid comparison.

6.4.1. Organ sensitivity based on histopathology of *Labeobarbus* spp. in the wetlands of Lake Tana

Organs were impacted in the following order of severity, i.e., gills > liver > spleen > gonad. The high total organ index in the gills could be explained by the severity of the regressive and progressive changes. The mild epithelial lifting and atrophy of secondary lamellae and congestion with the epithelial lifting of primary lamellae indicate regressive changes that terminate in a functional reduction or partial loss of an organ (Laurent et al., 1994; Figueiredo-Fernandes et al., 2007; Butchiram et al., 2009; Weli et al., 2013; Lebepe et al., 2020). Hyperplasia of secondary lamellae and its advancement to fusion indicate progressive changes that lead to increased activity of cells or tissues (Bernet et al., 1999; Figueiredo-Fernandes et al., 2007; Marinović et al., 2021). However, the liver's high total organ index could be due to severe damage and inflammation, such as the formation of granulomatous lesions with bacterial colonies at the center of granulomatous lesions. This is likely caused by an infection with *Edwardsiella tarda* (Ribeiro et al., 2006; van Dyk et al., 2009; Van Dyk et al., 2012a; Lebepe et al., 2020). In contrast, the low total organ index in the gonad could be explained by the lower circulatory disturbances and progressive changes. These indicate that the gonad was the least sensitive and that it was less impacted by environmental effects than the gill, liver, and spleen. The most prevalent lesions in the gonad were regressive and tumor changes, particularly structural alterations, necrosis, atrophy, and spermatic seminoma, respectively. Regressive alterations refer to processes that result in the functional reduction or loss of an organ. These changes encompass atrophy, deformity, or malfunction of cellular structures due to cell damage and necrosis (Bernet et al., 1999; Bernet et al., 2004).

The gills and liver exhibited higher regressive indices compared to the spleen and the gonads. For example, the percentage of fish in Class 3 with an organ index > 31, showing severe alterations of organ tissue, was highest in the gills for AV during the rainy season, in the liver for AV during the dry season, and in GRM during the early rainy and late rainy seasons. Regressive reaction patterns signify advanced pathological changes leading to functional decline or loss of the organ, contrasting with progressive changes indicating increased cellular or tissue activity (Bernet et al., 1999). Similarly, the gills and liver displayed higher circulatory disturbance indices relative to the gonads and spleen. Circulatory disruptions result from abnormal conditions affecting blood and tissue fluid flow. Alterations in tissue fluid composition due to inflammatory processes, such as exudate, are considered part of the response pattern (van der Oostet al., 2003; Bernet et al. 2004).

The gills and spleen exhibited higher progressive indices compared to the liver and gonads. Progressive changes depicted the increased activity of the splenic tissue (red and white pulp), interstitial tissues, and lymphoid tissue (Santhakumar et al., 2001; Olojo et al., 2005; Figueiredo-Fernandes et al., 2007; Abalaka, 2017; Lebepe et al., 2020). However, there were severe progressive changes like hypertrophy and hyperplasia of the melano-macrophage centers, which are processes that lead to increased activity of splenic tissues (Fänge & Nilsson, 1985; Sikhakhane, 2011; Marchand et al., 2012; Karim et al., 2016; Uğurlu et al., 2022). The marked increase in size and frequency of the melano-macrophage centers in *Labeobarbus* spp. from Lake Tana may suggest that the fish are experiencing adverse health effects due to the pollutants present in the lake's water. When exposed to adverse environmental conditions, melano-macrophage centers tend to enlarge or multiply, suggesting the potential of melano-macrophage centers as reliable indicators of water quality, including low oxygen levels and chemical pollutants (Agius & Roberts, 2003; Steinel & Bolnick, 2017). According to Steinel & Bolnick (2017), “the melano-macrophage centers consist of pigmented phagocytic cells in fish and are considered simple, cheap, and broadly applicable measures of innate and adaptive immunity in fish”. The melano-macrophage centers are nodular structures with delicate argyrophilic capsules, generally seen around the blood vessels (Stosik et al., 2019).

The inflammatory changes observed in the gill and liver were more pronounced compared to those in the gonads and spleen. Inflammation, often linked to different reaction patterns such as edema, can make it challenging to attribute these changes to a pattern. Bernet et al. (1999) provided a precise definition of inflammation, highlighting key features such as the presence of exudate, a high protein content in bodily fluids, and cellular remnants from blood and lymphatic vessels. Furthermore, the activation of the reticuloendothelial system involves the hypertrophy of endothelial cells and macrophages along small blood vessels, as well as the infiltration of leukocytes through blood vessel walls and into surrounding tissues.

The gills and reproductive organs displayed significant changes due to tumors compared to the liver and spleen. Tumors are abnormal growths of cells that can invade surrounding tissues or spread to other parts of the body (Grizzle and Goodwin, 2010; Sharma et al., 2012). These growths can be categorized based on the type of cells and the rate of proliferation (Bernet et al. 1999; Grizzle and Goodwin, 2010). Benign tumors are made up of specialized cells that grow abnormally but do not spread to other parts of the body. In contrast, malignant tumors consist of undifferentiated cells that multiply rapidly, invading and destroying surrounding tissues; malignant tumors have the potential to spread to other areas through metastasis. The primary malignant tumor was found in the secondary lamella of the gills, while a benign spermatic seminoma was detected in the testes. The spermatocyte seminomas showed important differentiation in germ cells, progressing through various developmental stages until sperm formation. The neoplasm was characterized by undifferentiated spermatocytes and a lower number of supporting cells. The spermatocyte seminoma, identified as a testicular benign tumor, was distinguished by the presence of melano-macrophage clusters, indicating poorly differentiated cells that multiply rapidly and invade the native testicular tissues. Typical features of spermatocyte seminomas include soft, white, multilobulated masses primarily confined in the testes (Grizzle and Goodwin, 2010; Sharma et al., 2012; Ren et al., 2019).

The higher histopathological sensitivity and lesion prevalence of the gill, followed by the liver and gonads, makes these three organs better indicators of contamination exposure than the spleen in this study. Previous research revealed severe health effects, including damage to the gills and liver, in fish exposed to contaminated environments (Olojo et al., 2005; González et al., 2006; Abdel-Moneim et al., 2012; Hadi & Alwan, 2012; Bhuvaneshwari et al., 2015; Dane & Şişman, 2015). Liver cell alterations can provide a reliable early indication of both fish health and water quality, leading to the widespread use of liver alterations in biomonitoring programs (Alaa, 2012; Reddy, 2012; Yancheva et al., 2016; Kumar et al., 2019; Nephale et al., 2021). Field-based studies have established the gill and liver as highly sensitive organs to contaminant exposure and toxicopathic lesion formation in many fish species exposed to different environmental conditions (McClain et al., 2003; Labadie & Chevreuil, 2011; Webb & Gagnon, 2013; Pattanayak et al., 2018; Ogbeide et al., 2019; Herrmann et al., 2023). In the livers of all three species, the most noticeable problems were damaged liver cells, disrupted bile ducts, and parasites (Ogbeide et al., 2019). Laboratory studies have established the liver as a highly sensitive organ to contaminant exposure and toxicopathic lesions (Sanad et al., 2015).

The high sensitivity of fish gills to environmental pollution can be attributed to the large external surface area of gills, making gills particularly vulnerable to chemical and physical changes in aquatic environments. (Evans, 1987; Poleksić & Mitrović-Tutundžić, 1994; Figueiredo-Fernandes et al., 2007; Flores-Lopes & Thomaz, 2011; Kostić-Vuković et al., 2021). Gills are responsible for regulating the exchange of salt and water and play a major role in the excretion of nitrogenous waste products. Moreover, the gills are important organs for respiration and acid-base balance (Hadi & Alwan, 2012). The damage to the gills could be attributed directly to the introduction of salts, heavy metals, pesticides, sewage, and fertilizers into the water (Temmink et al., 1983). The lesions are directly exposed to environmental contaminants, which frequently cause health problems in fish (Mallatt, 1985). The gills are highly susceptible to environmental factors due to the direct contact of gills with the water, making gills some of the most sensitive structures in teleost fish. Consequently, gills are prone to damage by any irritating substances present in the water, either dissolved or suspended (Roberts, 2012).

The liver is the primary organ for the biotransformation and excretion of xenobiotics (Neghab et al., 2020). The liver's high sensitivity could be attributed to its physiological functions. The liver is a key organ of overall homeostasis in terms of nutrition, defense against toxicants, and reproductive development (Bruslé & Anadon, 2017). The poor blood perfusion in the liver of fish as compared to mammals may enhance the stasis of toxicants, causing relatively more damage to the liver tissue (El-Nahhal, 2018; Hinton, 2021). Thus, the liver is the first organ to encounter absorbed nutrients, vitamins, metals, drugs, and environmental toxicants, as well as waste products of bacteria that enter the portal blood (Jaeschke, 2008; Ruch, 2020; Hinton, 2021). Several histological alterations such as mononuclear cell infiltration, congestion, and nuclear pyknosis were reported in the livers of fish exposed to industrial pollutants (Gül et al., 2004; Abdel-Moneim et al., 2012; Jabeen et al., 2018).

Although this study could not establish the presence or concentrations of some selected toxicants in the environment, some of the observed histological alterations have been shown to provide definite biological endpoints of exposure to several specific contaminants (van Dyk et al. 2009; Flores-Lopes and Thomaz 2011; Gaber et al., 2015; Reddy 2012; Yancheva et al. 2016). For example, epithelial lifting of primary lamellae to represent regressive changes and hyperplasia of secondary lamellae to represent early stages of fusion of secondary lamellae indicate progressive changes and are recommended as an excellent example of a gill histopathological biomarker for contaminant exposure (Olojo et al., 2005; Lebepe et al., 2020; Marinović et al., 2021). Similarly, granuloma formation with bacterial colonies or other infections in the liver tissue represents inflammatory changes and is suggested as the best example of a liver histopathological biomarker for contaminant exposure (Rodrigues and Fanta, 1998; Figueiredo-Fernandes et al., 2007; Sanad et al., 2015; Ruch, 2020; Topić Popović et al., 2023)

Pathological Grades 1, 2, and 3 were frequently observed in the gill and liver tissues. Conversely, only Grades 1 and 2 pathological changes were noted in the spleen and gonads. This finding aligns with previous research findings that suggest a higher occurrence of Grade 1 lesions compared to Grades 2, 3, and 4 in the less polluted areas (Abdel-Moneim et al., 2012; Naigaga, 2012; Liebelet al., 2013; Al-Mamoori et al., 2014; Hinton et al., 2018).

The gill lesions in WO and ZG were therefore less severe compared to the liver and gonads. The finding is in line with research that observed degenerative alterations and cell death in the gill epithelium of fish following exposure to heavy metals and pesticides by Santhakumar et al. (2001), Butchiram et al. (2009), and Hasan et al. (2015). Naigaga (2012) reported that regressive changes in the gills were mainly observed in the effluent-impacted sites of Lake Victoria. Ribeiro et al. (2006) found that examining the gills of fish can be a good way to measure general environmental pollution but preparing the tissue for this kind of study is time-consuming.

There were significant differences in median values of regressive and organ indices in gonads among wetlands ranging from low median values in WO and ZG to high values in MRM, GRM, and AV. The increased changes in the ovary and testis of fish in this study with increased postovulatory atresia, particularly of previtellogenic follicles, can indicate a pathological condition, and this has been associated with exposure to environmental contaminants (Blaze, 2002; Naigaga, 2012). Regressive reaction patterns are advanced pathological changes that depict processes that terminate in a functional reduction or loss of the organ, compared to other reaction patterns that reflect the increased activity of the cells or tissue (Moore and Simpson, 1992; Bernet et al., 1999; de Lima Cardoso et al., 2018; Doherty et al., 2019). Congestion and edema in the ovaries contribute to the highest circulatory disturbance index in gonads. However, this finding is not in line with the works of Naigaga (2012) who noted a low total organ index in the gonads, which could be explained by the absence of circulatory disturbances, such as hemorrhage and edema, which were not observed in the gonadal tissue.

Structural alterations were the most frequently encountered gill lesions, followed by circulatory disturbances. Atrophy in gills was observed in female fish, while malignant tumors on the lamellae were detected on the gills of male fish. Reaction patterns, including congestion/hemorrhage and thrombosis, hyperplasia, infiltration of inflammatory cells, necrosis, and malignant tumors were detected regularly in both female and male fish. Several other studies have found epithelial hyperplasia and curling of secondary lamellae on the gills, and swelling and thrombosis at the tips of several secondary lamellae (Yancheva, 2016). There are several reports noting histopathological alterations in the gills of *Labeoabarbus* species, which detected lesions such as hyperplasia, congestion and mucous cell proliferation of the gill epithelium, and damaged primary and secondary lamellae, the uplifting of respiratory epithelial wall and damaged pillar cells (Rességuier et al., 2020). Cellular alterations in the gills can provide a good early indication of overall fish health and water quality status in biomonitoring programs (Poleksic et al., 2010; Salamat et al., 2013; Abalaka, 2017; Sweidan et al., 2015; Lebepe et al., 2020). Laboratory and field-based studies have established the gills to be a highly sensitive organ to contaminant exposure and toxicopathic lesion formation in many fish species exposed to different environmental conditions (van Dyk et al., 2009; Stentiford et al., 2010; Lebepe et al., 2020; Corredor-Santamaría et al., 2021). The histopathological responses in six wetlands of Lake Tana are comparable to studies reporting such responses in fish from highly contaminated environments. For instance, the value of the fish index in the present study (14-106) is lower than the value of sea bass in Portugal

(Saraiva et al., 2015). However, it is higher than the report on a fish species in a Chinese fish farm (Li et al., 2020), carp exposed to Chlorpyrifos in Serbia (Banaee et al., 2013), and catfish from a polluted freshwater dam in South Africa (Marchand et al., 2012). The lower gill index values (0-18) in the present study compared to *Dicentrarchus labrax*, a farmed fish in Portugal (Saraiva et al., 2015). This suggests potentially healthier gills in *Labeobarbus* spp. of Lake Tana. However, the gill index in our study was higher than the values reported for other fish species in various environments. For example, (Marchand et al., 2012) found lower values in *Clarias gariepinus* from a polluted freshwater dam in South Africa. Additionally, studies reported lower gill indices in wetlands impacted by irrigation effluent (Abdel-Moneim et al., 2012) and a river affected by urban effluent (Nascimento et al., 2012).

The liver index in this study (0-15) was lower than in other studies around the world. This includes farmed fish in Portugal (Saraiva et al., 2015), fish in the Villarica River Basin (Portugal) exposed to agricultural effluent (Santos et al., 2022), and *C. gariepinus* in Roodeplaat Dam (South Africa) impacted by urban effluent (Marchand et al., 2012). However, the liver index in this study is higher than that reported for juvenile sole, *Solea* spp. in a Spanish estuary with high sediment levels (Briaudeau et al., 2019). The gonad index in our study (0-18) aligns with the findings of Pieterse et al. (2010) for *C. gariepinus* in a South African nature reserve impacted by urban effluent. The spleen index registered in this study (0-15) is higher than reported for *Hypothalmichthys molitrix* exposed to Deltamethrin in Pakistan (Karim et al., 2016). However, it falls below the values found in Lake Victoria (Waweru et al., 2024) for *O. niloticus*, likely impacted by industrial and agricultural waste. Table 6.4 provides a more detailed comparison of organ indices in this study with previous research.

The value of gill pathological severity of Grade I lesion in the present study (0-100%) is in agreement with findings by Naigaga (2012) for *O. niloticus* in Ugandan wetlands bordering Lake Victoria, also impacted by human activity. However, our results showed generally lower pathology compared to other studies. For instance, *Luciobarbus bocagei* in Portuguese rivers with industrial pollution (Pereira et al., 2013), *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012), and *O. andersonii* in Botswana's Okavango Delta exposed to ecotoxins (van Dyk et al., 2009). In contrast to gill Grade I lesions, the severity of Grade II lesions in our study (37.5-62.5%) is generally lower than those reported in other studies on fish from polluted environments. This includes *O. niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012) impacted by human activity, *L. bocagei* in Portuguese rivers with industrial pollution (Pereira et al., 2013), *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012), and *O. andersonii* in Botswana's Okavango Delta exposed to ecotoxins (van Dyk et al., 2009). The severity of Grade III lesions in the gill in this study (0-12.5%) was lower than what van Dyk et al. (2009) found in fish from Botswana's Okavango Delta exposed to ecotoxins. However, it was higher than findings in other studies on fish from less polluted environments. This comprises, *O. niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012), *L. bocagei* in Portuguese rivers with industrial pollution (Pereira et al., 2013), and *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012).

The severity of Grade I lesions in the liver in this study (30.6-87.5%) falls between the values reported in other studies. The value is higher than in fish from Botswana's Okavango Delta with no detectable lesions (van Dyk et al., 2009) on *O. andersonii*, likely due to ecotoxic exposure. However, it is lower than the findings for *Oreochromis niloticus* in Ugandan wetlands (Naigaga, 2012) bordering Lake Victoria and *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012). In contrast to the findings for Grade I lesions, the severity of Grade II lesions in the liver of *Labeobarbus* spp. in our study (43.3-48.3%) was higher than reported in other studies. This includes *Oreochromis niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012), *O. andersonii* in Botswana Okavango Delta exposed to ecotoxic effluent (van Dyk et al., 2009), and *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012). In contrast to findings for Grade I lesions, the severity of Grade II lesions in the liver in this study (12.5-37.5%) was generally lower than reported in other studies. This includes *O. niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012) and *O. andersonii* in Botswana's Okavango Delta exposed to ecotoxic effluent (van Dyk et al., 2009). However, it was comparable to findings for *O. niloticus* in Saudi Arabian wetlands impacted by irrigation runoff (Abdel-Moneim et al., 2012). The severity of Grade III lesions in the liver in our study (12.4-18.8%) falls between the values reported in other studies. The value is lower than the findings for *O. niloticus* in Ugandan wetlands (Naigaga, 2012) bordering Lake Victoria and wetlands in Saudi Arabia (Abdel-Moneim et al., 2012) impacted by irrigation runoff. However, it was higher than the findings for *O. andersonii* in Botswana's Okavango Delta exposed to ecotoxic effluent (van Dyk et al., 2009). The current study reveals a significantly higher prevalence (75-100%) of Grade I lesions in the gonads compared to findings in other studies on fish. This includes the value of *O. niloticus* from Ugandan wetlands bordering Lake Victoria (Naigaga, 2012) and Botswana's Okavango Delta exposed to ecotoxic effluent (van Dyk et al., 2009). A lower value is registered in *C. gariepinus* in a South African nature reserve impacted by urban effluent (Pieterse et al., 2010). In contrast to Grade I lesions, the severity of Grade II lesions in the gonads in this study (0-25%) was lower than some previously reported values. For instance, lower values were registered for *Oreochromis niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012) and for *Oreochromis andersonii* in Botswana's Okavango Delta exposed to ecotoxic effluent (van Dyk et al., 2009).

This study found a very high prevalence (87.5%-100%) of Grade I lesions in the spleen. This is higher than what other studies reported. This comprises values on *Oreochromis niloticus* in Ugandan wetlands bordering Lake Victoria (Naigaga, 2012), for *Oreochromis niloticus* exposed to Diazinon in Turkey (Uğurlu et al., 2022), and for *Gasterosteus aculeatus* in a creek impacted by a nuclear facility (Teh, 1997). The severity of Grade II lesions in the spleen in the present study (0-12.5%) was generally lower than reported in other studies on fish exposed to various pollutants. For instance, *O. niloticus* was exposed to Diazinon, a contaminant (Uğurlu et al., 2022). However, some minimal presence of Grade II lesions was observed in this study, which was higher than findings for fish in a creek impacted by a nuclear facility (Teh, 1997) and for *O. niloticus* in Ugandan wetlands impacted by human activity (Naigaga, 2012). Table 6.5 provides a more detailed comparison of pathological severity Grades in this study with previous research.

The prevalence of gill circulatory disturbances in the present study (20%) was lower than in previous findings on fish from polluted rivers in Serbia and Brazil (Lujić et al., 2015; HC et al., 2021). Conversely, it was higher than the prevalence observed in fish from rivers in Hungary, Russia, and South Africa (Sikhakhane, 2011; Yancheva et al., 2020; Sigacheva & Gavruseva, 2023). Similarly, the prevalence of gill regressive changes in our study (35%) was lower than in previous reports on fish from rivers in Hungary, South Africa, Serbia, and Brazil (Sikhakhane, 2011; Lujić et al., 2015; Yancheva et al., 2020; HC et al., 2021). However, it was higher than the prevalence observed in fish from Russia (Sigacheva & Gavruseva, 2023). The prevalence of gill progressive changes in this study (25%) was lower than that of the reports on previous studies on fish from rivers in Hungary, Russia, Serbia, and Brazil (Lujić et al., 2015; Yancheva et al., 2020; HC et al., 2021; Sigacheva & Gavruseva, 2023). The prevalence of gill inflammatory changes in our study (15%) was higher than that reported in studies on fish from rivers in Hungary, Russia, Serbia, and Brazil (Lujić et al., 2015; Yancheva et al., 2020; HC et al., 2021; Sigacheva & Gavruseva, 2023). The prevalence of gill tumor changes in our study (5%) was higher than the reports on previous studies on fish from rivers in Hungary, Serbia, and Brazil (Lujić et al., 2015; Yancheva et al., 2020; HC et al., 2021). Overall, the results suggest a relatively better gill health condition in the *Labeobarbus* spp. in Lake Tana compared to populations from heavily polluted environments, although some indicators of gill damage were more severe.

The prevalence of liver circulatory disturbances in our study (25%) was lower than in previous studies in fish from rivers in Hungary, Portugal, and Brazil (Yancheva et al., 2020; HC et al., 2021; Santos et al., 2022). Conversely, it was higher than the prevalence reported in fish from Russia and Türkiye (Kaptaner et al., 2014; Sigacheva & Gavruseva, 2023). The prevalence of liver regressive changes in the present study (32%) was lower than in previous reports in fish from rivers in Hungary, Russia, Brazil, and Portugal (Yancheva et al., 2020; HC et al., 2021; Santos et al., 2022; Sigacheva & Gavruseva, 2023). Conversely, it was higher than the prevalence reported in fish from Türkiye (Kaptaner et al., 2014). The prevalence of liver progressive changes in our study (10%) was lower than in previous reports on fish from rivers in Hungary, Portugal, Turkey, and Brazil (Kaptaner et al., 2014; Yancheva et al., 2020; HC et al., 2021; Santos et al., 2022). However, it was higher than the prevalence reported in fish from Russia (Sigacheva & Gavruseva, 2023). The prevalence of liver inflammatory changes in the present study (31%) was lower than in previous studies in fish from rivers in Hungary and Portugal (Santos et al., 2022; Yancheva et al., 2020). However, it was higher than the prevalence reported in fish from Russia and Türkiye (Kaptaner et al., 2014; Sigacheva & Gavruseva, 2023). No inflammatory changes were reported in the studies from Brazil (HC et al., 2021). The prevalence of liver tumorous changes in the present study was low (2%). This is in contrast to studies on fish from other locations, where no tumorous changes were reported (Kaptaner et al., 2014; Yancheva et al., 2020; HC et al., 2021).

The prevalence of gonad circulatory changes in the present study was 16% in our study. However, other research by van Dyk et al. (2009), Pieterse et al. (2010), Marchand et al. (2012), and Santos et al. (2022) did not report any circulatory changes in the gonads of studied fish populations in different water bodies. The prevalence of gonad regressive changes in this study (60%) was lower than the previous report by (van Dyk et al., 2009) for fish in an unpolluted area in Botswana. However, it was higher than the prevalence observed in fish from South Africa (Marchand et al., 2012; Pieterse et al., 2010) and lower than that found in fish from Portugal (Santos et al., 2022). The prevalence of gonad progressive changes in this study (4%) was generally lower than that observed in fish from South Africa and Portugal (Pieterse et al., 2010; Santos et al., 2022). However, it was higher than the prevalence reported in fish from Botswana (van Dyk et al., 2009) and South Africa (Marchand et al., 2012).The prevalence of gonad inflammatory changes in our study (16%) was higher than that observed in fish from South Africa (Pieterse et al., 2010; Marchand et al., 2012) but lower than that reported in fish from Portugal and Botswana (Santos et al., 2022; van Dyk et al., 2009). The prevalence of gonad tumor changes in our study was low (2%). While some tumor changes were observed in another study (Pieterse et al., 2010), no tumor changes were reported in the studies conducted by Marchand et al. (2012), Santos et al. (2022), and van Dyk et al. (2009).

