ASSESSMENT OF THE BIOTIC INTEGRITY AND WATER QUALITY OF LAKE ZIWAY USING BENTHIC MACROINVERTEBRATE AND DIATOM BASED MULTIMETRIC INDEX

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Assessment of the Biotic integrity and Water quality of lake Ziway using Benthic Macroinvertebrates and Diatom Based Multimetric index

By

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A Thesis Presented to the School of Graduate Studies of the Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Biology (Fisheries and Aquatic Science)

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DECLARATION

I hereby declare that this thesis, entitled: A*ssessment of the biotic integrity and water quality of Lake Ziway using benthic macroinvertebrate and diatom based multimetric index* has been composed entirely by myself and has not been previously submitted for any other degree or qualification and complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ABSTRACT

Development of macroinvertebrate and diatom based multimetric index for the assessment and monitoring of Lake Ziway, Ethiopia

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Addis Ababa University, 2019

Human-induced stressors (urbanization, agriculture, and industrial effluent) are common around Lake Ziway. As a result, the lake is threatened and ecological functions of the lake are in question, and this requires urgent monitoring. Therefore, this study was conducted to assess the ecological quality of Lake Ziway using a composite diatom and macroinvertebrate multimetric index. Physicochemical and biological data were collected from 9 sites in the littoral zone between September 2015 and April 2016 in 4 rounds. Candidate references were proposed through *a priori* selection criteria. Physicochemical parameters were different among sites, except for pH, soluble reactive phosphorus (SRP), and NH_4^+. pH showed no significant change among sites as well during the last two decades. DO, EC, Total Phosphorus (TP), and NO_2, showed variation among the sites as the mean values ranged from 3.51±0.27 to 9.33±0.30 mg/L, 405±0.5 to 592±75.6 µS/cm, 0.14±0.02 to 0.51±0.45 mg/L, and 0.03±0.02 to 0.22±0.21 mg/L, respectively ($p<0.01$). Lake Habitat Quality Assessment score (LHQAs) displayed significant difference across site categories ($p=0.003$). References displayed highest average LHQAs values, while stressed sites exhibited least values. Thirty-four invertebrate families which belonged to 12 orders and 39 diatom species which belonged to 24 genera were identified. Hemipterans contributed (25.77%) abundance followed by Snails (18.57%) and Bivalves

(17.29%). The diatom communities were dominated by *Achnanthidium* sp*.*, *Ulnaria ulna*, and *Encyonopsis microcephala* with percent abundance of 12.5, 11.7, and 10.3, respectively. There was a significant variation in number of diatom species among sites ($p=0.003$). Diatom species in references (37) with 94% was higher than test sites (31) confined 79%. Hydropsychidae, Philopotamidae, and Polymitarcyidae showed higher percent abundance in reference sites, whereas Hirudinidae, Chironomidae and Oligochaeta can be considered reliable indicators of highly-stressed sites typified by low concentration of DO (3mg/L). The association of Chironomidae, Physidae, and Hirudinidae with NO_2 and NO_3 was strong and positive (Spearman correlation $p<0.05$). Polymitarcyidae and Hydropsychidae showed strong but negative association with most nutrient variables. *Cymbella kappii*, *Gomphonema augur*, and *Ulnaria ulna* were positively related to SRP and TP; and *Aulacoseira granulata* with pH. The most abundant species at the test sites were *Ulnaria ulna* and *Nitzschia* sp. which tolerate organic pollution and high nutrient load. Shannon diversity (H′) values of invertebrates and diatoms were different among sites (Kruskal- Wallis t $p<0.05$), and a considerable large H′ values were observed in references (2.69 and 2.09), and low values were noted in multiple stressors, (1.97 and 1.89), respectively. For developing Multimetric Index of Lake Ziway (MMIZ), 32 macroinvertebrate and 18 diatom metrics were evaluated for interquartile range overlap (discrimination efficiency), response to water quality parameters (spearman correlation test) and redundancies within candidate metrics (box-and-whisker plots). Out of 50 candidate metrics tested, 16 met the three criteria and were compiled for the multimetric index development using discrete scoring system. Therefore, four levels of discriminatory bio-criteria for water quality were eventually obtained: 16-32, poor; 33-48, fair; 49-64, good; and 65-80, very good. The MMIZ showed that water quality of the reference sites were very good, 1 site from the intermediary stressors was good, whereas 2 sites from the intermediary and 1 site from the multiple stressors were fair, and 2 sites from the multiple stressors were poor. Ecological Quality Ratio (EQR) was calculated by dividing MMIZ value by the median value of reference, and five classes were developed to rate the ecological quality status as very good (≥ 0.96), good ($\geq 0.72 < 0.96$), moderate ($\geq 0.48 < 0.72$), poor ($\geq 0.24 < 0.48$), and bad (< 0.24). Two sites were rated high quality; 2 sites good quality; 3 sites moderate quality; and 2 sites poor quality. The MMIZ showed positive correlation with lake habitat quality scores ($r=0.959$, $p<0.01$), which indicated good responsiveness of the multimetric index to variables of stressors. Validation of MMIZ using independent data set of Lakes Ziway and Hawassa indicated the MMIZ had good performance to discriminate between reference and non-reference sites. Therefore, the study indicated the potential use of a multimetric index to assess and monitor the habitat integrity and water quality in similar ecoregions in Ethiopia.

Keywords: Benthic Diatoms; Benthic Macroinvertebrates; Bioassessment; Lake Ziway; Metrics; Multimetric Index; Physicochemical; Stressors; and Water Quality.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
APHA	American Public Health Association
CRVLs	Central Rift Valley Lakes
D	Dominance
DCA	Detrended Correspondence Analysis
DO	Dissolved Oxygen
EC	Electrical Conductivity
EQR	Ecological Quality Ratio
H'	Shannon Diversity Index
IS	Intermediate Stressors
J	Equitability Index
LHQA	Lake Habitat Quality Assessment
LHQAs	Lake Habitat Quality Assessment score
LHS	Lake Habitat Survey
MMIZ	Multimetric Index of Lake Ziway
MoWR	Ministry of Water Resources
MS	Multiple Stressors
PCA	Principal Component Analysis
RDA	Redundancy Analysis
RS	Reference Sites
RVLE	Rift Valley Lakes of Ethiopia
SRP	Soluble Reactive Phosphorus
TP	Total Phosphorus
TSS	Total Suspended Solids
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive

CHAPTER 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

Human-induced stressors (e.g. urbanization, agriculture, logging, sewage contamination, industrial effluent, and others) alter the aquatic environments and organisms by changing their habitat structure, water quality, energy source, and biotic interactions (Karr, 2006). According to Harte (2007), in recent years the cumulative effects of the establishment of increasing human populations and intense agricultural practices in catchments has resulted in significant degradation of aquatic ecosystems, which is in requirement of urgent monitoring, management and restoration activities. Conceptually such alterations may cause the biota to respond in a predictable way by changing its energetics, nutrient cycling, and community structure (Odum, 1985; Solimini *et al.*, 2006).

Most water quality monitoring programs document physical and chemical attributes of the aquatic environments, but accurate assessment of water quality requires examination of physical, chemical, and biological components of these ecosystems (Barbour *et al.*, 1999; McGoff *et al.*, 2013). Therefore, the assessment of biotic (ecological) integrity in streams, rivers, and lakes is an essential method for the comprehensive monitoring and management activities (Barbour *et al.*, 2000; Vugteveen *et al.*, 2006).

Bioassessment is mainly an evaluation of the biological integrity defined as “the ability to support and maintain a balanced, integrated community of organisms having species composition, diversity and functional organization comparable to that of the natural habitat” (Barbour *et al.*, 1999). Furthermore, bioassessment focuses on changes in biotic community structure in response to human disturbance on the aquatic environment which can contribute to understand the environmental changes (Jones *et al.*, 2007).

Benthic macroinvertebrates or simply “benthos” are animals without a backbone, bottom-dwelling live on rocks, debris, sediments, and aquatic plants (Vyas and Bhawsar, 2013). Aquatic macroinvertebrates are reflected as good indicators of water quality because they are a critical part of the stream food web (Basu *et al.*, 2018) and are affected by physical, chemical, and biological conditions (Poikane *et al.*, 2016a). Most benthic invertebrates are immobile and cling to their habitat, then they quickly react to environmental changes and die if the water quality is below their tolerance (Czerniawska-Kusza, 2004).

Macroinvertebrates can be grouped into three categories based on their response to pollution: pollution tolerant, those that can survive on a wide range of environmental conditions; facultative, those prefer clean waters but can survive in less polluted waters, and pollution intolerant, that are sensitive to changes and cannot survive in polluted water systems (Weber, 1973). Analysis of collected invertebrates is both time and cost efficient; show the cumulative impact of pollutions; display the effect of short and long-term environmental disturbances; can convey changes in community structure in response to disturbances, and measures the effect of pollution rather than the concentrations of the pollutants (Smith *et al.*, 2007; Trichkova *et al.*, 2013; Mangadze *et al.*, 2019).

Therefore, this aspect makes these organisms ideal candidate groups to monitor the water quality. Using macroinvertebrates as indicators of water quality is common and easy in temperate regions, which relate directly with the availability of well-defined standards developed for the assessment of the water quality (Bouchard *et al.*, 2005). Lack of these practices in the tropical waters, particularly in lakes is the major gap not to implement bioassessment and biomonitoring. Therefore, there is a need to evaluate the application of these organisms as indicators of water quality in the lakes of Ethiopia.

Diatoms, a group of algae, are good bio-indicators of water quality in streams and rivers (Stevenson *et al.*, 2010). Diatoms are also sensitive to conditions prevailing in different aquatic conditions (Bellinger *et al.*, 2006). As primary producers, they are at the base of the food web structure in aquatic ecosystems. Benthic diatom communities react rapidly to disturbances of water, such as physicochemical conditions of the water or to pollution-affected catchment areas (Passy, 2007). With short life cycles, they respond quickly to the environmental conditions such as organic pollution, siltation, pH, and eutrophication (Potapova and Charles, 2003). Often they change their species composition or diversity, which can vary from species-rich to monotonous communities based on the quality of the aquatic habitat (Pan *et al.*, 1996; Ács *et al.*, 2005).

The advantage in using benthic diatoms is that they can be found in every surface water. Moreover, collected diatom samples can be well-kept-up for an unlimited period of time in the form of preserved slides and/or acid digested samples, and can be investigated whenever necessary. However, a disadvantage in investigating diatoms is that it requires a thorough taxonomic knowledge (Ács *et al.*, 2005; Taylor *et al.*, 2007a).

There are visible works on using benthic macroinvertebrates as indicators of water quality in the tropics (Getachew Beneberu *et al.*, 2014). Eventhough fragmented reports on composition of invertebrate specimens were known, there was no detail systematic study of aquatic macroinvertebrates in Ethiopia, which further hindered their use and application in biomonitoring program (Seyoum Mengistou, 2006). Some works done on the assessment of macroinvertebrates in different water bodies of Ethiopia include that of Harrison and Hynes (1988); Tilahun Kibret and Harrison (1989); Amanuel Aklilu (2011); Habiba Gashaw and Seyoum Mengistu (2012); and most of these studies were inventory.

In Ethiopia, only a few studies have been done: example, Baye Sitotaw (2006) developed an index of biotic integrity using discrete type of scoring for some rivers in the Awash, Abay, and Omo-Gibe basins. Similarly Solomon Akalu *et al.* (2011) developed the same index but with a different approach for Akaki river. Getachew Beneberu (2013) also developed an index of biotic integrity for some selected rivers in Ethiopia, while Aschalew Lakew and Moog (2015) attempted to develop a new biotic score “ETHbios” for assessing the ecological conditions of some highland rivers in Ethiopia.

Several studies on the composition of algae specimens were reported, but there was no detailed study of diatoms as indicator of water quality in Ethiopia. Abebe Beyene *et al.* (2009) made an effort to apply single metrics to evaluate the ecological integrity of rivers using diatoms and macroinvertebrates as indicators of water pollution. Besides, majority of the studies done so far mainly focused on single metrics and physicochemical analysis to assess the health of the aquatic ecosystems, disregarding the multimetric approaches.

Biometrics of a single assemblage respond to limited stressors (Kane *et al.*, 2009). Assessment of ecological condition using multi-assemblages and multi-metrics approach, provides a comprehensive information about the biotic integrity and water quality status (Wang *et al.*, 2015; Poikane *et al.*, 2016a; Mangadze *et al.*, 2019). This study, therefore, attempts to assess the biotic integrity of Lake Ziway to determine whether the lakeshore is affected or not and to estimate the pollution level. In this study, a multimetric index based on multi-assemblages was developed to assess the biotic integrity and water quality of the lake and which can respond to different types of stressors.

1.2 OBJECTIVES OF THE STUDY

1.2.1 General objective

- ❖ Develop benthic macroinvertebrate and benthic diatom based multimetric index that can discriminate between stressor types in the littoral zone of Lake Ziway.

1.2.2 Specific objectives

The specific objectives of this research study are:

1. Assess the physical habitat quality and physicochemical parameters distribution among the study sites and identify reference condition and stressed sites;
2. Identify the critical abiotic factors that determine the benthic diatoms and benthic macroinvertebrates composition and diversity in Lake Ziway;
3. Evaluate the appropriate diatom and macroinvertebrate metrics/indices, that have the potential to discriminate reference and test sites;
4. Develop a composite diatom and macroinvertebrate multimetric index and validate its responsiveness to discriminate between reference and test sites;
5. Evaluate the water quality and ecological status of the study lake sites of Lake Ziway using the developed composite multimetric index (MMIZ).

1.3 RESEARCH QUESTIONS

1. Which physical and chemical factors influence the benthic macroinvertebrates and diatoms assemblage composition and diversity in Lake Ziway?

2. Which benthic macroinvertebrate and diatom metrics and/or indices developed for temperate regions are applicable in tropical lake, Lake Ziway?

3. Which metrics and/or indices can be developed into a multimetric index that can discriminate the study sites of Lake Ziway across the disturbance gradients?

1.4 DESCRIPTION OF THE STUDY AREA

Lake Ziway is one of the freshwater Rift Valley Lakes of Ethiopia (RVLE). It is at about 160 Kilometers south of Addis Ababa (7^0 52′ to 8^0 8′ N latitude and 38^0 43′ to 38^0 56′ E longitude) (Makin *et al.*, 1975). It is 31 Km long and 20 Km wide, at an altitude of 1636 meter above sea level (Wood and Talling, 1988). The lake has a surface area of 442 Km^2, a maximum depth of 7 meters, an average depth of 2.5 meters, and a volume of 1.1 Km^3 (UNEP, 2006). It has five Islands: Debre-Sina, Gelila, Tsedecha, Fundro and Tullu-Gudo. Lake Ziway is fed primarily by two rivers, Meki from the west and Ketar from the east, and is drained by the Bulbula River which empties into Lake Abijata. Batu town lies on the lake's western shore and most of human-induced pressures are from this direction.

The lake's catchment has an area of about 7414 Km^2 and bounded between Latitude of $7^0$20′54″ to $8^0$25′56″ and Longitude of $38^0$13′02″ to $39^0$24′01″ (Makin *et al.*, 1975). The catchment extends from Gurage Mountain in the west to Mount Chilalo and Galema in its eastern side. The climate is characterized by Semi-arid to Sub-humid with mean annual precipitation (650 mm) and temperature (25 ºC) (Lemma Abera *et al.*, 2018).

The Ziway-Abijata catchment in the Central Rift Valley Lakes (CRVLs) has the greatest potential for development due to its favorable agro-climatology and socio-economic conditions that attract investments mainly in agricultural sector (Tenalem Ayenew, 2004). More prominently, Lake Ziway catchment has been one of the extensively utilized parts of CRVL area (MoWR, 2002; Jansen *et al.*, 2007). The recently expanding over-irrigation practices and the floricultures can add a stress over the water resources in the catchment (Mulugeta Dadi *et al.*, 2016). According to Dessie Tibebe (2017), the hydraulic residence time for Lake Ziway was about 6.67 years, which is much higher than the reported 1.5 to 2 years by Spliethoff *et al.* (2009). Therefore, due to these interlinked influences, Lake Ziway is under different types of pressures which can deteriorate its ecological functions.

In Lake Ziway, 122 phytoplankton species were identified (Tsegaye Miheretab, 1988), of which 50 blue-green algae, 41 green algae, and 31 were diatoms. Girma Tilahun (2006), identified 58 phytoplankton species, of which 9 were diatoms. Zooplankton community of the lake was dominated by rotifer species, 49 rotifer, 7 cladoceran, and 3 copepod species were identified in the study of Adamneh Dagne (2010). Fourteen macrophyte species were identified and the macrophyte composition was dominated by *Arundo donax* (Girum Tamire and Seyoum Mengistou, 2013). Fish communities of the lake comprises *Barbus paludinosus, Carassius carassius, Clarias gariepinus, Cyprinus carpio, Garra dembecha, Garra makiensis, Labeobarbus ethiopicus, Labeobarbus intermedius, Oreochromis niloticus,* and *Tilapia zilli* (Lemma Abera *et al.*, 2018).

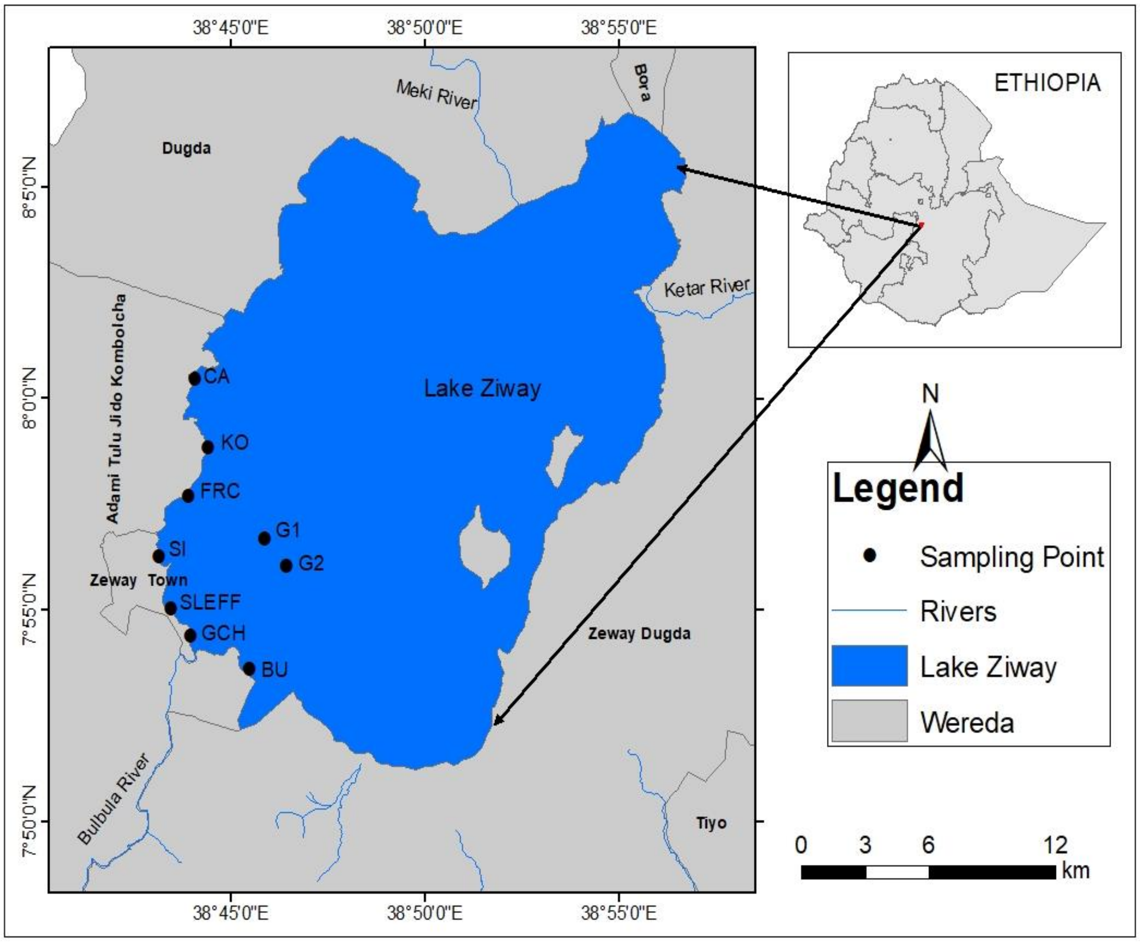

Figure 1.1 Map of Lake Ziway showing the lake segment study sites/sampling points. (Abbreviation: BU: Buchesa; GCH: Gabriel Church; ShEFF: SherEthiopia Flower Farm; SI: Sida; KO: Korokonch; CA: Cafeteria; FRC: Fishery Research Center; G1: Gelila 1; and G2: Gelila 2).

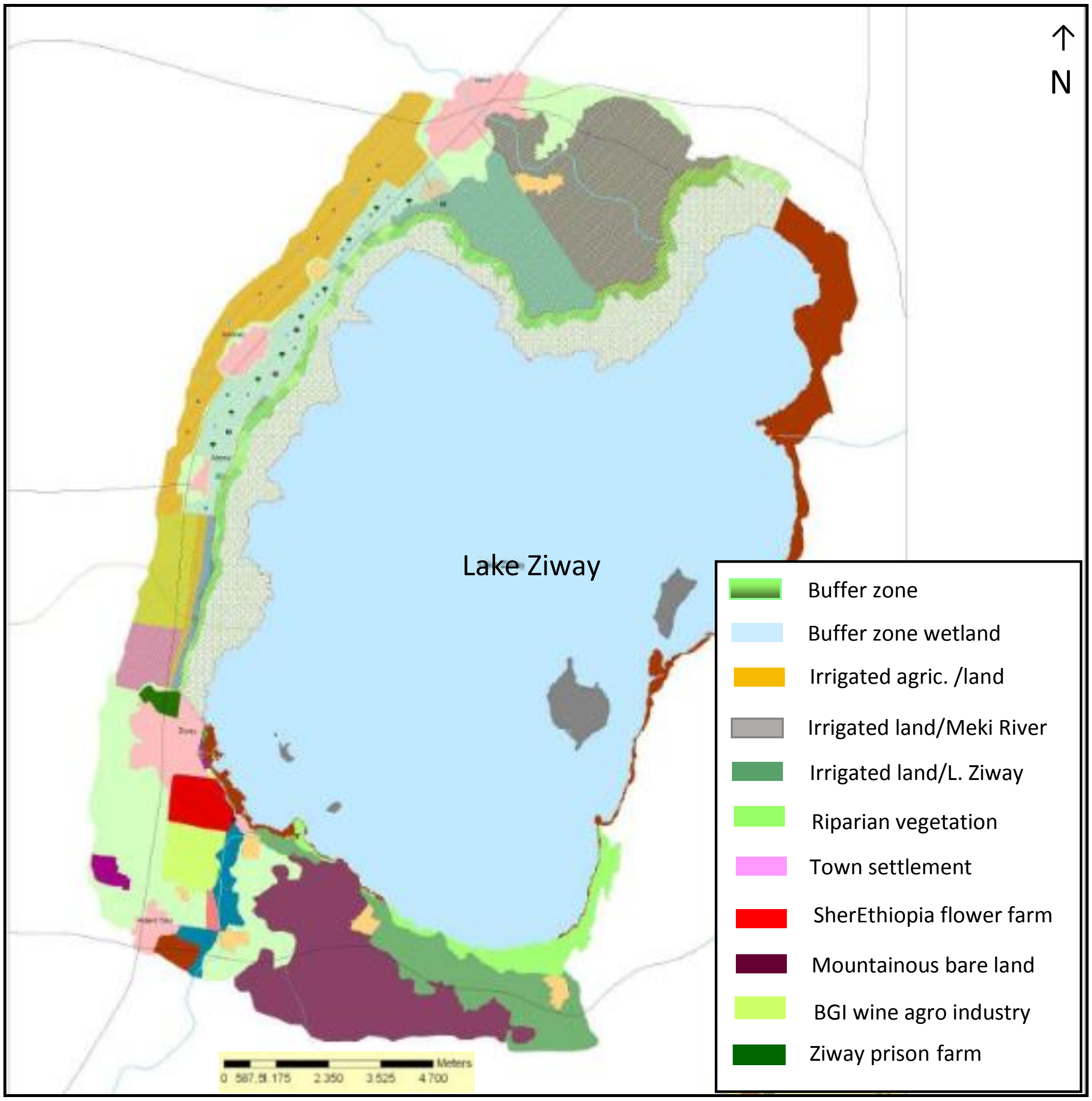

Figure 1.2 Map of Lake Ziway land use/land cover demonstration along the western shore and riparian zone (After Hengsdijk *et al.* (2008)).

Based on the land use/land cover map, SI and ShEFF sites appear to be influenced by the flower farms and CA and KO sites by the town settlements with significant shore modifications (Fig. 1.2). G1 and G2 sites were far away from the related stressors from the western side of the lake as they are situated at the Gelila Island.

1.4.1 Meteorological features of Lake Ziway region

There are two distinct seasons in Lake Ziway region: the main rainy season ranging from June to September, and the dry season from October to February. Based on the data gathered from 2000 to 2016, average annual precipitation and temperature were 800mm and 21.5ºC, respectively (Fig. 1.3). The rainy period extends from March to September with two peaks, one between March and May and the second from June to September. The second peak exhibited the wet months (July to September), in which the precipitation curve goes above 100 mm. The maximum temperature of the warmest month was 30.8ºC, and minimum temperature of the coldest month was 11.3ºC (Fig. 1.3).

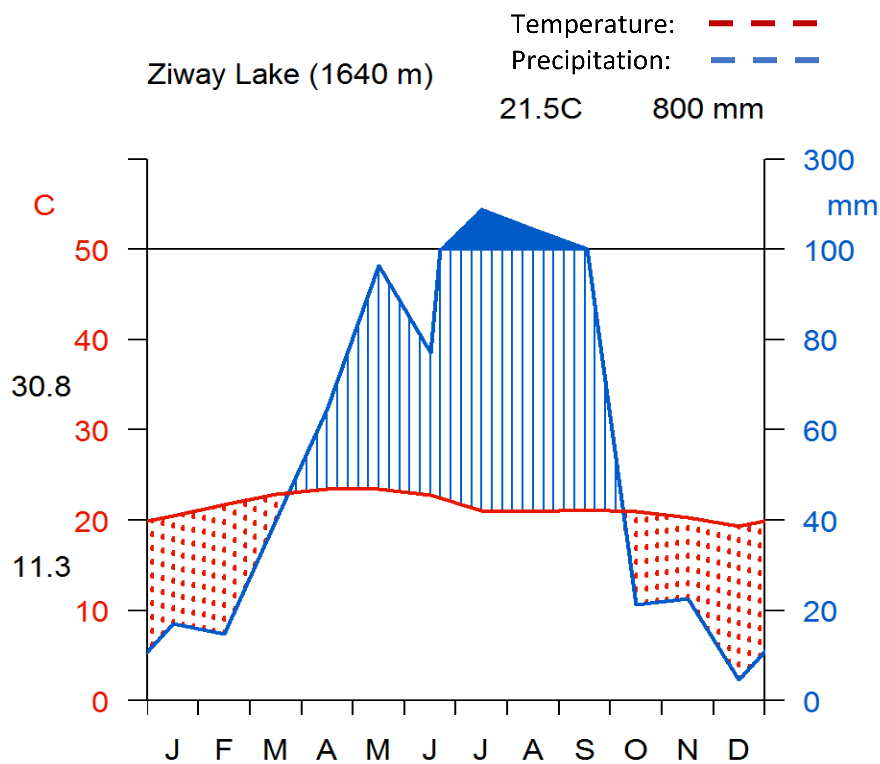

Figure 1.3 Walter and Lieth climate diagram showing a summary of climate conditions for Lake Ziway (Data source: Ethiopian National Meteorological Services Agency).

The diagram shows both mean daily temperature and precipitation for each month. The left Y axis shows temperature (ºC), while the right Y axis shows precipitation (mm). Months are shown along the X-axis, the temperature curve is shown in red, and the precipitation curve is shown in blue. The mean monthly temperature and precipitation values were analyzed based on data gathered between 2000 and 2016.

1.4.2 Habitat descriptions of the study sites

Buchesa (BU): study site is found in the west eastern part of the lake at 07º 53′ 57.4″ N latitude and 038º 44′ 55.4″ E longitude and is dominated by grassland, and has surface water inflow and outflow. This study site is located near to the mouth of River Bulbula which empties the lake water into Lake Abijata (located in Abijata-Shalla National Park). Serves mainly for livestock watering and bathing. Over grazing, agriculture, and road impact can be stated as stressors to this particular site.

Plate 1. 1 Buchesa (BU) study site (sampling point)

Gabriel Church (GCH): study site is found in the west eastern part of the lake at 07º 53′ 52.4″ N latitude and 038º 44′ 37.9″ E longitude and is dominated by grassland, modest coverage of macrophytes, and has surface water inflow and outflow. Located near to the mouth of River Bulbula on the side of Saint Gabriel Church, and serves mainly for livestock watering. Over grazing and agricultural activities (small scale irrigation) can be stated as stressors to the site.

Plate 1. 2 Gabriel Church (GCH) study site (sampling point)

SIDA (SI): study site is found in the west eastern part of the lake at 07º 54′ 46.7″ N latitude and 038º 44′ 06.9″ E longitude and is dominated by grassland, slight coverage of macrophytes, and has surface water inflow and artificial discharge. Located near to the corner of Batu town. Serves mainly as cattle watering, water abstraction, and bathing. Over grazing, agricultural activities (small scale irrigation) and artificial discharge from the flower farms and Batu town can be stated as the main stressors (Fig. 1.2).

Plate 1.3 Sida (SI) study site (sampling point)

Sher-Ethiopia Flower Farm (ShEFF): study site is found in the west eastern part of the lake at 07º 54′ 42.4″ N latitude and 038º 44′ 01.7″ E longitude, and is dominated by grassland, high macrophyte coverage, and has surface water inflow and agro industrial discharge. Located near to the flower farm industry. Serves mainly for water abstraction, livestock watering, and small scale tannery processing. Industrial activities, large scale irrigation, and industrial discharges can be stated as the main stressors (Fig. 1.2).

Plate 1. 4 SherEthiopia Flower Farm (ShEFF) study site (sampling point)

Korokonch (KO): study site is found in the western part of the lake at 07º 55′ 29.4″ N latitude and 038º 43′ 41.9″ E longitude and is dominated by construction, very slight coverage of macrophytes, and has surface water inflow and wastewater discharges. Serves mainly as recreational area, boat landing site, bathing, and water abstraction. Recreational activities, waste dumping, artificial discharges, and non-natural beach can be stated as the main pressures/stressors to the site.

Plate 1.5 Korokonch (KO) study site (sampling point).

Cafeteria (CA): study site is found in the western part of the lake at 07º 56′ 11.3″ N latitude and 038º 43′ 33.4″ E longitude and is dominated by construction, very slight coverage of macrophytes and has surface water inflow and wastewater discharges. The lake bed is dominated with having sand. Serves mainly as recreational area, boat landing site, and bathing. Recreation and fishing activities, artificial discharges, and non-natural beach can be stated as the main pressures or stressors to the site.

Fisheries Research Center (FRC): study site is found in the western part of the lake at 07º 55′ 14.7″ N latitude and 038º 43′ 43.2″ E longitude and is dominated by tree and average coverage of macrophytes. It is found near to Batu fisheries research center office. It is a closed lake segment site, and also free from agricultural and other human activities. Besides, characterized as having 20 meter distance protected buffer zone.

Plate 1. 6 Fisheries Research Center (FRC) study site (sampling point)

Gelila 1 (G1): study site is found in the eastern part of the lake at 07º 55′ 42.5″ N latitude and 038º 45′ 8.64″ E longitude, and dominated by tree and high coverage of macrophytes. Situated on the Gelila Island. It is closed for human activities as it is positioned in the Gelila Island, and also free from agricultural and other human activities. Characterized with having natural shore covered with grass and tree canopy riparian.

Plate 1. 7 Gelila 1 (G1) study site (sampling point) and the Gelila Island.

Gelila 2 (G2): study site is found in the eastern part of the lake at 07º 55′ 40.3″ N latitude and 038º 45′ 21.8″ E longitude, and dominated by tree and high coverage of macrophytes. Situated on the Gelila Island. It is closed for human activities as it is in the Gelila Island, and also free from agricultural and other human activities. Characterized with having natural shore covered with grass and tree canopy riparian zone.

Plate 1.8 Gelila 2 (G2) study site and its riparian vegetation condition

1.5 THESIS OUTLINE

This thesis compiles the research study done to develop a composite multimetric index for Lake Ziway. Environmental biomonitoring on lakes started far behind that of rivers, and most of the bioassessment metrics/indices developed refer to lotic temperate systems. This study has attempted to extend the few studies on lake bioassessment such as Delgado *et al.* (2010); Poikane *et al.* (2016a); Poikane *et al.* (2016b) and adopted the methodologies to a tropical lake condition.

The thesis is organized into six chapters. The first chapter is a general introduction more of introductory and provides information on the overall thesis introduction, objectives, thesis outline, and also describes the study area. The second chapter deals with the Lake Habitat quality and physicochemical parameters, which supported the characterization of the lake study sites across the disturbance gradients. The historical changes in the nutrient concentration of the lake water were also discussed by comparing with earlier reports.

The third chapter describes the abundance, diversity, composition, and seasonal variation of benthic macroinvertebrates in relation to some physicochemical factors in the study sites of Lake Ziway. The diversity indices were calculated for each study site and the macroinvertebrates diversity index values were also discussed in relation to the physical and chemical conditions of the study sites. A manuscript written from part of this chapter was published in the *International Journal of Advanced Research in Biological Sciences*.

The fourth chapter describes the abundance, diversity, composition, and the seasonal variation of benthic diatoms in relation to some physicochemical factors in the study sites of Lake Ziway. The diversity indices were calculated for each study site and the diatom diversity index values were also discussed in relation to the littoral physical and chemical conditions of the study sites. A manuscript extracted from the major part of this chapter has been submitted to the journal - *African Journal of Aquatic Sciences*.

The fifth chapter deals with developing a composite multimetric index (MMIZ) using macroinvertebrate and diatom assemblages. Various macroinvertebrate and diatom metrics were evaluated, and the potential ones were selected as core metrics to develop a composite multimetric index. Therefore, for each study site, an aggregated MMIZ score was calculated. The use of MMIZ was also validated based on well-established standard procedures and found useful to determine the ecological status of the lake. The power of the MMIZ to discriminate between the sites with different stressors guaranteed the capacity of the MMIZ to provide a clear picture about the quality of the water than that cannot be obtained by single metrics. This chapter has been submitted to the journal of *Ecological Indicators*. The last chapter concludes major findings of the research work and recommends some management options and research gaps for future studies.

Limitation of the study

The major challenge of Lake Bioassessment is that the natural spatial heterogeneity and temporal variations may mask effects of stressors on biota. Despite this, there are advantages of doing bioassessment on same lake considered in this study.

1. Confounding spatial effects are reduced, except those due to identified stressors;
2. Close-range sampling of abiotic and biological parameters can easily extract the factors and metrics that govern autecological and stressor traits;
3. Seasonal variances are easy to uncouple and the eco-regional influences on the metrics and/or indices are reduced.

