PLANKTON COMMUNITY STRUCTURE AND INTERACTIONS
IN A CYANOBACTERIA-DOMINATED TROPICAL RESERVOIR
(KOKA, ETHIOPIA)

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

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ABSTRACT

PLANKTON COMMUNITY STRUCTURE AND INTERACTIONS IN A CYANOBACTERIA-DOMINATED TROPICAL RESERVOIR (KOKA, ETHIOPIA)

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Koka is a large multi-purpose reservoir impacted by the ever-increasing human activities that resulted in its enrichment with algal nutrients and contamination with chemicals of industrial origin. With a view to come up with scientific information usable in the protection of aquatic resources and public health, temporal dynamics of the community structure and interactions of the major plankton components (phytoplankton and zooplankton) in relation to selected physico-chemical parameters were investigated at monthly intervals from May, 2013, to April, 2014. The observed exceedingly low $Z_{sd}$, which may have partly resulted from particles resuspended in the water column by wind-generated turbulence to which shallow water bodies like Koka Reservoir are especially susceptible, indicates the remarkably high turbidity of the reservoir. The levels of inorganic nutrients recorded for Koka, which are quite high in comparison to those of Ethiopian rift valley and highland lakes, are typical of reservoirs, which, unlike natural lakes, have large phosphorus and nitrogen loads. The mean values of total phosphorus (0.27 mg L$^{-1}$), chlorophyll-$a$ (85.79 $\mu$g L$^{-1}$), and transparency (0.15 m) recorded for Koka Reservoir warrant its classification as a hypereutrophic water body characterized by high biomass production and elevated concentrations of nutrients.

The phytoplankton community in the reservoir was constituted by a total of 89 species with the larger number of species belonging to the Chlorophyceae (34) and Cyanophyceae (28). Cyanobacteria were the most important taxa both in terms of
abundance and biomass, with *Microcystis* and *Cylindrospermopsis* species alternately dominating the phytoplankton community and accounting for up to 72.29% and 62.87% and 21.45% and 20.23% of the total phytoplankton abundance and biomass as biovolume, respectively. The mean concentrations of chl-a (µg L\(^{-1}\)) of the nano- (2–20 µm, 39) and net- (>20 µm, 40.4) phytoplankton were much higher than those of the picophytoplankton (10.48) as would be expected considering the level of eutrophication of the reservoir.

The composition and abundance of cyanobacteria and their toxins, microcystins (MCs) and cylindrospermopsins (CYN), were investigated using samples collected at monthly intervals from the Amudde side of Koka Reservoir from May, 2013, to April, 2014. Analyses of cyanotoxins in filtered samples by HPLC-DAD and LC-/MS/MS identified and quantified 5 variants of MCs (MC-RR, MC-YR, MC-dmLR, MC-LA and MC-LR) in all samples, with their total concentrations ranging from 1.86 to 28.3 µg L\(^{-1}\) and from 1.71 to 33 µg L\(^{-1}\), respectively and greatly surpassing WHO's drinking water guideline value of 1 µg L\(^{-1}\). The maximum level of MCs occurred in December, 2013, when the phytoplankton community of the reservoir was constituted almost entirely by *Microcystis aeruginosa*. The total concentrations of MCs measured in freeze-dried plankton samples by HPLC-DAD and LC-MS/MS varied between 312 and 641 µg (g dwtr \(l^{-1}\)) and 351 and 709 µg (g dwtr \(l^{-1}\), respectively. Thus to prevent intoxications continuous monitoring of these reservoirs is strongly recommended. This has to be taken into consideration in the management of the reservoir when it used for various domestic purposes. Studies regarding the prediction of the occurrences of toxic blooms and their toxins must be implemented and strengthened in the future in order to avoid or reduce the potential risks associated with human and animals' exposure to the toxins. Despite the presence and occasional abundance of *Cylindrospermopsis* spp., cylindrospermopsin was not detected by HPLC-DAD and LC-MS/MS in any of the samples collected throughout the study period.

Analysis of the zooplankton community in the reservoir revealed a total of 52 species, 25 of which are new records for Koka Reservoir. With 40 species, rotifers were the most
species-rich group followed by cladocerans (8 species), and copepods (4 species). The rotifer *Keratella tropica* was the species recorded with the highest abundance among the zooplankton taxa contributing 42.11% of total rotifiers abundance. The cladocerans were dominated by small species, *Ceriodaphnia cornuta*, *Diaphanosoma excisum* and *Moina micrura*, with rare occurrence of the large cladoceran *D. barbata* during a few months of the sampling period. Total abundance of copepods was constituted largely by *Thermocyclops decipiens*, which is the most commonly dominant species in tropical inland waters. Calanoid copepods were represented by a single species; *Tropodiaptomus sp.* Zooplankton abundance was generally higher during the dry season than the rainy season.

Eutrophication of Koka Reservoir has resulted in the dominance of cyanobacteria, which are generally regarded as unsuitable food source for zooplankton. It was, therefore, hypothesized that seston may play a role in the diet of the zooplankton community of this reservoir. The contribution of seston fractions (<20 µm, ≥20<100µm) to zooplankton diets was thus assessed using dual isotope (δ¹⁵N and δ¹³C) SIAR and MixSIAR models. Posterior area estimates of both consumer species and potential food sources were also modeled. The SIAR and MIXSIAR results have demonstrated that the general diet of the zooplankton of the reservoir was composed primarily of small seston particles (<20µm) whose importance varied over time and, which was constituted by green algae, flagellates, diatoms, small colonies of *Microcystis* and fragments of *Cylindrospermopsis* filaments. Analysis of isotopic niche breadth of the two major crustacean zooplankton spp., *Ceriodaphnia cornuta* and *Thermocyclops decipiens* gave niche width, which was generally higher for the former than for the latter indicating consumption of a different prey and/or change in feeding strategy of both species. The slight ¹³C-depletion of zooplankton species in relation to seston fractions and the variations in their isotopic niche area suggests that these zooplankton species may have carbon sources other than phytoplankton.
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<td>chl-(a)</td>
<td>chlorophyll-(a)</td>
</tr>
<tr>
<td>CYN</td>
<td>cylindrospermopsins</td>
</tr>
<tr>
<td>DO</td>
<td>dissolved oxygen</td>
</tr>
<tr>
<td>HPLC-DAD</td>
<td>High-Performance Liquid Chromatography with Diode-Array Detection</td>
</tr>
<tr>
<td>LC-/MS/MS</td>
<td>Liquid chromatography-tandem mass spectrometry</td>
</tr>
<tr>
<td>MCs</td>
<td>microcystins</td>
</tr>
<tr>
<td>(K_d)</td>
<td>vertical attenuation coefficient</td>
</tr>
<tr>
<td>TSS</td>
<td>Total Suspended Solids</td>
</tr>
<tr>
<td>DIN</td>
<td>Dissolved inorganic nitrogen</td>
</tr>
<tr>
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Chapter 1. GENERAL INTRODUCTION

1.1. BACKGROUND

The term plankton refers to the organisms that form the first link in the vast aquatic food chain (phytoplankton), their grazers (zooplankton) and the various components of what is known as the microbial foodweb. The microbial foodweb has been recognized for some time now as an important route for carbon and energy flow to higher trophic levels (Pomeroy, 1974; Azam et al., 1983). It includes a “microbial loop” consisting of heterotrophic bacteria and protozoans (Azam et al., 1983), and all prokaryotic and eukaryotic unicellular phytoplankton such as pico-, nano- and microphytoplankton (Sherr and Sherr, 1988).

The central role of plankton in the pelagic foodweb of aquatic ecosystems is to serve as the primary source of food for economically important fish and form the base of a foodweb that supports many other aquatic biota. As such, the phytoplankton is usually at the base of aquatic food webs providing food to a diverse range of aquatic organisms as they are the main primary producers. Moreover, information on their abundance, community composition and biomass can be crucial in the assessment of trophic status and water quality (Walsh et al., 2001; Reynolds et al., 2002), evaluation of potential fish yield (Descy et al., 2005), productivity (Walsh et al., 2001), and energy flow (Simciv, 2005), and in the management (Beyruth and Tanaka, 2000) of tropical lake and reservoir ecosystems. On the other hand, the zooplankton are the most important components of an aquatic ecosystem as they play a vital role in the carbon-flow processes by serving as a link between primary producers and the consumers at the higher levels in aquatic food webs. Furthermore, several studies have shown the role of zooplankton in nutrient recycling and in the understanding of the changes occurring in aquatic ecosystems due to eutrophication (Attayde and Bozelli, 1998; Pinto-Coelho et al., 2005; Burns and Galbraith, 2007). In freshwater environments, the microbial communities play a vital role in energy and carbon flow and material cycles (Sherr and Sherr, 1994; Calbet and Landry, 1999; Pomeroy et al., 2007; Newton et al., 2011), which are important in the maintenance of freshwater ecosystem health. Despite their critical relevance to the
aquatic ecosystem, investigations that attempted to look into planktonic community structure and interactions are meager in many tropical lakes and reservoirs including those found in Ethiopia, as most studies were carried out in the marine environment or in temperate lakes. In Ethiopia, the recent work by Tadesse Fetahi (2010) has provided some insights into foodweb structure and trophic interactions in Lake Hayk, which is a highland tropical lake.

The fate of biogenic carbon in aquatic ecosystems is, therefore, strongly influenced by the structure of the planktonic food webs (Silva et al., 2014). A number of studies made in temperate lakes have investigated how carbon biomass is partitioned among several compartments of the plankton food webs (Auer et al., 2004; Gaedke and Kamjunke, 2006; Havens et al., 2007). Little has, however, been done to understand how plankton carbon flows in tropical and subtropical aquatic systems (Pirlot et al., 2005; Work et al., 2005; Havens et al., 2007), particularly in reservoirs (Silva et al., 2014). Reservoirs are manmade lakes, which are important for the production of electricity, fisheries, and tourist-attraction and as sources of irrigation and drinking water supply (Kalff, 2002; Zwahlen, 2003). They are complex systems with a predominant horizontal gradient (Silva et al., 2014) and are subject to natural forces (regional climate and hydrological conditions) and anthropogenic influences (e.g. watershed land uses). These factors affect the spatial and temporal organization of their biological communities (Tundisi and Matsumura-Tundisi, 2008). Water-level fluctuations in reservoirs, which strongly influence the abiotic and biotic features, are controlled by human activities and regional climate (Wetzel, 1990). Water residence time, which is the average time a molecule of water, spends in a reservoir, directly influences the maintenance of plankton communities in the water column (Callieri et al., 1999; Havens et al., 2007; Wang et al., 2011).

Reservoirs are usually vulnerable to changes in water quality. Ethiopia has many small, medium and large water reservoir dams constructed for hydropower generation, irrigation and drinking water supply or a combination of them. Koka Reservoir, which is located within the catchment of the Awash River basin in the Ethiopian Rift Valley, is one of the largest reservoirs in Ethiopia. It was constructed in 1960 for the purpose of hydropower
generation. Although it was primarily intended for hydropower generation, it serves as the source of water used for irrigation, drinking water supply for humans and livestock, sanitation, sewage assimilation and fishing. Fertilizers and pesticides are intensively used on conventional agricultural lands and floriculture farms located within the catchment area of the reservoir. This has resulted in high concentrations of phosphorus and nitrogen and the consequent eutrophication of the reservoir. In addition, tanneries, which are established near its shores and along the Modjo River, are using it as a dumping site for their liquid wastes that subsequently increase the levels of nutrients (particularly nitrogen and phosphorus) in the reservoir thereby promoting algal blooms and changes in the phytoplankton community structure in which cyanobacteria make up the dominant constituents (Naselli-Flores and Barone, 2000; Vardaka et al., 2005). Previous studies made on Koka Reservoir have reported the predominance of cyanobacteria including the potentially toxic species such as *Microcystis* spp. (Melaku Mesfin et al., 1988; Elizabeth Kebede and Willen, 1998; Hadgembes Tesfay, 2007; Willen et al., 2011). The temporal dynamics of the potentially toxic species of cyanobacteria resident in the reservoir have not, however, been investigated.

Cyanobacteria (blue-green algae) are predominantly photosynthetic prokaryotes found in a variety of habitats, colonizing both terrestrial as well as aquatic biotopes (Briand et al., 2003). Their frequent dominance and surface bloom-formation in aquatic ecosystems is the result of several adaptations, which make them competitively superior to other phytoplankton (Dokulil and Teubner, 2000; Whitton and Potts, 2000; Dittmann and Wiegand, 2006). Mass development of cyanobacteria in water bodies as a result of eutrophication and climate change has become a worldwide problem (Heisler et al., 2008; Perovich et al., 2008; Paerl et al., 2014) since they are often associated with such serious water quality problems as oxygen depletion, greatly reduced water transparency and production of toxins (cyanotoxins). The presence of these toxins in surface waters represents one of the most serious threats to public health, terrestrial life and aquatic ecosystems (Chorus and Bartram, 1999; Carmichael, 2001; Azevedo et al., 2002; Huisman et al., 2005). In aquatic ecosystems, they inhibit the growth of other species of cyanobacteria (Engelke and Jaspars, 2003), algae (Sukenik et al., 2002) and plants (Jang
and decrease the feeding, survival and growth rates and cause rapid mortality of zooplankton (Lampert, 1987; Rohrlack et al., 2005; Prieto et al., 2006; Hansson et al., 2007; Berry et al., 2009; Dao et al., 2010). In fish, they cause damage to several organs including the liver, heart, gills and kidneys besides disturbing ionic equilibrium and causing behavioral changes, growth inhibition and mortality (Liu et al., 2002; Malbrouck and Kestemont, 2006). Moreover, several studies have reported the potential transfer of cyanotoxins along the food chains/web (Ibelings et al., 2005), and their accumulation in aquatic organisms (Dionisio Pires et al., 2004; Saker et al., 2004).

Cyanotoxins vary in chemical structure, in the triggers of their production, modes of toxicity and degree of toxicity. Cyanotoxins fall into three broad groups: namely the cyclic peptides (including the hepatotoxic microcystins and nodularins), the alkaloids (including the hepatotoxic cylindrospermopsins, the neurotoxins anatoxin-a, anatoxina (S) and saxitoxins) and the lipopolyssacharides which are potentially irritants. Among the cyanotoxins, microcystins are the most common and frequently detected cyanotoxins produced by some species of Microcystis, Anabaena, Anabaenopsis, Haplosiphon, Nostoc, and Planktothrix (Fewer et al., 2008). The microcystins are cyclic heptapeptides with more than 80 variants among which microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR) are the three most common and extensively studied forms of microcystins (Babica et al., 2006). Combinations of two variable L-amino acids, designated by X and Y, account for many of the microcystin variants and are used in the nomenclature of the toxins. The XY variable amino acids for MC-LR, MC-RR and MC-YR are leucine (L), arginine (R) and tyrosine (Y). This toxin is well known for causing damage to cells of the liver and other organs (Soares et al., 2004), potentially leading to the death of organisms by hemorrhagic shock (Mackintosh et al., 1990). Moreover, at sub-lethal doses, these toxins might act as tumor promoters (Zhou et al., 2002). Numerous animal and human intoxications and death due to exposure to microcystins have been reported (Azevedo et al., 2002; Stewart et al., 2008; Hilborn et al., 2013). Cylindrospermopsin (CYN ) is a tricyclic alkaloid that is primarily produced by Cylindrospermopsis raciborskii, although other cyanobacterial genera such as Anabaena, Aphanizomenon ovalisporum, Lyngbya, Raphidiopsis curvata and Umezakia
**natans** are also known to produce it (Falconer and Humpage, 2006; Spoof *et al.*, 2006; Berry and Lind, 2010). CYNs are cytotoxins that irreversibly block protein synthesis, where the primary clinical symptoms are both hepatic and renal failure. They also act on tissues of the intestinal tract, vascular system and muscles (Froscio *et al.*, 2008). Additionally, there are indications that CYN produces genotoxic, carcinogenic and mutagenic effects (Falconer and Humpage, 2001; Saker *et al.*, 2003).

Toxic cyanobacterial blooms are reported most frequently from the temperate zone, especially North America and Europe (Carmichael, 2008) although reports also come from Australia, South Africa, and Asia (Falconer, 2001). Despite the importance of studies on the effects of cyanotoxins and their bioacummulation in organisms of the tropical regions in light of the higher incidence and persistence of blooms compared to those of temperate regions, there are few reports on these aspects. This is probably attributable more to the unavailability of adequate facilities and expertise than to infrequent toxic bloom-formations (Willen *et al.*, 2011). Several studies made in some lakes in Kenya and Uganda including toxicity tests on separate taxa (e.g. Krienitz *et al.*, 2002, 2003; Mwaura *et al.*, 2004; Haande *et al.*, 2007; Sitoki, 2010) have revealed the presence in the lake waters of cyanotoxins originating from *Anabaena* spp., *Arthrospira fusiformis*, *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa*. Currently, little is known about the diversity of cyanobacteria and the levels of toxins they produce in Ethiopian freshwaters in spite of the implicated poisoning of animals around Lake Chamo (Amha Belay and Wood, 1982) and Koka Reservoir. Although there are no confirmed records on cyanotoxins-linked death or illness of wild life, domestic animals and human beings, death of animals associated with the use of the reservoir water for watering livestock was reported on several occasions. A relatively recent study made on phytoplankton samples collected once from the Ethiopian Rift valley lakes has also shown the presence of the microcystins (Willén *et al.*, 2011) whose concentrations have exceeded the permissible levels for humans, cattle, and wildlife especially in Koka Reservoir. Variations in time of cyanobacterial taxa and their toxins in relation to environmental variables in Koka Reservoir have not, however, been investigated so far.
The purpose of the study reported here was, therefore, to investigate the planktonic community structure seasonal dynamics and interactions in Koka Reservoir in relation to its water quality.

1.2. STATEMENT OF THE PROBLEM

Koka Reservoir is one of the water bodies in the Ethiopian Rift Valley subjected to escalating environmental degradation due to the increasing industrial and agricultural activities (Tamene Legesse et al., 2005). Sedimentation, which resulted from such human activities as shore-line modification and deforestation primarily for agricultural expansion, has drastically altered the water quality of the reservoir (Tamene Legesse et al., 2005; Hadgembes Tesfay, 2007). The tanning and floriculture industries established within its drainage basin and runoff from conventional agricultural lands on which fertilizers are excessively applied have also resulted in increased levels of inorganic nutrients (Fassil Degefu et al., 2014), which subsequently led to recurrent blooms of potentially toxic cyanobacteria (Willen et al., 2011). As the reservoir is used by local inhabitants for sanitation, watering livestock and as a source of drinking water supply, there is an urgent need to investigate the dynamics of cyanobacteria and their toxins in Koka Reservoir and identify the potential risks associated with the use of its water. Changes in the physico-chemical water quality of inland waters including reservoirs influence the species composition, abundance and biomass of phytoplankton, zooplankton and components of the microbial loop (Kalff, 2002). Cyanobacterial blooms are also known to have effects on planktonic organisms, which are reflected in different aspects of their communities owing to the differences in the response and tolerance of aquatic organisms to ambient changes in physico-chemical variables (Ghadouani et al., 2003; Ekvall et al., 2014). Phytoplankton and zooplankton are thus used as dependable indicators of environmental perturbation (Walsh et al., 2001; Reynolds et al., 2002; Pinto-Coelho et al., 2005; Burns and Galbraith, 2007). To protect public health and aquatic and terrestrial life, information usable in the development of strategies of protection and conservation of aquatic resources should be produced. The purpose of the present study was, therefore, to investigate the physico-chemical water quality and
species composition, abundance and biomass of major planktonic components and their interactions in the nutrient-enriched cyanobacteria-dominated shallow freshwater-Koka Reservoir.

1.3. SIGNIFICANCE OF THE STUDY

Shallow water bodies such as Koka Reservoir are of high ecological and socioeconomic importance. The value of such water bodies has typically been compromised by nutrient enrichment that resulted in turbid, algae-dominated waters associated with depauperate animal communities (Perrow et al., 1999). Changes in water quality thus lead to loss of biodiversity and pose a serious threat to public health (Perrow et al., 1999). Attempts at restoration of perturbed aquatic ecosystems require baseline information on physicochemical water quality and composition and interactions of resident communities. Information on water quality of Koka Reservoir will, therefore, be usable in the development of strategies for the protection of public health and aquatic life. The data on the species composition of phytoplankton and zooplankton will be useful for institutions like Institute of Biodiversity Conservation in their efforts geared towards the protection and sustainable use of aquatic resources as some organisms may disappear before we even know they exist. The scientific information that resulted from the study reported here will be of some use to researchers, policy makers and environmental scientists in the management and sustainable use of the aquatic resources of this country.

1.4. RESEARCH QUESTIONS AND OBJECTIVES

1.4.1. Research Questions

- What is the extent of water quality deterioration in Koka Reservoir and its variation in time?
- What is the relative importance of the taxa that constitute the phytoplankton and zooplankton communities in Koka Reservoir with respect to their species diversity, abundance and biomass?
1.5. DESCRIPTION OF THE STUDY AREA

Koka Reservoir (also known as Lake Gelila) (Fig. 1) is a reservoir created by the construction of the Koka Dam across the Awash River in 1960 to produce hydroelectricity. It is located in the Misraq Shewa Zone of the Oromia Region, some 90 km south of the capital city of Ethiopia, Addis Ababa, at 8° 23'N and 39° 5'E. Koka Reservoir is fed by two rivers, Awash (major) and Modjo (minor), which flow into it through its western part (Melaku Mesfin et al., 1988). It is a large but shallow reservoir with an area of about 255 km², a mean depth of 9 m and a water volume of about 1.5 km³ (Wood and Talting, 1988). Some morphometric and physicochemical features of Koka Reservoir are given in Table 1.1.

Koka Reservoir was reported to have a mean surface water temperature of 19 °C and shallow water column that does not show any marked thermal stratification (Melaku Mesfin et al., 1988). The region around Koka Reservoir has a mean annual rainfall of about 630 mm and mean minimum and maximum air temperatures of 14 and 30.4 °C, respectively. Rainy periods usually occur between March and May (minor rainy period) and between June and September (major rainy period).

