UNIVERSITEIT GENT
Faculty of Science

Vakgroep Biologie

Protistology & Aquatic Ecology

Krijgslaan 281-S8
9000 Ghent (Belgium)

Phytoplankton community structure and cyanobacterial blooms in the semi-arid highlands of Tigray, Ethiopia

Proefschrift voorgelegd tot het behalen van de graad van Doctor in de Wetenschappen: Biologie

Tsehaye Asmelash Dejene
2009

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Promoter:

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The research reported in this thesis was performed in the laboratory of Protistology and Aquatic Ecology, Biology Department, Ghent University, Krijgslaan 281-S8, B-9000 Ghent, Belgium. http://www.pae.ugent.be/

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Tsehaye Asmelash Dejene
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Chapter 1

1. Introduction and thesis outline

1.1. General introduction

Ethiopia is located in the easternmost part of the African continent. It stretches between 3° N and 15° N Latitudes and 33° E to 48° E Longitudes and is bordered in the north by Eritrea, in the south by Kenya, in the east and southeast by Djibouti and Somalia, and in the west by Sudan (Fig. 1.1). Ethiopia is the third largest country in Africa with an area of over one million km^2, and has more than 80 million inhabitants (CSA, 2007). The country is endowed with a variety of agro-ecological conditions ranging from desert to rainforest and from 120 meters below sea level (the Denakil Depression) to highlands with altitudes of over 4600 metres above sea level (m.a.s.l). Its complex topography and wide altitudinal variation also ensure a variety of temperature and rainfall patterns. The traditional Ethiopian classification of climate is based on elevation and recognizes at least three zones: 1) the Kolla zone (the hot lowlands) below 1800 metres, with mean annual temperatures of 20-28°C, 2) the Woina Dega (temperate) zone between 1800 and 2400 metres, with mean annual temperatures of 16-20°C, and 3) the Dega (cool) zone above 2400 metres, with mean annual temperatures of 6-16°C. Most of the population inhabits the Woina Dega and Dega zones that are cooler, healthier (lower incidence of parasitic diseases) and more suitable for agriculture.

The Ethiopian highlands (more than 1500 m.a.s.l) cover about 500,000 km^2. They represent about 43% of the country but support about 88% of the population (MNRDEP, 1994; Mohamed-Saleem, 1995), and account for 95% of the regularly cropped land, more than 70% of the livestock, and 90% of the economic activities of the country (Constable, 1984; FAO, 1986). The population density in the highlands is close to ten times that of the

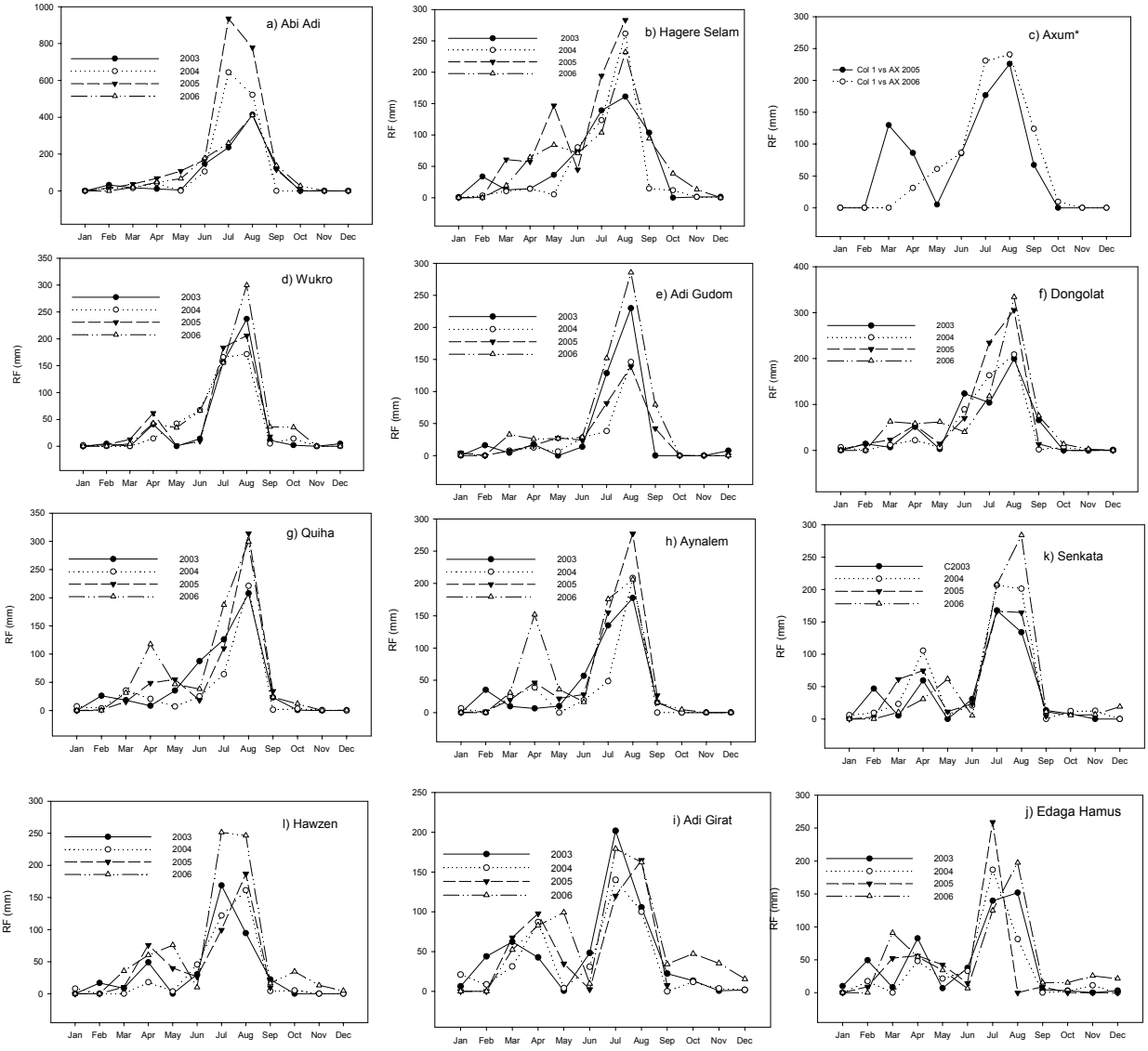
lowlands. The highlands have been settled for millennia and are known for a similar long-standing agricultural history (McCann, 1995). This long history of settlement and high population pressure has lead to un-sustainability in agriculture. The basis for the early development of agriculture and high human population densities in this agro-ecological zone likely has been the reasonably favourable climatic and ecological conditions, with sufficient rainfall, moderate temperatures, less incidence of tropical diseases, and well-developed soils. The highlands of Ethiopia are considered to be amongst the most degraded lands in Africa (El-Swaify and Hurni, 1996). The FAO (1986) estimated that 50% of the highlands are significantly eroded, 25% are seriously eroded and 4% have reached the point of no return.