The prevalence of spleen circulatory disturbances in our study (14%) was lower than that observed in fish from Uganda and Turkey (Naigaga, 2012; Uğurlu et al., 2022). Conversely, it was higher than the prevalence reported in fish from South Africa and Egypt (Mohamed et al., 2023; Taylor, 2019). The prevalence of spleen regressive changes in the present study (17%) was lower than that reported in studies conducted in Uganda and Turkey (Naigaga, 2012; Uğurlu et al., 2022). However, it was higher than the prevalence observed in fish from Egypt Mohamed et al. (2023). The prevalence of spleen progressive changes in the present study (50%) was lower than that observed in fish from Uganda and South Africa (Naigaga, 2012; Taylor, 2019). However, it exceeded the prevalence reported in fish from Egypt and Türkiye (Mohamed et al., 2023; Uğurlu et al., 2022). The rate of spleen inflammation in this study (17%) was lower than that found in fish from Ugandan wetlands (Naigaga, 2012). However, no inflammatory changes were detected in studies conducted in South Africa and Turkey (Taylor, 2019; Uğurlu et al., 2022). The prevalence of spleen tumor occurrence was low in this study (2%). While previous studies in Uganda, South Africa, Egypt, and Turkey reported minimal or no tumor changes in fish tissues (Naigaga, 2012; Taylor, 2019; Uğurlu et al., 2022; Mohamed et al., 2023), the present study observed moderate histopathological alterations in *Labeobarbus* spp., particularly in gill and liver tissues. These findings support the study's objective of identifying sub-lethal tissue-level responses to pollution in Lake Tana wetlands. Rather than listing multiple studies, this comparison emphasizes how histopathological indices in this study reflect localized pollution pressures and seasonal variability, which were not addressed in earlier research. A comparison of the prevalence of tissue reaction changes with previous research findings is indicated in Table 6.6

Table 6.4. Comparison of median and fish indices with previous research findings. Where, IC=index for circulatory disturbances; IR=index for regressive changes; IP=index for progressive changes; II =index for inflammation; and IT =index for tumor. Organ index (OI), and FHI=Fish histopathology index

Organ/ reaction pattern indices		**Range in the present study**	**Previous research findings**				**Reference**
			Range	**Fish species**	**Environment/Country**	**Contamination type**	
	*I*C		2.2-7.8	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-8	0-4	*Abramis brama*	Tamiš River/ Serbia	Irrigation effluent	2
			0-2	*Silurus glanis*	Tamis River/Serbia	Organic production	3
			2-8	*D. labrax*	Aquaculture /Portugal	Aquaculture	4
	*I*R		1.9-18.7	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-18	0-10	*D. labrax*	Aquaculture/Portugal	Aquaculture	4
			0-9	*Sander lucioperca*	Tamis River/Serbia	Organic production	3
	*I*P		11.5-27.9	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
Gill		0-12	0-10	*A. brama*	Tamiš River/ Serbia	Irrigation effluent	2
			0-13	*S. glanis*	Tamis River/Serbia	Organic production	3
	*I*I		0-3	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	5
		0-12	0-19.1	*O. niloticus*	Lake Victoria/Kenya	Industries and agriculture	6
			0-40	*Danio rerio*	Aquarium/Brazil	Industrial additive	8
	*I*T		-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-18	0-3	*Sciades herzbergii*	Mangrove /Brazil	Domestic effluent	9
			0-6	*Astyanax aff. fasciatus*	Lake Park Brazil	Urban effluent	10
			1.52-5.35	*O. niloticus*	Lake Victoria/Kenya	Industries and agriculture	6
			0-22	*A. brama*	Tamiš River/ Serbia	Irrigation effluent	2
	*O*I	0-18	27-78	*D. labrax*	Aquaculture/Portugal	Aquaculture	4
			3.9-17.4	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	5
			3-13	*Oligosarcus hepsetus*	Paraı´ba do Sul River/Brazil	Urban effluent	7
			4-12	*Hypostomus auroguttatus*	Paraı´ba do Sul River/Brazil	Urban effluent	7
			3-9	*Geophagus brasiliensis*	Paraı´ba do Sul River/Brazil	Urban effluent	7
			8.10-16.30	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	8

1= Naigaga (2012); 2= Marinović et al. (2021); 3=Lujić et al. (2015); 4=Saraiva et al. (2015); 5= Abdel-Moneim et al. (2012); 6=Waweru et al. (2024); 7= Nascimento et al. (2012); 8=_Libanio et al.,(2024); 9= HC et al. (2021); 10=Liebel et al. (2013).

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Organ/ reaction pattern indices		Range in the present study	Previous research findings				Reference
			Range	Fish species	Environment/Country	Contamination type	
	*I*C	0-6	1.4-5.6	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			0–6	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			0-1.5	*L. bocagei*	River/Portugal	Agricultural effluent	3
	*I*R	0-15	0.8-23.4	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			4–24	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			10-30	*L. bocagei*	Vilariça River Basin/Portugal	Agricultural effluent	3
			0-5-8	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	6
	*I*P	0-12	1.4-5.2	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
Liver			0-6	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			2.5-20	*L. bocagei*	Vilariça River Basin/Portugal	Agricultural effluent	3
			2.5-11	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	6
	*I*I	0-10	1.9-16.9	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			0-15.9	*O. niloticus*	Lake Victoria/Kenya	Industries and agriculture	4
			0-8	*L. bocagei*	Vilariça River Basin/Portugal	Agricultural effluent	3
	*I*T	0-4	-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			0-2	*S. herzbergii*	Mangrove /Brazil	Domestic/ fish farming	5
			1.70-6.38	*O. niloticus*	Lake Victoria/Kenya	Industries and agriculture	4
	*O*I	0-15	6-36	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			22-52	*L.bocagei*	Vilariça River Basin/Portugal	Agricultural effluent	3
			0-12	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	6
			12.21-29.39	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	7

1= Naigaga (2012); 2= Saraiva et al. (2015); 3= Santos et al. (2022); 4=Waweru et al. (2024); 5= HC et al. (2021); 6= Briaudeau et al. (2019); 7=Marchand et al. (2012)

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Organ/ reaction pattern indices		Range in the present study	Previous research findings				Reference
			Range	Fish species	Environment/Country	Contamination type	
	*I*C		2.6-10.2	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-6	0-1.5	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	2
			-	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
			-	*C. gariepinus*	Hartbeespoort Dam/ South Africa	Endocrine disrupting metals	5
	*I*R		35.5-56.0	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-18	0-8	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	2
			0-50	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
			0.7-2	*C. gariepinus*	Hartbeespoort Dam/ South Africa	Endocrine disrupting metals	5
Gonad	*I*P		11.25-11.35	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-3	3-11	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	2
			-	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
	*I*I		-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-8	0.5-2.5	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	2
			-	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
			-	*C. gariepinus*	Hartbeespoort Dam/ South Africa	Endocrine disrupting metals	5
	*I*T		-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		0-15	-	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
			-	*C. gariepinus*	Hartbeespoort Dam/ South Africa	Endocrine disrupting metals	5
			-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
	*O*I	0-18	0-7.5	Juvenile *Solea* spp.	Bilbao estuary/Spain	Sediment	2
			0-18.8	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	3
			0.75-8.15	*C. gariepinus*	Roodeplaat Dam / South Africa	Urban effluent	4
			0-13.25	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	6

1= Naigaga (2012); 2= Briaudeau et al. (2019); 3= Pieterse et al. (2010); 4= Marchand et al. (2012); 5= Botha (2011)

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Organ/ reaction pattern indices		Median/range in the present study	Previous research findings				Reference
			Range	Fish species	Environment/Country	Contamination type	
	*I*C	0-4	1.4-4.5	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*Hypophthalmichthys molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
	*I*R	0-15	1.2-9.8	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			1-2	*H. molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
	*I*P	0-8	4.7-16.8	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			1-4	*H. molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
			1-6	*Oncorhynchus mykiss*	aquatic ecosystem/Iran	Effects of diazinon	6
	*I*I	0-8	2.7-10.4	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*H. molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
	*I*T	0-2	-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
Spleen			-	*H. molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
			-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
	*O*I	0-15	1-5	*H. molitrix*	Sheikhupura/Pakistan	Exposure to Deltamethrin	5
			7.0-16,61	*O. niloticus*	Lake Victoria/Kenya	Industries and agriculture	3
Fish Index		14-106	59-130	*D. labrax*	Aquaculture/Portugal	Aquaculture	2
			16.1-52.9	*Ctenopharyngodon idella*	Shanghai/China	Fish farm	4
			0-45	*Cyprinus carpio*	Stream/Serbia	Exposure to chlorpyrifos	6
			38.96-59.84	*C.gariepinus*	Roodeplaat Dam/ South Africa	Hyper-eutrophic freshwater	8

1= Naigaga (2012); 2= Saraiva et al. (2015); 3=Waweru et al. (2024); 4= Li et al. (2020); 5 =Karim et al.,(2016), 6= Banaee et al. (2013); 7=Rašković et al. (2013); 8= Marchand et al. (2012)

Among the organs examined, the gills were the most affected, followed by the liver, spleen, and gonads. The high total organ index in the gills was primarily due to pronounced regressive and progressive changes, including epithelial lifting, lamellar atrophy, hyperplasia, and fusion. These lesions are consistent with acute and chronic exposure to irritants and suspended particulates, reflecting the gills' direct contact with the aquatic environment. The liver exhibited severe inflammatory responses and granulomatous lesions, likely linked to chronic pollutant exposure and potential bacterial infections. In contrast, the spleen and gonads showed lower index values, with fewer circulatory and progressive changes, indicating reduced sensitivity to environmental stressors. The spatial distribution of histopathological patterns closely matched the physicochemical profiles of the wetlands. Wetlands such as Megech River Mouth (MRM), Gumara River Mouth (GRM), and Avaj (AV), which exhibited elevated nutrient loads, conductivity, and ammonia levels, also showed the most severe tissue alterations. Conversely, Zewdie Girar (ZG) and Wonjeta (WO), which had better water quality, exhibited minimal histological damage. These findings confirm that histopathological responses are reliable indicators of water quality degradation. Based on sensitivity, diagnostic clarity, and ecological relevance, the gills and liver are recommended as the most suitable organs for routine biomonitoring in Lake Tana wetlands. Their consistent response to pollution gradients and ease of histological interpretation make them effective biomarkers for the early detection of ecological stress.

Table 6.5. Comparison of lesion severity grades (%) in fish organs between the present study and previous research. Organ-specific indices (IC, IR, IP, II, IT) are applied to evaluate pollution-related tissue damage.

Lesion severity grades (%)	Range in the present study	Previous research findings				Reference
		Value	Fish species	Environment/Country	Contamination type	
Gill						
	0-100	0-97.6	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
1		25-75	*L. bocagei*	Rivers/Portugal	Industrial effluent	2
		58.3	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3
		-	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
	37.5-62.5	61.2-67.8	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
2		25-90	*L. bocagei*	Rivers/Portugal	Industrial effluent	2
		41.7	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3
		67	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
	0-12.5	0-2.4	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
3		10.0	*L. bocagei*	Rivers/Portugal	Industrial effluent	2
		-	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3
		33	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
Liver						
1	30.6-87.5	0-71.4	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		-	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
		50	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3
2	12.5-37.5	43.3-48.3	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		53	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
		38.9	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3
3	12.4-18.8	0-3.3	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		47	*O. andersonii*	Okavango Delta panhandle/Botswana	Ecotoxic effluent	4
		11.1	*O. niloticus*	Wetlands/ Saudi Arabia	Irrigation effluent	3

1= Naigaga (2012); 2= Pereira et al. (2013); 3= Abdel-Moneim et al. (2012); 4= van Dyk et al. (2009)

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Lesion severity grades (%)	Range in the present study	Previous research findings				Reference
		Value	Fish species	Environment/Country	Contamination type	
Gonad						
1	75-100	71.4	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		60	*O. andersonii*	Okavango Delta /Botswana	Ecotoxic	2
		28	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	3
2	0-25	28.6	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		40	*O. andersonii*	Okavango Delta /Botswana	Ecotoxic	2
		72	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	3
Spleen						
1	87.5-100	0-95.3	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		11	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
		-	*G. aculeatus*	East Fork Poplar Creek/ Southern England	Nuclear weapons facility	5
2	0-12.5	0-4.7	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
		33	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
		-	*G. aculeatus*	East Fork Poplar Creek /Southern England	Nuclear weapons facility	5

1= Naigaga (2012); 2= van Dyk et al. (2009); 3= Pieterse et al. (2010); 4=Uğurlu et al. (2022); 5=Teh (1997)

Table 6.6. Comparison of prevalence of reaction indices with previous research findings. Where, IC=index for circulatory disturbances; IR=index for regressive changes; IP=index for progressive changes; II =index for inflammation; and IT =index for tumor. Organ index (OI), and FHI=Fish histopathology index

Organ/ reaction pattern indices		**Prevalence (mean, range)**	**Previous research findings**				**Reference**
			Value	**Fish species**	**Environment/Country**	**Contamination type**	
Gill	*I*C	(20, 11.76-41.18)	21.5-31.5	*Knipowitschia caucasica*	River Tisza/Hungary	Municipal wastewater	1
			-	*Neogobius melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			0-15	*C. gariepinus*	Hartbeespoort/South Africa	Mining and agriculture	3
			0-70	*Esox lucius and S. glanis*	Tamis River/ Serbia	Waterborne pollutants	4
			26.67-73.33	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*R	(35, 16.42-65.69)	42	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			15.28	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			40	*C. gariepinus*	Hartbeespoort/South Africa	Mining and agriculture	3
			30-80	*E. lucius and S. glanis*	Tamis River/ Serbia	Waterborne pollutants	4
			80-100	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*P	(25, 0-52.94)	87	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			66.67	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			10-100	*E. lucius and S. glanis*	Tamis River/ Serbia	Waterborne pollutants	4
			26.67-100	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*I	(15, 0-43.75)	-	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			2.78	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			-	*E. lucius and S. glanis*	Tamis River/ Serbia	Waterborne pollutants	4
			-	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*T	(5, 0-25)	-	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			-	*E. lucius and S. glanis*	Tamis River/ Serbia	Waterborne pollutants	4
			-	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5

1= Yancheva et al. (2020); 2= Sigacheva and Gavruseva (2023); 3= Sikhakhane (2011); 4=Lujić et al. (2015); 5= HC et al. (2021)

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Organ/reaction pattern indices		Prevalence (mean, range)	Previous research findings				Reference
			Value	Fish species	Environment/Country	Contamination type	
	*I*C	(25, 12.5-43.75)	45 ± 3	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			13.89	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			0-45	*L. bocagei*	Vilariça Rivers Basins/Portugal	Agricultural watershed	3
			23.75	*Chalcalburnus tarichi*	Lake Van/Türkiye	Sewage treatment plants	4
			60-66.67	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*R	(32, 18.75-81.25)	67 ± 2.5	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			35.42	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			0-100	*L. bocagei*	Vilariça Rivers Basins/Portugal	Agricultural watershed	3
Liver			28.05	*C. tarichi*	Lake Van/Türkiye	Sewage treatment plants	4
			13.3-86.8	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*P	(10, 2.08-29.18)	26 ± 4.5	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			-	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			0-50	*L. bocagei*	Vilariça Rivers Basins/Portugal	Agricultural watershed	3
			22.5	*C. tarichi*	Lake Van/Türkiye	Sewage treatment plants	4
			13.33-100	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*I	(31, 20.83- 60.42)	44 ± 3.5	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			-	*N. melanostomus*	Apollonova Bay/Russia	Shipping effluent	2
			0-90	*L. bocagei*	Vilariça Rivers Basins/Portugal	Agricultural watershed	3
			18.75	*C. tarichi*	Lake Van/Türkiye	Sewage treatment plants	4
			13.3-93.3	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5
	*I*T	(2, 0-6.5)	-	*K. caucasica*	River Tisza/Hungary	Municipal wastewater	1
			-	*C. tarichi*	Lake Van/Türkiye	Sewage treatment plants	4
			-	*S. herzbergii*	Mangrove regions /Brazil	Urban effluent	5

1= Yancheva et al. (2020); 2=Sigacheva and Gavruseva (2023);3= Santos et al. (2022) ;4= Kaptaner et al. (2014); 5= HC et al. (2021)

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Organ/ reaction pattern indices		Prevalence (mean, range)	Previous research findings				Reference
			Value	Fish species	Environment/Country	Contamination type	
	*I*C	(16, 6.25-31.25)	-	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	1
			-	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	2
			-	*L. bocagei*	Vilariça River/Portugal	Agricultural runoff	3
			-	*O. niloticus*	Okavango Delta /Botswana	Unpolluted/ pristine	4
	*I*R	(60, 31.25-85.42)	17.3-64.2	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	1
			25-50	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	2
			-	*L. bocagei*	Vilariça River/Portugal	Agricultural runoff	3
			100	*O. niloticus*	Okavango Delta /Botswana	Unpolluted/ pristine	4
	*I*P	(4, 0-18.75)	16.0	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	1
			-	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	2
Gonads			22-27	*L. bocagei*	Vilariça River/Portugal	Agricultural runoff	3
			0-80	*O. niloticus*	Okavango Delta /Botswana	Unpolluted/ pristine	4
	*I*I	(16,0-20)	6.2	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	1
			-	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	2
			7-22	*L. bocagei*	Vilariça River/Portugal	Agricultural runoff	3
			0-50	*O. niloticus*	Okavango Delta /Botswana	Unpolluted/ pristine	4
	*I*T	(2,0-6.25)	3.2	*C. gariepinus*	Nature Reserve / South Africa	Urban effluent	1
			-	*C. gariepinus*	Roodeplaat Dam/South Africa	Hyper-eutrophic freshwater	2
			-	*L. bocagei*	Vilariça River/Portugal	Agricultural runoff	3
			-	*O. niloticus*	Okavango Delta /Botswana	Unpolluted/ pristine	4

1= Pieterse et al. (2010); 2= Marchand et al. (2012); 3= Santos et al. (2022);4= van Dyk et al. (2009)

Continued----

Organ/ reaction pattern indices		Prevalence (mean, range)	Previous research findings				Reference
			Value	Fish species	Environment/Country	Contamination type	
	*I*C	(14, 6.25-37.5)	27-49	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*Oreochromis* spp.	Northwest and Limpopo/South Africa	Aquaculture systems	2
			-	*O. niloticus*	Qalyubia/Egypt	Aquaculture systems	3
			20	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
	*I*R	(17, 0-34.06)	5-90	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*O. niloticus*	Qalyubia/Egypt	Aquaculture systems	3
			46.7	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
	*I*P	(50, 26.83-68.75)	6-96	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
Spleen			0.6-89.9	*Oreochromis* spp.	Northwest and Limpopo/South Africa	Aquaculture systems	2
			25	*O. niloticus*	Qalyubia/Egypt	Aquaculture systems	3
			20	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
	*I*I	(17, 3.13-34.38)	55-98	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*Oreochromis* spp.	Northwest and Limpopo/South Africa	Aquaculture systems	2
			-	*O. niloticus*	Qalyubia/Egypt	Aquaculture systems	3
			-	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4
	*I*T	(2, 0-12.5)	-	*O. niloticus*	Wetlands /Uganda	Urban effluent	1
			-	*Oreochromis* spp.	Northwest and Limpopo/South Africa	Aquaculture systems	2
			-	*O. niloticus*	Qalyubia/Egypt	Aquaculture systems	3
			-	*O. niloticus*	Dicle University/ Türkiye	Exposure to Diazinon	4

1= Naigaga (2012); 2= Taylor (2019); 3= Mohamed et al. (2023); 4= Uğurlu et al. (2022)

The organ and reactive index values reported in this version of the thesis are accurate and reflect methodological refinements made during the final analysis. These include the incorporation of additional lesion categories, particularly for gonadal and splenic tissues, and the application of a more rigorous scoring protocol based on updated interpretations of (Bernet et al., 1999) and (Zimmerli et al., 2007). Furthermore, each histopathological slide was evaluated using the average of three independent blind readings to minimize observer bias and enhance reproducibility. These improvements resulted in slightly elevated but more precise index values that better represent the severity of tissue alterations observed in *Labeobarbus* spp. across the study wetlands.

6.4.2. Spatial and seasonal differences in the histopathology of *Labeobarbus* spp. in the wetlands of Lake Tana

Although multiple environmental stressors can damage critical tissues and organs in aquatic environments, this study links the frequency and prevalence of lesions to water quality. Fish from AV, MRM, and GRM wetlands showed higher levels of histopathology index grades, severe reaction indices, and advanced lesion prevalence than fish from WO and ZG wetlands. The three wetlands had the highest percentage of fish with pathological grades 2 and 3. Based on the protocol suggested by Bernet et al. (1999), 'circulatory', 'progressive', 'regressive', and 'inflammatory' reaction patterns had the highest mean reaction indices in AV, GRM, and MRM. This suggests that the degree and extent of lesions or alterations under these indices were mostly severe. These four wetlands had the highest prevalence of circulatory disturbance, which included congestion, regressive necrosis, structural alterations, and inflammatory changes in the study organs. It is thus suggested that the water quality in these four wetlands is more degraded compared to WO and ZG.

Low histopathology indices and lesion prevalence were observed in WO and ZG. However, *Labeobarbus* spp. from WO, which is a wetland not directly affected by effluent discharge, also exhibited a significant degree of organ histopathology. This difference may be attributed to the location of the bay, which may be affected by adjacent Qat cultivation. The pesticide and herbicide impacts entering the lake through the bays should also be considered. Additionally, since this study used passive biomonitoring methods, fish migration could have played a role, as the location where the fish was sampled may not have been the same as the site where it had been living before sampling. WO shares the same gulf with AV, so fish in the bay may have been exposed to different water quality before reaching WO.

The study on fish histopathology indices and lesion prevalence and wetland conditions found that the level of environmental damage decreased in this order: AV, GRM, MRM, RA, WO, and ZG. These results match previous findings from chapters three, four, and five, which identified AV, MRM, and RA as the most degraded areas due to poor water quality (chapter three). The findings in MRM, GRM, and AV were also associated with stenotopic and eutrophic macroinvertebrate taxa of pollution-tolerant *Chironomidae* and *Oligochaeta* (Chapter Four). However, the findings in WO and RA (chapter four) are associated with pollution-sensitive macroinvertebrate taxa of *Caenidae*, *Libellulidae*, *Lestidae*, *Baetidae*, and *Corduliidae*. Therefore, it can be concluded that the effects of environmental degradation were observed at both the community level and the cellular level.

6.5. Conclusions and recommendations

In conclusion, the results of this chapter described the effects of the water quality and some environmental deterioration in AV, GRM, MRM, and RA on the histology of the liver, gills, gonads, and spleen of *Labeobarbus* spp. The study on fish histopathology found that GRM and MRM were heavily polluted, while RA, WO, and AV had moderate pollution, and ZG was the least polluted. This was confirmed by higher levels of fish histopathology indicators and more severe damage in the heavily polluted areas. Gill damage, such as atrophy, necrosis, infiltration of inflammatory cells, and epithelial hyperplasia, were significantly affected by the wetland environments. Wetlands significantly influenced liver damage, including hyperplasia of melano-macrophage centers and bile ducts, necrosis, deposits, and inflammation. For instance, AV, MRM, RA, and GRM had the highest rates of hyperplasia of melano-macrophage centers and bile ducts, while ZG and WO had the lowest. The prevalence of spleen damage, including cell infiltration, melano-macrophage center enlargement, lymphoid hyperplasia, and lymphoid hypertrophy, varied across the wetlands. Differences between wetlands significantly affected the occurrence of gonad damage, including structural changes, melano-macrophage center enlargement, necrosis, and atrophy. For instance, AV caused the highest rate of structural changes, while ZG had the lowest.

AV, ZG, MRM, RA, and GRM had the highest rates of melano-macrophage center enlargement, while MRM, RA, and GRM had the lowest. The variation of histopathological indices, lesion severity grades, and prevalence may be linked to relatively higher values of electrical conductivity, salinity, nitrite, pH, temperature, ammonia, soluble reactive phosphorous, total dissolved solids, and total-nitrogen total-phosphorous ratio on the histology of gills, liver, gonads, and spleen of *Labeobarbus* spp. in these wetlands as described in chapter three. *Labeobarbus* spp. in MRM, GRM, AV, and RA wetlands showed higher histopathological indices. Over 50% of the fish were classified under the two pathological grades. This indicates that either irritants in the wastewater effluents (e.g. toxicants, insoluble substances), or the higher values of physicochemical conditions due to the effluents as was observed in chapter three (e.g. electrical conductivity, salinity, nitrite, pH, temperature, ammonia, soluble reactive phosphorous, total dissolved solids, and total-nitrogen total-phosphorous ratio) had a detrimental impact. The percentage of various types of fish organ damage differed depending on the wetland and season. The most severe damage was seen in the MRM, AV, GRM, and RA regions during the dry, rainy, and late rainy seasons. The study suggests that both wetlands and seasons significantly impacted the water quality of Lake Tana. Therefore, monitoring the aquatic environment in Ethiopia should be strategically planned during the dry, rainy, and late rainy seasons to serve as an early warning system for water quality deterioration caused by pollution from nearby farms and cities in Lake Tana. Relating the results observed in this study to the pollution assessment literature, the histopathological lesions are prominent as potential biomarkers of water quality deterioration in Lake Tana wetlands in Ethiopia using *Labeobarbus* spp. as an indicator species. Based on the observed histopathological patterns, regressive lesions such as necrosis, atrophy, and degenerative changes are recommended as the most appropriate pathology type for routine aquatic ecosystem monitoring. These lesions were consistently observed in gill and liver tissues from highly impacted wetlands and showed strong alignment with physicochemical degradation. Regressive changes reflect irreversible or semi-reversible cellular damage caused by chronic pollutant exposure, making them reliable indicators of long-term ecological stress. While progressive and inflammatory responses were also present, their variability and potential reversibility reduced their diagnostic specificity. Therefore, regressive pathology offers the most robust and ecologically meaningful signal for biomonitoring in Lake Tana and similar freshwater systems.

CHAPTER 7

Fish Health Assessment Index biomonitoring tool for aquatic environmental quality in wetlands of Lake Tana, Ethiopia

7.1. Introduction

In aquatic ecosystems, fish are regarded as representative indicators of overall system health. Thus, fish and fish health assessments have been used as a comparative animal model to generate data about the status of water bodies in various environmental studies (Adams et al., 1993; Schleiger, 2004; Zimmerli et al., 2007; Maita, 2007; Pont et al., 2007; Palm, 2011; Watson et al., 2012; Jia and Chen, 2013; Marzin et al., 2014; Blazer et al., 2018; Achieng et al., 2021; Kang et al., 2022). Some of the commonly used approaches for qualitatively assessing fish health are age, growth analysis, the hepatosomatic index (HSI), and the condition factor (Adams et al.,1993; Heath et al., 2004; Watson et al., 2012; Mehinto et al., 2021; Cabral et al., 2022). Other quantitative methods include multiple tissue weights and blood chemistry (Del Rio-Zaragoza et al., 2021; Mangold-Doring et al., 2021; Wang et al., 2022). Each of these has advantages, depending on the objectives of the study, but most fish cannot be rapidly and inexpensively applied to field studies. A field necropsy method is a quick and inexpensive way to assess fish health and condition as developed by Adams et al. (1993). This approach provides a health profile of fish based on the percentage of anomalies observed in the tissues and organs of individuals sampled from a population.

A modification of this method was developed to quantify variables of fish health in their environment (Heath et al., 2004; Crafford & Avenant-Oldewage, 2009; Madanire-Moyo & Barson, 2010; Watson et al., 2012). This approach assigns numerical values to index variables based on the severity of environmental damage. Organs that are visually inspected include the eyes, skin, fins, opercula, gills, liver, spleen, hindgut, kidneys, and pseudobranchs. Other variables include the presence of parasites, as well as the values of hematocrit and plasma proteins. All values are equally weighted and range from zero (no effect) to 30 (severely affected) (Watson et al., 2012).