CHAPTER 2: EVALUATION OF HABITAT QUALITY, PHYSICOCHEMICAL, AND BACTERIOLOGICAL PARAMETERS OF THE LITTORAL ZONE OF LAKE ZIWAY

2.1 INTRODUCTION

Freshwater ecosystems provide a broad variety of valuable goods and services for human society (Covich *et al.*, 2004), and also support several species (Gascon *et al.*, 2015). However, these ecosystems are threatened on a world-wide scale by a variety of pollutants and destructive land-use and water management practices (Harte, 2007). The extent of human activities that influence the environment has increased dramatically during the past few decades (Behmel *et al.*, 2016). Among several factors that contribute to the decline of water quality: exponential growth of human population, industry, and land use practices are at the heart of many aspects of pollution (Harte, 2007). As a result, continued increase in unwise resource use and the related environmental impacts can have a multitude of further negative effects leading to more ecological crises around the freshwater ecosystems (Magadza, 2010).

According to the US EPA (2002), water pollutants are divided into different general categories: the biodegradable wastes which consist of human and animal wastes; the plant nutrients, mainly phosphate and nitrates; sediment, washed off from construction sites, agricultural and livestock processes; and the hazardous and toxic chemicals, sourced from domestic and personal use of chemicals, industrial discharges and oil spills. Hence, the radioactive pollutants came from factories, hospitals, and mines are the most serious and has effect with the little amount (Hill, 2010).

Riparian vegetation that extends along the catchment landscape of water bodies provide a wide range of socio-economic, biophysical and ecological functions (Kachi *et al.*, 2015a). However, human-induced activities such as human settlements, livestock populations, poor agricultural practices, and uncontrolled mining activities, have degraded vegetation cover at the riparian zone of the freshwaters (Wantzen and Mol, 2013). These poor management practices around freshwaters risk the physical quality of the environment, the hydrological and ecological support systems, and the livelihoods of local inhabitants (Harte, 2007). Therefore, reduced vegetation cover along water bodies, coupled with increasing pollution from domestic and industrial wastes, has resulted in increased nutrient load and deterioration in water quality.

According to Wantzen and Mol (2013), in tropical countries soil erosion is often severe due to the high erodibility of geologically old and weathered soils; inappropriate land management; removal of forest vegetation cover; and mining activities; which also modify the habitat and food web structures. Therefore, conversion of native vegetation to agriculture and human settlements has significantly increased the human well-being at the expense of the degradation of the aquatic ecosystem services (Thomsen *et al.*, 2017).

In Ethiopia the rapid population growth and economic developments are degrading the aquatic environment through uncontrolled growth of urbanization and industrialization (Getachew Beneberu, 2013), expansion and intensification of agriculture, and destruction of natural habitats (Teklu Gebretsadik, 2016). Therefore, generally, the water pollution in Ethiopian freshwater bodies mainly comes from three main sources: domestic sewage, industrial effluents, and runoff from the agricultural lands. These anthropogenic activities have been increasingly affecting the freshwater ecosystems of the country.

Lake Ziway watershed is inhabited by about 2 million people and 1.9 million livestock (Hayal Desta *et al.*, 2015). Agriculture is the dominant land-use structure contributing to the livelihoods of the majority of the watershed population (Lemma Abera, 2016). The lake ecosystem is being affected by catchment degradation, siltation, and excessive water utilization (Tenalem Ayenew and Dagnachew Legesse, 2007; Girum Tamire, 2014). The agricultural sector is characterized by the small-scale farming and raising of livestock (Hayal Desta *et al.*, 2017). In addition, Lake Ziway water quality is degrading through the pressures from the large scale horticulture companies nearly situated to the lakeshore using pesticides and fertilizers at an increase rate, sewage contamination from Batu town, and the aggravated lakeshore modification related to recreational facilities.

Consequently, the cumulative negative effects result in a significant degradation of the aquatic ecosystems, which are in need of urgent monitoring and management activities. Earlier, Seyoum Mengistou (2006), indicated the increasing pressure on the country's natural environments in general and aquatic resources in particular, which require biological quality assessment. Thus, water quality monitoring is the most direct and valid method available to assess water quality and its response to management and restoration activities (Altenburger *et al.*, 2015). Therefore, this study chapter is geared towards evaluating physical, chemical and bacterial load parameters of Lake Ziway. It largely focuses on the major physicochemical characteristics, physical lake habitat quality condition, and lakeshore modifications of Lake Ziway. An attempt was also made in this chapter to cluster the study sites to design and/or classify the reference and non-reference/impaired sites and to develop Lake Habitat Quality Index.

2.2 MATERIALS AND METHODS

2.2.1 Physical habitat quality assessment

Data collection for the Lake Habitat Quality Assessment **(**LHQA) was done based on a standard 300 meter length of riparian and 20 meter transect of littoral zone. LHQA form modified after FDEPA (2017) was used to record the habitat information (Appendix 1). During field survey, features of the channel (hydrology), habitat features, complexity of bank vegetation structure, type of artificial modification to the channel and littoral zone, lakeside adverse alterations, upland buffer zone, shore structure naturalness, stormwater inputs and adverse watershed land use were recorded at each station. The lake segments were deliberately chosen based on the stressors gradient around the lake perimeter.

Measurements were constrained to an observation window representing three zones: riparian, shoreline, and littoral. The human pressure was assessed up to 300 m back from the waterline. These were designed to record the main lakeshore habitat characteristics over 300 m wide plots which extend from within the riparian zone to within the littoral zone following the criteria specified in Jones *et al.* (2007) and Kaufmann *et al.* (2014).

Six main components of habitat were measured using a scoring system of 1 to 20 points. Each of the component was ranked as “optimal” 20-16; “suboptimal” 15-11; “marginal” 10-6 and “poor” 5-1 following the method outlined in FDEPA (2017). The individual scores were added to give the overall score for the particular site, resulting in a total score out of 120. The study site with a high score on this portion of the assessment likely indicates higher habitat quality, whereas a low score indicates more human interference and lower habitat quality. The components were calculated using individual station-level measurements as described in the US EPA (2009) NLA Technical Report.

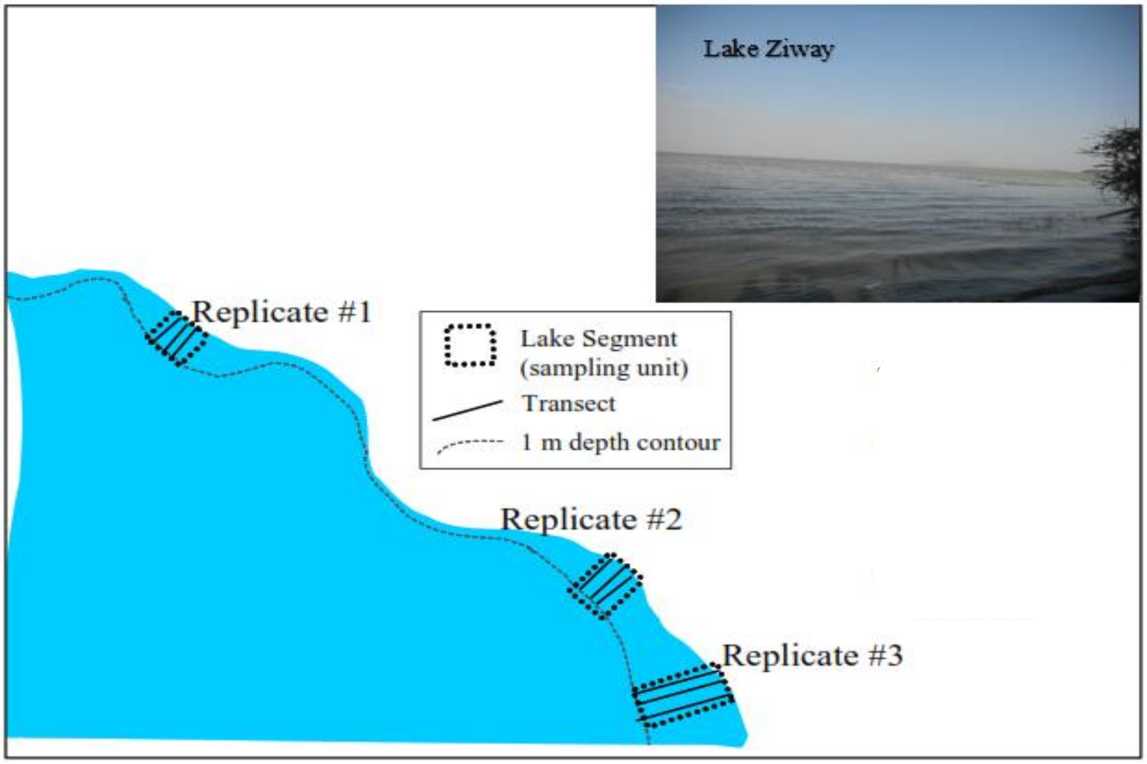

Figure 2.1 Sketch shows near shore littoral zone traveling kick for Lake Ziway benthic macroinvertebrates collection (Modified after Jones *et al.* (2007)).

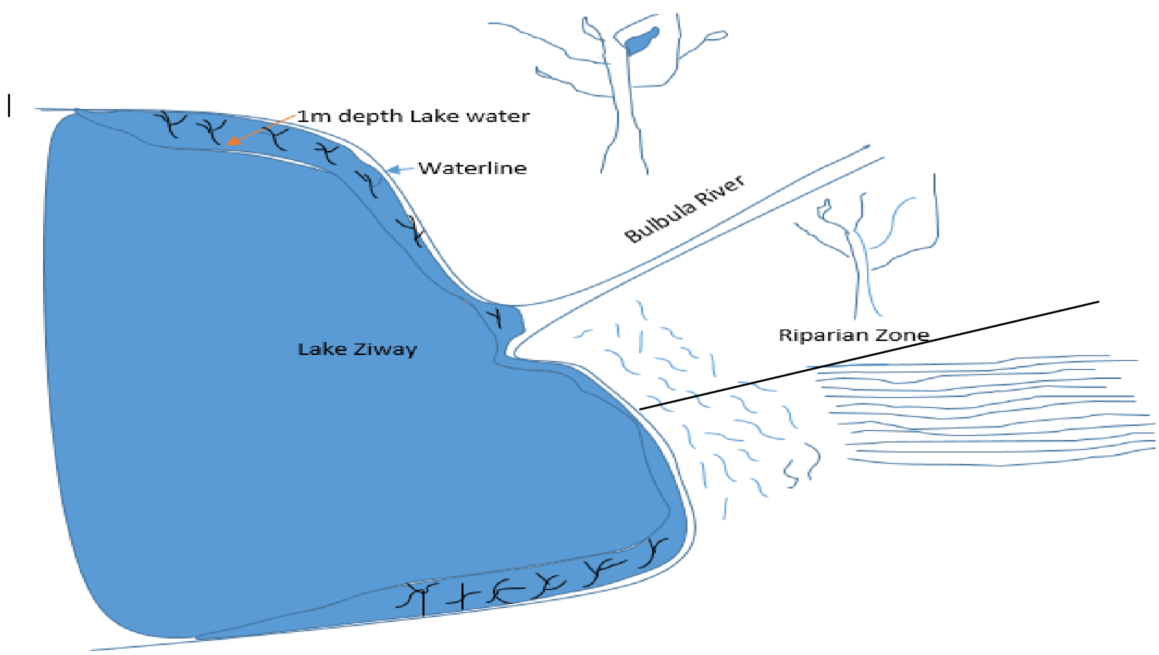

Figure 2.2 Sketch to show the studied 300 m length riparian zone of Lake Ziway.

2.2.2 Physicochemical variables analysis

Physical parameters (pH, dissolved oxygen, temperature, and electrical conductivity) of the lake water were measured *in situ* using a portable digital multi-parameter probe (Model HQ 9012 HACH Instruments) at the end of the month during the study periods between September 2015 and April 2016 (September-November represented the wet season and February-April for the dry season) at each sampling visit.

Water samples were collected from each sampling site to analyze for nitrate, nitrite, ammonium, silicon dioxide, soluble reactive phosphorus, and total phosphorus. The analysis of the nutrients was done monthly using spectrophotometric method in the limnology laboratory of the Addis Ababa University. Nitrate was measured with sodium salicylate method, ammonium with indophenol blue method (APHA, 1995), silica with the molybdosilicate method, soluble reactive phosphate and total phosphate with ascorbic method (APHA, 1999). Nitrite concentration was determined by the reaction between sulfanilamide and N-naphthyl-(1)-ethylenediamine-dihydrochloride (APHA, 1995).

Chlorophyll *a* concentrations were measured from each site, 200 mL of the lake water was filtered through Whatman GF/C filters. The filters were folded with aluminium foil, labelled and transported to the laboratory in an ice box which was stored for one night. Pigments were crushed and extracted in 90% acetone. After extraction the extract was clarified by centrifugation (the algal material was centrifuged). Then, the extract was decanted into 5 ml cuvette and the absorbance of a chlorophyll *a* was measured using the spectrophotometric method at wavelengths of 665 and 750 nm without the phaeophytin pigments correction according to Talling and Driver (1963).

2.2.3 Bacteriological load analysis

Evaluation of bacteriological contamination of water was carried out during the dry study season in order to avoid wet season flood-conveyed bacterial contamination. The wet season study periods were characterized with high runoff, and which could be the main reason for the transported wastes and contaminants. Further the wet season might be responsible for the bacteriological contamination transported from other areas. Total Coliform (TC) and Fecal Coliform (FC) parameters were tested. Sampling was carried out using sterilized sample bottles, which otherwise may interfere with fecal coliform growth (Entry and Farmer, 2001; Omezuruike *et al.*, 2008). Water sample (100mL) was collected using bottles containing sodium thiosulfate. The collected samples were transported with ice full icebox immediately to the Laboratory of Ethiopian Ministry of Water, Irrigation, and Electricity for the analysis of total and fecal coliform bacteria.

Bacteriological parameters that are colony forming and pollution indicator bacteria were analyzed using the Wage-tech bacteriological portable kits according to APHA (1999) procedure. Membrane-filtration (MF) methods were used to culture, and enumerate indicator colony forming bacteria according to APHA *et al.* (1998). Tests were conducted using 100mL of water sample filtered through a 47-mm, 0.45-µm pore size cellulose ester membrane filter that retains the bacteria (Gelman Sciences, Michigan, and the USA). The filters were then layered on membrane lauryl sulphate broth agar for TC and FC growth, and then colonies were counted after 24 hours of incubation period at 37ºC and 44ºC, respectively.

2.2.4. Data analysis

Data analysis was done with Statistical Package for Social Science Students (SPSS Inc., software version 20.0). The significant differences in the concentration of nutrients and bacteriological load between the study sites were compared using Kruskal-Wallis H test and Mann-Whitney U test. Comparative analysis was conducted to characterize sampling sites and was clustered based on their relative values. Principal Component Analysis (PCA) was employed to observe the difference among the study sites regarding their physicochemical properties using CANOCO for windows 4.5 software (Ter Braak and Smilauer, 2002). The PCA was carried out using correlation because the variables were on different scales. Cluster analysis using Wards linkage and Euclidean distance as a measure of similarity was employed to judge/set least stressed (reference condition) and stressed sites based on their habitat quality conditions and physicochemical parameters.

2.3 RESULTS

2.3.1 Lake physical habitat quality assessment

The observed hydrological conditions of the studied lake sites vary regarding surface water inflows, lake water outflow, and impounded artificially controlled inflows. Thus, Fishery Research Center (FRC), Gelila 1 (G1), and Gelila 2 (G2) study sites were characterized as having surface water inflows; Buchesa (BU) and Gabriel Church (GCH) study sites as having surface water inflows and lake water outflow; Sher-Ethiopia Flower Farm (ShEFF), Sida (SI), Korokonch (KO), and Cafeteria CA study sites as having surface water inflow and impounded artificially controlled inflows (Table 2.1).

Point scoring system for the LHQA was conducted based on a consensus of informed professional judgment. It was subjective but necessarily consisted comparisons between sites. LHQA score values showed a significant difference between the categories of the studied lake sites ($p<0.01$). G1 and G2 sites showed the highest LHQA score values, 105 and 107, respectively. KO, ShEFF, and CA sites displayed the least LHQA score values, 36, 37, and 41, respectively (Table 2.2).

Percent vegetation coverage (PVC) of the riparian zone and percent buffer zone (PBZ) structure of the lakeshore/lakeside varied between the study sites. G1 and G2 sites were dominated by over 80% tree coverage and >90% lakeshore with >18m buffer structure followed by the FRC site, indicated 55% of vegetation coverage in the riparian zone up to 100 m distance from the lake watermark and >75% lakeshore with >18m buffer structure. KO and CA sites were the least in their riparian vegetation coverage, 23% and 30%, respectively by having <29% lakeshore with >18m buffer structure (Table 2.1).

Table 2.1 Study sites characterization based on some major features and stressors type.

Study site	Hydrology	Buffer zone condition	Bottom Substrate	Major features of the riparian zone and Stressors
BU	Surface water inflow and outflow present	<29% shore with >18m buffer	Mixture of sand, clay, and detritus	Dominated by grassland and characterized by agricultural activities and livestock
GCH	Surface water inflow and outflow present	<29% shore with >18m buffer	Mixture of sand, clay, and detritus	Dominated by grassland and characterized by industrial activities and livestock
SI	Impounded, artificially controlled inflow.	<29% shore with >18m buffer	Mixture of sand, mud, and detritus	Man-made structures, (roads, buildings, and irrigation), other human disturbance visible
ShEFF	Impounded, artificially controlled inflow.	<29% shore with >18m buffer	Mixture of mud, clay, and detritus	Highly developed man-made structures mainly agricultural and industrial activities
KO	Impounded, artificially controlled inflow.	<29% shore with >18m buffer	Moderate layer of mud and sand	Highly developed man-made structures mainly recreational constructions
CA	Impounded, artificially controlled inflow.	<29% shore with >18m buffer	High composition of sand	Highly developed man-made structures mainly recreational constructions
FRC	Surface water inflow present.	>75% shore with >18m buffer	Cobble, sand, and detritus	Very few man-made structures mainly characterized by well-developed riparian vegetation
G1	Surface water inflow present.	>90% shore with >18m buffer	Cobble, sand, and detritus	Characterized by well-developed riparian vegetation and natural beach
G2	Surface water inflow present.	>90% shore with >18m buffer	Cobble, sand, and detritus	Characterized by well-developed riparian vegetation and natural beach

Percent natural shore zone (PNSZ) condition of the studied lake segments displayed different values, and G1 and G2 sites scored the highest values 16 and 18, respectively. The lowest PNSZ score (1) was recorded in KO study site. Storm water inputs (SWI), characterized the lake segments by scoring higher values for G1 and G2 sites 18 and 19, respectively, and scored the lowest values of SWI for KO and ShEFF study sites, 1 and 3, respectively (Table 2.2).

Percent agricultural land (PAL) within the riparian zone up to 300 m distance from the lake watermark was assessed with percent coverage. SI and ShEFF study sites were dominated with agricultural land use and scored the lowest PAL conditions analysis values 9 and 5, respectively. G1 and G2 study sites were the least in their agricultural land coverage, in which these sites were free from agricultural practices and scored the highest values 18 each (Table 2.2).

Lakeside adverse human alterations (LAHA) degree varied between the study sites, KO, ShEFF, and CA sites were categorized under the highly developed (existence of roads, constructions, direct pipe lines/ditches, small scale tannery processing, and other visible structures) or disturbed (>70% of the lakeside affected). BU, GCH, and SI study sites were categorized under moderate disturbance (10 - 49% of the lakeside affected). FRC, G1, and G2 sites were categorized under the very few man-made structures (absence of roads, artificial constructions, ditches, point-source contaminations, and other structures) or minimal disturbance adjacent to the lake segment (<10% of the lakeside affected) (Table 2.1 and Table 2.2).

Table 2.2 Habitat quality condition assessment of Lake Ziway study sites/lake segments gathered through observation and calculated LHQA scores (variables calculated out of 20 score and LHQA score out of 120).

Study sites	**PVC**	**PNSZ**	**PBZ**	**PAL**	**SWI**	**LAHA**	**LHQAs**
BU	16	8	5	14	11	14	68
GCH	16	5	5	14	13	14	67
SI	12	4	5	9	8	10	48
ShEFF	16	3	5	5	5	3	37
KO	14	1	5	12	3	1	36
CA	14	3	4	12	3	5	41
FRC	18	12	16	16	10	16	88
G1	18	16	16	18	19	18	105
G2	18	18	16	18	18	19	107

Abbreviations: PVC: percent vegetation coverage; PNSZ: percent natural shore zone; PBZ: percent buffer zone; PAL: percent agricultural land; SWI: storm-water inputs; LAHA: lakeside adverse human alteration; LHQAs: lake habitat quality assessment score.

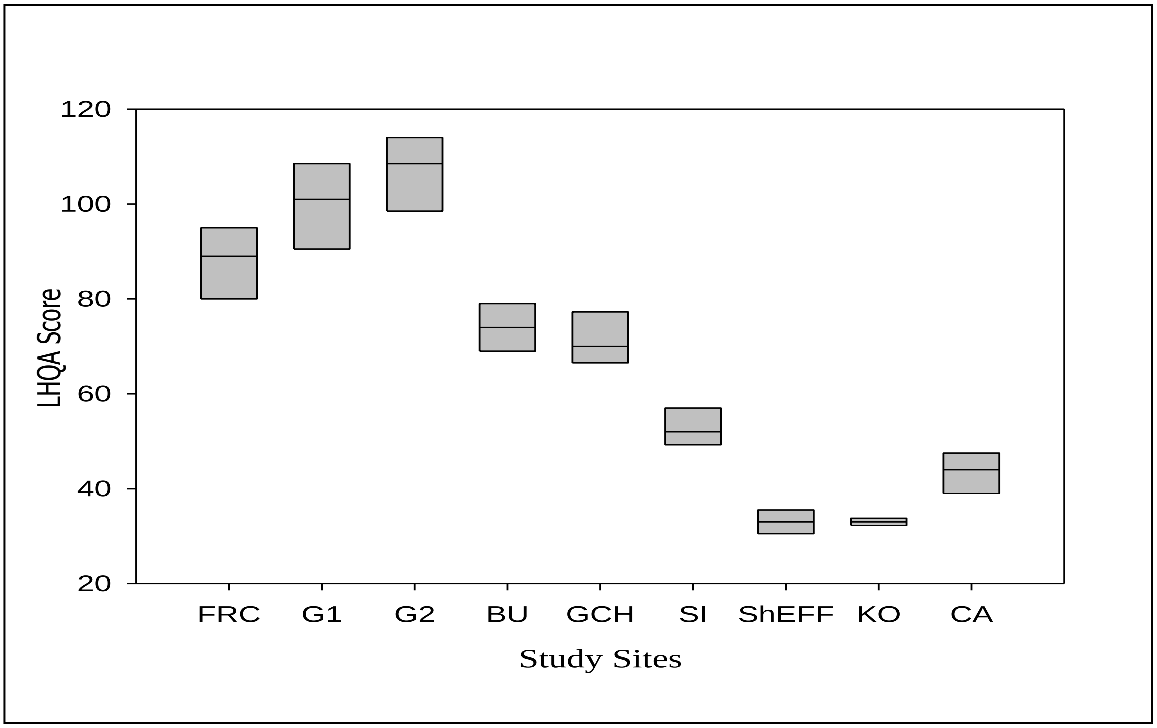

Figure 2.3 Distribution of the study sites with their lake physical habitat quality assessment score values. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles.

2.3.2 Physicochemical parameters

Physicochemical parameters indicated optimal values of ecological qualities of the study sites with increasing stressors load. The most substantial parameter related to the sustainability of aquatic life, Dissolved Oxygen (DO) was significantly different between the study sites ($p<0.01$). The maximum DO value was 9.33 mg/L in BU study site and the minimum recorded value was 3.51 mg/L in ShEFF study site (Table 2.3). Electrical Conductivity (EC) (a measure of water's capability to pass electrical flow directly related to the concentration of ions in the water) was significantly different among sites ($p<0.01$), and varied from 405 µS/cm and reached to a level of 592 µS/cm. The minimum and maximum mean pH values recorded were 7.61 and 8.74 (Table 2.3).

The nutrients (nitrite, total phosphorus, and silicon dioxide) showed significant variation among the study sites ($p<0.05$). Nitrate, ammonium, and soluble reactive phosphorus roughly displayed variation among sites, but statistically not significant ($p>0.05$). Mean values ranged between (0.03-0.22), (0.06-0.62), (0.02-0.27), (0.03-0.43), and (0.14-0.51) mg/L for nitrite, nitrate, ammonium, soluble reactive phosphorus, and total phosphorus, respectively (Table 2.4). Other physical and chemical parameters analyzed in this study displayed no more variation among the sites. Distribution of temperature, conductivity, turbidity, nitrite, ammonium, SRP, and chlorophyll *a* was significantly different across categories of the seasons ($p<0.05$) (Table 2.3 and Table 2.4).

Axis 1 and Axis 2 of the principal component analysis explained 82.6% of the total variance (Fig. 2.4) regarding the sites versus physicochemical variables association, where the first axis and the second axis contributed 57.0% and 25.6% of the variation, respectively. The result of the analysis discriminates G1 and ShEFF sites from the others by axis 1, remaining to higher value of nutrients and also positively correlated with this axis. ShEFF site was positively correlated with nitrite and nitrate, and the association was very strong. Dissolved oxygen, pH, and electrical conductivity were negatively correlated (-0.68, -0.27, and -0.04, respectively) with the first axis (Table 2.5), and the correlation of dissolved oxygen with this axis was strong. The other sites occurred on the opposite side of axis 1 (Fig. 2.4) indicating that they had lower values of these ions relatively.

Table 2.3 Spatial and seasonal variation of physical environmental variables along the studied littoral lake zone sites of Lake Ziway.

Site	**Season**	**pH**	**Temp. (^{0}C)**	**DO mg/L**	**EC µS/cm**	**TSS mg/L**	**Chl-*a* µg/L**
BU	DR	8.54±0.28	25.6±4.05	7.77±0.46	519±3.50	192±15.88	22.6±15.18
	WT	8.63±0.09	23.6±2.45	9.33±0.30	485±8.38	171±7.80	10.1±2.42
GCH	DR	7.71±0.23	26.1±1.27	3.82±2.10	512±21.24	206±2.31	13.2±0.01
	WT	8.48±0.14	21.9±2.03	8.29±1.05	484±3.77	256±118.07	43.5±27.56
SIDA	DR	8.31±0.32	23.8±0.80	6.42±0.25	561±20.12	143±1.15	13.3±1.01
	WT	8.46±0.08	22.2±1.27	8.11±0.16	486±2.31	249±147.22	30.9±6.62
CA	DR	8.38±0.27	27.3±1.45	8.15±0.50	527±6.24	279±5.77	12.5±0.01
	WT	8.74±0.14	24.9±1.22	8.91±0.16	476±4.99	289±106.52	43.2±29.55
KO	DR	8.25±0.13	25.1±0.60	6.35±0.36	567±7.90	218±13.86	13.1±1.01
	WT	8.37±0.13	23.4±0.63	6.33±0.79	492±1.71	200±93.83	43.7±19.49
ShEFF	DR	8.50±0.34	27.1±4.95	6.28±1.18	592±75.63	216±12.53	20.1±1.68
	WT	7.61±0.18	24.2±3.04	3.51±0.27	483±33.79	109±6.65	17.2±3.10
FRC	DR	8.52±0.31	23.1±1.36	5.59±0.75	470±62.07	244±97.59	22.7±18.04
	WT	8.55±0.21	21.9±2.11	6.13±0.06	405±0.50	193±64.09	20.6±1.01
G1	DR	8.35±0.50	24.2±0.62	5.81±0.51	464±68.42	240±42.73	21.7±1.57
	WT	8.61±0.02	21.6±1.31	8.25±1.15	407±1.50	217±102.48	39.9±15.44
G2	DR	8.39±0.54	21.7±0.28	6.08±0.51	480±82.28	192±57.16	17.1±14.83
	WT	8.50±0.11	22.8±2.04	6.46±1.61	407±1.15	257±66.70	37.1±2.02

Abbreviations: Chl-*a*: chlorophyll *a*; DO: dissolved oxygen; DR: dry season; EC: electrical conductivity; Temp: temperature; TSS: total suspended solids; TU: turbidity; WT: wet season; BU: Buchesa; GCH: Gabriel church; ShEFF: SherEthiopia flower farm; KO: Korokonch; CA: Cafeteria; FRC: Fishery research center; G1: Gelila 1; G2: Gelila 2.

Table 2.4 Spatial and seasonal variation of chemical environmental variables along the studied littoral lake zone sites of Lake Ziway.

Site	Season	NO_2^- mg/L	NO_3^- mg/L	NH_4^+ mg/L	SiO_2 mg/L	SRP mg/L	TP mg/L
BU	DR	0.04±0.02	0.06±0.01	0.02±0.01	0.14±0.12	0.03±0.01	0.25±0.02
	WT	0.06±0.03	0.08±0.01	0.02±0.01	0.15±0.12	0.04±0.02	0.26±0.23
GCH	DR	0.05±0.01	0.12±0.07	0.04±0.03	0.14±0.02	0.17±0.01	0.25±0.02
	WT	0.15±0.06	0.18±0.02	0.29±0.21	0.15±0.08	0.27±0.13	0.26±0.63
SIDA	DR	0.04±0.01	0.16±0.01	0.24±0.26	0.22±0.01	0.13±0.11	0.24±0.02
	WT	0.14±0.07	0.18±0.01	0.23±0.02	0.26±0.02	0.25±0.26	0.28±0.04
CA	DR	0.06±0.03	0.15±0.01	0.05±0.03	0.13±0.12	0.12±0.07	0.14±0.02
	WT	0.13±0.15	0.17±0.01	0.15±0.12	0.23±0.23	0.28±0.13	0.28±0.04
KO	DR	0.03±0.02	0.21±0.06	0.19±0.03	0.22±0.01	0.17±0.01	0.36±0.28
	WT	0.15±0.06	0.18±0.01	0.27±0.05	0.24±0.06	0.41±0.14	0.46±0.31
ShEFF	DR	0.22±0.21	0.22±0.09	0.26±0.03	0.24±0.79	0.36±0.93	0.47±0.40
	WT	0.05±0.01	0.62±0.31	0.02±0.01	0.28±0.05	0.43±0.17	0.51±0.45
FRC	DR	0.06±0.08	0.09±0.08	0.13±0.11	0.16±0.41	0.15±0.16	0.17±0.02
	WT	0.03±0.06	0.05±0.01	0.05±0.01	0.16±0.35	0.06±0.03	0.14±0.03
G1	DR	0.03±0.01	0.05±0.08	0.06±0.04	0.29±0.71	0.15±0.16	0.18±0.12
	WT	0.03±0.01	0.07±0.01	0.08±0.06	0.25±0.31	0.12±0.03	0.13±0.03
G2	DR	0.03±0.02	0.05±0.08	0.06±0.05	0.24±0.18	0.14±0.13	0.17±0.10
	WT	0.06±0.05	0.07±0.02	0.10±0.06	0.24±0.14	0.16±0.18	0.19±0.15

Abbreviations: DR: dry season; SRP: soluble reactive phosphorus; TP: total phosphorus; WT: wet season; BU: Buchesa; GCH: Gabriel church; ShEFF: SherEthiopia flower farm; KO: Korokonch; CA: Cafeteria; FRC: Fishery research center; G1: Gelila 1; G2: Gelila 2.

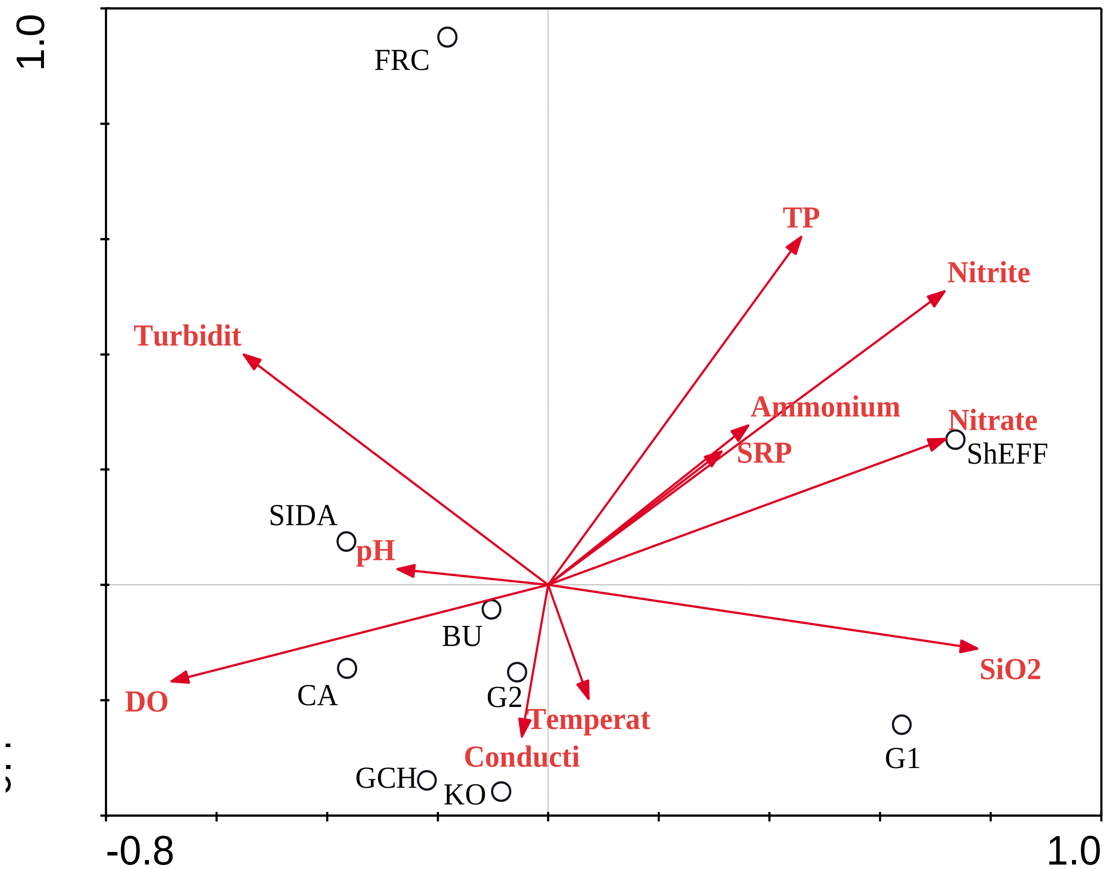

Figure 2.4 Principal Component Analysis (PCA) ordination diagram of physicochemical factors at the study sites (**Abbreviations:** SRP: soluble reactive phosphorus; TP: total phosphorus; BU: Buchesa; GCH: Gabriel church; ShEFF: SherEthiopia flower farm; KO: Korokonch; CA: Cafeteria; FRC: Fishery research center; G1: Gelila 1; G2: Gelila 2).

Table 2.5 Correlation coefficient of the environmental variables used in this study with the first two principal component axes (strong correlations are marked bold at p<0.05).