Fig.1.1 Map of Ethiopia (from: http://www.fao.org/nr/water/aquastat/countries/ethiopia/ethiopia.pdf)

Most farmers in the Ethiopian highlands depend on rain-fed agriculture. Rainfall in Ethiopia is highly erratic with an extreme spatial and temporal variability. The country is often divided into three regions according to seasonal rainfall patterns with distinctive characteristics: 1) an extended single wet season in the south-west 2) a shorter single wet season further north and 3) a bi-modal pattern in the east with a short wet season in March-May preceding the main wet season. The erratic nature and extreme spatial and temporal variability of rainfall causes major socio-economic problems in the country (Shanko and Chamberlain 1998). As a result, Ethiopia has become known by the recurring droughts and the resulting famine that have occurred in 1888 – 1892 and more recently, in 1984 – 1985 (Pankhurst, 1966; Mehari and Vahlquist, 1976). There are recurrent periods of drought every 3-5 years in the northern parts of Ethiopia and every 6-8 years over the whole country (Haile, 1988). Part of the variability of seasonal and annual rainfall across time and space in the drought prone tropical African regions is known to be associated with the El Nino-Southern Oscillation (ENSO) phenomenon (Quinn, 1993; Seleshi and Demaree, 1995; Camberrlin, 1997; Camberlin, et al., 2001; Comenetz and Caviedes, 2002). The main wet season droughts in Ethiopia, for example, are more likely to occur during the years of warm ENSO events (Seleshi and Demaree, 1995; Seleshi and Zanke, 2004). The El Nino phase of the cycle is reported to relate to a low index phase of the Southern Oscillation and to be associated with an eastern and northern Australian drought, an east monsoon drought over Indonesia, deficient monsoon rainfall over India, and deficient summer monsoon rainfall over the highlands of Ethiopia (Quinn, 1993; Camberlin, 1997). El Nino has been related to a decrease in discharge of the Nile River, which mainly originates from the Ethiopian highlands (Griffiths, 1972; Quinn, 1993).

Extreme spatial and temporal variation in rainfall is also characteristic for the Tigray region, located in the northern tip of the country (Fig. 1.2). Realising the problems

and the potentials, agricultural development through irrigation has been a priority for the Ethiopian Government for the last two decades.



* Only two year data was available for Axum station.

Fig. 1.2 Monthly rainfall for selected meteorological stations around the study area (Tigray, North Ethiopia) for a period of 4 years (2003-2006), showing variation among years and sites [Source: MU - IUC, 2007: Digital database of climatological and stream flow data of Geba catchment, obtained from National Meteorological Services Agency and Ministry of Water Resources. VLIR (Belgium) - Mekelle University Institutional University Cooperation Programme, Mekelle (Ethiopia) and Leuven (Belgium)].

To achieve their goal, the Regional Government of Tigray established the 'Commission for Sustainable Agriculture and Environmental Rehabilitation for the Tigray Region (Co-SAERT)' in 1994. The commission's target was to achieve food self-sufficiency to the area

mainly by development of irrigated agriculture through the planning, design and construction of about 500 dams within ten years (SAERT, 1994, Sustainable agriculture and environmental rehabilitation in Tigray, unpublished), and accomplished only about 14%.

Reservoirs provide various services to the local people, such as the production of electricity, an increased availability of water for irrigation and drinking purpose, and/or benefits with respect to flood protection, navigation, fisheries, the attraction of tourists, etc. (Zwahlen, 2003). Ethiopia has many small, medium and large reservoir dams constructed for hydropower generation, irrigation and drinking water supply or a combination of them. Koka reservoir, constructed in the 1950's on the Awash River in central Ethiopia is used for commercial fisheries (Tudorance *et al*., 1999) in addition to its function for hydropower generation and irrigation water supply. According to Frenken (2005), small dams are defined to be less than 15 meter high and to have a capacity of less than 3 million m^3. The height of the medium and large dams in Ethiopia is 15 – 50 meters and their capacity ranges from 4 to 19000 million m^3. Currently several (seven) medium and large reservoirs are under construction for hydropower generation, irrigation and/or water supply in Ethiopia.

Multi-purpose small dams (micro-dams) constructed for irrigation supply are concentrated in the Amhara and Tigray regional states. The micro-reservoirs are intended to be permanent bodies of standing water between five and 50 hectares in size, established by the government and used by villages near the reservoirs. In Tigray regional state, there are more than 70 reservoirs (see fig. 1.3) and their ages vary between five and 20 years (Hurni, 1993; De Wit, 2003; Nigussie *et al.*, 2006; Tsehaye *et al*. 2007).