The fish health index (HAI) developed by Adams et al. (1993) for use in aquatic environments compares different measures of fish health (related to contaminants, bioindicators, and reproduction) to assess the health of a freshwater ecosystem. Parasites can serve as a marker for the deteriorating health of fish in the environment, and the presence or absence of parasites will be recorded in the original HAI. In addition, a method developed by Crafford & Avenant-Oldewage (2009), and Heath et al. (2004) recommended the use of fish ecto-and endoparasites as an indicator of environmental health as a refined HAI. According to Palm (2011) and Santoro et al. (2020), parasite communities can be used as effective indicators of environmental stress and biodiversity. Recent studies carried out in South Africa (Madanire-Moyo and Barson, 2010; Riddell et al., 2019; Kadiru et al., 2022; Ouma et al., 2022; Selwe et al., 2022) went a step further than Adams et al. (1993) and incorporated ecto-and endoparasites as separate variables in the HAI.

Crafford & Avenant-Oldewage (2009) used three parasite indices: the original parasite index by Adams et al. (1993), the inverted Parasite Index (IPI) by Marx (1996), and the refined parasite index by Madanire-Moyo & Barson (2010) and Mokonyane (2020). The ratio of ecto- to endoparasites is calculated by dividing the number of ectoparasite species by the number of endoparasite species. The IPI assumes that ectoparasites are more directly affected by water quality. So, we expect to find relatively more ectoparasite species in areas with good water quality. Because ectoparasite species are a sign of good water quality, IPI should have a lower score, as good water quality is associated with low HAI values.

This chapter aims to evaluate the Fish Health Assessment Index (HAI) as a measure of water quality in the wetlands of Lake Tana. Wetlands were assessed and compared using the HAI and the prevalence of abnormalities. Objective 4 focuses on the use of the HAI and parasite indices as bioindicators of water quality. External and internal anomalies, hematocrit levels, and parasite loads were examined across wetlands and seasons. The results highlight that fish health metrics are reliable biomarkers for detecting environmental stress and pollution in aquatic ecosystems.

7.2. Specific Methods

7.2.1. Field sampling

Fish were collected from six wetland ecotones along the shoreline of Lake Tana. *Labeobarbus* spp. was chosen as the genus. During each seasonal survey, fish were collected at each wetland by the use of gillnets of varied mesh sizes: multi-mesh monofilament gillnets with small mesh sizes (5, 8, 10, 15, and 19 mm bar mesh) and multifilament gillnets with large mesh sizes (25, 30, 38, 45 and 55 mm bar mesh) (Vijverberg et al., 2009). Fish were kept alive in the field by continuous water exchange of running water from a mobile tap unit in the field. The water used in the mobile tap was collected from the studied wetland.

In addition to health and parasite assessments, the sex of each *Labeobarbus* specimen was recorded during necropsy to determine male-to-female (M: F) ratios across wetlands and seasons. This sex ratio data was used to explore potential ecological or physiological stressors influencing population structure. Although not directly included in the Health Assessment Index (HAI), sex ratio patterns were considered in the interpretation of wetland conditions, particularly where skewed ratios may reflect environmental pressures, reproductive disruption, or sampling bias. The ratios were later compared across wetlands to assess whether effluent exposure or habitat quality influenced sex distribution.

7.2.2. Health Assessment Index (HAI) and Parasite Index

Fish were transferred from the boat's live well to a holding tank, after which the HAI examination was performed in the field. After parasites had been collected, fish were killed by severing the spinal cord, while covering the eyes of fish with a damp cloth.

Different types of skin abrasions were distinguished based on the cause of skin abrasion (Blazer et al., 2018). Abrasions caused by gill nets were characterized by visible bleeding and narrow, linear cuts or marks, especially on the head. These gill net-induced abrasions were not included in the overall health assessment calculation. Abrasions with unknown origins lacked these specific characteristics and were included in the calculation of HAI (Adams et al., 1993).

Fish were checked for mobile external parasites (body surface, gill cavity, and buccal cavity) as soon as fish were removed from the gill net. This was done to reduce the chance for external parasites to escape by detaching themselves from the skin or gill tissue of the fish. External parasites were removed using a brush, placed in sampling bottles containing lake water, and then recorded. Fish from which parasites were obtained were marked using numbered plastic tags. The fish were placed in separate holder tanks for further examination in the laboratory. Each fish was subsequently weighed measured and labeled. The total length (TL in mm) and weight (in g) of each fish were measured and recorded (Cabral et al., 2022) before the blood was collected. Then, blood samples were collected by caudal vein puncture using disposable sterile plastic syringes fitted with a needle. The blood sample was placed in microhematocrit capillary tubes and used for hematological analysis. The remaining blood was transferred to tubes coated with 3% ethylene diamine tetra-acetate (EDTA) in 0.7% NaCl, an anticoagulant agent (Ferri et al., 2022). The microhematocrit capillary tubes were filled to three-quarters with blood and plugged at one end with critoseal clay for hematological analysis. These blood samples were centrifuged in a hematocrit centrifuge for five minutes at 15,000 revolutions per minute at the parasitology laboratory of the School of Animal Science and Veterinary Medicine at Bahir Dar University. A hematocrit reader was used to determine the hematocrit value for each fish by expressing the volume of red and white blood cells as a percentage of the total plasma and RBC volume.

The gills were removed and inspected for external parasites using a dissecting microscope. For gill clip examination, the left operculum was removed, and a sample of primary lamellae tips with a size of less than 2–3 mm was amputated with fine scissors from the most lateral gill arch. These were chosen to potentially yield a higher parasite burden due to maximized exposure to water and the external environment. The lamellae tips were immediately placed onto a drop of lake water on the slide and covered with a coverslip. Parasite numbers were recorded, and parasites were collected, and preserved. Skin smears were obtained by firmly grasping the fish's head and scraping the skin on both sides with glass slides. The slides were examined for parasites using a stereomicroscope. Ectoparasites were identified based on the appearance of parasites, as described by Untergasser and Axelrod (1989) and Woo and Buchmann (2012). The prevalence of parasite infestation was calculated using the method described by Margolis et al. (1982).

The scoring system for the inverted parasite index (IPI) followed the methods used by Heath et al. (2004); Crafford & Avenant-Oldewage (2009); Watson et al. (2012). A detailed description of these methods is available in a user manual developed by Heath et al. (2004). The HAIs for sample populations were calculated by adding up all the individual fish health index values and dividing the total by the number of fish examined.

The internal organs and tissues (mesenteric fat, hindgut, kidney, liver, gall bladder, and spleen) were examined as described by Adams et al. (1993) and Heath et al. (2004). The fish were opened ventrally, and the body cavity and mesenteries were examined for metazoan parasites. Designated characteristics were assigned to the organs. Portions of the skin between the lateral line and dorsal fin of each fish were peeled off with forceps and fillets of muscle tissue were cut and examined for encysted parasitic forms. The liver, spleen, gall bladder, and kidneys of each fish were also examined for parasites or cysts. Photographs were taken to show abnormalities on external and internal body parts. The Inverted Parasite Index was used to quantify the number of external and internal parasites (Crafford & Avenant-Oldewage, 2009; Palm, 2011a; Watson et al., 2012).

Table 7. 1. Presents the fish health variables assessed during necropsy-based evaluations along with their normal conditions, observed deviations, and the corresponding numerical values used in the Health Assessment Index (HAI) adapted from Adams et al. (1993) and Heath et al. (2004). Each variable reflects a specific anatomical or physiological feature, such as eye condition, fin integrity, or organ anomalies. Deviations from the norm are categorized and assigned a severity score based on their potential impact on fish health. For example, exophthalmia, hemorrhaging, or blindness in the eyes are each assigned a value of 30, indicating a significant deviation. These values are then summed to generate an overall HAI score for each fish, allowing for comparative assessment across wetlands and seasons.

Variables	Variable condition	Original field designation	Substituted value for the HAI
Length	Total length (mm)	mm	-
Weight	Weight (g / fish)	g	-
Eyes	Normal	N	0
	Exophthalmia	E1/E2	30
	Hemorrhagic	H1/H2	30
	Blind	B1/B2	30
	Missing	M1/M2	30
	Other	OT	30
Fins	No active erosion, or previous erosion healed over	0	0
	Mild active erosion with no bleeding	1	10
	Severe active erosion and hemorrhage / secondary infection	2	20
Skin	Normal, no aberrations	0	0
	Mild skin aberrations	1	10
	Moderate skin aberrations	2	20
	Severe skin aberrations	3	30
Opercula	Normal/no shortening	0	0
	Mild/slight shortening	1	10
	Severe shortening	2	20
Gills	Normal	N	0
	Frayed	F	30
	Clubbed	C	30
	Marginate	M	30
	Pale	P	30
Pseudobranch	Normal	N	0
	Swollen	S	30
	Lithic	L	30
	Swollen and lithic	P	30
	Inflamed	I	30
	Other	OT	30
Thymus	No hemorrhage	0	0

Continued---

	Mild hemorrhage	1	10
	Moderate hemorrhage	2	20
	Severe hemorrhage	3	30
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30
Mesenteric fat	(Internal body fat expressed about amount present) None	0	-
	Little, where less than 50% of each caecum is covered	1	-
	50% of each caecum is covered	2	-
	More than 50% of each caecum is covered	3	-
	Caeca are completely covered by a large amount of fat	4	-
Spleen	Black	B	0
	Red	R	0
	Granular	G	0
	Nodular	NO	30
	Enlarged	E	30
	Other	OT	30
Hindgut	Normal, no inflammation or reddening	0	0
	Slight inflammation or reddening	1	10
	Moderate inflammation or reddening	2	20
	Severe inflammation or reddening	3	30
Kidney	Normal	N	0
	Swollen	S	30
	Mottled	M	30
	Granular	G	30
	Urolithic	U	30
	Other	OT	30
Liver	Red	A	0
	Light red	B	30
	"Fatty" liver, "coffee with cream" color	C	30
	Nodules in liver	D	30
	Focal discoloration	E	30
	General discoloration	F	30
	Other	OT	30
Bile	Yellow or straw color, bladder empty or partially full	0	-
	Yellow or straw color, bladder full, distended	1	-
	Light green to "grass" green	2	-
	Dark green to dark blue-green	3	-

Continued----

Blood (hematocrit)	Normal range	30-45%	0
	Above normal range	>45%	10
	Below normal range	19-29%	20
	Below normal range	<18%	30
Parasites	No observed parasites	0	0
	Few observed parasites	1	10
Endoparasites	No observed endoparasites	0	0
	Observed endoparasites < 100	0	10
	100–500	1	20
	>500	2	30
Ectoparasites	No observed ectoparasites	0	0
	Observed ectoparasites 1–10	1	10
	11–20	2	20
	> 20	3	30

7.2.3. Inverted Parasite Index (IPI)

The Inverted Parasite Index (IPI) was used to quantify the severity of parasite infections in fish by assigning numerical scores to observed ectoparasites and endoparasites. The scoring system was adapted from Crafford & Avenant-Oldewage (2009), Palm (2011a), and Watson et al. (2012). For ectoparasites, scores ranged from 0 (none observed) to 30 (more than 20 parasites). For endoparasites, scores ranged from 0 (none observed) to 30 (more than 1000 parasites). The IPI was calculated by summing the parasite scores and inverting the scale to reflect better health with lower parasite loads. This index was integrated into the overall Health Assessment Index (HAI) to evaluate fish condition across wetlands and seasons. As a result, both external and internal parasites were categorized based on the severity of parasites, as shown in Table 7.2.

Table 7.2. Numerical scaling system in the use with Inverted Parasite Index (IPI) adapted from Crafford & Avenant-Oldewage (2009), Palm (2011a), and Watson et al. (2012)

Ectoparasites	**PI**	**IPI**	**Endoparasites**	**PI**
Zero parasites observed	0	30	Zero parasites observed	0
1 – 10	10	20	< 100	10
11 – 20	20	10	101-1000	20
> 20	30	0	>1000	30

7.3. Results

7.3.1. *Labeobarbus* spp. attributes in the wetlands of Lake Tana

Table 7.3 shows the fish species found in different wetlands of Lake Tana. A total of 180 fish were collected, with *Labeobarbus* spp. being the most abundant, comprising 80.6% of the catch. *C. gariepinus* and *O. niloticus* were less common, accounting for 6.7% and 12.8% of the fish, respectively. Due to the low numbers of other fish species, only *Labeobarbus* spp. was used for further analysis, including health assessments, parasite studies, and growth rate calculations.

Using contingency analysis, the results of the statistical test were based on frequencies for sex M: F ratio (%: %) did not differ by combination of wetland and season (χ^2=1.61, df=23, p>0.05), among wetlands (χ^2=2.13, df=5, p>0.05), and seasons (χ^2=2.86, df=3, p>0.05) (Table 7.3). The M: F ratio was slightly higher in GRM, RA, and AV (≥ 40:60) while in WO, MRM, and ZG the M: F ratio was lower (≤40:60) (Figure 7.1). The adult male-to-female ratio (M: F) did not differ significantly across wetlands or seasons (χ^2=1.61, df=23, p>0.05) and therefore should not be presented as a key ecological metric in this study.

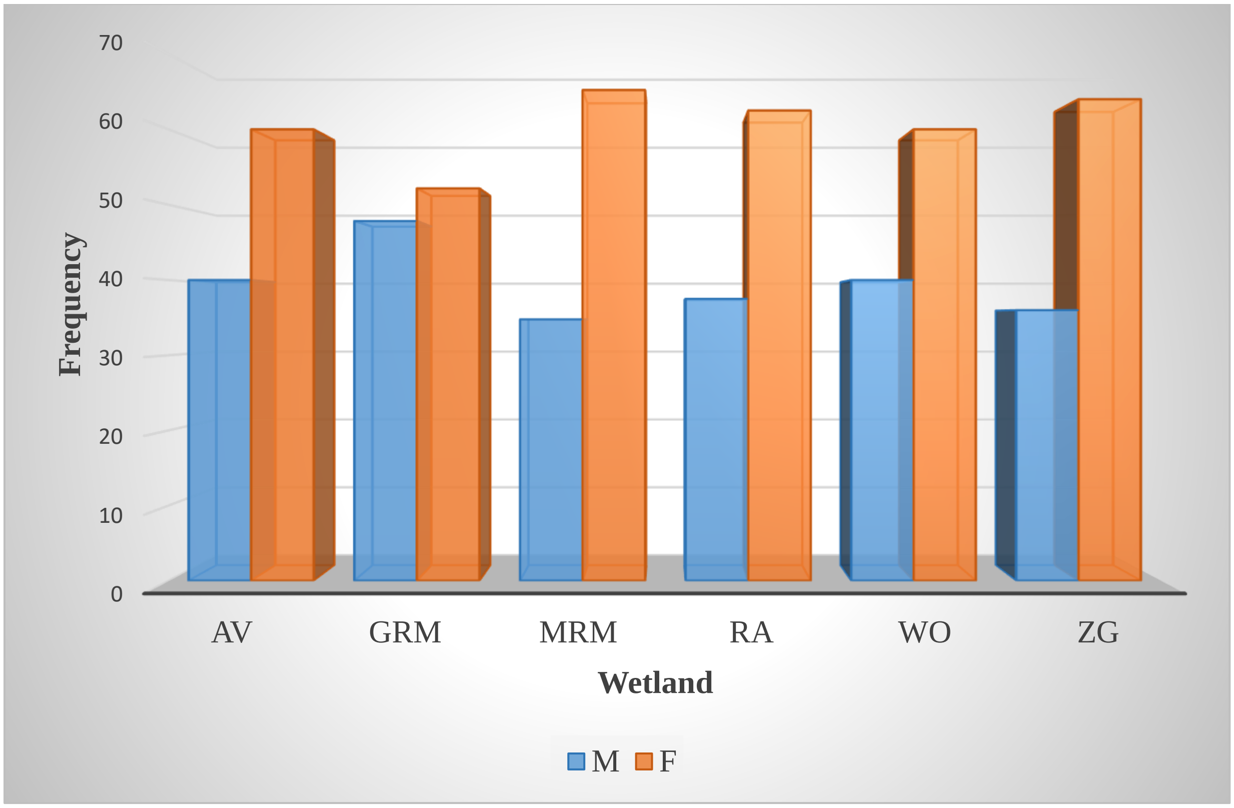


Figure 7. 1. Male-to-female ratio in *Labeobarbus* spp. in six wetlands of Lake Tana. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wongeta, ZG-Zewdie Girar

Table 7.3. Presents the total number of fish collected across wetlands and seasons, comprising *Labeobarbus* spp., *O. niloticus*, and *C. gariepinus*. While all species were recorded to ensure transparency in reporting catch composition, only *Labeobarbus* spp. were selected for health assessments, parasite analysis, and growth rate calculations. The inclusion of other species in the table reflects the overall catch diversity but does not imply their involvement in the Health Assessment Index (HAI) or related analyses. This distinction maintains clarity in the study's analytical focus while providing a comprehensive overview of the sampled fish community. AV-Avaj, RA-Ras Abbbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early Rainy, R-Rainy, LR-Late rainy season, M-male, F-female, T-total.

Season	**Genera or species**	**GRM**			**MRM**			**AV**			**RA**			**WO**			**ZG**			**Total**		
		M	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**	**M**	**F**	**T**
D	*Labeobarbus* spp.	3	4	7	2	3	5	1	2	3	4	1	5	5	9	14	3	2	5	18	21	39
	O. niloticus	0	2	2	0	0	0	5	2	7	3	2	5	1	1	2	0	0	0	9	7	16
	C. gariepinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ER	*Labeobarbus* spp.	1	4	5	2	3	5	1	4	5	2	4	6	1	2	3	1	4	5	8	21	29
	O. niloticus	0	0	0	1	0	1	1	2	3	0	0	0	0	0	0	0	0	0	2	2	4
	C. gariepinus	0	0	0	1	1	2	0	0	0	1	0	1	0	0	0	1	0	1	3	1	4
R	*Labeobarbus* spp.	5	1	6	2	4	6	4	3	7	2	6	8	4	4	8	2	6	8	19	24	43
	O. niloticus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	C. gariepinus	2	0	2	1	1	2	1	0	1	0	0	0	0	0	0	0	0	0	4	1	5
LR	*Labeobarbus* spp.	2	3	5	2	5	7	2	3	5	1	4	5	2	3	5	3	4	7	12	22	34
	O. niloticus	0	0	0	0	0	0	0	1	1	0	1	1	1	0	1	0	0	0	1	2	3
	C. gariepinus	0	0	0	0	0	0	1	0	1	0	1	1	0	0	1	0	0	0	1	2	3

7.3.2. Length-weight Relationship and Condition Factor of *Labeobarbus* spp. in the wetlands of Lake Tana

The body weight and length of *Labeobarbus* spp. in six wetlands of Lake Tana are presented in Figure 7.2.

Body weight

The overall median body weight was 68.21 ± 62.11 g. The median of the body weight of fish did not differ among wetlands; it ranged from 29.63 to 149.05 g (median: 64.39 ± 31.04) in MRM and from 34 to 190 g (median: 112.94 ± 53.31) in GRM (K-W ANOVA, $P > 0.05$) (Figure 7.2 a). However, the mean value of body weight differed among seasons, it ranged from 33.20 to 185.40 g (median: 54.26 ± 33.43) during the dry season and from 66.97 to 318.30 (median: 170.13 ± 60.87) during the late rainy season (K-W ANOVA, $P < 0.05$).

Body length

The overall median body length was 190 ± 41.66 mm. The median of body length of fish did not differ among wetlands, it ranged from 148.00 to 298.77 mm (median: 181.00 ± 41.06) in MRM and from 153.00 to 271.00 mm (median: 210.00 ± 34.41) in ZG (K-W ANOVA, $P > 0.05$, Figure 7.2 b). However, the median value of body length differed among seasons, it ranged from 139 to 271 mm (median:181.16 ± 31.76) during the dry season and from 165 to 365 mm (median: 239.86 ± 48.45) during the late rainy season (K-W ANOVA, $P < 0.05$).

The length-weight relationship (LWR)

The overall mean of b was 2.66. The median of b did not differ among wetlands, ranging from 1.00 to 1.71 (mean:1.245 ± 0.328) and from 3.23 to 4.74 (mean: 3.835 ± 0.642). Likewise, the median value of b did not differ across seasons, ranging from 0.80 to 4.74 (mean:1.87±1.44) during the late rainy season and from 1.03 to 4.24 (mean:3.04 ± 1.34) during the early rainy season.

Although b values ranged from 1.245 to 3.835 across wetlands, these differences were not statistically significant (K-W ANOVA, $p > 0.05$). Consequently, emphasis was placed on the interpretation of growth pattern types derived from the b values. Fish from AV and MRM exhibited negative allometric growth (b < 3), indicating relatively slower weight gain in relation to length, which may reflect suboptimal environmental conditions or limited food resources. In contrast, fish from RA and GRM displayed positive allometric growth (b > 3), suggesting better energy assimilation and more favorable environmental conditions. Meanwhile, fish from WO and ZG showed isometric growth (b ≈ 3), indicative of balanced growth patterns. These growth trends provide ecologically meaningful insights into habitat quality, despite the absence of statistically significant differences in b values across wetlands. The group and growth pattern of each fish are summarised in Table 7.5.

Table 7.4. Log-transformed values on the estimated parameters of the length-weight relationship of *Labeobarbus* spp. in wetlands of Lake Tana. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, a-intercept of the regression line, b- the slope of the regression line, CI -Confidence Level, R^2- Regression Coefficient

Wetland	**a**	**95% CI of a**	**b**	**95% CI of b**	**R^2**
AV	0.720	0.428- 1.012	1.245	0.071-2.419	0.905
GRM	0.088	0.205- 0.379	3.835	2.661-5.009	0.972
MRM	0.351	0.059- 0.644	2.767	1.593-3.942	0.880
RA	0.233	0.058- 0.526	3.055	1.881-4.229	0.957
WO	0.353	0.061- 0.646	2.542	1.368-3.717	0.900
ZG	0.306	0.013- 0.598	2.540	1.366-3.714	0.810

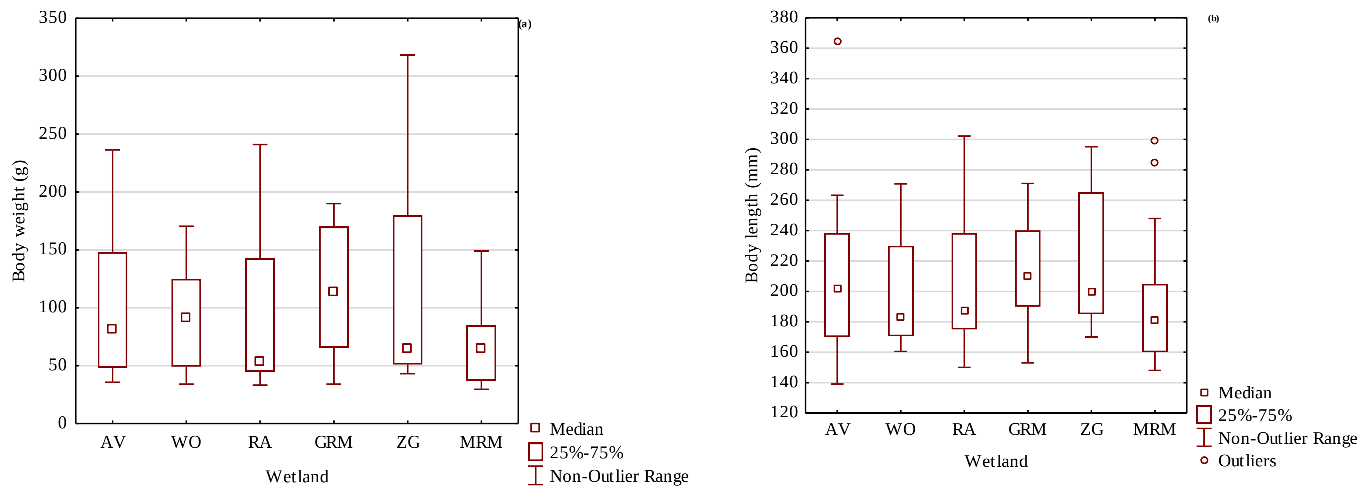


Figure 7. 2. Box plot of body weight (a) and body length (b) of *Labeobarbus* spp. grouped by the wetland of Lake Tana. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar

Table 7.5. Fish grouping in six wetlands of Lake Tana based on the log-transformed value of b, the slope of the linear regression. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, NA- Negative Allometric (b<3), IS-Isometric (b=3), PA-Positive Allometric (b >3)

Wetland	**Group**	**b, the slope of the regression**	**Growth pattern**	**W=aL^{b}**
AV	Light	1.245	NA	$0.720L^{1.245}$
GRM	Heavy	3.835	PA	$0.088L^{3.835}$
MRM	Light	2.767	NA	$0.351L^{2.767}$
RA	Isometric	3.055	IS	$0.233L^{3.055}$
WO	Light	2.542	NA	$0.353L^{2.542}$
ZG	Light	2.540	NA	$0.306L^{2.540}$

7.3.3. Health assessment index values in the wetlands of Lake Tana

Health Assessment Indices (HAI)

The median of HAI differed among wetlands; it ranged from 30.0 to 84.0 (median: 31.0 ± 13.96) in ZG and from 30.0 to 161.0 (median: 90 ±38.09) in MRM (K-W, ANOVA, $P < 0.05$). Multiple comparisons mean ranks for all groups indicated the highest HAI value recorded in MRM, GRM, and RA, moderate HAI value in AV, and the lowest HAI value in WO and ZG (Figure 7.3a). Similarly, the median value of HAI differed among seasons, it ranged from 30.0 to 104.0 (median: 34.0 ± 18.79) during the rainy season and from 30.0 to 161.0 (median: 63.0 ± 40.36) during the dry season (K-W ANOVA, $P < 0.05$).