Environmental variables	**Axis 1**	**Axis 2**
Temperature	0.073	-0.197
pH	-0.273	0.027
DO	**-0.681**	-0.167
Conductivity	-0.048	-0.263
Nitrate	**0.718**	0.253
Nitrite	**0.717**	**0.509**
Ammonium	0.361	0.276
SRP	0.313	0.231
TP	0.457	**0.604**
SiO2	**0.776**	-0.110

2.3.3 Bacteriological characteristics

Colony forming bacteria (total coliform and fecal coliform) testing was conducted at all study sites during the dry season study period. The total coliform and fecal coliform bacteria load showed a significant difference between the categories of sites ($p<0.01$). The total coliform and fecal coliform load average values ranged from 415 to 2595, and 300 to 1250 CFU/100 mL, respectively. Relatively FRC, G1, G2, and BU sites were the least concentrated in microbial load and among the severely contaminated study sites, KO and CA sites were highly concentrated in microbial load (Fig. 2.5).

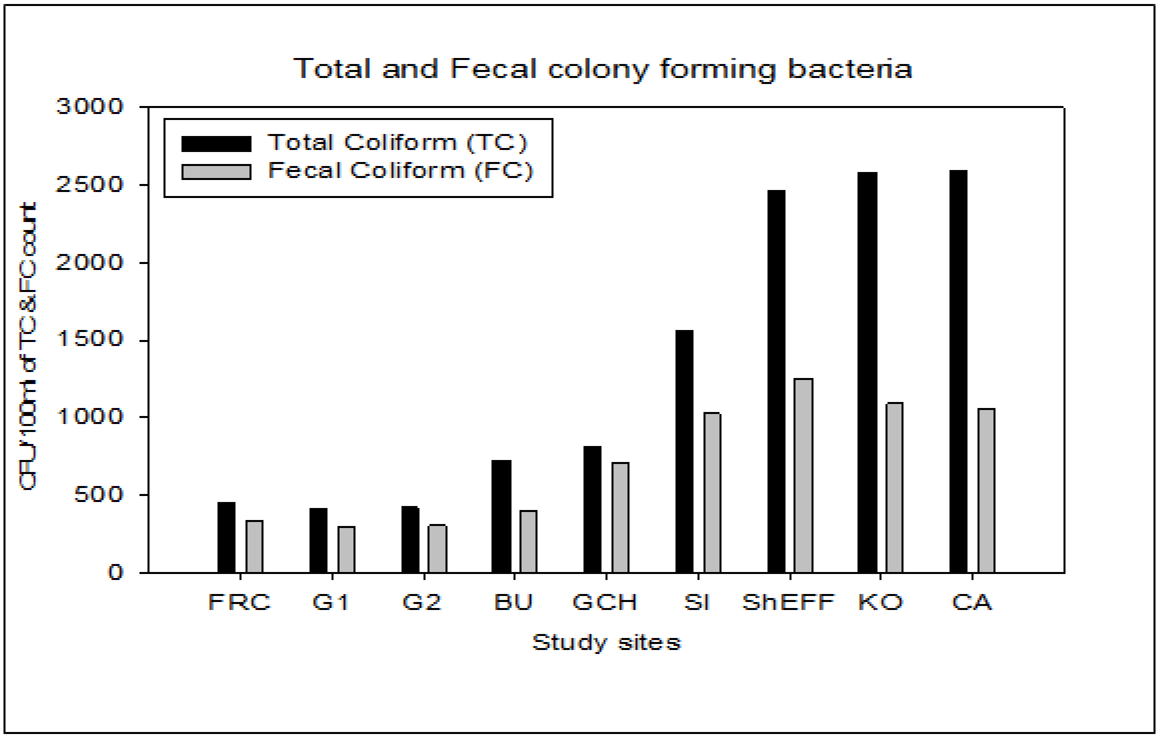

Figure 2.5 Colony forming bacteria of total coliform and fecal coliform count values of the study sites in Lake Ziway (CFU/100 mL of sample water).

2.3.4 Study sites/lake segments clustering

Based on physical habitat quality, physicochemical and bacteriological data the study sites were clustered into three groups. Sites classified as "reference" were considered representative of near natural or least disturbed conditions in their respective study sites (FRC, G1, and G2). Conversely, sites classified as "most disturbed" were considered representative of the most modified by human activities (ShEFF, KO, and CA). The third class "intermediate" represented sites altered to intermediate degree (BU, GCH, and SI). Sites were classified using lake screening process primarily based on physicochemical data following the criteria designed for lake habitat assessment (Jones *et al.*, 2007; Whittier *et al.*, 2007b; US EPA, 2009). Cluster analysis was used with Ward's method and Euclidean distance as similarity measure.

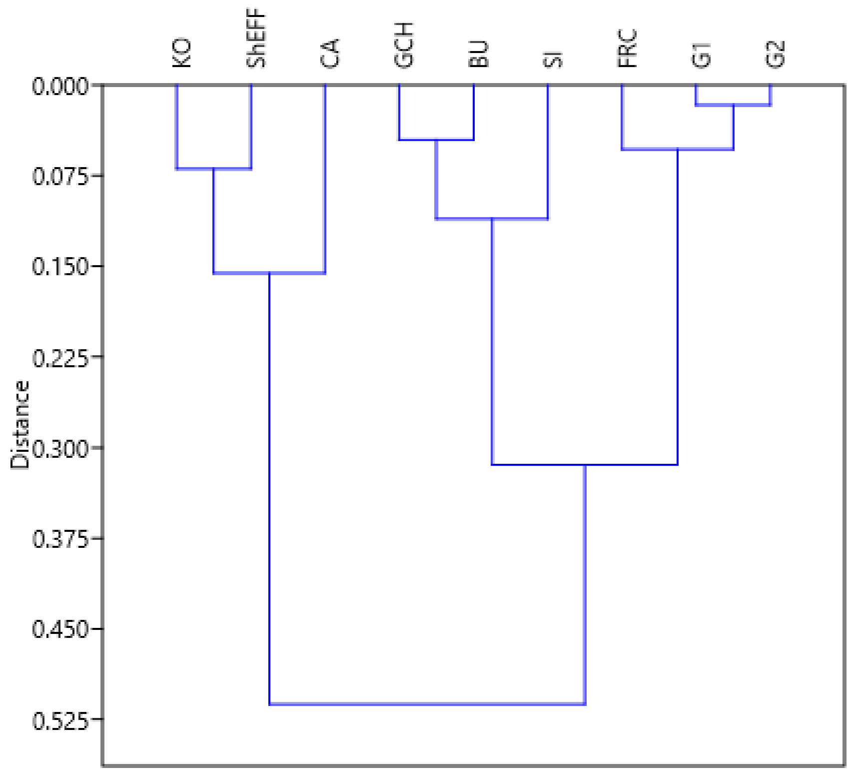

Figure 2.6 Hierarchical cluster analysis of the study sites (Dendrogram) using Ward's method and Euclidean distance as similarity measure based on physicochemical and lake habitat quality assessment score values.

2.4 DISCUSSION

2.4.1 Physical habitat quality and physicochemical conditions

The lack of standard assessment methods to support judgements of lake habitat quality led to developing Lake Habitat Survey (LHS) (Rowan *et al.*, 2006); and LHQA could be useful for evaluating lake habitat quality status (McGoff and Irvine, 2009). G1 and G2 study sites showed the highest LHQA score values, this might be possibly because these sites are free from agricultural and other human activities. KO, ShEFF, and CA sites displayed the least LHQA score values which could be attributed with the observed high human alteration on these lake segment sites.

The components of the habitat quality measurements were ranked as optimal, suboptimal, marginal, and poor. Reference sites (G1 and G2) were ranked the optimal rank for the components and FRC site was ranked optimal for most components, resulting in a higher total score which, indicates negligible human interference. Sites from the intermediate stressors were ranked suboptimal and marginal rank for most components. Sites from the multiple stressors were ranked marginal and poor rank for most components, resulting in a lower total score which indicates high human interference.

Percent vegetation coverage of the riparian zone and percent buffer zone of the littoral lakeside varied between the study sites. The differences in these features could be attributed to the specific land use/cover dissimilarity. G1 and G2 sites were dominated by over 80% tree coverage and > 90% lakeshore with >18m buffer structure followed by the FRC site, which indicated 55% of vegetation coverage. This could be most probably because G1 and G2 sites are far away from human interventions, and the FRC site is a closed littoral zone for any human alteration. KO and CA sites were the least in their riparian vegetation coverage, which is due to excessive modification.

Recent efforts have gone beyond basic lake morphometry, focusing on the structure and complexity of physical habitat in the nearshore environments (Kaufmann *et al.*, 2014). According to the study of US EPA (2009) in lakes of the United States, of the stressors in National Lakes Assessment (NLA), poor lakeshore habitat is the biggest problem in the nation’s lakes; over one-third exhibited poor shoreline habitat condition. Poor biological health is three times more likely in lakes with poor lakeshore habitat (US EPA, 2009). Therefore, these reports indicated that poor lakeshore physical habitat structure in lake environments is the primary decisive factor.

Water quality parameters of Lake Ziway in the nine sampling sites were variable, except for pH, NO_3^-, SRP, and NH_4^+ (Table 2.3 and Table 2.4). The significant variations in these factors could be attributed to the specific environmental stressors dissimilarity, land use influences, and straight discharges. pH showed almost no change during the last two decades as the mean value found in this study (8.38) were comparable with the value (8.50) reported by Elizabeth Kebede *et al.* (1994); (8.65) reported by Girma Tilahun and Ahlgren (2010); (8.44) reported by Girum Tamire and Seyoum Mengistou (2013); and (8.21) reported by Lemma Abera (2016).

Electrical conductivity reading increased considerably when compared with some recent results. The average EC reading (410 µS/cm) reported by Elizabeth Kebede *et al.* (1994) and (419.1 µS/cm) reported by Girum Tamire and Seyoum Mengistou (2013) were less than the result in this study (489.8 µS/cm), excluding the result (478 µS/cm) reported by Girma Tilahun and Ahlgren (2010) nearly comparable to the present study (Table 2.6). The increasing trend in EC of the lake can be largely attributed to catchment degradation, multiple stressors around the lake, and water level reduction, which all make possible the influx of ions to the lake.

The mean SRP and nitrate values found in this study 190 and 230 µg/L, respectively were higher than values 10.1 and 3.17 µg/L reported by Girma Tilahun and Ahlgren (2010), 29.6 and 56.7 µg/L reported by Girum Tamire and Seyoum Mengistou (2014), 49.8 and 44.05 µg/L reported by Lemma Abera (2016) (Table 2.6). However, values varied between (200-950 µg/L for nitrate) and (20-380 µg/L for SRP) reported by Getachew Beneberu and Seyoum Mengistou (2009) is comparable to this study values varied between (120-920 µg/L for nitrate) and (20-270 µg/L for SRP).

The higher values of nitrate and SRP for this study might be because all the study sites are in the littoral zone of the lake; the purposeful selection of the sampling sites in correlation with stressors gradient; the possibility to comprise straight discharges from anthropogenic sources which directly relate with poor vegetation cover in most of the test sites of the lake. It was similarly reported by Getachew Beneberu and Seyoum Mengistou (2009) that the mean values of nitrate and SRP were high in the littoral than the offshore of Lake Ziway. Girum Tamire and Seyoum Mengistou (2014), also reasoned that the increase in SRP is possibly because of nutrient enrichment of the littoral zone of the lake from anthropogenic sources visibly situated near to the lakeside littoral areas, this situation was also reported by Lemma Abera *et al.* (2018).

2.4.2 Bacteriological load

Colony forming bacteria (total and fecal coliform) load average values ranged from 415 to 2595, and 300 to 1250 CFU/100 mL, respectively. Relatively FRC, G1, and G2 sites were the least in bacterial load. The least concentration of bacterial load in these sites agrees with their limited human interference. Among the highly contaminated sites, KO and CA sites were greatly concentrated in bacterial load (Fig. 2.5). The high concentration in bacterial load in these sites is probably because of the high human and animal contact and the artificial inflow, which release wastewater from Batu town to these lake adjacent (Table 2.1). According to Kachi *et al.* (2015b), the concentration in microbial load was positively correlated with high human and livestock population. The present study results also displayed high human interference and disturbance scores recorded through the habitat quality assessment on these study sites, which might be the primary reason for the higher concentration of their bacterial load.

Table 2.6 Previous and current physicochemical parameters of Lake Ziway, and trends since 20 years back from the present study. (**Abbreviation:** NC- No Change).

Parameters	Elizabeth Kebede *et al* (1994)[a] Elizabeth Kebede and Willen (1998)[b]	Adamneh Dagne (2010)[a] Girma Tilahun (2006)[b]	Girum Tamire (2014)	Lemma Abera (2016)	Present study (2017)	**General trend**
pH	8.5[b]	8.7[a]	8.4	8 - 8.4	8.38	NC
EC (µS/cm)	410[b]	425.4[a] , 478[b]	419.1	361.5 - 484.5	489.8	Increasing
Chl-*a* (μgL^{-1})	(48 - 334)[a], 154[b]	23.4[a]	-	37 - 54.5	10.1 - 43.7	Decreasing
TP (μgL^{-1})	219[b]	68.5[b]	-	-	262.2	Increasing
SRP (μgL^{-1})	< 1[b]	10.1[b]	14 - 64.5	38.2 - 64.7	30.1 - 430	Increasing
NO_3- (μgL^{-1})	-	3.2[b]	33.7 - 89.1	30.1 - 61.7	50 - 620	Increasing
NH_4+ (μgL^{-1})	36.3[a]	111[b]	56.5 - 212.1	64.2 - 258.9	20.3 - 290	Increasing

2.4.3 Characterization of reference and test (affected) sites

Developing multimetric index as part of a bioassessment program requires establishment of reference conditions (Barbour *et al.*, 1996). So sites can be classified based on *a priori* or *a posteriori* approach to ascertain reference site conditions (Barbour *et al.*, 1999). The reference condition approach (RCA) has recently emerged as a broadly applicable protocol to monitor streams, rivers, and lakes (Tall *et al.*, 2008; Wang *et al.*, 2015). In the present study, sites were pre-assigned or designated based on a professional judgment, and classified on *a posteriori* approach based on a measured lake habitat quality and physicochemical variables using cluster analysis. Study sites classification is one of the common practice during a multimetric index development (Wang *et al.*, 2015); and such classification is a common practice in many parts of the world (Thorne and Williams, 1997). Getachew Beneberu (2013) and Seid Tiku *et al.* (2013) have also used the same approach to distinguish the reference sites from test sites in their studies.

Reference state is relatively with little modification by humans which can be the bench mark for several biodiversity surrogates (Gibbons *et al.*, 2008). Usually, it is difficult to define actual reference sites in a homogeneous ecological region (Wang *et al.*, 2015), and as well due to frequent occurrence of pollution in Ethiopian Rift Valley Lakes. Thus, in this study, the classification of reference and impaired sections used a relative category. The presence of five islands in Lake Ziway (3 of which are free from human settlements) was considered as an option for the relative better references. Therefore, the reference and impaired sites were differentiated based on the physicochemical and habitat quality information (land use pattern, habitat degradation, and lakeshore modification) of the studied lake segments.

Subsequently, based on the lake habitat quality assessment score, physicochemical, and bacteriological data, study sites were clustered into three levels of overall dissimilarity. Sites classified as "references" were considered representative of the least disturbed in their respective lakeshore study sites. The result obtained through the cluster analysis using Dendrogram which showed the similarity of the three sites in their environmental data distribution. Besides, these sites were characterized with relative lower conductivity reading and nitrate concentration (Table 2.3 and Table 2.4), and also exhibited better natural shore and riparian vegetation coverage (Table 2.1 and Table 2.2).

Studies of inland freshwaters indicate that streams supporting diverse aquatic life have a conductivity range between 150 and 500 µmhos/cm, and conductivity outside this range could indicate that the water is not suitable for certain species of fish or invertebrates (US EPA, 2012). All reference sites showed <500 µS/cm (between 405 and 470) EC reading. Therefore, the possible reason for the lower EC and ions concentration in the references could be related with the relative better natural land use/land cover conditions observed and absence of significant lakeshore modification (Fig. 1.2 and Table 2.2).

Conversely, sites classified as "most disturbed" were more similar in the disturbance category of components score, and were characterized with higher relative conductivity reading and nitrate concentration. Therefore, most likely the overall study sites clustering was appropriate as evaluated through physical, chemical, and bacteriological data. However, agreed threshold for the chemical values in the reference sites within the ecoregion should be standardized, and can be a criterion for determining the reference condition in upcoming bioassessment studies.

CHAPTER 3: BENTHIC MACROINVERTEBRATES DIVERSITY AND DISTRIBUTION IN RELATION TO ABIOTIC FACTORS IN THE LITTORAL ZONE OF LAKE ZIWAY

3.1 INTRODUCTION

In lentic freshwaters, macroinvertebrates play an indispensable role in key ecosystem processing, such as food chain dynamics, productivity, nutrient cycling, biochemical breakdown, and decomposition (Covich *et al.*, 1999; Smith *et al.*, 2007). They are crucial part of the stream food web (Mandaville, 2002). Their distribution and abundance are directly related to different environmental factors such as food availability and quantity, sediment type, substrate, and water quality (Barbour *et al.*, 1999; Mackie *et al.*, 2013). In aquatic ecosystems, the assessment of the diversity and distribution of macroinvertebrates in relation to abiotic factors will provide a desirable particular information about the status of the ecosystem (Butler and Bird, 2010).

Benthic macroinvertebrates most preferably used in biomonitoring studies (Barbour *et al.*, 1999; Covich *et al.*, 1999), and are important ecological tools to describe spatial and temporal changes in the aquatic ecosystems (Vyas and Bhawsar, 2013). Conversely, various human influences used for recreational purposes, bathing for personal hygiene, irrigation, artificial beach construction, domestic and industrial wastewater discharges etc., cause habitat impairment and affect the benthic biodiversity of streams, rivers, and lakes (Rak *et al.*, 2011; Trichkova *et al.*, 2013). Thus, bioassessment focuses on changes in biotic community structure and function in response to a human disturbance on the aquatic environments, to assess and comprehend the environmental changes of the aquatic ecosystems (Jones *et al.*, 2007; Smith *et al.*, 2007).

Different biological attributes of macroinvertebrates can be used as indicators of water quality such as: (i) abundance: used as a tool in trophic status; (ii) richness: an attribute whose variation is associated with several environmental factors/stressors; (iii) diversity: contemplates the presence and absence of taxa as a system evaluation criterion; (iv) biomass: used in developing metrics and/or indices for environmental quality assessment.

In lakes, the macroinvertebrates community may be susceptible to water level changes that alter sediment exposure and redistribution, and temperature regime (Blakely and Harding, 2005; Butler and Bird, 2010). They also show considerable spatial variation with lake depth and across habitats (Trichkova *et al.*, 2013; Bazzanti *et al.*, 2017). However, macroinvertebrates respond sensitively to pollution, and to several other human impacts (hydrological, morphological and recreational), their potential use for a holistic indication system for lake ecosystem health has been considered to support bioassessment and biomonitoring applications (Solimini *et al.*, 2006).

Eventhough lake water quality assessment was made using physical and chemical parameters in Lake Ziway, no earlier study has been done on the macroinvertebrates. This chapter, therefore, will attempt to assess the diversity of benthic macroinvertebrates and their distribution in relation to physicochemical parameters and lakeshore habitat quality mainly based on the gradation of the human pressures. Consequently, this study is geared towards 1) providing a comprehensive list of macroinvertebrates taxa and their distribution in relation to physicochemical factors; 2) identifying the main factors driving macroinvertebrates diversity and distribution; 3) assessing the ecological condition of the lake through macroinvertebrates diversity metrics.

3.2 MATERIALS AND METHODS

3.2.1 Benthic macroinvertebrates sample collection and identification

Benthic macroinvertebrates were collected based on the method outlined in Ontario Benthos Biomonitoring Network Protocol Manual (Jones *et al.*, 2007). On the representative lake segments, a series of transects were established from the water's edge to 1 meter depth (nearly 10 m distance) with 3 replicates. A 500 µm mesh net, D-frame Traveling Kick (30cm x 28cm in diameter) were used. Macroinvertebrate samples were collected actively for 10 minutes per replicate, or until at least 100 animals were collected, using a multi-habitat approach. Physicochemical parameters were measured using standardized measuring methods (Materials and methods - Chapter 2).

After samples were collected, net contents of macroinvertebrates were transferred to sampling bottle, preserved with 10% buffered formalin and transported for later sorting and identification to the Limnology Laboratory of the Addis Ababa University. The preservative was replaced with 70% ethanol to prevent hard body parts from dissolving per the recommendation of Barbour *et al.* (1999). In the laboratory, samples were washed through a 500µm sieve again and were rinsed well with water to remove the preservative, to make the samples suitable for examination. Sorted and picked/selected in a white tray, identified and counted under dissecting microscope.

Taxonomic identification was made to family level using standard keys (Macan, 1979; Edington and Hildrew, 1981; Gerber and Gabriel, 2002; Bouchard, 2004). Animals that cannot be identified were recorded as unknown, but their count was not considered part of the 100-animals. Diversity metrics representing richness, composition, diversity, and abundance measures were considered. The standard metrics like Shannon Diversity Index

(H′) (a commonly used diversity index that considers both abundance and evenness of species present in the community), Family-level taxa Richness (RICH), and Equitability Index (J) were used for evaluating benthic macroinvertebrates diversity of the lake at different spatiotemporal scales.

3.2.2 Data analysis

The association between macroinvertebrates distribution and physicochemical variables was evaluated with canonical multivariate analysis using CANOCO for windows 4.5 version software (Ter Braak and Smilauer, 2002). Detrended correspondence analysis (DCA) was employed to check the response of the data, and it was found that the length of the longest gradient was 1.137. Therefore, redundancy analysis (RDA) was used as the benthic macroinvertebrates taxa data showed a linear response to the environmental variables. Benthic macroinvertebrates taxa with total percent abundance < 0.1 were not included in the assessment of the association between benthic macroinvertebrates taxa distribution and physicochemical variables.

Benthic macroinvertebrates diversity in Lake Ziway was computed with Shannon-Wiener diversity index, Dominance, and Equitability index by using PAST software. Percent abundance of each taxon was calculated on excel spreadsheet Microsoft 2007. Significant differences in the diversity index values and the abundance of invertebrates between the study sites were compared using Kruskal-Wallis test. Later, environmental parameters and macroinvertebrates diversity measures were incorporated to find the most appropriate information on the assessment of benthic macroinvertebrates distribution in relation to the environmental variables of Lake Ziway.

3.3 RESULTS

3.3.1 Benthic macroinvertebrates diversity and distribution

Thirty-four benthic macroinvertebrate taxa were identified, which belonged to 12 orders (Table 3.1), and their relative frequency of occurrence and abundance were determined (Appendix 1). The results were based on 10,276 individuals, collected in 36 sampling rounds in 9 sampling sites. Hemipterans attained highest relative number of families (9) with percent abundance of 25.77 followed by Gastropods (Snails) and Bivalves (4 each) contributed percent abundance of 18.57 and 17.29, respectively (Table 3.5).

Benthic macroinvertebrate communities were dominated by Sphaeridae, Chironomidae and Physidae families which contributed a total percent abundance of 19.1, 14.5 and 13.3, respectively, while Philopotamidae, Aeshnidae, Corbiculidae, Unionidae, and Tabanidae families were sparse relatively by contributing a total percent abundance of < 0.1 each (Table 3.1).

Percent abundance of the benthic macroinvertebrates was different between the study sites ($p<0.05$), FRC site contributed the highest abundance value (17.75%) and KO with the lowest value (6.23%) (Fig. 3.1). Percent abundance of benthic macroinvertebrates in the reference study sites was highest by contributing (43.59%) than both test sites, sites with intermediate and multiple stressors contributed 24.91% and 31.49%, respectively. Percent of Hydropsychidae, Philopotamidae, and Polymitarcyidae varied considerably between test and reference sites, their percent abundance was higher in the reference sites, while percent Oligochaete was higher in test sites (55.4) than reference sites (21.6) (Appendix 2).

Table 3.1 Percent abundance of benthic macroinvertebrates identified in the littoral zone of Lake Ziway (* class and ** Subclass).

Order	Family	Number	Percent Abundance	Shannon-Weiner Diversity Index (H')
Trichoptera	Hydropsychidae	150	1.46	0.643
	Philopotamidae	9	0.09	0
Ephemeroptera	Baetidae	516	5.01	1.284
	Caenidae	87	0.85	1.727
	Polymitarcyidae	369	3.59	0.944
Odonata	Coenagrionidae	695	6.42	1.915
	Aeshnidae	3	0.03	0.146
	Libellulidae	72	0.69	1.831
Hemiptera	Belostomatidae	1020	9.91	1.958
	Corixidae	972	9.43	1.833
	Gerridae	23	0.22	0.621
	Mesoveliidae	42	0.41	0.692
	Naucoridae	77	0.71	0.076
	Nepidae	15	0.15	1.512
	Notonectidae	395	3.14	1.774
	Veliidae	85	0.53	1.317
	Cicadellidae	19	0.18	1.868
Coleoptera	Hydrophilidae	189	1.53	2.083
	Notoridae	41	0.29	0
Trombidiformes	Hydrachnidae	14	0.14	0.991
Bivalvia*	Corbiculidae	5	0.05	1.928
	Sphaeriidae	1755	19.1	1.928
	Unionidae	3	0.03	0
	Dreissenidae	14	0.14	0.891
Gastropoda*	Physidae	1373	13.3	1.418
	Lymnaeidae	34	0.33	1.745
	Planorbidae	83	0.79	0.071
	Pleuroceridae	418	4.01	1.778
Diptera	Chironomidae	1492	14.5	2.026
	Tabanidae	9	0.09	0.245
Hirudinea**	Hirudinidae	16	0.16	1.488
Araneae	Pisauridae	113	1.09	1.597
	Tetragnathidae	21	0.20	0.214
Oligochaeta**	Oligochaetes	147	1.43	1.923

Shannon-Wiener diversity index (H′) values of Hemiptera were high (2.031) followed by Coleoptera (2.016) and Diptera (2.002), whereas Trichoptera displayed the least value (0.776) (Table 3.1). H′ value of the macroinvertebrates was different between the study sites ($p<0.05$), and a considerable larger values were observed in references (Table 3.2). H′ value in FRC site was high (2.415), while low in CA sampling site (1.779). Similarly, H′ value in reference sites was high (2.692) than both test sites, sites with intermediate stressors and multiple stressors (2.224) and (1.977), respectively. Equitability index (J) value of the macroinvertebrates displayed no significant difference between the study sites and clustered sites ($p> 0.05$).

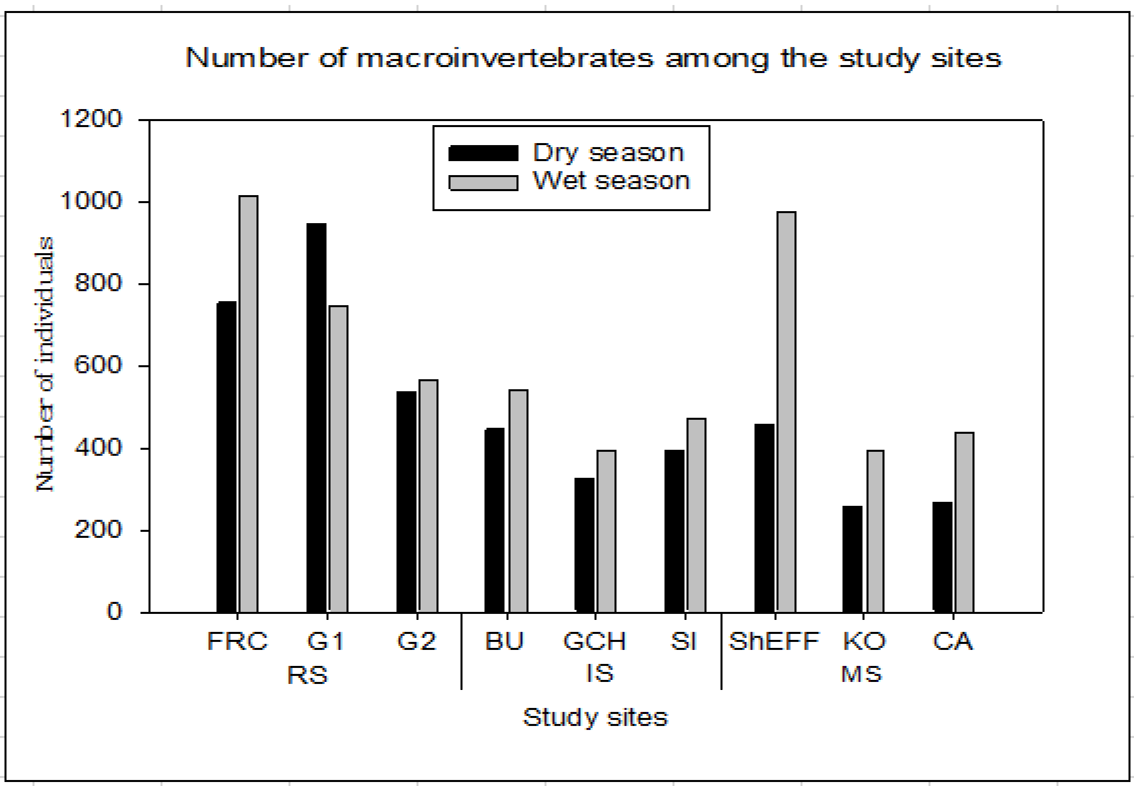

Figure 3.1 Macroinvertebrates abundance distribution among the study sites and clustered sites across categories of season. (**Abbreviations:** RS: Reference Sites; IS: Intermediate Stressors; and MS: Multiple Stressors).

3.3.1.1 Benthic macroinvertebrates taxa richness

Benthic macroinvertebrates taxa richness was different between the study sites. FRC site showed the highest number of taxa (29) and KO the lowest (17) (Table 3.2). The total of macroinvertebrate families collected from the littoral zone of Lake Ziway declined from 34 in reference sites to 23 in the test sites. The taxa richness of macroinvertebrates were lower in all sites with multiple stressors (Table 3.2). Number of families identified within the insect orders of Ephemeroptera and Trichoptera were high in G1 site (5) which is a cluster of reference sites and low in ShEFF site (1), a cluster of test sites or multiple stressors site (Appendix 5).

Table 3.2 Determination of macroinvertebrates taxa richness, Shannon diversity index, Dominance, and Equitability index values of the study sites of Lake Ziway.

Site Clusters	Sites	Taxa Richness	Dominance (D)	Shannon-Weiner Diversity Index (H′)	Equitability (Evenness) Index (J)
Intermediate stressors	BU	23	0.179	2.221	0.780
	GCH	19	0.179	2.242	0.845
	SI	20	0.286	1.934	0.663
Multiple stressors	ShEFF	18	0.247	2.008	0.716
	KO	17	0.200	2.004	0.769
	CA	18	0.347	1.779	0.614
Reference sites	FRC	29	0.215	2.415	0.701
	G1	24	0.219	2.276	0.654
	G2	24	0.194	2.281	0.729

Table 3.3 Abundance of benthic macroinvertebrates in the clustered study sites.

Order	Multiple stressors		Intermediate stressors		Reference sites	
	Number	Percent	Number	Percent	Number	Percent
Trichoptera	6	3.8	9	5.6	144	90.6
Ephemeroptera	198	20.4	141	14.5	631	65.1
Odonata	293	39.3	209	28.0	244	32.7
Hemiptera	703	27.0	559	21.5	1341	51.5
Coleoptera	56	26.5	59	28.0	96	45.5
Trombidiformes	0	0.0	2	14.3	12	85.7
Bivalvia	489	26.1	849	45.4	532	28.4
Gastropoda	852	44.6	297	15.5	762	39.9
Diptera	435	29.0	257	17.1	809	53.9
Hirudinea	5	31.3	1	6.3	10	62.5
Araneae	28	24.3	27	23.5	60	52.2
Oligochaeta	35	23.8	82	55.8	30	20.4

3.3.2 Seasonal composition of benthic macroinvertebrates

Number of individuals and families were high in the wet season (5,803 and 33) than the dry season (4,473 and 29), respectively (Table 3.4). Total number of organisms under Hemipterans were higher in the wet season (1,665) with 28.69% composition than the dry season (983) with 21.98%, while Dipterans were higher in the dry season (1,050) than a wet season (451), and thus the Chironomidae alone contributed 99.61% composition of the dipterans in the dry season (Fig. 3.2).

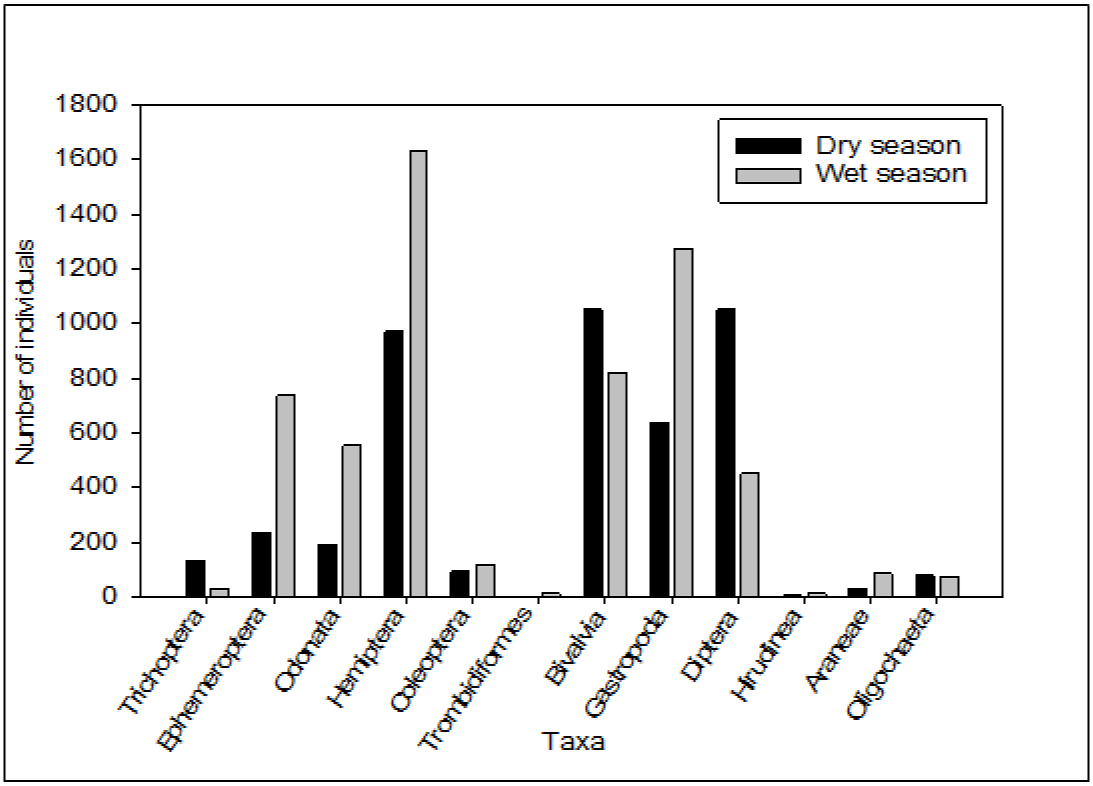

Figure 3.2 Seasonal variation in the density of macroinvertebrates in the assortment of their orders in the littoral zone of Lake Ziway.