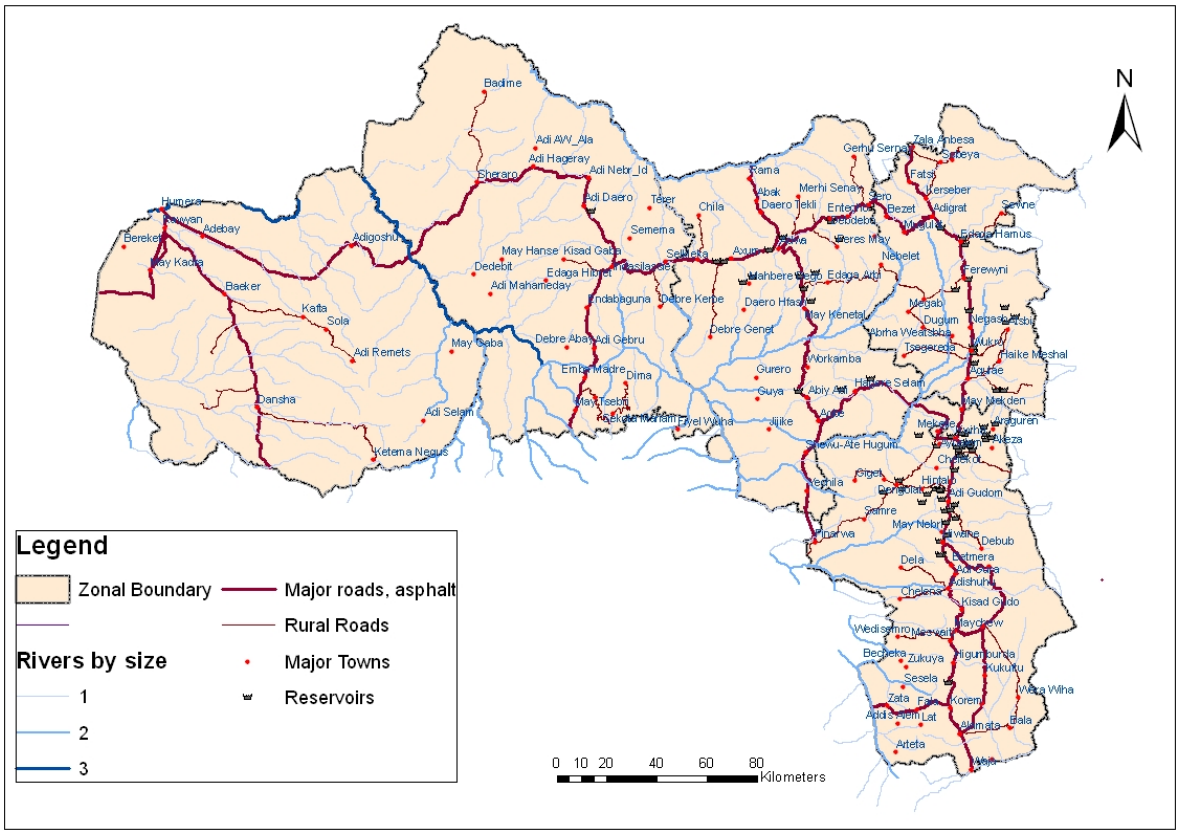


Fig. 1.3 Map of Tigray with distribution of reservoirs and lakes (Map from the Geographical & Statistical Information Office, Regional National State of Tigray, Ethiopia)

In addition to the role of these reservoirs in boosting agricultural development and preventing erosion, they have also potential to create added value for local residents, local nature value and biodiversity: 1) the water from the reservoirs can directly be used for household purposes, 2) some reservoirs hold the promise of enabling the culture of fish as a source of proteins and 3) they can also function as sites attracting bird life, amphibians, aquatic invertebrates and aquatic plants. In addition to its intrinsic value, biodiversity is an important indicator of ecosystem health and sustainability (Wilson, 1988) and shows important relationships with ecosystem functioning and ecosystem services (Myers, 1996; Holmlund & Hammer, 1999; Moss, 2000; Tilman, 1999; Luck *et al.*, 2003; Worm et al., 2006).

1.2. Reservoirs and cyanobacterial blooms in semi-arid regions

Reservoirs are man-made lakes. The requirement to store water to supply a wide range of human needs, and the operational procedures to fulfil that demand, may influence their ecosystem functioning and make them different from natural lakes (Straskraba & Tundisi, 1999). Reservoirs frequently differ from natural lakes in the shape of their longitudinal profiles. Natural lakes are normally deepest near the middle; reservoirs are almost always deepest just upstream from the dam (Baxter, 1977). As a result, reservoirs have been referred to as "half-lakes". Reservoirs often have a limited diversity because they are young and artificial systems, and because they show less development of vegetation along the littoral zone because of artificial changes in water level. Some authors consider reservoirs to occupy an intermediate position between rivers and natural lakes by combining numerous features of these two types of ecosystems (Kimmel *et al*., 1990). The extent of shoreline modification in a reservoir is likely to be greater than in comparable natural lakes because the annual drawdown exposes a large area to the effects of shoreline development (Baxter, 1977).

One key characteristic of reservoirs is that they experience pronounced changes in water level. The water level consistently rises during the rainy season(s), and goes down when the water is used without being refilled during the dry season. Also year-to-year variations can be very pronounced in the African semi-arid sub/tropics as a result of the large inter-annual variability in rainfall, river flow and lake level (Dagnachew *et al.*, 2002). The seasonal and inter-annual fluctuations of water level are significantly larger in shallow lakes and reservoirs (Vallet-Coulomb *et al.*, 2001). Different authors associate eutrophication and development of blooms of specific phytoplankton species with water level variations of reservoirs (Harris & Baxter, 1996; Bouvy *et al*., 1999, 2000). Dominance of a particular species within a phytoplankton community depends upon a

complex interplay of factors that include physical (e.g. retention time), chemical (e.g. nutrient loading) and biological (e.g. grazing) processes. Cyanobacteria are commonly encountered in many eutrophic reservoirs, and are known to contribute by their blooms to the deterioration in the aquatic environment (Chorus and Bartram, 1999). Harris & Baxter (1996) reported that cyanobacterial blooms are linked to a decrease in total water capacity in a subtropical reservoir.

Because of massive erosion linked to land degradation (Nyssen *et al.*, 2005), there is an excessive nutrient loading associated with sediment influx (Nigussie *et al*. 2006) to the reservoirs in Tigray. Many reservoirs are therefore expected to be characterized by high nutrient loads and phytoplankton blooms, including cyanobacteria blooms. This has indeed been observed in a field survey of reservoirs (Tsehaye *et al*. 2007; Dejenie *et al*. 2008).