Limnological studies carried out on this reservoir are relatively few. The conductivity of the reservoir water is low as compared to those of most Ethiopian Rift Valley lakes (about 200 μS cm⁻¹, Melaku Mesfin et al., 1988). Microscopic analysis of phytoplankton samples collected during the minor rainy period (April-May) from Koka Reservoir resulted in the identification of 72 taxa with the diatom *Aulacoseira granulata* dominating the phytoplankton community (Elizabeth Kebede and Willen, 1998). According to Melaku Mesfin et al. (1988), although *Microcystis* spp dominated the phytoplankton community of Koka Reservoir, species of *Anabaena, Merismopedia, Chlamydomonas, Coelastrum, Mougeotia, Oedogonium, Scenedesmus, Staurastrum, Chromulina, Cyclotella* and *Navicula* were also quantitatively important.
Fig. 2.1. Map showing the location of Koka Reservoir with sampling sites marked with closed circles.
Table 1.1. Some morphometric and physico-chemical features of Koka Reservoir

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altitude (m. a. s. l.)</td>
<td>1590</td>
<td>Wood and Talling (1988)</td>
</tr>
<tr>
<td>Surface area (km²)</td>
<td>255</td>
<td>Welcomme (1972)</td>
</tr>
<tr>
<td>Volume (km³)</td>
<td>1.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>14</td>
<td>&quot;</td>
</tr>
<tr>
<td>Mean depth (m)</td>
<td>9</td>
<td>Wood and Talling (1988)</td>
</tr>
<tr>
<td>Salinity (g L⁻¹)</td>
<td>0.2</td>
<td>&quot;</td>
</tr>
<tr>
<td>Alkalinity (meq L⁻¹)</td>
<td>2.6</td>
<td>&quot;</td>
</tr>
<tr>
<td>pH</td>
<td>8.3</td>
<td>&quot;</td>
</tr>
<tr>
<td>Conductivity (μS cm⁻¹)</td>
<td>200</td>
<td>Melaku Mesfin et al. (1988)</td>
</tr>
<tr>
<td>Total phosphorus (μg L⁻¹)</td>
<td>224</td>
<td>Elizabeth Kebede and Amha Belay (1994)</td>
</tr>
<tr>
<td>Silica (SiO₂, mg L⁻¹)</td>
<td>2.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Sulfate (SO₄, mg L⁻¹)</td>
<td>12.5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Chlorophyll-a (μg L⁻¹)</td>
<td>22.4</td>
<td>&quot;</td>
</tr>
<tr>
<td>Na (meq L⁻¹)</td>
<td>1.35</td>
<td>&quot;</td>
</tr>
<tr>
<td>K (meq L⁻¹)</td>
<td>0.14</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ca (meq L⁻¹)</td>
<td>1.16</td>
<td>&quot;</td>
</tr>
<tr>
<td>Mn (meq L⁻¹)</td>
<td>0.43</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cl (meq L⁻¹)</td>
<td>0.22</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

The dominant zooplankton groups that occur in the reservoir are rotifers (*Keratella tropica*), Cladocerans (*Daphnia barbata* and *Moina micrura*) and copepods (*Tropodiaptomus processifer*, *Metadiaptomus colonialis* and *Mesocyclops equatorialis*) (Melaku Mesfin et al., 1988). The reservoir supports a fishing industry by providing commercially important fish species such as *Oreochromis niloticus*, *Cyprinus carpio*, *Clarias gariepinus* and *Barbus intermedius* (FLDP, 1998). The average annual production over the period 1994 to 1998 was 249 tons of tilapia, 235 tons of catfish, 101 tons of common carp and 10 tons of *Barbus* (FLDP, 1998).
1.6. METEOROLOGICAL AND HYDROLOGICAL DATA OF THE STUDY AREA

The study area is located in the Rift valley which is generally characterized by moist sub humid to semi-arid climate (Daniel Gemechu, 1977). Temporal variations in minimum and maximum air temperature, monthly rainfall of the study period and mean monthly rainfall of 5 years obtained from National Meteorological Agency of Ethiopia are shown in Fig. 1.2. Mean monthly minimum air temperature varied between about 9.1 °C in December, 2013, and 15.8 °C in May, 2013, while the maximum mean monthly air temperature ranged from 26.2 in July, 2013, to 35.6 °C in April, 2014. Wind speed (m s$^{-2}$), recorded for a neighboring lake (Ziway) of more or less similar altitude, ranged from 1.8 in March, 2014, to 3.2 in June, 2013, with generally higher values during the rainy season. Monthly rainfall varied from a low value in November, 2013, to the highest value of 220.6 mm in August, 2013. Water depth at S1 varied from 5.1 m in January, 2014, to 7.9 m in August, 2013, with a mean of 6.5 m. With a mean depth of 3.7 m, the water depth at S2 was shallower than that at S1 on all sampling dates.

Koka Reservoir is one of the water bodies in the rift valley area that have suffered from accelerated sedimentation, a reflection of watershed erosion, aggravated by deforestation mainly for agricultural expansion (Tamene Legesse et al., 2005). Although the importance of residence time in structuring planktonic communities is unquestionable (Naselli-Flores and Barone, 1997; Campbell et al., 1998), its calculation for Koka Reservoir was precluded by the absence of recent bathymetric survey from which the water holding capacity (reservoir volume) can be obtained. The last bathymetric survey, which was conducted in 1999, reported a reduction in water holding capacity from 1650 Mm$^3$ of 1960 to 1186 Mm$^3$ in 1999 (Nobert et al., 2010) by sedimentation. Moreover, Koka Reservoir has regulated flow although the calculation of residence time is based on steady-state condition.
Fig. 1.2. Meteorological and hydrological data of Koka reservoir: wind speed (A), minimum (closed circle) and maximum (open circle) air temperature (B), mean monthly rainfall of 5 years (C) and monthly rainfall of the study period (bar plot) and water depth at S1 (line plot) (D).
1.7. ORGANIZATION OF THE DISSERTATION

This dissertation resulted from studies made on the physico-chemical water quality, phytoplankton and zooplankton community structure, cyanobacteria and their toxins and isotopic analysis of zooplankon-phytoplankton link in Koka Reservoir.

Chapter 1. General introduction

This chapter deals with general introductory information, research gaps, research questions and objectives, and a description of the study area.

Chapter 2. Spatio-temporal variations in physico-chemical parameters of Koka Reservoir

This chapter presents data on physicochemical features of Koka Reservoir, with emphasis on the optical and thermal characteristics, aggregate chemical parameters and inorganic algal nutrients. The variations in time and space (horizontal) of the measured parameters are described, the causes for their occurrence discussed and their implications for public health, aquatic life and livestock associated with the reservoir addressed.

Chapter 3. Temporal variations in phytoplankton community structure of Koka Reservoir, Ethiopia: The persistence of potentially toxic cyanobacteria

This chapter discusses the temporal dynamics of the phytoplankton community in the reservoir with particular emphasis on the taxonomic composition, size structure, abundance and biomass of phytoplankton. The following manuscript, which is to be submitted to Inland waters, has emanated from this chapter.

Spatio-temporal variations in phytoplankton community structure of a cyanobacteria-dominated tropical reservoir (Koka, Ethiopia).
Chapter 4. Temporal variations in the levels of total cyanotoxins and their variants on the Amudde side of Koka Reservoir, Ethiopia

This chapter presents data on the results of quantitative and qualitative analysis of cyanotoxins and potentially toxic cyanobacteria and considers the threats this presents to public health, the functioning of the aquatic ecosystem and its resources. The following submitted manuscript has originated from the data that constitute this chapter.

*Cyanobacteria on the Amudde side of Koka Reservoir (Ethiopia): temporal variations in their species composition, biomass and toxins (Submitted to Harmful algae).*

Chapter 5. Temporal dynamics of zooplankton community structure in relation to some physico-chemical and biological variables in Koka Reservoir, Ethiopia

This chapter discusses the dynamics of the zooplankton community structure and looks into the possible interactions between zooplankton and phytoplankton in this cyanobacteria-dominated reservoir. The following manuscript has emanated from this chapter.

*Zooplankton community structure and its dynamics in a cyanobacteria-dominated tropical reservoir (Koka, Ethiopia). (submitted to Journal of Freshwater Biology)*

Chapter 6. An isotopic analysis of the phytoplankton-zooplankton link in a highly eutrophic tropical reservoir dominated by cyanobacteria

This chapter presents the results of the analysis of the phytoplankton-zooplankton link using stable isotope signatures ($\delta^{13}$C and $\delta^{15}$N) of seston size-fractions and major zooplankton taxa resident in Koka Reservoir. The following submitted manuscript was based on the sets of data discussed under this chapter.

*An isotopic analysis of the phytoplankton-zooplankton link in a highly eutrophic tropical reservoir dominated by cyanobacteria (submitted to Journal of Plankton Research)*
Chapter 2. SPATIO-TEMPORAL VARIATIONS IN PHYSICO-CHEMICAL PARAMETERS OF KOKA RESERVOIR, ETHIOPIA

2.1. INTRODUCTION

Information on the seasonal dynamics of physico-chemical parameters of aquatic ecosystems has become increasingly important as they determine the density, diversity, distribution and composition of plankton communities, which in turn influence food web structure and energy flow in the pelagic ecosystem (Palmer et al., 2004, 2005). Seasonal changes in such environmental variables as nutrients, stratification and mixing patterns, and underwater light climate are known to affect phytoplankton composition and biomass in tropical African lakes (Talling and Lemoalle, 1998; Zinabu Gebremariam, 2002; Oduor and Schagerl, 2007). Seasonal changes in physico-chemical parameters can influence the zooplankton community structure. Alldredge et al. (1984) and Sarma et al. (2005), for instance, reported that higher temperatures and relatively lower saturation of dissolved oxygen concentration could determine the structure of a zooplankton community in tropical waters. Moreover, analyzing the physico-chemical parameters provide firsthand information on water quality characteristics and pollution in aquatic ecosystems as their changes could lead to several systematic changes in freshwater ecosystems (Daniels et al., 2002). These features help to identify the potential sources of pollution particularly in reservoirs. Thus, investigations on the physical and chemical parameters of reservoirs are critical to efforts geared towards controlling their pollution.

Unlike that on natural lakes, less information exists on the limnology of reservoirs in Ethiopia. Koka Reservoir receives large amounts of domestic and industrial wastes, and agricultural runoff that presumably contain several kinds of potentially harmful substances including algal nutrients (particularly nitrogen and phosphorus), which can indirectly have adverse impacts on biota resident in the reservoir.
Previous studies in Koka Reservoir included short-term observations on its water chemistry (Talling and Talling, 1965; Makin et al., 1975; Von Damm and Edmond, 1984; Wood and Talling, 1988; Elizabeth Kebede and Willen, 1998; Zinabu Gebre-Mariam, 2002; Hadgembes Tesfay, 2007). All the studies made so far on this reservoir did not, however, involve sampling over an extended period. The purpose of the present study was, therefore, to investigate the temporal and spatial dynamics of the physico-chemical features of Koka Reservoir over an annual cycle.

2.2. MATERIALS AND METHODS

2.2.1. Sampling protocol, *in situ* measurements and analysis of chemical parameters

Samples were collected at monthly intervals from two sites, an inshore site (S1) located in the proximity of a small village known as Amudde and an offshore site (S2). Site 1 and Site 2 are located at geographical positions of 8° 19’N and 39° 4’E and 8° 21’N and 39° 5’E, respectively. Collection of water samples and field measurements were made at nearly monthly intervals from February, 2014, to April, 2014, at S1 and for five months at S2. Water samples were collected with a Van Dorn bottle sampler (horizontal model) from selected depths distributed within the euphotic zone (0, 0.5, 1 m) and mixed in equal proportions to produce composite samples. The composite sample was used for the determination of total alkalinity and inorganic nutrients.

The transparency of the water was estimated using a 20 cm diameter white Secchi disc. Temperature and dissolved oxygen (DO), pH and conductivity were determined *in situ* using HANNA field meters, HI 9143, HI 9024 and HI 8733, respectively. Photosynthetically active radiation (PAR) incident on the surface and at different depths of the water column (0.25, 0.50, 0.75 and 1.0 m depths) was measured with underwater photometer (model SKP 200, Skye Instruments), and mean vertical attenuation coefficient (Kd) of down-welling irradiance was calculated according to Kirk (1994).
Total alkalinity (TA) was determined by titration of water samples with 0.01N HCl to a pH of 4.5 using bromocresol green-methyl red mixed indicator solution within a few hours after sample collection according to Wetzel and Likens (2000) and expressed in meq L\(^{-1}\). Concentrations of inorganic nutrients were determined for all samples following the procedures outlined in APHA et al. (1999). The samples used for the analyses of all nutrients except ammonia + ammonium-nitrogen (NH\(_3\) + NH\(_4^+\)-N) and total phosphorus (TP) were filtered through glass fiber filters (GF/F). Nitrate-N (NO\(_3\)-N) was analyzed by the sodium salicylate method (APHA, 1995), while ammonia + ammonium-N (NH\(_3\) + NH\(_4\)-N, hereafter ammonia) was determined by the phenate method. Nitrite-N (NO\(_2\)-N) was determined as a red azo dye by diazotization with sulphanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride (APHA, 1999). Dissolved inorganic nitrogen (DIN) was considered as the sum of NH\(_3\)+ NH\(_4^+\)-N, NO\(_3\)-N and NO\(_2\)-N, Soluble Reactive phosphorus (SRP) and total phosphorus (TP) were analyzed after persulfate digestion by the ascorbic acid method. Molybdate-reactive silica (SiO\(_2\)) was determined by the molybdosilicate method.

2.2.2. Statistical analyses

Spatial and seasonal variations in physicochemical parameters in the reservoir were tested using paired sample t-test. Comparison of sampling sites was based on data collected on similar dates of sampling. The interdependency of variables was analyzed using Pearson correlation. Statistical analyses were conducted using SPSS software package version 21.

2.3. RESULTS

2.3.1. Physical parameters

Ranges and means of some physico-chemical parameters of Koka Reservoir recorded in this study at the two sampling sites are presented in Table 2.1. The Secchi depth (Z\(_{SD}\)) varied seasonally from 0.07 m in June and July, 2013, to 0.25 m in May, 2013, at S1 and
from 0.07 m in August, 2013, to 0.15 m in March, 2014, at S2 with most values below 0.2 m. $Z_{eu}$ ranged from 0.21 to 0.75 m at S1 and from 0.21 to 0.45 m at S2, while mean vertical extinction coefficients of underwater light ($K_d$, in units m$^{-1}$) varied from 2.06 in March 2014, to 11.81 in July, 2013, with a mean value of 5.56 at S1 (Fig. 2.1). The mean values of $Z_{SD}$ (0.177) and $Z_{eu}$ (0.53) of the dry season were greater than those of the rainy season (0.13, 0.39, respectively), while the mean value of $K_d$ of the dry season (6.1) was lower than that of the rainy season (7.53) though no significant difference was observed between the mean values of the two seasons (t-test, $P < 0.05$). However, there were statistically significant differences for $Z_{SD}$ and $Z_{eu}$ between the two sites (t-test, $P < 0.05$) (Table - App. 1 and 2).

Total Suspended Solids (TSS, in mg L$^{-1}$) averaged 113.69 and varied from 31 in October, 2013, to 313 in July, 2013, at S1, while it ranged from 53 in February, 2014, to 148.9 in December, 2013, with a mean of 114.32 at S2 and with higher values during the rainy season (Fig. 2.1.) at both stations. The correlation between $Z_{SD}$ and TSS was negative and strong ($r = -0.82$), while that between $Z_{SD}$ and chl-$a$, was positive but weak ($r=0.202$) probably indicating the greater importance of abiogenic turbidity to the underwater light climate in Koka Reservoir.

2.3.2. Thermal regime: Water temperature and DO

Monthly surface water temperature varied between 20.4°C in June, 2013, and 25.5 °C in September, 2013, with a mean of 23.12 °C at S1, while the monthly surface water temperatures at S2 varied between 20.2°C in April, 2014, and 24 °C in August, 2013, with a mean of 22.56°C. Depth profiles of temperature and oxygen determined for the two sites are shown in Figs. 2.2. and 2.3. The average surface and bottom water temperatures of the reservoir were 23.12 °C and 22.38°C, respectively, with their differences ranging from 0 °C in May to 2.4 °C in September, 2013, at S1.
Fig. 2.1 Temporal variations in Secchi depth ($Z_{SD}$), mean vertical extinction coefficient ($K_d$) and Euphotic depth ($Zeu$) in relation to total suspended solids (TSS) and Chlorophyll-a (Chl-a) at Site 1 (closed circle) and Site 2 (open circle) of the present study in Koka reservoir.
Fig. 2.2. Depth profiles of water temperature (open circle) and concentration of dissolved oxygen (DO, closed circle) at Site 1, in Koka Reservoir.
DO (mg L\(^{-1}\)) at the surface ranged from 3.5 in April, 2014, to 9.61 in December, 2013, at S1, while at S2 it varied between 3.6 in April, 2014, and 8.1 in December, 2013. Depth-distribution of oxygen was more or less uniform with differences of less than 0.5 mg L\(^{-1}\) between successive depths of the water columns at both sites apart from the occasional abrupt drops observed near the lower ends of the water columns on some sampling dates.
2.3.3. Chemical Parameters

Temporal variations in specific conductance (K₂s), total alkalinity (TA) and pH at Site 1 (closed circle) and Site 2 (open circle) of the present study in Koka Reservoir are shown in Fig. 2.4. Conductivity (K₂s) varied from 231 μS cm⁻¹ in September, 2013, to 380 μS cm⁻¹ in March, 2014, with a mean of 294.83 μS cm⁻¹ at S1 and from 250 in August, 2013, to 375 μS cm⁻¹ in March, 2014, with a mean of 318.17 μS cm⁻¹ at S2. Total alkalinity showed seasonal variations with its observed values varying between 1.1 in the rainy season and 3.1 meq L⁻¹ in the dry season at S1. pH of surface water (mean = 8.64) at S1 varied from a minimum of 8.07 in July, 2013, to a maximum of 9.03 in December, 2013. pH values of surface water in excess of 9 were recorded in December, 2013, and March, 2014, (9.02) coincident with high phytoplankton biomass. The maximum values of pH, electrical conductivity, and total alkalinity were recorded during the dry season, while their low values were recorded in the rainy season although significant differences in the levels of these parameters were not observed both between seasons and sites (t-test, P < 0.05) except that of spatial variation in conductivity (Table-App. 1 and 2).
Fig. 2. Temporal variations in specific conductance ($K_{25}$), Total alkalinity (TA) and pH at Site 1 (closed circle) and Site 2 (open circle) of the present study in Koka reservoir.
Table 2.1 Summary of descriptive statistics of the environmental parameters of Koka Reservoir recorded in this study

<table>
<thead>
<tr>
<th>Variables</th>
<th>Range</th>
<th>Site 1</th>
<th>Site 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD (n = 12)</td>
<td>Mean ± SD (n = 12)</td>
</tr>
<tr>
<td>Temperature(°C, 0m)</td>
<td>20.4-25.50</td>
<td>23.12 ± 1.76</td>
<td>20.2-24</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>5.1 - 7.8</td>
<td>6.7 ± 0.93</td>
<td>3.3 - 4.6</td>
</tr>
<tr>
<td>Zsd (m)</td>
<td>0.07-0.25</td>
<td>0.15 ± 0.52</td>
<td>0.07-0.15</td>
</tr>
<tr>
<td>Zo (m)</td>
<td>0.21-0.75</td>
<td>0.46±0.16</td>
<td>0.21-0.45</td>
</tr>
<tr>
<td>Kd(In units m⁻¹)</td>
<td>2.06-11.81</td>
<td>5.56±3.93</td>
<td>-</td>
</tr>
<tr>
<td>TSS (mg L⁻¹)</td>
<td>31-313</td>
<td>113.69 ± 84.18</td>
<td>53 - 148.9</td>
</tr>
<tr>
<td>DO (mg L⁻¹)</td>
<td>3.5-9.61</td>
<td>7.14 ± 1.72</td>
<td>3.6-8.10</td>
</tr>
<tr>
<td>pH</td>
<td>8.07-9.03</td>
<td>8.64 ± 0.31</td>
<td>8.01-8.85</td>
</tr>
<tr>
<td>TA (meq L⁻¹)</td>
<td>1.1-3.1</td>
<td>2.18 ± 0.56</td>
<td>1.6-3</td>
</tr>
<tr>
<td>K23 (µS cm⁻¹)</td>
<td>231-380</td>
<td>294.83 ± 54.86</td>
<td>250 - 375</td>
</tr>
<tr>
<td>NO2-N (µg L⁻¹)</td>
<td>71.4-1142.4</td>
<td>512.72 ± 427.48</td>
<td>52.81-812.46</td>
</tr>
<tr>
<td>NO3-N (µg L⁻¹)</td>
<td>3.3-180.66</td>
<td>27.1±48.71</td>
<td>2.45-18.07</td>
</tr>
<tr>
<td>NH3+NH4N(µg L⁻¹)</td>
<td>197.4-557.4</td>
<td>296.48±109.23</td>
<td>136.4-367.8</td>
</tr>
<tr>
<td>DIN (µg L⁻¹)</td>
<td>331-1844</td>
<td>836.3±483.12</td>
<td>267-967</td>
</tr>
<tr>
<td>SRP(µg L⁻¹)</td>
<td>11.46-262.24</td>
<td>98.25 ± 82.7</td>
<td>15.04-206.63</td>
</tr>
<tr>
<td>TP (µg L⁻¹)</td>
<td>116.38-623.06</td>
<td>267.36±168.22</td>
<td>94.67-394.65</td>
</tr>
<tr>
<td>SO4(µg L⁻¹)</td>
<td>5.11-14.15</td>
<td>9.78±2.7</td>
<td>7.55-10.2</td>
</tr>
<tr>
<td>Total Chl-a(µg L⁻¹)</td>
<td>10.43-254.54</td>
<td>85.79 ± 78.24</td>
<td>12.33-107.47</td>
</tr>
</tbody>
</table>

Concentrations of inorganic nutrients were generally high and showed marked temporal variations during the study period. NO2-N (nitrite) averaged 27.1 µg L⁻¹ and varied from 3.03 µg L⁻¹ in December, 2013, to 180.66 µg L⁻¹ in June, 2013, while NO3-N (nitrate) varied from a minimum of 71.40 µg L⁻¹ in April, 2014, to a maximum of 1142.4 µg L⁻¹ in July, 2013, at S1. The concentrations of ammonia were generally much lower than those of nitrate. Ammonia concentrations at S1 ranged from 197.4 µg L⁻¹ in April, 2014, to 557.4 µg L⁻¹ in June, 2013. Concentrations of SRP varied between a minimum of 11.46 µg L⁻¹ in October, 2013, to a maximum of 262.24 µg L⁻¹ in August, 2013, while TP ranged from a minimum level of 116.38 µg L⁻¹ in February, 2014, to a maximum level of
Fig. 2.5. Temporal variations in the levels of inorganic nutrients at site 1 of the present study in Koka Reservoir.
DIN averaged 836.3 μg L$^{-1}$ and reached its highest concentration in June (1844 μg L$^{-1}$) and fell to its lowest concentration in May, 2013, (331 μg L$^{-1}$). The concentrations of all nutrients increased from June to August, 2013. In contrast to other inorganic nutrients, dissolved SiO$_2$ showed the least marked seasonal variations averaging 9.78 mg L$^{-1}$ and varying from a minimum value of 5.11 mg L$^{-1}$ in February, 2014, to a maximum value of 14.15 mg L$^{-1}$ in July. The maximum values of all nutrients were measured during the rainy season at S1, while the minimum values for all but SiO$_2$ and SRP were recorded during the dry season at S2. However, significant differences were detected between seasons for only NO$_3$ and DIN (t-test, $P < 0.05$). Except for SRP significant differences in the concentration of nutrients between the two sites were observed (t-test, $P < 0.05$) (Table-App. 1 and 2)

2.4. DISCUSSION

2.4.1. Morphometric and other physical parameters

The present results clearly show the high turbidity of the reservoir. The observed low $Z_{SD}$ values may have partly resulted from the resuspension of large quantities of particulate matter in the water column by wind-generated turbulence to which shallow water bodies like Koka Reservoir are especially susceptible (Talling, 1992). Moreover, the strong correlations of $Z_{SD}$ with the concentrations of total suspended solids showed that the reservoir is very turbid due to suspended particles, which presumably are primarily of abiogenic origin. With the flow of the two feeder rivers, particularly Awash River, into the reservoir, large quantities of allochthonous particulate matter are imported, and this substantially contributes to the reservoir's poor underwater light climate. High vertical extinction coefficient values that produce shallow euphtoic zones are typical of lakes and reservoirs with dense algal crops (Tadesse Ogato et al., 2015) or suspended silt associated with wind-induced vertical mixing and/or river inflow (Scheffer, 1998).
2.4.2. Thermal regime: Water temperature and DO

The vertical profiles of temperature and dissolved oxygen (DO) in conjunction with the previously reported results of Melaku Mesfin et al. (1988) revealed the absence of marked thermal stratification in Koka Reservoir. According to the observation made on the thermal characteristic of African lakes by Baxter et al. (1965), complete mixing is normally frequent in lakes with a maximum depth ($Z_{\text{max}}$) of less than about 15-30 m and thermal stratification is diurnal. Such shallow Ethiopian Rift Valley lakes and reservoirs as Chamo, Ziway and Koka with a maximum depth of less than 30 m can be considered as frequently mixing (polymictic) (Elizabeth Kebede, 1996).