The density of the macroinvertebrates was higher in wet season except for Trichoptera, Bivalvia, and Diptera (Fig. 3.2), percent abundance values of Bivalvia and Diptera were higher in the dry study season and contributed 24.12 and 23.47, respectively (Table 3.5). Baetidae, Belostomatidae, Coenagrionidae, Corixidae, and Hydrophilidae were relatively abundant in the wet season whereas Sphaeriidae, and Chironomidae were relatively abundant in the dry season. Coleopterans, Hydrachnidae, Hirudinidae, and Oligochaeta showed no variation across categories of season in abundance. The lowermost abundance values in both dry and wet seasons were that of Hirudinidae 0.11% and 0.19%, respectively and Hydrachnidae 0.07% and 0.19%, respectively (Table 3.5).

Table 3.4 Determination of diversity metrics/indices across the categories of season.

Diversity parameters	Dry Season	Wet Season
Number of taxa	29	33
Number of individuals	4,472	5,791
Dominance (D)	0.136	0.098
Shannon (H′)	2.379	2.651
Evenness (e^ H/S)	0.372	0.429
Equitability (J)	0.706	0.758

Table 3.5 Seasonal composition and percent abundance of benthic macroinvertebrates.

	Dry Season		**Wet Season**		**Total**	
Taxa	**Number**	**Percent Abundance**	**Number**	**Percent Abundance**	**Number**	**Percent Abundance**
Trichoptera	131	2.93	28	0.48	159	1.55
Ephemeroptera	267	5.97	705	12.15	972	9.46
Odonata	136	3.04	634	10.93	770	7.49
Hemiptera	983	**21.98**	1,665	**28.69**	2,648	**25.77**
Coleoptera	54	1.21	176	3.03	230	2.24
Trombidiformes	3	0.07	11	0.19	14	0.14
Bivalvia	1,079	**24.12**	698	12.03	1,777	**17.29**
Gastropoda	645	14.42	1,263	**21.76**	1,908	**18.57**
Diptera	1,050	**23.47**	451	7.77	1,501	14.61
Hirudinea	5	0.11	11	0.19	16	0.16
Araneae	43	0.96	91	1.57	134	1.30
Oligochaeta	77	1.72	70	1.21	147	1.43
Total	**4473**	**100%**	**5803**	**100%**	**10276**	**100%**

3.3.3 Relationships between physicochemical variables and distribution of benthic macroinvertebrates

The RDA-triplot of samples, macroinvertebrates, and environmental variables indicated that axis 1 and axis 2 contributed 81.7% of the cumulative percentage of variance in a species-environmental relationship (Fig. 3.3). The RDA ordination of macroinvertebrates taxa association indicated that nitrate, silicon dioxide, nitrite, ammonium, soluble reactive phosphorus, total phosphorus, temperature, and electrical conductivity were positively correlated with the first axis and contributed 59.2% of the variance. The first four nutrient variables were strongly correlated with this axis (Table 3.6).

Density of Hirudinidae, Physidae, and Chironomidae was positively related with nitrogen and water temperature, besides Oligochaeta and Coenagrionidae were positively related to electrical conductivity, soluble reactive phosphorus, and ammonium. Philopotamidae and Planorbidae were positively related to silicon dioxide, besides Sphaeridae and Lymnaeidae showed positive correlation with total suspended solids and turbidity. Baetidae, Veliidae, Mesoveliidae, Pisauridae, Corixidae, Notonectidaae, and Nepidae showed a positive correlation with total phosphorus (Fig. 3.3).

The association of Chironomidae, Physidae, and Hirudinidae with nitrite and nitrate, Baetidae, Veliidae and Mesoveliidae with TP was strong ($p<0.05$). Polymitarcyidae, Hydropsychidae, and Naucoridae showed strong and negative association with TP, and similarly displayed negative association with most of the nutrient variables but was not strong. Hirudinidae, Chironomidae, and Oligochaeta showed a negative association with pH and DO. The second axis was positively correlated with all environmental variables except DO, pH and silicon dioxide, but not strongly.

Table 3.6 Results of redundancy analysis (RDA) of benthic macroinvertebrates taxa versus physicochemical variables relationship including eigenvalues and percentage variance explained by the first two axes (**Abbreviations:** DO: Dissolved oxygen; EC: Electrical Conductivity; SRP: Soluble Reactive Phosphate; TP: Total Phosphate; and TSS: Total Suspended Solids), (strong correlations are marked bold).

Axes	**1**	**2**
Eigenvalues	0.592	0.225
Cumulative percentage variance of species- environment relation	59.2	81.7
DO	**-0.6996**	-0.0354
pH	-0.4925	-0.0313
EC	0.2406	0.1305
Temperature	0.3417	0.0815
Turbidity	**-0.7098**	**0.4257**
Nitrate	**0.9101**	0.1984
Nitrite	**0.7481**	0.1854
Ammonium	**0.6258**	0.3411
SRP	0.2752	0.018
SiO_2	**0.7708**	-0.4176
TP	0.3908	**0.6495**
TSS	**-0.7064**	**0.4262**

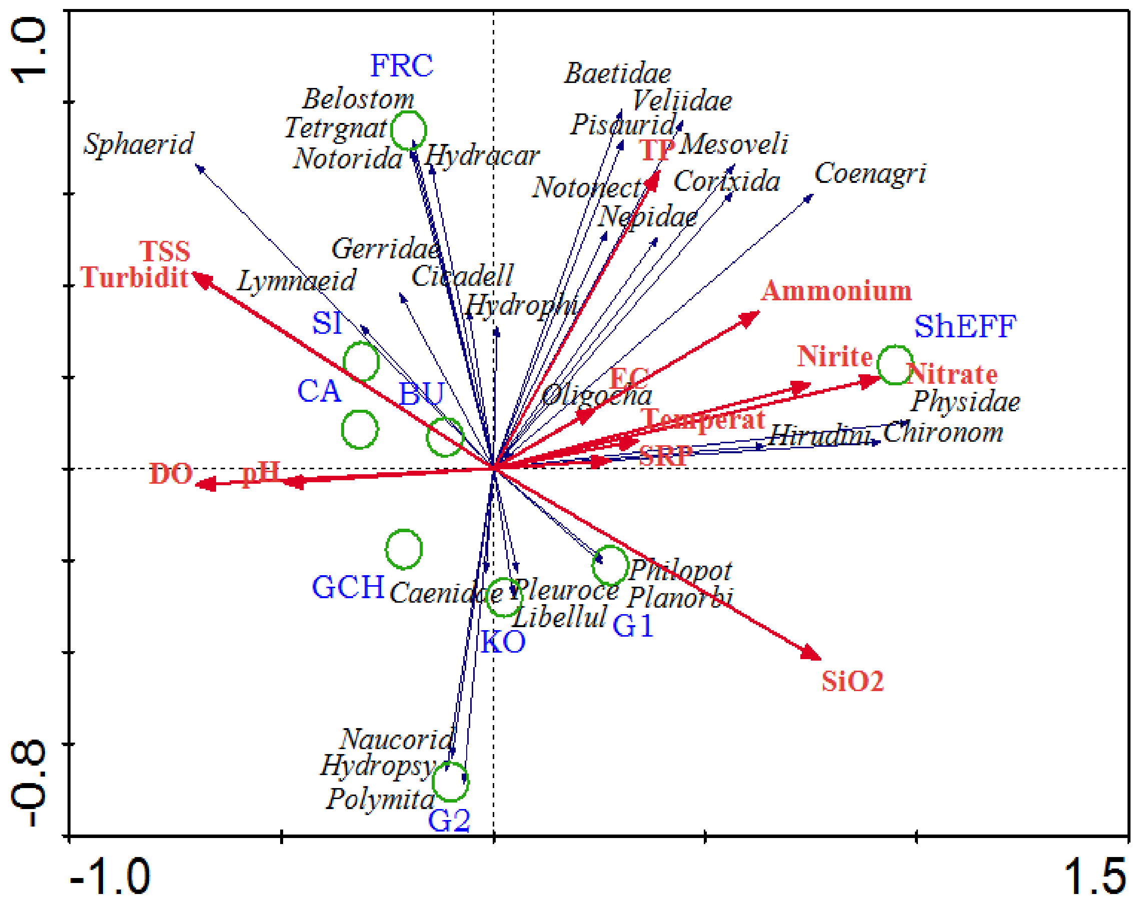

Figure 3.3 RDA-triplot of samples, macroinvertebrates and environmental variables based on the first two axes. (**Abbreviations:** BU: Buchesa; GCH: Gabriel Church; ShEFF: Sher Ethiopia flower farm; KO: Korokonch; CA: Cafeteria; FRC: Fishery research center; G1: Gelila 1; G2: Gelila 2; DO: Dissolved Oxygen; EC: Electrical Conductivity; TP: Total Phosphorus; SRP: Soluble Reactive Phosphorus; and TSS: Total Suspended Solids) (Full name of macroinvertebrates taxa - **Table 3.1**).

3.4 DISCUSSION

3.4.1 Diversity and distribution of benthic macroinvertebrates

Hemipterans contributed the highest relative percent abundance (25.41) followed by Snails (18.56) and Bivalves (18.16). The highest abundance of Hemipterans was possibly because of their high number of families and their cosmopolitan performance. Barman and Gupta (2016), reported that Hemipterans are known with their broad range of habitats with a water body. Snails colonize quickly; are tolerant to habitat variability due to a strong and thick shell (Turner *et al.*, 2016); most are parthenogenetic females so only one snail is needed to produce more in short time (Flores and Zafaralla, 2012).

The macroinvertebrate communities were dominated by Sphaeriidae, Chironomidae, and Physidae and this is most likely because of the lake bed habitat type, their rapid life cycle and most likely over 50% of the sites were stressed. According to Galdean *et al.* (2001) clayed bed with sand is the preference of a large community of Bivalves such as Sphaeriidae with reports of >300ind/m^2 in Tanque River, Brazil. The least number of Hydrachnidae and Hirudinidae families recorded in this study is possibly because of the small size of the organisms in relation to the mesh size of the net used.

The Shannon diversity and taxa richness values were maximum in FRC site which fits in the reference sites and minimum in CA and KO sites, respectively which are from the multiple stressors sites. Maximum Simpson's Dominance value (0.347) indicated that CA site was occupied by dominant species and, therefore, justified the lowest H' value. This is most likely because of the multiple stressors pressure on the CA site as it is a shore dominated by multiple human activities like waste dumping, boat transport landing site and the high percentage of non-natural habitat conditions.

Since diversity values for real communities are often found to fall between 1.0 and 6.0 (Stirling and Wilsey, 2001), diversity in all study sites of Lake Ziway was relatively low - moderate since none had an H' value higher than 2.5. Wilhm and Dorris (1968), proposed a relationship between species diversity and pollution status of water: H > 3 clean water; H = 1 to 3 moderately polluted; and H < 1 heavily polluted. Staub *et al.* (1970), had set the diversity index of <1 for poor diversity; 1-2 for moderate diversity; 2<3 for average diversity; and >3 for high diversity. Thus, all study sites in Lake Ziway would come out between moderately and averagely diversified (had a value of H' between 1.77 and 2.41). Accordingly, benthic macroinvertebrates diversity in Lake Ziway could be affected by the presence of environmental stressors around the riparian and littoral zones.

Macroinvertebrates taxa richness exhibited a gradual, significant decline from reference sites to test sites. Bode *et al.* (2005), categorized sites with greater than 26 taxa as non-impacted, 19-26 as slightly affected, 11-18 as moderately affected, and 0-10 as severely affected. Because of this, the study sites in Lake Ziway fit between non-impacted and moderately affected. One site from the reference group was categorized as non-impacted; two sites of reference and all sites from the intermediate stressors as slightly affected; and all the sites from multiple stressors group as moderately affected.

3.4.2 Seasonal composition of benthic macroinvertebrates

Shannon diversity and Simpson's Dominance values were not different, and found 2.65 and 0.90, respectively, in wet season and 2.37 and 0.86, respectively, in the dry season. The apparent lack of significant seasonality in the diversity of macroinvertebrates in Lake Ziway could be because of the presence of active and growing benthic invertebrate populations during the entire year. Flores and Zafaralla (2012), in their study of benthic invertebrates in lowland tropical river Mananga in the Philippines also observed a lack of seasonality, suggesting that benthic communities have similar favorable conditions and stresses throughout the year. Yuen and Dudgeon (2016), have also reported the absence of a distinct cold season in tropical areas which allows many species to reproduce and present all year round.

There was a variation in the density of benthic macroinvertebrates in Lake Ziway during the two seasons. Possibly the seasonal difference in the density of the macroinvertebrates would be the variation of the availability of the food. This result agrees with the findings of Basu *et al.* (2018), in that the difference in the availability of food resources was the determinant factor for the macroinvertebrates density variation between the two seasons. Other workers have suggested factors such as microclimatic changes (Barman and Gupta, 2016; Gogoi and Gupta, 2017); water quality changes (Barman and Gupta, 2016; Basu *et al.*, 2017) and variation in the availability of food resources (Basu *et al.*, 2017; Basu *et al.*, 2018) as causes for fluctuation in abundance of macroinvertebrates.

Dipterans were higher in the dry season (1,050) than the wet season (451), and thus the Chironomidae alone contributed 99.61% of the dipterans in the dry season. This is most likely because of the Chironomidae has diverse feeding habits and the larvae occur in almost every conceivable aquatic habitat. Silveira *et al.* (2013), reported larvae of Chironomidae have highly diverse feeding habits (collector-gatherers, collector-filterers, scrapers, and shredders) and ingest a wide variety of foods such as algae, detritus, macrophytes, debris, and animal matter (Mazão and da Conceição Bispo, 2016). They also may go into latency or produce eggs resistant to unfavorable environmental conditions (Montalto and Paggi, 2006; Cañedo-Argüelles *et al.*, 2016).

Besides, due to limited studies on benthic communities of Lake Ziway except the research work conducted by Martens and Tudorancea (1991), data for comparison of seasonal changes and diversity pattern is scarce. Therefore, the macroinvertebrates data recorded in the present study will serve as a benchmark information for future studies.

3.4.3 Distribution of benthic macroinvertebrates in relation to the physicochemical variables

RDA indicated that macroinvertebrate assemblages in the littoral were influenced by environmental vectors. In the ordination diagram, macroinvertebrates and environmental variables indicated that axis 1 and axis 2 make 81.7% of the cumulative percentage of variance in a species-environmental relationship, whereas the remaining 18% of variance might be due to biotic and abiotic factors not considered in this study such as BOD_5, COD, heavy metals, pesticides, etc. Thus, for better verification of the species-environmental relationship it will be necessary to include many other environmental parameters.

Density of Hirudinidae, Physidae, and Chironomidae was positively related with nitrogen and temperature. This might be possibly because most of these families diversity increased with increases in hardness and nutrients. Hirudinea (leech) can live in different trophic levels in lakes (Capítulo *et al.*, 2001; Cortelezzi *et al.*, 2015), Physidae can tolerate less than optimal water quality due to eutrophication, resulting in increasing nitrate concentration (Zeybek *et al.*, 2012). Oligochaeta was positively related to electrical conductivity and ammonium, most likely because of its tolerance capacity. According to the findings of Ragonha *et al.* (2014), Oligochaeta is resistant to hard and nutrient-rich water and also abundant in sites with high organic matter concentrations.

Polymitarcyidae showed strong and negative association with TP and similarly displayed negative association with most of the nutrient variables. This is most likely because of the high sensitivity of this family to environmental variables. Hirudinidae, Chironomidae, and Oligochaeta showed a negative association with DO, and this result is comparable with the general observation of Rosa *et al.* (2014), Oligochaeta and Chironomidae tolerate low levels of DO. According to Hamburger *et al.* (1995), the possibility of Chironomidae species survival in low oxygenation condition is due to hemoglobin, which allows the maintenance of aerobic metabolism despite the low oxygen concentrations and gradual reduction of this metabolism until they go into latency under anoxia condition.

Abundance of tolerant organisms such as Physidae, Sphaeridae, and Chironomidae, and the total absence of sensitive order Plecoptera could be an indication of deterioration of lake water quality and/or pollution in Lake Ziway. However, there were few pollution-sensitive (Philopotamidae and Polymitracyidae), and some moderately-sensitive families (Baetidae, Hydropsychidae, and Hydrophilidae), which implies that the reference sites were not as polluted as the test sites. Benthic invertebrates' diversity and taxa richness decreased from the reference to test sites indicating better water quality and favorable habitat conditions for macroinvertebrate communities in the references. This observation was confirmed by the diversity index where references came as none to slightly-impacted, and the test sites as moderately-impacted, showing that benthic macroinvertebrates were useful indicators of water quality in Lake Ziway.

CHAPTER 4: BENTHIC DIATOMS DIVERSITY AND DISTRIBUTION IN RELATION TO THE ABIOTIC FACTORS IN THE LITTORAL ZONE OF LAKE ZIWAY

4.1 INTRODUCTION

Diatoms are microscopic algae having distinct geometrical shapes (Gutierrez *et al.*, 2017); occur where photosynthesis is possible (Chen *et al.*, 2016a); believed to be of global significance in biogeochemical cycles and the functioning of aquatic food webs (Malviya *et al.*, 2016). They constitute a large component of aquatic biomass, particularly during visible seasonal phytoplankton blooms and mainly possess photosynthetic pigments such as chlorophyll a, c, and fucoxanthin (Taylor and Cocquyt, 2016). They are planktonic (free-floating) or benthic (attached to a substratum) and also occur as endosymbionts in dinoflagellates and foraminifers (Stevenson *et al.*, 2010). Some benthic diatoms have a specialized raphe system that secretes mucilage to attach along a surface (Malviya *et al.*, 2016).

The most distinctive feature of this unicellular algae is its extracellular coat or frustule, which comprises of two overlapping valves, composed of silica (hydrated silicon dioxide) fitting into each other connected by girdle bands (Taylor and Cocquyt, 2016). Diatoms growth and composition may be influenced by nutrients like nitrate, phosphate, and silicate (Taylor and Cocquyt, 2016; Dalu *et al.*, 2017). Modifications in the nitrogen, phosphorus and silicate concentrations affect the cellular composition, fatty acid profile of the lipid fraction and the final growth rate of the diatoms (Agusti *et al.*, 2015). The relationship and composition between the nutrients were also an important factor for the growth rate of the diatoms (Malviya *et al.*, 2016).

The diversity and taxonomic composition of local diatoms result from a balance between processes operating on a regional scale, such as allopatric species formation and geographic dispersal (Cox, 2014); which both add species to communities, and processes capable of promoting local extinction, including environmental degradation, size and nature of nutrients, and pollution (Kulikovskiy and Kuznetsova, 2014). They should also be associated with factors including basin morphometry, mixing dynamics, water clarity, and alkalinity (Reynolds, 1998; Mellard *et al.*, 2011).

Diatoms are good bio-indicators of water quality (Stevenson *et al.*, 2010); react rapidly to disturbances, such as physicochemical conditions of the water or to pollution-affected catchment areas (Chen *et al.*, 2014); with short life cycles they respond quickly to the environmental conditions (Chen *et al.*, 2016b). Diatoms typically have relatively narrow preferences for several environmental variables and their community structure responds to changing environmental conditions rapidly (Taylor and Cocquyt, 2016; Dalu *et al.*, 2017). Using diatoms for biomonitoring is preferable due to their cosmopolitan nature, rapid cell cycle, and the ability to provide a relatively rapid indicator for disturbance (Dalu *et al.*, 2017; Dell'Aquila *et al.*, 2017).

Benthic diatoms from the littoral can indicate major environmental gradients (Kelly *et al.*, 2014). Periphytic diatom assemblages in the littoral are important components of benthic assemblages (Chen *et al.*, 2016a), and sensitive bioindicators of chemical conditions (Bennion and Simpson, 2011). Benthic diatoms respond sensitively to the hydro-morphological modification (Hering *et al.*, 2006b; Delgado *et al.*, 2010). Often they change their species composition or diversity, which can vary from species-rich to monotonous communities based on the aquatic habitat quality (Ács *et al.*, 2004).

In addition, benthic diatoms are an adequate reference for measuring biodiversity because of their high species richness in relative to other benthic communities (Chen *et al.*, 2014; Siqueiros-Beltrones *et al.*, 2017). The relative abundance of diatom species can calculate and describe the assemblages through joint analyses of classical parameters such as species richness, diversity, dominance, and equitability, which can help to detect patterns in the ecological status of the particular water body (Chen *et al.*, 2014; Dalu *et al.*, 2017).

The advantage in benthic diatoms is that they can provide a useful marker for detecting localized, short to medium-term changes and patchiness at small spatial scales, for example, pollution resulting from the intermittent release of sewage and shoreline development (Passy, 2007; Dalu *et al.*, 2017). In the form of permanently prepared slides or acid digested samples, collected diatom samples can be archived easily for long periods of times for future analysis and long-term records, and also can be investigated whenever necessary (Stevenson *et al.*, 2010).

There have been studies regarding phytoplankton diversity and distribution in Lake Ziway (Gasse *et al.*, 1983; Girma Tilahun, 1988; Elizabeth Kebede and Willén, 1998; Girma Tilahun, 2006; Adamneh Dagne, 2010; Girma Tilahun and Ahlgren, 2010; Mesfin Gebrehiwot *et al.*, 2017). However, these studies recorded composition and distribution, whereas the present study investigated the abiotic factors that determine the structure of the diatom assemblages across both spatial and temporal gradients. The relationship among diatom communities and human disturbance factors was also studied. In addition, this study provides one more reference to identifying the freshwater benthic diatom communities of Lake Ziway.

4.2 MATERIALS AND METHODS

4.2.1 Benthic diatoms sample collection and processing

Benthic diatoms, epilithic (from cobbles) and epiphytic (from macrophytes) were sampled. Epilithic diatoms were collected from three cobbles obtained from the littoral zone. Loosely attached surface contaminants were removed by washing the cobbles slightly with lake water, samples were collected by brushing a measured square area of 25cm^2 on the upper surface of each cobble with a hard toothbrush in a white plastic tray, then were pooled to form a single sample according to Kelly *et al.* (1998).

Epiphytic diatom samples were collected from five pieces of permanently growing submerged macrophytes of about 5 cm each on which a layer of diatoms/biofilms were visible. Pieces of macrophytes were placed together in a plastic zip bag with 50 mL of lake water, closed and rubbed firmly between hands, epiphyton were removed by vigorously shaking the plastic bag for 2 minutes according to Zimba and Hopson (1997) and Taylor *et al.* (2007a) . The resulting brown-greenish liquid was poured into a sample bottle, were preserved with 20% ethanol, and transported for cleaning in the Limnology Laboratory of Addis Ababa University. Physicochemical parameters were also measured using standardized measuring methods (Materials and methods - Chapter 2).

In the laboratory, diatom frustules were cleaned with concentrated sulfuric acid and acid-cleaned samples were washed with distilled water. Cleaned frustule samples were filled to a volume that provides an adequate density of diatom valves, mounted on permanent slides using Naphrax mounting medium (refractive index 1.73) and transported to the Laboratory of Research Unit for Environmental Sciences of North-West University, Potchefstroom, South Africa, for taxonomic analysis.

Diatom frustules were examined with Nikon Eclipse 80i light microscope at 1000x magnification, under oil immersion objective using bright-field illumination with a green filter to increase contrast. Diatom frustules were identified to the species level with standard identification manuals and publications (Gasse, 1986; Kelly, 2000; Sonneman *et al.*, 2000; Krammer, 2003; Taylor *et al.*, 2007a; Taylor and Cocquyt, 2016). Diatom valves were counted using OptiCount and the total counts of 400 cells (200 from the cobbles and 200 from the macrophytes) were examined in random microscope fields to determine the relative abundance of each taxon as recommended by Prygiel *et al.* (2002). Therefore, the enumerated diatom taxa were used to summarize the diversity parameters.

4.2.2 Data analysis

Benthic diatoms species diversity in the lake was computed through the Shannon-Weiner diversity index (H′), Dominance index (D), Simpsons (1-D), Evenness, and Equitability index (J) by using PAST software. Percent abundance of each benthic diatom taxon was calculated on excel spreadsheet Microsoft 2007. Significant differences in the diversity index values and the abundance of diatoms between the study sites were compared using Kruskal-Wallis test. The association between benthic diatoms taxa distribution and physicochemical variables was evaluated by canonical multivariate analysis using CANOCO for windows 4.5 version software program (Ter Braak and Smilauer, 2002). Detrended correspondence analysis (DCA) was employed to check the response of the data, and it was found that the length of the longest gradient was 1.179. Therefore, redundancy analysis (RDA) was used as the diatom taxa data showed a linear response to disturbance gradient.

4.3 RESULTS

4.3.1 Benthic diatoms species diversity and composition

Thirty-nine diatom species were identified, which belonged to twenty-four genera, and their relative frequency and abundance were determined (Table 4.1). The results were based on 7,200 diatom frustule individuals' count, from 36 samples of 9 sites. The genera *Gomphonema* and *Nitzschia* attained the highest relative number of species (5 each) followed by *Aulacoseira* (4 species). The diatom communities were dominated by *Achnanthidium* sp., *Ulnaria ulna* and *Encyonopsis microcephala* with relative percent abundance of 12.57, 11.79 and 10.35, respectively, whereas *Pinnularia grunowii, Pinnularia viridiformis, Rhopalodia operculata, Caloneis aequatoriales, Craticula ambigua, Nitzschia clausii,* and *Surirella angusta* were sparse relatively with percent abundance contribution of less than 0.1 (Table 4.1).

Percent of *Ulnaria ulna* and *Nitzschia* sp. were higher in the test sites (24.17 and 14.00) than reference (2.42 and 6.75), respectively, while percent composition of *Encyonopsis microcephala* and *Stephanodiscus* sp. were higher in the reference sites (11.67 and 1.17) than test sites (6.18 and 0.25), respectively. Shannon diversity index (H′) values of the diatoms community was different among the species ($p<0.05$). H′ values of *Cymbella kappii* was high (2.08) followed by *Navicula* spp. (2.07), *Gomphonema* spp. (2.06), and *Aulacoseira* spp. (2.04), whereas *Craticula ambigua* and *Surirella angusta* showed the minimum H′ values (0.01 each) which correlated with their higher values of dominance (1.0) (Table 4.2). In addition, a considerable site-specific composition of *Craticula ambigua* and *Surirella angusta* was observed in the reference sites.

Table 4.1 Benthic diatoms species identified from the littoral zone of Lake Ziway with their number distribution, and percent abundance.

Diatom Species	**Number**	**Percent Abundance**
Achnanthidium sp.	905	12.57
Afrocymbella beccarii (Grunow) Krammer	39	0.54
Anomoeoneis sphaerophora (Ehr.) Pfitzer	18	0.25
Aulacoseira ambigua (Grunow) Simonsen	84	1.17
Aulacoseira granulata (Ehr.) Simonsen	187	2.60
Aulacoseira granulata (Ehr.) Simonsen var.angustissima (O.M.)Simonsen	44	0.61
Aulacoseira muzzanensis (Meister) Krammer	30	0.42
Caloneis aequatorialis Hustedt	6	0.08
Craticula ambigua (Ehrenberg) Mann	6	0.08
Cyclotella meneghiniana Kützing	30	0.42
Cyclotella ocellata Pantocsek	21	0.29
Cymbella kappii (Cholnoky) Cholnoky	719	9.99
Diploneis ovalis (Hilse) Cleve	30	0.42
Encyonema volkii (Rumrich. Krammer and Lange-Bertalot) Krammer	409	5.68
Encyonopsis microcephala (Grunow) Krammer	745	10.35
Gomphonema aequatoriale Hustedt	301	4.18
Gomphonema affine Kützing	345	4.79
Gomphonema augur Ehrenberg	13	0.18
Gomphonema gracile Ehrenberg	105	1.46
Gomphonema parvulum (Kützing) Kützing var. parvulum f. parvulum	150	2.08
Hantzschia amphioxys (Ehr.) Grunow in Cleve et Grunow	5	0.07
Navicula subrhynchocephala Hustedt	43	0.60
Navicula zanoni Hustedt	475	6.60

… Continued **Diatom Species**	**Number**	**Percent Abundance**
Nitzschia acicularis (Kützing) W.M.Smith	40	0.56
Nitzschia amphibia Grunow f.amphibia	445	6.18
Nitzschia clausii Hantzsch	6	0.08
Nitzschia intermedia Hantzsch ex Cleve and Grunow	487	6.76
Nitzschia sp.	557	7.74
Pinnularia grunowii Krammer	1	0.01
Pinnularia subgibba Krammer var. subgibba	4	0.06
Pinnularia viridiformis Krammer var. minor Krammer	2	0.03
Pleurosigma salinarum (Grunow) Cleve and Grunow	8	0.11
Rhopalodia operculata (Agardg) Hakansson	3	0.04
Sellaphora pupula (Kützing) Mereschkowksy	10	0.14
Stephanodiscus sp.	38	0.53
Surirella angusta (Kützing)	7	0.10
Thalassiosira baltica (Grunow) Ostenfeld	25	0.35
Tryblionella calida (grunow in Cl. and Grun.) D.G. Mann	8	0.11
Ulnaria ulna (Nitzsch.) Compère	849	11.79

Shannon diversity index (H′) values of the benthic diatom species was different across sites ($p<0.01$). H′ value of the diatoms was highest in FRC sampling site (2.724) followed by G2 (2.667), and low in KO sampling site (1.802) which is a cluster of the most stressed sites (Table 4.3). KO sampling site displayed the highest dominance value (0.237), which was under its lower H′ value. Besides, H′ value was high in reference sites (2.692) than both test sites, sites with intermediate and multiple stressors (2.214) and (1.899), respectively. Equitability index (J) values exhibited a gradual non-significant decline from the reference to test sites ($p>0.05$).

Table 4.2 Determination of Shannon diversity index and Dominance values of benthic diatoms calculated by the assortment of their genera.

Genera	**Omnidia Code**	**Dominance (D)**	**Shannon-Weiner Diversity Index (H')**
Achnanthidium Kützing	ACHD	0.15	1.98
Afrocymbella Krammer	AFCY	0.36	1.06
Anomoeoneis Pfitzer	ANOM	0.43	0.96
Aulacoseira Thwaites	AULA	0.14	2.04
Caloneis Cleve	CALO	0.50	0.69
Craticula Grunow	CRAT	1.00	0.01
Cyclotella (Kützing) Brebisson	CYCL	0.14	2.03
Cymbella C. Agardh	CYMB	0.14	2.08
Diploneis (Ehrenberg) Cleve	DIPL	0.56	0.64
Encyonema Kützing	ENCY	0.15	1.99
Encyonopsis Krammer	ENCP	0.16	1.99
Gomphonema Ehrenberg	GOMP	0.14	2.06
Hantzschia Grunow	HANT	0.68	0.50
Navicula Bory	NAVI	0.14	2.07
Nitzschia Hassall	NITZ	0.16	2.01
Pinnularia Ehrenberg	PINN	0.38	1.04
Pleurosigma W. Smith	PLSG	0.50	0.69
Rhopalodia O. Muller	RHOP	0.50	0.69
Sellaphora Mereschkowsky	SELL	0.36	1.06
Stephanodiscus Ehrenberg	STEP	0.27	1.34
Surirella Turpin	SURI	1.00	0.01
Thalassiosira Cleve	THAL	0.28	1.31
Tryblionella W.Smith	TRYB	0.31	1.28
Ulnaria Kützing Compère	ULNA	0.20	1.82

There was a significant variation in the number of diatom species across categories of sites ($p<0.01$). The species identified in the reference sites (37) with 94.87% was higher than the test sites (31) that comprised 79.48% of the total species. *Surirella angusta, Pinnularia viridiformis, Pinularia subgibba, Nitzschia clausii, Nitzschia acicularis and Craticula ambigua* were the species that characterized the reference sites. The highest relative number of species were observed in FRC site (33) which accounted for 84.61% of the total species followed by G2 site (25) and comprised 64.11% and the lowest species number was observed in ShEFF site (17) and accounted 43.58% composition of the total species (Table 4.3).

Table 4.3 Determination of the species number richness, Dominance, Shannon diversity index, and Equitability index in the clustered study sites of Lake Ziway.

Site Clusters	Sites	Species Number	Dominance (D)	Shannon-Weiner Diversity Index (H′)	Equitability Index (J)
Intermediate stressors	BU	18	0.145	2.183	0.741
	GCH	18	0.148	2.207	0.763
	SI	21	0.097	2.525	0.829
Multiple stressors	ShEFF	17	0.147	1.875	0.782
	KO	21	0.237	1.802	0.660
	CA	18	0.134	2.322	0.775
Reference sites	FRC	33	0.090	2.724	0.802
	G1	22	0.102	2.577	0.833
	G2	25	0.091	2.667	0.828

4.3.2 Seasonal composition of benthic diatom species

There was no significant variation in the diatom species number in Lake Ziway during the two seasons ($p>0.05$). However there was a difference in the diatom species density. The density of *Aulacoseira ambigua, Aulacoseira granulata, Diploneis ovalis, Surirella angusta, Gomphonema parvulum,* and *Ulnaria ulna* were higher in the wet season, while *Encyonema volkii, Encyonopsis microcephala, Nitzschia intermedia, Nitzschia* sp., and *Tryblionella calida* were higher in the dry season, other diatom species in this study has indicated negligible difference between the two seasons (Fig. 4.1).

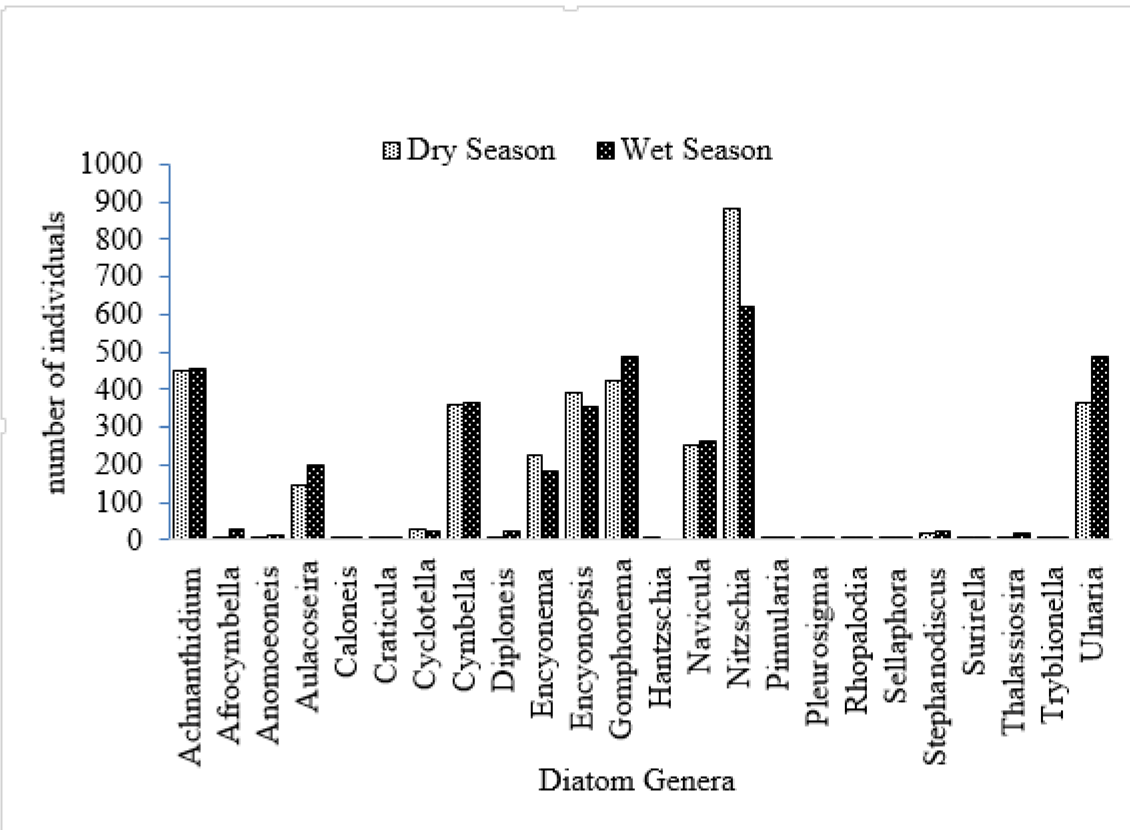

Figure 4.1 Seasonal variation in the density of benthic diatoms in the assortment of their genera in the littoral zone of Lake Ziway.