Phytoplankton blooms are transient departures from quasi-equilibrium when the primary productivity temporally exceeds the losses and transports and the population grows rapidly and reaches exceptionally high biomass (Paerl, 1988). Typically, only one or a small number of phytoplankton species are involved, and some blooms may be recognized by discoloration of the water resulting from the high density of pigmented cells. Phytoplankton blooms usually are not single discrete events but rather are a series of fluctuations in which the biomass and the species composition of the phytoplankton population change rapidly. Blooms can be very generally classified into three types (Cloern, 1996): 1) recurrent seasonal events that usually persist over periods of weeks, 2) aperiodic events that often persist for periods of days and 3) exceptional events that are typically dominated by a few species and persist for months. Cyanobacterial blooms in the tropical regions fall into the persistent bloom type (3) (Sommer et al., 1986, Zohary & Robarts, 1989, Sivenon & Jones, 1999, Akin-Oriola, 2003.

Although there is no officially recognized threshold level, algae can be considered to be blooming at concentrations of hundreds to thousands of cells per millilitre, depending on the severity. Algal bloom concentrations may reach millions of cells per millilitre. Algal blooms are often green, but they can also be yellow-brown or red, depending on the species of algae. Algal blooms are monitored using biomass measurements coupled with the examination of species present. A widely-used measure of algal and cyanobacterial biomass is the chlorophyll concentration. Peak values of chlorophyll *a* for an oligotrophic lake are about 1-10 µg/l, while in a eutrophic lake they can reach 300 µg/l. In cases of hypertrophy, maxima of chlorophyll a can be as high as 3,000 µg/l. (Zohary and Roberts, 1990).

The considerations of cyanobacterial bloom threshold have been different in different studies. In marine systems, cyanobacterial blooms have been defined as surface accumulations of cyanobacterial filaments by analysing satellite images (e.g. Kahru et al., 1994) and the intensity of bloom is characterised by spatial coverage. When microscopic analysis is conducted the biomass of species or group of species is used. Bloom intensity can be described as the maximum biomass observed. Wasmund (1997) took the biomass value 0.2 mg l^{-1} as a threshold in his analysis as this "differences from 'normal' levels of species abundance" and because at that concentration aggregated cyanobacteria became visible in the water and appeared as a "bloom" to the beholder. Lips (2005) characterized the intensity of cyanobacterial blooms by integrated biomass values of *Nodularia*, *Aphanizomenon* and Anabaena over a bloom period defined bloom period as the period when cyanobacterial biomass exceeds 0.5 mg l^{-1} at one or more sampling points in the Baltic Sea. In this study we defined blooms of *Microcystis* when *Microcystis* accounts for 65% or above of the total phytoplankton community. In doing so, we look at the total intensity of bloom across the water column rather than surface accumulations.

1.3. Cyanobacteria and cyanotoxins

Cyanobacteria or blue green algae are among the oldest and most ubiquitous oxygenic photosynthetic organisms (Falconer 1989; Carmichael, 1994; Chorus & Bartram, 1999; Castenholz, 2001). Their habitats range from hot springs to temporarily frozen ponds in Antarctica (Whitton, 1992). They are common members of the plankton of marine, brackish and freshwaters throughout the world. They also occur on rocks and soils and in symbiosis with plants and animals. Cyanobacteria possess characteristics similar to both bacteria and algae. They are similar to algae in size; they contain blue-green and green pigments and can perform photosynthesis and produce oxygen (Castenholz, 2001). Like eubacteria they are prokaryotes, and lack a nucleus and other membrane bound organelles.

Cyanobacteria are morphologically diverse. Unicellular and filamentous forms are commonly found, with both morphotypes able to form large colonies and bundles of filaments (fig. 2) (Whitton and Potts, 2000). Cells and filaments in colonies may be arranged in different ways, e. g. radially, in strict planes, or irregularly. Filaments can be branching, coiled, or straight. Cells growing in colonies may be packed in a mucilaginous sheath (e.g. *Microcystis* species) or in the case of filamentous species grow as floating mats (e.g. *Aphanizomenon*) or as free-floating strands (e.g. *Oscillatoria*). Further differentiation amongst the cyanobacteria includes the ability of certain filamentous genera, such as *Anabaena, Aphanizomenon, Gloeotrichia, Planktothrix,* and *Nodularia*, to enzymatically fix atmospheric nitrogen (called biological nitrogen fixation) in specialized cells called heterocysts. Several of the filamentous genera also produce other differentiated cells called akinets (spore stages) which are useful for survival in periods of harsh conditions like cold and drought.

Blooms of cyanobacteria are increasingly frequent in aquatic ecosystems around the world as a result of eutrophication (Chorus & Bartram 1999, FRW, 2000; Huisman et al.

2005). Cyanobacteria have evolved many adaptations to survive, compete and achieve dominance in freshwater environments. Several explanations have been forwarded for the formation of cyanobacterial blooms in water bodies. These include: elevated temperature, nutrient enrichment, low N/P ratios, low light energy requirements, high pH and/or low carbon dioxide concentration, selective zooplankton grazing, excretion of compounds that suppress the growth of competing algae, and the capacity to float and form scums (Porter, 1973; Shapiro, 1973, 1984, 1990; Murphy *et al.,* 1976; Keating, 1978; Flett *et al.,* 1980; Tilman *et al.,* 1982, 1986; Smith, 1983, 1986; Haney, 1987; McQueen & Lean, 1987; Varis, 1993; Chorus & Bartram, 1999; Huisman et al., 2005). Shapiro (1990) suggested that a low carbon dioxide concentration and associated high pH is the key important factor for cyanobacteria dominance, the other factors (high temperature, low available light, lower N/P ratios, buoyancy, and low grazing losses) being related to CO_2 in some aspects. However, all factors may play a role in determining cyanobacteria dominance, and taking one factor in isolation may not help to understand the complex phenomenon of cyanobacterial bloom formation. Although the initiation of the blue-green maximum does not depend upon conditions of low CO_2 concentration or high pH (Shapiro, 1997), once they become abundant they may ensure their dominance by reducing concentrations of CO_2 to levels available only to themselves. The large growths and accumulations of cyanobacteria are often aesthetically undesirable because they discolour the water and cause turbidity. In addition, cyanobacteria are able to synthesize a number of low molecular weight compounds that cause taste and odour problems. These substances, including geosmin and methyl isoborneol often result in complaints regarding recreational and other water bodies and the quality of raw and treated drinking water. Production of low molecular weight compounds with high toxicity to vertebrates, including mammals by some taxa of cyanobacteria causes more concern. A number of common cyanobacteria can

produce a wide range of toxins in water, called cyanotoxins, causing a potential risk to livestock and human health (Chorus & Bartram 1999). The cyanotoxins are classified functionally into hepato-, neuro-, and cytotoxins. In addition to the cyanotoxins, cyanobacteria also produce lipopolysaccharides (LPS) as well as secondary metabolites that are potentially pharmacologically useful.