To support the interpretation of parasite metrics, representative photographs of ectoparasites and endoparasites observed in *Labeobarbus* spp. are presented in Plate 7.1. These images illustrate the typical external and internal parasite forms encountered during necropsy, including gill-attached copepods, skin flukes, and intestinal helminths.

Inverted endoparasite index

The value of the inverted endoparasite index did not differ among wetlands, it ranged from non-detectable to 10.0 in GRM, WO, ZG, and RA and from non-detectable to 20.0 in AV and MRM (K-W ANOVA, $p > 0.05$). Similarly, the value of the inverted endoparasite index did not differ among seasons, it ranged from non-detectable to 10.0 during the dry season and was non-detectable during early rainy, rainy, and late rainy seasons (Figure 7.3b).

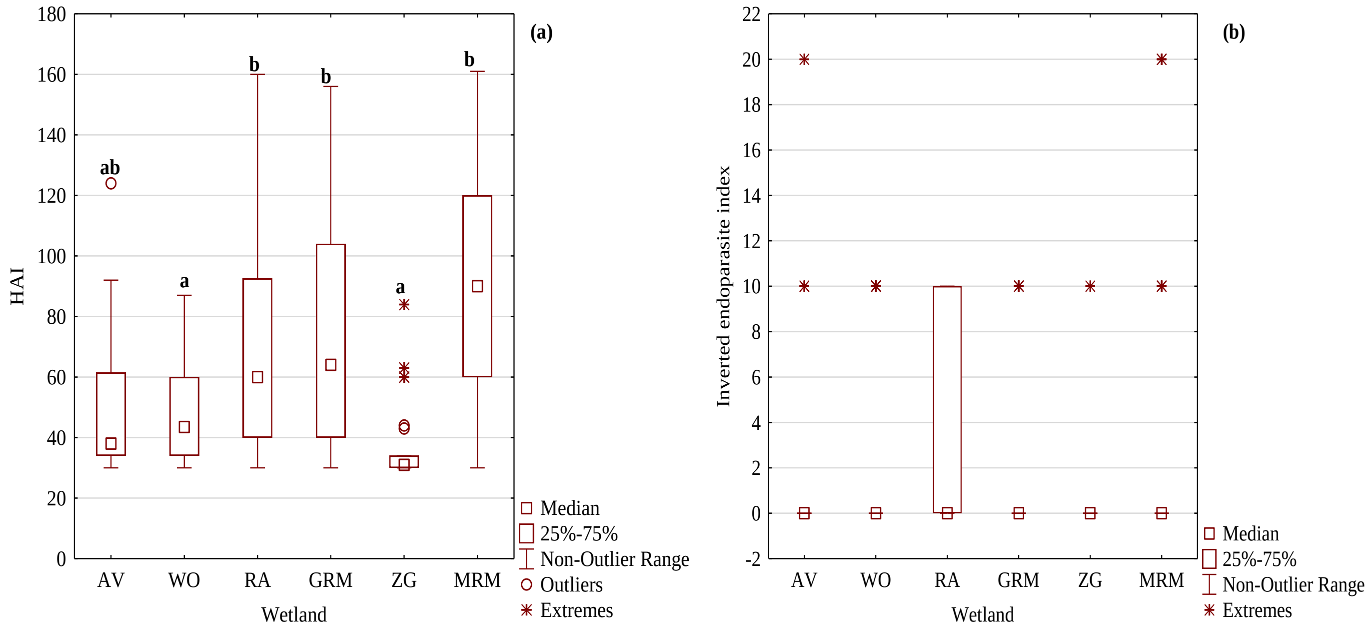
Inverted ectoparasite index

The value of the inverted ectoparasite index did not differ among wetlands, it ranged from non-detectable to 30.0 (median: 30.0) in AV, WO, RA, and GRM and from 10.0 to 30.0 (median 30) in GRM and ZG (Figure 7.3c). Similarly, the season did not affect the value of the inverted ectoparasite index.

7.3.4. Prevalence of anomalies of fish in the wetlands of Lake Tana

Hematocrit value

The proportion of abnormal hematocrit values did not differ among wetlands, these differences were not statistically significant ($\chi2=2.04$ df=5, $p > 0.05$). Similarly, hematocrit values did not differ across seasons ($\chi2=4.39$, df=3, $p > 0.05$). Abnormal hematocrit values were rarely observed, with the highest prevalence found at WO with a value of 6.7%. In contrast, these abnormalities were absent in wetlands AV, MRM, and ZG. Similarly, seasonal variations in hematocrit levels were minimal, with the highest occurrence during the dry season.



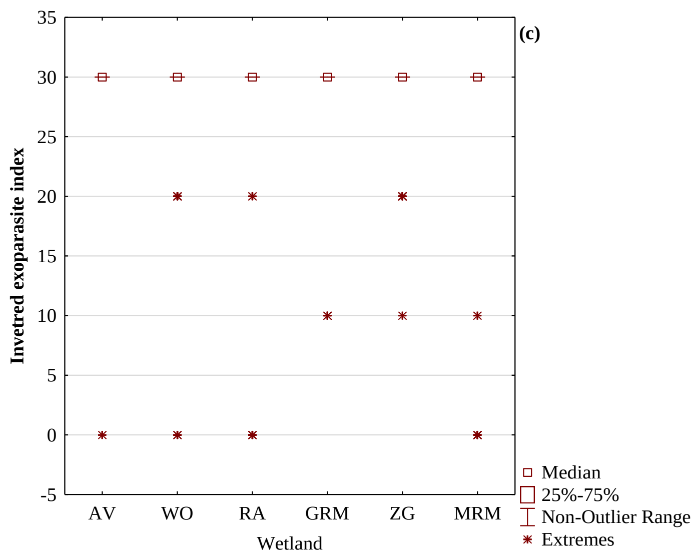


Figure 7.3. Box plot of (a) fish health assessment index (HAI) (b) inverted endoparasite index and (c) inverted ectoparasite index of *Labeobarbus* spp. grouped by the wetland of Lake Tana. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar

External organs

Eye

The prevalence of ocular abnormalities did not vary significantly across wetlands ($\chi^2 = 10.44$, df = 5, $p > 0.05$) and seasons ($\chi^2 = 4.11$, df = 3, $p > 0.05$). Therefore, no qualitative comparisons are made between wetlands or seasons, as the observed differences were not statistically significant. On the other hand, the prevalence of blindness varied significantly among the wetlands ($\chi^2 = 15.09$, df = 5, $p < 0.05$), with AV showing no cases and RA exhibiting the highest rate at 29.2%. The prevalence of blindness was not influenced by season ($\chi^2 = 4.22$, df = 3, $p > 0.05$). Hemorrhage and yellowish eyes were significantly more common in MRM and GRM compared to ZG ($\chi^2 = 18.74$, df = 5, $p < 0.05$), with ZG reporting no cases and both MRM and GRM having a 34.8% prevalence. However, the occurrence of these conditions did not vary across seasons ($\chi^2 = 3.69$, df = 3, $p > 0.05$). The prevalence of exophthalmia varied significantly among wetlands ($\chi^2 = 8.94$, df = 5, $p < 0.05$), with values ranging from 8.35% in RA to non-detectable in other wetlands. In contrast, the prevalence of exophthalmia did not differ across seasons ($\chi^2 = 1.09$, df = 3, $p > 0.05$). The prevalence of missing eyes varied among wetlands (χ^2=11.12, df = 5, $p < 0.05$), ranging from non-detectable to 8.7% in GRM, but did not differ across seasons ($\chi^2 = 1.09$, df = 3, $p > 0.05$) (Figure 7.7, Plate A).

Skin

The prevalence of overall skin abrasions in the present study was 16.55%. The prevalence of mild abrasion did not differ among wetlands ($\chi2$=10.51, df =5, $p > 0.05$). However, the prevalence of mild skin abrasion differed among seasons ($\chi2$=8.36, df =3, $p <0.05$) ranging from non-detectable during the rainy season to 23.53% during the late rainy season. The prevalence of moderate skin abrasion did not differ ($\chi2$=5.15, df =5, $p > 0.05$) among wetlands and seasons (0.89, df =3, $p > 0.05$) (Figure 7.7 Plate B).

Fins

The prevalence of mild and severe active erosion of fins in the present study was 8.27%. The prevalence of mild and severe active erosion did not differ among wetlands ($\chi2$=4.01, df =5, $p > 0.05$) and seasons ($\chi2$=2.88, df =3, $p > 0.05$). The prevalence of mild active erosion with no bleeding did not differ among wetlands ($\chi2$=5.56, df =5, p > 0.05) and seasons ($\chi2$=2.83, df =3, p > 0.05) (Figure 7.4 d). Similarly, the prevalence of severe active erosion with bleeding did not differ among wetlands ($\chi2$=5.21, df =5, p > 0.05) and season ($\chi2$=1.23, df =3, p > 0.05).

Opercula

The prevalence of slight/mild shortening of opercula in the present study was 0.07%. The prevalence of slight/mild shortening of opercula did not differ among wetlands ($\chi2$=5.05, df =5, p > 0.05) (Figure 7.4e), and among seasons ($\chi2$=0.77, df =3, p > 0.05).

Gills

The prevalence of clubbed /pale lesions on the gill in the present study was 16.55%. The prevalence of clubbed /pale lesions differed among wetlands ($\chi2$=24.69, df =5, p < 0.05) ranging from non-detectable in WO to 52.2% in MRM (Figure 7.4 f). Similarly, the prevalence of clubbed/ pale lesions differed ($\chi2$=12.73, df =3, p < 0.05) among seasons ranging from non-detectable during the rainy season to 28.20% during the dry seasons.

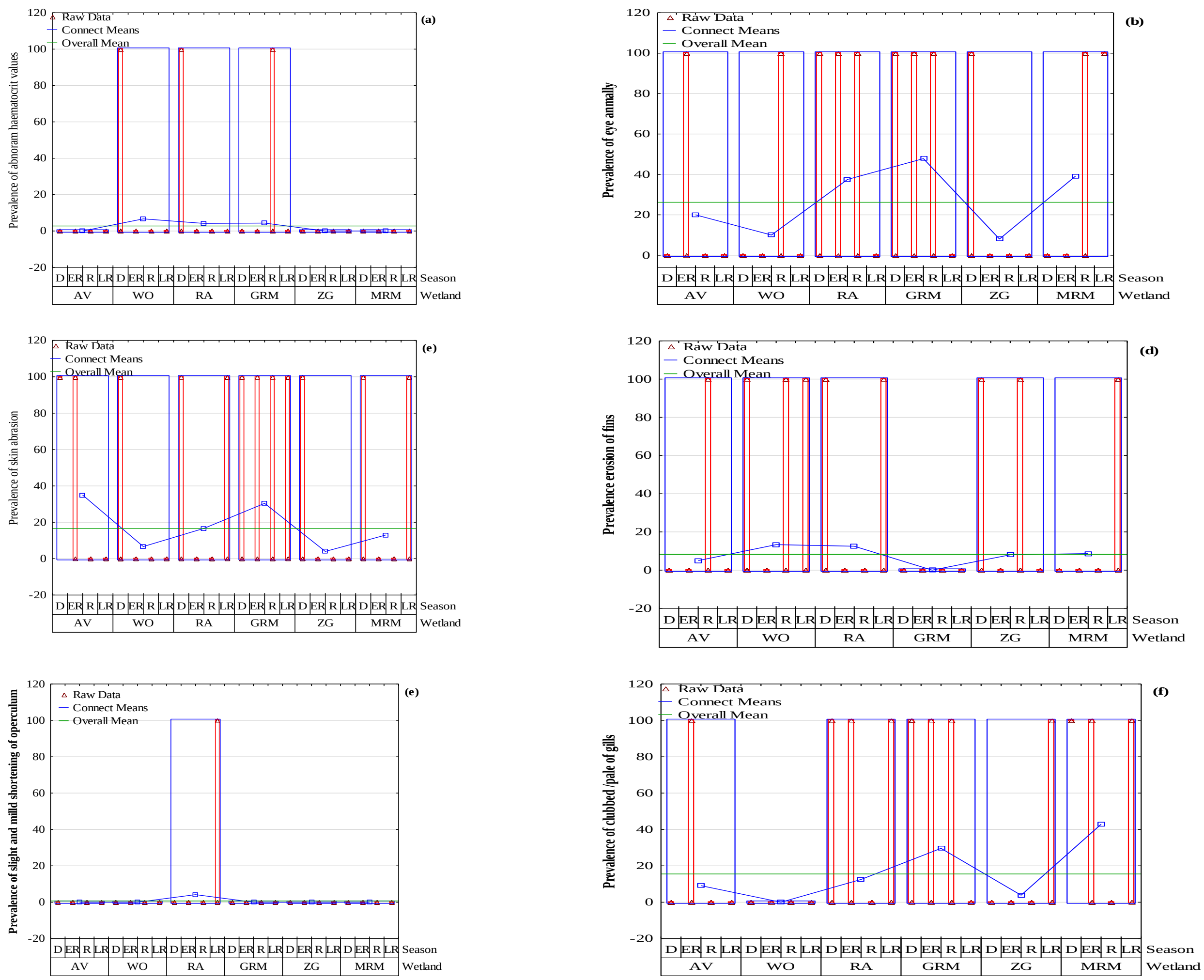
Pseudobranch

In this study, 4.83% of the fish had abnormal pseudobranchs. The prevalence of inflamed and swollen pseudobranchs did not differ among seasons ($\chi2$=0.77, df =3, p > 0.05). However, the prevalence of inflamed and swollen pseudobranchs differed ($\chi2$=18.21, df =5, p < 0.05) among wetlands ranging from non-detectable AV, GRM, WO, and ZG to 21.74% in MRM (Figure 7.4 g).

Internal organs

Thymus

The study found that 2.76% of the fish exhibited mild or moderate hemorrhagic thymus. The prevalence of mild and moderate hemorrhage differed ($\chi2$=21.32, df =5, p <0.05) among wetlands, ranging from non-detectable in AV, MRM, RA, WO, and ZG to 17.39% in GRM. Moreover, the prevalence of mild and moderate hemorrhagic thymus was influenced by seasonal variations ($\chi2$=21.44, df =3, p <0.05) (Figure 7.5a).



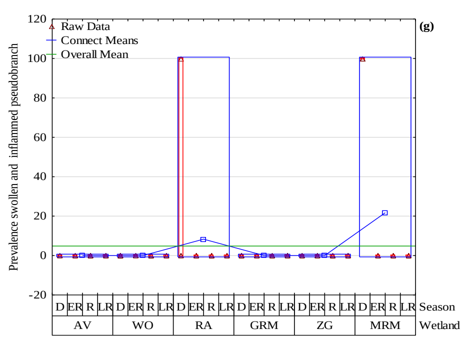


Figure 7.4. Variability plot of prevalence of fish hematocrit value and external organ anomalies in wetlands of Lake Tana. (a) Abnormal hematocrit value (b) Eye anomaly (c) Skin abrasion (d) Erosion of fins (e) Slight and mild shortening of opercula (f) Clubbed gills (g) Swollen and inflamed pseudobranch. AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, and LR-Late rainy season

Mesenteric fat

The overall prevalence of mesenteric fat anomaly was 45.2%. The prevalence of aberrant mesenteric fat accumulation varied with the season ($\chi2=13.31$, df =3, $p < 0.05$), ranging from 17.64% in the late rainy season to 74.36% in the dry season (Figure 7.5b). The maximum and minimum prevalence of less than 50% of caeca-covered mesenteric fat was recorded in AV and MRM, respectively. The maximum prevalence of 50% of the ceca covered by mesenteric fat was found in AV while the minimum and maximum were recorded in WO and ZG. ZG had the highest prevalence of caeca with over 50% coverage by mesenteric fat, whereas MRM and RA had the lowest prevalence. Similarly, the maximum and minimum prevalence of caeca completely covered by mesenteric fat was recorded in GRM and MRM, respectively (Figure 7.5c).

Spleen

The overall prevalence of spleen anomaly in the present study was 0.07%. The prevalence of splenomegaly did not differ among wetlands ($\chi2=5.05$, df =5, $p >0.05$) (Figure 7.5d) and among seasons ($\chi2=2.50$, df =3, $p >0.05$).

Hindgut

The overall prevalence of hindgut anomaly in the present study was 3.45%. The prevalence of slight or mild inflammation (reddening) of the hindgut did not differ among wetlands ($\chi2=5.25$, df =5, $p >0.05$) (Figure 7.5e) and season ($\chi2=6.96$, df =3, $p >0.05$).

Kidneys

There were no obvious kidney gross lesions detected in *Labaeobarbus* spp. in this study.

Liver

The overall prevalence of liver anomaly in the present study was 16.6%. The prevalence of general or focal discoloration of the liver differed among wetlands ranging from non-detectable in ZG to 39.13% in MRM ($\chi2=4.13$, df =5, $P< 0.05$). Similarly, the prevalence of general or focal discoloration of the liver differed among seasons ranging from 2.32 % during the rainy season to 35.89% during the dry season ($\chi2=18.40$, df =3, $P<0.05$) (Figure 7.7 Plate C).

Bile

The prevalence of light green or dark green bile did not differ among wetlands ($\chi2=5.77$, df =5, p >0.05) (Figure 7.5g). However, the prevalence of light green or dark green bile differed among seasons ranging from non-detectable during early rainy, rainy, and late rainy seasons to 17.9% during the dry season ($\chi2=18.27$, df =3, $p < 0.05$).

7.3.5. Prevalence of endo/ ectoparasites of fish in the wetlands of Lake Tana

Endoparasites

The study found that 2.2% of fish had endoparasites. There was no significant difference in the prevalence of endoparasites between different wetlands ($\chi2= 3.44$, df =5, p >0.05). However, the prevalence varied between seasons ($\chi2=12.22$, df =3, $p < 0.05$), with the highest rate in the dry season and the lowest in the late rainy season (Figure 7.6a).

The prevalence of *Contracaecum* spp. did not differ among wetlands ranging from non-detectable in GRM, WO, and ZG to 8.33% in RA ($\chi2=4.67$, df=5, p>0.05) (Figure 7.7 Plate D). Similarly, the prevalence of *Contracaecum* spp. did not differ among seasons ranging from non-detectable during the rainy season to 6.89% during the early rainy season ($\chi2=4.67$, df =3, $p > 0.05$).

The prevalence of fish with *L. intestinalis* did not differ among wetlands ($\chi2= 6.04$, df =5, p >0.05) (Figure 7.7 Plate E). However, the prevalence of *L. intestinalis* differed across seasons ($\chi2=12.67$, df =3, $p < 0.05$). The highest rate was found in the dry season and the late rainy season had the lowest.

Ectoparasites

Approximately 19.3% of the fish had external parasites. The prevalence of ectoparasites varied among wetlands, with AV having the lowest prevalence at 5% and WO having the highest at 43.33% ($\chi2= 6.05$, df = 5, p <0.05). In contrast, the prevalence of ectoparasites did not differ across seasons, which ranged from 5.71% in the late rainy season to 30.8% in the dry season ($\chi2= 7.27$, df =3, p >0.05) (Figure 7.7 Plate F).

Ectoparasite-endoparasite ratio

The prevalence of ectoparasite-endoparasite ratio did not differ among wetlands ($\chi2$=3.95, df =5, p >0.05) and season ($\chi2$=2.59, df =3, p >0.05) (Figure 7.7 Plate C).

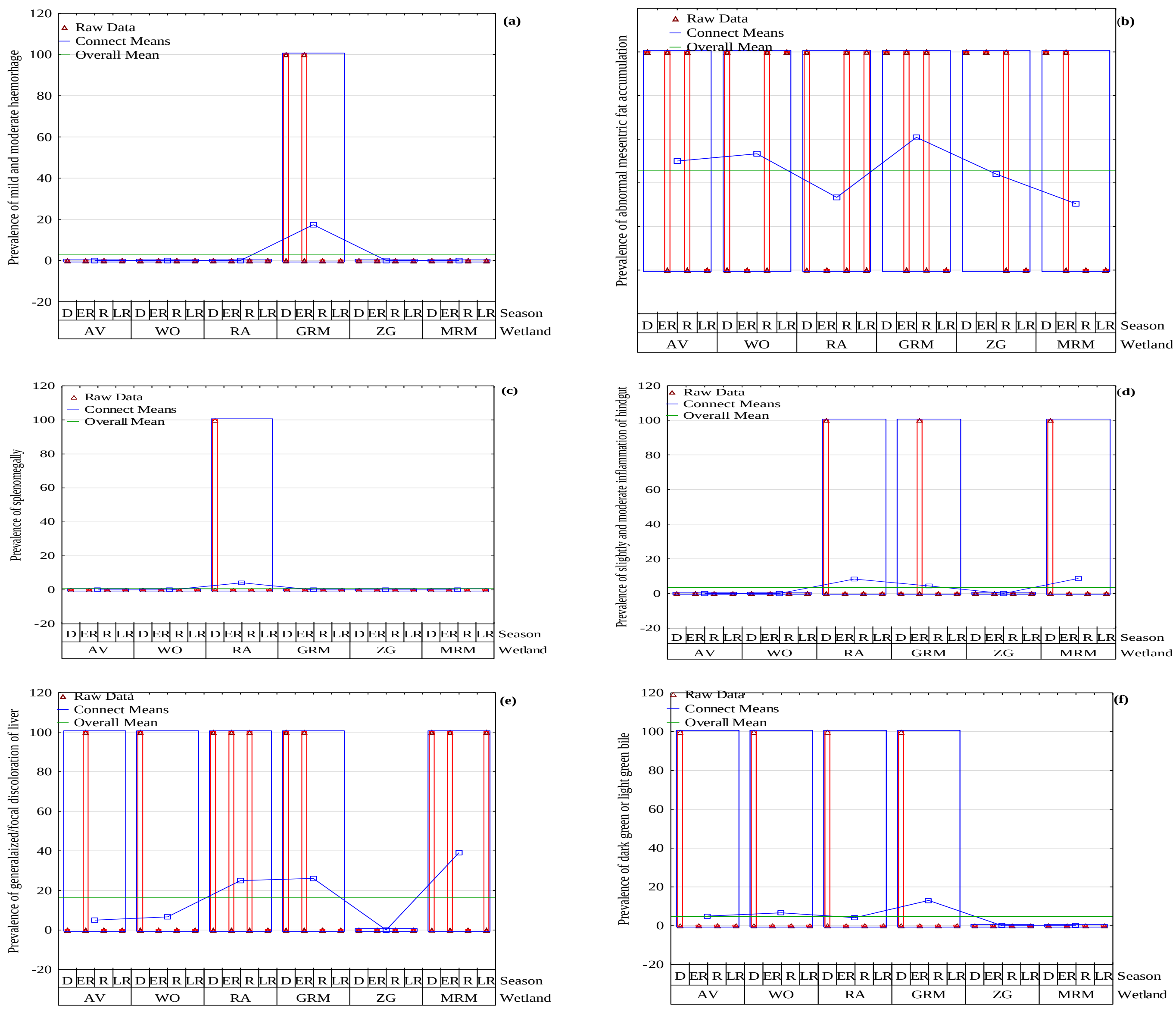
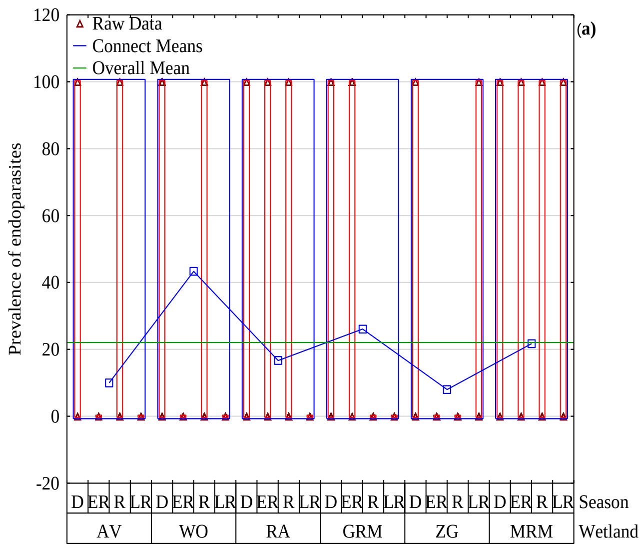
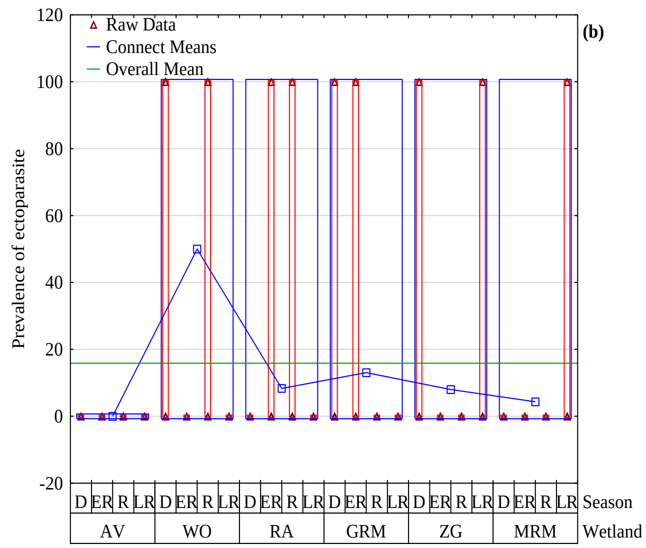


Figure 7.5. Variability plot of prevalence of fish internal organ anomalies by wetland and season in Lake Tana. (a) Mild and moderate hemorrhagic thymus (b) Abnormal mesenteric fat accumulation (c) Splenomegaly (d) Slight and moderate inflammation of hindgut (e) Generalized/focal discoloration of the liver (f) Dark green or light green bile. AV-Avaj,

GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, and LR-Late rainy season





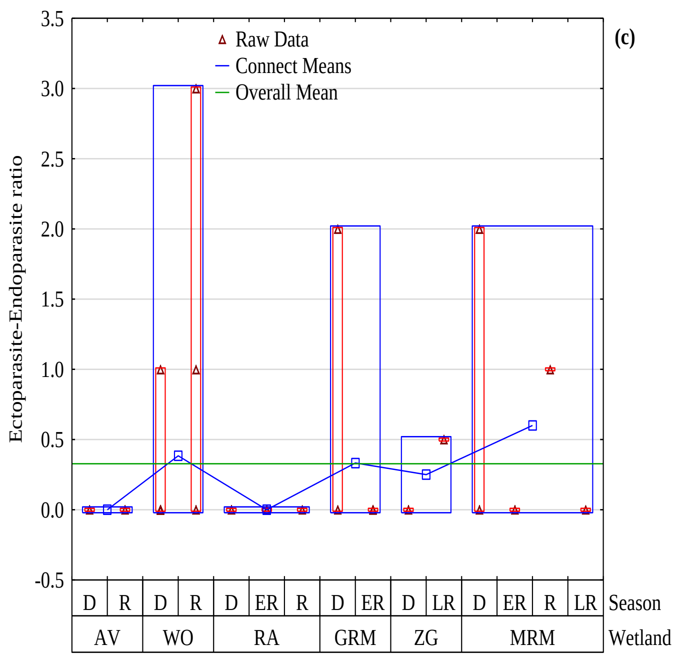


Figure 7.6. Variability plot of prevalence of fish endoparasite (a) Ectoparasite (b) Ectoparasite-endoparasite ratio (c) by wetland and season in Lake Tana, Ethiopia, AV-Avaj, GRM-Gumara River Mouth, MRM-Megech River Mouth, RA-Ras Abbay, WO-Wonjeta, ZG-Zewdie Girar, D-Dry, ER-Early rainy, R-rainy, and LR-Late rainy season

The prevalence of *Argulus* spp. infestation did not differ among wetlands ranging from non-detectable in AV and MRM to 40% in WO ranging from non-detectable in MRM and AV to 40% in WO ($\chi 2$= 7.58, df = 5, p >0.05). However, the prevalence of *Argulus* spp. infestation differed among seasons ranging from non-detectable during the late rainy season to 25.64% during the dry season ($\chi 2=9.11$, df =3, $p < 0.05$).