In the dry season, abundance of *Achnanthidium* sp. (12.49%), *Encyonopsis microcepha* (10.92%), *Ulnaria ulna* (10.08), *Cymbella kappii* (9.92%), and *Nitzschia* sp. (9.06%) contributed 52.47% of the total diatoms species composition. *Ulnaria ulna* (13.50%), *Achnanthidium* sp. (12.69%), *Cymbella kappii* (10.06%), *and Encyonopsis microcepha* (9.78%) were relatively abundant in the wet season by contributing 46.03% of the total diatoms species composition (Appendix 6). The lowermost abundance values in both dry and wet seasons were that of *Rhopalodia operculata* (0.06 and 0.03%), *Pinnularia viridiformis* (0.03 and 0.03%), and *Pinnularia grunowii*, (0.03 and 0.00%), respectively. The diversity parameter values displayed no difference among the seasons (Table 4.4).

Table 4.4 Determination of the values of diversity indices of the diatom species among the study seasons (Paleontological Statistics).

Diversity parameters	**Dry Season**	**Wet Season**
Number of Species	39	37
Individuals	3600	3600
Dominance (D)	0.079	0.076
Simpson (1-D)	0.920	0.923
Shannon (H′)	2.751	2.830
Evenness (e^ H/S)	0.391	0.445
Margalef	4.763	4.518
Equitability (J)	0.745	0.777
Berger-Parker	0.124	0.135

4.3.3 Relations between physicochemical variables and diversity of diatom species

The RDA-triplot of samples, diatoms, and environmental variables indicated that axis 1 and axis 2 contributed 55.3% of the cumulative percentage of variance in a species-environmental relationship (Fig. 4.2). The first axis contributed 33.8% of the variance, was positively correlated with physical factors (pH, DO, temperature and conductivity), the second axis revealed a gradient primarily associated with nutrients (nitrite, silicon dioxide, and total phosphorus), and turbidity factors (total suspended solids).

The density of *Caloneis aequatorialis*, *Pinnularia grunowii*, and *Tryblionella calida* was positively related with nitrate. *Achnanthidium* sp., *Cymbella kappii*, *Gomphonema augur* and *Ulnaria ulna* were positively related to SRP and TP, and *Aulacoseira granulata* and *Navicula zanonii* with pH. *Navicula subrhynchocephala*, *Nitzschia amphibia*, and *Nitzschia* sp. showed a positive association with ammonium, temperature, and electrical conductivity. *Aulacoseira muzzanensis*, *Gomphonema affine* and *Gomphonema gracile* were positively correlated with Silicon dioxide and total suspended solids, whereas the density of other species had negative but not strong species-environment association with most variables (Fig. 4.2).

The association of *Caloneis aequatorialis*, *Tryblionella calida* and *Pinnularia grunowii* with NO^{-}_{3}, and *Navicula subrhynchocephala*, *Nitzschia amphibia* and *Nitzschia* sp. with NH^{+}_{4} was positive and strong. *Afrocymbella beccarii*, *Cyclotella ocellata*, *Cyclotella meneghiniana and Gomphonema aequatoriale* displayed negative association with most of the nutrient variables but not strong. Temperature and electrical conductivity showed a strong and negative association with *Aulacoseira ambigua*, *Aulacoseira granulata var. angustisima*, *Nitzschia acicularis*, and *Thalassiosira baltica*.

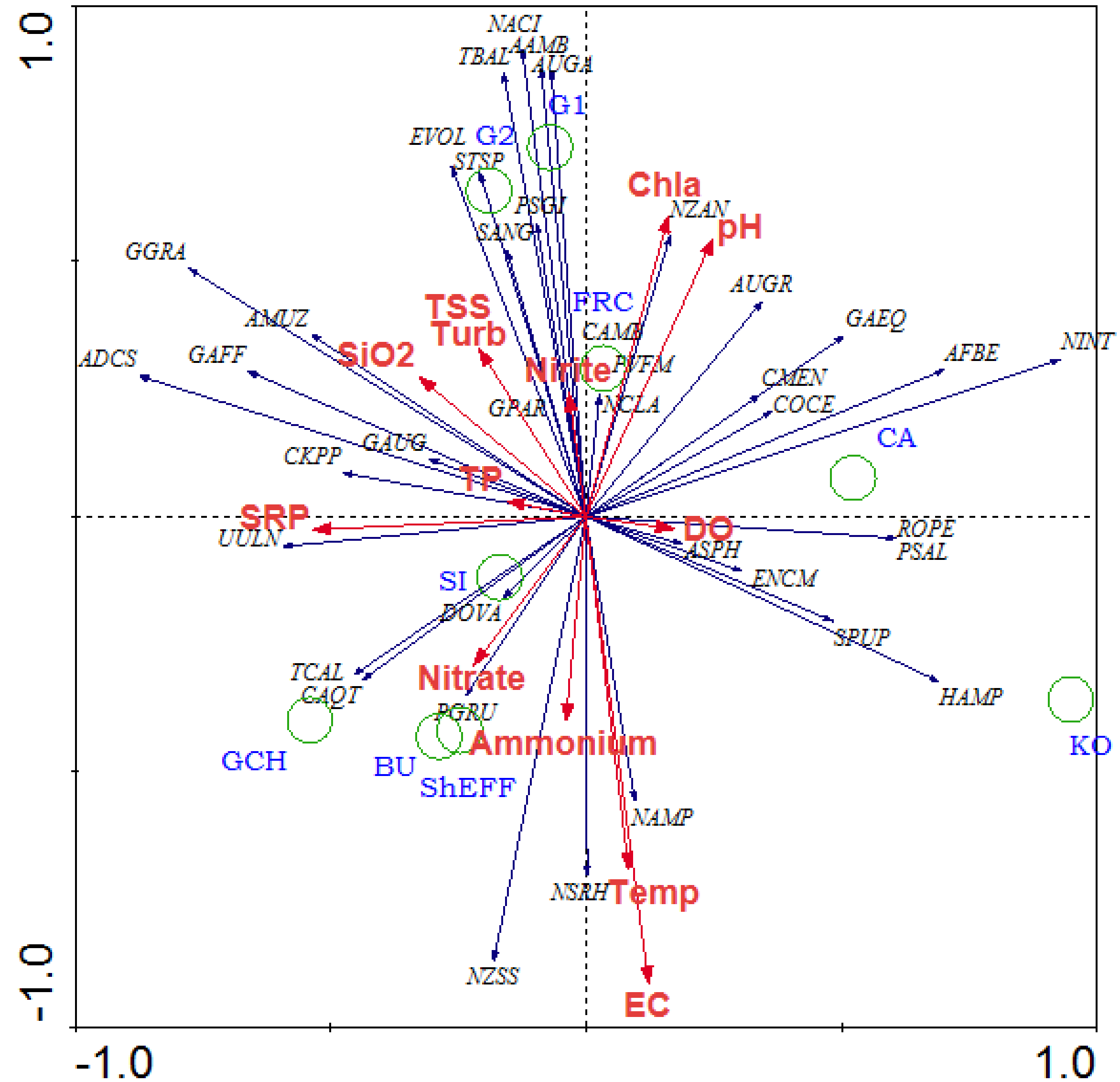

Figure 4.2 RDA-triplot of sites, benthic diatoms and environmental variables based on the first two axes (diatom species code: Appendix 3) (**Abbreviations:** EC: Electrical Conductivity, TP: Total Phosphorus, SRP: Soluble Reactive Phosphorus, TSS: Total Suspended Solids, Turb: Turbidity and Temp: Temperature). Sites are shown in blue and species in black.

4.4 DISCUSSION

4.4.1 Diversity and distribution of benthic diatoms species

Achnanthidium sp. was the most abundant in Lake Ziway followed by *Ulnaria ulna*. This might be because *Achnanthidium* sp. prefers to survive in water bodies with wave-swept littoral zones and their broad range of microhabitats (Cantonati *et al.*, 2009; Cantonati *et al.*, 2014). In addition, this result might be related to the observation that *Achnanthidium* sp. is stimulated by exposure to UV radiation (Francoeur and Lowe, 1998; Winslow *et al.*, 2014) and also according to Peterson *et al.* (1998), this taxon is a typically grazer-resistant which can cause its constant presence/dominance.

Pinnularia grunowii, *Pinnularia viridiformis*, *Nitzschia clausii*, *Caloneis aequatoriales*, *Craticula ambigua*, *Rhopalodia operculata*, and *Surirella angusta* were sparse with low percent abundance contribution. This could be because these species are generally scarce and of low abundance. *Pinnularia* spp. usually occur in low abundance, and *Nitzschia clausii* although widespread, is not usually abundant (Sonneman *et al.*, 2000).

Percent of *Ulnaria ulna*, *Encyonopsis microcephala*, *Stephanodiscus* sp. and *Nitzschia* sp., varied considerably in test and reference sites, which could be explained by the observed differences in environmental variables. The most abundant species at the test sites were *Ulnaria ulna* and *Nitzschia* sp. which tolerate organic pollutions and relatively high nutrient load (Çiçek and Yamuç, 2017). The high abundance of *Stephanodiscus* sp. in the reference than test sites in relative might be because of its ecological preferences of low to medium conductivity (Taylor and Cocquyt, 2016). Therefore, this outcome in the present study connoted with the lowest relative electrical conductivity reading observed in the reference sites.

Shannon diversity index was found maximum in the FRC site (2.724) which fits in the reference sites group, and minimum in KO site (1.802) which was categorized in the multiple stressors group. This is most likely because of the multiple stressors pressure on this site as it is a shore dominated by multiple human activities like waste dumping, boat transport landing site, fish processing, and non-natural habitat constructions. Therefore, maximum Simpson's index of dominance value (0.237) indicated that KO sampling site was habitated by dominant species which justified the lowest H' value.

Diversity values for real communities are often found to fall between 1.0 and 6.0 (Stirling and Wilsey, 2001). Diversity of Lake Ziway was relatively moderate since all sampling sites were arranged in an H' value between 1.802 and 2.724. Wilhm and Dorris (1968), proposed a relationship between species diversity and pollution status of water: H >3 clean water; H =1-3 moderately polluted; and H <1 heavily polluted. Staub *et al.* (1970), had set diversity index of <1 for heavy pollution, 1-2 for moderate pollution, 2<3 for light pollution, and >3 for slight/unpolluted water bodies.

All sites in Lake Ziway would come out as moderately-to-lightly polluted (had a value of H' between 1.802 and 2.724). Therefore, diatoms diversity in the lake could be affected by environmental stressors around the riparian zone. Besides, the overall moderate diatom diversity observed in the lake might be because of the homogenous environmental conditions of the lake. Even then an expected decrease of diversity values along the degraded study sites was observed, in which the diatoms community attribute showed a positive correlation with conservation status and protected riparian buffer. The number of diatom species and H′ were identified as potential diversity metrics since they displayed significant differences among the studied sites.

4.4.2 Seasonal composition of benthic diatoms

Shannon Diversity Index value of 2.751 was found in the dry season and 2.830 in the wet season, Simpson's Dominance value of 0.920 in the dry season and 0.923 in the wet season displayed no difference across the categories of seasons. Therefore, the apparent lack of seasonality in the diversity of diatoms in Lake Ziway could be because of the absence of distinct seasonal contrasts in the region and presence of more or less similar light intensity and water temperature during the entire year.

Ahmed and Wanganeo (2015), reported that growth of algae was limited chiefly by physical factors, such as light and temperature, high water temperatures supported the growth of diatoms, whilst low temperature inhibited their growth. Thus, the absence of seasonality in the present study can be attributed with no significant variation in water temperature between the seasons. Egge and Aksnes (1992) showed that in experiments diatoms were dominant despite the season if the concentration of silicate was high enough, this understanding was also reported by Mooij *et al.* (2016).

Even though there was no seasonality in diatoms diversity, the seasonal influence was observed on some diatoms species abundance in Lake Ziway (Fig. 4.1). Possibly the seasonal difference in the abundance of some benthic diatoms would be the availability of the nutrients. According to Sahoo and Ansari (2018), the causes of fluctuations in benthic diatoms density include mainly water quality changes and variation in the concentration of nutrients, this finding was also reported by Yang *et al.* (2014). Thus, the abundance of some diatom species in the wet season of the study in Lake Ziway could be attributed with the nutrient enrichment through runoff and turbulence.

Besides, due to limited studies on benthic diatom communities of Lake Ziway, data for comparison of seasonal changes and diversity pattern is scarce. However, some previous studies on phytoplankton of the lake documented diatoms taxa. In the study of Gasse *et al.* (1983) and Gasse (1986), 19 diatom species were reported. Elizabeth Kebede and Willen (1998) and Girma Tilahun (2006), reported 8 and 9 species, respectively. Adamneh Dagne (2010), also identified 42 phytoplankton genera, of which 16 were diatoms. In the present study 39 species fit to 24 genera were identified, of which 11 species in the study of Gasse *et al.* (1983) and Gasse (1986); 4 species in the study of Elizabeth Kebede and Willén (1998); 6 species in the study of Girma Tilahun (2006); and 8 genera in the study of Adamneh Dagne (2010) were also reported (Appendix 8).

Accordingly, 37.5% of the diatom species identified in the present study are new records, with a maximum of 28% overlap in species between this and previous studies. The observable percent diatom species difference between the present and reported works might be due to extensive sampling done in this study as opposed to limited studies earlier. Therefore, diatom species identified and recorded in this study will serve as a benchmark information for future studies.

4.4.3 Distribution of diatom species in relation to physicochemical variables

Most of the diatom species were associated directly with silicon dioxide, nitrite, and TSS. These results relate to the autecology of these species where formation of diatoms frustule depends on silicon (Martin-Jézéquel *et al.*, 2000), and the light intensity that relates directly to the water turbidity which controls phytoplankton photosynthesis and diatoms growth pattern (Karl *et al.*, 2002; Saunders *et al.*, 2016).

The association of a relative many diatoms species with nitrite was positive but not strong and is possibly related to high nitrogen requirement of diatoms for their growth (Yang *et al.*, 2014; Dell'Aquila *et al.*, 2017). According to Tuo *et al.* (2014) diatoms require the same amounts of nitrogen and silicon for growth, and changes in the relative availability of N and Si may influence the abundance of various diatom species (Gilpin *et al.*, 2004).

The direct association of nitrate, ammonium, and electrical conductivity with the density of *Caloneis aequatorialis, Diploneis ovalis*, *Nitzschia* sp. and *Tryblionella calida* implies that most of these species density increased with increases in nutrients. According to Taylor *et al.* (2007a), *Caloneis aequatorialis* favors high nutrient loads, *Diploneis ovalis* and *Nitzschia* sp. commonly found with moderate to elevated electrolyte contents. *Tryblionella calida* most commonly recorded in epipelic and epilithic assemblages, as narrow nutrient and conductivity tolerance (Sonneman *et al.*, 2000).

The relation of pH with distribution of A. *granulata* might be that A. *granulata* colonies have high growth rates at pH between 7 and 9 (Bicudo *et al.*, 2016). Thus, the recorded pH values between 7.61 and 8.74 of the lake might favor the distribution of this taxon. This taxon occurs in a wide range of trophic conditions (Taylor and Cocquyt, 2016); high flood conditions (Costa-Böddeker *et al.*, 2012); a rapidly sinking planktonic diatom and one of the most adaptable to low light conditions (Reynolds *et al.*, 1994). It is the most widely distributed species with the genus in Africa (Kilham, 1990). Elizabeth Kebede and Willén (1998), reported a wider distribution of the genus *Aulacoseira* in Lake Ziway. A. *granulata*, is the most common diatom species in shallow mixing lakes, in tropic (Hecky and Kling, 1987), Thus, its high abundance in the wet season might be because of the nutrient availability enhanced by the mixing factor in this season.

Combined effects of the environmental factors created habitat conditions that eliminated most of the pollution-sensitive species. Accordingly, abundance of less-sensitive species such as *Ulnaria ulna* and *Aulacoseira granulata*, and the relative sparse distribution of the sensitive species such as *Pinnularia grunowii* and *Pinnularia viridiformis,* could be an indication of deterioration of lake water quality in Lake Ziway. However, the relative high abundance of the pollution-sensitive species in the reference sites implies that the reference sites were not as polluted as the test sites.

The moderate diversity of diatoms in Lake Ziway might be because of the lake water turbidity and the homogenous environmental conditions observed in the lake ecosystem. The average TSS values recorded in this study were between 143 – 289 mg/L. This result is much higher than the average TSS value less than 40 mg/L for freshwaters in different countries, and the growth of benthic diatom species might be limited, as benthic species rely upon light penetrating the water column to reach for photosynthesis. Girum Tamire (2014), also reported that, the lake is highly turbid (42-70 NTU). This is further verified by the lower Secchi depth recorded in Lake Ziway (Mean value = 0.19m) by Girma Tilahun (2006), and (0.11 – 0.16m) by Girum Tamire (2014), then the turbidity of the lake is mainly non-algal as confirmed by the low percentage of light attenuation due to algae (Girma Tilahun, 2006). But some benthic diatoms are adapted to poor light, which is why they are dominant over when turbidity increases.

Additionally, benthic diatoms difficult to identify below the genus level due to their fragile frustules (*Achnanthidium, Stephanodiscus*, and *Nitzschia*) were recorded at genus level, which undervalues species richness. This phenomenon was also reported by Elizabeth Kebede and Willén (1998). However, we found an expected decrease of diversity values along degraded study sites, in which the benthic diatom communities showed a positive correlation with conservation status and protected riparian buffer. Therefore, this can be evidence that benthic diatoms are useful indicators of habitat quality status in Lake Ziway and other similar aquatic environments.

The RDA ordination diagram showed that diatom assemblages in the littoral zone were influenced by multiple environmental vectors (pH, nitrite, silicon dioxide, and TSS). Diatom and environmental variables accounted for 55.3% of the cumulative percentage of variance in a species-environmental relationship, whereas the rest of the variance might be due to biotic and abiotic factors not considered in this study such as light intensity, pesticide contaminations, BOD_5, COD, and other environmental variables. Therefore, for better verification of the species-environmental relationship studies, investigating all the possible environmental factors might be supportive.

In addition, in this study, the number of species within most diatom genera was low, with 21 genera having fewer than four species. The small number of species within each genus effectively reduces the chances of differing responses of several species with the same genus to environmental variables (Saunders *et al.*, 2016). Therefore, detailed studies must develop a refined diatom-based lake water quality monitoring tool in the region. Studies are also recommended to include the autecology of the diatom species to determine the environmental factors that determine the diatom composition of Lake Ziway.

CHAPTER 5: A MULTIMETRIC BENTHIC MACROINVERTEBRATE AND DIATOM BASED INDEX DEVELOPMENT TO ASSESS THE WATER QUALITY STATUS OF LAKE ZIWAY

5.1 INTRODUCTION

Water resources globally are affected by complex mixture of stressors, and understanding how stressors interfere upon ecosystem services is essential for developing effective management plans (Hering *et al.*, 2015). Most lake monitoring programs document physical and chemical attributes of the lake environment (Coffey and Smolen, 1990), but accurate assessment of water quality requires examination of physical, chemical, and biological components of the ecosystem (Barbour *et al.*, 1999; Jones *et al.*, 2007). Thus to refine these monitoring and management plans, it is strongly necessary to assess the physical, chemical and biological states of the aquatic ecosystems (Harte, 2007).

The biological assemblages integrate the effects of different stressors such as nutrient enrichment, toxic chemicals, and morphological alterations, thus provide an overall measure of the aggregate impacts (Odum, 1985). Since different biotic assemblages are sensitive to different impacts (Hughes, 2000), which is related to diversity, life cycle, mobility, and position in the food web (Barbour *et al.*, 1999), use of multiple biological assemblages can enhance the ability to detect the ecological impairments (Karr and Chu, 1999). However, biotic index development should be partitioned into ecoregions to minimize the degree of climatic, geologic, and biotic variability within a large area (Whittier *et al.*, 2007a; Stoddard *et al.*, 2008). Minimizing the among-site natural variation in physical, chemical, and biological attributes increases the ability to detect differences due to human impacts (Hering *et al.*, 2006a).

Ecological assessment of rivers and lakes is usually based on evaluating phytoplankton, phytobenthos, invertebrates, and fishes (Delgado *et al.*, 2010). Methods to assess the phytobenthos have tended to focus on diatoms which form a large part of the algal diversity in freshwaters (Kelly *et al.*, 2016), whereas macroinvertebrates have been frequently used in the assessment of rivers (Hering *et al.*, 2006b; Seid Tiku *et al.*, 2013). Assessment methods based on fishes have disadvantages because these organisms can migrate, escape from adverse conditions, and rarely have a rapid response to some environmental changes (Park *et al.*, 2014). Many biological water quality assessment methods have been developed based on the assessment of diatom and macroinvertebrates (Kelly and Whitton, 1995; Barbour *et al.*, 1999; Park *et al.*, 2014).

A significant body of research has established diatom species optima for organic pollution (Lange-Bertalot, 1979), nutrients and trophic status, and species tolerance to acidification (Van Dam *et al.*, 1994), sudden and minor changes up in water chemistry (Pan *et al.*, 1996), and sedimentation (Stevenson and Bahls, 1999). These attributes can be quantified with pollution and trophic indices, and combined with other measures of assemblage attributes, such as diversity and abundance, to yield a multimetric index that is both responsive to general environmental degradation and indicative of specific causes (Hering *et al.*, 2006a). Different research studies established macroinvertebrates as an ideal organisms for the assessment of different levels of pollution in the aquatic ecosystems (Gupta and Sharma, 2005). Benthic macroinvertebrates respond sensitively not only to pollution, but also to several other human impacts (hydrological, morphological, recreational, and others) (Solimini *et al.*, 2006).

Using macroinvertebrates as an indicator of water quality has been getting attention in tropical countries recently. Raburu *et al.* (2009) and Aura *et al.* (2010) developed IBI for rivers in Kenya; Getachew Beneberu (2013) developed IBI for some rivers in Ethiopia; Seid Tiku *et al.* (2013) developed a multimetric index for the assessment of wetlands in Ethiopia; Solomon Akalu *et al.* (2011) and Aschalew Lakew and Moog (2015) used macroinvertebrate based biotic scores for the assessment of river conditions in Ethiopia; and the effort which used diatoms and macroinvertebrates together for the water quality assessment has been done by Abebe Beyene *et al.* (2009) on Kebena and Akaki rivers, Ethiopia. Here we investigated a composite multimetric index to assess the water quality status of Lake Ziway because using multi-taxon and multimetric indices have many advantages (Wang *et al.*, 2015; Giorgio *et al.*, 2016), besides, such indices may be the only biological tools able to monitor disturbances or pollution gradients in lake littorals sampled at close spatial scale and exposed to multiple stressors.

Benthic diatoms and benthic macroinvertebrates are logical assemblages for Lake Ziway bioassessment and biomonitoring practices: they are relatively quick and easy to sample (Stevenson *et al.*, 2010), and a large diversity of species occur in the lake in relative to macrophytes and fishes. Furthermore, the relationship between diversity indices and environmental variables is stronger in case of small sized organisms, such as diatoms and invertebrates, compared to macrophytes and fishes (Springe *et al.*, 2006). Therefore, the primary objective of this chapter was to characterize the potential macroinvertebrate and diatom metrics/indices suitable for the conditions of the central rift valley lake, Lake Ziway and to develop a composite diatom and macroinvertebrate based multimetric index, which has the quality to assess the ecological status of the lake.

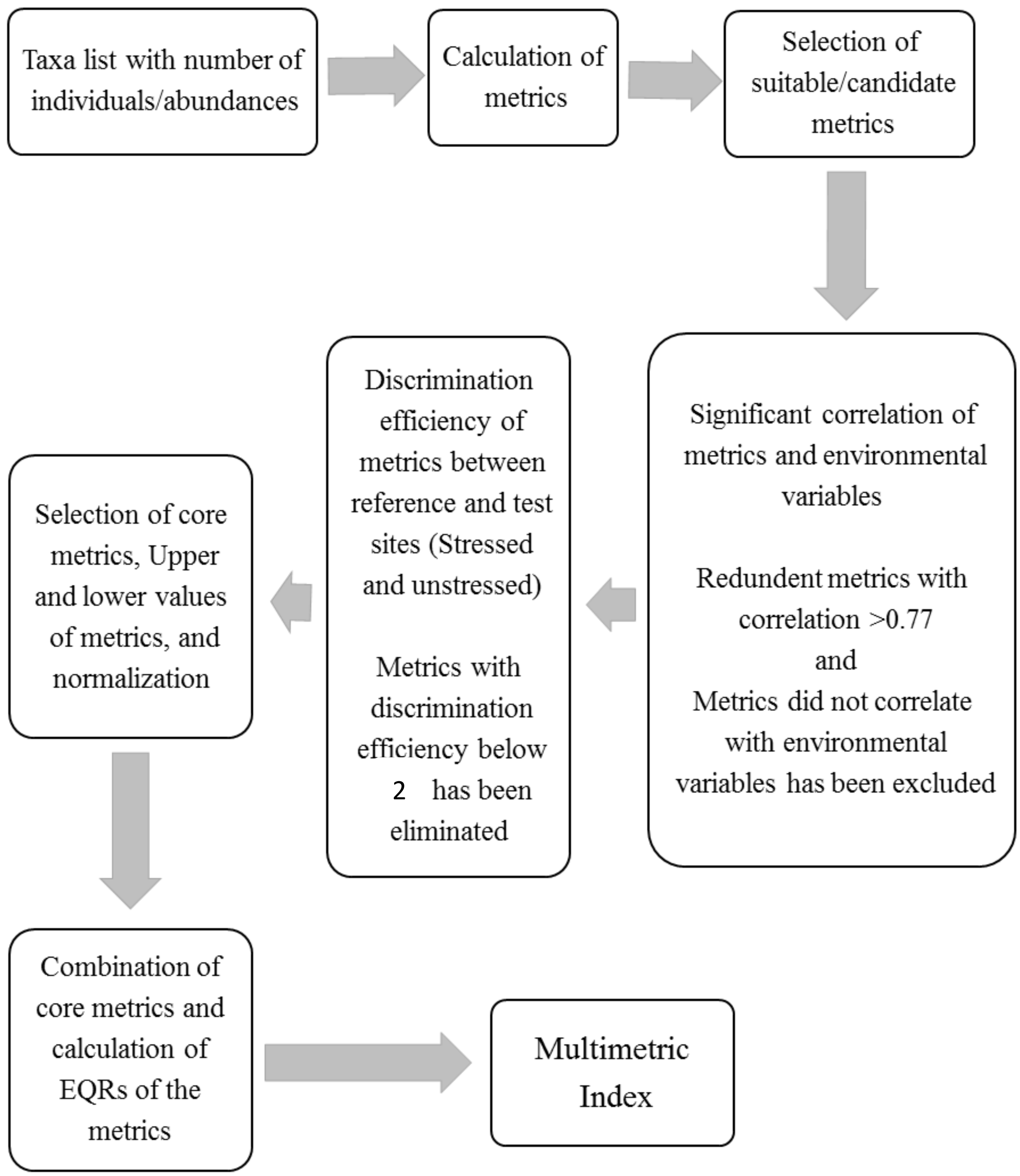

Figure 5.1 Schematic overview of the steps applied to develop a multimetric index for Lake Ziway based on benthic diatoms and benthic macroinvertebrates.

5.2 MATERIALS AND METHODS

5.2.1 Sampling design and selecting the reference and test sites

The study sites were differentiated with their riparian and littoral zone human pressures gradient using LHQAs following the method outlined in Ontario Benthos Biomonitoring Protocol Manual (Jones *et al.*, 2007). Reference Condition Approach (RCA) also used (12 physicochemical factors considered) and a visual-based lake habitat assessment was implemented to predict a test site to reference per the recommendation of Rowan *et al.* (2006). (Materials and methods - Chapter 2). Physicochemical parameters were also measured using standardized measuring methods (Materials and methods - Chapter 2), benthic macroinvertebrate samples were collected, processed, identified, and counted (Materials and methods - Chapter 3), benthic diatom samples were collected, cleaned, processed, identified, and enumerated (Materials and methods - Chapter 4).

5.2.2 Data analysis

The relationships between the environmental variables and biotic metrics data (diatom and macroinvertebrate metrics and indices) were evaluated or assessed by the Spearman correlation test using SPSS version20 software. SIMPER (Similarity Percentage) analysis was used to estimate the similarity between the reference and test samples using PAST. Discrimination efficiencies were judged to evaluate the most suitable metrics and indices those can discriminate between the reference and non-reference sites, by examining their distributions in the box-and-whisker plots using Sigma Plot 10.0. For the scoring of the selected metrics for developing lake multimetric index, the 75^{th} and 25^{th} percentile values were used per the recommendation of Barbour *et al.* (1999). Each metric was scored on a discrete scoring method using a trisection range.

5.2.2.1 Benthic macroinvertebrate and diatom metrics calculation

Benthic macroinvertebrates abundance data was used to calculate 32 metrics (Table 5.1). The excel spreadsheet 2016 was used to calculate the 28 macroinvertebrate metrics, and PAST software v.2.17 was used to calculate the diversity indices. Diatoms abundance data was used to calculate 18 diatom indices (Table 5.2). The software OMNIDIA v.5.3 Species list version 2009; Lecointe *et al.* (1993) was used to calculate 14 diatom indices. Each diatom index differed in the number of species used for the evaluated ecological relevance (Van Dam *et al.*, 1994).

For both benthic diatoms and macroinvertebrates, four new metrics were calculated for the present study based on the reference taxa: (1) relative abundance (ARSI) and (2) richness (RRS) of reference taxa (3) the ratio of reference taxa to the total taxa expressed as percentages of abundance (PARS) and (4) richness (PRRS). Statistical analyses for screening candidate metrics were performed with SPSS version 20 software. The metrics were summarized by determining the average of four sampling results.

5.2.2.2 Benthic macroinvertebrate and diatom metrics selection

Diatom and macroinvertebrate metrics representing ecologically relevant aspects of the assemblages, and responding to the targeted stressors tested were potential metrics to be combined in a multimetric index of Lake Ziway. The selection of core metrics followed the procedure described by Barbour *et al.* (1999). Redundancy analysis was employed to identify pairs of metrics with significant Spearman rank correlations. When a pair had a correlation coefficient >0.7, one of the two metrics was excluded from further analyses following the approach of Ofenböck *et al.* (2004). Box-and-whisker plots were used to evaluate the metrics and/or indices discrimination efficiency.

Discrimination efficiency of metrics was evaluated through the sensitivity values of the metrics to discriminate reference sites from test sites using box-and-whisker plots. The sensitivity of metrics was evaluated based on the degree of the overlap of interquartile in box-and-whisker plots following the method described in Barbour *et al.* (1996). Metrics were evaluated to have one of 4 sensitivity values: a sensitivity of 3 was given when there was no overlap existed in the interquartile range; 2 when there was some overlap that did not extend to the medians; 1 if there was a moderate overlap of interquartile range but at least one median was outside the range; and 0 when extensive overlap of interquartile range or both medians within the overlap existed. Therefore, metrics with a sensitivity value of 3 and 2, were considered for further analysis.

5.2.2.3 Multimetric index development and validation

Selected metric values were normalized to metric scores of 5, 3, or 1 depending on their proximity to the optimal values to make the final scores. Discrete type of scoring was identified as good measure of variability (Blocksom, 2003). A final multimetric index for Lake Ziway (MMIZ) was created by summing the values of the selected core metrics. The sensitivity of the multimetric index to discriminate the reference sites from test sites was determined using box-and-whisker plots. Pearson correlation analysis was also used to identify relationships between the index scores and environmental variables. Principal component analysis (PCA) was used to examine the distribution of the MMIZ scores between the sites and clustered site groups. The MMIZ scores were divided into several ranges corresponding to various levels of impairment (poor, fair, good, and very good). The Ecological Quality Ratio score (EQRs) was calculated by dividing each value of the multimetric by the MMIZ median value of the references.

Table 5.1 Macroinvertebrate metrics tested in the present study (Mandaville, 2002; Baptista *et al.*, 2007; Wang *et al.*, 2015). (ARTI, RRT, PARTI, and PRRT metrics were calculated based on the abundance of macroinvertebrate taxa those characterized the reference sites in the present study).

Codes	**Metrics and/or Indices**	**Expected Responses**
TNI	Total Number of Individuals	Decrease
NT	Number of Taxa (Family)	Decrease
NEI	Number of Ephemeroptera Individuals	Decrease
NTI	Number of Trichoptera Individuals	Decrease
NETT	Number of Ephemeroptera and Trichoptera Taxa	Decrease
NETI	Number of Ephemeroptera and Trichoptera Individuals	Decrease
NOI	Number of Oligochaeta Individuals	Increase
NCI	Number of Chironomidae Individuals	Increase
NDI	Number of Diptera Individuals	Increase
NTTI	Number of Tolerant Taxa Individuals	Increase
NITI	Number of Intolerant Taxa Individuals	Decrease
PEI	% Ephemeroptera Individuals	Decrease
PTI	% Trichoptera Individuals	Decrease
PETI	% Ephemeroptera and Trichoptera Taxa Individuals	Decrease
PDI	% Diptera Individuals	Increase
PCOI	% Chironomids and Oligochaeta Individuals	Increase
PDT	% Dominant Taxa	Increase

…Continued		**Expected**
Codes	**Metrics and/or Indices**	**Responses**
PToI	% Tolerant Individuals	Increase
PII	% Intolerant Individuals	Decrease
ET/C	Ephemeroptera and Trichoptera /Chironomidae Ratio	Decrease
TC/TI	Total Chironomidae/Total Individual Ratio	Increase
MI	Margalef's Index	Decrease
H'	Shannon Diversity Index	Decrease
D	Dominance Index	Increase
FBI	Family Biotic Index	Decrease
NETO	Number of Ephemeroptera, Trichoptera and Odonata families	Decrease
IBI	Index of Biological Integrity	Decrease
CLI	Community Loss Index	Increase
ARTI	Abundance of Reference Taxa Individuals (PS)	Decrease
RRT	Richness of Reference Taxa (PS)	Decrease
PARTI	Percent Abundance of Reference Taxa Individuals (PS)	Decrease
PRRT	Percent Richness of Reference Taxa (PS)	Decrease

Abbreviation: PS: Present Study

Table 5.2 Diatom metrics/indices tested in the present study (Kelly and Whitton, 1995; Taylor *et al.*, 2007b; Wang *et al.*, 2015). (NRSI, RRS, PARS, and PRRS were calculated based on diatom species abundance characterized the reference sites in the present study).