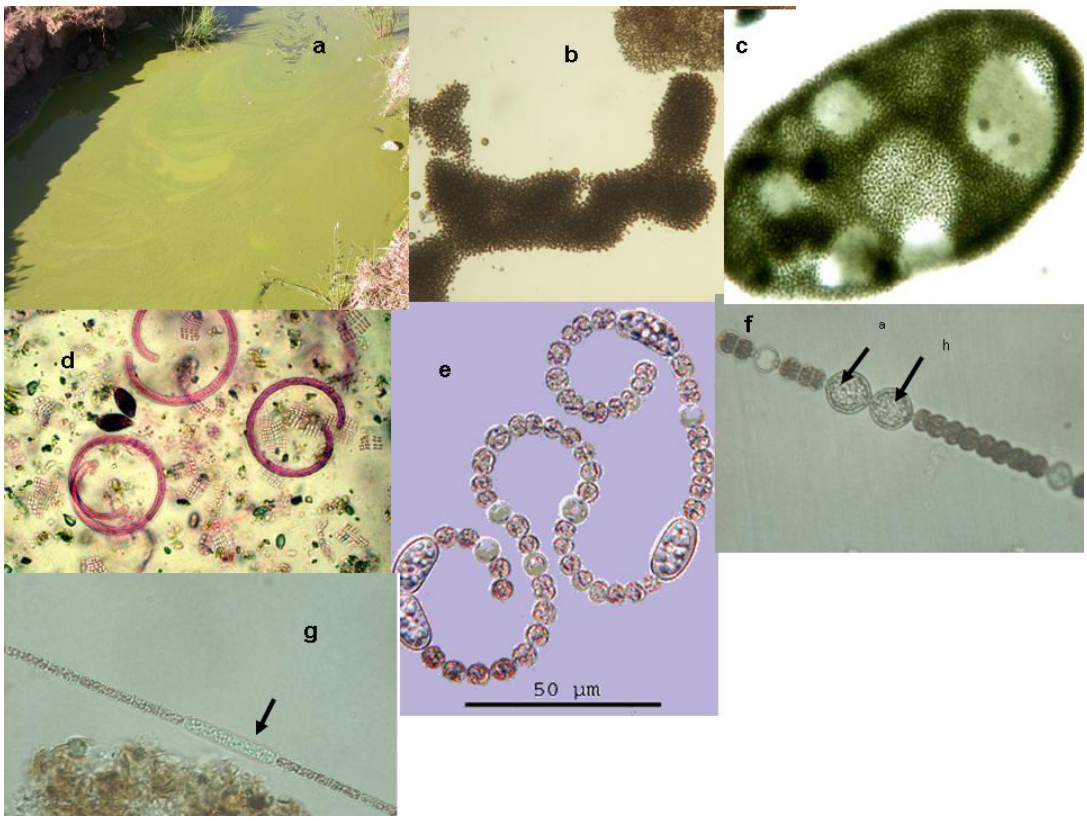


Fig. 1.4 Photograph of a cyanobacterial bloom in one of the study reservoirs (Mai Nigus) (a) and microphotographs of cyanobacterial colonies and filaments showing some of their morphological diversity (b-g). (b-c) colonies of *Microcystis sp.* (d) colonies of *Merismopedium* and filaments of *Anabaenopsis. (d)* coiled trichome of *Anabaena sp. (e) straight* trichome of *Anabaena sp.* and (f) trichome of *Aphanizomenon.* Heterocystis (h) and akinets (a) are indicated by arrows. Photos a and d are taken by Tsehaye Asmelash and photos b, c, a, d, e-g are taken by Jeroen Van Wichelen.

Defined by their chemical structure, cyanotoxins fall into three groups: cyclic peptides (the hepatotoxins microcystins and nodularin), alkaloids (the neurotoxins anatoxin and saxitoxins), and lipopolysaccharides (LPS). Of the cyanobacterial genera that include toxin forming species, the ones of particular concern when mass populations occur include

Microcystis, *Anabaena*, *Planktothrix (*formerly know as *Oscillatoria*), *Aphanizomenon*, *Cylindrospermopsis*, *Pharmidium, Nostoc, Anabaenopsis* and *Nodularia (*NHMRC 1994, Chorus and Bartram, 1999; FWR, 2004). These genera of cyanobacteria can produce a wide range of cyanobacterial toxins that vary in structure, toxicity and mode of action. Examples of toxic mass populations of cyanobacteria and their toxic effects on animal and/or humans have been reported in fresh, brackish and marine waters on a global basis (see Table 1.1 and 1.2)

Cyanobacterial toxins are generally divided into categories based on their modes of action in mammalian test systems (Codd, 1995; Sivonen and Jones, 1999) as hepatotoxins and neurotoxins. Although the most studied cyanobacterial toxins are the hepatotoxins and neurotoxins, cyanobacterial endotoxins (LPS) have also been implicated in human illness.