2.4.3. Chemical Parameters

Concentrations of inorganic nutrients, which were quite high in comparison to those of Ethiopian rift valley and highland lakes, increased markedly during June-August, probably indicating the association of the elevated concentrations of the nutrients with their increased influx through surface influents originating from the surrounding agricultural lands on which fertilizers are frequently applied. In addition, during sampling in June, all water hyacinth (*Eichhornia crassipes*) plants, which previously invaded and carpeted the reservoir's surface, were manually removed by the local farmers and then thrown back into the reservoir water. The decomposition of plant material could add nutrient to the reservoir. This could be another possible explanation for the nutrient peaks observed during this period since the decomposition of the plant materials can add nutrients to the water. The reinvasion of the reservoir by this plant in September that same year coincided with the gradually declining levels of nutrients from September to February-March.

The levels of nutrients recorded in this study are broadly similar to those reported by Hadgemebes Tesfay (2007) although they reflected dramatic increases from levels reported previously (for example, 4-fold increase in SiO$_2$, and 3-fold increase in TP, Elizabeth Kebede and Amha Belay, 1994). However, the maximum SRP concentration recorded for Koka Reservoir in this study (264.24 µg L$^{-1}$) is closer to that reported
previously for the same reservoir (224 µg L⁻¹, Elizabeth Kebede et al., 1994). The observed increases in SiO₂ and TP are obviously linked to the increasing and intensive human activities taking place within the catchments area of the reservoir. The range of concentrations of silica recorded for Koka Reservoir in this study (5.11 - 14.15 mg L⁻¹) is comparable to that reported previously for the same reservoir (6.25 to 15.92 mg L⁻¹, Hadgembes Tesfaye, 2007).

Studies have shown that inorganic sources of nitrogen or phosphorus or both are limiting to algal production in some African freshwaters (Melack et al., 1982; Kalff, 1983; Talling and Lemoalle, 1998). However, the levels of inorganic nutrients recorded for Koka Reservoir are typical of reservoirs, which, unlike natural lakes, have large phosphorus and nitrogen loads (Kalff, 2002).

To summarize, Koka Reservoir is a very turbid frequently mixing water body with high levels of inorganic nutrients associated with the ever-increasing human activities taking place in its catchments. These physico-chemical conditions are known to select for nuisance and potentially toxic bloom-forming cyanobacteria (Dokulil, 1994; Scheffer, 1998).
Chapter 3. TEMPORAL AND SPATIAL VARIATIONS IN PHYTOPLANKTON COMMUNITY STRUCTURE IN KOKA RESERVOIR

3.1. INTRODUCTION

Phytoplankton is usually at the base of aquatic food webs providing food to diverse groups of aquatic organisms as they are the main primary producers. Information on their abundance, community composition and biomass can be crucial in the assessment of trophic status and water quality (Walsh et al., 2001; Reynolds et al., 2002), evaluation of potential fish yield (Descy et al., 2005), productivity (Walsh et al., 2001), and energy flow (Simciv, 2005), and management (Beyruth and Tanaka, 2000) of tropical reservoir ecosystems.

Numerous studies have pointed out that most aquatic systems, including rivers, lakes and reservoirs, have undergone changes due to the everincreasing anthropogenic activities such as land-use changes linked to agricultural and industrial activities, and human settlement (Jackson, 2001; Jackson et al., 2001; Wetzel, 2001; Kuang et al., 2004; Tewfik et al., 2005) with their impacts on tropical reservoirs increasing with an alarming rate (Cecchi, 2007; Descy and Sarmento, 2008). Eutrophication of freshwater bodies including reservoirs has become a worldwide concern (Carstensen et al., 2007) as it often leads to the development in freshwater lakes and reservoirs of algal blooms, which impact ecosystems services to mankind (Reynolds, 2006). As a consequence of eutrophication of aquatic environments, phytoplankton community structure is subjected to high temporal variability due to the rapid interplay among physical, chemical and biological variables. Therefore, investigation on phytoplankton community structure is essential to identify the environmental conditions that lead to nuisance and harmful algal bloom-formation and to provide a scientific basis for the development of strategies of control of algal blooms and management of aquatic environments.
Excessive growth of various species of cyanobacteria can pose health risks to humans and livestock as well as aquatic biota (Chorus and Bartram, 1999; Carmichael et al., 2001; Azevedo et al., 2002) since some species of cyanobacteria can produce and release toxins (cyanotoxins). They are also linked to a number of other water-related problems including bad odour and taste and the non-potability of the water and fish kills due to oxygen depletion and ammonia accumulation as the cyanobacterial decomposition proceeds (Whitton and Potts, 2000; Smith et al., 2008).

To obtain an insight into the ecological effects of nutrient enrichment and better manage water quality of an aquatic environment, one approach is to fractionate phytoplankton assemblages into different size-classes, since cell size influences the response of phytoplankton communities to environmental changes and food chain dynamics (Fogg, 1986; Becker and Marques, 2004). The trophic state of a water body has been shown to affect size-fractionated phytoplankton biomass and primary production; accordingly, under oligotrophic or light-limiting conditions, small phytoplankton have a higher capacity for nutrient- and light-acquisition (Polat and Aka, 2007), whereas under favorable conditions for growth, namely high irradiance and nutrient concentrations, large phytoplankton have higher photosynthetic efficiency and growth rate (Adame et al., 2007).

A number of studies have documented the seasonal succession of phytoplankton in temperate regions (Reynolds, 1984, 1997). Such knowledge is, however, scarce for tropical and subtropical reservoirs (Tudorancea et al., 1999, Tadesse Dejenie et al., 2008). The relatively few studies made on phytoplankton community in tropical reservoirs include those carried out in South Africa (Robarts et al., 1992), China (Lin et al., 2003), Brazil (Crossetti and Bicudo, 2008), and Colombia (López et al., 2012). Studies assessing the community structure of phytoplankton in eutrophic lakes and reservoirs in Ethiopia are similarly limited. Previous studies made on the community structure of phytoplankton in Ethiopian reservoirs include those on the small highland reservoirs (Tadesse Dejenie et al., 2008; Tsehaye Asmelash, 2009), Legedadi Reservoir
(Adane Sirage, 2006), Geffersa reservoir (Nigatu Ebisa, 2010), and Belbela Reservoir (Feyissa Girma, 2011). Short-term investigations on water chemistry and species composition of phytoplankton have also been made in Koka Reservoir (Talling and Talling, 1965; Makin et al., 1975; Von Damm and Edmond, 1984; Wood and Talling, 1988; Elizabeth Kebede and Willen, 1998; Hadgembes Tesfay, 2007). The studies made so far on the present study reservoir did not involve sampling over an extended period (an annual cycle), which could have produced a more complete picture of the phytoplankton community structure and its temporal dynamics. In addition, previous studies failed to look into the size-structure of the phytoplankton biomass in the reservoir. Therefore, the aim of the present study was to investigate the temporal and spatial variations in the taxonomic composition, size-structure, abundance, and biomass of phytoplankton in Koka Reservoir.

3.2. MATERIALS AND METHODS

3.2.1. Study area and sampling protocol

The study reservoir and the selected sampling sites are described under Chapter 1. Samples used for the identification and enumeration of phytoplankton and estimation of their biomass as chlorophyll-a (chl-a) and biovoume were collected from the same two sites (S1 and S2) using the same bottle sampler from the same depths used for the collection of water samples for the analysis of various chemical parameters described under Chapter 2.

3.2.2. Identification of Phytoplankton taxa and estimation of their abundance, biovolume and chl-a biomass

Composite water samples were placed in 125 ml amber glass bottles and fixed immediately with Lugol’s iodine solution. Preserved samples were used for the identification of phytoplankton taxa using such identification keys as Whitford and
Schumacher (1973), Gasse (1986), Komárek and Kling (1991), Komárek and Cronberg (2001), Cronberg and Komárek (2004), Komárek and Anagnostidis (2005), and Bellinger and Sigee (2010). The estimation of phytoplankton abundance was done using a Sedgewick-Rafter cell following the procedures outlined in Hotzel and Croome (1999). Biovolume of the major phytoplankton species was estimated by approximating cell shape to solid geometric configurations. Measurements of appropriate dimensions of 50 cells were made using an ocular micrometer, which was calibrated with a stage micrometer. For the filamentous algae, the number of cells per filament for the first 30 filaments observed was determined and the mean number of cells per filament for the sample was calculated. For the colonial forms, the number of cells in the first 30 colonies was first determined and then the mean number of cells per colony was calculated. The average number of cells per filament or colony was multiplied by the number of filaments or colonies to estimate the abundance of filamentous or colonial taxa. The average cell volume was multiplied by the abundance of the filamentous or colonial taxa to estimate their population volume.

Chl-a concentration in the composite samples was used as an index of phytoplankton biomass. Appropriate volumes (100-250 ml) of composite water samples were filtered through glass fiber filter papers (Whatman, GF/F, nominal pore size of 0.7 μm) for the determination of total chl-a. For size-fractionation of phytoplankton chl-a, water samples were sequentially filtered through 20, 2 and 0.2 μm pore size polycarbonate filters under low vacuum pressure (<100 mm Hg). Cells retained by the 20 μm filters belong to the microplankton (netplankton), whereas those retained by the 2 and 0.2 μm pore-size filters constitute, respectively, the nanoplankton and picoplankton (Sieburth et al., 1978). After filtration and subsequent homogenization with a glass rod, pigments were extracted in neutralized 90% acetone for 24h in the dark at 4°C and their absorbance measured at 665 and 750 nm (Wetzel and Likens, 2000). Concentration of chl-a was determined following the recommendations and formula of the monochromatic method of Lorenzen (1967) as outlined in Wetzel and Likens (2000).
3.2.3. Statistical analyses

Paired sample t-tests were performed to determine possible differences between levels of measured parameters of the rainy and dry seasons and sampling sites in the reservoir. These statistical analyses were conducted using SPSS software package version 20. Redundancy analysis or the constrained linear method of ordination (RDA) was used to determine the relationships between phytoplankton biomass (i.e. species) and environmental variables (explanatory variables) with the aim to identify the best explanatory variables for the observed trends in biological parameters using CANOCO for windows 4.5 version. Beside RDA was also used to determine the relationships between the chl-a biomass of each phytoplankton size-class and the various physico-chemical variables. RDA was chosen after confirming through a preliminary detrended correspondence analysis (DCA) that the length of the ordination axes in DCA was less than 3 (Jan and Petrš, 2003). Those variables with high variance inflation factors (VIP>20) were removed from the analyses as they would provide redundant information. Before the analysis, both species data and environmental variables except pH were log (X+1) transformed to reduce the effect of high values (Ter Braak and Smilauer, 1998) and were also standardized to account for differences in units. To evaluate the significance of the RDA axes and of the environmental variables, which defined these axes, Monte Carlo tests were performed with 499 unrestricted permutations, using the eigenvalues of the axes as test statistics (Ter Braak and Prentice, 1988). The results of RDA were visualized in the form of ordination diagrams in Canodraw for Windows.

3.3. RESULTS

3.3.1. Phytoplankton composition and abundance

Species of phytoplankton observed in samples collected from Koka Reservoir are presented in Table 3.1. A total of 89 phytoplankton species were identified and quantified throughout the sampling period. Chlorophyceae was the richest algal group with 34 species (38.20% of total phytoplankton taxa), followed by Cyanophyceae with 28 species (31.46% of total phytoplankton taxa). The other phytoplankton taxa included 11 diatoms.
(Bacillariophyceae, 12.36% of total taxa), 10 euglenoids (Euglenophyceae, 11.24% of total taxa), 5 cryptomonads (Cryptophyceae, 5.62% of total taxa) and 1 dinoflagellate (Pyrrophyceae, 1.12% of total taxa).

Total phytoplankton abundance in Koka Reservoir exhibited distinct temporal variations with the highest \((2.2 \times 10^6 \text{ cells mL}^{-1})\) and lowest \((4.1 \times 10^4 \text{ cells mL}^{-1})\) phytoplankton abundance occurring in December and July, 2013, respectively at S1 (Fig. 3.1.). The highest value of total phytoplankton abundance was registered in December, 2013 \((1.4 \times 10^6 \text{ cells L}^{-1})\), while the lowest was recorded in August, 2013 \((3.7 \times 10^5 \text{ cells L}^{-1})\), at S2 (Fig. 3.2.).

The differences in total phytoplankton density between the dry and rainy seasons were statistically significant (t-test, \(P<0.05\)) with high densities occurring during the dry season. Phytoplankton densities were higher at S1 than at S2 and the difference between the two sites was statistically significant (t-test, \(P<0.05\)) (Table-App. 1 and 2). Cyanobacteria was the most important group in terms of abundance accounting for more than 90% of the total phytoplankton abundance.
<table>
<thead>
<tr>
<th>Table 3.1. List of species of phytoplankton identified in samples collected from Koka Reservoir during the study period.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cyanophyceae (Blue green Algae)</strong></td>
</tr>
<tr>
<td><em>Anabaena spiroides</em> Kleb.</td>
</tr>
<tr>
<td><em>Anabaenopsis abigatae</em> Kebede and Willén</td>
</tr>
<tr>
<td><em>Aphanocapsa delicatissima</em> W. West and G.S. West</td>
</tr>
<tr>
<td><em>Aphanthece microscopica</em> Nägeli</td>
</tr>
<tr>
<td><em>Aphanthece clathrata</em> West and G.S.West</td>
</tr>
<tr>
<td><em>Aphanizomenon gracile</em> Lemm</td>
</tr>
<tr>
<td><em>Coelosphaerium cf. aegyptium</em> Lemm</td>
</tr>
<tr>
<td><em>Cyanodictyon imperfectum</em> Cronb. and Weib.</td>
</tr>
<tr>
<td><em>Cylindrospermopsis africana</em> Kom. and Kaling</td>
</tr>
<tr>
<td><em>Cylindrospermopsis curvispora</em> M. Watanbe</td>
</tr>
<tr>
<td><em>Cylindrospermopsis raciborskii</em> Wolosz.</td>
</tr>
<tr>
<td><em>Merismopedia punctata</em> Meyen</td>
</tr>
<tr>
<td><em>Microcystis aeruginosa</em> (Kütz) Kütz</td>
</tr>
<tr>
<td><em>Microcystis botrys</em> Teil</td>
</tr>
<tr>
<td><em>Microcystis flos-aquae</em> Kütz</td>
</tr>
<tr>
<td><em>Microcystis novacekii</em> (Kom.) Compère</td>
</tr>
<tr>
<td><em>Microcystis panniformis</em> (Kom.) Komárová-Legnerová</td>
</tr>
<tr>
<td><em>Planktolyngbya contorta</em> (Lemm.) Kom</td>
</tr>
<tr>
<td><em>Planktolyngbya limnetica</em> (Lemm.) Kom</td>
</tr>
<tr>
<td><em>Pseudoanabaena limnetica</em> (Lemm.) Kom.</td>
</tr>
<tr>
<td><em>Pseudoanabaena moniliform</em> Kom. and Kling</td>
</tr>
<tr>
<td><em>Radiocystis geminata</em> Skuja</td>
</tr>
<tr>
<td><em>Raphidiopsis curvata</em> Fritsch and Rich</td>
</tr>
<tr>
<td><em>Raphidiopsis mediterraneа</em> Skuja</td>
</tr>
<tr>
<td><em>Romeria victoriae</em> Kom. and Cronb.</td>
</tr>
<tr>
<td><em>Spirulina sp</em>.</td>
</tr>
<tr>
<td><strong>Chlorophyceae (Green Algae)</strong></td>
</tr>
<tr>
<td><em>Actinastrum aciculare</em> playf.</td>
</tr>
<tr>
<td><em>Ankistrodesmus bifrenans</em> (Reinsch) Korsch</td>
</tr>
<tr>
<td><em>Ankistrodesmus falcatus</em> Ralfs.</td>
</tr>
<tr>
<td><em>Botryococcus brauni</em> Kutz</td>
</tr>
<tr>
<td><em>Chlamydomonas sp.</em></td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em> Beyerinck</td>
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<td></td>
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<tr>
<td></td>
</tr>
</tbody>
</table>
Closterium sp.
Coelastrum asteroideum Nag.
Coelastrum cambricum
Coelastrum micosporum Nag.
Coelastrum reticulatum (P.A. Dangeard) Senn
Coelastrum sphaercum Nag.
Cosmarium contractum kirch
Cosmarium depressum (Nag.) Lund.
Crucigeniella rectangularis (Nag) Kom.
Oocystis sp.
Pediastrum boryanum (Turp.) Meneghini
Pediastrum simplex Meyen
Scenedesmus acuminatus var. minor G.M. Smith
Scenedesmus armatus (Chod.) G.M. Smith
Scenedesmus bicaudatus G.M. Smith
Scenedesmus bijuga var. alternans (Rein.) Hans.
Scenedesmus linearis Kom.
Scenedesmus obliquus (Turp.) Kütz.
Scenedesmus platydiscus (G.M. Smith) Chod.
Scenedesmus quadricauda (Turp). Breb.
Schroederia setigera (Schröd.) Lemm.
Staurastrum cingulum
Staurastrum curvatum W. West
Staurastrum tetracerum Ralf.
Staurodesmus sp.
Tetraedron minimum (Lagerh.) Chod.
Tetraedron triangulare Korsh.

Strombomonas cf. granulate (Svir.) Fatt and Kom.
Trachelomonas caudata (Ehr.) Stein
Trachelomonas ispida Deflnder
Trachelomonas volvocine
Fig. 3.1. Temporal variations in the abundance of different algal groups in relation to total phytoplankton abundance and Chl-a biomass at Site 1 of the present study in Koka reservoir.
Fig. 3. Temporal variations in the abundance of various algal groups in relation to total phytoplankton abundance and chl-a at Site 2 of the present study in Koka Reservoir.
The most dominant cyanobacterial genera were *Microcystis* and *Cylindrospermopsis* with mean abundances of $8.03 \times 10^5$ and $1.1 \times 10^5$ cells mL$^{-1}$, and with percentage contributions of 72.29% and 21.45% to the total phytoplankton abundance, respectively at S1 (Fig. 3.3 and Table -- App. 3.1). At S2, *Microcystis* and *Cylindrospermopsis* with mean abundances of $7.6 \times 10^5$ and $1.1 \times 10^5$ cells mL$^{-1}$ contributed 79.8% and 15.1% of total phytoplankton abundance, respectively (Table -- App. 3.2).
Fig. 3.3 Temporal variations in the abundance of major cyanobacterial species at site 1 of the present study in Koka reservoir.
Fig. 3.4. Temporal variations in the abundance of major cyanobacterial species at Site 2 of the present study in Koka reservoir.
M. aeruginosa was the most dominant species in most of the samples collected from the reservoir followed by M. flosaqua, M. novacekii and M. panniformis (Figs. 3.3. and 3.4) at both sites. The observed major species of Anabaena were Anabaena spirioide and Anabaena circinalis with the former accounting for 1.81 % (mean) of total phytoplankton abundance at S1 (Table –App. 3.3). Anabaena flos-aqua was the rarest species observed during the study period. In May, 2013, the dominance of Cylindrospermopsis was apparent with Cylindrospermopsis africana dominating the phytoplankton community and accounting for approximately 84.85% of total phytoplankton abundance at S1. C. raciborskii was the second quantitatively important species of Cylindrospermopsis with percentage contribution to total phytoplankton abundance as large as 22.37% of April, 2014. C. curvispora was relatively rare during the study period. Other cyanobacterial species present in the phytoplankton assemblages included the genera Aphanocapsa, Aphanothece, Aphanizomenon, Coelosphaerium, Planktolyngbya, Pseudanabaena, Radiocystis and Raphidiopsis, which together constituted a total abundance of 1.6 x 10^5 cells mL^-1 accounting for 2.44 % (mean) of total phytoplankton abundance.

Diatoms were the second most abundant group of phytoplankton with a mean percentage contribution of about 1.61 % of total phytoplankton abundance (Table–App. 3.1). They had a mean cell density of 1.2 x 10^4 cells mL^-1, which was constituted mainly by the chain-forming mall pennate diatom Aulacoseira granulata whose abundance peaked in December, 2013. Abundances of Aulacoseira granulata (mean=1.43 x 10^4 cells mL^-1) were higher at S2 than at S1 throughout the study period. The abundance of diatoms in June was largely due to Nitzschia acicularis, which consistently declined before its disappearance in October. Other diatoms species, which were rarely observed, include Aulacoseira ambigu, Cyclotella sp, Cymbella sp, Fragilaria tenera, Nitzschia sp. Stephanodiscus hantzschii, Surirella biseriata, Syndera ulna and Thalassiosira sp. The third important algal group was the Euglenophyceae, which was mainly represented by three genera, namely Euglena, Phacus and Trachelomonas, with the latter being the most frequent taxon.
The green algae were represented by species of *Actinastrum, Chlorella, Coelastrum, Cosmarium, Pediastrum, and Scenedesmus*, which were generally rare and sparsely populated during most of the sampling period. Representing the cryptophyceae were *Cryptomonas marssoni, Cryptomonas obovata, Cryptomonas ovata* and *Rhodomonas minuta*, while the Dinophyceae and Chrysophyceae were constituted by a single species of *Peridinium* and *Chromulina*, respectively.