Codes	Metrics/ Indices	Responses
CEE	Commission for Economic Community (Descy and Coste, 1991)	Decrease
DESCY	Descy’s pollution metric (Descy, 1979)	Decrease
EPID	Pollution metric based on diatoms (Dell'Uomo, 1996)	Decrease
IBD	Biological Diatom Index (Lenoir and Coste, 1996)	Decrease
IDAP	Indice Diatomique Artois Picardie (Prygiel and Coste, 1993)	Decrease
IDP	Pampean Diatom Index (Gómez and Licursi, 2001)	Decrease
IDSE	Diatom Index of Saprobity-Eutrophication (Louis-Leclercq, 2008)	Decrease
IPS	Specific Pollution Sensitivity Index (Cemagref, 1982)	Decrease
PTV	Percent of Pollution Tolerant Valves (Kelly and Whitton, 1995)	Increase
SID	Saprobic Index (Rott *et al.*, 2003)	Decrease
SHE	Steinberg and Schiefele trophic (Steinberg and Schiefele, 1988)	Decrease
SLA	Sladecek’s pollution index (Sládeček, 1986)	Decrease
TDI	Trophic Diatom Index (Kelly and Whitton, 1995)	Decrease
WAT	Watanabe Index (Lecointe *et al.*, 1993)	Decrease
NRSI	Number of Reference Species Individuals (PS)	Decrease
RRS	Richness of Reference Species (PS)	Decrease
PARS	Percentage Abundance of Reference Species individuals (PS)	Decrease
PRRS	Percentage Richness of Reference Species (PS)	Decrease

Abbreviation: PS: Present Study

5.3 RESULTS

5.3.1 The reference benthic macroinvertebrate taxa

Five benthic macroinvertebrate taxa (Hydropsychidae, Polymitarcyidae, Philopotamidae, Naucoridae, and Hydrachnidae), which belong to the orders Ephemeroptera, Trichoptera, and Hemiptera were considered the core of the reference invertebrates assemblage. Their presence, abundance, and percent contribution were used to build the new metrics (ARTI, RRT, PARTI, and PRRT**)**. Benthic macroinvertebrate families those characterized the reference assemblages were dominated by Hydropsychidae, Polymitarcyidae, and Hydrachnidae by contributing 91.17% of the total reference macroinvertebrate families. These three macroinvertebrate families of the reference community appeared as well in the non-reference group sites (Hydropsychidae, Polymitarcyidae, and Hydrachnidae), but at lower percentage contribution (Table 5.3).

The SIMPER routine compared the three site groups, 'intermediary stressors', 'multiple stressors' and 'reference' groups. The overall average dissimilarity between reference and intermediary stressors was 31.09%, in which Sphaeriidae, Physidae, Hydropsychidae, Polymitarcyidae, and Hydrachnidae accounted 35.46% of the difference between these groups. The overall average dissimilarity between reference and sites with multiple stressors was 33.14%, in which Sphaeriidae, Physidae, Chironomidae, Hydropsychidae, Philopotamidae, and Polymitarcyidae accounted 49.81% of the difference. The overall average dissimilarity between sites with intermediary and multiple stressors was 24.65%, in which Physidae, Philopotamidae, Polymitarcyidae, and Naucoridae families accounted 25.55% of the difference. The within-group percentage of similarity in the references was 58.51%, and in the test sites was 42.82%.

Table 5.3 Percentage contribution and accumulative percentage of the macroinvertebrate taxa characterized the reference and non-reference/test sites.

	Non-Reference group		**Reference group**	
Family	**Percent (%) Contribution**	**Percent (%) Accumulative**	**Percent (%) Contribution**	**Percent (%) Accumulative**
Hydropsychidae	0.13	10.00	3.42	90.00
Polymitarcyidae	0.60	11.95	7.41	88.05
Hydrachnidae	0.02	14.28	3.51	85.71
Philopotamidae	0	0.00	0.21	100.00
Naucoridae	0	0.00	1.25	100.00

5.3.2 The reference benthic diatom species

Eight diatom species (*Aulacoseira ambigua, Aulacoseira granulata, Encyonema volkii, Nitzschia acicularis, Pinnularia subgibba, Stephanodiscus* sp., *Surirella angusta* and *Thalassiosira baltica*), and three species more abundant in reference sites than test sites (*Achnanthidium* sp., *Encyonopsis microcephala* and *Navicula zanonii*), were considered core of the benthic diatom reference assemblage. The diatom reference assemblage was dominated by species: *Aulacoseira ambigua, Aulacoseira granulata, Encyonema volkii,* and *Nitzschia acicularis* by contributing 78.74% of the total reference diatom species. Four species of the reference community (*Aulacoseira ambigua*, *Aulacoseira granulata*, *Encyonema volkii*, and *Thalassiosira baltica*) appeared as well in the non-reference sites, but at lower percentage (Table 5.4).

Table 5.4 Percentage contribution and accumulative percentage of the diatom species characterized the reference and non-reference/test sites. (Species Code: Table 5.5).

Species	**Non-Reference group**		**Reference group**	
Code	**% Contribution**	**% Accumulative**	**% Contribution**	**% Accumulative**
AAMB	1.5	19.35	6.25	80.65
AUGA	1.0	18.18	4.5	81.82
EVOL	19.5	42.16	26.75	57.84
NACI	0	0.00	3.25	100.00
PSGI	0	0.00	3.0	100.00
STSP	0	0.00	3.5	100.00
SANG	0	0.00	2.75	100.00
TBAL	0.25	12.50	1.75	87.50

The SIMPER routine compared the three site groups, ‘intermediary stressors’, ‘multiple stressors’ and ‘reference’ groups. The overall average dissimilarity between reference and intermediary stressors was 30.03%, in which *Encyonopsis microcephala, Encyonema volkii, Navicula zanonii, Nitzschia* sp., and *Ulnaria ulna* accounted 55.76% of the difference. The average dissimilarity between reference and multiple stressors sites was 36.44%, in which *Achnanthidium* sp., *Encyonopsis microcephala, Navicula zanonii, Nitzschia intermedia,* and *Nitzschia sp.* accounted 69.83% of the difference. The average dissimilarity between intermediary stressors and multiple stressors was 34.33% (Fig. 5.2), in which *Achnanthidium* sp., *Gomphonema affine,* and *Nitzschia intermedia* accounted 65.53% of the difference. The within-group percentage of similarity in the references was 68.84% and in the test sites 48.28%.

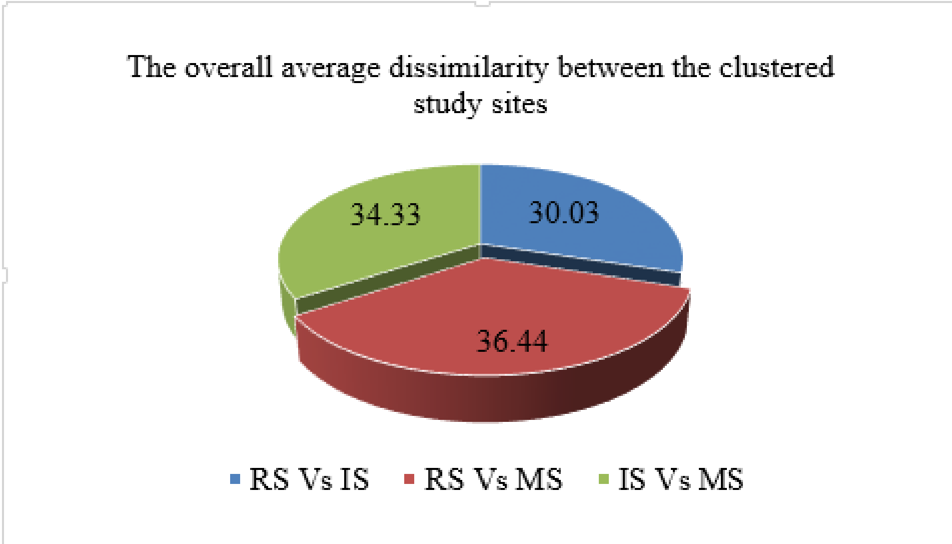

Figure 5.2 Average dissimilarity of diatom species distribution between the clustered study sites (RS: Reference Sites; IS: Intermediary Stressors; MS: Multiple Stressors).

Table 5.5 Species name of the reference diatom taxa (eight species) for the littoral zone of Lake Ziway.

Code	Reference Diatom Species (Taxa)
AAMB	*Aulacoseira ambigua* (Grunow) Simonsen
AUGA	*Aulacoseira granulata* (Ehr.) Simonsen var. angustissima (O.M.) Simonsen
EVOL	*Encyonema volkii* (Rumrich. Krammer and Lange-Bertalot) Krammer
NACI	*Nitzschia acicularis* (Kützing) W. M. Smith
PSGI	*Pinnularia subgibba* Krammer var. subgibba
STSP	*Stephanodiscus* sp. Ehrenberg
SANG	*Surirella angusta* (Kützing)
TBAL	*Thalassiosira baltica* (Grunow) Ostenfeld

5.3.3 Metrics and indices selection

5.3.3.1 Benthic macroinvertebrate metrics and indices selection

Macroinvertebrate metrics and/or indices were compared based on their correlations, discriminatory efficiency and response to the physicochemical variables. The metrics TNI, NITI, NEI, NETI, NTI, NETT, NETO, NCI, H′, MI, PEI, and PII were excluded due to their high correlation coefficient with other metrics such as NT, PETI, PTI, IBI, PDT, NTTI, PToI, CLI, FBI, PARTI, and PRRT (macroinvertebrate metrics - Table 5.1).

The NETI was strongly correlated with TNI and NITI (r = 0.845 and 0.993, respectively), IBI with NEI and NETT (r = 0.936 and 0.936, respectively). PTI was strongly correlated with NTI and NETT (r = 0.949 and 0.738, respectively), FBI was strongly correlated with H′ and PTI (r = 0.852 and 0.936, respectively), NDI was strongly correlated with NCI (r = 1.000), PTI was strongly correlated with NTI (r = 0.949), and PDT was more strongly correlated with TNI (r = 0.801). (Spearman's correlation is significant at the 0.01 level). D, TC/TI, ET/C, PCOI, and PDI were excluded because they were not correlated with the physicochemical variables (Table 5.6).

The rest of the macroinvertebrate metrics and/or indices were selected based on the discrimination efficiency value ≥ 2: NT, PETI, PTI, IBI, PDT, and CLI (Fig. 5.3 and Table 5.6). From the four metrics developed based on the reference taxa, PARTI and PRRT were selected due to their strong correlation with the physicochemical variables and their higher discriminatory efficiency (3 each) (Table 5.6). Therefore, NT, PETI, PTI, IBI, PDT, CLI, PARTI, and PRRT were macroinvertebrate based metrics considered for the multimetric index construction in the present study.

Table 5.6 Spearman correlation among macroinvertebrate metrics and physicochemical variables, and metrics discrimination sensitivity values, (** significant correlation at the level of 0.01 and * at the level of 0.05). (Temp.: Temperature; TP: Total phosphorus).

Metrics/ Indices	**Temp.**	**DO**	**pH**	**EC**	**NO_3^-**	**PO_4^{3-}**	**TP**	**Sensitivity Value**
TNI	-.168	-.246*	.063	-.364**	.276**	.146	.353**	1
NT	-.320**	-.009	.178	-.388**	-.061	.184	.293**	3
NEI	-.219*	-.230*	.017	-.377**	.125	.189	.325**	1
NTI	-.252*	.053	.069	-.126	-.342**	.107	.083	2
NETT	-.312**	.134	.198	-.214*	-.274**	.019	.068	2
NETI	-.219*	-.230*	.017	-.377**	.125	.189	.325**	2
NOI	-.051	-.245*	-.246*	.204*	-.215*	-.214*	-.228*	2
NCI	-.139	-.353**	.011	-.328**	.252*	.207*	.368**	1
NDI	-.139	-.353**	.011	-.328**	.252*	.207*	.368**	2
NTTI	-.046	-.224*	.004	-.125	.278**	-.104	.082	1
NITI	-.251*	-.223*	.042	-.401**	.096	.195	.343**	1
PEI	-.203*	-.098	.034	-.307**	-.013	.189	.245*	2
PTI	-.223*	-.024	.039	-.128	-.254*	.117	.096	3
PETI	-.266**	-.149	.017	-.328**	-.057	.153	.219*	3
PDI	-.022	-.078	.026	-.065	-.050	.122	.132	1
PCOI	-.110	-.053	-.011	.004	-.227*	.098	.060	0
PDT	.138	.435**	.064	.318**	-.332**	-.115	-.306**	3
PTI	.223*	.127	-.044	.341**	.004	-.156	-.237*	2

… Continued

Metrics/ Indices	**Temp.**	**DO**	**pH**	**EC**	**NO_3^-**	**PO_4^{3-}**	**TP**	**Sensitivity Value**
PII	-.223*	-.127	.044	-.341**	-.004	.156	.237*	2
ET/C	-.143	-.066	-.034	-.187	.067	.112	.111	2
TC/TI	-.016	-.072	.043	-.078	-.030	.139	.161	1
MI	-.364**	.063	.220*	-.368**	-.126	.177	.260*	0
H′	-.276**	-.299**	-.017	-.359**	.085	.150	.247*	1
D	-.001	.136	.121	.015	.089	-.113	-.061	0
FBI	.197	.228*	-.040	.388**	-.108	-.171	-.315**	2
NETO	-.271**	.076	.142	-.237*	-.338**	.118	.168	2
IBI	-.300**	-.223*	.000	-.355**	.007	.144	.250*	3
CLI	.317**	.117	-.144	.385**	-.024	-.232*	-.322**	3
ARTI	-.265**	.025	.152	-.314**	-.231*	.225*	.301**	3
RRT	-.357**	.095	.204*	-.326**	-.151	.113	.150	3
PARTI	-.357**	.095	.204*	-.326**	-.151	.113	.150	3
PRRT	-.288**	.021	.119	-.294**	-.257*	.195	.256*	3

Note: ARTI, RRT, PARTI, and PRRT metrics were developed for the present study based on the macroinvertebrate taxa those characterized the reference groups. (For the full names of the metrics Table 5.1).

5.3.3.2 Benthic diatom metrics and indices selection

Diatom metrics and indices were compared based on their correlations, discriminatory efficiency and response to the physicochemical variables. The indices CEE, DESCY, and EPID were excluded due to their high correlation coefficient with other indices such as IBD, IPS, and TDI. The CEE was strongly correlated with IBD ($r = 0.72$), DESCY with IPS ($r = 0.81$), and EPID with IPS ($r = 0.86$). Similarly, DESCY was strongly correlated with TDI ($r = 0.81$), and EPID was more strongly correlated with TDI ($r = 0.83$). (Spearman's correlation is significant at the 0.01 level). IDAP, IDSE, SLA, and WAT indices were excluded because they were not correlated with selected physicochemical variables/criteria (Table 5.7).

The rest of the diatom metrics and/or indices were selected based on the discrimination efficiency value ≥ 2: IBD, IPS, CEE, SHE, PTV and TDI (Fig. 5.4 and Table 5.7). From the four metrics developed based on the diatom reference species, PARS and PRRS were selected due to their higher discriminatory efficiency value (3) (Table 5.7). Therefore, IBD, IPS, CEE, SHE, PTV, TDI, PARS, and PRRS were metrics and indices considered for the multimetric index development (Fig. 5.4) (diatom metrics/indices - Table 5.2). The selected metrics/indices (eight macroinvertebrate and eight diatom metrics) were potential metrics. Therefore, these potential metrics were used for the development of new macroinvertebrate and diatom based multimetric index (MMIZ) for the lake water quality assessment of Lake Ziway.

Table 5.7 Spearman correlation among diatom metrics/indices and physicochemical variables and indices discrimination sensitivity values, (** significant correlation at the level of 0.01; * at the level of 0.05). (Temp.: Temperature; TP: Total phosphorus).

Metrics/ Indices	**Temp.**	**pH**	**EC**	**NO_3^-**	**PO_4^{3-}**	**TP**	**Sensitivity Value**
CEE	-0.180	-0.039	-0.228*	0.029	0.057	0.207*	3
DESCY	-0.327**	-0.080	-0.294**	0.170	0.106	0.197	1
EPID	-0.237*	-0.083	-0.161	-0.221*	0.111	0.117	1
IBD	-0.257*	0.082	-0.346**	0.009	0.185*	0.281**	2
IDAP	-0.073	-0.204*	0.063	0.189	-0.054	-0.068	1
IDP	-0.222*	-0.020	-0.211*	0.196*	0.089	0.153	2
IDSE	-0.157	0.018	-0.266**	0.028	0.072	0.203*	1
IPS	-0.346**	0.037	-0.276**	-0.263**	0.182*	0.205*	2
PTV	-0.310**	-0.053	0.337**	-0.310**	0.212*	0.228*	3
SID	-0.128	0.167	-0.249**	0.304**	0.215*	0.180*	2
SHE	-0.052	0.134	-0.309**	-0.344**	0.250**	0.278**	2
SLA	0.044	-0.054	0.021	-0.042	-0.099	-0.055	0
TDI	-0.343**	0.049	-0.366**	0.110	0.184*	0.290**	3
TID	-0.136	0.264**	-0.163	-0.058	0.159	0.123	1
WAT	0.056	-0.088	0.022	0.009	-0.114	-0.059	0
NRSI	-0.197	0.200*	-0.332**	0.101	0.151	0.283**	1
RRS	-0.159	0.233*	-0.378**	0.064	0.204*	0.315**	2
PARS	-0.219*	0.233*	-0.359**	0.071	0.186*	0.340**	3
PRRS	-0.221*	0.235*	-0.400**	0.050	0.185*	0.322**	3
MMIZ	-0.268**	-0.314*	-0.401**	-0.254*	0.223*	0.365**	3

5.3.4 Generating a composite multimetric index (MMIZ)

The potential eight diatom and eight macroinvertebrate metrics/indices were combined in a multimetric index, the MMIZ. Diatom indices, calculated with the OMNIDIA software, had values between 0 and 20, while the metrics PARS and PRRS calculated with percentage and ranged from 0 to 100 with values decreasing with increased degradation. The PTV index is an exception, with values between 0 and 100 explained by percentage and a positive value correlations with pressure variables. Macroinvertebrate metrics, calculated with an excel spreadsheet, had values between 0 and >100, while the metrics PARTI and PRRT calculated with percentage and ranged from 0 to 100 with values decreasing with increased degradation. The PDT and CLI, indices are the exception, explained by positive value correlations with increased degradation.

5.3.4.1 Scoring the potential metrics and indices

For the scoring of the selected potential metrics, the 75th and 25th percentile values of the reference sites were used (Barbour *et al.*, 1996). Metric values were normalized to metric scores of 5, 3 or 1 depending on their proximity to the optimal values (Table 5.8). Metrics whose values decreased with the increase of stress (positive metrics), metric value above the 25th percentile were scored as 5, a metric value between and including the 25th and 5th percentile were scored 3, and metric values below the 5th percentile were scored as 1. Metrics whose values increased with the increase of stress (negative metrics), metric values below the 75th percentile were scored as 5, metric values between and including the 75th and 95th percentile were scored 3, and metric values above the 95th percentile were scored as 1. Therefore, a final multimetric index value was created by summing the normalized scores of the selected potential metrics and indices.

Table 5.8 Frequency distribution statistics of the potential metrics and indices selected for developing a multimetric index and their scoring criteria based on the reference lower (25%ile) and upper (75%ile) quartiles (Min= Minimum; Max= Maximum).

Potential metrics/ indices	**Frequency distribution**					**Score**		
	Min.	**5th %**	**25th %**	**75th %**	**Max.**	**5**	**3**	**1**
NT	17	24	26	28	29	>26	24 - 26	<24
PTI	0	0.82	1.47	10.6	12.8	>1.47	0.82 - 1.47	<0.82
PETI	1.68	6.75	11.1	32.6	39.7	>11.1	6.75 - 11.1	<6.75
PDT	0.19	0.19	0.21	0.24	0.52	<0.24	0.24 - 0.37	>0.37
IBI	23.5	41.9	52.2	64.1	73.2	>52.2	41.9 - 52.2	<41.9
CLI	0	0	0.08	0.10	0.88	<0.10	0.10 – 0.82	>0.82
PARTI	0	3.38	4.47	28.4	46.2	>4.47	3.38 - 4.47	<3.38
PRRT	0	60	60	80	80	>60	60	<60
IPS	3.6	11.3	11.7	13.2	13.7	>11.7	11.3 - 11.7	<11.3
SHE	13.4	14.6	14.9	16	16.4	>14.9	14.6 - 14.9	<14.6
TDI	7.6	11.2	11.4	11.8	12.6	>11.4	11.2 - 11.4	<11.2
IBD	10.2	11.8	12.2	13.3	14.6	>12.2	11.8 - 12.2	<11.8
CEE	4.2	8.1	8.4	9.8	11.5	>8.4	8.1 - 8.4	<8.1
PTV	3.8	4.1	4.9	11.8	58.3	<11.8	11.8 - 14.6	>14.6
PARS	1.0	9.0	11.2	18.6	20.5	>11.2	9.0 - 11.2	<9.0
PRRS	12.5	75	75	87.5	100	>75	75	<75

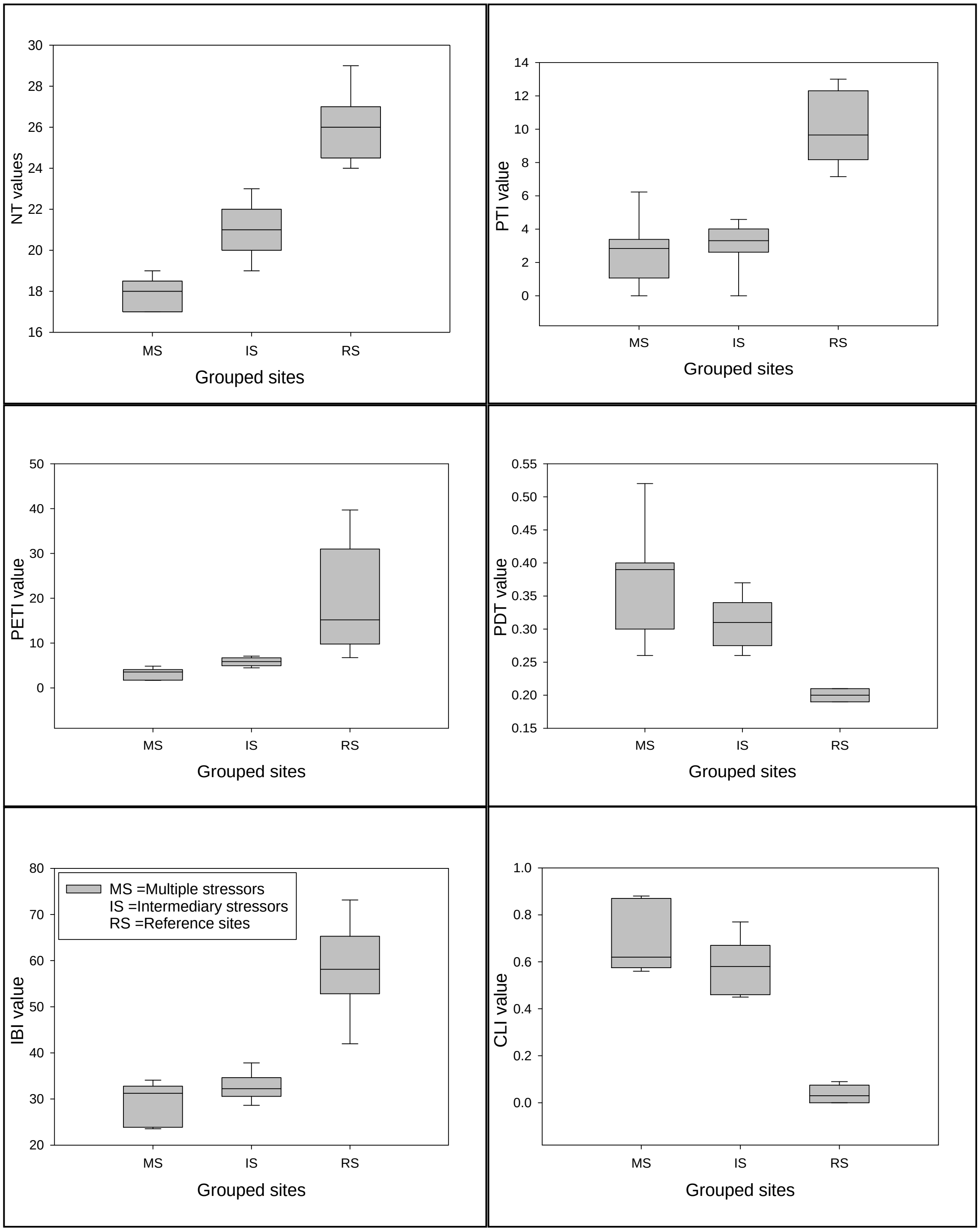

Figure 5.3 Distribution of the macroinvertebrate metrics values across the clustered study site classes. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile.

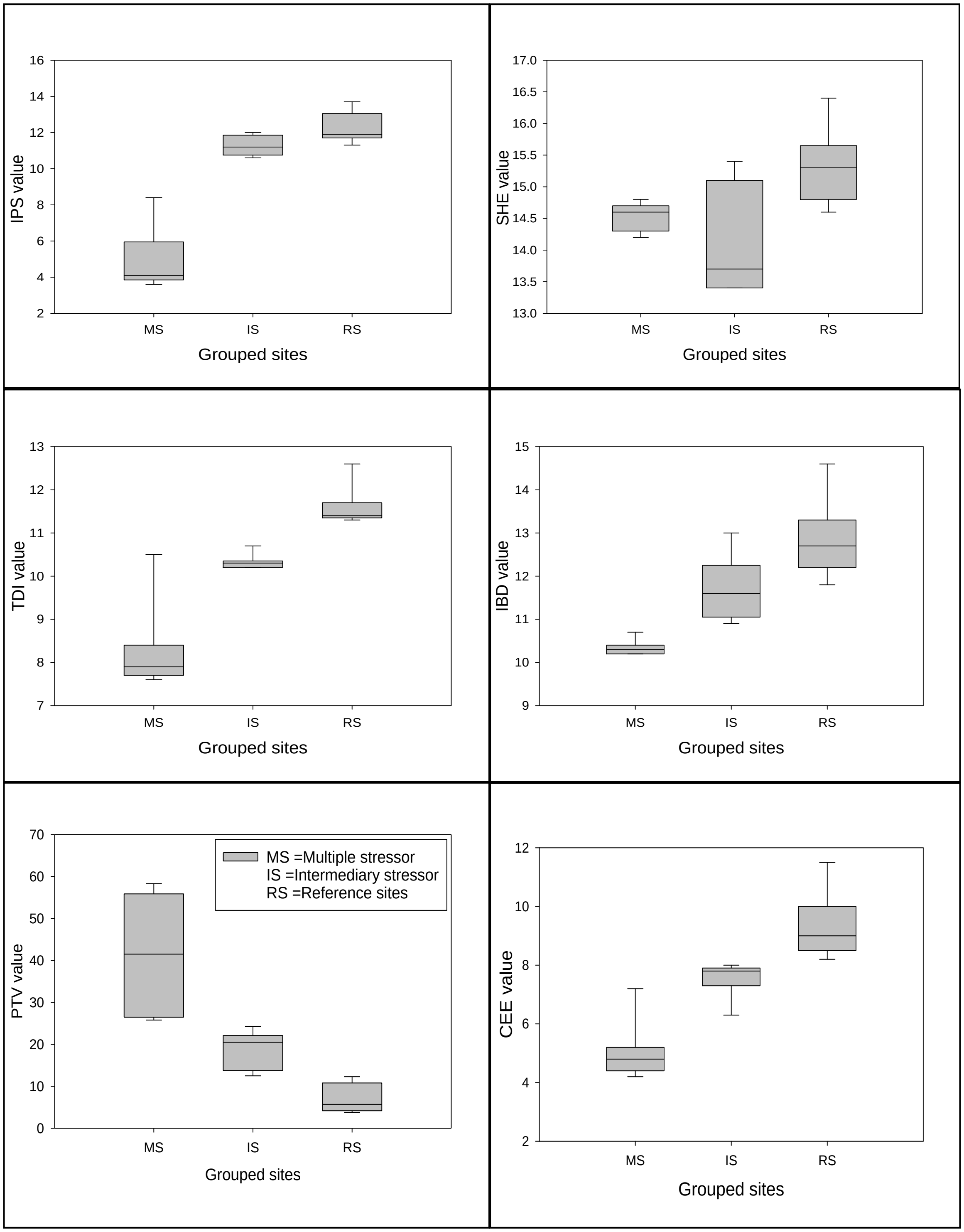

Figure 5.4 Distribution of diatom metrics values across the clustered study site classes. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile.

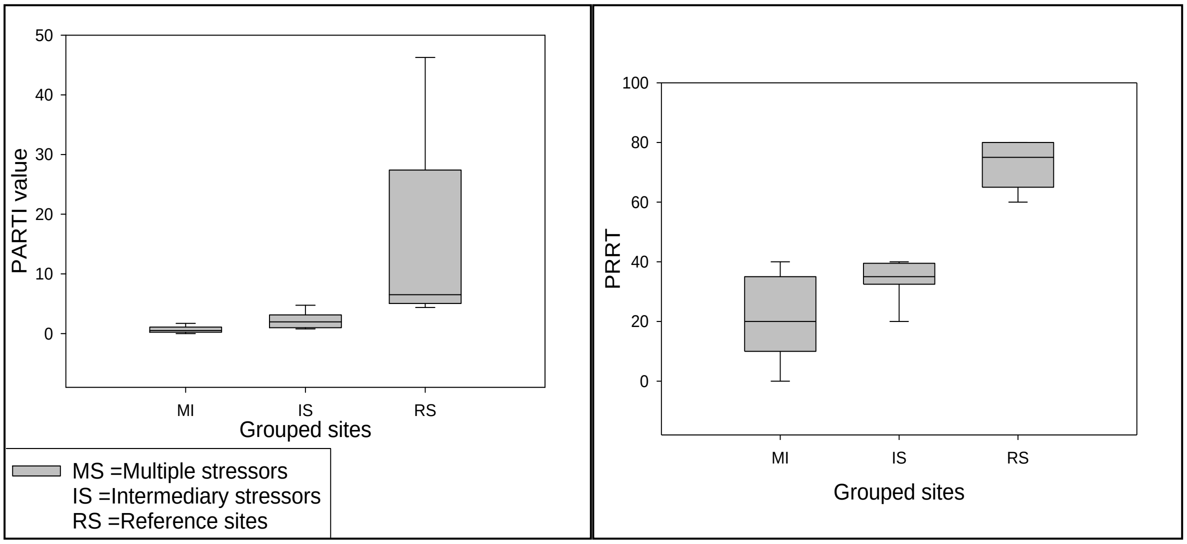

Figure 5.5 Distribution of the present study new macroinvertebrate metrics values for the clustered site classes. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile. (PARTI: Percent Abundance of Reference Taxa Individuals, PRRT: Percent Richness of Reference Taxa).

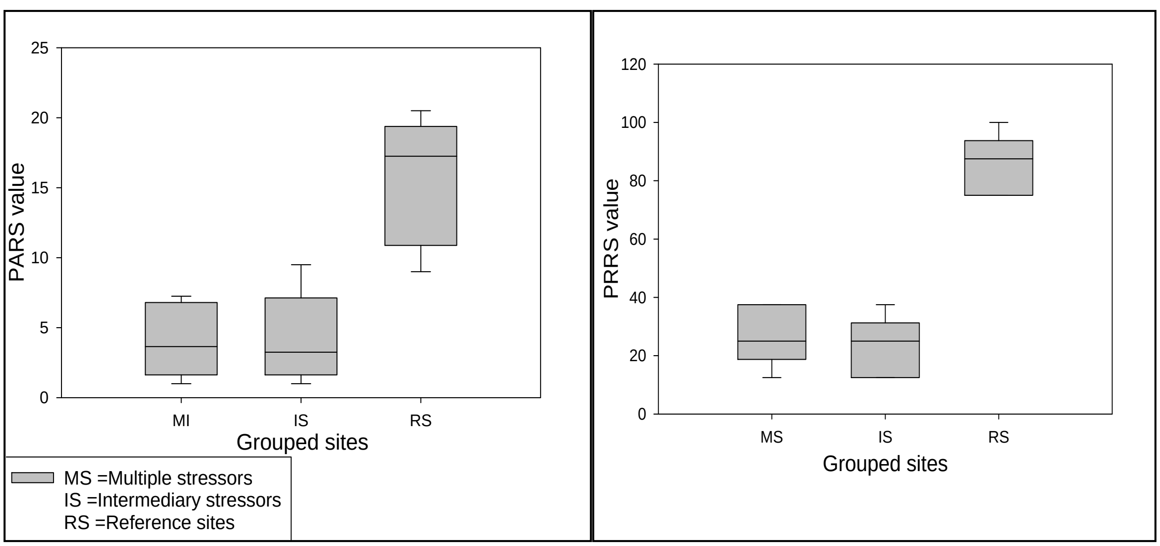

Figure 5.6 Distribution of the present study new diatom metrics values for the clustered site classes. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile. (PARS: Percent Abundance of Reference Species, PRRS: Percent Richness of Reference Species).

Table 5.9 Minimum (Min.), Maximum (Max.), Mean, and Standard Error (SE) values of the potential metrics, MMIZ, and EQR-MMIZ of reference and all study sites.

Metrics/ Indices	Reference sites				All study sites			
	Min.	**Max.**	**Mean**	**SE**	**Min.**	**Max.**	**Mean**	**SE**
MMIZ	66.0	72.0	68.0	2.0	24.0	72.0	47.8	5.68
EQR-MMIZ	0.95	1.09	0.99	0.03	0.36	1.09	0.72	0.08
NT	24	29	25.6	2.08	17	29	21.3	1.79
PTI	6.81	12.8	9.32	0.73	0	12.8	1.77	1.38
PETI	6.75	39.7	20.5	9.8	1.68	39.7	9.99	3.9
PDT	0.19	0.21	0.20	0.06	0.19	0.52	0.29	0.03
IBI	41.9	73.2	55.8	9.15	23.5	73.2	39.4	4.99
CLI	0	0.09	0.03	0.03	0.00	0.88	0.47	0.12
IPS	11.3	13.7	12.4	0.21	3.6	13.7	9.59	0.57
SHE	14.6	16.4	15.6	0.52	13.4	16.4	14.9	0.34
TDI	11.2	12.6	11.9	0.37	7.60	12.6	10.3	0.55
IBD	11.8	14.6	13.3	0.81	10.2	14.6	11.8	0.51
CEE	8.1	11.5	9.56	0.99	4.2	11.5	7.08	0.59
PTV	3.8	12.3	7.96	2.45	3.8	58.3	22.9	5.8
PARTI	3.38	46.2	15.26	5.01	0.00	46.2	8.53	2.94
PRRT	60	80	66.6	6.6	0.00	80	37.5	9.87
PARS	9.0	20.5	15.4	1.44	1.00	20.5	8.50	2.18
PRRS	75	100	87.5	7.21	12.5	100	48.6	10.9

5.3.4.2 Setting ecological status class boundaries

Frequency distribution statistics and scoring criteria of the potential metrics are listed in Table 5.8. Based on the sum scores of potential metrics (normalized metric values), a multimetric possible value between scales ranging from 16 to 80 was developed for each studied lake segment site. Then based on the values of the MMIZ, four levels of discriminatory bio-criteria for lake water quality were eventually obtained by quartation: 16-32, poor; 33-48, fair; 49-64, good; 65-80, very good. The MMIZ and EQR-MMIZ values of the study sites were listed in Table 5.11.