Table 1.1 Mass mortalities of flamingo species (*Phoenocopterus*) after exposure to ingestion of cyanobacterial biomass (from Codd et al., 2005)

Location	***Flamingo species***	***Numbers dying***	***Candidate toxigenic cyano-bacteria[b]***	***Presence of cyanobacterial toxins[c] in:***		***Reference***
				Blooms, mats, scum	***Bird tissues, excreta***	
Zoo pond, Orlando, Florida, USA	Chilean (*P. chilen-sis*)	10 (adults)	*M*	MC	MC	Chittick et al. (2002)
Wetland lagoon, Doñana National Park, Spain	Greater (*P. ruber*)	579 (out of 943 chicks)	*M, Ana, Osc*	MC	MC	Alonso-Andicoberry et al. (2002)
Lakes Nakuru and Bogoria, Kenya	Lesser (*P. minor*)	Thous-ands[a]	*Ana, An, Osc, Ph,* (others ?)	MC, ANA	MC, ANA	Ballot et al. (2002) Krienitz et al. (2003)

[a] Tens of thousands, 1991-1999 (see Krienitz et al., 2003); [b] *M, Microcystis; Ana, Anabaena; Osc, Oscillatoria; An, Anabaenopsis; Ph, Phormidium;* [c] MC, microcystins; ANA, anatoxins.

Table 1.2 Examples of human exposures to cyanobacterial blooms and toxins, with associated health outcomes (from Codd et al., 2005)

Year	*Location (source[a])*	*Cyano-bacteria[b]*	*Toxins[c]*	*Health outcomes[d]*	*References*
Drinking water					
1. 1975	USA (DWR)	*S, L, Ph*	?	GI	Lippy and Erb (1976), Keleti (1981)
2. 1979	Australia (DWR)	*C*	CYN	GI, LD, KD, ID	Byth (1980), Griffiths et al. (1998)
3. 1981	Australia (DWR)	*M*	MC	LD	Falconer et al. (1983)
4. 1972 – ca. 1990	China (SWR)	*M*	MC	PLC, D	Yu (1989, 1995)
5. 1988	Brazil (DWR)	*M, Ana*	?	GI, D	Teixeira et al. (1993)
6. 1994	Sweden (DWR, CR)	*Pl*	MC	GI, F, AP, MP	Annadotter et al. (2001)
Recreational/occupational water contact					
7. 1989	UK (FR, SW, KY)	*M*	MC	GI, ST, BM, V, AP, F, PC	Turner et al. (1990)
8. 1995	Australia (FR, SW, BA)	*M, Ana, Aph, Nod*	Hepato -toxins	GI, FLS, BM, F, EE	Pilotto et al. (1997)
9. 1996	UK (FR, BO)	*Pl*	MC	R, F	G. A. Codd & K. A. Beattie, Environment Agency report
10. 1996-1998	Australia (MR, SW, BA, FI)	*L*	?	CD, EE, RI	Dennison et al. (1999)
Haemo-dialysis					
11. 1974	USA (DC)	present	LPS	F, MY, C, V	Hindman et al. (1975)
12. 1996	Brazil (DC)	present	MC, CYN	VID, TIN, N, V, LD, D	Jochimsen et al. (1998), Pouria et al. (1998), Carmichael et al. (2001), Azevedo et al. (2002)
13. 2001	Brazil (DC)	*Ana, Mic*	MC	Same as in 12 (no D reported)	Carmichael et al. (2002)

[a] DWR, treated water from drinking water reservoir; SWR, raw water from surface sources; CR, contaminated with raw river water; FR, freshwater; SW, swimming; KY, kayaking; BA, bathing; BO, boating; MR, marine; FI, fishing; DC, dialysis clinic water.
[b] *S, Schizothrix*; *L, Lyngbya*; *Ph, Phormidium*; *C, Cylindrospermopsis*; *M, Microcystis*; *Ana, Anabaena*; *Pl, Planktothrix*; *Aph, Aphanizomenon*; *Nod, Nodularia*.
[c] CYN, cylindrospermopsin; MC, microcystin(s); LPS, lipopolysaccharide endotoxin(s).
[d] D, deaths; GI, gastroenteritis; LD, liver damage; KD, kidney damage; ID, intestinal damage; PLC, primary liver cancer; F, fevers; AP, abdominal pain; MP, muscular pain; ST, sore throat; BM, blistered mouth; V, vomiting; PC, pulmonary consolidation; FLS, flu-like symptoms; EE, eye and/or ear irritation; R, rashes; CD, contact dermatitis; RI, respiratory irritation; MY, myalgia: C, chills; VID, visual disturbance: TIN, tinitus: N, nausea.

1.3.1. *Hepatotoxins*

The hepatotoxins are low molecular weight cyclic peptide toxins. These are the toxins that have been most frequently linked to animal intoxication. Cyanobacterial hepatotoxines are reported the most common toxins worldwide and can cause gastro-enteritis, liver-damage and hepatocellular carcinoma's (MacKintosh *et al*., 1990; Repavich *et al*., 1990; Ohtani *et al*., 1992; Elder *et al*., 1993; Carmichael 1994; Chorus and Bartram, 1999; Humpage & Falconer, 1999). Some of the hepatotoxins (e.g. cylindrospermopsin) may also cause kidney damage in addition to liver damage. Based on their chemical structure, hepatotoxins can be divided into three categories: microcystins, nodularins and cylindrospermopsins.

Microcystins have been described from the genera *Microcystis, Anabaena, Planktothrix, Nostoc,* and *Anabaenopsis* (Botes et al., 1982; Carmichael et al., 1988a; Meriluoto et al., 1989; Namikoshi et al., 1990; Namikoshi et al., 1992). They are cyclic molecules (Fig. 1.5) that contain unusual amino acids and are synthesized nonribosomally via peptide synthetases (Meissneret al., 1996; Dittmann et al., 1997). Their molecular weight ranges from 800 to 1,000 Da (Botes et al., 1982a; 1982b).

Microcystin is a 7 amino acid ring, of which two, N-methyl-dehydroalanine (Mdha) and a 3-amino-9-methoxy-2, 6, 8-trimethyl-10-phenyldeca-4, 6-dienoic acid (ADDA), are almost unique to microcystin (Fig. 1.5). The structure of microcystin was first described from an isolate of *M. aeruginosa*. Meanwhile, more than 70 structural variants of this toxin are known (Botes et al., 1982a; 1982b, 1985; Rinehart et al., 1994; Sivonen, 1996) (which differ in their L amino acid components and the presence of certain methyl groups.) The current nomenclature names the most common structural variants, for instance microcystin LR (L, L-leucine; R, L-arginine) or microcystin-LW (W, L-tryptophane) (Carmichael et al., 1988b). Microcystin LR is the toxin most frequently associated with documented cases of animal poisoning (Eriksson *et al*., 1990; MacKintosh *et al*., 1990; De Mott & Dhawale,

1995; Zurawell *et al*., 2005). Nodularins are structurally similar to the microcystins (Fig. 1.5). They are cyclic peptides that also contain ADDA, but with only 5 amino acids in total. Unlike microcystins that are produced by a number of freshwater cyanobacterial genera, nodularins have only been documented in cyanobacterial blooms and strains of *Nodularia,* a bloom forming cyanobacteria typical of brackish and marine waters.