The prevalence of *Dactylogyrus* spp. infestation did not differ among wetlands ranging from non-detectable in AV, GRM, MRM, RA, and ZG to 10% in WO ($\chi 2= 3.0$, df = 5, $p >0.05$). Similarly, the prevalence of *Dactylogyrus* spp. infestation did not differ among seasons ranging from non-detectable during the early rainy and late rainy seasons to 5.12% during the dry season ($\chi 2=2.43$, df =3, $p > 0.05$).

The prevalence of *Gyrodactylus* spp. infestation did not differ among wetlands ranging from non-detectable in AV, RA, WO, and ZG to 4.34% in MRM ($\chi 2= 4.39$, df = 5, $p >0.05$). Similarly, the prevalence of *Gyrodactylus* spp. infestation did not differ among seasons ranging from non-detectable during dry, early rainy, and rainy seasons to 5.88% during the late rainy season ($\chi 2=1.55$, df =3, $p > 0.05$).

The prevalence of leech infestation did not differ among wetlands ranging from non-detectable in AV, MRM, WO, and ZG to 4.35% in GRM ($\chi 2= 4.39.$, df = 5, $p >0.05$). Similarly, the prevalence of leech infestation did not differ among seasons ranging from non-detectable during the dry and late rainy seasons to 3.45% in the early rainy season.

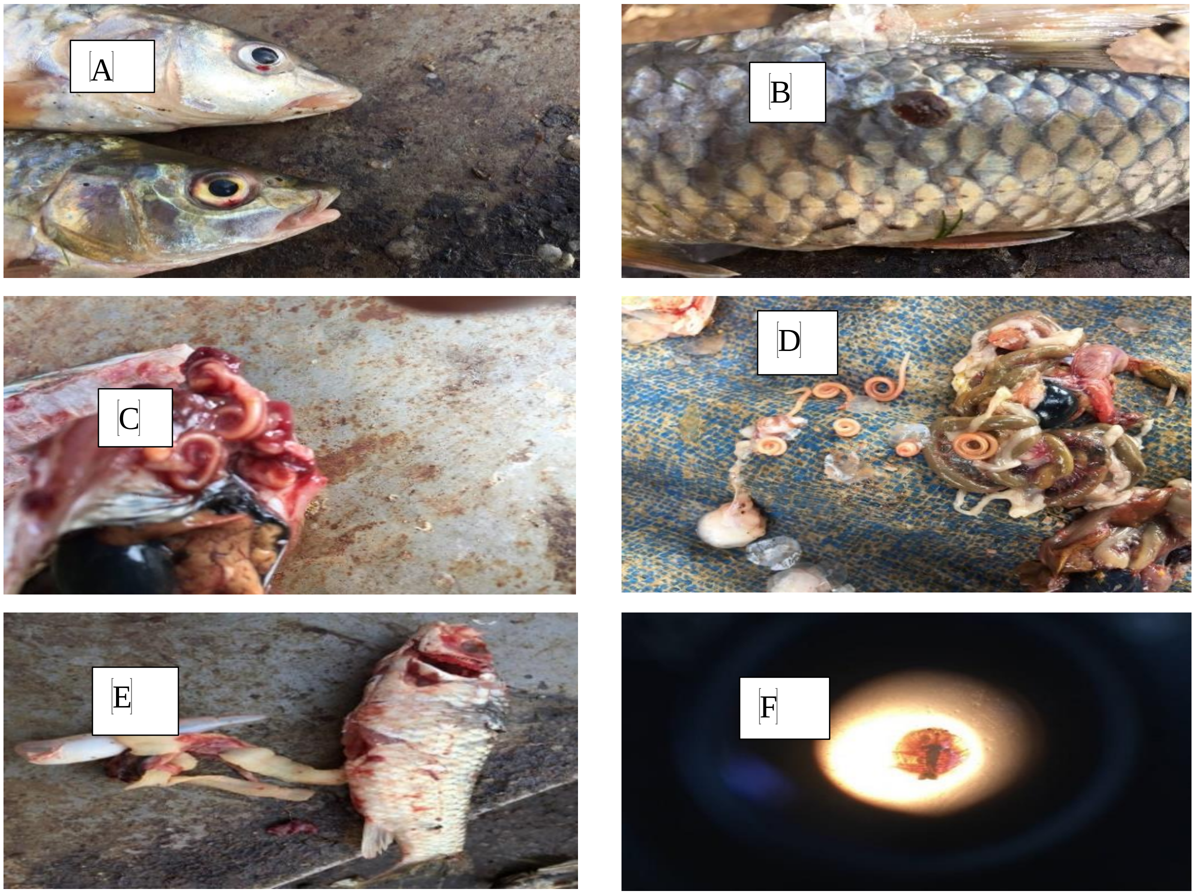


Plate 7. 1. Anomalies of *Labeobarbus* spp. in wetlands of Lake Tana, Ethiopia, (A) Exophthalmia with hemorrhage (above) and yellowish discoloration of the eye (below) (B) Severe skin abrasion (C) General 'discoloration' of the liver (D) Nematode parasites, *Contracaecum* spp. (E). *Ligula intestinalis* (F) Ectoparasite (*Argulus* spp.)

7.4. Discussion

7.4.1. Fish attributes in wetlands of Lake Tana

The most abundant genus was *Labeobarbus* (80.6%), while *O. niloticus* and *C. gariepinus* accounted for 12.8% and 6.7%, respectively. This finding is in line with previous reports (Nagelkerke et al., 1994; de Graaf et al., 2008; Anteneh et al., 2012a; Gebremedhi et al., 2013; Dejen et al., 2017), in which it was noted that the largest fish family in the lake is *Cyprinidae*, represented by three genera, *Garra*, *Varicorhinus*, and *Barbus*. Several reports state that

Labeobarbus spp. is the most dominant genus of the Cyprinidae in Lake Tana, comprising 18 species (Mequanent et al., 2022). Cyprinid fish species dominate Lake Tana, making up nearly 90% of the total fish population. Other fish families are much less diverse, with only one genus and species each (Mengistu et al., 2017).

The adult male-to-female ratio (M: F) did not differ across the wetlands of Lake Tana. In wetlands GRM, AV, and RA, the ratio was slightly higher towards males. However, this is contrary to previous findings for Lake Tana, which reported a female-biased sex ratio due to higher male mortality and longer female lifespans (Arendt et al., 2014; Mequanent et al., 2022). These prior studies also suggested minimal offspring sex ratios.

The current study suggests a different scenario in some wetlands. Wetlands WO, MRM, and ZG showed a lower M: F ratio, favoring females. This could be related to migration patterns. Several reports indicate that a significant portion of Lake Tana fish migrate to the river mouth for spawning (de Graaf et al., 2008; Anteneh et al., 2012a; Anteneh et al., 2017; Shkil et al., 2017; Zelalem et al., 2022). The M: F ratio observed in some wetlands might be influenced by the timing of the study relative to these migrations. Further investigations are needed to understand the reasons behind the observed M: F ratio variations across wetlands and reconcile these findings with previous reports on sex ratio patterns in Lake Tana. Several studies have found that female fish prefer larger food (Homski et al., 1994; Bence & Murdoch, 1986), feed more frequently (Reddy, 1975), and spend more time foraging when other females are around (Pilastro et al., 2003; Arrington et al., 2009).

7.4.2. The length-weight relationship

The fish in this study varied from juveniles to adults. Isa et al. (2010) and Sekitar et al. (2015) indicated that all age groups can be used to determine the length-weight relationship. The LWR from six wetlands conformed to b values of 1.245 to 3.835. However, these values did not vary significantly among wetlands. The overall state of wetlands is reflected in high b values (Pervin and Mortuza 2008; Sekitar et al. 2015). The weight of fish increases when fish utilize the food items that are available for growth and energy (Offem et al., 2007; Kamaruddin et al., 2011). The values may additionally range substantially in line with different elements providing sex, and gonad development (Hossain et al., 2006; Leunda et al., 2006; Pervin and Mortuza, 2008; Sekitar et al., 2015). Furthermore, b values depend on geographical, temporal, and sampling characteristics as well as biological and ecological characteristics (Bagenal & Tesch, 1978; Froese, 2006).

The higher b value in RA and GRM showed that it provided a more favorable environment for *Labebobarbus* spp. as compared to AV, MRM, WO, and ZG. The result for WLR may indicate that AV and MRM might be a relatively less healthy environment for *Labeobarbus* spp. This might be associated with higher values of SRP, TDS, pH, temperature, and ammonia in AV from the hospital effluent discharged from the nearby hospital. Similarly, less favorable conditions of MRM might be correlated higher with higher values of electrical conductivity, salinity, and nitrite from agricultural and urban effluents discharged into the lake. Several reports indicated that sedimentation had an effect on badly managed logging activities that may affect water quality (Kaba et al., 2014; Abate et al., 2015a).

The differences in LWR between some wetlands could be due to could be due to the availability of food at a particular time as well as the difference in gonad development or a combination of factors that require further investigation. Since the LWR has a significant correlation, the exponent b plays a critical role in determining the health of the fish. Since there has never been any prior research on the length-weight relationship (LWR) of fish in these biologically rich waters, this study offers new insights into the fish population in the wetlands of Lake Tana.

7.4.3. Health assessment index mean values in the wetlands of Lake Tana

The overall health status of the fish, as indicated by the HAI, was better in WO and ZG compared to AV, GRM, MRM, and RA. The fish from GRM exhibited the worst health condition, followed by MRM and RA. The best health status was observed in fish from ZG followed by WO. The higher the HAI calculation for a population being assessed, the poorer the health profile of the fish in that aquatic system (Adams et al., 1993; Schleiger, 2004; Crafford and Avenant-Oldewage, 2009; Botha, 2011; Watson et al., 2012). The average fish health index in this study was 31.78, indicating a moderate level of overall health. This value falls between those reported in studies from North America and South Africa. The index was higher than those found in fish populations from polluted rivers in North America and South Africa but lower than those reported for fish from other regions in South Africa, particularly those exposed to industrial and agricultural effluents. The overall fish health assessment index calculated in this study was higher than those reported in previous studies conducted on fish populations exposed to polluted environments in North America (Adams et al., 1993) and South Africa (Botha, 2011; Sara et al., 2014). Although the fish in this study were considered moderately healthy, the overall health of fish was lower than that of fish populations exposed to polluted environments in other regions (Crafford and Avenant-Oldewage, 2009; Sara et al., 2014; Watson et al., 2012). Table 7.6 provides a comparison of the median fish health assessment indices from this study and previous research.

Table 7.6. Comparison of median Fish Health Assessment Index (HAI) values between the present study and previous research. This table contextualizes fish health in Lake Tana wetlands relative to other contaminated aquatic environments.

Percent age in the present study	**Previous research findings**				**Reference**
	Percentage	**Fish species**	**Environment/Country**	**Contamination type**	
	17-79	*Micropterus nigricans*	Tennessee River Basin/North Carolina	Polychlorinated biphenyls	1
	42-72	*M. nigricans*	Hartwell Reservoir/South Carolina	Polychlorinated biphenyls	1
	21-61	*M. nigricans*	Pigeon River, North Carolina	Polychlorinated biphenyls	1
30-161 (median: 32)	70.3	*M. nigricans*	West Point/ Georgia	Municipal and industrial runoff	2
	54.6	*M. nigricans*	Sinclair/Georgia	Animal waste generation	2
	58.0	*M. nigricans*	Juliette/Georgia	Municipal and residential runoff	2
	46.5	*M. nigricans*	Tobesofkee/Georgia	Agricultural sedimentation	2
	57.3	*Lepomis macrochirus*	West Point/ Georgia	Municipal and industrial runoff	2
	55.9	*L. macrochirus*	Sinclair/Georgia	Animal waste generation	2
	58.0	*L. macrochirus*	Juliette/Georgia	Municipal and residential runoff	2
	50.4	*L. macrochirus*	Tobesofkee/Georgia	Agricultural sedimentation	2
	101.8-113.8	*C. gariepinus*	Loskop Dam/South Africa	Flood impact	3
	72-105.3	*C. gariepinus*	Bronkhorstspruit Dam/South Africa	Flood impact	3
	105	*C. gariepinus*	Mamba/South Africa	Flood impact	3
	55.5-93.3	*C. gariepinus*	Balule/South Africa	Flood impact	3
	17-79	*C. gariepinus*	Hartbeespoort Dam/South Africa	Agricultural and industrial impact	4
	87.18	*C. gariepinus*	The Vaal River system/South Africa	Urban/industrial/ agricultural effluent	5
	38	*C. gariepinus*	Hout River Dam/South Africa	Industrial and agricultural effluent	6
	41	*O. mossambicus*	Hout River Dam/South Africa	Industrial and agricultural effluent	6
	35	*C. carpio*	Hout River Dam/South Africa	Industrial and agricultural effluent	6

1= Adams et al. (1993), 2= Schleiger .(2004), 3= Watson et al. (2012), 4= Botha (2011), 5=Crafford & Avenant-Oldewage (2009), 6=Sara et al. (2014)

7.4.4. Prevalence of anomalies of fish in the wetlands of Lake Tana

Hematocrit value

The hematocrit value describes the volume of precipitated erythrocytes following the centrifugation of blood treated with an anticoagulant (Chen et al., 2023). It is a reliable measure of the erythrocytes' packed cell volume in blood (Blaxhall, 1972). It is the most widely used, commonly measured, and readily ascertained hematological variable according to (Houston, 1997). Madanire-Moyo (2011) reported that hematocrit was one of the most sensitive variables, showing distinctive differences among sites. Several research findings show that changes in the hematological indices of fish are predetermined both by the concentration of heavy metals in water and the duration of exposure, and both these factors can cause reversible and irreversible changes in the homeostatic system of fish. Hematocrit values > 45% are considered a mild abnormality, a value between 30% and 45% is considered normal, values between 19% and 29% are deemed moderately abnormal, and values below 18% severely abnormal (Heath et al., 2004; Crafford & Avenant-Oldewage, 2009). The prevalence of abnormal hematocrit values in this study was low at 0.07%. This is significantly lower than the rates reported in previous studies on fish populations exposed to polluted waters in South Africa (Crafford & Avenant-Oldewage, 2009; Sara et al., 2014; Watson et al., 2012). The prevalence of the highest hematocrit readings at WO during the dry season may be suggested to be due to the favorable conditions of the environment. On the other hand, the lower hematocrit values at RA and GRM during the rainy season could be due to the beginning of the gonad maturation period. Several reports (Mukherjee & Bhattacharya, 1982; Blazer, 2002; Hasan et al., 2015) indicated that when the gonads are fully developed fish consume relatively less food.

Several pathological indicators, including hematocrit values and ocular abnormalities, did not differ significantly across wetlands or seasons. Therefore, no ecological trends or site-specific interpretations were drawn from these results. While minor variations were observed, they are likely attributable to natural variability or sampling effects rather than meaningful environmental differences. This approach avoids overinterpreting non-significant findings and ensures that conclusions are based on statistically robust patterns.

External organs

Eyes

The eyes are highly vascularized organs that can indicate the well-being of a fish in several ways (Novotny & Beeman, 1990; Watson et al., 2012; Breitmeyer et al., 2022). The prevalence of eye anomalies in this study was high at 26.2%. This figure surpasses the rates reported in previous studies conducted on fish populations exposed to polluted waters in South Carolina (Adams et al., 1993) and South Africa (Crafford & Avenant-Oldewage, 2009; Sara et al., 2014; Watson et al., 2012). In GRM, MRM, and RA *Labeobarbus* spp. showed exophthalmia, hemorrhage/yellowish discoloration of the eye, which can lead to blindness and missing eye. The eye abnormalities observed in *Labeobarbus* spp are consistent with previous findings in other fish species. Novotny & Beeman (1990) reported similar anomalies in chinook salmon (*Oncorhynchus tshawytscha*), and Douellou (1992) observed the in *Oreochromis mortimeri*. Yılmaz et al. (2004) suggest that environmental, biological, and ecological factors may contribute to these eye abnormalities. Blindness was more prevalent in one or both eyes in the RA, MRM, and GRM, probably resulting from parasite infestation or any other infectious diseases that reduce the eyesight of the fish as the intermediate host. Similar reports by Karvonen et al. (2004) and Seppälä et al. (2004) indicated that *Diplostomum spathaceum* (Trematoda) metacercariae lodge in the eyes of fish and induce cataract formation.

Skin

The highest prevalence of mild and moderate skin abrasions in AV during the dry season could be associated with the discharge of effluents from the Felege-Hiwot Hospital adjacent to this wetland. This finding is in line with several reports. For example, Laurent et al. (2022) and Watson et al. (2012) indicated that mild, moderate, and severe skin lesions can be caused by environmental stress, irritants, bacterial infection, parasites, netting-related damage, surface abrasions or predator attacks. The prevalence of skin abnormalities found in this study (16.55%) was higher than the rates reported in previous studies conducted on fish populations exposed to polluted environments in South Africa (Crafford & Avenant-Oldewage, 2009; Sara et al., 2014) and the United States (Adams et al., 1993). Nevertheless, the prevalence observed in this study is lower than that found in a previous study conducted on different fish species within the Olifants River system in South Africa (Watson et al., 2012)

The only abnormalities found at AV and GRM were surface lesions observed during the dry season, while abnormalities were observed in MRM and RA in the early and late rainy seasons. Higher incidences of skin lesions were recorded during the dry season in AV. The cause of the lesions was undetermined. *Argulus* spp. was observed on the skin during the dry season, causing a severe inflammatory response at the site of attachment.

Fins

Mild active erosion with no bleeding and severe active erosion with bleeding recorded in WO, RA, and MRM might be the result of extreme exposure of the dorsal fins to ectoparasite infestations, such as *Argulus* spp. in the dry and late rainy seasons. Crafford & Avenant-Oldewage (2009) reported that drought and flood conditions did not lead to deformed fin rays in any of the three fish species they studied. However, the authors observed mild fin fraying. The prevalence of fin bleeding and severe erosion in this study (8.27%) was higher than that observed in previous studies conducted on fish populations exposed to polluted environments in South Carolina (Adams et al., 1993) and South Africa (Crafford & Avenant-Oldewage, 2009; Sara et al., 2014). The prevalence observed in this study is lower than those reported in a previous study on various fish species within the Olifants River system in South Africa (Watson et al., 2012).

Opercula

Slight to mild shortening of opercula was observed in RA during the late rainy season. This might be associated with congenital defects (Watson et al., 2012). Several reports state that opercula, which serve to protect the gills, react to vitamin deficiencies, resulting in edge necrosis, bulging, and deformation (Fryxell et al., 2015a). However, the prevalence of slight and mild shortening of the opercula was extremely low (0.07%). This finding aligns with observations from a previous study conducted in South Africa (Crafford & Avenant-Oldewage, 2009; Sara et al., 2014; Watson et al., 2012), where a similarly low prevalence was reported. However, such abnormalities were not documented in a study conducted in the United States (Adams et al., 1993).

Gills

The prevalence of clubbed and pale lesions in this study (16.55%) was similar to findings in some studies conducted in South Africa (Sara et al., 2014; Watson et al., 2012) and the United States (Adams et al., 1993). However, it was lower than the prevalence reported in other studies from these countries, particularly those involving fish populations exposed to polluted waters (Adams et al., 1993; Watson et al., 2012). Environmental perturbations or parasitic infections affect the physiology and appearance of lamellae, resulting in anemia of the gills (Currie et al., 2022; Novotny & Beeman, 1990; Weli et al., 2013). Clubbed gills were commonly observed in fish from MRM wetlands, particularly during dry seasons. This condition may be associated with sublethal exposure to nitrite and ammonia, as reported in studies on fish in the United States (Riddell et al., 2019; Kadiru et al., 2022). The highest nitrate concentration was recorded in MRM during the dry season. Total nitrogen from sediment should have affected the bottom-dwelling residents more in this wetland. Parasitic infections on the gills were predominantly observed with pale-gill abnormalities which were observed only during drought conditions. Parasite-induced lesions, compounded by pollution, which result in hyperplasia, have been recorded in Narragansett Bay young-of-the-year winter flounder (MacLean, 1993) and the Olifants River system in South Africa in *C. gariepinus*, *O. mossambicus* and *Labeobarbus marequensis* during drought and flood conditions (Watson et al., 2012).

Pseudobranch

The prevalence of inflamed and swollen pseudobranchs was low at 4.53%. This figure is lower than rates reported in previous studies conducted on fish populations in South Africa (Crafford & Avenant-Oldewage, 2009; Watson et al., 2012; Sara et al., 2014) and the United States (Adams et al., 1993). MRM, characterized by inflamed and swollen pseudobranchs, is a health issue that damages the pseudobranch, an organ involved in various functions such as regulating salt levels, breathing, producing hormones, and sensing the environment (Dunel-Erb et al., 1993; Laurent et al., 1994).

Internal organs

Thymus

The thymus is a primary lymphoid organ that appears as a paired, ovoid structure located subcutaneously in the dorsal commissure of the operculum. The prevalence of mild and moderate thymus inflammation was very low in this study (2.76%). This finding is not in agreement with thymus inflammation in previous studies conducted on fish populations in South Africa (Sara et al., 2014; Watson et al., 2012) and the United States (Adams et al., 1993). Mild and moderate inflammation of the thymus in the current study may be the result of chemical and bacterial contamination in the GRM wetland (Untergasser & Axelrod, 1989; Novotny & Beeman, 1990; Lopes, 2021).

Spleen

Enlargement of the spleen (splenomegaly, seen in all fish species at RA during the dry season), may suggest physiological stress exerted on the immune system of these fish. A weakened immune system may have contributed to the higher *Contracaecum* spp. number observed at RA during the dry season. The spleen stores blood, produces blood cells (Fänge & Nilsson, 1985), disintegrates erythrocytes, and releases hemoglobin (Goede & Barton, 1990; Johansen et al., 2006). Enlargement or swelling of the spleen could indicate disease or weak immunity (Adams et al., 1992). The prevalence of spleen anomalies was very low (0.07%). This finding aligns with previous research conducted in South Africa (Sara., 2014) and the United States (Adams et al., 1993), where such anomalies were also rarely reported.

Hindgut

The prevalence of mild to moderate gut inflammation in this study was low at 3.45%. This figure is lower than the rates reported in previous studies conducted on fish populations in South Africa (Sara et al., 2014; Watson et al., 2012). Mild to moderate inflammation was observed in the gut of fish from RA, GRM, and MRM during the dry season. This inflammation, primarily affecting the hindgut, might be linked to stress-induced infections. Similar inflammatory responses have been observed in other fish species during dry periods, suggesting that this condition may not have a specific underlying cause (Crafford & Avenant-Oldewage, 2009; Marx, 2012; Pal & Maiti, 2018; Faheem et al., 2021).

Kidney

The absence of obvious kidney gross lesions on *Labaeobarbus* spp. in this study suggests that no pathogenic infection existed in the populations studied. The kidney of freshwater fish excretes water (Won et al., 2003) and is also the primary hematopoietic organ in fish (Heath et al., 2004). Pale, mottled color, excessive swelling, or granulation of the kidney probably reflects a pathogenic infection (Goede & Barton, 1990). No kidney abnormalities were observed in this study, aligning with findings from previous research conducted in South Africa (Sara et al., 2014; Watson et al., 2012). However, these results contrast with those from studies in the United States (Adams et al., 1993), which reported a significant prevalence of kidney abnormalities in fish populations exposed to polluted waters.

Liver

Abnormalities such as neoplastic changes, reduction in size and weight, lipoid degeneration, fatty liver, and detection of bright spots or hemorrhages may result from the activation of carcinogenic substances (Laurent et al., 1994) or vitamin deficiencies (Schäperclaus et al., 1992; Corredor-Santamaría et al., 2021). The prevalence of liver abnormalities, such as discoloration and nodular lesions, was 16.6%. The prevalence of liver abnormalities was lower than reported in certain studies from the United States (Adams et al., 1993) and South Africa (Sara et al., 2014) but higher than the rates observed in some South African studies (Watson et al., 2012). Abnormalities included general or focal liver discoloration and nodular lesions, with MRM-affected fish being particularly susceptible during dry seasons. Focal and general discoloration of the liver indicates an aberration or response at a specific focus of the liver and may be induced by focal infections, inflammation, or necrosis. Histopathological examination was made of *Labeobarbus* spp. livers collected from the six wetlands in Chapter 6, and circulatory disturbances, particularly hemorrhage, hyperemia, aneurysm, and vascular degeneration were most frequently observed, followed by infiltration of inflammatory cells in the hepatic tissue. No microorganism isolation was made in this study. Lesions are associated with malnutrition, which is categorized as disorders that result from either insufficiency or relative nutrient imbalance, but may also be induced by a variety of irritants and toxicants (Goede & Barton, 1990; Harper & Wolf, 2009).