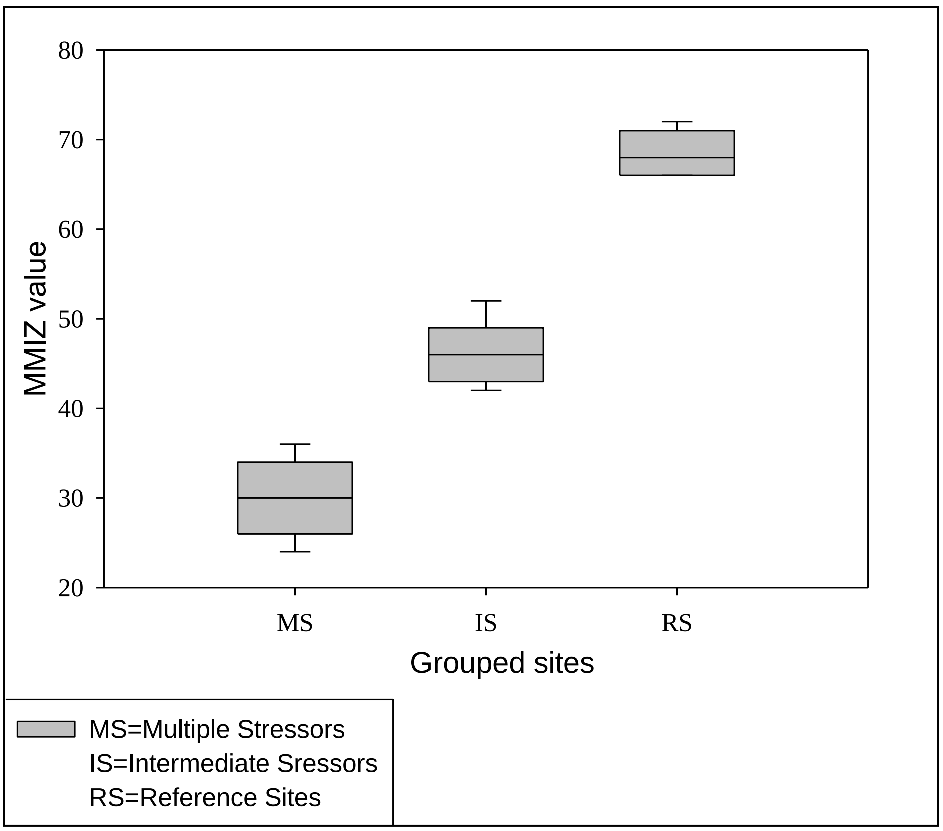

Figure 5.7 Distribution of the MMIZ score for the clustered site classes of Lake Ziway. Horizontal lines represent median values, gray boxes represent 25^{th} and 75^{th} percentiles, and whiskers represent 5^{th} and 95^{th} percentile.

Ecological Quality Ratio (EQR) was calculated by dividing each value of the multimetric by the MMIZ median value of the reference data, and each result was translated into a value between 0 and 1 (EQR-MMIZ). The ranges of values obtained for the selected potential metrics, MMIZ and EQR-MMIZ in the reference and test sites are summarized in Table 5.9. The values of the MMIZ ranged from 24.0 to 72.0 and EQR-MMIZ from 0.364 to 1.091 (values > 1 were set to 1). The EQR values were expressed as a numerical value between zero (0) and one (1): high ecological status was represented by values close to one and bad ecological status by values close to zero.

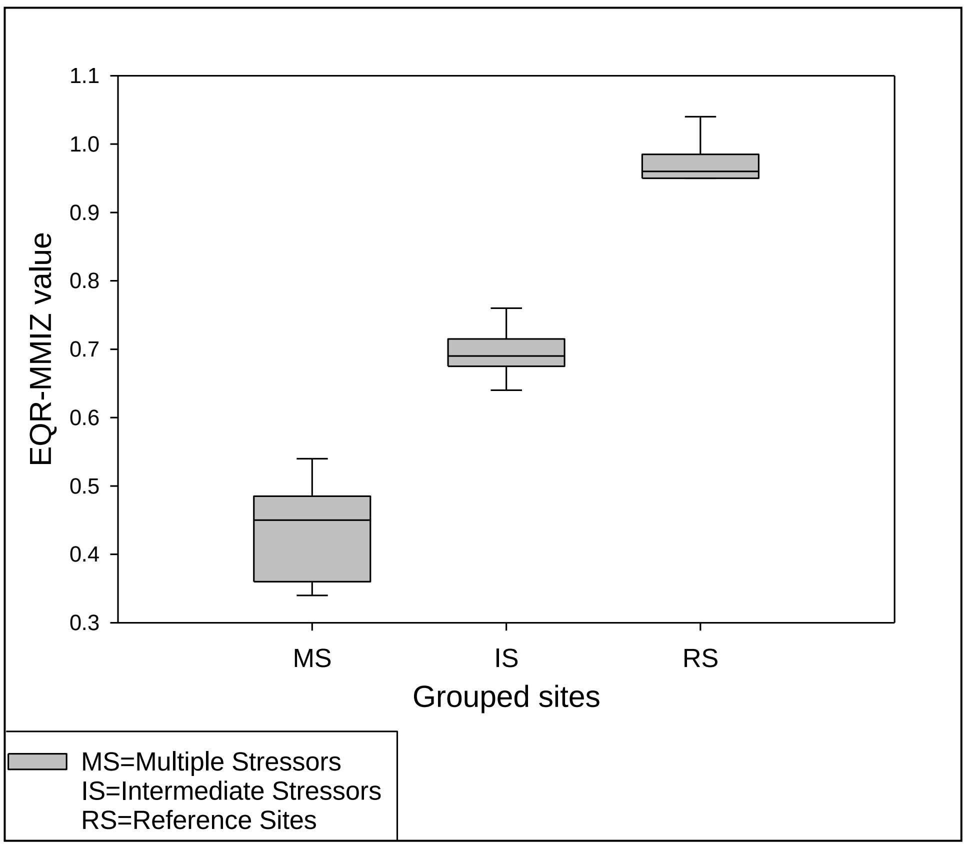

Figure 5.8 Distribution of EQR-MMIZ score for the clustered site classes of Lake Ziway. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile.

EQR-MMIZ values were categorized into five classes: high, good, moderate, poor, and bad, using the upper P_{25} of the reference values as the limit between high and good. The remaining values from the upper P_{25} to 0 were divided into four equal intervals following the criteria stated in WFD (2007). The final EQR-MMIZ values were 0.96, 0.72, 0.48, and 0.24, respectively (Table 5.10). The upper 25% percentile of the EQR-MMIZ values from reference sites were used as the class boundary for reference/high quality condition.

Table 5.10 Quality class boundaries established based on the EQR-MMIZ values.

Quality Classes	**EQR-MMIZ Values**
Reference/High quality	≥ 0.96
Good quality	$\geq 0.72 < 0.96$
Moderate quality	$\geq 0.48 < 0.72$
Poor quality	$\geq 0.24 < 0.48$
Bad quality	< 0.24

Assessment of the study lake segments within the clustered study sites was carried out based on the established MMIZ water quality bio-criteria. The results showed that the water quality of the reference sites of Lake Ziway was very good, and one site from the intermediary stressors was good, whereas two sites from intermediary and one site from multiple stressors were fair, and two sites from multiple stressors section were poor (Table 5.11). The EQR-MMIZ showed that the water quality of the two sites from the reference was high, and one site from reference and one site from intermediary stressors were good; two sites from intermediary and one site from the multiple stressors were fair; and two sites from multiple stressors were under poor quality.

Table 5.11 Multimetric index of Lake Ziway (MMIZ) and Ecological Quality Ratio of the MMIZ (EQR-MMIZ) values with the water quality class and ecological status interpretations of the study sites of the littoral zone of Lake Ziway.

Site Clusters	Study Sites	MMIZ Score	Water quality class	Ecological quality status	EQR-MMIZ Score	Water quality class	Ecological quality status
Intermediary stressors	**BU**	50.0	II	Good	0.76	II	Good
	GCH	42.0	III	Fair	0.64	III	Moderate
	SI	44.0	III	Fair	0.67	III	Moderate
Multiple stressors	**ShEFF**	36.0	III	Fair	0.54	III	Moderate
	KO	24.0	IV	Poor	0.36	IV	Poor
	CA	30.0	IV	Poor	0.45	IV	Poor
Reference sites	**FRC**	66.0	I	Very good	0.95	II	Good
	G1	68.0	I	Very good	0.96	I	High
	G2	72.0	I	Very good	1.00	I	High

5.3.4.3 Validation of the multimetric index (MMIZ)

Validation of multimetric index (MMIZ) was carried out through different approaches, (1) based on the quality classification power of the MMIZ using box-and-whisker plots, (2) Principal Components Analysis (PCA) was used to test the sensitivity of the MMIZ how it distinguished the site groups, and (3) independent data set was used to validate the MMIZ (data from Lake Hawassa). In the first case, the MMIZ was evaluated by comparing its quality class classification versus determined quality class based on pre-classification, and 88.8% of the study sites were classified fittingly with the pre-classification prepared based on the environmental data and stressors gradient.

The multimetric index discriminated between the reference and test site groups (Fig.5.7). The MMIZ classified two sampling sites of the reference group (66.6%) as having very good quality, and one sampling site (33.3%) as of good condition, which followed the initial site classification. The study sites initially considered impaired were categorized in fair and poor conditions (100%). One site from the multiple stressors group and all the intermediary stressors sites were classified as having fair conditions, whereas two sites from the multiple stressors group were classified as of poor condition.

Principal Components Analysis (PCA) was used to examine the responsiveness or efficiency of the multimetric index scores exactly how discriminated the reference sites from the test sites. The first PCA axis explained 76.8% of the inter-site variance, with eigenvalues >1. The reference sites grouped closely together on the right side of the PC axis 1, while the test sites were scattered to the left side (Fig. 5.9).

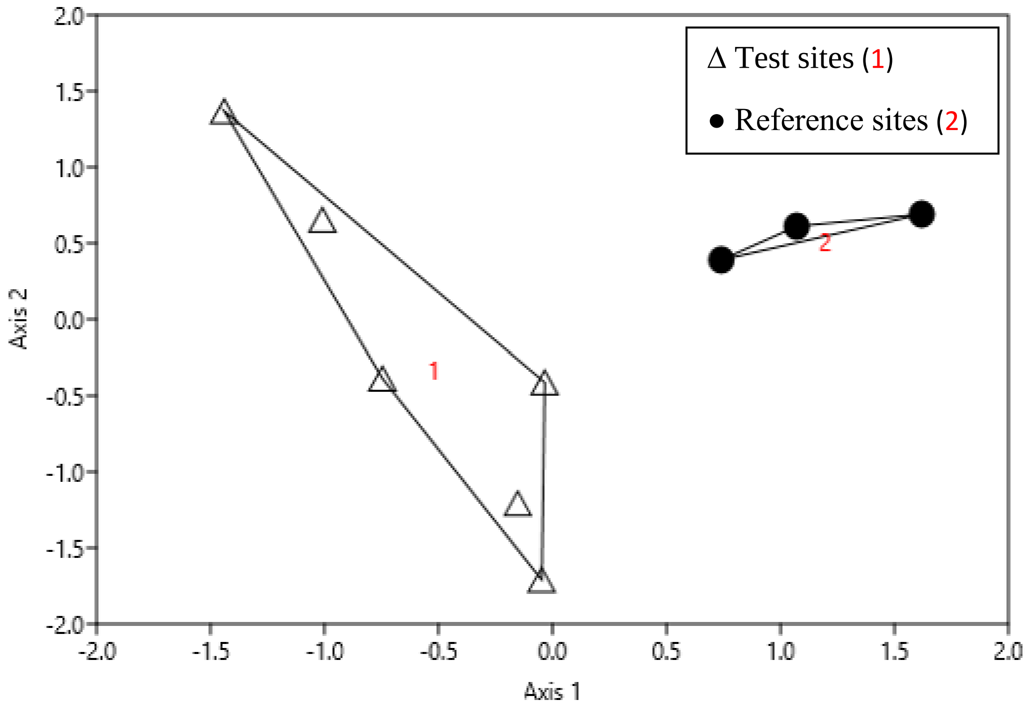

Figure 5.9 Distribution of reference and test sites by the first two principal components (PC1 and PC2) based on the multimetric index scores of Lake Ziway (MMIZ).

The multimetric index discriminated between reference and test site groups of Lake Hawassa (Fig. 5.10), and 83% of the sites were classified correctly. The MMIZ classified two sampling sites of the reference group (66.6%) as having very good quality class, one sampling site (33.3%) as having good, which followed the initial site classification. All of Lake Hawassa sampling sites initially considered impaired (100%) were categorized as in the fair and poor conditions (Fig. 5.10), which also followed the initial classification. All moderately disturbed sites and one site from the disturbed sites were classified as having fair conditions, whereas two sites from the disturbed site's group were classified as poor condition. These sites have also near similar typologies in the studied lake sites as stated in the LHQA investigation.

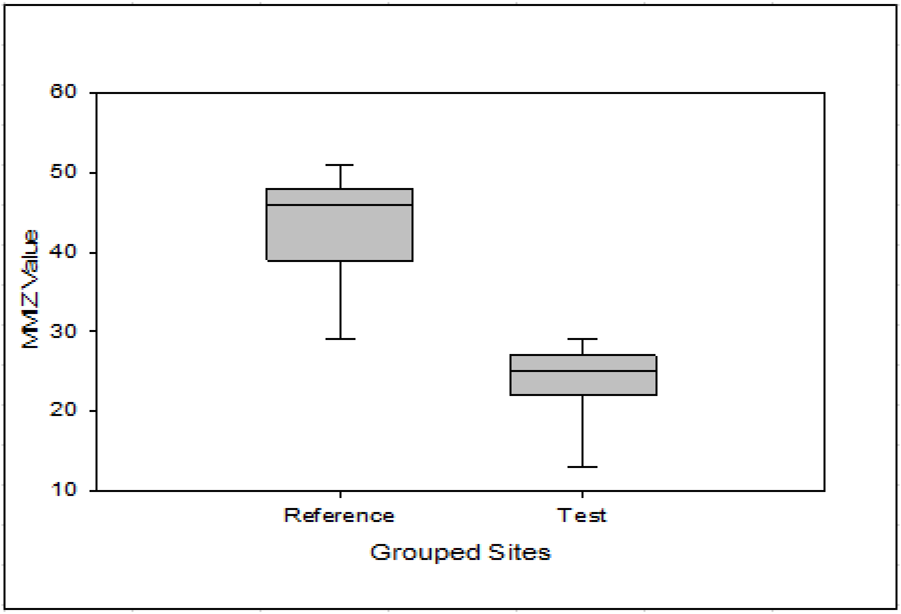

Figure 5.10 Distribution of MMIZ score between the reference and test site groups of Lake Hawassa. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile.

For the calibration of the MMIZ, only clearly degraded sites in Lake Ziway (3 sites from multiple stressors group, which were not included in the multimetric index) were used. Therefore, the MMIZ classified two of these sites as having poor quality class, and one site as having fair condition, which followed the initial site classification.

Table 5.12 Spearman correlation among lake habitat conditions and the MMIZ values (** significant correlation at the level of 0.01). (PVQ: Percent Vegetation Quality; LAHA: Lake Adverse Human Alteration; PAL: Percent Agricultural Land; PNSZ: Percent Natural Shore Zone; LHQS: Lake Habitat Quality Score).

	PVQ	**LAHA**	**PAL**	**PNSZ**	**LHQS**
MMIZ	.756**	-.822**	-.722**	.685**	.959**

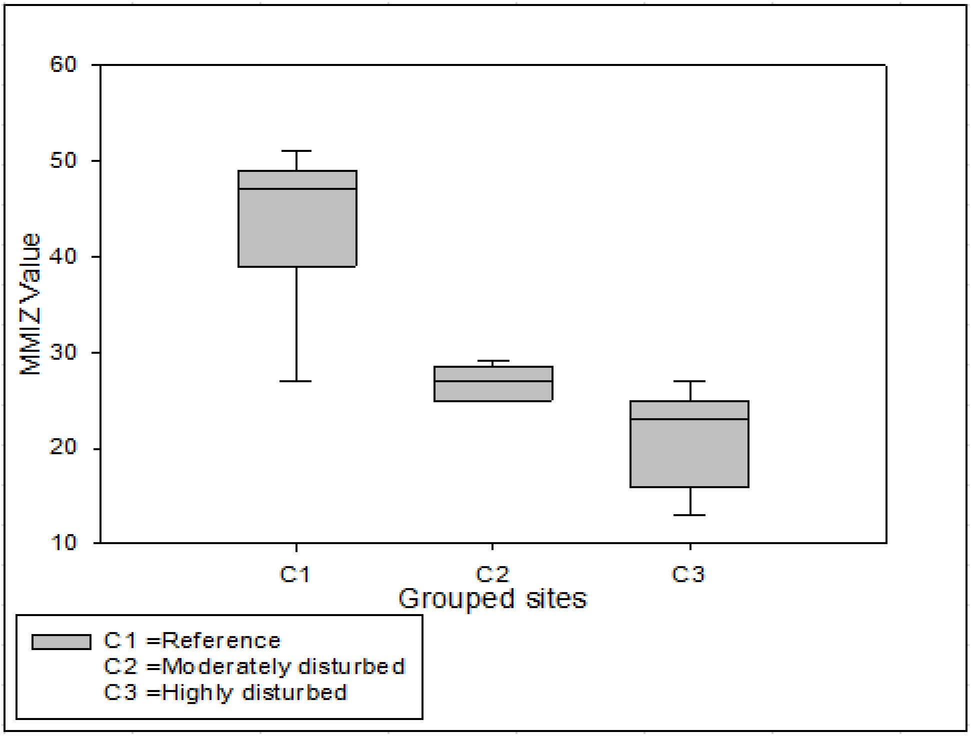

Figure 5.11 Distribution of MMIZ score for the clustered site classes of Lake Hawassa. Horizontal lines represent median values, gray boxes represent 25th and 75th percentiles, and whiskers represent 5th and 95th percentile.

The MMIZ index showed good responsiveness to all selected environmental factors, as evidenced by a significant statistical correlations (Table 5.7). The factors indicating the anthropogenic activities such as lake adverse human alteration, percent agricultural land, and some water quality parameters were negatively correlated with the MMIZ ($p<0.01$), reflecting the impact of human disturbance on the biological integrity of the lake. In contrast there were positive correlations with percent vegetation quality of riparian zone ($r=0.756$, $p<0.01$), percent of natural shore zone ($r=0.685$, $p<0.01$), and lake habitat quality score ($r=0.959$, $p<0.01$) (Table 5.12). These correlation results indicated good responsiveness of the multimetric index to variables of disturbance/stressors along the different study sites of Lake Ziway.

5.4 DISCUSSION

5.4.1 The reference benthic macroinvertebrate and diatom communities

Five benthic macroinvertebrate taxa and eight diatom species were considered the core of the reference assemblages. Three macroinvertebrate and four diatom taxa of the reference assemblage also appeared in test sites with moderate level of disturbance, but at lower relative abundances. Therefore, we considered these assemblages to be sensitive because they disappear with increasing levels of human disturbances.

Macroinvertebrate and diatom assemblages found in Lake Ziway showed high similarity, this could be because of the ecological and geographical similarity between sites. The within-group percentage of similarity on invertebrate and diatom communities in the references was 58.51 and 68.84% and in test sites 42.82 and 48.28%, respectively, which indicated that sites in the reference group had similar biological integrity rather than test sites and this result agrees with the reports of Delgado *et al.* (2010). Therefore, we considered the observed stressors to be responsible for the differences.

The overall average dissimilarity of macroinvertebrates and diatoms distribution between reference and intermediary stressor sites was 31.09 and 30.03%, and it was 33.14 and 36.44%, between reference and multiple stressor sites and 24.65 and 34.33% between intermediary and multiple stressor sites. Three macroinvertebrate taxa and five diatom species that characterized the reference assemblage contributed large in the dissimilarity percentage, as they disappeared in the disturbed sites. Therefore, the highest value of dissimilarity between the reference and multiple stressors sites were possibly because of the environmental variation formed through several stressors pressure in the test sites, this is also argued in the results of Mabidi *et al.* (2017) in South Africa.

5.4.2 Selection of core metrics and indices

In the present study, 50 candidate metrics (32 macroinvertebrate and 18 diatom metrics) were tested to include in a multimetric index development. Most metrics are widely recognized as being sensitive to a range of anthropogenic stressors (Verdonschot *et al.*, 2012; Wang *et al.*, 2015). When we selected the potential metrics, the obtained metrics were compared based on their correlations, discriminatory efficiency, and response to the environmental variables. And four metrics were generated based on the reference community which further strengthens the discrimination efficiency of the MMIZ as also applies in the approach of Delgado *et al.* (2010). Low sensitive metrics were canceled by boxplot method based on the reference and impaired sites information (Barbour *et al.*, 1996). Therefore, most of the candidate metrics were eliminated because they did not discriminate well among reference and impaired sites.

The macroinvertebrate metrics (NETI, NT, NTI, IBI, PDT, CLI, PARTI, and PRRT) and diatom metrics/indices (IBD, IPS, CEE, SHE, PTV, TDI, PARS, and PRRS) relatively indicated better ecological quality in the reference sites than the test sites. The selected potential metrics showed strong correlation with most of the physicochemical variables. However, most metrics did not discriminate against the intermediary stressors group from multiple stressors (Fig. 5.3 and 5.4). This might be because of the similarity of the stressors in both stressed site groups. Besides, weak power of the single metrics to discriminate narrow differences between these groups, and the presence of numerous stressors in the intermediary and multiple stressors sites might be a reason. Ferreira *et al.* (2011), also reported that most of the single metrics were designated to detect a specific single stressor in aquatic environments.

5.4.3 A multimetric index development and bioassessment

Differences that existed in water quality of the studied sites of Lake Ziway corresponded to changes in macroinvertebrate and diatom assemblages, and to the values of the selected core metrics. The responses to pressures that some macroinvertebrate and diatom metrics provided when applied to the study sites of Lake Ziway could not discriminate intermediary stressor sites from multiple stressor sites, and so it was necessary to develop new local metrics using the conceptual framework proposed by the WFD, to distinguish the reference assemblages that have the potential to discriminate between these sites.

The new metrics calculated based on benthic macroinvertebrate and diatom reference assemblages were more sensitive indicators than the macroinvertebrate and diatom metrics used and developed for European countries. Our observations follow findings from studies that show some diatom indices developed in certain parts of Europe were not effective when they were used in other areas (Tan *et al.*, 2017); we incorporated the new metrics formulated based on the reference assemblages.

It is demonstrated that metrics selected for the multimetric index development satisfied three basic requirements: they were not strongly correlated with other selected core metrics (Fore and Grafe, 2002); they responded to disturbances in the predicted ecological direction (Hering *et al.*, 2006a); and they were also associated with the physicochemical variables (Wang *et al.*, 2015). Therefore, the MMIZ constitutes a good tool to evaluate the ecological status of Lake Ziway. MMIZ values were better correlated with the physicochemical variables than the individual metrics, and better discriminated the test sites from reference sites, integrating the effects of the cumulative pressures.

From selected core metrics, three of macroinvertebrate metrics were directly related with the families that belonged to Ephemeroptera and Trichoptera. Individuals belonged to ET taxa were absent in most of the stressed sites and rarely occurred in some of the stressed sites. These taxa are good indicators of water quality and are usually incorporated in studies dealing with multimetric index development (Flores and Zafaralla, 2012; Getachew Beneberu, 2013). The problem with these taxa is that they are not sensitive to narrow ranges of class boundaries. For instance, in the present study ET taxa individuals were observed in both reference and intermediary stressed sites. However, Philopotamidae family was absent from all stressed sites and abundant in reference sites. Raburu *et al.* (2009), suggested separation of individual families will give better results than lumping them since the families in the ET taxa respond differently to degradation.

Based on the MMIZ values, four levels of discriminatory bio-criteria for water quality were eventually obtained: 16-32, poor; 33-48, fair; 49-64, good; 65-80, very good. Thus, 3 sites (33%) were rated as very good; 1 site (11%) as good; 3 sites (33%) as fair; and 2 sites (22%) as poor condition (Table 5.11). Based on EQR-MMIZ values, five classifications were categorized: ≥0.96, High; ≥0.72< 0.96, Good; ≥0.48<0.72, Moderate; ≥0.24<0.48, Poor; and <0.24, bad using the upper P_{25} of the reference values as the limit between High and Good following the criteria stated in WFD (2007). Accordingly, 2 sites (22%) were rated high quality; 2 sites (22%) good; 3 sites (33%) moderate; and 2 sites (22%) poor (Table 5.13). WFD (2007) stated that the purpose of expressing results as an EQR (standardizing to values between 0 and 1) is to ensure comparability between different assessment methods and to provide a common scale of ecological quality.

Furthermore, all previously assigned reference sites based on cluster analysis were rated as very good condition on the MMIZ score categories, besides 2 sites were rated as having high quality and 1 site as good based on the EQR-MMIZ score categories. Two sites assigned to impaired condition based on cluster analysis were rated as poor condition based on both the MMIZ and EQR-MMIZ score categories, and 1 site from this group was rated as having fair condition or moderate quality. This is most probably because of the high density of macrophytes in this site that might support benthic macroinvertebrates and the relative high amount of plant growth nutrients (nitrogen families) which might support the diatom growth.

Based on the MMIZ score categories, only 22.2% of the sites were rated as having poor condition, of which > 66% of the sites were found in the multiple stressors sites group. Lower MMIZ score (24) was recorded within multiple stressors site, whereas high score (72) was recorded at reference sites. In general, from these results, it can be concluded that the MMIZ responded in a predictable way to impairments and hence can be used to monitor the water quality in lowland lakes of Ethiopia.

Table 5.13 Range of MMIZ scores to categorize sites into various levels of ecological quality for sites under study and percentage distribution.

MMIZ range	**Ecological Quality**	**Number of sites**	**Percent (%)**
16 - 32	Poor	2	22.2
33 - 48	Fair	3	33.3
49 - 64	Good	1	11.1
65 - 80	Very good	3	33.3

5.4.4 Validation of the multimetric index

The developed multimetric index is a summation of sixteen metrics reflecting different aspects of the structure and functioning of macroinvertebrate and diatom assemblages. Correlation analysis revealed this MMIZ was negatively correlated with both major disturbance measures, to percent agricultural land ($r=0.72$, $p<0.01$) and to lake side adverse human alteration ($r=0.82$, $p<0.01$). And the MMIZ was positively correlated with quality measures, to percent vegetation quality ($r=0.75$, $p<0.01$) and to percent natural shore zone ($r=0.69$, $p<0.01$) (Table 5.12). This validation method was also found successful in the study of Seid Tiku *et al.* (2013), in their macroinvertebrates based MMI development for the assessment of natural wetlands in Southwest Ethiopia. Therefore, this validation indicated that the MMIZ index responded appropriately to the over-all disturbances, and represents an appropriate tool to detect environmental degradation.

The sensitivity of the MMIZ was also evaluated by comparing its quality class classification versus the pre-classification done based on the environmental data using box-and-whisker plots (Fig. 5.7). Hence, 88.8% of the sampling sites were classified properly with the pre-classification. This would be also an indicator that the MMIZ responded appropriately to general measurements of disturbance, representing a wide variety of combined stressors, being an appropriate tool to detect environmental impacts. The efficiency of the MMIZ scores on how they discriminated the reference sites from the test sites were determined using PCA analysis. Reference sites grouped closely together on the right side of PC axis 1, while the test sites were scattered to the left side (Fig. 5.9), showing that the responsiveness of the MMIZ was explained by the distribution of the sites in the ordination space.

The MMIZ also discriminated between the reference and test site groups of Lake Hawassa (Fig. 5.10), 83% of the sites were classified correctly or in agreement with the cluster analysis done based on the environmental variables. Therefore, the observed discrimination power of the MMIZ on Lake Hawassa could indicate that the MMIZ can be implemented in other water bodies within the ecoregion. The differences in the correctly classified study sites between the lakes were not caused by differences in the collection of samples since the collection of samples was performed in a similar way and time for both lakes. The differences in the classification for Lakes Ziway and Hawassa can be explained by the different metrics selected as potential for developing the multimetric index.

For the calibration of the MMIZ index, only clearly degraded sites (3 sites) in Lake Ziway were used, while the classification of moderately degraded sites created the biggest problem in the classification (Vlek *et al.*, 2004). The MMIZ classified two of these sites as having poor quality class, and one site as having fair condition, which followed the initial classification by over 66%.

When comparing results of water quality assessment by using established biocriteria with water quality assessment by using physicochemical quality parameters, it was found that the two assessment results were consistent in Lake Ziway. Therefore, the established benthic macroinvertebrate and diatom-based biocriteria can be suitable for water quality assessments in the regions of Lake Ziway. This result was agreeable with the finding of Wang *et al.* (2015) in the development and evaluation of Lake Multi-biotic Integrity Index for Dongting Lake, China. Besides, all the validation or accuracy analyses indicated that the MMIZ could reflect the water quality status of Lake Ziway.

Overall, the multimetric index discriminated the reference and impaired site groups more clearly than the individual single metrics selected for developing the MMIZ, which could be an indicator for the quality of the MMIZ's responsiveness to the combined stressors. And the MMIZ developed for Lake Ziway demonstrated the feasibility of conducting bioassessment at eco-regional scales, but more biometrics need to be developed at eco-regional levels and should be incorporated for developing MMIs for the regional and national level.

Generally, the factors of climate, lakebed substrate, water level, light intensity, etc., which can influence the power and accuracy of the assessment results were not considered into the MMIZ. Moreover, the number of diatom candidate metrics and reference sites were relatively low in number. Therefore, other possible diatom metrics need to be screened and incorporated in to the MMIZ (especially those considered in other Ethiopian bioassessment studies), and an appropriate number of reference sampling sites should be expanded. An appropriate number of sampling sites would be critical in obtaining more accurate water quality information of lakes (Wang *et al.*, 2015; Giorgio *et al.*, 2016). So for future studies, it is crucial to include the factors not considered in the present study such as pesticides analysis, heavy metals analysis, predator-prey dynamics, and species rarity; and also it is recommended to contemplate numerous sampling sites to be used as both reference and test sites.

CHAPTER 6: GENERAL CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

Lake Ziway has shown some adverse changes in terms of some physicochemical factors. For example, SRP and nitrate levels of the lake increased in recent years, while pH didn't show a reliable increase. Most probably the increasing trend of SRP and nitrate level might be due to the significant changes of land use influences, sewage discharges, agro industrial activities, and other different environmental stressors. These alterations, along with the littoral and riparian zones of the lake might have caused changes in the nutrient level of the lake over the past few decades.

The macroinvertebrates community has been taken over by Hemipterans, whereas in the past Sphaeriidae, Chironomidae, and Physidae families used to be dominant. Percent abundance of macroinvertebrates was different between the study sites and seasons, while taxa richness was different among the sites. It is likely that the taxa richness difference between sites and the difference in the abundance of macroinvertebrates between seasons might be related with the availability of the food and other factors associated with the influx of nutrients in to the lake.

Overall, macroinvertebrates diversity in Lake Ziway was average and could be interpreted as moderately polluted waterbody, and this also reflects the presence of environmental stressors near around the littoral regions of the lake. Although, there was a relative higher diversity values in the reference sites, the overall diversity values recorded for the lake was moderate, which might be most likely because of the homogeneity of the lake environment and the narrow range/uniformity of ecological conditions.

The benthic diatom species community composition of Lake Ziway was dominated by *Gomphonema* and *Nitzschia* (in number of species) and *Achnanthidium* sp., *Ulnaria ulna* and *Encyonopsis microcephala* (in relative percent abundance). This is probably because *Achnanthidium* sp. prefer to survive in waterbodies with wave-swept littoral zones and their broad range of microhabitats. The most abundant species at the test sites were *Ulnaria ulna* and *Nitzschia* sp., which tolerate organic pollutions and high nutrient load.

Percent abundance of the diatoms was different between the study sites and seasons, while species diversity was different only among the sites. Therefore, the apparent lack of significant seasonality in the diversity of diatoms in Lake Ziway could be because of the stability of environmental conditions such as temperature and light throughout the year in this tropical environment. However, the difference between the species richness among the sites is related to the presence of different stressors near around the lake.

The diversity of diatoms in all sites would come out as moderately diversified, which implies that diatoms diversity could be affected by environmental stressors. Moreover, the overall average diversity detected in the lake might be because of the higher lake water turbidity (light is one of the major factor for the growth of diatoms). However, diatoms diversity showed trend of decreasing along the gradient of disturbed sites.

The selected core macroinvertebrate and diatom-based biometrics for the condition of Lake Ziway indicated better ecological quality in reference sites than test sites. Some of the single metrics did not distinguish between the intermediary and multiple stressors sites, while the MMIZ discriminated the three clustered study sites more clearly. The better discriminatory efficiency of the MMIZ could be due to its composition from different biometrics, with multi-assemblages which are responsible for different stressors.

The validation of MMIZ was carried out through different approaches: PCA was used to validate the sensitivity of MMIZ to distinguish the site groups, independent data set from Lakes Ziway and Hawassa was used to test the MMIZ. Again, for the MMIZ calibration only clearly degraded 3 sites in Lake Ziway (not included in the MMIZ) were used. Therefore, the MMIZ was found useful by classifying two of these sites as having poor quality class, and one site as having fair condition. The MMIZ also discriminated between the reference and test site groups of Lakes Ziway and Hawassa. Then the MMIZ showed good responsiveness to all selected environmental factors, as evidenced by significant statistical correlations ($p<0.01$). Overall, MMIZ was found useful to classify the reference and impaired site groups more clearly than individual single metrics, which could be an indicator for MMIZ's responsiveness to the multiple stressors.

In general, the factors of climate, lakebed substrate, water level, light intensity, and etc., which can influence the power and accuracy of the assessment results obtained here were not considered in the MMIZ. However, the established macroinvertebrate and diatom-based biocriteria are basically suitable for water quality assessment in the studied lake segments of Lake Ziway. The validation or accuracy analysis indicated that the MMIZ can discriminate between near reference and impaired sites, and therefore, could reflect the water quality status of Lake Ziway. Besides, developing regionally appropriate biometrics for the assessment of the aquatic ecosystem health is a pressing need for several Ethiopian tropic aquatic ecosystems.

6.2 RECOMMENDATIONS

1. The present study documented the existing benthic macroinvertebrate and diatom communities of Lake Ziway, and exhibited variation among the studied sites along the disturbance gradient. Further changes in benthic macroinvertebrate and diatom communities composition may continue in the future and therefore, biological monitoring and monitoring of stressors should be carried out on regular basis. Besides, current practice of Batu fisheries research center of protecting 20m distance buffer zone should be implemented all over the lake shoreline.