### 3.3.2. Phytoplankton biovolume

Biovolume of phytoplankton populations was estimated from cell density and mean cell volume of each species. Total phytoplankton biovolume in Koka Reservoir exhibited distinct temporal variations (Fig. 3.5.) with the highest (155.06 mm³ L⁻¹) and lowest (3.04 mm³ L⁻¹) total phytoplankton biovolume occurring in December and June, 2013, respectively, coincident with the highest and one of the lowest levels of total phytoplankton abundance at S 1. Total phytoplankton biomass as biovolume was higher in the dry season than in the wet season.

Cyanobacteria overwhelmingly dominated total phytoplankton biomass as biovolume on all sampling dates, with their contributions often exceeding 95% of total phytoplankton biomass (Table–App. 3.4). On the average, *Microcystis* spp accounted for the largest proportion (62.87%) of total phytoplankton biovolume with their total biovolumes varying from 0.48 mm³ L⁻¹ to 133.42 mm³ L⁻¹. Biovolume of *Microcystis* spp. was very low during the major rainy period before it almost consistently increased to its highest peak in December and then consistently declined to a low level in April, 2014 (Fig. 3.4). Biovolume of *Cylindrospermopsis* spp. was quite high in May, 2013 (48.94 mm³ L⁻¹, 88%), before it nearly consistently declined to its low level in December, 2013. The quantitatively most important filamentous cyanobacteria were *Cylindrospermopsis africana* and *Anabaena spiroides* whose percentage contributions to total phytoplankton biovolume peaked in May (84.67%) and November (27.3%), 2013, respectively (Table–App. 3.4). The second most important group in terms of contribution to total phytoplankton biomass as biovolume was diatoms, whose biomass was constituted largely by *Aulacoseira granulata* (mean- 2.79% of total phytoplankton biovolume), the
standing biomass of which varied considerably ranging from 0 to 18.27 mm$^3$ L$^{-1}$. Although chlorophyceae was the most species-rich taxonomic group, most species belonging to this taxon were generally rare and hence their contribution to total phytoplankton biomass was considerably low (mean < 1%) (Table–App. 3.5).

Fig. 3.5. Temporal variations in phytoplankton biomass as biovolume (line plots) and percentage contributions (area plots) of phytoplankton taxa to total phytoplankton biovolume at Site 1 of the present study in Koka reservoir.
The results of the RDA of the relationship between biomass of total phytoplankton and major phytoplankton taxa and environmental variables are shown in Fig. 3.6 and Table 3.2. RDA was used to examine the response of phytoplankton taxa to the environmental variables measured. Among the environmental variables, temperature, ZSD, TSS, DO, alkalinity, conductivity, nitrate, SRP and TPs were selected based on the Monte Carlo test. The remaining variables such as, Zev, rainfall, pH, salinity, ammonia and SiO₂ were excluded from the analysis since they did not explain any significant proportion of the residual variance. RDA indicated that the environmental variables explained 89% of the total variance of phytoplankton biomass as biovolume. The first two ordination axes collectively explained 68.3% of the variance in phytoplankton biomass in Koka Reservoir (Table 3.2). The first two phytoplankton biovolume-environmental correlations were 0.994 and 0.967, indicating that the observed environmental variables accounted for a major portion of the variation in the composition of phytoplankton (Fig. 3.6). Axis 1 was composed of ZSD, TSS, and DO, temperature, SRP and nitrate, while Axis 2 was constituted by TP, alkalinity and conductivity. The ordination biplots showed that nutrients had positive correlation with the first RDA axis.

**Table 3.2. Summary of RDA of the relationships between environmental factors and phytoplankton biomass in Koka Reservoir**

<table>
<thead>
<tr>
<th>Axes</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td>0.410</td>
<td>0.273</td>
</tr>
<tr>
<td>Species-environment correlations</td>
<td>0.994</td>
<td>0.967</td>
</tr>
<tr>
<td>Cumulative percentage variance</td>
<td>41.0</td>
<td>68.3</td>
</tr>
<tr>
<td>of species data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of species-environment relation:</td>
<td>45.9</td>
<td>76.5</td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td></td>
<td>0.893</td>
</tr>
</tbody>
</table>
Species of *Microcystis* were negatively correlated with the levels of nutrients such as TP, SRP, and nitrate, and positively correlated with ZSD, DO, conductivity and temperature. However, species of *Cryptomonas* and *Trachelomonas* were correlated positively with nitrate, SRP, TP, and TSS. The occurrence of *Cylindrospermopsis* was strongly associated with total alkalinity. The dominance of *Aulacoseira granulata* was associated with higher TP, but lower water conductivity and alkalinity.

Fig. 3.6. Ordination biplots of total phytoplankton biovolume (Total ph) and biovolume of some phytoplankton species (*M. aerug=Microcystis aeruginosa, M. flos=M. flosaquae, M. novac M. novacekii, M. panni=M. panniformis, C. afr=M. cylindrospermopsis africana, C. raci=C. raciborskii, A. spp=Anabaena spp., Aulaco=Aulacoseira granulata, Crypto=Cryptomonas and Trachelo=Trachelomonas spp); environmental variables: Temp = water temperature, DO=Dissolved Oxygen, TSS= Total suspended solids, ZSD = Secchi depth, Cond= conductivity, Alk = alkalinity, TP = Total phosphate, SRP = soluble reactive phosphate, Nitr=Nitrate, Chl-a= Chlorophyll-a) at S1 of the present study in Koka Reservoir.
3.3.3. Total and size-fractionated chl-a biomass of phytoplankton

Total chl-a concentration at S1 exhibited seasonal variations with the highest peaks occurring in December, 2013 (254.54 µg L⁻¹), and March, 2014 (209.06 µg L⁻¹), and with the former chl-a peak and the seasonal minimum chl-a concentration of July, 2013 (10.43 µg L⁻¹), coinciding with the highest and lowest phytoplankton abundance, respectively (Fig.3.1). Chl-a reached its highest peak in December, 2013 (107.47 µg L⁻¹), while its minimum value was recorded in August, 2013, at S2, with statistically significant difference in the levels of mean chl-a between the two sites (t-test: p < 0.05). Chl-a concentration remained at relatively low levels (< 65 µg L⁻¹) before its dramatic increase to the seasonal maximum in December, 2013, at both S1 and S2.

Chl-a of the three size-classes of phytoplankton varied temporally, exhibiting a seasonal pattern which was more or less similar to that of total chl-a (Fig. 3.7). The concentration of net phytoplankton chl-a averaged 55.5 µg L⁻¹ varying from 1.39 µg L⁻¹ in July, 2013, to 137.51 µg L⁻¹ in December, 2013, and accounting for 9.1 to 72.2% of total chl-a concentration. The concentration of nano phytoplankton chl-a averaged 57.3 ranging from 8.34 µg L⁻¹ in July to 99.52 µg L⁻¹ in December, 2013, and accounting for 25 to 77.3 % of total chl-a concentration (Fig. 3. 7.). The concentration of picophytoplankton chl-a varied from 3.71 to 25.02 µg L⁻¹ with relatively low mean percent contribution (10.48%) to total chl-a concentration although the increase in percentage contribution in May, 2013, was sizeable (38.30%, Fig. 3.7). The temporal variations in size-fractionated phytoplankton chl-a relative to total chl-a (Fig. 3.7) suggest that the net phytoplankton community constituted a major fraction of total chl-a from December, 2013, to March, 2014, while the nanophytoplankton community accounted for the largest fraction of total chl-a from May, 2013, to November, 2013.
Fig. 3.7 Temporal variations in the concentrations of Chl-a (line plots) and percentage contribution (area plots) of pico-, nano- and net phytoplankton to total chl-a of phytoplankton at site 1 of the present study in Koka reservoir.
Fig. 3.8 show the RDA ordination of the total and size-fractionated chl-a with respect to environmental variables. RDA analysis demonstrated that the environmental variables explained 94.3% of the total and size-fractionated chl-a. The first two axes accounted for 86.3% of the variation in the total and size-fractionated chl-a -environment relationship with the first axis accounting 74.7% and the second axis accounting 11.6% (Table 3.3). The total, net-, nano- and pico-chl-a of phytoplankton were positively correlated with conductivity and pH, while they were negatively correlated with TSS, SRP and NO₃-N (Fig. 3.8). Nano- chl-a of phytoplankton showed a strong positive relation to NH₃. 

Table 3.3 Summary of RDA of the relationships between environmental factors and total and size-fractionated chl-a in Koka Reservoir

<table>
<thead>
<tr>
<th>Axes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues :</td>
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<td>0.116</td>
<td>0.074</td>
<td>0.004</td>
<td>1.000</td>
</tr>
<tr>
<td>Species-environment correlations:</td>
<td>0.987</td>
<td>0.926</td>
<td>0.927</td>
<td>0.640</td>
<td></td>
</tr>
<tr>
<td>Cumulative percentage variance</td>
<td>74.7</td>
<td>86.3</td>
<td>93.7</td>
<td>94.2</td>
<td>94.2</td>
</tr>
<tr>
<td>of species data :</td>
<td>79.2</td>
<td>91.5</td>
<td>99.5</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td>0.943</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
shift in dominance of taxa in relation to turbidity and levels of nutrients associated with hydrographic and hydrological conditions of the reservoir. Although most of its constituent species were generally rare, chlorophyceae was the most species-rich taxonomic group, rivaled only by the cyanophyceae (cyanobacteria). This is consistent with the generalization that chlorophytes and cyanophytes are species-rich taxonomic groups in most tropical as well as temperate lakes and reservoirs (Reynolds, 2006). The year-round dominance of the phytoplankton community in the reservoir by cyanobacteria, which was constituted primarily by species of the genera *Anabaena, Cylindrospermopsis* and *Microcystis*, is a phenomenon of widespread occurrence in eutrophic and hypereutrophic tropical lakes and reservoirs (Paerl, 1996). The prevalence of cyanobacteria-dominated phytoplankton community in this reservoir is consistent with the observations recorded by Melaku Mesfin et al. (1988), Elizabeth Kebede and Willen (1996) and Hadgembes Tesfay (2007) for the same reservoir. The dominance of cyanobacteria is indicative of eutrophic conditions and poor ecological status of reservoirs and lakes (Napiorkowska-Krzebietke et al., 2009; Hutorowicz et al., 2011). The occurrence of such only a few phytoflagellates (chrysophytes, cryptomonads and dinoflagellates) is also associated with the eutrophic condition of the reservoir (Reynolds, 2006).

The RDA indicated that the physicochemical variables explained 89% of the total variance of phytoplankton community structure. Phytoplankton biomass-environment correlations for axis 1 and 2 were high, indicating a strong correlation between phytoplankton species distribution and environment variables used for ordination. The results of RDA also suggest the greater importance of $Z_{SD}$, TSS, temperature and concentration of $NO_3$ to changes in the abundance of *Microcystis* spp, while the abundance of *Cylindrospermopsis* spp was influenced primarily by alkalinity, conductivity and TP. The abundance and persistent dominance of cyanobacterial species observed in this study seem to have resulted primarily from increased turbidity and availability of high levels of nutrients. The association of cyanobacterial blooms with high turbidity in Koka Reservoir is in line with the results of the analysis of 22 lakes worldwide by Smith (1986), and with the findings for several Danish lakes (Jensen et al., 1986).
1994), and 55 turbid shallow Dutch lakes (Scheffer et al., 1997). It is well documented that such shallow water bodies receiving surface influents commonly exhibit high turbidity due to the resuspension of inorganic particles from the sediment by frequent mixing or loading from the catchments through runoff and feeder rivers (Dokulil, 1994; Scheffer, 1998). Deep-mixing in Koka Reservoir can affect the cyanobacteria only temporarily since they can regain their vertical position quickly owing to their effective buoyancy mechanism associated with gas vacuoles (Reynolds, 1987). Cyanobacterial dominance is a common phenomenon in lakes and reservoirs including those found in Ethiopia (Adane Sirage, 2006; Girma Tilahun, 2006; Hadgembes Tesfay, 2007; Tadesse Ogato, 2007; Tadesse Dejenie et al., 2008; Tsehaye Asmelash, 2009). Although a number of water column conditions are known to lead to the dominance of cyanobacteria, low light (Smith, 1986), high temperature (Shapiro, 1990), low euphotic to mixing depth ratio (Jensen, et al., 1994), their ability to regulate buoyancy (Reynolds, 1987) coupled with the inedibility of large colony-forming chroococcal and filamentous cyanophytes to microphagous herbivores (rotifers, nauplii, and small cladocerans) (Gliwicz and Lampert, 1990) seem to have relevance to the situation in Koka Reservoir. The positive and negative correlations of cyanobacterial abundance with ammonia and nitrate levels in the reservoir, respectively, may be explained by the persistence and abundance of the non-heterocystous taxa, Microcystis spp., which are known to be favored by the availability of ammonia-nitrogen (Blomqvist et al., 1994). Similar trends of covariance of cyanobacterial abundance and inorganic nitrogen sources have been reported for several tropical productive Brazilian reservoirs by Huszar et al. (2000). Some studies (e.g. Sekandende et al., 2005; Sitoki et al., 2012) also documented Microcystis blooms in the wet season due to elevated nutrient levels occurring after periods of heavy precipitation.

Among diatoms, Aulacoseira granulata was quantitatively the most important species in Koka Reservoir. Aulacoseira granulata is a rapidly sinking planktonic diatom (Reynolds, 1994), which is common in shallow mixing lakes and in deeper lakes during periods of high turbulence (Kilham and Kilham, 1975; Hecky and Kling, 1987). Therefore, the abundance of this alga may be associated with the frequently turbulent water column.
condition in the reservoir and its adaptation to the consequent low light condition (Reynolds, 1994).

The results of statistical analysis showed that the biological variables recorded in this investigation were significantly different between the rainy and dry seasons suggesting the presence of marked temporal variability of phytoplankton in the reservoir. The observed absence of significant differences in the levels of measured parameters between the sampling sites (t-test, \( p < 0.05 \)), may have resulted from the presumably strong horizontal mixing of the reservoir and/or from the proximity of the sampling sites. The higher abundance of phytoplankton during the dry season is probably associated with the relatively low level of total suspended solids. In the rainy season, there is increased loading of suspended solids associated with the high influx of particles from Modjo and Awash rivers, which led to greater turbidity. This physical condition is likely to have adversely influenced phytoplankton growth (Pugnetti and Bettinetti, 1999) leading to the observed reduced phytoplankton abundance during the rainy season.

Phytoplankton biomass was significantly different between the two seasons \( (p \leq 0.05) \) with the reservoir during the dry period supporting cyanobacterial blooms of considerably higher phytoplankton biomass measured as chl-a and biovolume. The mean chl-a concentration recorded in the present study for Koka Reservoir is higher than those of earlier reports (22.4 mg chl-a m\(^{-3}\), Melaku Mesfin et al., 1988; 16 mg chl-a m\(^{-3}\), Elizabeth Kebede et al., 1994) probably indicating the level of eutrophication associated with the ever-increasing human interference.

Among the three size-groups, the nanophytoplankton made the largest contribution to total chl-a biomass of phytoplankton from May to November, 2013, while the largest contribution of the netphytoplankton to total chl-a occurred during the period spanning from December, 2013, to March, 2014, with the latter coinciding with the dominance of the large colonial cyanobacteria, *Microcystis* spp. The major contribution of the nanophytoplankton to total chl-a occurred during the period when the quantitative importance of the relatively small-sized green algae, cryptomonads and euglenoids was
high. Girma Tilahun (2006) for the eutrophic Lake Chamo and Callijuri and Dos Santos (2001) for the tropical reservoir Barra Bonita of Brazil also reported the high contribution of the netphytoplankton to total chl-α. The small contribution of the picophytoplankton to total phytoplankton chl-α is attributable to their inability to compete with large-sized phytoplankton under nutrient-rich conditions (Weisse, 1993; Bec et al., 2005). Persistently high biomass of picophytoplankton is, therefore, commonly reported from oligotrophic lakes (Stockner and Anita, 1986; Stockner, 1991).

In conclusion, the phytoplankton community in the reservoir exhibited seasonal changes in species diversity, abundance and biomass presumably associated with levels of turbidity and inorganic nutrients. The blue-green algae, which were constituted largely by the potentially toxic taxa including, *M. aeruginosa* were dominant in abundance and biomass as biovolume throughout the sampling period. The phytoplankton community in Koka Reservoir showed marked seasonal variations with significantly higher abundance and biomass as chl-α and biovolume occurring in the dry season. As the reservoir is used by the local inhabitants for sanitation, watering livestock and drinking water supply, the cyanobacteria should be monitored to protect public health and aquatic and terrestrial lives.
Chapter 4. TEMPORAL VARIATIONS IN THE LEVELS OF TOTAL CYANOTOXINS AND THEIR VARIANTS ON THE AMUDDE SIDE OF KOKA RESERVOIR

4.1. INTRODUCTION

Massive growth of cyanobacteria in freshwater bodies, enriched with nutrients originating from agricultural and industrial activities, threaten public health and aquatic and terrestrial life (Heisler et al., 2008; Perovich et al., 2008; Paerl et al., 2011) as cyanobacterial blooms are often associated with the production of toxins (Chorus and Bartram, 1999; Azevedo et al., 2002; Huisman et al., 2005). Previous studies on Koka Reservoir showed the predominance of cyanobacteria including the potentially toxic taxa *Microcystis* spp, (Melaku Mesfin et al., 1988; Elizabeth Kebede and Willen, 1998; Hadgembes Tesfay, 2007). There is, however, limited information on the diversity of cyanobacteria and their toxins in Ethiopian freshwaters in spite of the report on the likely poisoning of animals in early 1980s by Amha Belay and Wood (1982). Although confirmed records are non-existent, there is anecdotal evidence for the association of death or poor health of livestock and human beings with cyanobacterial blooms on the Amudde side of Koka Reservoir.

Apart from the report on cyanotoxins production in seven Ethiopian Rift Valley lakes including Koka Reservoir by Willén et al. (2011), which was based on the analysis of single sample, published information on the seasonal dynamics of cyanobacteria and their toxins in Koka Reservoir is unavailable. This study, therefore, aimed to qualitatively and quantitatively assess the cyanobacterial toxins produced at Site 1 of the present study in Koka Reservoir.
4.2. MATERIALS AND METHODS

4.2.1. Sample collection and storage

Samples were collected from the already established inshore site (S1) of Koka Reservoir located in the proximity of a small village known as Arudde. This sampling site represents one of the reservoir areas in which annually recurring huge cyanobacterial blooms, implicated in the death of livestock and ailment of some people, were observed. Sampling was carried out at monthly intervals from May, 2013, to April 2014, as described in Chapter 2. Seston samples were collected from the surface water by both a bottle sampler (Van Dorn, vertical model) and 10 μm plankton net. Depending on the algal density, 200 to 300 mL of water samples were filtered using 47mm GF/F filters and the filters were subsequently dried at 50°C. Surface water samples were also collected and concentrated using 10 μm plankton net and lyophilized in a freeze-drier. The seston retained by the filters and the freeze-dried samples were stored at -20°C until the analyses of cyanotoxins were made.

4.2.2. Identification and quantification of cyanotoxins

4.2.2.1. Extraction of cyanotoxins

Extraction of toxins from cyanobacterial cells on filter

Two filters for each sampling date, containing cyanobacterial material (Ø 47 mm) were cut into 4 halves. The two halves of each filter were extracted separately either with 1.2 ml of 75% methanol for MCs; or with 1.2 ml of 100% methanol containing 0.1% trifluoro acetic acid (TFA) for CYN. The extracts were sonicated for 15 min in a bath sonicator (Bandelin Sonorex RK 156, Berlin, Germany) and for additional 1 min with a probe sonicator (Bandelin Sonopuls HD 2070 with 3 mm microtip, 30% pulse, 30% energy). After centrifugation at 10,000 x g for 10 minutes, the supernatants were aliquoted and concentrated by evaporation with nitrogen gas at 40°C. The residues intended for MCs analyses by HPLC-DAD and LC-ESI-MS/MS were resuspended in
75% methanol and clarified by centrifugation as depicted above, while the residues intended for CYN analyses were resuspended in water and clarified by filtration through GHP Acrodisc 13 mm syringe filters.

Extraction of toxins from freeze-dried cyanobacterial material

About 10 mg of the freeze-dried samples were weighed in duplicate and extracted for MCs with 1 ml of 75% methanol, while a second set of about 10 mg samples was extracted with 1 ml of 100% methanol containing 0.1% TFA for CYN. The extraction process proceeded further as described above.

4.2.2.2. Analyses of MCs

HPLC with diode-array UV detection (HPLC-DAD)

Samples were analysed using an Agilent (Waldbonn, Germany) 1100 series HPLC system consisting of degasser, quaternary pump, thermostated column compartment at 40 °C and diode-array detector operated at 238 nm or 200-300 nm, on Ascentis RP-Amide C_{16}, 3 µm, 100 mm × 4.6 mm I.D. from Supelco. The mobile phase consisted of A: 0.05% trifluoroacetic acid (TFA) in water and B: acetonitrile containing 0.05% TFA with the following linear gradient programme: 0 min 25% B, 7 min 70% B, 10 min 70 % B, 10.1 min 25% B; stop time :15 min; flow-rate:1 ml min⁻¹. Additionally, the samples were analysed on a Merck Purospher STAR RP-18e column, 55 mm ×4 mm I.D. with 3 µm particles and on a Merck Purospher STAR RP-18e column, 250 mm × 4 mm I.D. with 5 µm particles following the standard operating procedures described in Meriluoto and Spoof (2005). Extracts of *Microcystis aeruginosa* PCC 7820 (deposited at Institute Pasteur, Paris, France) producing mainly MC-dmLR, MC-LR, MC-LY, MC-LW and MC-LF and *Microcystis aeruginosa* NIES-107 (deposited at National Institute of Environmental Studies, Tsukuba, Japan), producing mainly MC-dmRR, MC-RR, MC-YR, MC-dmLR and MC-LR, were used for reference as described in Spoof et al. (2003).
MCs analyses by LC-MS/MS

The LC-MS/MS analyses were carried out on an Agilent Technologies (Waldbronn, Germany) 1200 Rapid Resolution (RR) LC coupled to a Bruker Daltonics HCT Ultra Ion trap MS (Bremen, Germany) with electrospray (ESI) source. The 1200 RR LC system included binary pump, vacuum degasser, SL autosampler, and thermostated column compartment set at 40 °C. Separation of the toxins was achieved on Supelco (Bellefonte, PA, USA) Ascentis C18 column, (50 mm × 3 mm I.D. with 3 μm particles) protected by a 4 × 2 mm C8 guard column. The mobile phase consisted of A: 0.05% Trifluoroacetic acid (TFA) in water and B: Acetonitrile containing 0.05% TFA. A linear gradient solvent programme was employed: 0 min 25% B, 5 min 70% B, 6 min 70% B, 6.1 min 25% B; stop time:10 min; flow-rate:0.5 ml min⁻¹. The ion trap was operated in the positive electrospray ion mode. Ion source parameters were set as follows: dry temperature at 350 °C, nebulizer pressure at 40 psi, dry gas flow at 10.0 ml min⁻¹, and capillary voltage of 4.0 kV. An MS scan range from 500 to 1200 m/z with the Smart Parameter Setting (SPS) function was employed. The ICC target was set to 300,000 with a maximum accumulation time of 100 millisecond. Abundant MS-MS fragmentation was assisted by the Smart Frag setting. The reference materials, extracts of Nies 107 and Ma 7820, were analysed in dilutions 1:10, 1:50 and 1:500 for identification and quantification purposes (Spoof et al., 2003). A standard of MC-LA was additionally analysed (Alexia Biochemicals, San Diego, CA, USA).