Among the organs assessed during necropsy-based fish health evaluations, the gills and liver proved most diagnostically useful for rapid field-based monitoring. Gills consistently exhibited regressive changes such as lamellar fusion, epithelial lifting, and necrosis are early indicators of waterborne pollutant exposure. The liver, as a primary detoxification organ, showed visible signs of inflammation and discoloration associated with chronic contaminant exposure. These organs were most frequently affected and demonstrated the strongest association with degraded water quality. Accordingly, gill and liver assessments are recommended as priority targets for rapid fish health monitoring in Lake Tana wetlands. Regressive pathologies, particularly necrosis and atrophy, emerged as the most ecologically relevant and consistent indicators of chronic environmental stress.

7.4.5. Prevalence of endo/ ectoparasites of fish in the wetlands of Lake Tana

Endoparasites

There was a significant difference in the prevalence of endoparasites across the four seasons, with a higher prevalence of endoparasite infections in the dry season compared to the other seasons. Poulin (2020) reported that seasonal infection dynamics characterize many host-parasite interactions, as abiotic conditions drive fundamental biological processes in both hosts and parasites. These include seasonal variations, such as between winter and summer in temperate areas and wet and dry seasons in tropical regions. A study on the diversity and seasonal distribution of parasites in *O. niloticus* fish in semi-arid reservoirs revealed that the prevalence of parasites was higher in the rainy season compared to the dry season (Yamba et al., 2016). According to the report by Omeji et al. (2022) on African lungfish in Nigeria's Upper River Benue, it was found that 51% of parasites were observed during the dry season and 48% during the wet season. However, Önalan et al. (2022) reported that *L. intestinalis* infestations, based on annual examinations, were seen in any month of any season. Mgwede & Msiska (2018) noted that the endoparasites identified in *Barbus paludinosus* in Malawi during the dry season were *Contracaecum* spp. and *L. intestinalis*, with no clear seasonal differences.

The relatively higher prevalence of *L. intestinalis* in WO during the dry season than AV could be associated with the activity of migratory birds (definitive host) in WO. This is in line with several reports in Lake Tana where the overall prevalence of *L. intestinalis* was 29.00% in *Barbus* species (Tizie et al., 2014). Gebremedhn & Tsegay (2017) reported that digenean trematodes, nematodes, and cestodes are the most common internal parasites in Ethiopian water bodies. Research from other countries shows that *L. intestinalis* larvae have caused significant economic losses in fish production due to high rates of sickness, death, reduced production, and expensive treatment (Gabagambi & Skorping, 2018; Song & Park, 2018; Gabagambi et al., 2019).

The prevalence of endoparasites in this study was very low at 2.2%. This is significantly lower than rates reported in previous research conducted in South Africa (Sara et al., 2014; Watson et al., 2012). Interestingly, no endoparasites were detected in studies conducted in the United States (Adams et al., 1993). The relatively higher prevalence of *Contracaecum* spp. in RA, MRM, and AV than in WO, ZG, and GRM could be associated with feeding habits, and a diverse diet may put fish into contact with potential intermediate hosts of the parasite. In RA and MRM, the most common fish species were zooplankton eaters like *L. brevicephalus*. A study by Ageze & Menzir, (2018), (Kaba et al., 2014), and (Guta et al., 2024) found that *Contracaecum*, a nematode, was the most prevalent parasite in *O. niloticus* and small *Barbus* species, with infection rates of 49.5% and 31%, respectively. The existence of parasites of the gonads and abdominal cavity may lead to complete castration, and reductions in egg and spermatozoa numbers have so far been found to be due only to parasites of the body cavity.

The prevalence of ectoparasite infection in this study was 19.31%, which is lower than that reported in studies conducted in Ethiopia (Tesfaye et al., 2017), Egypt (Abd-Elrahman et al., 2023), and South Africa (Watson et al., 2012). No data on ectoparasite prevalence were available from a study conducted in the United States (Adams et al., 1993). The greatest number of ectoparasites (*Argulus* spp. *Dactylogyrus* spp., *Gyrodactylus* spp., leeches) were collected from the skin and gills of fish in WO and GRM, while ectoparasite numbers were lower at the AV and MRM in the dry season. The fact that more ectoparasites were found in WO and GRM is consistent with the parasite index, which suggests that areas with better water quality will have higher ectoparasite intensity. This is supported by previous research that found lower intensity in fish from polluted areas by Gelnar et al. (1994) and Crafford & Avenant-Oldewage, (2009). Additionally, ectoparasites were more common during dry seasons and less common during late wet seasons.

Ectoparasite-endoparasite ratio

The prevalence of ectoparasite-to-endoparasite ratio did not differ among wetlands, although slightly higher levels were observed in WO, ZG, GRM, and MRM compared to AV and RA. The ratio of ecto- versus endoparasites is used to indicate the health status of aquatic ecosystems in the tropics. Several studies have used ectoparasites as bioindicators of deteriorating water quality due to organic pollutant sources (Crafford & Avenant-Oldewage, 2009; Watson et al., 2012; Ferri et al., 2022). The highest number of endoparasites collected from the study wetlands was represented by the tapeworm *L. intestinalis* and the adult nematodes of *Contracaecum* spp. This finding is in line with previous studies on Lake Tana fish (Ageze & Menzir, 2018), which reported prevalence rates of 22.5% for cestodes *L. intestinalis* and 49.5% for nematodes *Contracaecum*.

7.5. Conclusion and recommendations

The results of the length-weight relationship and HAI did not show similar patterns to the results of the previous chapters on water quality, macroinvertebrates, and fish histopathology. This indicated that LWR is not a factor for aquatic environmental quality and further investigation is required. The highest HAI values were recorded in MRM, GRM, and RA, moderate HAI value in AV, and the lowest HAI value in WO and ZG. As with physicochemical evaluations, macroinvertebrate diversity and biotic indices, and histopathology reaction patterns and organ indices, HAI showed a similar pattern of fish health status among wetlands. It is recommended to read the outcomes of this chapter along with Chapters 3, 4, and 6. The decline in water quality in MRM, AV, and RA is linked to elevated levels of conductivity, salinity, soluble reactive phosphorus, total dissolved solids, pH, temperature, and ammonia. This indicates that either irritants in the wastewater effluents (e.g., toxicants, insoluble substances), or the changed physicochemical conditions due to the effluents, as was observed in Chapter 3 (e.g., conductivity, salinity, higher values of soluble reactive phosphorous, total dissolved solids, pH, temperature, and ammonia), had a detrimental impact on the health of the fish. However, the study could not establish a causal link between fish health anomalies and specific contaminants in the water. Given the severity of anomalies observed in MRM, GRM, and RA in all organs and tissues except for the fins, mesenteric fat, hematocrit values, spleen, hindgut endoparasites, and ecto/endoparasite ratio, we recommend an analysis of effluent at these wetlands for toxicant-specific assessment.

The prevalence of eye abnormalities, including blindness, exophthalmia, and missing eyes, varied among the different wetlands. The highest rates of these abnormalities were seen in RA, while AV, GRM, MRM, WO, and ZG had the lowest. Other health issues, such as mild active erosion with no bleeding in fins, slight or mild shortening of opercula, clubbed or pale lesions on gills, inflamed and swollen pseudobranch, mild and moderate hemorrhage on thymus, general or focal discoloration of the liver, and ectoparasite infestation differed among wetlands. Anomalies of gill, liver, eye, and skin contributed more towards the fish health assessment index in terms of water quality deterioration, as these are most likely to be caused by environmental deterioration. The study found that the four seasons differed in fish health assessment indices including body weight, body length, overall fish health assessment index, skin mild abrasion, clubbed/pale gill lesion, mesenteric fat accumulation, general and focal liver lesion, and endoparasite occurrence. The dry season had the highest overall fish health assessment index, followed by the early and late rainy season with the rainy season having the lowest. Both wetland and season had an effect on the fish health assessment index and some of the anomalies in the external and internal bodies of fish including clubbed or pale gill, mesenteric fat accumulation, general or focal liver lesions, and mild or moderate hemorrhage in the thymus. Therefore, monitoring the aquatic environment in Ethiopia should strategically consider location, and season to assess deterioration caused by pollution in Ethiopian water bodies. Relating the results observed in this study to the pollution assessment literature and using the fish health assessment indices as an indicator, there is a clear deterioration of the aquatic environmental quality in the Lake Tana wetlands in Ethiopia. This study recommends the use of the fish health assessment index in the assessment of aquatic ecosystem contamination as part of comprehensive biomonitoring programs. The use of this index as an effective biological monitoring tool in Ethiopia has been recommended.

CHAPTER 8

General discussion and application of fish and macroinvertebrate bioindicators and fish health assessment biomarkers to assess aquatic environmental quality in wetlands of Lake Tana, Ethiopia

To establish the foundation for the general discussion, Figure 8.1 is presented to summarize the integrated biomonitoring strategy used in this study, which combines physicochemical measurements, macroinvertebrate and fish community assessments, and biomarker diagnostics such as histopathology and health indices.

The illustration visually demonstrates how each assessment layer contributes to a progressively deeper understanding of aquatic ecosystem health across the six wetlands of Lake Tana. It also highlights the spatial variation in pollution levels as detected by different indicators, reinforcing the value of a multi-tiered approach. Placing this figure at the beginning of the discussion provides a conceptual roadmap for interpreting the results and supports the development of region-specific monitoring frameworks.

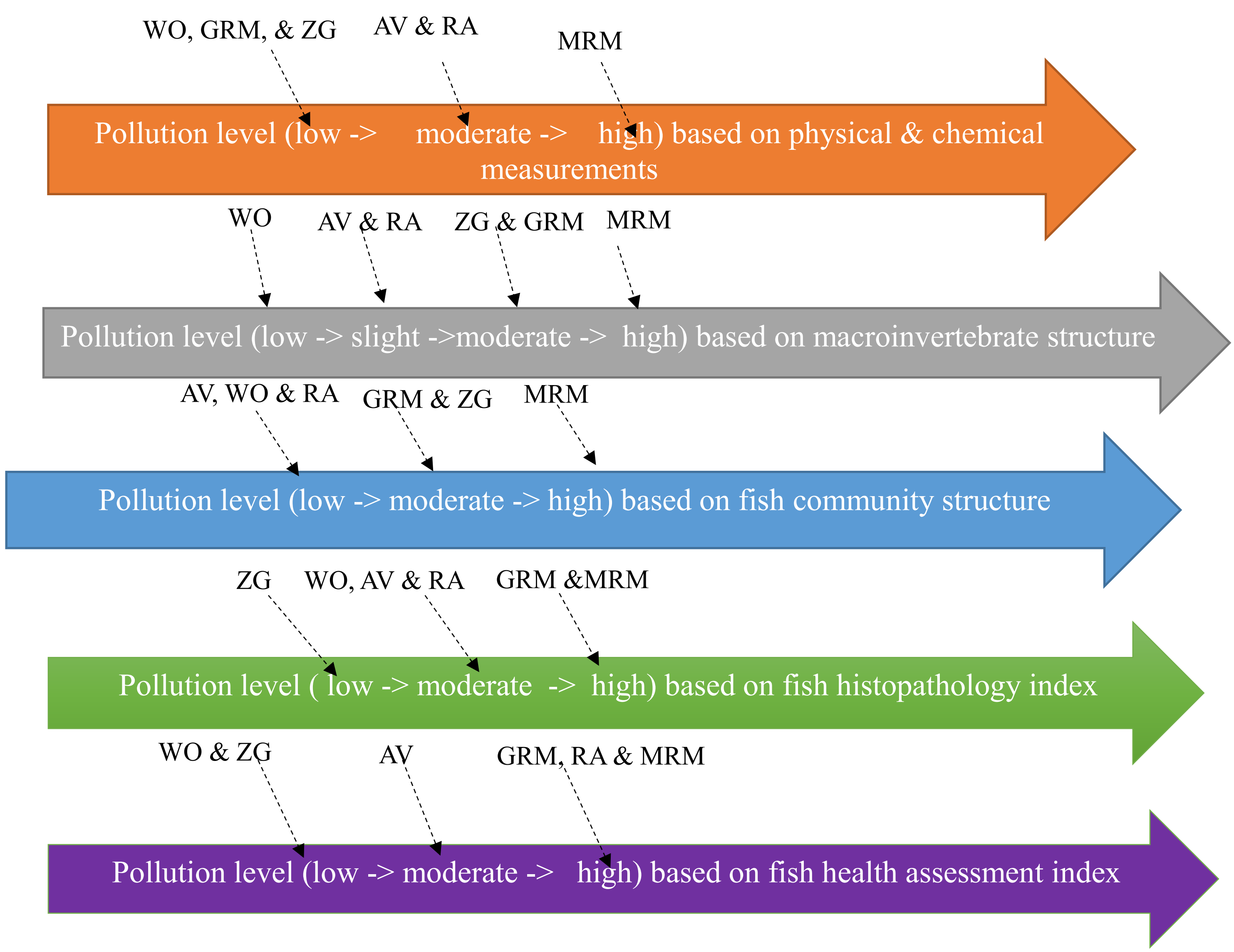


Figure 8. 1. Summary of integrated biomonitoring strategy using physicochemical and biological measures in six wetlands of Lake Tana. Arrows indicate how the physicochemical measure drops on different biological measures for each wetland.

This chapter summarizes the thesis findings in relation to the contemporary understanding of pollution impacts on water quality, biodiversity, and fish health. It evaluates the potential for integrating fish and macroinvertebrate bioindicators, fish histopathology, fish health assessments, and inverted parasite biomarkers into water quality monitoring. Together, these tools provide organ- and organism-level evidence of environmental stress, validating and complementing community-level and physicochemical assessments.

The study employed a multi-indicator approach to assess ecological integrity across six wetlands in the Lake Tana watershed. By combining seasonal and spatial variability with physicochemical and biological data, the research offers a comprehensive framework for evaluating aquatic environmental quality. These findings provide practical guidance for developing sustainable, region-specific monitoring and management strategies in Ethiopian freshwater ecosystems.

In assessing the aquatic environment of Lake Tana, important considerations in method selection are critical. While physical and chemical measurements offer insights into water quality, focusing solely on physicochemical properties may overlook key biological factors such as biodiversity and fish health assessments (Sikhakhane, 2011; Ogidi & Akpan, 2022; Castro et al., 2023; Zehra et al., 2023). A combined approach integrating both physical and chemical measurements with biological monitoring provides a more comprehensive understanding, as these components are often interrelated (Bartell, 2006; Wepener, 2008; Ferizi et al., 2020; Calisi, 2023).

Ethiopia lacks integrated approaches for assessing overall aquatic ecosystem health. Incorporating bioindicators and biomarkers with traditional water quality assessments offers a more sensitive and early-warning system for monitoring ecological health (Moore et al., 2004; Hook et al., 2014a; Ji et al., 2019; Carere et al., 2021). These methods evaluate the molecular and physiological impacts of pollutants, enabling better conservation and management of freshwater resources. Through combining these tools, environmental monitoring becomes more adaptable, improving the protection and management of aquatic ecosystems.

Despite challenges such as limited resources and the complexity of chemical monitoring, this thesis pioneers the use of macroinvertebrate and fish bioindicators, along with fish histopathology and health assessments, to assess aquatic environments in Ethiopia. By advancing methods for detecting pollutant impacts, the research provides valuable tools for monitoring ecosystem health, identifying pollution hotspots, and informing regulatory policies, contributing to the long-term sustainability of freshwater ecosystems in Ethiopia. The study also examines macroinvertebrates in both the surface and benthic zones across wetlands and seasons, offering a nuanced evaluation of aquatic ecosystem health. This approach deepens our understanding of environmental quality, pollutant impacts, habitat-specific dynamics, and the role of macroinvertebrates in food webs. Additionally, the fish histopathology and health assessment indices were used to refine the evaluation, providing precise, sensitive, and species-specific insights into the effects of pollution. Biomarkers play a crucial role in the early detection of stressors, long-term monitoring, and guiding effective management and conservation efforts, contributing to the preservation of biodiversity and the sustainability of fisheries. Focusing on *Labeobarbus* spp**.**, a common fish species in African freshwater systems, this study examines fishes' health biomarkers to assess pollution impacts and detect environmental stressors. By measuring a range of biomarkers, the research offers valuable insights into the health of aquatic ecosystems in Ethiopia and helps identify risks to biodiversity.

Biological assessment methods like species counts, biodiversity indices, and fish histopathology are essential, albeit labor-intensive, for evaluating ecosystem health (Schmutz et al., 2015; Parmar et al., 2016b; Zhuang et al., 2024). The choice of metrics, such as species richness versus abundance, significantly influences the interpretation of ecosystem health and pollution impacts. Using multiple biological indicators and biomarkers broadens the assessment and helps identify pollution sources (Adimasu, 2015; Atamanalp et al., 2024; da Silva Rosa et al., 2024). Monitoring frequency and duration are also key factors. Short-term studies may fail to capture seasonal or long-term trends, such as nutrient fluctuations or species population changes. Long-term monitoring is essential for detecting gradual changes like eutrophication (Rocha et al., 2015; Rathore et al., 2016; Zhuang et al., 2024). This study covered four seasons and six wetlands adjacent to Lake Tana, aligning sampling frequency with environmental variability. In conclusion, method selection directly affects the accuracy, scope, data reliability, and management strategies. A well-designed study integrating diverse methods tailored to the lake's characteristics ensures a holistic understanding of the lake's environment (Bhateria & Jain, 2016; Xu et al., 2016; Karr, 2020).

In bioindicator and biomarker-based aquatic environmental assessments, important methods include biomarker analysis**,** histopathological examination**,** bioaccumulation studies**,** macroinvert ebrate bioassessment**,** and phytoplankton analysis. These techniques are crucial for detecting early signs of environmental stress and pollutant impacts before visible degradation occurs (Ferizi et al., 2020; Lomartire et al., 2021). Biomarker analysis, for instance, reveals physiological changes at the molecular level, while histopathological examination identifies tissue damage from contaminants (Hasan et al., 2015; Yancheva et al., 2016; Sula et al., 2020). Bioaccumulation studies assess pollutant concentrations in organisms, while macroinvertebrate and phytoplankton surveys offer ecological insights into water quality and ecosystem health (Kadim & Risjani, 2022; Salunke et al., 2024). Combining these methods provides a comprehensive approach to understanding and managing aquatic ecosystem health (Carere et al., 2021; Zebral et al., 2021; Calisi, 2023). To address this knowledge gap, the thesis identifies key water quality variables, bioindicators such as fish and macroinvertebrates, alongside biomarkers for fish histopathology and health assessment, that were used to evaluate water quality in Ethiopian lakes. These findings provide valuable insights for developing more region-specific water quality assessment tools.

The methods used in this study focused on bioindicators, biomarkers, and seasonal variations, may not be directly applicable to ecosystems in the northern hemisphere due to significant geographical and environmental differences, particularly in terms of the factors driving seasonal changes. One of the most important distinctions between northern and southern hemisphere ecosystems lies in the primary drivers of seasonal variation. In the northern hemisphere, temperature fluctuations play a dominant role in seasonal changes, which significantly influence the metabolic rates and activity of aquatic organisms, as well as the overall health and biodiversity of the ecosystem (Jeppesen et al., 2000, 2005; Xenopoulos & Lodge, 2006; Ogidi & Akpan, 2022). In temperate climates, seasonal temperature changes directly affect water temperature, which in turn influences the physiology of aquatic species. For instance, lower temperatures during the winter months slow down the metabolic processes of ectothermic organisms, such as fish and macroinvertebrates, reducing organism's activity levels, feeding rates, and reproductive potential (Shuter et al., 2012). These temperature-induced changes can have cascading effects on community dynamics, as slower metabolic rates may reduce predation pressures, alter competition, and modify species interactions. Moreover, colder temperatures can also affect the solubility and availability of oxygen, which is a critical factor influencing species distribution and health (Rice, 2003; Sokolova & Lannig, 2008; Pörtner & Peck, 2010; Shuter et al., 2012). In contrast, warmer summer temperatures typically increase metabolic rates, leading to higher rates of oxygen consumption, and can cause oxygen depletion in the water column, further influencing aquatic health (Baxa et al., 2021).

The seasonal variations in temperature in the northern hemisphere can also influence nutrient cycling. For example, in temperate regions, the thermal stratification of lakes in the summer and winter can result in distinct vertical temperature layers, with colder, denser water at the bottom and warmer water at the surface. This stratification can limit nutrient exchange between the surface and bottom waters, affecting the productivity of the ecosystem. In winter, ice cover can further restrict light penetration, impacting primary productivity and altering food availability for higher trophic levels (Jeppesen et al., 2005; Smol, 2009). These seasonal dynamics are quite different from those in tropical regions like Ethiopia, where temperature changes are less pronounced, and ecological responses are more driven by rainfall and water availability. Moreover, seasonal shifts in precipitation patterns are another crucial distinction. In temperate northern hemisphere ecosystems, seasonal precipitation typically manifests as snow and rain, which affects runoff, nutrient loading, and sediment transport into aquatic ecosystems. Snowmelt in the spring can lead to pulses of nutrients and contaminants entering freshwater systems, which may result in temporary increases in water quality degradation. This contrasts with the tropical wet-dry seasonal cycle, where rainfall-driven nutrient loading can result in more predictable fluctuations in water quality, often with intense nutrient pulses during the rainy season and drier conditions in the low-water period.

Therefore, while the methodologies used in this study, including the use of bioindicators and biomarkers for assessing seasonal changes in aquatic ecosystems, are valuable, the physiological and ecological responses of aquatic organisms in the northern hemisphere may differ significantly from those in regions such as Ethiopia. As such, adjustments to monitoring techniques, bioindicator selection, and the interpretation of seasonal variations may be necessary to account for these geographic and climatic differences. Furthermore, the integration of temperature, ice cover, and snowmelt dynamics into the analysis of northern hemisphere ecosystems would improve the accuracy of ecological assessments and make the methodologies more universally applicable across diverse environmental contexts.

Ethiopian freshwater ecosystems are primarily influenced by seasonal rainfall patterns rather than temperature variations. Rainfall significantly affects not only water temperature but also nutrient dynamics, hydrological flow, and pollutant dispersal in aquatic systems (Wondie et al., 2007b; Jemberie et al., 2016; Moges et al., 2017). The seasonal influx of rain leads to shifts in nutrient loading, which can trigger algal blooms, eutrophication, and changes in the distribution of aquatic organisms (Moss et al., 2003). Moreover, rainfall also impacts fish reproduction and migration patterns, as many species rely on specific rainy seasons for spawning (Anteneh et al., 2012b; Gebremedhin et al., 2012). These water quality and biological responses are more closely linked to precipitation dynamics than temperature fluctuations, necessitating the development of bioindicator and biomarker methods that consider these distinct environmental drivers.

These geographic and seasonal differences highlight the importance of tailoring bioindicator and biomarker-based monitoring methods to the specific ecological conditions of each region. While bioindicators and biomarkers are valuable tools globally, the application of bioindicators and biomarkers must be adapted to account for the unique environmental processes that govern ecosystem health in different regions. In Ethiopia, bioindicators such as native fish species and macroinvertebrates respond to factors like nutrient pulses from rainfall and the effects of localized pollution, which may not be relevant to northern hemisphere systems (Dalu et al., 2021; Naigaga, 2012; Ochieng et al., 2019). Therefore, a localized approach, focusing on the region's specific climatic and ecological dynamics, is critical to ensuring the accuracy and efficacy of ecological monitoring and conservation efforts.

Bioindicator-biomarker research in Lake Tana wetlands provides real-time insights into the health of the ecosystem, guiding effective lake management and conservation strategies. These findings enhance understanding of Lake Tana's health and inform broader ecological monitoring and policy-making efforts. To ensure successful application in other lakes, research must be adapted to each lake’s unique ecological and environmental conditions. Future research should focus on refining methodologies, selecting appropriate species, and establishing long-term monitoring systems. A collaborative, region-wide approach, supported by advanced technologies, will ensure comprehensive lake health management across various ecosystems.

In this study effects of effluent exposure were determined using a range of approaches.

1. Physicochemical variables are commonly available to water resource managers. This study examined six wetlands based on the physicochemical properties of study wetlands, trophic state indices, water quality indices, and possible underlying factors that influenced these variables across four seasons.
2. Macroinvertebrate families and fish species were related to physicochemical variables. The macroinvertebrate families and fish species were explored as biological indicators of environmental quality deterioration in wetlands in Lake Tana. Macroinvertebrate families and fish species were evaluated and compared across the six wetlands and four seasons.
3. Histopathology of the liver, gills, gonads, and spleen of *Labeobarbus* spp. was evaluated and compared across the six wetlands and four seasons using the modified protocol developed by Bernet et al. (1999)
4. Standardized fish health assessment indices by Adams et al. (1993) and Crafford & Avenant-Oldewage (2009) were used for comparisons across the six wetlands and four seasons.

Several studies in Ethiopia have physical and chemical water property analysis with benthic macroinvertebrate evaluation to assess aquatic ecosystem health (Gezie et al., 2017a; Zelalem & Prokin, 2017; Gezie et al., 2020; Mohammed et al., 2022). The combined physicochemical and benthic macroinvertebrate approach provides valuable insights into water quality and pollution effects, as benthic macroinvertebrates are sensitive indicators of environmental change, revealing long-term ecological trends. Such studies are essential for managing freshwater resources in Ethiopia, where agricultural, urban, and industrial pressures impact water bodies (Awoke et al., 2016; Wondim et al., 2016; Zelalem & Prokin, 2017; Getnet et al., 2022).