Cylindrospermopsins (Fig. 1.5) are alkaloids produced by *Cylndrospermopsis raciborskii, Umezkia natans* and *Aphanizomenon ovalisporum* (Sivenon & Jones, 1999). This toxin induces pathological changes in the liver but also in the kidneys, spleen, thymus and heart (Hawkins et al., 1985, 1997).

Cyanotoxins are usually found inside the cell, and are degraded very slowly after cell death (Sivonen, 1990; Rapala et al., 1997; Orr and Jones; 1988). Even cyanobacterial scums that accumulate and dry out on lake shores may retain high levels of microcystins for a few months (Jones et al., 1995). Animal intoxication may occur either by direct ingestion of living cells or by drinking contaminated water after the bloom collapses and toxins are released from the cells. Hepatotoxins are absorbed across the ileum and are then transported to the liver and taken up by hepatocytes. These toxins are potent inhibitors of protein phosphatases type 1 and 2A, enzymes that are crucial to cell growth and tumour suppression (MacKintosh et al., 1990; Matsushima et al., 1990; Yoshizawa et al., 1990; Runneger et al., 1995). Acute poisoning by hepatotoxins causes weakness, anorexia, pallor of the mucous membranes, vomiting, cold body extremities and diarrhoea (Carmicahel, 1992). Death due to intrahepatic haemorrhage and hypovolaemic shock may occur within a few hours. The LD_{50} for microcystin LR in mice is 50-160 μg Kg^{-1} body weight (bw) by intraperitoneal injection and about 5000 μg Kg^{-1} bw by oral route. Humans and animal mortalities due to cyanobacterial hepatotoxins have been reported from many countries

including Africa (Joshimsen *et al*., 1998; Ginkel, 2004; Jayatissa, *et al*. 2005; Kreintz *et al*. 2003).

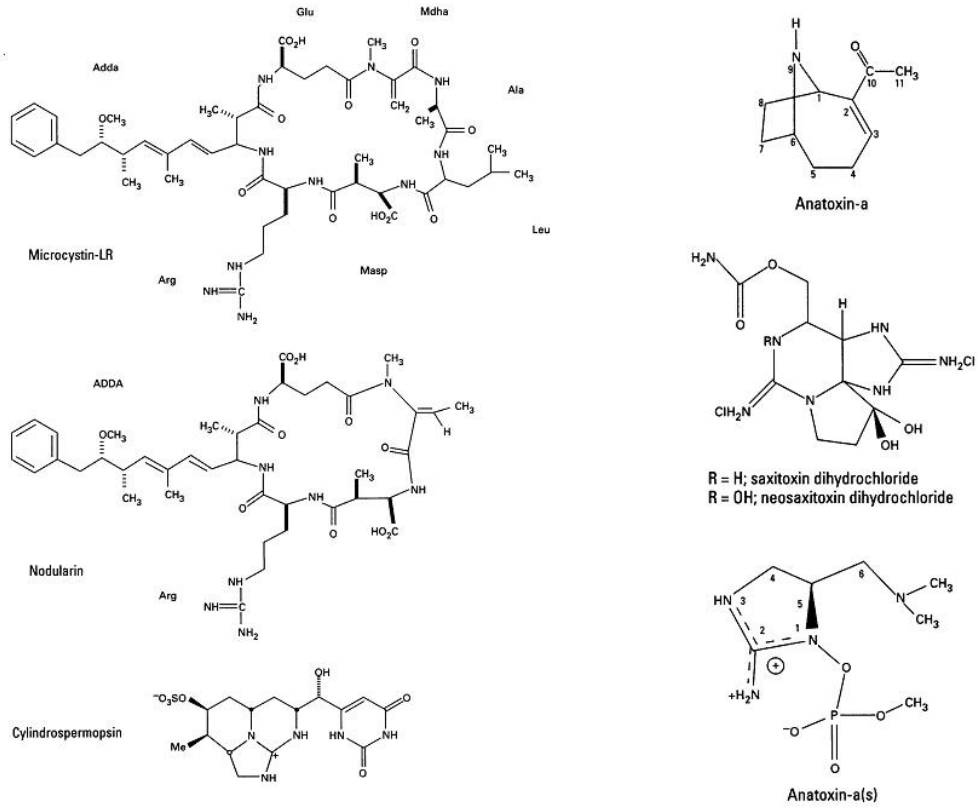


Fig.1.5. Structure of the toxic cyclic peptides (microcystin, nodularin, and cylindrospermopsin) and alkaloids (anatoxin-a, saxitoxins, and anatoxin-a(s) produced by cyanobacteria

1.3.2. Neurotoxins

There are three known cyanobacterial neurotoxins (fig. 1.5): anatoxin-a, anatoxin-a(s), and paralytic shellfish poisons (PSPs, also known as saxitoxins) (Carmichael, 1994; Sivonen & Jones, 1999).

Cyanobacterial neurotoxins disrupt the normal propagation of neural impulses to muscles, causing paralysis and death via respiratory failure and asphyxia in animals (Carmichael, 1994). Anatoxin-a is a low molecular-weight secondary amine that mimics acetylcholine. It binds to the nicotinic-acetylcholine receptors with higher affinity than acetylcholine, and is not susceptible to hydrolysis by acetylcholinesterase. It induces

muscle twitching and cramping, followed by fatigue and paralysis (Gorham & Carmichael, 1988a; Carmichael, 1994). Anatoxin-a(s) is an organophosphate that also causes muscle fatigue and failure, yet through a different mechanism. It binds to acetylcholinesterase, rendering it incapable of breaking down acetylcholine, which results in overstimulation of the muscle cells (Carmichael, 1994). Saxitoxins inhibit nerve impulse propagation along axons by blocking sodium ion entry into nerve cells through sodium channels, effectively suppressing stimulation of muscles, including those of the respiratory system (Adelman et al., 1982; Sivonen & Jones, 1999).