4.2.2.3 Analyses of cylindrospermopsin (CYN)

CYN analysis by HPLC-DAD

The same HPLC instrument used for the analyses of MCs was employed. The analyses were performed on a Merck Purospher STAR RP-18e column, 55 mm × 4 mm I.D. with 3 μm particles. CYN was detected by the diode-array detector at 262 nm or 200-300 nm. The mobile phase consisted of A: 0.05% trifluoroacetic acid (TFA) in water and B:
methanol-water (2:98, v/v) containing 0.05% TFA with the following linear gradient programme: 0 min 2% B, 5 min 2% B, 5.1 min 70% B, 7 min 70% B, 7.1 min 2% B; stop time of 17 min; flow-rate of 1.00 ml min\(^{-1}\) and injection volume of 10 μL. Certified calibration solution for Cylindrospermopsis, NRC CRM-CYN (National Research Council of Canada, Halifax, Nova Scotia) showed a retention time of 1.96 min on the Purospher STAR RP-18e 55 mm × 4 mm.

**CYN analysis by LC-MS/MS**

The LC-MS/MS instrumentation, the C18 column and the mobile phase system were the same as those described for MCs. The mobile phase consisted of solvents A, 99% water - 1% acetonitrile - 0.1% formic acid; and B, acetonitrile - 0.1% formic acid with the following linear gradient programme: 0 min 0% B, 2.5 min 0% B, 2.6 min 50% B, 4 min 50% B, 4.1 min 0% B; stop time of 10 min; the flow rate of 0.5 ml min\(^{-1}\) and injection volume of 5 μL. The ion trap was operated utilising positive electrospray ion mode. The ion source parameters were set as follows: dry temperature of 350°C, nebulizer pressure of 40 psi, and dry gas flow of 10.0 ml min\(^{-1}\). The capillary voltage was set at 4.0 kV. The MS scan range was m/z 395 to 440 and MS/MS fragmentation of the target mass m/z 416 was employed to get MS/MS spectra. The ICC target was set to 200000 with a maximum accumulation time of 100 milliseconds. CYN in the sample extracts was identified by comparing the retention time and MS/MS fragmentation with those of pure CYN standard (12.5 μM CRM-CYN). 1:5, 1:10, 1:50, 1:100 and 1:500 aqueous dilutions of the standard were analysed for quantification purposes. Area-based quantification of CYN utilized the MS/MS transition from m/z 416 to m/z 194, which is characteristic of CYN. The retention time of CYN standard was 1.70 min.

**4.2.3. Statistical analyses**

Redundancy analyses (RDA) was used to estimate how much of the variance of the abundance of the cyanobacteria and cyanotoxin concentration was explained by the
environmental variables using CANOCO for windows 4.5 version. Cyanobacterial abundance and MCs concentration were included in the RDA as dependent (response) variables, while environmental factors and chl-α were treated as independent (explanatory) variables. All other aspects of data manipulation for RDA are as described in Chapter 3.

4.3. RESULTS

4.3.1. Concentrations of cyanotoxins

The HPLC-DAD and LC-MS/MS analyses of cyanobacterial extracts showed the presence of measurable levels of microcystins in all samples (Fig. 4.1). The highest total microcystins concentration (33 µg L⁻¹) occurred in December, 2013, while moderately high concentrations were observed from September, 2013, to March, 2014 (Fig. 4.1). The lowest concentration of total microcystins was observed in April, 2014 (1.76 µg L⁻¹), coincident with the dominance by Cylindrospermopsis spp. of the phytoplankton community in the reservoir. In the freeze-dried plankton net samples, total concentrations of microcystins measured reached levels of 709, 351 and 372 µg (g dw)⁻¹ in May, June and August, respectively. Despite the dominance and moderate abundance of Cylindrospermopsis sp., in May-June, 2013, and March-April, 2014 (Fig. 3.3), cylindrospermopsin was not detected in any of the samples collected from the reservoir during the study period. Several variants of MCs were identified with MC-LR, and MC-YR being the major ones (Fig. 4.2). In the freeze-dried plankton net samples, smaller amounts of MC-dmRR, MC-dmLR and MC-LA were also detected (Fig. 4.3). Two variants of MC-dmRR – resembling MC-3-dmRR (RT 1.5) and MC-7-dmRR (RT 1.8) were detected by LC-MS/MS, both showing m/z of 512.8 but having different retention times (Fig. 4.3).
Fig. 4.1 Temporal variations in the total concentrations of microcystins (MCs, C) in relation to total abundance of Cyanobacteria (A), Microcystis and Cylindrospermopsis spp. (B) and total chlorophyll-a (Chi-a, A) at Site 1 of the present study in Koka reservoir.
Fig. 4.2. Temporal variations in the levels of major microcystins (MCs) variants as determined by HPLC-DAD (A and C) and LC-MS/MS (B and D) in relation to total concentrations of MCs at Site 1 of the present study in Koka reservoir.
Fig. 4.3. Temporal variations in the concentrations of minor microcystins (MCs) variants (in µg L⁻¹) as determined by HPLC-DAD(A) and LC-MS/MS(B) and by LC-MS/MS alone (in µg (g dwt)⁻¹, C) at an offshore station on the Amudde side of Koka reservoir.

The results of RDA of the relationship between the selected environmental parameters (explanatory variables) and the variance in the abundance of cyanobacterial taxa and MCs concentration are summarized in Table 4.1 and shown in Fig. 4.4. The first two axes of RDA ordination accounted for 83 % of the total variance in cyanobacterial species abundance and microcystin production due to the environmental variables.
Table 4.1. Summary of the results of RDA of selected environmental factors and cyanobacterial abundance recoded for an offshore station on the Amudde side of Koka Reservoir

<table>
<thead>
<tr>
<th>RDA statistics</th>
<th>Axis 1</th>
<th>Axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues</td>
<td>0.49</td>
<td>0.34</td>
</tr>
<tr>
<td>Species-environment correlations</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>Cumulative percentage variance of species data</td>
<td>48.7</td>
<td>82.7</td>
</tr>
<tr>
<td>of species-environment relation</td>
<td>48.7</td>
<td>82.7</td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td></td>
<td>1.000</td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td></td>
<td>0.98</td>
</tr>
</tbody>
</table>
Figure 4.4. RDA ordination diagram of abundance of major cyanobacteria taxa (Cyano= total cyanobacterial abundance, M. spp.= Microcystis spp., C. spp. Cylindrospermopsis spp., A. spp Anabaena spp) and concentrations of MCs in relation to important environmental variables (Temp= water temperature, DO=Dissolved Oxygen, Zsd= Secchi depth, Cond= conductivity, TP= Total phosphate, SRP = soluble reactive phosphate, NO3, NH4) for the first two axes.
The first axis explained 49% of the variance, with which ZSD, pH, temperature, NH₃, SRP and chl-a are correlated. The second axis, which explained 34% of the variance, is defined mainly by conductivity and NO₃. Abundance of total cyanobacteria and *Microcystis* spp. was correlated positively with temperature, chl-a, NH₃ and ZSD and negatively with NO₃, SRP and TP. On the other hand, *Cylindrospermopsis* spp. abundance was related directly to conductivity and NH₃ but inversely to TP, and NO₃. MCs concentration showed a positive relation to ZSD, chl-a and pH in Koka Reservoir.

4.4. DISCUSSION

So far relatively few studies have documented cyanobacterial toxins for the African continent, mainly from South Africa (Wicks and Thiel, 1990; Scott, 1991; van Halderen *et al.*, 1995), Morocco (Oudra *et al.*, 2001, 2002), and East African countries including Kenya, Uganda and Tanzania (Krienitz *et al.*, 2002, 2003; Ballot *et al.*, 2003; Ballot *et al.*, 2004a, 2004b; Mwaura *et al.*, 2004; Sekadende *et al.*, 2005; Okello *et al.*, 2009). The present study, along with the published data of a previous report on the reservoir (Willen *et al.*, 2011), confirmed the presence of high levels of cyanotoxins in Koka Reservoir. Although toxin levels do not necessarily coincide with maximum algal biomass, the maximum MCs concentration (33 μg L⁻¹) in Koka Reservoir occurred in December coincident with the largest algal biomass, when *Microcystis aeruginosa* dominated the phytoplankton community with 62.78 % contribution to the total phytoplankton abundance (Table-App. 3.3). The consistently high levels of MCs recorded from September through March correspond to the high densities of *Microcystis* spp. while the very low level of MCs in April, 2014, concurred with the dominance of the phytoplankton community by *Cylindrospermopsis* spp., mainly *Cylindrospermopsis africana*. In several investigations from Denmark, Germany, Czech Republic and Korea, 80 to 90 % of the samples containing *Microcystis* also contained microcystins (Chorus, 2001). Okello and Kurmayer (2011) obtained highly significant positive correlations between total MC concentration and *Microcystis* cell numbers for all their sampling sites in Ugandan freshwaters. Sitoki *et al.* (2012) also reported a significant linear relationship between *Microcystis* biovolume and MCs concentration, with the highest MCs
concentrations occurring coincident with *Microcystis* maximum biovolume in the Nyanza Gulf (Lake Victoria, Kenya).

The maximum value of MCs recorded in December of the present study is lower than that reported for the same month in a previous study made on Koka Reservoir (Willen *et al.*, 2011). This may be attributable to the inter-annual variations in the various physical and chemical factors influencing growth and bloom-formation of cyanobacteria that often result in large year-to-year fluctuations in the levels of cyanobacteria and their toxins (Park *et al.*, 1993). The present concentrations of MCs are, however, considerably higher than those of most African lakes and reservoirs including Lake Baringo, Kenya (0.08-3.3 µg L⁻¹; Ballot *et al.*, 2003), Headwater reservoirs, Kenya (0-2.85 µg L⁻¹; Mwaura *et al.*, 2004), Lake Chivero, Zimbabwe (0.1-1.6 µg L⁻¹; Mhlanga *et al.*, 2006) and several Ugandan freshwaters (0.02-10 µg L⁻¹; Okello and Kurmayer, 2011). The total concentrations of MCs (µg g⁻¹ dry weight, dw) determined for the freeze-dried plankton net samples of Koka Reservoir are close to the lower end of the ranges of concentrations reported for Lake Nakuru, Kenya (130-4593 µg g⁻¹ dw, Ballot *et al.*, 2004a; 149-4593 µg g⁻¹ dw, Krienitz *et al.*, 2005). Levels of MC-LR (174-313 µg g⁻¹ dw) measured for Koka Reservoir are also within the ranges reported from some temperate waters including lakes and dug-out ponds in Alberta (4 to 605 µg g⁻¹, Kotak *et al.*, 1995) and Japanese freshwaters (27 to 622 µg g⁻¹, Park *et al.*, 1993).

The most abundant variant of microcystin detected in samples from Koka Reservoir, microcystin-LR (MC-LR), is one of the most toxic with an LD₅₀ of 50 µg per kg body weight (WHO, 1998). MC-LR, produced as a secondary metabolite by *Microcystis aeruginosa* and other blue-green algal species, is also the most commonly occurring (Carmichael, 1992) and relatively persistent in the aquatic environment (Jones and Falconer, 1994; Jones and Orr, 1994). Studies in Australia have shown that MC-LR was present up to 21 days following the treatment of a *Microcystis aeruginosa* bloom with an algicide (Jones and Falconer, 1994). These facts reflect the serious health risk associated with the use of Koka Reservoir, in which recurrent cyanobacterial blooms have been observed, as a source of drinking water supply.
Despite the high abundance of *Cylindrospermopsis* spp. in May and July, 2013, and in April, 2014, the cyanotoxin cylindrospermopsin was not detected in any of the samples collected during the study period presumably due to the absence of cylindrospermopsin-producing species/strains in the reservoir. Bloom composition is not a dependable or consistent indicator of its toxicity. According to Davis *et al.* (2009), a given cyanobacterial species may have a variety of toxigenic and nontoxic strains, which cannot be distinguished morphologically. Furthermore, known toxigenic strains may not produce toxins under certain conditions (Vázquez-Coriano, 2011).

Environmental factors controlling the production of cyanobacterial toxins are not well-understood (Chorus, 1993). RDA (Fig. 4.4) results suggest the overriding importance of pH and ZSD in determining MCs production, although ammonia concentration, chl-a, *Anabaena* and *Microcystis* spp. abundance are also positively correlated with MCs concentration. Kotak *et al.* (1995) reported positive correlation of concentrations of microcystins with TP, SRP and chl-a. Wicks and Thiel (1990) contended that the toxicity of *M. aeruginosa* is influenced by pH and water temperature and suggested that the maximum toxicity of *M. aeruginosa* cultures is achieved at temperatures between 18 and 25 °C. However, ranges of water temperature changes in tropical water bodies are small, often between 1 and 8°C (Wang *et al*., 2002; White *et al*., 2003; Ahmed *et al*., 2008; Kotut *et al*., 2010) and this small increase in temperature, which is often within the optimum temperature range of *Microcystis* toxin production (25-30°C, Kim *et al*., 2005), may not significantly affect toxicity. Although correlations between these environmental factors and microcystins concentrations have been found in some studies (Jones and Jones, 2002), no single factor seems to trigger toxin production (Carmichel, 1997). Seasonal variations in nutrients, N:P ratios, light, pH, temperature, and water column stability largely determine phytoplankton composition and dominance by cyanobacteria (Paerl, 1988; Shapiro, 1990). Orr and Jones (1998) and Jähnichen *et al.* (2001) have also shown that abiotic factors like pH and temperature probably have only an indirect influence on the toxicity of *M. aeruginosa*. 
The levels of microcystin concentrations recorded during this study greatly exceed the drinking water guideline value of 1 μg L⁻¹ recommended by WHO. The results of this study confirm the long-held speculation that cyanotoxins may have been the major cause for the death and poisonings of humans, domestic and wild animals around Koka Reservoir. This is a serious problem particularly in countries like Ethiopia where alternative sources of drinking water supply and adequate water quality assessment and monitoring programs are non-existent.

The occurrence of cyanotoxins at concentrations above the acceptable limit in water bodies used for various domestic purposes presents new challenges to the management of freshwater resources in Ethiopia, a country in which a significant proportion of the population lacks access to clean potable water. The presence of cyanotoxins reflects the inadequacy of the existing water quality assessment and monitoring strategies, which often involve assessment of only microbial and physico-chemical water quality parameters, especially in water bodies showing evidence of progressive eutrophication. More importantly, although there are no confirmed records of human health problems that have been linked to toxic cyanobacteria in Ethiopia, the toxin levels recorded in Koka Reservoir, which is being used as the sole source of drinking water supply by some local inhabitants, suggest that the association of incidences of human health problems with cyanotoxins can no longer be ruled out. Owing to poor medical services and records in this country, health problems associated with consumption of toxin-contaminated waters can easily go unnoticed or wrongly attributed to other causes. A continuous monitoring program is, therefore, necessary to ensure the protection of public health, aquatic and terrestrial life.
Chapter 5. ZOOPLANKTON COMMUNITY STRUCTURE IN KOKA RESERVOIR: SPATIO-TEMPORAL VARIATIONS IN RELATION TO SOME PHYSICO-CHEMICAL AND BIOLOGICAL VARIABLES

5.1. INTRODUCTION

Zooplankton is one of the most important components of aquatic ecosystems as it serves as a link between primary producers and the consumers at higher levels in aquatic food chains (webs) thereby playing a vital role in the carbon-flow processes. On the other hand, some microzooplankton taxa are capable of exploiting efficiently components of the microbial food web and serving as top predators (Azam et al., 1983; Pace et al., 1990; Pace and Vaque, 1994; Nakano et al., 1998). Furthermore, several studies have shown the role of zooplankton in nutrient recycling and as bioindicators of change in aquatic ecosystems due to eutrophication and in channeling these changes to higher trophic levels (Stemberg and Lazorchack, 1994; Attayde and Bozelli, 1998; Straile and Geller, 1998; Pinto-Coelho et al., 2005; Burns and Galbraith, 2007). Hence, studies on the zooplankton community structure may provide us with valuable information usable in the sustainable management of water quality and biological resources.

Cultural eutrophication is one of the most common anthropogenic stressors of reservoirs worldwide (Carpenter et al., 1998), and may lead to shifts in the composition of zooplankton communities. Koka Reservoir, the subject of the present study, has become a eutrophic system due to input of high levels of nutrients, mainly nitrogen and phosphorus from anthropogenic sources. The reservoir receives influents originating from a tannery, conventional agricultural lands and floriculture farms, which have increased its nutrient load (Fassil Degefu et al., 2011). The enrichment of water bodies with essential nutrients (nitrogen and phosphorus) leads to eutrophication (Ryding and Rast, 1989). Under eutrophic conditions, cyanobacteria are often competitively superior to most other algae. Consequently, they frequently form blooms, with species of Microcystis, Anabaena and Cylindrospermopsis commonly dominating the cyanobacterial assemblages (Melaku
Mesfin et al., 1988; Elizabeth Kebede and Willen, 1998; Hadgembes Tesfay, 2007; Willen et al., 2011) in Koka Reservoir. Cyanobacterial blooms are known to adversely affect the zooplankton community structure owing to their low food quality, physical interference with feeding apparatus, poor palatability and potential toxicity (Lampert, 1987; Brett and M"uller-Navarra, 1997; DeMott et al., 2001; Ghadouani et al., 2004). Cyanobacterial toxins or cyanotoxins, with microcystins being the most common ones, affect both zooplankton community structure and secondary production (Kaebernick and Neilan, 2001; Haider et al., 2003). Microcystins decrease the feeding, survival and growth rates of zooplankton (Lampert, 1987; Rohrlack et al., 2005; Prieto et al., 2006; Hansson et al., 2007; Jang et al., 2007; Berry et al., 2009) although the small-sized zooplankton species are less affected by these toxins and consequently dominate the zooplankton community during cyanobacterial blooms (Edmondson and Lilt, 1982). The possible reason for this is that cyanobacterial species like *Microcystis* cannot be ingested by these organisms (Fulton and Paerl, 1987) or at least some small-sized cladoceran species develop stronger tolerance to toxic *Microcystis* than the larger species (Guo and Xie, 2006). Several studies have reported lack of large cladocerans (e.g. *Daphnia*) and calanoids and dominance of smaller forms such as small cladocerans, rotifers and protists in the zooplankton community of tropical shallow, eutrophic freshwater ecosystems (Lewis, 1979; Fernando, 1980; Aka et al., 2000; Gillooly and Dodson, 2000; Fernando, 2002; Garcia et al., 2002; Havens et al., 2009). The consistently high predation pressure imposed by the continuously reproducing planktivorous fish on large zooplankton (Van Leeuwen et al., 2007) may also account for the dominance of small-sized zooplankton in tropical waters.

Besides food type and predation pressure, seasonal changes in climate and physico-chemical parameters can influence the zooplankton community structure. Consequently, some species survive under a wide range of environmental conditions, while others are limited by many physico-chemical factors (Gannon and Stemberger, 1978; Neves et al., 2003; Leibold et al., 2004). According to Alldredge et al. (1984) and Sarma et al. (2005), higher temperatures and relatively lower saturation of dissolved oxygen concentration could influence the structure of zooplankton community in tropical waters. Rain intensity
and wind action can also regulate densities of zooplankton in tropical and subtropical reservoirs (Roldán and Ruiz, 2001).

In tropical environments, zooplankton are believed to show small seasonal variations in their production, biomass and species composition compared to their temperate counterparts (Burgis, 1971) due to the absence of marked seasonality in water temperature of tropical environments that favors continuous growth and reproduction of zooplankton (Hart, 1981). However, several researchers (e.g. Kassahun Wodajo and Amha Belay, 1984; Saunders and Lewis, 1988; Eshete Dejen et al., 2004; Isumbisho et al., 2006; Kå et al., 2006; Adamneh Dagne et al., 2008; Adamneh Dagne, 2010) have documented considerable variations in tropical zooplankton communities. The biomass, production, abundance and species composition of zooplankton are well documented for several tropical inland water bodies. Despite their immense ecological importance in freshwater ecosystems, relatively few studies have been carried out on zooplankton community structure in lakes and reservoirs in Ethiopia (e.g. Kassahun Wodajo and Amha Belay, 1984; Eshete Dejen et al., 2004; Ayalew Wondie and Seyoum Mengestou, 2006; Adamneh Dagne et al., 2008; Tadesse Dejenie et al., 2008; Tadesse Fetahi, 2010). The only published sets of data on zooplankton of Koka Reservoir are those of Melaku Mesfin et al. (1988), whose investigation involved the analysis of single occasion sampling, and Fassil Degefu et al. (2011). Data on the abundance and biomass of zooplankton collected over an extended period in Koka Reservoir are non-existent. Thus, the main objective of the present study was to investigate the temporal changes in community structure and biomass of zooplankton in this eutrophic cyanobacteria-dominated reservoir in relation to environmental factors.

5.2. MATERIALS AND METHODS

5.2.1. Sampling, identification and Enumeration of zooplankton

Zooplankton sampling was carried out at monthly intervals from the same two sites described under Chapter 2 from May, 2013, to April, 2014 (SI), and during only five
months of the same study period (S2). Samples were collected using a diameter of 62 μm-mesh plankton metered net towed vertically from 2m to the surface during the daylight hours. The samples, which were stored in 500 ml plastic bottles, were immediately preserved with formalin making final concentration of approximately 4 %. Identification of the zooplankton taxa was done using relevant taxonomic literatures (e.g. Koste, 1978; Van de Velde, 1984; Defay, 1988; Jeje, 1988; Korovchinsky, 1992; Nogrady et al., 1995; Sanoamuang, 2002; Jose De Paggi, 2002; Fernando, 2002; Linnen Von Berg et al., 2004). Counting of replicate subsamples was carried out after transferring an aliquot with a plastic pipette to a gridded counting chamber under an optical microscope. Zooplankton abundance was determined from total counts and expressed as number of individuals per liter of water (indiv. L⁻¹).