2. The reference sites information is used as a measure of biological integrity for the particular aquatic ecosystem settings. Therefore, it is advisable to establish criteria for the reference condition on geographic/ecoregion bases, as the response of biological communities to environmental degradation is determined by comparison to reference. Moreover understanding the spatial distribution of macroinvertebrates and diatoms is an important step in knowing how to use the biological information as a guide for resource management. Therefore, more research should follow on how to standardize biocriteria for near reference sites in tropical water bodies.

3. It would also be appropriate to use all the possible physicochemical parameters to determine the precise factors influencing the distribution of benthic diatoms and macroinvertebrates. More metrics belonging to habitat, hydrology, morphology, and chemical parameters should also be considered into the MMIZ. Future studies should focus on comprising broadened dataset which should include some modification to the sensitivity values of the organisms based on their ecological distribution. In part, such information is also important to strengthen the accuracy of the biometrics.

4. The MMIZ index and the component metrics should be tested over time, as they are applied to a new reference and test sites, and therefore, based on the results obtained can be used to establish assessment methods. Consequently, the establishment of the biological communities' dataset should be done to ease understanding of the changes of the aquatic ecosystem. Furthermore, the MMIZ developed for Lake Ziway demonstrated the feasibility of conducting bioassessment for Lake Hawassa and provides a basis for calibrating MMIs at eco-regional and national level through the combination of several locally developed MMIs.

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APPENDIXES

Appendix 1: Lake Habitat Quality Assessment Recording Field Sheet (Modified after FDEPA, 2017).

LAKE NAME	DATE (D/ M/ Y)	SAMPLING SITE NUMBER:		FIELD ID / NAME:
LATITUDE__________ LONGITUDE___________	INVESTIGATORS	SAMPLING LOCATION/DESCRIPTION:		LAKE SEGMENT SIZE:
PARAMETER **HYDROLOGY**	No surface inflow or outflow present, long water residence time, groundwater seepage dominates ☐	Surface water inflow present, but flow is rare, moderate to long water residence time ☐	Surface water inflow and outflow present sometimes with visible flow, short water residence time ☐	Impounded, hydrology of system artificially controlled ☐
Color	Very clear, uncolored water (benthic sampling appropriate) ☐	Water somewhat tannin stained (benthic sampling appropriate) ☐	Dark, discolored water (water color 20 PCU or higher) ☐	Visibility reduced due to high color ☐
	Optimal	**Suboptimal**	**Marginal**	**Poor**
Vegetation Quality ☐	Diverse, expected native vegetation (emergent or submersed), less than 5% nuisance taxa	Mostly expected native plants, but moderate growths (6%-20% of lake segment) of nuisance macrophytes, or over 50% of lake covered with plants	Large masses (21%-40%) of nuisance macrophytes (e.g., Hydrilla, hyacinth, cattail, etc.) or algal mats	Lake choked (>40%) with nuisance macrophytes (duck-weed, hyacinth, etc.) or algal mats, or few plants present at all (e.g., plants removed)
	20 | 19 | 18 | 17 | 16	15 | 14 | 13 | 12 | 11	10 | 9 | 8 | 7 | 6	5 | 4 | 3 | 2 | 1
Storm water Inputs ☐	Storm water enters system via sheet flow over non-cultivated and/or natural vegetation	Some direct storm water inputs (ditches, pipes, cultivated vegetation < 10%) but good BMPs in place	Moderate direct inputs of storm water (ditches, pipes, cultivated vegetation 11%-50%) but few BMPs in place	Much direct input of storm water (ditches, pipes, cultivated vegetation > 51%) and no or ineffective BMPs in place

	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Bottom Substrate Quality	Diverse mixture of sand, detritus, with small amounts of CPOM/mud/muck. SAV may be present					Mixture of sand or clay and detritus with higher % CPOM/mud/muck content. SAV may be present					Moderate layer of CPOM/ mud/muck, or hard packed sand only, or moderate algal growth (mats) on bottom					Thick deposits of CPOM, or fine detritus and anaerobic muck/mud/silt, or algal growth or nuisance plants cover bottom				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Lakeside Adverse Human Alternations	Very few man-made structures, roads, or other disturbance adjacent to lake (<10%)					Moderate disturbance visible (structures, roads or other), 10%-49% lakeside affected					Many structures, roads or other human disturbance visible (50%-70%) lakeside affected)					Highly developed or disturbed (>70% of lakeside affected)				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Upland Buffer Zone	Expected native vegetation between uplands and littoral zone, greater than 90% of shore with >18 m buffer					89%-51% of shoreline with >18m buffer or >75% with 10m to 18m buffer					50%-30% of shoreline with >18m buffer or 50%-74% with 10m to 18m buffer					< 29% of shoreline with >18m buffer				
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Adverse Watershed Land Use	Score the potential effects from adverse human land uses, based on a continuum of amount and type, with least to most adverse: Native vegetation, Pasture or Citrus, Low Density Residential, Row Crops, Commercial, High Density Residential, Urban, Industrial																			
	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1

TOTAL SCORE		**COMMENTS:**

Appendix 2: Macroinvertebrates family list and tolerance values (Mandaville, 2002).

Taxa	Family	Tolerance value
Trichoptera	Hydropsychidae	4
	Philopotamidae	3
Ephemeroptera	Baetidae	4
	Caenidae	7
	Polymitarcyidae	2
Odonata	Coenagrionidae	9
	Aeshnidae	3
	Libellulidae	9
Hemiptera	Belostomatidae	5
	Corixidae	5
	Gerridae	5
	Mesoveliidae	5
	Naucoridae	5
	Nepidae	5
	Notonectidae	5
	Veliidae	5
	Cicadellidae	5
Coleoptera	Hydrophilidae	5
	Notoridae	5
Trombidiformes	Hydrachnidae	-
Bivalvia	Corbiculidae	6
	Sphaeriidae	8
	Unionidae	8
	Dreissenidae	8
Gastropoda	Physidae	8
	Lymnaeidae	6
	Planorbidae	7
	Pleuroceridae	6
Diptera	Chironomidae	8
	Tabanidae	8
Hirudinea	Hirudinidae	10
Araneae	Pisauridae	-
	Tetragnathidae	-
Oligochaeta	Oligochaetes*	8

(* Subclass)

Appendix 3: Diatom species list, sensitivity, and indicator values (Lecointe *et al.*, 1993).

Diatom species found in the present study	**Omnidia code**	**IPS**	
		Sensitivity	**Indicator**
Achnanthidium sp.	ADCS	4.8	2
Afrocymbella beccarii (Grunow) Krammer	AFBE	5	3
Anomoeoneis sphaerophora (Ehr.) Pfitzer	ASPH	2	3
Aulacoseira ambigua (Grunow) Simonsen	AAMB	3	1
Aulacoseira granulata (Ehr.) Simonsen	AUGR	2.9	1
Aulacoseira granulata (Ehr.) Simonsen var.angustissima (O.M.)Simonsen	AUGA	2.8	1
Aulacoseira muzzanensis (Meister) Krammer	AMUZ	3.8	1
Caloneis aequatorialis Hustedt	CAQT	4	1
Craticula ambigua (Ehrenberg) Mann	CAMB	3	3
Cyclotella meneghiniana Kützing	CMEN	2	1
Cyclotella ocellata Pantocsek	COCE	3	1
Cymbella kappii (Cholnoky) Cholnoky	CKPP	4	2
Diploneis ovalis (Hilse) Cleve	DOVA	4	2
Encyonema volkii (Rumrich. Krammer and Lange-Bertalot) Krammer	EVOL	4.9	2.4
Encyonopsis microcephala (Grunow) Krammer	ENCM	4	2
Gomphonema aequatoriale Hustedt	GAEQ	4	1
Gomphonema affine Kützing	GAFF	4	3
Gomphonema augur Ehrenberg	GAUG	3	3
Gomphonema gracile Ehrenberg	GGRA	4.2	1
Gomphonema parvulum (Kützing) Kützing var. parvulum f. parvulum	GPAR	2	1
Hantzschia amphioxys (Ehr.) Grunow in Cleve et Grunow	HAMP	1.5	3
Navicula subrhynchocephala Hustedt	NSRH	3	2
Navicula zanoni Hustedt	NZAN	3.4	1.9
Nitzschia acicularis (Kützing) W.M.Smith	NACI	2	2
Nitzschia amphibia Grunow f.amphibia	NAMP	2	2
Nitzschia clausii Hantzsch	NCLA	2.8	3
Nitzschia intermedia Hantzsch ex Cleve and Grunow	NINT	1	3
Nitzschia sp.	NZSS	1	2
Pinnularia grunowii Krammer	PGRU	4.7	2.3
Pinnularia subgibba Krammer var. subgibba	PSGI	5	2
Pinnularia viridiformis Krammer var. minor Krammer	PVFM	5	2
Pleurosigma salinarum (Grunow) Cleve and Grunow	PSAL	2	3
Rhopalodia operculata (Agardg) Hakansson	ROPE	5	2
Sellaphora pupula (Kützing) Mereschkowksy	SPUP	2.6	2
Stephanodiscus sp.	STSP	3	2
Surirella angusta (Kützing)	SANG	4	1
Thalassiosira baltica (Grunow) Ostenfeld	TBAL	2.6	1
Tryblionella calida (Grunow in Cl. Grun.) D.G. Mann	TCAL	2.3	2
Ulnaria ulna (Nitzsch.) Compère	UULN	3	1

Appendix 4: Benthic macroinvertebrates distribution in the dry and wet seasons in the littoral zone of Lake Ziway.

Taxa	Family	Dry Season	Wet Season	Total	Abundance
Trichoptera	Hydropsychidae	131	19	150	1.46
	Philopotamidae	0	9	9	0.09
Ephemeroptera	Baetidae	106	410	516	5.01
	Caenidae	50	37	87	0.85
	Polymitarcyidae	111	258	369	3.59
Odonata	Coenagrionidae	118	577	695	6.72
	Aeshnidae	2	1	3	0.03
	Libellulidae	16	56	72	0.69
Hemiptera	Belostomatidae	318	702	1020	9.91
	Corixidae	322	650	972	9.43
	Gerridae	7	16	23	0.22
	Mesoveliidae	3	39	42	0.41
	Naucoridae	73	4	77	0.75
	Nepidae	5	10	15	0.15
	Notonectidae	250	145	395	3.84
	Veliidae	5	80	85	0.83
	Cicadellidae	0	19	19	0.18
Coleoptera	Hydrophilidae	54	135	189	1.83
	Notoridae	0	41	41	0.39
Trombidiformes	Hydrachnidae	3	11	14	0.14
Bivalvia	Corbiculidae	0	5	5	0.05
	Sphaeriidae	1071	684	1755	19.1
	Unionidae	3	0	3	0.03
	Dreissenidae	5	9	14	0.14
Gastropoda	Physidae	358	1015	1373	13.3
	Lymnaeidae	11	23	34	0.33
	Planorbidae	71	12	83	0.79
	Pleuroceridae	205	213	418	4.01
Diptera	Chironomidae	1047	445	1492	14.5
	Tabanidae	3	6	9	0.09
Hirudinea	Hirudinidae	5	11	16	0.16
Araneae	Pisauridae	43	70	113	1.09
	Tetragnathidae	0	21	21	0.20
Oligochaeta	Oligochaetes*	77	70	147	1.43
Total Number of Organisms		**4473**	**5803**	**10276**	**100%**
No of Families		**28**	**33**	**34**	

(***** Subclass)

Appendix 5: Benthic macroinvertebrates distribution and total composition in the study sites of Lake Ziway.

Family/Taxa	BU	GCH	SI	ShEFF	KO	CA	FRC	G1	G2	Total
Hydropsychidae	0	5	4	0	3	3	1	5	129	150
Philopotamidae	0	0	0	0	0	0	0	9	0	9
Baetidae	27	12	19	168	3	13	262	8	4	516
Caenidae	12	15	9	0	5	7	3	34	2	87
Polymitarcyidae	27	12	3	0	0	2	5	56	264	369
Coenagrionidae	81	38	68	209	41	10	131	78	39	695
Aeshnidae	1	1	0	0	0	0	0	0	1	3
Libellulidae	5	7	8	4	27	5	2	12	2	72
Belostomatidae	194	91	109	90	12	65	299	77	83	1020
Corixidae	139	57	144	193	92	63	149	57	78	972
Gerridae	0	0	0	0	0	0	11	0	12	23
Mesoveliidae	0	0	0	22	0	0	20	0	0	42
Naucoridae	0	0	0	0	0	0	10	0	67	77
Nepidae	3	0	3	5	0	3	1	0	0	15
Notonectidae	11	10	1	68	41	20	147	59	38	395
Veliidae	7	2	6	32	0	0	36	1	1	85
Cicadellidae	0	0	0	0	0	0	19	0	0	19
Hydrophilidae	31	15	13	26	13	17	36	6	31	188
Notoridae	19	0	0	0	0	0	22	0	0	41
Hydrachnidae	1	0	1	0	0	0	9	3	0	14
Corbiculidae	0	0	0	0	0	0	5	0	0	5
Sphaeridae	214	201	387	19	92	328	371	110	33	1755
Unionidae	0	0	0	0	0	0	0	0	3	3
Dreissenidae	0	0	2	0	2	8	0	2	0	14
Physidae	105	20	20	687	100	25	42	341	33	1373
Lymnaeidae	9	4	0	11	0	0	6	4	0	34
Planorbidae	0	0	0	0	0	0	0	74	9	83
Pleuroceridae	62	69	21	8	10	34	42	128	44	418
Chironomidae	130	83	44	171	195	69	135	424	241	1492
Tabanidae	0	0	0	0	0	0	3	4	2	9
Hirudinidae	1	0	0	4	0	1	2	7	1	16
Pisauridae	5	16	5	26	2	0	38	10	11	113
Tetragnathidae	4	0	0	0	0	0	17	0	0	21
Oligochaeta	15	34	33	16	15	3	6	24	1	147
Total Individuals	**1103**	**692**	**900**	**1759**	**653**	**676**	**1830**	**1533**	**1129**	**10276**
No of taxa	**23**	**19**	**20**	**18**	**17**	**18**	**29**	**24**	**24**	

***Note*: BU**: Buchesa, **GCH**: Gebreal Church, **SI**: Sida, **ShEFF**: Sher Ethiopia flower farm, **KO**: Korokonch, **CA**: Cafeteria, **FRC**: Fishery research center, **G1**: Gelila 1 and **G2**: Gelila 2.

Appendix 6: Benthic diatoms distribution in the dry and wet seasons in Lake Ziway.

Species name	Seasons		Total	Percent Abundance
	Dry	**Wet**		
Achnanthidium sp.	448	457	905	12.57
Afrocymbella beccarii (Grunow) Krammer	9	30	39	0.54
Anomoeoneis sphaerophora (Ehr.) Pfitzer	7	11	18	0.25
Aulacoseira ambigua (Grunow) Simonsen	31	53	84	1.17
Aulacoseira granulata (Ehr.) Simonsen	71	116	187	2.60
Aulacoseira granulata (Ehr.) Simonsen var.angustissima (O.M.)Simonsen	22	22	44	0.61
Aulacoseira muzzanensis (Meister) Krammer	20	10	30	0.42
Caloneis aequatorialis Hustedt	2	4	6	0.08
Craticula ambigua (Ehrenberg) Mann	1	5	6	0.08
Cyclotella meneghiniana Kützing	17	13	30	0.42
Cyclotella ocellata Pantocsek	13	8	21	0.29
Cymbella kappii (Cholnoky) Cholnoky	357	362	719	9.99
Diploneis ovalis (Hilse) Cleve	6	24	30	0.42
Encyonema volkii (Rumrich. Krammer and Lange-Bertalot) Krammer	225	184	409	5.68
Encyonopsis microcephala (Grunow) Krammer	393	352	745	10.35
Gomphonema aequatoriale Hustedt	172	129	301	4.18
Gomphonema affine Kützing	167	178	345	4.79
Gomphonema augur Ehrenberg	10	3	13	0.18
Gomphonema gracile Ehrenberg	47	58	105	1.46
Gomphonema parvulum (Kützing) Kützing var. parvulum f. parvulum	29	121	150	2.08
Hantzschia amphioxys (Ehr.) Grunow in Cleve et Grunow	5	0	5	0.07
Navicula subrhynchocephala Hustedt	23	20	43	0.60
Navicula zanoni Hustedt	231	244	475	6.60
Nitzschia acicularis (Kützing) W.M.Smith	13	27	40	0.56
Nitzschia amphibia Grunow f.amphibia	219	226	445	6.18
Nitzschia clausii Hantzsch	1	5	6	0.08
Nitzschia intermedia Hantzsch ex Cleve and Grunow	321	166	487	6.76
Nitzschia sp.	326	231	557	7.74
Pinnularia grunowii Krammer	1	0	1	0.01
Pinnularia subgibba Krammer var. subgibba	2	2	4	0.06
Pinnularia viridiformis Krammer var. minor Krammer	1	1	2	0.03
Pleurosigma salinarum (Grunow) Cleve and Grunow	6	2	8	0.11
Rhopalodia operculata (Agardg) Hakansson	2	1	3	0.04
Sellaphora pupula (Kützing) Mereschkowksy	5	5	10	0.14
Stephanodiscus species	17	21	38	0.53
Surirella angusta (Kützing)	1	6	7	0.10
Thalassiosira baltica (Grunow) Ostenfeld	9	16	25	0.35
Tryblionella calida (grunow in Cl. and Grun.) D.G. Mann	7	1	8	0.11
Ulnaria ulna (Nitzsch.) Compère	363	486	849	11.79

Appendix 7: Benthic diatom species distribution in the study sites of Lake Ziway.

Omnidia Code	Study sites									Total No.
	BU	GCH	SI	ShEFF	KO	CA	FRC	G1	G2	
ADCS	123	121	97	86	27	32	99	157	163	905
AFBE	0	0	0	0	10	11	0	12	6	39
ASPH	3	1	6	2	1	1	4	0	0	18
AAMB	3	8	9	0	2	8	10	11	33	84
AUGR	10	20	48	6	21	19	11	28	24	187
AUGA	5	0	2	0	0	4	6	13	14	44
AMUZ	4	4	1	0	0	2	7	4	8	30
CAQT	0	2	4	0	0	0	0	0	0	6
CAMB	0	0	1	2	0	0	3	0	0	6
CMEN	5	0	9	0	3	1	4	4	4	30
COCE	3	3	3	0	3	2	3	4	0	21
CKPP	123	65	46	36	46	159	31	159	54	719
DOVA	4	1	3	15	0	0	7	0	0	30
EVOL	43	26	39	30	20	51	31	88	81	409
ENCM	107	42	41	109	124	113	100	75	32	743
GAEQ	15	17	56	14	45	32	46	27	49	301
GAFF	26	88	84	21	7	31	24	11	53	345
GAUG	0	0	3	3	0	0	3	0	4	13
GGRA	4	29	11	5	2	3	9	16	26	105
GPAR	0	0	34	39	23	0	50	0	4	150
HAMP	0	0	1	0	4	0	0	0	0	5
NSRH	5	9	11	6	7	2	2	0	1	43
NZAN	61	27	27	41	52	34	88	65	80	475
NACI	1	0	0	0	0	0	2	13	24	40
NAMP	86	73	45	60	33	64	54	12	18	445
NCLA	0	0	0	0	0	0	4	2	0	6
NINT	9	6	21	19	213	134	42	17	26	487
NZSS	121	65	66	63	48	64	87	17	28	559
PGRU	1	0	0	0	0	0	0	0	0	1
PSGI	1	0	1	0	0	0	1	0	1	4
PVFM	0	0	0	0	0	0	2	0	0	2
PSAL	0	0	0	0	3	0	5	0	0	8
ROPE	0	0	0	0	1	0	2	0	0	3
SPUP	1	1	0	0	3	1	4	0	0	10
STSP	0	5	4	3	3	2	7	8	6	38
SANG	0	0	0	0	1	0	0	0	6	7
TBAL	0	0	1	0	0	0	16	4	4	25
TCAL	1	3	0	1	0	0	3	0	0	8
UULN	35	184	126	239	98	30	34	53	51	850

Appendix 8: Benthic diatoms identified in Lake Ziway, and reported in previous studies.

Diatom species found in the present study	**Gasse *et al.*(1983)**	**Gasse (1986)**	**Girma Tilahun (2006)**
Achnanthidium sp.			
Afrocymbella beccarii (Grunow) Krammer			
Anomoeoneis sphaerophora (Ehr.) Pfitzer	√		
Aulacoseira ambigua (Grunow) Simonsen			
Aulacoseira granulata (Ehr.) Simonsen	√	√	√
Aulacoseira granulata (Ehr.) Simonsen var. angustissima			
Aulacoseira muzzanensis (Meister) Krammer		√	
Caloneis aequatorialis Hustedt			
Craticula ambigua (Ehrenberg) Mann			
Cyclotella meneghiniana Kützing	√		√
Cyclotella ocellata Pantocsek		√	
Cymbella kappii (Cholnoky) Cholnoky			
Diploneis ovalis (Hilse) Cleve			
Encyonema volkii (Rumrich Krammer and Lange-Bertalot)			
Encyonopsis microcephala (Grunow) Krammer		√	
Gomphonema aequatoriale Hustedt			
Gomphonema affine Kützing			
Gomphonema augur Ehrenberg			
Gomphonema gracile Ehrenberg		√	
Gomphonema parvulum (Kützing) Kützing var. parvulum			
Hantzschia amphioxys (Ehr.)Grunow in Cleve et Grunow			
Navicula subrhynchocephala Hustedt			
Navicula zanoni Hustedt			
Nitzschia acicularis (Kützing) W.M.Smith			√
Nitzschia amphibia Grunow f.amphibia	√	√	
Nitzschia clausii Hantzsch			
Nitzschia intermedia Hantzsch ex Cleve and Grunow		√	
Nitzschia sp.	√	√	
Pinnularia grunowii Krammer			
Pinnularia subgibba Krammer var. subgibba		√	
Pinnularia viridiformis Krammer var. minor Krammer			
Pleurosigma salinarum (Grunow) Cleve and Grunow			
Rhopalodia operculata (Agardg) Hakansson			
Sellaphora pupula (Kützing) Mereschkowksy			
Stephanodiscus sp.	√		
Surirella angusta (Kützing)			
Thalassiosira baltica (Grunow) Ostenfeld			
Tryblionella calida (grunow in Cl. and Grun.) D.G. Mann			
Ulnaria ulna (Nitzsch.) Compère			√

Appendix 9: Some benthic macroinvertebrate families identified from Lake Ziway.

Belostomatidae

Beatidae

Caenidae


Corixidae

Coenagrionidae

Chironomidae

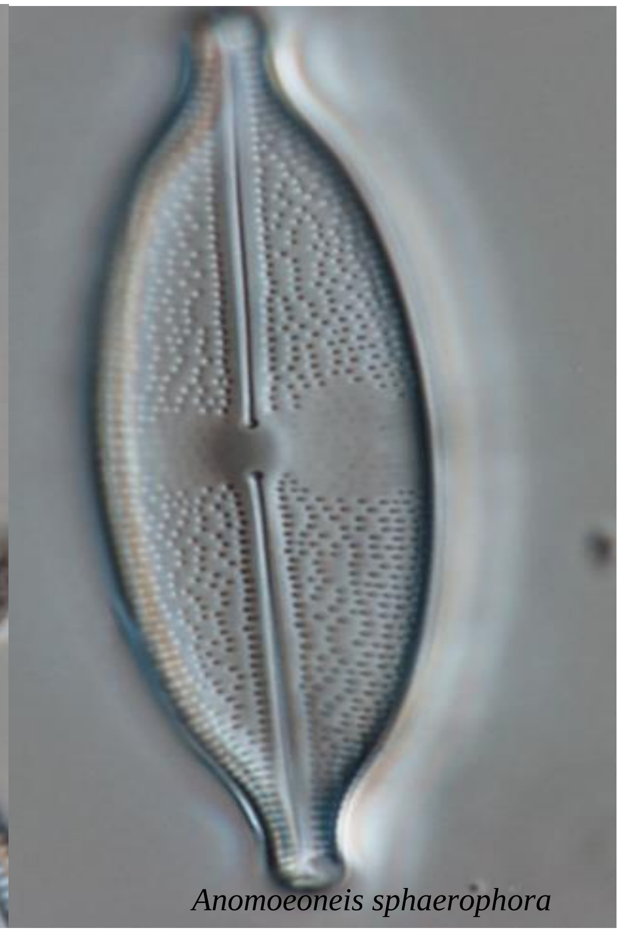
Hydrophilidae

Libellulidae

Nepidae

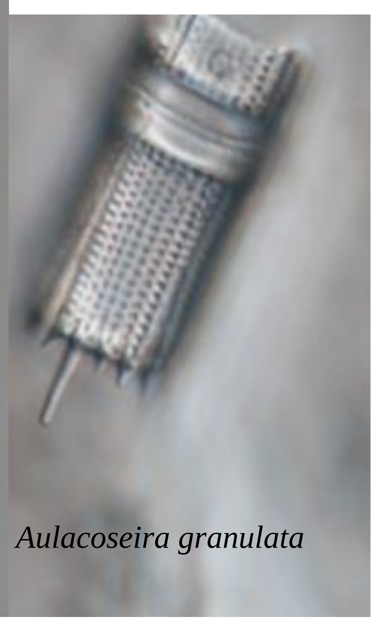
Appendix 10: Some diatom species identified from the littoral zone of Lake Ziway

Afrocymbella beccarii



Anomoeoneis sphaerophora

Aulacoseira granulata var. angustissima



Aulacoseira granulata


Cyclotella meneghiniana

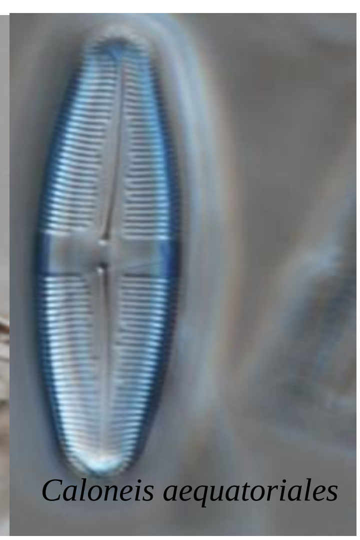

Cyclotella ocellata

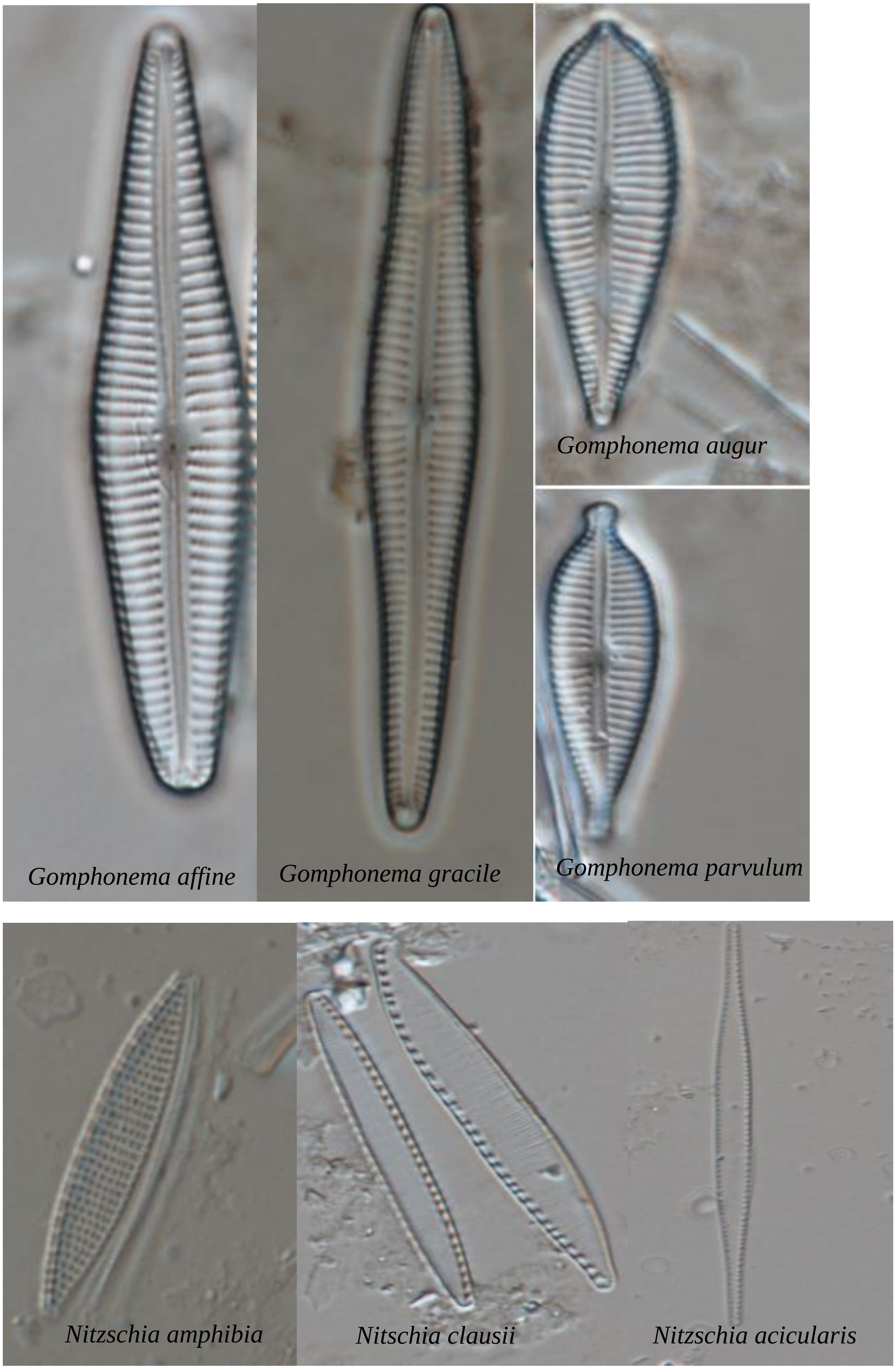

Cymbella kappii


Encyonema volkii


Diploneis ovalis


Hantzschia amphioxys

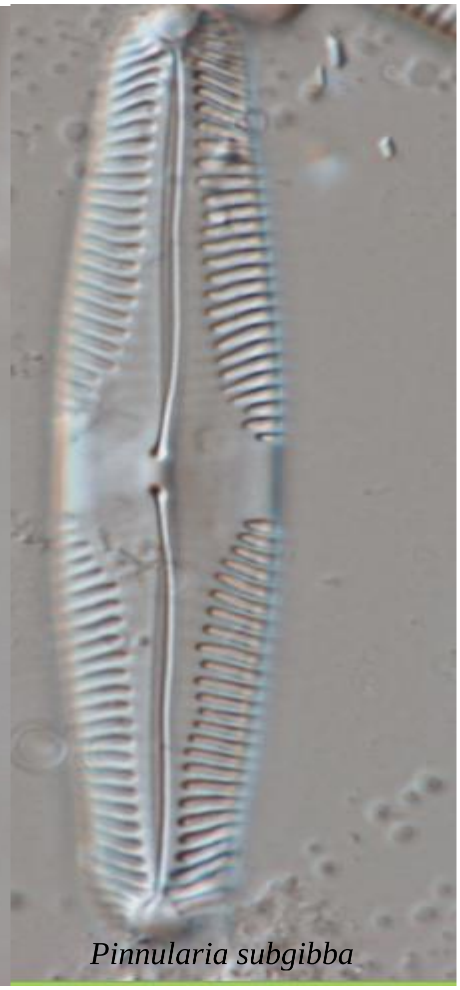

Caloneis aequatoriales



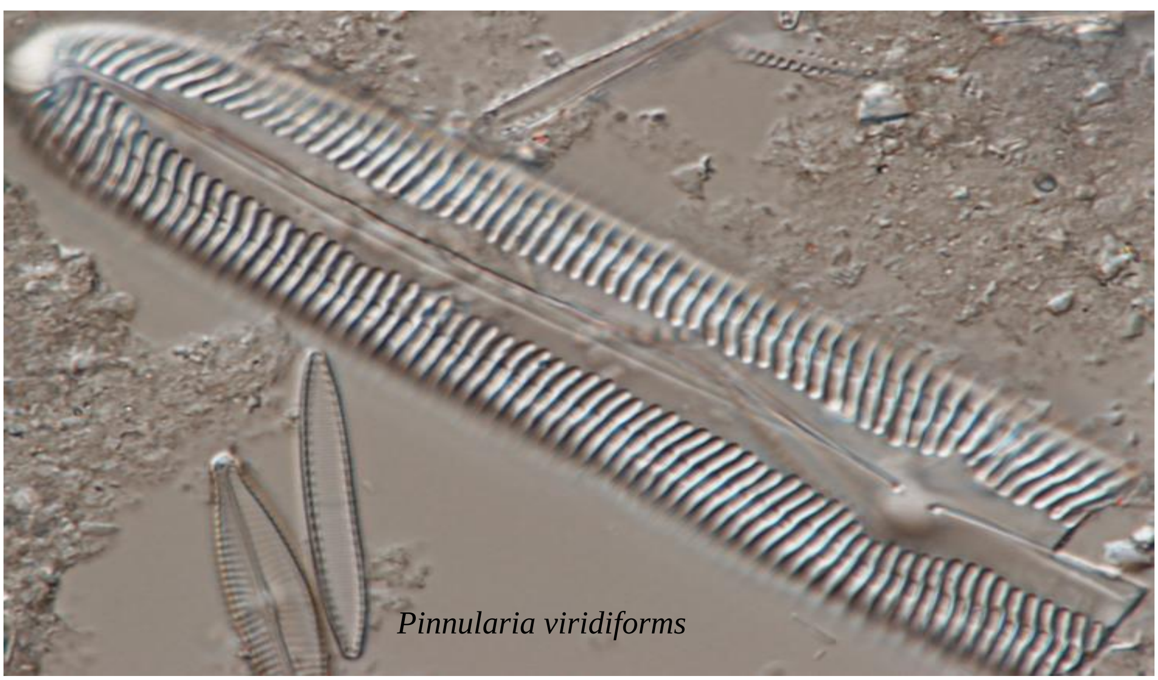
Gomphonema affine *Gomphonema gracile* *Gomphonema augur* *Gomphonema parvulum*

Nitzschia amphibia *Nitschia clausii* *Nitzschia acicularis*

Pinnularia grunowii



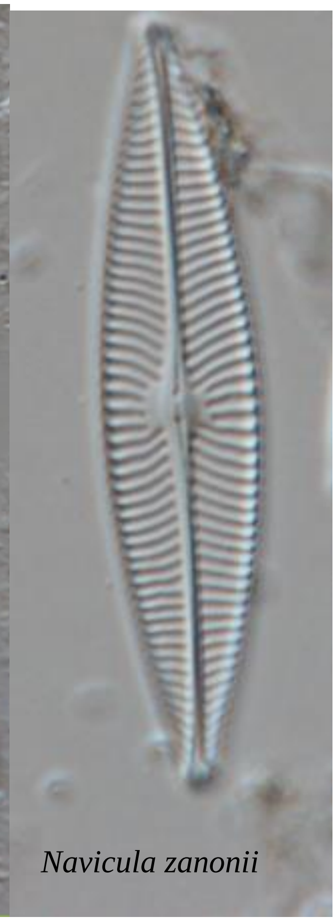
Pinnularia subgibba



Pinnularia viridiforms

Pleurosigma salinarum

Sellaphora pupula



Navicula zanonii