Many of the previous studies on Lake Tana have made significant contributions but often overlooked or insufficiently explored critical aspects, such as the interaction between physical, chemical, and biological factors. Prior studies frequently employed single-dimensional approaches, missing the complex relationship between water quality and biological health. Furthermore, the limited focus on seasonal assessments failed to address long-term pollution trends or the seasonal variability of biological indicators (Mohammed et al., 2022). The integration of fish histopathology and health biomarkers, which detect sub-lethal effects of pollutants, was rarely used in earlier research, leaving many subtle impacts on aquatic life overlooked. Moreover, past studies did not account for the role of pollutant mixtures and the cumulative effects of pollutant mixtures, a gap this study addresses by incorporating both chemical and biological indicators for a dynamic evaluation of Lake Tana's health. Moreover, previous studies focused water quality assessment only on dry and wet assessments (Zelalem & Prokin, 2017; Tibebe et al., 2019b; Abegaz et al., 2023).

This study differs from previous aquatic assessments of Lake Tana by adopting a more comprehensive approach, integrating a range of methods, including water quality indices, multivariate techniques, and bioindicators such as fish and macroinvertebrate species, along with fish histopathology and health biomarkers across multiple seasons and wetlands. Unlike prior studies, which often focused on seasonal variations or single parameter assessments (Gezie et al., 2017a; Zelalem & Prokin, 2017), this research provides a holistic evaluation of the lake's health by considering both physical and biological factors. By combining traditional water quality assessments with advanced biomarkers, this study offers deeper insights into pollutant impacts and ecological dynamics (Mohammed et al., 2022). This integrated approach highlights the need for long-term, multi-faceted assessments to inform better environmental management. Differences between this study and prior research can be attributed to variations in methodology, scope, and temporal coverage. Previous studies typically focused on seasonal water quality or single biological indicators (Mehari et al., 2014; Gebresllassie et al., 2014; Adimasu, 2015; Wondim et al., 2016; Gezie et al., 2017a; Zelalem & Prokin, 2017), while this study adopted a more integrated approach, including biological and chemical parameters, multivariate analyses, and biomarkers. The four-season, multi-wetland temporal span of this study allows for a more robust analysis of seasonal and spatial variations. Advanced tools like fish histopathology and health biomarkers provide species-specific insights into environmental stressors, which may explain discrepancies in findings.

This study contributes to a nuanced understanding of environmental quality by demonstrating how different wetlands respond to pollution gradients across seasons. For example, Megech River Mouth consistently exhibited high nutrient loads and histopathological damage in fish, indicating chronic pollution, while Zewdie Girar and Wonjeta showed stable physicochemical profiles and healthier biological indicators. The study also revealed that macroinvertebrate diversity declined in wetlands with elevated ammonia and nitrite levels, while fish health indices were lowest in areas with high parasite prevalence and organ anomalies. These findings highlight the interplay between water chemistry, biological responses, and habitat-specific stressors, offering a detailed picture of how pollution affects ecosystem structure and function in Lake Tana.

This study also effectively utilized the Shannon-Wiener Diversity Index (SWDI) and Carlson's Trophic State Index (TSI) to assess biodiversity and lake health. The SWDI, which measures species diversity and community composition, and the TSI, which quantifies eutrophication and nutrient levels, offer complementary insights into the lake's ecological health (Zelalem & Prokin, 2017). Combining macroinvertebrate diversity with fish health biomarkers provides a powerful, multi-dimensional approach to monitoring pollution. Macroinvertebrates, sensitive to water quality changes, act as an early warning system for habitat degradation, while fish health biomarkers help detect sub-lethal pollution effects on higher trophic levels and species integral to food webs (Gezie et al., 2020; Mohammed et al., 2022). The histopathological biomarker tool provides valuable insights but is invasive, complex to interpret, and costly (Mohammed et al., 2022), making it less suitable for routine monitoring. In contrast, simpler indices like TSI or SWDI are more practical for long-term studies, especially in resource-limited settings like Lake Tana. Combining these methods offers a balanced approach, integrating practical tools with more detailed data on ecosystem health.

This study highlights the severe impacts of agricultural and urban wastewater pollution on wetland health, leading to declines in macroinvertebrate and fish diversity, abundance, and overall health. Fish populations, particularly in MRM and GRM wetlands, exhibited anomalies in internal and external organs (e.g., liver, gonads, spleen, gills), indicating disrupted growth and reproductive potential. Similarly, degraded water quality in MRM, AV, ZG, and RA wetlands negatively affected macroinvertebrates, reducing the abundance and diversity of the wetlands. These impacts were corroborated by lower biodiversity indices (Shannon-Wiener, Hilsenhoff's Index) and classifications such as BMWP and EPT, which identified the wetlands as polluted.

The observed decline in macroinvertebrate genera, particularly in the ZG, GRM, and MRM wetlands, disrupted the structure of the aquatic food web and signaled marked ecological imbalances. For *Labeobarbus* species, deteriorating water quality in these wetlands contributed to habitat degradation, as reflected by lower values in Menhinick's and Hill's diversity indices, alongside a reduced fish-based Index of Biotic Integrity (IBI). The integrated use of macroinvertebrate and fish bioindicators, complemented by histopathological biomarkers, provided a more robust and holistic assessment of ecological health in the Lake Tana wetlands. Among the surveyed sites, the WO wetland exhibited comparatively lower pollution levels and maintained stronger ecological integrity, in contrast to the more severely impacted GRM and MRM wetlands, which demonstrated critical ecological stress and thus warrant immediate restoration and management interventions.

In the case of *Labeobarbus* spp., poor water quality in ZG, GRM, and MRM wetlands contributed to reduced habitat quality, as reflected by lower Menhinick's and Hill's indices and a diminished fish-based index of biotic integrity. The integrated use of macroinvertebrate and fish bioindicators, combined with biomarkers, provided a comprehensive evaluation of the wetlands' ecological health. WO, the least impacted wetland, showed lower pollution levels and better ecological integrity compared to the highly polluted GRM and MRM wetlands, which urgently need intervention.

Multivariate techniques proved more effective than traditional indices (TSI and WQI) for classifying wetlands by water quality, distinguishing TSI and WQI into the least, moderately, and most polluted groups. Combining multivariate techniques with biodiversity and macroinvertebrate-based biotic indices further refined rankings based on macroinvertebrate community structure. The high diversity in ZG, AV, WO, and RA wetlands, likely linked to indigenous tree forests and Cyperus papyrus, indicated healthier ecological conditions and more stable, biodiverse habitats.

This study also revealed that multivariate techniques applied to fish bioindicators offer a more precise and effective approach for ranking wetlands, providing superior insights into ecosystem health compared to traditional diversity and biotic indices. The use of multiple bioindicators enables a more comprehensive assessment, capturing a broader spectrum of ecological conditions. Significant variations in indices such as the Habitat Quality Index (HQI) underscore the need for further investigation into the underlying drivers of species diversity and community composition. Commercial fishing in the Southern Gulf of Lake Tana wetlands (WO, AV, and RA) has led to a decline in fish diversity and biotic indices, whereas more remote and less accessible wetlands (MRM, ZG, and GRM) exhibit higher biodiversity and biotic integrity, likely due to reduced fishing pressure and more intact habitats.

This study demonstrated that multivariate techniques, when applied to fish bioindicators, offer a more accurate assessment of wetland health compared to traditional diversity and biotic indices. These techniques, combined with multiple bioindicators, provide a broader and more precise evaluation of ecological conditions. Significant variations in indices such as the Habitat Quality Index (HQI) highlight the need for further research into the drivers of species diversity and community composition. Commercial fishing in the Southern Gulf of Lake Tana wetlands (WO, AV, RA) has contributed to declines in fish diversity and biotic indices, while less accessible wetlands (MRM, ZG, GRM) show higher biodiversity, likely due to reduced fishing pressure and intact habitats.

The study also established tissue reaction patterns and histopathology indices, revealing that GRM and MRM are under severe ecological stress from pollution, while RA, WO, and AV are moderately impacted, and ZG remains the least affected. Variations in tissue damage across these wetlands underscore the value of histopathology as a tool for detecting ecological stress not evident through conventional water quality metrics. Fish Health Assessment Indices (HAI) also revealed similar patterns, with MRM, GRM, and RA showing the highest values, indicating significant pollution, while WO and ZG exhibited the lowest HAI scores. Abnormalities in critical organs such as the gills, liver, eyes, and skin were strong indicators of environmental stress and habitat degradation. These findings emphasize the importance of using integrated biomonitoring strategies, combining physical, chemical, and biological measures, to evaluate the health of aquatic ecosystems across Lake Tana's wetlands. Summary of integrated biomonitoring strategy using physical chemical and biological measures in six wetlands of Lake Tana in Figure 8.1

This study reveals significant seasonal variability in the health of Lake Tana, with water quality assessments showing marked fluctuations depending on both wetland and season. The interaction between wetland characteristics and seasonal factors is pivotal, driving temporal changes that either enhance or degrade water quality. Macroinvertebrate and fish populations, including abundance and diversity of macroinvertebrate and fish populations, also exhibit seasonal changes, reflecting the dynamic and adaptive nature of wetland ecosystems. Seasonal variations in fish health indicators, such as gill and gonadal damage, along with alterations in spleen size, were linked to environmental variables including water levels, temperature, food availability, and pollutant concentrations. These findings align with previous reports on seasonal fluctuations observed in the Tana sub-basin (Wondie et al., 2007b; Jemberie et al., 2016; Tesfaw et al., 2024). The Lake Tana sub-basin, located between latitudes 9°N and 13°N, is characterized by a complex seasonal pattern influenced by the region's topography, elevation gradient, and the dynamics of the Intertropical Convergence Zone (ITCZ). This region, with its tropical climate, experiences four distinct seasons: the main rainy season (June–September), the dry season (December–February), the early rainy season (March-May), and the late rainy season (October–November). The varying altitudes, ranging from 1,800 meters in the highlands to lower lake-side elevations, create localized microclimates and orographic effects that modulate rainfall distribution. Additionally, the seasonal migration of the ITCZ plays a critical role in regulating precipitation patterns, with its northward shift initiating the main rainy season and its southward retreat ushering in the dry season (Conway & Hulme, 1993; Camberlin et al., 2009).

This study highlights the multifaceted nature of aquatic ecosystem health assessment, asserting that water quality alone, while critical, does not provide a comprehensive evaluation of lake and wetland conditions. Water quality indicators, such as nutrient concentrations and chemical pollutants, are vital for understanding environmental stressors; however, the macroinvertebrates only represent a subset of the ecological dynamics within these ecosystems. These water quality parameters are inextricably linked to other ecological components, including biodiversity, fish health, habitat quality, and hydrological conditions, all of which influence the overall integrity of the ecosystem. Provided that these factors interact in complex ways, relying solely on water quality metrics may obscure crucial insights into the broader ecological health of an aquatic system. For instance, shifts in biodiversity and alterations in fish health or habitat quality can reflect underlying environmental changes that are not captured by traditional water quality measures alone (Postel & Carpenter, 1997; Carpenter et al., 1998; Nguyen et al., 2015; Gezie et al., 2020). Therefore, an integrated, multi-indicator approach, encompassing not only water quality but also biodiversity assessments, fish health indices, habitat quality evaluations, and hydrological factors, provides a more comprehensive and nuanced understanding of ecosystem health (Carr & Neary, 2006; Duarte et al., 2008; Dalzochio et al., 2016b). This approach is essential for identifying the full scope of ecological stressors and formulating effective conservation and management strategies.

The study found variability in wetland health rankings when using different biological assessments. This variability stems from the fact that biological indicators, such as fish and macroinvertebrate diversity, habitat quality, and fish health, may respond differently to environmental pressures and seasonal changes. For instance, macroinvertebrates are highly sensitive to changes in water quality and can provide early signals of environmental stress (Barbour, 1999), while fish health may reflect longer-term trends (Schwaiger et al., 2004). This variability indicates the need for an integrative approach to wetland health assessments, which accounts for the diversity of biological indicators and the responses of biological indicators to both short-term and long-term environmental factors (Plisnier et al., 2022). The idea of using a multi-indicator approach is comprehensive, but there may be instances where this approach could be too resource-intensive or difficult to implement at large scales. The need for careful data integration and interpretation is crucial, but practical limitations such as time, funding, and personnel may impede such comprehensive assessments. While this is acknowledged, it would be useful to discuss potential strategies or alternatives for mitigating these challenges. Consider exploring the development of cost-effective monitoring frameworks that incorporate a subset of key bioindicators that can be easily assessed, allowing for more widespread use without compromising the overall ecological insight.

The study also addresses the limitations of multivariate techniques used in aquatic environmental quality assessments. While such methods can be valuable for analyzing complex datasets, the methods are sensitive to data assumptions, outliers, and ecological relevance, and may not detect all types of stressors, such as emerging pollutants or endocrine disruptors (Mukherjee & Bhattacharya, 1982; Moss et al., 2003). The effectiveness of multivariate assessments relies heavily on careful data interpretation and integration with ecological knowledge, emphasizing the need for complementary monitoring techniques (Hellawell, 1991). The study acknowledges the limitations of multivariate techniques such as sensitivity to data assumptions, outliers, and failure to detect emerging pollutants. This is an important point, but the critique could be expanded by emphasizing the potential trade-offs between the complexity of multivariate analyses and the ecological interpretability of multivariate analysis. Future studies could consider combining multivariate analysis with simpler, more intuitive metrics to balance complexity and interpretability.

Macroinvertebrates and fish are commonly used as bioindicators of aquatic ecosystem health. Macroinvertebrates are particularly useful because they respond rapidly to changes in water quality, and macroinvertebrate diversity can reflect shifts in the overall health of the ecosystem (Barbosa et al., 2001; López-López & Sedeño-Díaz, 2015a; Sumudumali & Jayawardana, 2021; Hu et al., 2022). However, both macroinvertebrates and fish may fail to detect subtle environmental stressors or pollutants, such as endocrine disruptors or pharmaceuticals, which can go undetected in traditional assessments (Mukherjee & Bhattacharya, 1982; Van der Oost et al., 2003). Furthermore, the study highlights the resource-intensive nature of fish and macroinvertebrate studies, which require specialized equipment and trained personnel and can yield inconsistent results due to the different sensitivities of species to environmental changes (Barbour, 1999). The study points out the failure of bioindicators, particularly macroinvertebrates and fish, to detect subtle stressors like endocrine disruptors and pharmaceuticals. While this is an important consideration, it may seem like an overly broad critique, especially considering that bioindicators are still valuable for detecting many other types of stressors. A more nuanced suggestion could be made regarding the integration of bioindicators with more specific molecular or chemical assessments to address the detection gaps for these subtle pollutants. Additionally, exploring which bioindicators are most effective for detecting different stressors would improve the clarity of the argument.

The study points out that fish and, macroinvertebrate studies can be resource-intensive, requiring specialized equipment and trained personnel. However, this point may need further exploration, as it could imply that such monitoring is inherently impractical. This could deter future researchers or policymakers from considering bioindicator-based approaches. A possible direction could be to suggest more efficient methodologies or technological advances, such as remote sensing or automated systems for macroinvertebrate and fish monitoring, that could reduce the resource burden. Another suggestion would be to explore the possibility of citizen science or community-based monitoring programs that could complement more detailed scientific efforts.

Fish histopathology indices are a valuable tool for detecting the sub-lethal effects of environmental stressors. These indices provide high-resolution insights into the impacts of pollutants and stressors on fish tissue, offering important information on aquatic ecosystem health (Schwaiger et al., 2004). However, histopathology is costly, time-consuming, and may not establish a direct link between observed tissue damage and specific environmental factors. To improve the utility of histopathology, the study recommends combining these assessments with other monitoring techniques, such as molecular biomarkers or water quality testing, to create a more robust and comprehensive understanding of aquatic health (Dalu et al., 2021; Calisi, 2023). While combining histopathology with other monitoring tools such as molecular biomarkers is a strong suggestion, there is an underlying assumption that all such approaches would be equally effective across different wetlands and environmental conditions. It might be helpful to discuss the potential challenges and limitations of combining these methods, especially in terms of applicability to different ecosystems and the cost-benefit trade-offs. Some methods may be more suitable for certain types of pollutants, while others may excel in different conditions.

The study categorizes wetlands based on pollution severity using an integrated approach. The results show that wetlands with the lowest pollution levels (WO) had stable water quality, high biodiversity, and good habitat quality. Wetlands with moderate pollution levels (ZG, RA, AV) exhibited reduced biodiversity and elevated nutrient concentrations, while severely polluted wetlands (MRM, GRM) showed poor water quality, high pollutant concentrations, and significantly reduced biodiversity, signaling advanced ecological degradation. This classification underscores the utility of integrated assessments that combine water quality, biological indicators, and habitat conditions to determine the pollution severity and ecological status of wetlands. The classification of wetlands based on pollution severity using an integrated approach is a sound conclusion, but the study might face resistance in its application across different regions or types of wetlands, especially when baseline data or access to certain metrics (e.g., fish health and histopathology) is lacking. Future work could focus on developing scalable, universally applicable methods for wetland classification that could be used in a variety of geographical locations and that require fewer resources. Additionally, expanding the ecological classification system to incorporate more dynamic ecological factors (such as seasonal variations and long-term ecological trends) could further refine the classification accuracy.

This study emphasizes the importance of using a comprehensive, multi-indicator approach when assessing the health of wetlands and lakes. Relying solely on water quality can obscure critical ecological components such as species diversity and fish health. The variability observed in biological assessments across different wetland sites highlights the complexity of aquatic ecosystems and the need for integrative, multi-faceted evaluations. Moreover, the limitations of bioindicator approaches, particularly regarding the detection of emerging pollutants and the need for resource-intensive methods, suggest the necessity of combining traditional monitoring techniques with more advanced molecular and diagnostic tools. Through integrating water quality, biological indicators, and habitat assessments, researchers can obtain a more accurate understanding of ecosystem health, which is essential for the development of effective conservation strategies. Future research should continue to refine these methods and focus on improving the detection of subtle environmental stressors to better protect aquatic ecosystems. The suggestion for future research to focus on improving the detection of subtle environmental stressors is an excellent one. However, it could benefit from more specific recommendations on how to refine these methods. Broad statements about future directions may not sufficiently guide future studies in terms of practical application or method refinement. It would be useful to outline specific research areas or techniques for advancing the detection of emerging pollutants, such as the development of more sensitive molecular assays, or the incorporation of nanotechnology for pollutant detection in aquatic systems.

To strengthen the integration of findings, this study proposes a tiered aquatic monitoring framework that links the different classification methods used. Physicochemical parameters provide baseline water quality conditions, while macroinvertebrate indices offer early biological responses to pollution gradients. Fish community structure and biotic integrity indices reflect broader ecological impacts, including habitat degradation and fishing pressure. Histopathological and health assessment biomarkers serve as sensitive indicators of chronic and sub-lethal stress.

Together, these tools form a complementary system: physicochemical and macroinvertebrate data guide routine monitoring, while fish-based and histological assessments are used for diagnostic confirmation and long-term ecological evaluation. This integrated approach enhances the resolution and reliability of wetland classification and supports adaptive management strategies for aquatic ecosystems.

This general discussion integrates the study's four objectives into a unified aquatic monitoring framework. Objective 1 established baseline environmental conditions using physicochemical parameters. Objective 2 demonstrated how macroinvertebrates and fish communities respond to pollution gradients, biologically validating water quality classifications. Objective 3 identified organ-level responses through fish histopathology, offering sensitive biomarkers of chronic stress. Objective 4 applied fish health indices for practical, field-based monitoring. Together, these objectives support a tiered strategy: physicochemical and macroinvertebrate data for routine assessments, fish-based indices for ecological integrity, and histopathology for diagnostic confirmation. This integration enhances wetland classification resolution and supports adaptive management of Lake Tana's aquatic ecosystems.

To illustrate the application of this framework, the outcomes of the *Labeobarbus* health biomarker examination revealed clear spatial and seasonal patterns in organ-level responses to pollution. Fish from Megech River Mouth (MRM), Gumara River Mouth (GRM), and Avaj (AV) exhibited the most severe histopathological alterations, particularly in gill and liver tissues, including necrosis, hyperplasia, and inflammatory infiltration. In contrast, Zewdie Girar (ZG) and Wonjeta (WO) showed minimal tissue damage, indicating better ecological conditions. Health assessment indices further confirmed these patterns, with a higher prevalence of external anomalies and internal organ discoloration in more polluted wetlands. These findings demonstrate the diagnostic value of *Labeobarbus* spp. as a sentinel species and underscore the utility of histopathology and health indices in detecting sub-lethal and chronic environmental stress.

Building on these integrated findings, this study recommends a combined monitoring strategy that leverages the strengths of each assessment method. Physicochemical assessments should serve as the first-tier screening tool for identifying potential pollution hotspots. Macroinvertebrate indices can then be used to validate these findings through biological responses. In areas where stress is suspected or confirmed, fish-based indices and histopathological biomarkers should be applied for deeper diagnostic insight. This tiered approach allows for efficient resource allocation, early detection, and long-term ecological evaluation. The integration of these methods not only enhances classification accuracy but also supports a scalable, adaptive monitoring framework for freshwater ecosystems like Lake Tana.

This study makes a significant contribution to the field of aquatic environmental health in Ethiopia by pioneering the application of a bioindicator-biomarker approach for the first time. Bioindicators and biomarkers serve as crucial tools for assessing the health of aquatic ecosystems, offering insights into the environmental conditions affecting aquatic organisms. By incorporating these methodologies, this research provides a more comprehensive and precise means of evaluating water quality and ecological integrity in the context of Ethiopia’s freshwater systems. This innovative approach will be instrumental in informing policy development, shaping future research agendas, and guiding the design of effective environmental management strategies not only within Ethiopia but also across the broader Eastern African region, where freshwater ecosystems face increasing threats from climate change, population growth, and industrialization.

The study’s comprehensive design, which includes data collection across four distinct seasons, is another key strength. By incorporating seasonal variability in both the physical and chemical properties of water as well as biological measures, the research presents a detailed and dynamic understanding of how seasonal changes influence aquatic ecosystem health in Ethiopian highland lakes. This seasonal approach is crucial, as it allows for the identification of temporal patterns and potential seasonal stressors that may impact the aquatic biota, which has often been overlooked in prior studies. The study’s findings offer valuable insights into the temporal variability in aquatic health, facilitating better-informed environmental management practices tailored to specific seasonal conditions.

Moreover, unlike previous studies that have primarily focused on benthic macroinvertebrates as indicators of water quality, this study innovatively includes both water-column and benthic macroinvertebrates. This broader scope enhances the accuracy of the bioassessment and reflects a more holistic view of the ecosystem's overall health. The inclusion of both habitats allows for a more nuanced understanding of the interrelationships between water-column organisms and benthic communities, which are both vital components of freshwater ecosystems. The use of both benthic and water-column macroinvertebrates, alongside water quality indicators, will contribute to the development of applied diversity and biotic indices that can be used to assess aquatic environmental health in Ethiopia and the wider Eastern African region. These indices are essential for establishing reliable monitoring frameworks to assess the long-term health of aquatic environments, offering a foundation for future environmental policies and conservation efforts.

Through utilizing regionally relevant species like fish and macroinvertebrates, the research tailors bioindicator methods to Ethiopia's unique ecological conditions. This approach not only advances the understanding of local freshwater health but also enhances the practical application of these techniques in other regions, such as sub-Saharan Africa, where data on aquatic biodiversity is scarce (Holt & Miller, 2011; Mgwede & Msiska, 2018; Dalu et al., 2021; Pastorino & Barceló, 2024). Furthermore, the development of predictive models using bioindicators offers a proactive way to forecast ecological changes and manage freshwater systems more effectively (Moss et al., 2003; Duarte et al., 2008). This study promotes for using bioindicators and biomarkers in regions with limited resources, but this could be perceived as an ambitious goal considering the potential cost and complexity involved in setting up these monitoring programs. In regions where infrastructure and technical capacity are lacking, implementing advanced ecological tools could face significant barriers. To address this challenge, the study could propose strategies for lowering the costs associated with these techniques, such as through collaboration with local universities, non-governmental organizations, or international agencies. Another avenue could involve developing low-cost, mobile kits for field-based analysis that require minimal training and equipment.

The introduction of biomarkers enhances the detection of sub-lethal impacts of pollutants, providing an additional layer of sensitivity in monitoring aquatic ecosystems. These biomarkers, together with bioindicators, offer a comprehensive framework for assessing ecological health, crucial in areas where ecosystems are under threat from anthropogenic activities (Lusk et al., 2004; Ferizi et al., 2020; Calisi, 2023) The concept of using predictive models based on bioindicators to forecast ecological changes is promising, but it may not always be feasible in regions with limited data or where ecological understanding is still in its infancy. The accuracy of predictive models depends heavily on having sufficient baseline data and well-understood ecological dynamics, which may not be readily available. The study could suggest a phased approach, beginning with data collection and baseline ecological assessments before implementing predictive models. Additionally, collaboration with local environmental authorities to improve data accuracy and model calibration could be emphasized to ensure more reliable outcomes.

Incorporating seasonal dynamics into water quality assessments further improves the precision of monitoring, allowing for more context-sensitive management strategies. Seasonal variations, such as nutrient loading or temperature fluctuations, significantly affect aquatic organisms, and understanding these changes is essential for adaptive management (Carpenter et al., 1998; Schindler et al., 2016). The emphasis on incorporating seasonal dynamics into water quality assessments is crucial, but it may add another layer of complexity to monitoring programs, especially in settings where regular monitoring is already a challenge. Seasonal variations can create unpredictable and inconsistent data that may make it harder for local authorities to act on time. Consider recommending a more targeted approach to seasonal assessments. For example, conducting focused seasonal studies on specific critical periods including peak agricultural runoff seasons or temperature extremes) could provide clear insights without overburdening monitoring systems.

Through developing cost-effective, field-based biomarkers using native species like *Labeobarbus* spp., this research provides a practical solution for in-situ water quality assessments in regions with limited resources. It empowers local communities, policymakers, and researchers to independently monitor water quality and make informed decisions (Naigaga, 2012; Chirwa & Chilima, 2017; Dalu et al., 2021; Mulenga et al., 2024; Kitaka et al., 2024). This approach not only enhances the sustainability of freshwater management in Ethiopia but also offers a scalable model that can be applied globally, contributing to the protection of freshwater ecosystems in similar ecological and environmental contexts (Carpenter et al., 1998; Jeppesen et al., 2005). In conclusion, this research introduces innovative methods for ecological monitoring, including predictive modeling and the use of bioindicators and biomarkers, to improve the sustainable management of freshwater resources. It provides a scalable framework for environmental monitoring and contributes to informed decision-making in regions facing ecological challenges. Through capacity-building and the integration of local knowledge, this work supports long-term freshwater conservation globally.