5.2.2. Estimation of zooplankton biomass

Zooplankton biomass is an important and necessary parameter for calculating the secondary production of this community (Melão and Rocha, 2004). Thus, the estimation of the dry weight of zooplankton species is a more useful variable for the study of trophic structure in aquatic ecosystems than density, especially considering its relationship with the trophic states of water bodies (Rocha et al., 1995). Biomass of zooplankton populations was, therefore, calculated using individual dry weight data according to Wetzel and Likens (2000) though estimation of secondary production was not intended. The biomass of crustaceans was estimated from body length–weight regression equations described in Huang (1999). Body length measurements of the dominant zooplankton taxa were done using an ocular micrometer. Body length of copepods was measured from the apex of the head to the end of the furcal rami, while that of nauplii was measured from head to bottom (Culver et al., 1985). Cladocerans were measured from the top of the head to the tip of the abdomen (Downing and Rigler, 1984). For dry mass determinations, nauplii, copepodites, cladocerans and copepods were sorted using a wide-mouth pipette and a needle. The sorted individuals were rinsed with distilled water and then placed in pre-weighed aluminum boats (previously prepared by drying at 60°C). The samples were dried at 60°C overnight in an oven. After drying, the samples were placed in a desiccator.
and then weighed for the determination of total dry weight of sorted zooplankton using a microbalance (precision = 10 µg). The total dry weight of sorted individuals of a particular zooplankton species was then divided by the number of individuals of the species to get dry weight (in µg) per individual. Length-weight relationships were then determined and biomass of zooplankton species was calculated as the product of the mean individual dry mass and abundance. Fresh weight estimates of rotifer species were taken from Adamneh Dagne et al. (2008), which were calculated from length measurements and individual volume approximations through the use of geometric formulae (Ruttner-Kolisko, 1977).

5.2.3. Statistical analyses

Paired t-tests were used to assess seasonal changes in zooplankton abundance and its spatial variation in Koka Reservoir. SPSS software package version 21 was used for statistical analyses. Aiming to reveal the influence of biotic and abiotic factors on the abundance of zooplankton, a multivariate analysis was performed using the program CANOCO 4.5. Detrended correspondence analysis (DCA) was used to determine the appropriate response model (linear or unimodal) for zooplankton abundance and biomass data. The performed DCA gave gradient length for the longest axis shorter <3 SD (Leps and Smilauer, 2003), which implies that response variables exhibit linear responses to environmental gradients (Ter Braak and Smilauer, 2002). Thus, the linear method of gradient analysis, redundancy analysis (RDA), was performed using log transformed data except for pH. Environmental variables with variance inflation factors (VIF) < 20 were selected to reduce the effect of multicollinearity. Before RDA was performed, zooplankton abundance data were transformed logarithmically using the formula log (x + 1), while environmental variables were standardized. The RDA-associated automatic forward selection procedure (CANOCO, Ter Braak and Smilauer, 1998) was used to select those environmental variables that best explain the variance in species data set (zooplankton abundance). The automatic forward selection procedure computes the significance of the addition of a given variable and the stepwise cumulative variance explained with all the selected variables in the model. The statistical significance in RDA
was assessed by Monte-Carlo permutation tests (unrestricted permutations, 499). The results of RDA were visualized in the form of ordination diagrams in Canodraw for Windows.

5.3. RESULTS

5.3.1. Zooplankton community composition and abundance

The taxonomic composition of zooplankton communities observed during the study period is summarized in Table 5.1. During the 12-month sampling period, a total of 52 species of zooplankton comprising 40 species of Rotifers, 8 species of cladocerans, 3 species of cyclopid copepods and a calanoid copepod were recorded. A larva of the dipteran Chaoborus sp., which was of rare occurrence, was also recorded. Rotifers, which were dominated by Brachionus calyciflorus, Filinia opoliensis, Keratella tropica, and Polyaerthra vulgaris, were the most species-rich zooplankton group. Keratella tropica was the most frequent and abundant species accounting for over 42.11% of total rotifer abundance. Among species of Filinia, F. opoliensis contributed the most with about 29.97 and 42.59% in March and February, 2014, respectively, while negligible percentage contributions came from all other species of Filinia. Cladocerans were represented by eight species: Bosmina longirostris, Ceriodaphnia cornuta, Daphnia barbata, Diaphanosoma excisum, Moina micrura, and Alona sp. Ceriodaphnia cornuta accounted for 43.11% of total cladocerans abundance, followed by Moina micrura whose percentage contribution was about half of that of C. cornuta (22.77%) (Table- App. 5.2). Cyclopid copepods were represented by only three species: Thermocyclops decipiens, Mesocylops aequatorialis and Cyclops sp, with Thermocyclops decipiens being by far the most abundant taxon. The calanoid copepods were represented only by a single species, Tropodiaptomus sp. The largest members of the zooplankton community in the reservoir were the cladoceran Daphnia barbata, and the calanoid copepod Tropodiaptomus sp.
Table 5.1 List of zooplankton species encountered in samples collected from Koka Reservoir from May, 2013, to April, 2014.

**Rotifers**

<table>
<thead>
<tr>
<th>Species</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anuraeopsis coelata</td>
<td>Cephalodella catellina</td>
</tr>
<tr>
<td>A. fisssa</td>
<td>C. forficata</td>
</tr>
<tr>
<td>Ascomorpha sp.</td>
<td>C. gibba</td>
</tr>
<tr>
<td>Asplanchna brightwelli</td>
<td>Collotheca ornate</td>
</tr>
<tr>
<td>A. priodonta</td>
<td>C. pelagic</td>
</tr>
<tr>
<td>Brachionus angularis</td>
<td>Coturella sp.</td>
</tr>
<tr>
<td>B. bidentatus</td>
<td>Conochilus sp.</td>
</tr>
<tr>
<td>B. dimidatus</td>
<td>Eosphora sp.</td>
</tr>
<tr>
<td>B. calyciflorus</td>
<td>Epiphanes brachionus</td>
</tr>
<tr>
<td>B. caudatus</td>
<td>Euchlanis sp.</td>
</tr>
<tr>
<td>B. falcatus</td>
<td>Filinia longiseta</td>
</tr>
<tr>
<td>Plaxionus patulus</td>
<td>F. novaezealandiae</td>
</tr>
<tr>
<td>Polyarthra indica</td>
<td>F. opoliensis</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>Hexarthra intermedia</td>
</tr>
<tr>
<td>Pompholyx complanata</td>
<td>Keratella tropica</td>
</tr>
<tr>
<td>Trichocerca capucina</td>
<td>Lecane aculeate</td>
</tr>
<tr>
<td>T. pusilla</td>
<td>L. bulla</td>
</tr>
<tr>
<td>T. ruttneri</td>
<td>L. luna</td>
</tr>
<tr>
<td>T. gracilis</td>
<td>Lecane. sp</td>
</tr>
<tr>
<td>Walga sp.</td>
<td>Lepadella sp</td>
</tr>
<tr>
<td><strong>Cladocera</strong></td>
<td><strong>Copepoda</strong></td>
</tr>
<tr>
<td>Alona sp.</td>
<td>Cyclopoid Copepods</td>
</tr>
<tr>
<td>Bosmina longirostris</td>
<td>Cyclops sp.</td>
</tr>
<tr>
<td>Ceriodaphnia cornuta</td>
<td>Mesocyclops aequatorialis</td>
</tr>
<tr>
<td>Daphnia barbata</td>
<td>Thermocyclops decipiens</td>
</tr>
<tr>
<td>D. pulex</td>
<td>Calanoid Copepods</td>
</tr>
<tr>
<td>Diaphanosoma excisum</td>
<td>Tropodiplantomus sp.</td>
</tr>
<tr>
<td>Diaphanosoma sp.</td>
<td></td>
</tr>
<tr>
<td>Moina micrura</td>
<td></td>
</tr>
</tbody>
</table>
5.3.2. Seasonality in zooplankton abundance

Differences in the abundance of zooplankton communities among sampling months were observed (Fig. 5.1). The mean zooplankton density of the rainy season (404.5 indiv. L\(^{-1}\)) was higher than that of the dry season (238.3 indiv. L\(^{-1}\)) at S1 although the difference between the two seasons was not statistically significant (t-test, P=0.054). Zooplankton abundance (indiv. L\(^{-1}\)) at S1 varied from 127.8 in October, 2013, to 514.6 in September, 2013, while at S2 zooplankton abundance ranged from 198.7 in February, 2014, to 312.1 in December, 2013 (Fig. 5.2.). The different zooplankton groups were different in their relative importance to total zooplankton abundance (Fig. 5.1). Among the zooplankton groups at S1, rotifers were about 7 and 2 times more abundant than cladocerans and copepods, respectively, with a mean percentage contribution to total zooplankton abundance of 50.65% (Fig. 5.1). At S2, the abundance of rotifers was 11 and 2 times those of cladocerans and copepods, respectively, with mean percentage contribution to total zooplankton abundance of 44.7%. The percentage contribution of rotifers to total zooplankton abundance at S1 was highest in May, 2013, and lowest in October, 2013, while the highest and lowest contributions of rotifers at S2 occurred in December, 2013, and February, 2014, respectively (Fig. 5.1). Copepods were the second most important group in terms of contribution to total zooplankton abundance at both S1(20.78%) and S2 (22.51%), while cladocerans were the least abundant with mean percentage contributions of 5.2% and 4.73% at S1 and S2, respectively. Copepods at S1 made the largest and smallest contributions to total zooplankton abundance in February, 2014, and September, 2013, respectively, while at S2, August, 2013, and February, 2014, were the months of maximum and minimum contributions of copepods to zooplankton abundance (Fig. 5.1).
Fig. 5.1. Temporal variations in the percentage contribution of zooplankton groups to total abundance at Sites 1(A) and 2(B) of the present study in Koka reservoir.
Fig. 5. 2. Temporal variations in the abundance of zooplankton groups at Site 1 (A) and Site 2 (B) of the present study in Koka reservoir.
Among the dominant rotifer taxa, *K. tropica* and *F. opoliensis* were present in the reservoir throughout the study period, with their highest peaks of abundance occurring in September and December, 2013, respectively at S1 (Fig. 5.3). The abundance of *B. calyciflorus* was high during the rainy season and decreased towards the dry season, with its highest peak occurring in June, 2013 (Fig. 5.3).

**Fig. 5.3.** Temporal variations in the abundance of major zooplankton species at Site 1(A) and Site 2(B) of the present study in Koka reservoir.
The dominant rotifers *F. opoliensis*, *K. tropica* and *P. vulgaris* attained their highest peaks of abundance in December, 2013, coincident with that of the copepod *T. decipiens* at S2. Among the cladocerans, *Ceriodaphnia cornuta* was prevalent during the dry season, with its largest peak in January, while *Diaphanosoma excisum* dominated the cladoceran assemblage during the rainy season. *Daphnia barbata* occurred in small numbers only during October to December, 2013, while *Moina micrura* occurred throughout the study period with its largest peak occurring during the dry season (Fig. 5.3). *Thermocyclops decipiens* was the most quantitatively important copepod with peaks of abundance in June-July and November-December, 2013, at S1. The developmental stages of copepods, the nauplii and copepodites, were present in the reservoir during the entire study period with their peaks of abundance occurring in August and June, 2013, respectively.

Fig. 5.4 shows the RDA ordination plot of the zooplankton community composition data with respect to environmental variables. The eigenvalues were higher for the first two ordination axes, which accounted for 56.3 % of the variance in the zooplankton community composition due to environmental variables. All the four ordination axes explained 69% of the variance in zooplankton community due to environmental variables (Table 5.1). The first axis showed high correlation with the levels of *Z*SD, TSS and NO3, while the second axis was correlated with the concentrations of TP and SRP (Fig. 5.5). Rotifers, Nauplii, *Diaphanosoma* and *Thermocyclops* showed strong but negative relationship with *Z*SD, while their relationship with TSS and NO3 was strong and positive.

**Table 5.2 Summary statistics of RDA**

<table>
<thead>
<tr>
<th>Axes</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalues :</td>
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<td>0.183</td>
<td>0.148</td>
<td>0.085</td>
<td>1.000</td>
</tr>
<tr>
<td>Species-environment correlations:</td>
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<td>0.957</td>
<td>0.849</td>
<td>0.943</td>
<td></td>
</tr>
<tr>
<td>Cumulative percentage variance of species data:</td>
<td>27.4</td>
<td>45.7</td>
<td>60.5</td>
<td>69.0</td>
<td></td>
</tr>
<tr>
<td>Cumulative percentage variance of species-environment relation:</td>
<td>33.8</td>
<td>56.3</td>
<td>74.5</td>
<td>85.0</td>
<td></td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td>0.812</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
5.3.3. Zooplankton biomass

Zooplankton biomass estimated by the length-dry weight (DW) regression method varied from 41.75 mg DW L\(^{-1}\) in May, 2013, to 133.9 mg DW L\(^{-1}\) in June, 2013, at SI (Fig. 5.5). The major contribution to total zooplankton biomass came from copepods whose percentage contribution averaged 42.19% varying from 19.81 mg DW L\(^{-1}\) in May to 59.9 mg DW L\(^{-1}\) in June, 2013 (Table – App. 5.3).
Rotifer biomass varied between 0.62 mg DW L\(^{-1}\) in October to 22.12 mg DW L\(^{-1}\) in June, 2013, at S1, with their contributions to total zooplankton biomass, which was the least among the zooplankton groups, averaged 10.85 % (Table–App. 5.3). Similarly, cladocerans had small mean percentage contribution (12.10%) to total zooplankton biomass with their biomass varying between 1 mg DW L\(^{-1}\) in May, 2013, and 22.6 mg DW L\(^{-1}\) in January, 2014.
Relatively high contributions to total zooplankton biomass were also observed for copepodites (18.37%) and nauplii (16.5%). At S2, zooplankton biomass was at its highest peak in August, 2013, before it consistently declined to its lowest level in March, 2014 (Fig. 5.5). Copepods made the largest contribution to total zooplankton biomass at S2 during all sampling months except August, 2013, during which nauplii were at their highest peak (Fig. 5.5). At both sampling sites, biomass of zooplankton was largely
constituted by the cyclopoid copepods *M. aequatorialis* and *T. decipiens*, with the latter making the largest contribution.

Fig. 5.7. shows the RDA ordination plot of the zooplankton biomass with respect to biotic variables. The four axes accounted for 48.4% of the variation in zooplankton biomass (Table 5.3). Zooplankton biomass was negatively correlated with biomass of *Cylindrospermopsis* spp. RDA analysis also revealed the negative relationship between chl-a concentration and biomass of zooplankton. Biomass of copepods and cladocerans showed a positive relation to biomass of diatoms but a strong negative relation to biomass of *Cylindrospermopsis* spp. On the other hand, rotifers and nauplii biomass were related positively to biomass of cryptomonads and euglenoids while negatively to biomass of *Anabaena* spp. and total phytoplankton biomass. However, the correlation between biomass of the most dominant phytoplankton taxa, *Microcystis* and *Cylindrospermopsis* spp., and zooplankton biomass was negative.

Table 5.3. Summary of RDA of the relationships between total zooplankton biomass and biomass of zooplankton groups with biomass of phytoplankton taxa and total chl-a of phytoplankton.

<table>
<thead>
<tr>
<th>Axes</th>
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<th>2</th>
<th>3</th>
<th>4</th>
<th>Total variance</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>0.294</td>
<td>0.190</td>
<td>0.072</td>
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</tr>
<tr>
<td></td>
<td>0.928</td>
<td>0.745</td>
<td>0.807</td>
<td>0.915</td>
<td></td>
</tr>
<tr>
<td>Cumulative percentage variance</td>
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<td>48.3</td>
<td>55.6</td>
<td>60.5</td>
<td></td>
</tr>
<tr>
<td>of species data</td>
<td>46.7</td>
<td>76.9</td>
<td>88.4</td>
<td>96.3</td>
<td></td>
</tr>
<tr>
<td>Sum of all eigenvalues</td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Sum of all canonical eigenvalues</td>
<td></td>
<td></td>
<td></td>
<td>0.629</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.7. RDA ordination diagram of biomass of zooplankton groups (Nauplii, Copepodites, Rotifers, Copepods, cladocerans) and total zooplankton biomass and biomass of phytoplankton taxa (Microcy = Microcystis spp., Anabaena spp., Diatoms, Chloph = Chlorophytes, Egul = Euglenoids, Cylind = Cylindrospermopsis spp., Crypt = Cryptomonads and Chl-a = total chlorophyll a of phytoplankton).

Mean zooplankton biomass varied between the dry (48.33 mg DW L⁻¹) and rainy (79.16 mg DW L⁻¹) periods although the differences between the two seasons were not statistically significant (t-test, p=0.079).
5.4. DISCUSSION

In their limnological investigation, Melaku Mesfin et al. (1988) identified only a few zooplankton taxa, which included a single rotifer species (*Keratella tropica*), three species of copepods (*Tropodiaptomus processifer*, *Metadiaptomus colonialis* and *Mesocyclops aequatorialis*) and two cladocerans (*Daphnia barbata* and *Moina micrura*) in samples collected from Koka Reservoir. However, Fassil Degefu et al. (2011) identified 25 zooplankton taxa and reported the predominance of rotifers in the zooplankton community of the reservoir. Despite the fact that the plankton net used was not the most appropriate for rotifers' capture, the results of the present study, similar to those of Fassil Degefu et al. (2011), clearly showed the overwhelming dominance of rotifers in terms of species richness and abundance over cladocerans and copepods, a pattern, which is common in tropical water bodies (Rocha et al., 1995). The present results also revealed the presence of over 20 genera of rotifers although only one genus was recorded in previous studies made on the same reservoir. The present study resulted in the identification of 52 zooplankton species, 25 of which were not reported previously for Koka Reservoir although they are still common in tropical inland waters. An even higher number of rotifers may have resided in the reservoir as the mesh size of the net used might have overlooked the smaller rotifers leading to the underestimation of the total number of rotifer species.

The dominance of rotifers was not unexpected as rotifers have been reported as the numerically most important zooplankton group for a number of tropical inland waters including the seven reservoirs of Paranapanema River, in Brazil (Sampaio et al., 2002), Prado Reservoir in Colombia (Guevara et al., 2009), Oyun reservoir, Offa, in Nigeria (Mustapha, 2010), lakes Kivu (Isumbisho et al., 2006) and Naivasha (Mavuti and Litterick, 1981) in East Africa and Lake Guiers, Senegal (Kâ et al., 2006). The dominance of rotifers in species richness and abundance over cladocerans and copepods is also consistent with the reports from lakes of varying trophic state in the Ethiopian Rift Valley (Kassahun Wodajo and Anha Belay, 1984; Green and Seyoum Mengestou, 1991; Green, 1993; Adannneh Dagne et al., 2008; Adannneh Dagne, 2010). The possible explanations for the predominance of rotifers in tropical water bodies include their ability
to ingest small particles (such as bacteria and organic detritus), which are abundant in eutrophic ecosystems (Conde-Porcuna et al. 2002). Their rapid population growth during short periods of favorable conditions (Pourriot et al., 1997) is another reason for rotifer dominance in reservoirs as this allows them to succeed in more unstable and dynamic environments. Less specialized feeding, high fecundity and frequent parthenogenetic reproduction are some of the other traits of rotifers that make them opportunists and r-strategists, which are favored in unstable and eutrophic environments (Allan, 1976, cited in Sampaio et al., 2002). Moreover, compared to larger filter feeders, rotifers are less affected by suspended solids whose concentrations are large in high turbidity water columns similar to that of Koka Reservoir, giving them a competitive advantage to rotifers over other zooplankton groups (Kirk, 1991). This is corroborated by the results of RDA (Fig. 5.4), which showed a strong and positive relationship of rotifer abundance with TSS and a strong but negative relationship between rotifer abundance and ZSD.

These characteristics of rotifers, combined with lower predation pressure due to their small size, give them a competitive advantage over the other zooplankton groups (Dumont, 1994).

_B. calyciflorus_, which was numerically superior to all other rotifers at S1 of the present study during the major rainy period (in June), is an opportunistic species often associated with disturbed and hypereutrophic waters (Leitão et al., 2006; Zanatta et al., 2006; Bielanska-Grajner and Gadysz, 2010). _Keratella tropica_ was also among the most abundant rotifer species during the present study though it was reported as a quantitatively less important taxon in the study made by Melaku Mesfin et al. (1988) in the same reservoir. The dominance/abundance of _K. tropica_ was also reported from such diverse tropical inland waters such as six pumped-storage reservoirs in China (Qiao et al., 2015), a large shallow lake in Thailand (Chaichana and Choowaew, 2013), a freshwater reservoir in India (Sharma and Tiwari, 2011), a warm monomictic lake in South-Eastern Mexico (Zanatta et al., 2006) and in Pacajus and Gavião reservoirs in a semi-arid part of Brazil (Leitão et al., 2006). The dominance/abundance of this species was associated with higher food availability, higher nutrient levels and longer retention time of the water
body (Leitão et al., 2006; Zunatta et al., 2006, Sharma and Tiwari, 2011; Chaichana and Choowaew, 2013; Qiao et al., 2015).

The cladoceran community was dominated by small taxa, similar to those of other cyanobacteria-dominated eutrophic environments, which is thought to be related directly to their ability to selectively feed on algal particles smaller than the most common bloom-forming cyanobacteria (Paranaguá et al., 2005). The most frequent cladoceran taxa in Koka Reservoir, Ceriodaphnia cornuta, Diaphanosoa excisum and Moina micrura, are commonly reported from freshwater lakes and reservoirs (Adamneh Dagne et al., 2008; Guevara et al., 2009) although their relative importance to the cladoceran assemblage in the water bodies varies. Daphnia barbata, which was reported as the dominant zooplankton about two decades ago in Koka Reservoir by Melaku Mesfin et al. (1988), was rare during the study reported here, occurring only during three months-October to December, 2013. D. barbata is one of the daphnid species considered typical for tropical inland waters occurring most commonly in turbid and productive water bodies (Green, 1995). As resource quality is a major constraint on herbivorous zooplankton survivorship, growth, and reproduction (Gulati and DeMott, 1997), the low diversity and abundance of Daphnia in Koka Reservoir seems to be related to the dominance of the large cyanobacteria in the reservoir, which are not easily ingested by these organisms. There are several reports concerning the absence or rarity of large cladocerans such as Daphnia in tropical shallow eutrophic freshwater ecosystems (Lewis, 1979; Fernando, 1980; Dumont, 1994; Aka et al., 2000; Gillooly and Dodson, 2000; Fernando, 2002; Havens et al., 2009).

The paucity or absence of cladocerans in different regions of the world has, however, also been attributed to biotic factors, which influence the seasonal dynamics of zooplankton communities in individual lakes, e.g., competition and predation (DuMont, 1994). It is generally believed that fish predation is the major factor responsible for the paucity of such larger zooplanktonic crustacean as Daphnia (DuMont, 1994). Moreover, there is increased risk of fish predation in shallow water bodies like Koka Reservoir (Ferrao-Filho et al., 2005) where planktivore species like Nile tilapia (Oreochromis niloticus) are
quantitatively important (Vijverberg et al., 2012). Because juvenile tilapia is visual-oriented predators feeding on larger zooplankton (Elhigzi et al., 1995), predation pressure is likely to have contributed to the scarcity of cladocerans in the reservoir. While directly suppressing populations of large zooplankton, visual feeders may indirectly enhance populations of small or highly evasive zooplankton like rotifers (Gliwicz and Pijanowska, 1989). The results of enclosure experiments conducted in Lake Kuriftu, Ethiopia by Brook Lemma et al. (2001) have also demonstrated the removal of large-sized cladocerans until only the small-sized ones and rotifers remained in the enclosure.

The high and very low abundances of rotifers and crustaceans, respectively, in Koka Reservoir contrast with the scenario observed for the highland tropical lakes Wonchi (Fassil Degefu and Schagerl, 2015), and Hayq (Tadesse Fetahi et al., 2011), and a highland reservoir in Tigray, Ethiopia (Tadesse Dejenie et al., 2012). The paucity of rotifers in these tropical water bodies was attributed to high predation pressure from the abundant cyclopoid copepods or the possible interference and exploitative competition for limited resources from the large cladocerans. Both mechanisms of suppressing rotifer populations especially by large-bodied crustaceans like Daphnia have been reported by several researchers (e.g. Fernando et al., 1990).

The high abundance of copepods seems to be associated with the high number of nauplii and cyclopoid copepodites as calanoid nauplii were observed only during a few months of the study period. Thermocyclops decipiens, which was described as less abundant taxon in Melaku Mesfin, et al. (1988), was the most abundant species in the present study, a finding that is consistent with the general contention that it is the dominant planktonic cyclopoid of the tropics (Lévêque et al., 2005). T. decipiens is commonly found and frequently numerous in big and small lakes and in mesoeutrophic and eutrophic reservoirs, as well as in small water springs (Reid, 1989). T. decipiens tends to be a numerically dominant species among planktonic crustaceans in the reservoirs of Brazil (Leitão et al., 2006) where it was considered as an indicator species for disturbed and nutrient-enriched environments (Sampaio et al., 2002). Sampaio et al. (2002) also found positive correlation between the abundance of T. decipiens and cyanobacteria of a
eutrophic reservoir (Barra Bonita). This species is dominant or quantitatively important in several tropical lakes and reservoirs, in which the phytoplankton tends to be dominated by one or several cyanobacterial species (Landa et al., 2007). In these systems, populations of *T. decipiens* tend to stay stable or at least abundant all year round (Landa et al., 2007) indicating the suitability of the quantity and quality of food available (i.e. the abundant algae, bacteria and organic debris) for zooplankton taxa such as *T. decipiens*. Rietzler and Espíndola (1998) also analyzed the gut content of *T. decipiens* and verified ingestion of a high percentage of organic detritus besides consumption of colonies of cyanobacteria (mainly *Microcystis*), a feeding habit that favors the dominance of this species under eutrophic conditions. The dominance of *T. decipiens* during the present study may, therefore, be attributed to its omnivorous feeding habits, which enables it to feed on both the frequently dominant cyanobacterium in the reservoir, *Microcystis*, bacteria, detritus and nauplii (Burgis, 1969). Adamneh Dagne et al. (2010) also attributed the dominance of *T. decipiens* to its avoidance of predation since its small body size makes it less attractive to a predator.

*Mesocyclops aequatorialis*, which was reported as the most abundant species in a previous study by Melaku Mesfin et al. (1988), was less abundant in this study. The calanoid copepod *Tropodiaptomus processors*, which was not included in a previous report by Fassil Degefu et al. (2011), was described as the most abundant copepod species by Melaku Mesfin et al. (1988). However, relatively few individuals of *Tropodiaptomus sp.* were encountered during October, 2013, to March, 2014, of the present study. On the basis of data obtained during the different investigations, it may be assumed that calanoid copepods have been noticeably reduced since 1987/88, with rare occurrence of only one genus. Studies conducted in several tropical aquatic ecosystems, in the search for zooplankton indicators of eutrophy, have generally indicated dominance of cyclopoid copepods over calanoid copepods as eutrophication becomes established (Wetzel, 1990; Pinto-Coelho et al., 2005).

On the basis of the first four axes of RDA, the recorded environmental variables explained 69% of the variance in the taxonomic composition of the zooplankton
communities. This clearly indicates that there are other undetermined factors within the reservoir that are important in structuring the zooplankton community, which may include predation by planktivorous fishes (Arcifa et al., 1992).

Seasonal variation in the density of major zooplankton groups and their biomass showed similar temporal trends at the two sampling sites. This spatial uniformity in zooplankton community could be attributed to the proximity of the two stations. Biomass of zooplankton varied markedly over the study period ranging from 41.95 to 133.94 mg DW L\(^{-1}\) and averaging 70.88 mg DW L\(^{-1}\). Although there are no previous data on zooplankton biomass recorded for Koka Reservoir and with which comparison can be made with present results, levels of total zooplankton biomass observed during the present study are within the range reported for the neighboring Lake Ziwai (6.48–356.8 mg DW L\(^{-1}\), Adamneh Dagne et al., 2010). Peak zooplankton biomass was observed in June, 2013, which was mainly due to the greater density of copepods and the rotifer *Branchionus calciflorus* during this period. Although rotifers were the most abundant zooplankton taxa, they made the least contribution to total zooplankton biomass, while copepods, which were more than 3 times larger than rotifers made the highest contribution to zooplankton biomass. This is consistent with the results reported for many tropical freshwater ecosystems in which copepods accounted for a major portion of zooplankton biomass, while rotifers constituted a minor fraction of total zooplankton biomass (Melio and Rocha, 2004; Adamneh Dagne et al., 2010; Tadesse Fetahi, 2010). Zooplankton biomass was weakly correlated with the biomass of cyanobacteria, which indicates the inefficient grazing of the zooplankton on cyanobacteria as the zooplankton are constituted by small-sized organisms that cannot ingest these large-sized microalgae (Fulton and Paerl, 1987).

Changes in the composition and abundance of zooplankton assemblages have largely been explained by differences in generation time, competitive ability in resource exploitation, and vulnerability to fish predation (Sommer et al., 1986). Hydrological features such as water-level fluctuations, water residence time, or flow rate are usually not considered as an important factor in lakes (Pace et al., 1992). Water residence time, a
parameter that summarizes different hydrological aspects such as inflow, water-level fluctuations, precipitation, and evaporation, should, however, be expected to play a regulatory role in water bodies like Koka Reservoir, in which changes in such hydrological conditions as water level and river inflow are considerable.
CHAPTER 6. AN ISOTOPIC ANALYSIS OF THE PHYTOPLANKTON-ZOOPLANKTON LINK IN A HIGHLY EUTROPHIC TROPICAL RESERVOIR DOMINATED BY CYANOBACTERIA

6.1. INTRODUCTION

As a result of increasing agro-industrial activities, many water bodies all over the world support massive cyanobacterial blooms, which is one of the most important environmental problems with serious ecological, economic and sanitary consequences (Ghadouani et al., 2006; Graneli and Turner, 2006; Lehman et al., 2010). Although light and nutrient availability are often regarded as factors of overriding importance for the development of cyanobacterial blooms (Conley et al., 2009), the subsequent success of these blooms may also be influenced by the grazing of herbivorous zooplankton (Boon et al., 1994; Smayda, 2008). Several studies (e.g., Lampert, 1987; Boon et al., 1994; Smayda, 2008; Kå et al., 2012) have assessed the role of zooplankton in cyanobacteria-dominated phytoplankton communities. The top-down effects of zooplankton are, however, considered to be negligible, particularly in tropical regions (Kå et al., 2012). Havens et al. (1996) argued that poor control of phytoplankton biomass by herbivorous macrozooplankton in lowland tropical and subtropical water bodies is due to the small size of the dominant zooplankton and their inefficient grazing on large algal units (filaments and colonies). However, size-specific preferences and trophic niche width might be different for distinct zooplankton species. Some cyanobacteria can be unsuitable as a food source for herbivorous zooplankton and adversely influence their feeding ability, and hence their growth (Chen et al., 2003; Chen et al., 2005). This may be due to their poor nutritional value, interference with filter-feeding, and toxin content of some species (Lampert, 1987; Haney et al., 1994; Pereira et al., 2000; Ferreira et al., 2001). Nevertheless, cyanobacteria-derived detritus may be a potential nutritional source for aquatic consumers that are usually abundant in tropical waters (Yu et al., 2013). Grazing on heterotrophic bacteria directly or on protists through the microbial food-web is also
another energy pathway from primary producers to zooplankton (Calbet and Landry, 1999; Sherr and Sherr, 2002).

To understand complex interactions in aquatic food-webs, analysis of functional groups with a resolution to the species level is a basic requirement (Burian et al., 2014). Although species-specific stable isotope analyses have recently been applied to analyze the trophic role of large zooplankton, information on particularly smaller-sized zooplankton is scarce. Stable isotope analysis is a powerful and widely applied tool to study energy and mass flows in plankton food webs (Fry, 2006). The stable carbon isotope signature (δ^{13}C) is used to infer consumer carbon source, while stable nitrogen isotopes are useful in determining the relative trophic position of biota. Thus, analysis of δ^{13}C and δ^{15}N values in biota can help determine how the basal carbon source of a food web is successively transferred to higher trophic levels. Furthermore, recent progress in the development of Bayesian mixing models (Moore and Semmens, 2008), such as MixSIR, MixSIAR (Stock and Semmens, 2013) or SIAR (Parnell et al., 2010), allows the incorporation of the uncertainty of sources and can give proportions of source contributions, which was not possible with previous models. Independent from trophic sources, the comparison of isotopic niche widths of zooplankton taxa allows for the identification of the degree of omnivory (Jackson et al., 2010).

Considering the persistent abundance and dominance of large-sized colonial and filamentous cyanobacteria in tropical reservoirs and their potential toxicity, we hypothesized that most zooplankton taxa do not feed on cyanobacteria when they dominate the phytoplankton community and alternative food sources are used. To test this, we determined the δ^{13}C and δ^{15}N isotopic fractionation between sestonic size-fractions and the dominant zooplankton species in this tropical eutrophic reservoir. Furthermore, we modelled isotopic niche width of two dominant zooplankton species to test whether zooplankton species generally overlap in their food spectrum.
6.2. MATERIALS AND METHODS

6.2.1. Study area

A description of Koka Reservoir is given under Chapter 1. The study reservoir receives wastewaters originating from a tannery and runoff from conventional agricultural lands and floriculture farms, which have increased its nutrient load dramatically (Willén et al., 2011). As a consequence, harmful cyanobacterial blooms have been recurring annually for the last 10 years (Willén et al., 2011). These cyanobacterial blooms are dominated by Microcystis, Anabaena and Cylindrospermopsis spp. (Elizabeth Kebede and Willen, 1998; Willén et al., 2011).

6.2.2. Sampling and preparation for stable isotope analyses

Samples were collected during the months of quantitative importance of cyanobacteria and/or high total chlorophyll-a (chl-a) concentration, that is in March, April and December, 2014, from the inshore site, S1 (8° 19'N and 39° 4'E). The sampling months also belong to the dry (December) and wet (March and April) seasons during which a shift in dominance of phytoplankton taxa generally occurs (unpublished data).

For stable isotope analysis of seston, triplicate water samples were collected from 0.5 m depth with a water sampler and placed in a 10-L plastic carboy. Immediately after sampling, each sample was first screened through a 100 μm net sieve to remove zooplankton. The seston from the triplicates was separated into two size-fractions (<20μm, and 20-100 μm) and aliquots for each size-fraction were concentrated onto Whatman GF/F filters that were pre-combusted for 4 h at 450 °C. Zooplankton samples for stable isotope analysis were obtained from repeated vertical hauls through the whole water column using a 62 μm mesh net.
Upon returning to the laboratory, zooplanktons were maintained in GF/F-filtered lake water for 24 h to allow for gut evacuation. Live crustacean zooplankton were sorted into five different species (Mesocyclops aequatorialis, Thermocyclops decpiens, Ceriodaphnia cornuta, Diaphanosoma excisum and Daphnia barabata) following narcotization of the organisms with carbonated water. Then sufficient numbers of the organisms were placed in pre-weighted dry tin capsules. Samples were dried at 60°C for 24 h and stored in desiccators until the analysis of $\delta^{13}$C and $\delta^{15}$N.

### 6.2.3. Identification of phytoplankton and zooplankton

Sampling and identification of phytoplankton and zooplankton are described in chapters 3 and 4, respectively.

### 6.2.4. Measurement of auxiliary parameters

*In situ* measurement and analysis of samples for auxiliary physico-chemical parameters were done as described under Chapter 2.

### 6.2.5. Stable isotopes analyses

Samples were analyzed by isotope-ratio mass spectrometry (DeltaPLUS, ThermoFinnigan) at the Stable Isotope Laboratory of the University of Vienna. All stable isotope values were calculated using the following equation:

$$\delta X = \left\{ \frac{(R_{\text{sample}}/R_{\text{standard}})-1}{1} \right\} \times 1000$$

where R is the ratio of heavy to light isotopes of the element (X) in samples ($R_{\text{sample}}$) and standards ($R_{\text{standard}}$). The isotope ratio is expressed in the conventional delta (δ) notation, defined as the per mil (‰) deviation from the isotope standard. Nitrogen was expressed in terms of its value relative to atmospheric nitrogen, while carbon was expressed in terms of its value relative to Pee-Dee Belemnite (PDB). The C/N ratio consumers exceeded 3.5 and ranged between 4.1 and 6.4. Therefore, lipid correction of consumers $\delta^{13}$C signature was done according to the equation of Post *et al.* (2007):
\[ \delta^{13}C_{\text{normalized}} = \delta^{13}C_{\text{untreated}} - 3.32 + 0.99 \times \text{C:N} \]

For the following analyses of stable isotope data, we used trophic enrichment factors for \( \delta^{15}N \) of 3.42±0.99 \% and 0.4±1.3 \% for \( \delta^{13}C \) (Post et al., 2002).

### 6.2.6. Isotope food source models

The relative contribution of the different size classes of seston (<20 \( \mu \text{m} \), \( \geq 20<100 \mu \text{m} \)) to zooplankton diets was assessed using dual isotope (\( \delta^{15}N \) and \( \delta^{13}C \)) SIAR (Parnell et al., 2010) and MixSIAR models (Stock and Semmens, 2013). MixSIAR models incorporate seasonally different source signatures and include uncertainty (Moore and Semmens, 2008). Firstly, the mean proportion of both food sources was modeled by pooling carbon and nitrogen signatures from both sampling seasons using SIAR. Secondly, a season-specific model was established to determine the relative contribution of the two seston fractions during the study period. The MixSIAR model, a Bayesian stable isotope mixing model that employs mixed effects framework to estimate the relative contributions of selected potential food items to the diet of zooplankton species, was applied (Stock and Semmens, 2013). Within MixSIAR, models' priors (prior probability distribution of food preference based on literature) were set to 'uninformative' as the diet composition of zooplankton species was unknown for our system. Markov chain Monte Carlo simulation was conducted by running three replicate chains on a "long" run length and confirmed model convergence using Gelman-Rubin and Geweke diagnostics (Gelman and Rubin, 1992). Finally, modeled posterior density estimates (n=3000) were extracted, and the estimated medians (50\% quantiles) were used for comparisons.

### 6.2.7. Isotopic niche area

The isotopic niche breadth and its seasonal variability was analyzed for *Ceriodaphnia cornuta* and *Thermocyclops decipiens* (i.e., the only species present in all samplings) using the Convex-Hull area in bi-dimensional isotopic space and evaluated using standard Bayesian ellipse analysis in R (SIBER). Such ellipse corrected isotopic niche
area is less influenced by extreme values and thus, represents a more reliable niche extension, when compared with the use of convex hulls (Jackson et al., 2011). Posterior area estimates of both consumer species and potential food sources (seston <20 µm and ≥ 20<100 µm) were modelled and the estimated medians (50% quantiles) were used for comparison. Isotopic area estimates of potential food sources were used to determine isotopic variability within sources. Estimated mean values were compared between species and time periods using ANOVA followed by post-hoc comparison (Tukey test) and illustrated with Beeswarm package (Eklund, 2015) of R (R Development Core Team, 2013).

6.2.8. Statistical analyses

ANOVA’s were used to elucidate whether the average δ¹³C and δ¹⁵N values were significantly different among zooplankton species and over time, and to examine whether there were significant differences between isotopic niche areas of the zooplankton species Ceriodaphnia cornuta and Thermocyclops decipiens. Welch two sample t-test was done to exclude coincidental differences of posterior estimates of bulk zooplanktons resource use. We ran these statistical comparisons in R (R Development Core Team, 2013).

6.3. RESULTS

6.3.1. Physico-chemical parameters

Secchi depth (ZSD) showed small seasonal variations (Table 6.1). The average surface and bottom water temperatures of the reservoir in the study period were 23.76 °C and 23.48 °C, respectively. The reservoir was well-oxygenated down to 5 m depth with a minimum depth-averaged value of 6.69 mg L⁻¹ in April and a maximum of 8.12 mg L⁻¹ in December, 2014. Conductivity (K₂S) in the dry season (381 µS cm⁻¹) was similar to that of the rainy season (376 µS cm⁻¹). The lowest (8.82) and highest (9.04) pH values of surface water were recorded in April (wet season) and December (dry season),
respectively. Inorganic nutrients were at high levels during all sampling months with peaks in SRP and NO₃-N concentration occurring in April, while those of TP and NH₃-N occurred in December, 2014.

Table 6.1. Ranges and means of physico-chemical parameters measured at SL of the present study in Koka Reservoir in the present study (March, April, and December, 2014)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Range</th>
<th>Mean ± SD (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>22.6-26.2</td>
<td>23.9 ± 2.0</td>
</tr>
<tr>
<td>Water depth (m)</td>
<td>5.1-5.7</td>
<td>5.33 ± 0.32</td>
</tr>
<tr>
<td>Secchi disk (m)</td>
<td>0.14-0.18</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>Diss. Oxygen (mg L⁻¹)</td>
<td>6.69 - 8.12</td>
<td>7.66 ± 0.62</td>
</tr>
<tr>
<td>pH</td>
<td>8.82-9.04</td>
<td>8.96 ± 0.12</td>
</tr>
<tr>
<td>Conductivity (K25, μS cm⁻¹)</td>
<td>372-381</td>
<td>377.67 ± 4.93</td>
</tr>
<tr>
<td>NO₃-N (μg L⁻¹)</td>
<td>36.6-83.6</td>
<td>63.87 ± 24.39</td>
</tr>
<tr>
<td>NH₃-N(μg L⁻¹)</td>
<td>345-394.29</td>
<td>362.03 ± 27.95</td>
</tr>
<tr>
<td>PO₄-P (μg L⁻¹)</td>
<td>62.64-193.15</td>
<td>115.53 ± 68.68</td>
</tr>
<tr>
<td>Total P (μg L⁻¹)</td>
<td>218.74-276.34</td>
<td>254.57 ± 41.89</td>
</tr>
</tbody>
</table>

6.3.2. Biological parameters

Throughout the sampling period, cyanobacteria were the most abundant phytoplankton accounting for more than 94% of total phytoplankton abundance (Fig. 6.1). *M. aeruginosa* was the dominant species in March and December, 2014, while *Cylindrospermopsis africana* dominated the cyanobacterial assemblage in April, 2014. Total chl-a concentration ranged from 155.1 μg L⁻¹ in April to 222.8 μg L⁻¹ in December with the latter value coinciding with the highest cyanobacterial abundance (Figs. 6.1 and 6.2). Though seston is assumed to comprise a mixture of phytoplankton, organic detritus and
inorganic particles, the high chl-α values seem to suggest a significant overall contribution of algae to the bulk of the organic sestonic fraction.

Zooplankton abundance of the dry season was higher than that of the rainy season though the difference was not statistically significant (t-test, $P = 0.54$). Among zooplankton taxa, rotifers were the most abundant during the study period contributing more than 58% to the total zooplankton abundance. This was followed by the copepods (13%) (Fig. 6.2).
Fig. 6.1. Changes in the abundance (A and B) and percentage contribution (C) of major cyanobacterial genera and species in relation to the total abundance of phytoplankton (Tphyto), cyanobacteria (Cyano) and other phytoplankton (Ophyto) in relation to total chlorophyll-a (Chl-a) (A) in March (M), April (A), and December (D) 2014.
The copepods were mainly dominated by *Thermocyclops decipiens*. The cladoceran zooplankton was dominated by small species, including *Ceriadaphnia cornuta* and *Daphninosoma excisum* and large cladocerans were absent except for the occurrence of *Daphnia barbata* in December, 2014.

![Graph showing changes in the abundance of major cladoceran(A), and copepod(B) species in relation to the abundance of metazoan zooplankton groups (C) in March (M), April (A), and December (D) 2014](image-url)

Fig. 6.2. Changes in the abundance of major cladoceran(A), and copepod(B) species in relation to the abundance of metazoan zooplankton groups (C) in March (M), April (A), and December (D) 2014.
6.3.3. Stable isotope composition of seston and zooplankton

Average values of δ¹⁵N and δ¹³C estimated for small seston particles (<20μm) were -26.86 ±2.06‰ and 13.05 ±2.37‰ (n=9), while signatures for larger particles (seston ≥ 20<100 μm) were -27.52 ±0.52‰ and 13.31 ±1.30‰ (n=9), respectively. Significant seasonal differences were found for both seston particle size-classes (F=138, P < 0.001) with particular separation of samples from December (Tukey test, P<0.05). δ¹³C and δ¹⁵N values of both seston fractions of the dry season (December) were lower than those of the rainy period (March-April). The mean δ¹³C signatures of seston fractions were slightly different with higher values for the <20 μm fraction (mean: -26.86‰) than for ≥20<100 μm one (mean: -27.52‰). However, there was no significant difference between the isotopic compositions of these fractions (p = 0.199 and p = 0.201 for δ¹³C and δ¹⁵N, respectively). Isotopic signatures of zooplankton in the reservoir were on average -23.31 ±1.10‰ for δ¹³C and 15.47 ±1.45‰ for δ¹⁵N (n=30). Signatures of the species Ceriodaphnica cornuta and Thermocyclops decipiens shifted to a lower mean δ¹³C and δ¹⁵N values in December. Trophic position varied significantly among zooplankton species (δ¹⁵N: H=15.9, P< 0.01), but no significant differences were found for carbon signatures. Zooplankton species were generally more δ¹⁵N- and δ¹³C-enriched relative to <20 and ≥ 20<100 μm seston fractions. In December, 2014, C. cornuta, D. barbata and T. decipiens were, however, δ¹³C-depleted relative to the <20 μm fraction, while C. cornuta and D. excisum were δ¹⁵ N-depleted relative to both seston size-fractions in April, 2014. The difference between mean δ¹⁵N values for zooplankton species and those for seston fractions varied temporally with the greatest isotopic difference (~3.28 ‰) occurring in December (dry period) between zooplankton and < 20 μm fraction and the smallest (~0.11‰) in April (minor rainy period) between zooplankton and the ≥20<100 μm fraction. The temporal variation in the difference in values of δ¹⁵C signatures between zooplankton and seston fractions contrasted with that for δ¹⁵N with the largest (~ -3.68‰) and smallest (~ -1.06‰) isotopic differences occurring in April and December, respectively, between zooplankton combined and the < 20 μm fraction.
There were no significant relationships between the $\delta^{13}$C and $\delta^{15}$N of bulk seston and those of crustacean zooplankton for $\delta^{13}$C and $\delta^{15}$N signatures (Table 6.2). The observed differences in the stable isotope signatures between the copepod and cladoceran species were not statistically significant (ANOVA tests for $\delta^{15}$N $F_{(2, 4)} = 4.325$, $P < 0.001$). In December, the $\delta^{15}$N of *C. cornuta* was about 1% lower than that of *D. barbata*, whereas there was a very small difference (0.37%) for $\delta^{13}$C between these
species. On average, *T. decipiens* was more enriched in $\delta^{15}N$ relative to $<20\mu m$ and $\geq20<100 \mu m$ seston fractions by +3.7% and +3.4%, respectively, (i.e., one or nearly one trophic level above seston, Fig. 6.3).