Even though the development of cost-effective field-based biomarkers is an admirable goal, there may be limitations in terms of how universally these can be applied, especially when considering the variety of environmental conditions in Ethiopia. *Labeobarbus* spp., for instance, may not be representative of other regions or ecosystems within the country or sub-Saharan Africa. The study could propose developing a broader toolkit of native species or generalizable biomarkers that can be applied across different regions, rather than focusing solely on *Labeobarbus* spp. or other species that might not be ubiquitous. Additionally, exploring the use of molecular or remote sensing technologies could help broaden the applicability of in-situ assessments. While the research advocates for empowering local communities to monitor water quality and make informed decisions, this approach may face resistance due to low community engagement or insufficient local capacity to understand or act on monitoring results. The effectiveness of citizen science or community-based monitoring programs can vary significantly depending on local education levels and resource availability. The study could include a more detailed discussion of capacity-building strategies that go beyond training programs, such as involving local stakeholders in the design and implementation of monitoring programs, ensuring that the knowledge generated is accessible, and integrating local knowledge into ecological assessments. Additionally, community-level programs should be paired with local infrastructure support and long-term funding to ensure sustainability.

During this study, some challenges and limitations were encountered, which may have influenced the quality and interpretability of the data. The primary challenges encountered during this study included the difficulty in clearly delineating the boundaries of each sampling site prior to initiating data collection. The lack of precise and well-defined demarcation led to uncertainties regarding the spatial extent of the study areas. Additionally, the required unit area for sampling within the six wetlands was not specified, which further complicated the establishment of consistent sampling protocols. These ambiguities in site definition and spatial area requirements posed significant hurdles in ensuring uniformity and accuracy in the data collection process, thereby impacting the overall reliability of the study's findings. While water samples were preserved consistently throughout the study periods, the delay in conducting water quality tests at the end of data collection may have introduced potential biases. In-situ measurements offer a more accurate representation of real-time water quality conditions, as the in-situ measurements minimize the effects of storage and handling. Consequently, the accuracy of the laboratory-based analyses may be compromised to some extent, potentially leading to less precise and reliable results compared to immediate, on-site testing.

Though histopathological alterations and anomalies observed in different organs and tissues of *Labeobarbus* spp. in this study suggest potential physiological stress, a definitive causal link to pollutants discharged into the lake and its adjacent wetland remains inconclusive. Further controlled studies are necessary to establish a direct correlation between these observed lesions and specific pollutant(s). Additionally, expanding the scope of investigation to include other fish species within the lake ecosystem would provide a more comprehensive understanding of the potential impacts of environmental stressors on the overall aquatic community.

The data collected for fish bioindicator studies can be significantly influenced by seasonal variations, particularly during breeding periods. For instance, species like *Labeobarbus* spp., known to aggregate in river mouths for spawning from July to September, may exhibit altered behavior and distribution patterns during this time. Similarly, *O. niloticus* and *C. gariepinus*, with peak breeding seasons in March-April and early July, respectively, may also show seasonal fluctuations in abundance and activity. These seasonal variations can impact key bioindicator parameters such as catch per unit effort, relative abundance, fish diversity, and biotic indices, potentially leading to biased interpretations if not accounted for in the analysis. To mitigate this, it is crucial to consider the reproductive cycles of target species and design sampling strategies that account for seasonal variability.

This thesis established that macroinvertebrate diversity data offer a more accurate and long-term assessment of lake health compared to traditional water quality metrics, as macroinvertebrates reflect the cumulative impacts of environmental stressors over time. While water quality measurements, such as pH, dissolved oxygen, and nutrient concentrations, capture the immediate state of the environment, macroinvertebrates may fail to account for the chronic and cumulative effects of pollution, habitat degradation, and other stressors (Cairns & Pratt, 1993). Macroinvertebrates, being sensitive to fluctuations in water quality, integrate these stressors over extended periods, providing valuable insights into the lasting impacts on aquatic ecosystems (Clements & Kiffney, 1994; Flores & Zafaralla, 2012). The diversity of macroinvertebrates, including the relative abundance of sensitive and tolerant species, can serve as an indicator of overall water quality, habitat degradation, and pollution levels (Barbour, 1999; Bass, 2007). Therefore, macroinvertebrate data are essential for understanding the long-term ecological health of a lake, as macroinvertebrates reflect not only the immediate water quality but also its historical and ongoing environmental changes.

In contrast, fish health assessments can provide more detailed information on the direct effects of environmental stressors. Fish, being more complex organisms than macroinvertebrates, have a range of organ systems, including gills, liver, spleen, kidneys, and gonads, which are directly affected by pollutants and environmental disturbances (Osman et al., 2010; Reddy, 2012; Yancheva et al., 2016; Jaffer et al., 2017; Abdallah et al., 2024). Histopathological analyses of these organs can reveal specific damages caused by toxins, nutrient overload, or habitat degradation, thus offering insights into the impacts of pollution at a more granular level (Liebel et al., 2013). Fish also tend to have longer lifespans than macroinvertebrates, allowing fish to accumulate the effects of environmental stressors over a more extended period, thus serving as a bioindicator for long-term ecological changes (López-López & Sedeño-Díaz, 2015a; Gouda et al., 2024). This capacity to reflect the chronic impacts of pollution and habitat degradation makes fish health assessments an important tool for understanding ecological trends that may not be immediately apparent from macroinvertebrate data alone.

This thesis further demonstrates the benefits of combining macroinvertebrate and fish health assessments as a robust, integrated approach for evaluating lake health. Using multivariate techniques, diversity indices such as Shannon-Wiener Diversity and Margalef's richness index are ideal for analyzing macroinvertebrate data, as macroinvertebrates capture the richness and evenness of species present, which are sensitive to environmental changes (Magurran, 2005).

Biotic indices, including the BMWP (Biological Monitoring Working Party), EPT (Ephemeroptera, Plecoptera, and Trichoptera), and Hilsenhoff's richness index, are also effective in assessing macroinvertebrate communities and the relationship of biotic indices with water quality (Hilsenhoff, 1988). For fish health, indices such as the Habitat Quality Index, Hill's Index, and Fish-Biotic Integrity Index (FBI) are commonly used to assess the ecological status of fish populations in relation to habitat conditions (Hill, 1973; Karr, 1981). These indices consider various factors such as species composition, the percentage of sensitive species, and overall fish health, providing a comprehensive assessment of aquatic ecosystems. Additionally, the percentage of Cyprinid individuals, a commonly used metric, can indicate the level of pollution, as these species are often more tolerant to degraded conditions (Abdissa et al., 2022).

This thesis suggests ensuring reliable and statistically significant results, sample sizes for macroinvertebrates should include at least 100-300 individuals per site, and for fish, at least 20-30 individuals per species. These sample sizes allow for more accurate biodiversity measures and health assessments, ensuring that the data is representative of the overall ecosystem (Needham et al., 2007; Zhou et al., 2008; Friberg et al., 2011). Beyond the indices mentioned, other fish health indicators, such as fin erosion, shortening of opercula, gill lesions, inflamed pseudobranch and thymus, liver discoloration, and the presence of ectoparasites, provide further insight into the health of fish populations and the quality of aquatic environment (Nachev & Sures, 2016; Jerônimo et al., 2022; Omeji et al., 2022; Sures et al., 2023). These additional indicators offer a more nuanced understanding of how environmental stressors affect aquatic organisms, making indicators indispensable for a holistic assessment of wetland health. In integrating both macroinvertebrate and fish health assessments, this thesis emphasizes the importance of using multiple ecological indicators to assess the overall health of aquatic ecosystems. This dual approach not only provides a more accurate and holistic evaluation of environmental conditions but also contributes to the development of effective management and conservation strategies for freshwater ecosystems in Ethiopia.

In this thesis, it was explored that when parasite intensity and prevalence are low, fish pathology becomes a crucial tool for assessing the health of aquatic ecosystems. While parasitological data provide valuable insights into the presence and abundance of specific parasites, the parasites often focus on acute, high-intensity infections and do not fully capture sublethal, long-term stressors that may affect fish populations and overall ecosystem health. In contrast, fish pathology allows for a more detailed understanding of the physiological and biochemical impacts of environmental stressors, pollutants, and habitat degradation (Jerônimo et al., 2022). Pathological changes in fish organs such as lesions, tissue inflammation, or organ degeneration are indicative of sublethal effects that are not immediately apparent through parasitic load alone, thus providing a more comprehensive picture of the ecological impacts on aquatic organisms (Koskivaara, 1992; Santoro et al., 2020).

Fish pathology can reveal a range of sublethal effects, such as damage to internal organs, which are directly influenced by pollutants and environmental stressors (Liebel et al., 2013; Yancheva et al., 2016; Moreira et al., 2021). These effects often occur before visible signs of disease or mortality are detected, thus serving as early indicators of ecosystem decline. For example, chronic exposure to contaminants such as heavy metals, pesticides, or excess nutrients can impair liver function, disrupt immune responses, or cause reproductive failures (Rani et al., 2022). Furthermore, fish subjected to environmental stressors such as habitat degradation or temperature fluctuations may exhibit signs of immune suppression, making fish more susceptible to opportunistic infections (Yada & Tort, 2016; Weeks et al., 2018). Such physiological impairments can lead to reduced fish populations, altered community structures, and, ultimately, diminished ecosystem stability (Palm, 2011b; Roberts, 2012).

Fish pathology can also identify chronic stress responses that parasitological assessments alone may miss. While parasites may provide a snapshot of infection intensity, parasites are not always reflective of ongoing, low-level stressors that accumulate over time. For instance, studies have shown that fish exposed to suboptimal water quality may not exhibit high parasitic loads but could suffer from chronic internal damage, immune dysfunction, or endocrine disruption (Chen et al., 2017; Raibeemol & Chitra, 2020; Xu et al., 2021; Yang et al., 2021). These chronic impacts, although not immediately lethal, can have long-term consequences for fish populations and the broader ecosystem. Thus, when parasite prevalence is low, fish pathology offers a more nuanced assessment of ecological health by identifying the often-overlooked sublethal effects of pollution and environmental stress.

Combining both parasitological data and fish pathology provides a more reliable and comprehensive approach to monitoring aquatic ecosystems. Parasite data can serve as an initial indicator of ecological stress, revealing areas of potential concern where fish populations may be more vulnerable to infections. However, pathology adds another layer of depth, enabling the detection of more delicate, sublethal effects that could compromise long-term ecosystem health (Ogidi & Akpan, 2022; Sures et al., 2023). Integrating these two approaches ensures that a broader range of environmental impacts is captured, offering a more holistic view of ecosystem health. For instance, while parasites may thrive in areas with nutrient pollution, pathology can reveal underlying damage to fish organs that results from prolonged exposure to contaminants, even in the absence of high parasite loads. This combined methodology allows for a more robust assessment of both the immediate and long-term ecological effects of environmental changes, leading to more effective management and conservation strategies (Rice, 2003; Münzel & Daiber, 2018). In conclusion, while parasites provide useful insights into aquatic ecosystem health, fish pathology should be prioritized when parasite intensity and prevalence are low. Combining both parasite data and pathological assessments enhances the reliability and depth of ecosystem health evaluations, leading to more informed conservation and management strategies.

8.1. Proposed strategy for the integrated application of fish and macroinvertebrate bioindicators and fish and fish health assessment biomarkers to assess the aquatic environmental quality in wetlands of Lake Tana, Ethiopia

As outlined in Chapter 1, Ethiopia's current approach to assessing aquatic environmental quality primarily relies on physicochemical analysis. However, this method often faces limitations due to irregular monitoring caused by financial constraints (Eriksson, 2012; Pinna et al., 2023). Additionally, the complex interplay of various pollutants and the potentially toxic effects of the pollutants poses challenges to water quality management solely based on chemical or physicochemical data (Atumo Ante et al., 2023; Mekonnen et al., 2023; Woretaw et al., 2023; Tadesse & Lakew, 2024). To bolster the restoration and preservation of biological integrity in Ethiopia's aquatic ecosystems, this study proposes the integration of biological surveys alongside physicochemical assessments within national water quality programs. This integrated approach will provide a more comprehensive and ecologically relevant evaluation of water quality and ecosystem health.

Biomarkers are effective early detection tools for contaminant-induced impacts at the sub-organismal level, providing proactive insights into environmental stress before significant changes in community structure occur (Adams et al., 1993; Van der Oost et al., 2003; Crafford & Avenant-Oldewage, 2009; Wepener, 2008; Naigaga, 2012; Gouda et al., 2024). In contrast, shifts in community composition reflect more advanced ecosystem degradation and thus do not serve as early warning indicators. While physicochemical measurements offer essential snapshots of water quality, physicochemical lack the biological specificity required to assess ecosystem health comprehensively. These variables do not capture critical biological processes that drive ecosystem function. Therefore, integrating biomarkers, community structure assessments, and physicochemical data in a multi-indicator approach enhances the sensitivity of water quality monitoring, providing a more nuanced understanding of environmental stressors and informing more effective management of aquatic ecosystems.

Sustainable water quality and productivity in Lake Tana require an integrated management approach that combines regular monitoring of physicochemical variables, macroinvertebrate and fish diversity, and comprehensive fish health assessments. This study introduces the use of macroinvertebrate and fish health biomarkers as a novel tool for assessing water quality in Ethiopian freshwater ecosystems, marking the first application of such an integrated framework in the country. To ensure its broader applicability, the strategy should be tested in other ecosystems within the ecoregion, with adjustments made to reflect local ecological and geographical variations.

Implications for Region-Specific Assessment Tools

The findings of this study offer several valuable insights for developing region-specific aquatic assessment tools in Ethiopia and similar tropical freshwater systems:

Ecological Relevance of Local Species: Native fish (*Labeobarbus* spp., *Oreochromis niloticus*) and macroinvertebrate families (e.g., Baetidae, Chironomidae, Tubificidae) demonstrated predictable responses to pollution gradients. These taxa are ecologically adapted to local conditions and should be prioritized in future monitoring frameworks.

Sensitivity to Seasonal and Spatial Variability: The study revealed that pollution impacts varied significantly across seasons and wetland types. This underscores the need for assessment tools that incorporate seasonal sampling and account for hydrological and land-use differences across regions.

Biomarker Utility in Low-Resource Settings: Histopathological and fish health assessment biomarkers provided early-warning signals of sub-lethal stress, even in wetlands with similar physicochemical profiles. These tools are cost-effective and scalable for use in data-limited regions.

Framework for Indicator Selection: The integrated approach used in this study through combining physicochemical, bioindicator, and biomarker data can serve as a model for designing region-specific indices of biotic integrity and ecological health.

These insights support the development of tailored monitoring protocols that reflect Ethiopia's unique ecological, climatic, and socio-economic contexts. They also highlight the importance of adapting global biomonitoring tools to local realities for more accurate and actionable environmental assessments.

This study provides a valuable approach by offering early detection and prediction of the potential long-term effects of current or past exposure.

- It is crucial to continually assess the effectiveness of the monitoring strategies in place. This includes reviewing how well current programs capture changes in the lake's ecosystem and water quality. Regular evaluations help identify any gaps in data or areas where methods may need adjustment to better reflect the true state of the lake's health. By keeping a consistent check on the performance of monitoring efforts, researchers can ensure the strategies remain responsive to changes and provide timely insights into the environmental conditions of the lake.

- Based on periodic reviews of monitoring data, it is necessary to adjust the techniques and tools used for data collection. Refining methods may involve upgrading sensors, incorporating new technologies, or adopting different sampling approaches to enhance the accuracy of measurements. As lake conditions evolve, so too must the monitoring techniques to ensure the monitoring remains effective in capturing the most relevant and up-to-date information. This process helps maintain the precision of the data collected, ensuring that monitoring is both accurate and meaningful.

- Regularly reviewing and revising monitoring protocols is key to maintaining the quality and consistency of the data collected. This involves assessing both the procedural aspects of the monitoring process and the scientific frameworks behind the protocols. By making adjustments to protocols—whether it's refining data collection frequencies, improving sampling locations, or updating analysis methods, researchers can ensure that the data remains reliable over time. Continually updating protocols also helps address emerging environmental concerns, such as new pollution sources or changes in local ecosystems.

- Updated, reliable data is essential for making informed management decisions. By ensuring that monitoring programs are producing high-quality and current data, decision-makers can better understand the challenges facing Lake Tana and the broader impacts on the local environment. Accurate and up-to-date information serves as the foundation for evidence-based decisions, guiding actions that are both appropriate and effective in preserving the health of the lake. This ensures that management efforts are well-targeted and capable of addressing the most pressing environmental issues.

- Refined and continuously updated monitoring methods play a central role in improving the management and conservation of Lake Tana's ecosystem. By adapting monitoring strategies to reflect the most recent environmental data, managers can more effectively identify trends, anticipate potential threats, and implement timely interventions. This proactive approach helps safeguard the lake's ecological balance, improve water quality, and protect biodiversity, ultimately contributing to the long-term sustainability of the lake's ecosystem.

- This thesis highlights the significance of integrating macroinvertebrate and fish health assessments, employing a multi-indicator approach to provide a more precise and comprehensive evaluation of aquatic ecosystem health. By combining these ecological indicators, this methodology offers enhanced sensitivity to both short-term and cumulative environmental stressors, thereby improving the robustness of environmental monitoring. Furthermore, this integrated approach facilitates the formulation of more informed and effective management and conservation strategies for freshwater ecosystems in Ethiopia and similar regions.

8.2. Conclusions

Although eutrophication is widespread, the most severe pollution from agricultural and urban effluents was found to be localized, primarily affecting the lake's eastern, northern, and southern regions. This localized pollution has a differential impact on the health of the lake's ecosystems. Analysis of the wetlands around Lake Tana revealed varying levels of impact. Wetlands such as WO were minimally impacted, while ZG, AV, and RA experienced moderate degradation, and GRM and MRM were highly impacted by pollution. This variation indicates the spatial complexity of pollution's effects on water quality and ecosystem health.

The study highlights that agricultural and urban effluents contribute to the deterioration of key physicochemical parameters, leading to a loss of macroinvertebrate and fish species diversity, and severe histopathological damage in fish populations. These pollutants are likely responsible for changes in community structure and a decline in the overall ecosystem health of the lake.

This study employed a multi-indicator approach, integrating physicochemical parameters, macroinvertebrate and fish community structures, alongside fish health assessments, which proved effective in determining pollution levels and their ecological effects. The use of fish histopathology and health assessment indices offered valuable insights into the biological impacts of pollutants, enabling the detection of early signs of environmental stress and ecosystem degradation.

The significant histopathological responses observed in MRM and GRM wetlands call for immediate intervention to mitigate pollution levels and restore the health of these critical ecosystems. These areas are highly impacted, and timely mitigation actions are essential to prevent further ecological decline.

Although biomonitoring efforts in Ethiopian aquatic ecosystems are limited, this study establishes a valuable framework for the future. It proposes the integration of multiple indicators such as water quality parameters**,** biological diversity**,** and fish health assessments to provide a more comprehensive understanding of pollution impacts and support the development of effective management strategies**.**

The study underscores the importance of long-term monitoring and integrated biomonitoring to assess and manage pollution impacts in Lake Tana. The findings emphasize the need for sustained efforts to mitigate agricultural and urban effluent pollution, protect aquatic biodiversity, and develop management strategies tailored to local environmental conditions.

This thesis highlights the importance of integrating macroinvertebrate and fish health assessments through a multi-indicator approach, providing a more accurate evaluation of aquatic ecosystem health. This methodology improves monitoring by capturing both short-term and cumulative stressors and supports the development of effective management and conservation strategies for freshwater ecosystems in Ethiopia and beyond.

8.3. A summary of recommendations

Key Indicators for Future Monitoring in Ethiopian Lakes

Water Quality Variables

- Nutrients: Total Nitrogen (TN), Total Phosphorus (TP), Nitrate (NO_3^-), Ammonia (NH_4^+), Soluble Reactive Phosphorus (SRP)
- Physicochemical Parameters: Dissolved Oxygen (DO), pH, Electrical Conductivity (EC), Salinity, Temperature, Secchi Depth, Total Dissolved Solids (TDS), Chlorophyll-a.

Bioindicators

- Macroinvertebrates: Families such as Baetidae, Caenidae, Aeshnidae, Chironomidae, and Tubificidae, assessed using diversity indices (Shannon-Wiener, Simpson, Margalef) and biotic indices (BMWP, HFBI, EPT Index)
- Fish Communities: Species such as *Labeobarbus* spp., *O. niloticus*, and *C. gariepinus*, were evaluated using the Fish Index of Biotic Integrity (FIBI), Habitat Quality Index (HQI), and trophic guild composition.

Biomarkers

- Histopathological Markers: Lesions in gill (e.g., hyperplasia, atrophy), liver (e.g., necrosis, granulomas), gonads (e.g., atrophy), and spleen (e.g., melano-macrophage hyperplasia)
- Health Assessment Indices: External anomalies, hematocrit levels, parasite load (inverted parasite index), and organ condition scores

These indicators offer a robust framework for early detection of sub-lethal stress and long-term ecosystem degradation. Their integration into national monitoring programs will enhance the effectiveness of freshwater conservation and management in Ethiopia. Overall, this chapter underscores the importance of incorporating seasonal variability and biological indicators into freshwater biomonitoring. The integration of macroinvertebrate and fish health assessments provides a holistic evaluation of aquatic ecosystem integrity and supports evidence-based conservation strategies.

This study recommends incorporating biomonitoring data into water pollution management decisions in Ethiopian water bodies. To enhance biomonitoring approaches for aquatic environmental quality assessment in Ethiopia, the following recommendations are proposed:

1. A major challenge observed during this study was the absence of the necessary equipment and facilities required for a standard monitoring program. To address this, the study proposes:

- Adopting the multi-lake monitoring framework used for the African Great Lakes in Lake Tana**.** Three stations are suggested for this purpose: Bahir Dar Fisheries and Aquatic Research Center (southern Gulf), Addis Zemen (eastern part), and Gorgora Fisheries and Research Center (northern part).
- Collecting long-term environmental data, including meteorological and biogeochemical information from both the surrounding areas and within the lake itself. Such data is crucial for developing and evaluating models of lake hydrology, hydrodynamics, and ecosystems, which are essential for sustainable management.
- Research stations on lake shores require (electricity, and water), bots and vehicles, hand-held probes – temperature - conductivity, pH, DO, CTD (conductivity, temperature, and depth probe), mooring and thermistors/possibly other sensors, underwater light sensors (automatic) and photometers and different kits for water quality tests are needed.
- At least one of the research stations on the lake shore requires an automated tissue processor with its accessories and chemicals, A Digital Haemoglobin Analysis Meter and hematocytometres should be available.
- Research stations require equipment for biodiversity monitoring (gears etc.) and automatic weather stations.

2. Another significant challenge observed during the study was the lack of taxonomic expertise for both fish and macroinvertebrates, often requiring direct consultations with experts. To enhance biomonitoring approaches in Ethiopia, efforts should be made to provide the necessary fundamental knowledge and expertise. To this effect, this study recommends:

    - Local macroinvertebrate and fish fauna should be inventoried and described**.** Many macroinvertebrate taxa and Cyprinidae fish species remain undescribed. User-friendly field guides and manuals with illustrations and photographs should be published. These resources will help biomonitoring technical teams address taxonomic challenges. Taxonomic and systematic descriptions should be conducted for the existing species flocks of the 18 large *Labeobarbus* spp. in Lake Tana. These descriptions should utilize morphometric, meristic, and DNA barcoding techniques.

- Train and equip personnel in biomonitoring techniques. This will ensure the availability of skilled technicians who can conduct water quality monitoring and inform management and regulatory decisions.
- Create a database on the distribution, abundance, and temporal characteristics of fish species and macroinvertebrate taxa in Ethiopia. The results of this study have been limited to one ecoregion. Macroinvertebrate and fish bioindicators have ecological relevance. With further testing, modification, and refinement, the strategy could be applied in assessment programs to evaluate environmental water quality deterioration in both agricultural and urban impacted ecosystems.
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3. With the growing interest in biomonitoring aquatic environments in Ethiopia, it's essential to test and refine existing biomonitoring protocols, including those developed in this study**.** This will improve the predictive power of monitoring and ensure accurate applicability of monitoring to various aquatic systems. Additionally, active biomonitoring using *Labeobarbus* spp. as a sentinel species should be conducted to evaluate and confirm the predictive capabilities of the observed histopathology biomarkers.

4. The study observed that larvae of *Ligula intestinalis* were widespread and caused significant pathological damage to the gonads of certain *Labeobarbus* spp., potentially leading to the extinction of some cyprinid fishes in Lake Tana and its tributary rivers. The study recommends further research on

- The epidemiology and genetic diversity of *Ligula intestinalis*
- The pathological changes associated with the parasite
- The relationship between ecological features of host species and *Ligula intestinalis* plerocercoid in cyprinid fishes within Lake Tana and its tributary rivers.

This information would be valuable for understanding the parasite's impact and developing effective management strategies.

5. Regular monitoring of WO wetlands is recommended to maintain its less polluted status. Moderately polluted wetlands (ZG, AV, and RA) and highly polluted wetlands (MRM and GRM) require immediate intervention to mitigate further degradation and restore degraded habitats. Toxicant-specific assessments should be conducted in highly polluted wetlands, such as MRM and GRM. Proper implementation and enforcement of existing wastewater discharge standards should be enforced.
6. The study observed that the combination of wetland type and season significantly impacted the water quality of wetlands, with higher pollution levels recorded in RA, AV, GRM, and MRM during the late rainy, rainy, and dry seasons. This highlights the importance of considering both wetland type and season in biomonitoring programs for Ethiopian water bodies.
7. This thesis highlights the importance of integrating macroinvertebrate and fish health assessments through a multi-indicator approach, providing a more accurate evaluation of aquatic ecosystem health.
8. This study highlights future biomonitoring efforts in Ethiopia incorporating genetic characterization of selected macroinvertebrates and *Labeobarbus* spp. using tools such as DNA barcoding and molecular markers.

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