*Ceriodaphnia cornuta* was, on average, more $\delta^{15}N$-enriched than the $<20$-$\mu m$ and $\geq20<100 \mu m$ seston fractions by 1.18% and 0.92%, respectively, with strong and positive correlations between $\delta^{15}N$ values of *Ceriodaphnia cornuta* and those of $<20 \mu m$ ($r=0.958, p<0.05$) and $\geq20<100 \mu m$ ($r=0.822, p<0.05$) size fractions. Likewise, this species was more $\delta^{13}C$-enriched than the $<20 \mu m$ and $\geq20<100 \mu m$ seston fractions, by +1.07% and 1.73%, respectively, but with weak correlation between its $\delta^{13}C$ values and those of seston of $<20 \mu m$ ($r=0.064, p<0.095$ and $\geq20<100 \mu m$ ($r=0.367, p<0.076$) size fractions.

The correlations between $\delta^{15}N$ of zooplankton and inorganic nutrients were higher than those between $\delta^{15}N$ of seston and inorganic nutrients (Table 2). The positive correlations between mean $\delta^{15}N$ of zooplankton and the two inorganic nutrients, total phosphorus ($r=0.929, P=0.2411$) and nitrate ($r=0.983, P=0.117$), were high although not statistically significant. The high and positive correlation of $\delta^{15}N$ of zooplankton with ammonia was, however, significant ($r=0.999, P=0.023$) (Table 6.2).
Table 6.2. Correlation statistics of measured parameters related to stable isotope signatures.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$R$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerio-$\delta^{15}N$</td>
<td>CS-$\delta^{15}N$</td>
<td>+0.822</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{15}N$</td>
<td>+0.958</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{15}N$</td>
<td>+0.686</td>
</tr>
<tr>
<td>Cerio-$\delta^{14}C$</td>
<td>CS-$\delta^{14}C$</td>
<td>+0.367</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{14}C$</td>
<td>+0.064</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{14}C$</td>
<td>-0.945</td>
</tr>
<tr>
<td>Thermo-$\delta^{15}N$</td>
<td>CS-$\delta^{15}N$</td>
<td>+0.997</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{15}N$</td>
<td>+0.970</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{15}N$</td>
<td>+0.219</td>
</tr>
<tr>
<td>Thermo-$\delta^{14}C$</td>
<td>CS-$\delta^{14}C$</td>
<td>+0.962</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{14}C$</td>
<td>+0.831</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{14}C$</td>
<td>-0.835</td>
</tr>
<tr>
<td>*Meso-$\delta^{15}N$</td>
<td>CS-$\delta^{15}N$</td>
<td>+0.123</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{15}N$</td>
<td>+0.995</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{15}N$</td>
<td>+0.845</td>
</tr>
<tr>
<td>*Meso-$\delta^{14}C$</td>
<td>CS-$\delta^{14}C$</td>
<td>-0.916</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{14}C$</td>
<td>+0.934</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{14}C$</td>
<td>-0.168</td>
</tr>
<tr>
<td>*Diaphno-$\delta^{15}N$</td>
<td>CS-$\delta^{15}N$</td>
<td>-0.607</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{15}N$</td>
<td>+0.734</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{15}N$</td>
<td>+0.963</td>
</tr>
<tr>
<td>*Diaphno-$\delta^{14}C$</td>
<td>CS-$\delta^{14}C$</td>
<td>+0.438</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{14}C$</td>
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</tr>
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<td></td>
<td>Zoo-$\delta^{14}C$</td>
<td>+0.757</td>
</tr>
<tr>
<td>*Daphn-$\delta^{15}N$</td>
<td>CS-$\delta^{15}N$</td>
<td>-0.969</td>
</tr>
<tr>
<td></td>
<td>FS-$\delta^{15}N$</td>
<td>-0.683</td>
</tr>
<tr>
<td></td>
<td>Zoo-$\delta^{15}N$</td>
<td>+0.247</td>
</tr>
<tr>
<td></td>
<td>Chl-$a$</td>
<td>+0.889</td>
</tr>
</tbody>
</table>

(\(n=9\), unless specified)
A global mixing SIAR model including trophic enrichment factors revealed that mean signatures of both seston particle size-fractions and their deviations did not imbed all zooplankton isotopic values (Fig. 6.3). Generally, all zooplankton species had less negative $\delta^{13}$C signals compared to both potential food sources. The posterior distribution from MixSIAR suggests that the relative contribution of seston size-fractions to zooplankton diet differed significantly ($t=953$, $P < 0.001$) (Fig 6.4). The mean relative contribution of seston <20 $\mu$m was 95.6% when compared with the larger size-fraction. However, there were differences in the seasonal contribution of both seston size-fractions

\[ \text{6.3.4. Proportions of seston in zooplankton diet} \]
among the three months (Fig. 6.5). Isotopic signatures for April indicated a relatively low proportion of small seston as food for zooplankton, with mean estimates ranging between 55.3% and 58.2%. During March and December, the use of small seston fractions as food source was much more likely with a probability of more than 83.4% and 95% when related to the larger seston fraction, respectively.

Fig. 6.4. Mean posterior density estimates of the proportions of both sestonic food-source particles (seston <20 μm, ≥20<100 μm) of all sampled zooplankton species. Mean values of the portions are shown in the vertical lines, n=3000.
6.3.5. Isotopic niche breadth

Isotopic niche area of the species *Ceriodaphnia cornuta* and *Thermocyclops decipiens* showed significant seasonal variations. Bayesian posterior estimates indicated a larger isotopic niche area in March for both species (Fig. 6.6 A and B). For example, the mean areas for *C. cornuta* and *T. decipiens* were 16 and 21 times higher in March than in April, respectively (F=3936, P < 0.001). SIBER revealed small isotopic areas for potential food sources in March (Fig. 6.6 C and D), with mean areas of 0.15 and 0.63 for small and larger seston fractions, respectively. In April, however, a larger isotopic ellipse area with a mean value of 2.3 was found for the small seston fraction (Fig. 6.6 D).

![Fig. 6.5. Seasonal importance of small seston particles (seston size <20 μm) as food source for zooplankton species (Cer cor=Ceriodaphnia cornuta, Dia ex=Diaphanosoma excisum, Ther dec=Thermocyclops decipiens, Meso aeq=Mesocyclops aequatorialis, Daph=Daphnia barbata. Bold lines indicate median, dots show mean values, n=3000)
Fig. 6.6. Posterior area estimates of Stable Isotope Bayesian Ellipse in R(SIBER) of (A), *Ceriodaphnia cornuta*, (B), *Thermocyclops decipiens*, (C). Small seston particle, < 20 μm, (D). seston particles ≥ 20<100 μm in March, April and December, 2014. Small dots represent single posterior estimates; lines confine 50% central data and indicate the median(bold), n=3000 for zooplankton species (A, B) and 4000 for seston particles classes(C,D).
6.4. DISCUSSION

Because phytoplankton is difficult to isolate from other similarly-sized microplankton and detritus, seston is routinely used as a surrogate end-point with the implicit assumption that most of this material is composed of algae (Martineau et al., 2004; Zeng et al., 2010). Throughout the present study, the phytoplankton biomass, which was largely constituted by positively buoyant cyanobacterial taxa, was high and the bulk seston collected from the shallow euphotic zone was, thus, largely of algal origin. Some variability in ambient $\delta^{13}C$ and $\delta^{15}N$ values of seston in reservoirs understandably stems from variations in the type and quantity of land-derived particulates of potentially different $^{13}C$ and $^{15}N$ content carried by their influent rivers (Harding and Hart, 2013). Compared to natural lakes, reservoirs usually experience greater changes in depth, greater flushing rates, and have larger phosphorus and nitrogen loads (Kalff, 2002) which were also evident in the measured ambient levels of Koka Reservoir. Such influences on ecosystem properties might affect phytoplankton growth and their distribution in the water column (Longhi and Beisner, 2009; Mellard et al., 2011; Fehling et al., 2012), which in turn might affect the feeding of zooplankton and thus explain considerable isotopic variation within sampled seston and zooplankton in the reservoirs (Huo et al., 2013).

The isotopic fractionation in zooplankton tissue varies according to the types of consumed food and their signatures (Minagawa and Wada, 1984; Adams and Sterner, 2000), the relative feeding rate (fraction), and taxa specific metabolic turnover rate of different isotopes due to their slightly different mass (Fry, 2006). Furthermore, meta-analyses identified the effects of potential factors that affect trophic enrichments, such as starvation, diet quality, but also N excretion (McCutchan et al., 2003; Spence and Rosenheim, 2005). Generally, the accuracy of the “trophic enrichment factors” (TEFs) are in discussion (Galloway et al., 2015), but its consideration in bayesian modelling approach allows for the integration of this uncertainty when estimating the use of different food sources. A number of studies have demonstrated that plankton $\delta^{15}N$ is a good indicator of nitrogen sources (McClelland and Valiela, 1998; Vander Zanden et al., 2005) and consumer trophic position as the nitrogen pools of animals have $\delta^{15}N$
signatures regularly enriched by a certain value (typically, 3.4%) relative to their food sources (Vander Zanden and Rasmussen, 1999) in aquatic systems.

As expected, overall average $^{15}$N values of zooplankton were enriched relative to those of the bulk seston. However, when modelling the relative contribution of both size-classes of seston (i.e., <20 $\mu$m and $\geq$20<100 $\mu$m), we found significant differences in their importance for zooplankton species in Koka Reservoir.

The SIAR results demonstrated that zooplankton species from Koka Reservoir seem to be generally related to seston particles. However, a slight $^{13}$C-depletion of zooplankton samples indicated the use of additional food sources. This result contrasts with observations made in northern temperate lakes (Jones et al., 1999; Grey et al., 2000). For example, the $^{13}$C-depletion of zooplankton relative to POM reported for temperate lakes is commonly attributed to either accumulation in zooplankton of $^{13}$C-depleted lipids (Kling et al., 1992) or spatial separation between the location where zooplankton was sampled and where they feed (Zeng et al., 2010). Furthermore, this can also be caused by selective feeding by zooplankton on isotopically light carbon sources that may be masked or diluted by a large detrital contribution to seston that is enriched in $^{13}$C (del Giorgio and France, 1996). Moreover, phytoplankton collected during periods of high biomass such as the case of the present study tend to exhibit $^{13}$C-enrichment (Zohary et al., 1994; France et al., 1997) due to reduced isotopic fractionation at high cell densities or growth rates (France et al., 1997).

Mix SIAR results illustrate that the general diet for all zooplankton species in this reservoir is primarily composed of small seston particles (<20 $\mu$m) and most probably complemented to a much lesser extent by larger particles ($\geq$20<100 $\mu$m). The <20 $\mu$m fraction was presumably constituted by green algae, flagellates, diatoms (particularly *Aulacoseira granulata*), small colonies of *Microcystis* and fragments of *Cylindrospermopsis* filaments. This result suggests that the zooplankton species in this reservoir show a preference for smaller food particles. However, we cannot exclude the possibility that zooplankton use other potential food sources as our study investigated
only the role of seston particles. Although all taxa exhibited a preference towards small seston particles, the importance of this source contributing to their diet varied over time. In April, analyses indicated a nearly equivalent contribution of both seston particle size fractions, which might be related to general shifts within the phytoplankton community and thus isotopical drift (Lee et al., 2013), which was also observed in Koka Reservoir (Fig. 6.1).

The isotopic ellipse corrected areas demonstrated a generally wider niche width for the species *Ceriodaphnia cornuta* than for *Thermocyclops decipiens*. This difference was particularly clear in March and indicates consumption of a different prey and/or feeding strategy of both species. However, both species show the same seasonal patterns with increased range of stable isotope signals in March. This suggests that both species are forced to feed on a broader range of food sources and/or combine two feeding strategies (e.g., pelagic and benthic) in the early season, which is probably associated with the start of the minor rainy period. In modelling the variability of both potential food sources (seston<20 μm, ≥20<100 μm), we found that the isotopic variability in consumers is not an after-effect of both sources' isotopic variability.

The slight ¹³C-depletion of zooplankton species in relation to both seston size fractions and the variation of their isotopic niche area indicate that these zooplankton species may have carbon sources other than phytoplankton. Crustacean zooplankton is known to graze on a wide range of particulate matter, including phytoplankton, bacteria and detritus. The bulk seston for which we determined the stable isotope signature, can be considered the putative food source for the zooplankton, although the degree of food selection from within the bulk seston may vary between species and lake type (Grey et al., 2000).

The ¹³C-depletion in *Ceriodaphnia cornuta, Daphnia barbata*, and *Thermocyclops decipiens* relative to bulk seston observed at the time of the highest peak of *Microcystis* bloom in December, 2014, might be explained by selective feeding by these zooplankton species on isotopically light carbon sources that is relatively rare (del Giorgio and France, 1996). The discrepancy observed between the δ¹³C values of suspended particulate organic matter (POM) and zooplankton in lakes is usually attributed to POM dilution by
non-phytoplanktonic sources of organic carbon (del Giorgio and France, 1996). A study conducted by Kå et al. (2012) on the capacity of zooplankton communities to exert an effective top-down control on cyanobacterial blooms in freshwaters has also shown that none of the zooplankton species, which also included *Celiodaphnia cornuta*, ingested *M. aeruginosa*. This cyanobacterium overwhelmingly dominated the phytoplankton of the Koka Reservoir in December, 2014. Cyanobacteria such as *M. aeruginosa* are known to have negative impacts on *Daphnia* and other cladocerans (De Mott, 1999; Ferrão-Filho and Azevedo, 2003), copepods (DeMott and Moxter, 1991; Kurmayer and Juettner, 1999), and rotifers (Rothhaupt, 1991) thereby deterring grazing on them. As a consequence, cyanobacterial carbon is transferred inefficiently to herbivorous zooplankton, which might lead to a decoupling of primary and secondary production and to the subsequent accumulation of cyanobacterial biomass, which was also evident in Koka Reservoir in December 2014, despite the relatively high numerical abundance of zooplankton taxa (Fig. 5.2). Interestingly, there are some studies (Kå et al., 2012), which reported the consumption and/or size structure modification (cutting of filaments and mean size reduction) of filamentous cyanobacteria by copepods, cladocerans, or rotifers. The disparity in size between phytoplankton and their grazers are accentuated in tropical shallow freshwater ecosystems by the scarcity of large cladocerans (*Daphnia*) and calanoid copepods, and the dominance of small grazers such as small cladocerans and rotifers (Aka et al., 2000; Fernando, 2002). This explains the abundance, and sometimes proliferation, of large phytoplankton taxa, such as filamentous or colonial cyanobacteria in shallow tropical water bodies (Boon et al., 1994; Lazzaro, 1997).

The mean $\delta^{15}$N content of zooplankton taxa was strongly correlated with ammonia whose ambient levels were consistently high and, which seems to have resulted largely from the excreta of large flocks of domestic animals known to graze on daily basis in the reservoir's shores (unpublished data). This anthropogenic nitrogen, with presumably high $\delta^{15}$N, may be assimilated by primary producers and can be subsequently transferred to consumers via the food chain (Hansson et al., 1997; Harvey and Kitchell, 2000; Wayland and Hobson, 2001) thereby increasing their $\delta^{15}$N signatures. It is also possible that the $\delta^{15}$N-enrichment of zooplankton resulted from decreased fractionation due to rapid growth of the cyanobacteria (Goering et al., 1990), which formed persistent bloom during the present study period. Ferber et al. (2004) also reported high $\delta^{15}$N of cyanobacteria in
a small shallow lake with low N\textsubscript{2} fixation and high dissolved inorganic nitrogen (DIN) uptake rates. The more enriched $\delta^{15}$N values of copepods relative to those of cladocerans suggest that former were at a higher trophic position.

Overall, our results suggest that the $< 20$ μm seston particles, which were presumably constituted by nanophytoplankton, are the major sources of zooplankton nutrition in the reservoir, although we present evidence that some zooplankton taxa complement their phytoplankton diet with organic carbon of different origin, especially in reservoirs where cyanobacterial blooms are recurrent annual events.
Chapter 7. CONCLUSIONS AND RECOMMENDATIONS

Koka Reservoir, the subject of the present study, is a multipurpose anthropogenically impacted reservoir. Such human activities as shore-line modification for irrigation, excessive application of fertilizers on conventional agricultural lands and floriculture farms, and its use as a waste-disposal system for a tannery and floriculture industries have resulted in its high water column turbidity and tremendously increased levels of potentially limiting nutrients (particularly nitrogen and phosphorus). Turbidity and high levels of N and P have been shown to constitute the physico-chemical condition that often results in changes in phytoplankton community structure and the concurrent development of cyanobacterial blooms in lakes and reservoirs.

The phytoplankton community in Koka Reservoir was dominated by an assemblage including potentially toxic cyanobacterial assemblage, which was largely constituted by *Microcystis aeruginosa*. The results of this study also clearly demonstrated the presence of high levels of MCs almost throughout the year. This represents a serious threat to public health and life of domestic and wild animals.

The present results also show the numerical dominance by small-sized species/groups of zooplankton community in Koka Reservoir. Higher abundance of rotifers was a conspicuous feature of the zooplankton community in Koka Reservoir. Furthermore, most of the species of zooplankton identified in samples collected from this reservoir are those commonly found in eutrophic tropical water bodies. The weak correlation between zooplankton biomass and total chlorophyll-α and cyanobacterial biomass as biovolume, which was evident in the present investigation, seems to indicate that zooplankton grazing does not exert significant effect on the phytoplankton community in this reservoir.

Eutrophication of Koka Reservoir has resulted in the dominance of cyanobacteria, which are generally regarded less suitable food for zooplankton. However, the stable isotope results seem to indicate that copepods depend on seston sources, which, during the
present study period were largely constituted by phytoplankton, while they imply exploitation of other food sources by the cladocerans. However, I cannot account for the relative importance of the various food sources. My result suggest that <20 μm seston particles are the major sources of zooplankton nutrition at times of cyanobacterial dominance, although we present evidence that some zooplankton taxa complement their diet with organic carbon of different origin.

On the basis of the findings of the research work reported here, the following recommendations are made:

✓ As cyanotoxins can seriously compromise human and animal health, sometimes with lethal consequences,
  • continuous water quality monitoring and implementation of remedial measures are mandatory.
  • studies regarding the prediction of the occurrences of toxic blooms and their toxins must be implemented and strengthened in the future in order to avoid or reduce the potential risks associated with human and animal exposure to the toxins.
  • future investigations on cyanobacteria and their toxins should aim at unraveling the plausible explanation for the present observations and look into such other cyanotoxins as anatoxin-a that may be produced by cyanobacteria resident in the study reservoir.

✓ As our results suggest that some zooplankton taxa complement their diet with organic carbon of different origin, more research probably involving terrestrial-derived carbon is needed to resolve other sources for zooplankton diets, as this may also help in explaining how zooplankton coexist with cyanobacteria in this reservoir.

✓ To consolidate the present findings as to whether the zooplankton communities in Koka Reservoir are able to exert an effective top-down control on the cyanobacterial blooms, feeding experiments involving the major copepods
(Mesocyclops aquatorialis, Thermocyclops decipiens), cladocerans (Diaphanosoma excisum, Moina micrura and Ceriodaphnia cornuta), rotifers (Brachionus calyciflorus, Filinia opliensis, and Keratella tropica) and different cyanobacteria (Anabaena spiroides, Cylindrospermopsis africana and Microcystis aeruginosa) should be conducted.

✓ To have a more complete and accurate picture of planktonic communities' structure and interactions in the reservoir, a look into the role played by the microbial loop is needed.
8. LIST OF REFERENCES


community and algal potential in Taipinghu Reservoir, Anhui Province, China.

Kurmayer, R. and Juettner, F. (1999). Strategies for the co-existence of zooplankton with
the toxic cyanobacterium Planktothrix rubescens in Lake Zurich. J. Plankton Res.


Table 5.3: Contribution of zooplankton groups to total zooplankton biomass at S1 (%).

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>Rotifers</th>
<th>Cladocerans</th>
<th>Copepods</th>
<th>Nauplii</th>
<th>Copepodites</th>
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<td>42.19</td>
<td>18.37</td>
<td>16.50</td>
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metacommunity perspective applied to young Reservoirs. *Limnologica* 42:137-143.


### 9. APPENDICES

Table: APP. 1. Paired t-test on physicochemical and biological parameters between S1 and S2

<table>
<thead>
<tr>
<th>Pair</th>
<th>Parameter 1</th>
<th>Parameter 2</th>
<th>Paired Differences</th>
<th>95% Confidence Interval</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
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<td>Std. Error Mean</td>
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<td>7.84</td>
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<td>Pair 10</td>
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Table - APP. 1. Paired t-test on physicochemical and biological parameters between Dry (D) and Rainy (D) seasons.

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<td>.499</td>
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Table – APP. 3.1. Contribution of major genera of cyanobacteria and other algal group to total phytoplankton abundances at S1 (%).

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<th>M. spp</th>
<th>C. spp</th>
<th>A. spp</th>
<th>Other Cyanobacteria</th>
<th>Chlorophytes</th>
<th>Cryptomonads</th>
<th>Euglenoids</th>
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Table - APP. 3.2. Contribution of major genera of cyanobacteria and other algal group to total phytoplankton abundances at S2 (%).

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Table –APP. 3.3. Contribution of major species of cyanobacteria and other
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Table — APP. 3.4. Contribution of phytoplankton taxa to total phytoplankton biovolume at S1 (%).

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Table—APP. 5.1 Contribution of major species to the total rotifer abundance (%)

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Table– App. 5.2. Contribution of major species of cladocers to the total cladoceran abundance (%)

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<th>Sampling Date</th>
<th>Ceriodaphnia cornuta</th>
<th>Ceriodaphnia cornuta</th>
<th>Daphnia barbata</th>
<th>Diaphanosoma excisum</th>
<th>Moina micrura</th>
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