Anatoxin-a has been described in members of *Anabaena*, *Planktothrix Oscillatoria, Aphanizomenon*, *Cylindrospermum* (Devlin et al., 1977; Carmichael *et* al., 1990; Sivonen *et* al. 1989; Edwards et al., 1992; Skulberg *et* al. 1992; Mahmood & Carmichael 1986a,), and in lower concentrations in *Microcystis* (Carmichael et al., 1975; Park et al, 1993; Sivonen and Jones, 1999). Anatoxin a(s) is the only naturally occurring organophosphate and has first been isolated from *Aanbaena flos-aquae* and *A. lemmermannii* (Matsunaga et al., 1989; Mahmood & Carmichael, 1987; Henriksen et al., 1997; Onodera et al., 1997). Saxitoxins are well known from marine dinoflagellates (red tide) where they are responsible for paralytic shellfish poisoning after consumption of contaminated shellfish. However, they have also been detected in relevant amounts in freshwater cyanobacteria such as *Aphanizomenon flos-aquae*, *A. circinalis, Cylindrospermopsis raciborskii*, and *Lyngbya wollei* (Mahmood and Carmichael, 1986; Humpage et al. 1994; Negri and Jones, 1995; Lagos et al., 1999; Kaas & Henriksen, 2000; Pereira et al., 2000; Pomati et al., 2000; Ferreira et al., 2001; Li et al., 2003). Anatoxin-a, anatoxin-a(s), and saxitoxins are all lethal to animals, with LD50 based on intraperitoneal (ip) injection of mice of 200, 20, and 10 µg/kg, respectively (Carmichael, 1992; Monserrat et al., 2001).

1.3.3. Lipopolysaccharides (LPS)

Lipopolysaccharides, also called endotoxins, are structural components of the outer layer of the cell envelope of all gram negative bacteria. LPS is heat stable and toxic to mammals, with its toxicity varying among different bacterial and cyanobacterial isolates. The LPS molecule consists of three main parts: the O antigen, a core polysaccharide and lipid A. The lipid A is responsible for the biological effects and responses.

Unlike LPSs of most bacteria, cyanobacterial LPS lack phosphate in the lipid A (Keleti and Sykora, 1982). In animal experiments, cyanobacterial LPS is about 10 times less toxic than enterobacterial LPS (Codd, 1984). There are conflicting results on the effect of the endotoxins on mice. Some studies report lethal effects upon injection of the toxin (Keleti et al., 1979; Keleti and Sykora, 1982), while others report no effect (Weise et al., 1970; Keleti et al., 1979).

1.4. The aquatic food web

Knowledge on how species interact within ecosystems is necessary to understand how natural and anthropogenic pressures will affect ecosystem structure and functioning (Lampert & Sommer, 1997, Moss, 1998, 2000; Scheffer, 1998, Begon et al., 2006). We need information on abiotic environmental conditions as well as on the structure of the food web in the system to understand the processes and factors that cause algal blooms in reservoirs, for example. Below, we provide a brief overview of the aquatic food web structure in lakes and reservoirs and the interaction between the different food web components.

1.4.1. ***Planktonic food webs in standing freshwater systems***

The classic concept of food webs states that energy and nutrients flow up through well defined trophic levels (Krebs, 1994; Begon *et al*., 1996). In lakes, phytoplankton cells at

the bottom of the food web fix carbon; hence they are primary producers that support the upper trophic levels of secondary producers. The principal grazers, such as copepods and cladocerans, feed on phytoplankton and are in turn consumed by larger predators, such as insects and fish (Fig. 1.6). In many lakes secondary carnivores, including piscivorous fish, are at the top of the food web (Valiela, 1991).

The efficiency of the transfer of energy from one trophic level to the next is low. In most cases only 10% or less of the energy flowing into one trophic level is transferred to the next (Begon *et al*., 1996). As a result, a limited number of trophic steps can be sustained in open water food webs. So much energy is dissipated by respiration of organisms at each trophic level that by the fifth trophic step, virtually no energy remains to be passed on to an additional trophic level (Begon *et al*., 1996). In this classic view of food webs, bacteria are assumed to have only the role of degrading the dead organic matter that was not consumed. Bacteria (and fungi) are indeed the principal remineralizers of the nutrients that are sequestered in the organic matter synthesized by primary and secondary producers. These remineralized nutrients, plus externally supplied nutrients, keep the system going (Valiela, 1991).

The traditional view of the role of bacteria in plankton communities as remineralizers of nutrients became modified during the 1970s and 1980s. New techniques revealed that heterotrophic bacteria in the plankton were much more abundant and productive than previously thought. Moreover, several studies showed that in marine environments there was an alternative pathway of carbon flow that led from bacteria to protozoa to metazoa, with dissolved organic matter (DOM) being utilized as substrate by the bacteria (Steele, 1974; Pomeroy, 1974). This food web paradigm was called the 'microbial loop' (Azam *et al*., 1983). The microbial loop was first defined as a separate 'loop' in which bacteria utilize dissolved organic matter and are consumed by protozoa,

with energy and nutrients thereby returning to the phytoplankton-zooplankton-fish food chain (Azam *et al*., 1983). However, the current view of the microbial food webs is one of a complex interacting community, including phytoplankton, bacteria, and protozoans, which collectively account for primary carbon fixation, nutrient regeneration and production to support metazoans (Sherr & Sherr, 1991). In addition to bacterial uptake of nonliving organic matter, many direct links exist among algae, bacteria, and heterotrophic protozoa. By these numerous pathways, fixed organic carbon can be recovered, often into larger-sized microorganisms that may be more available for consumption by metazoans (Sherr & Sherr, 1988). Metazoans consume organisms of the microbial food web by grazing heterotrophic flagellates and ciliates (Wetzel, 2001).

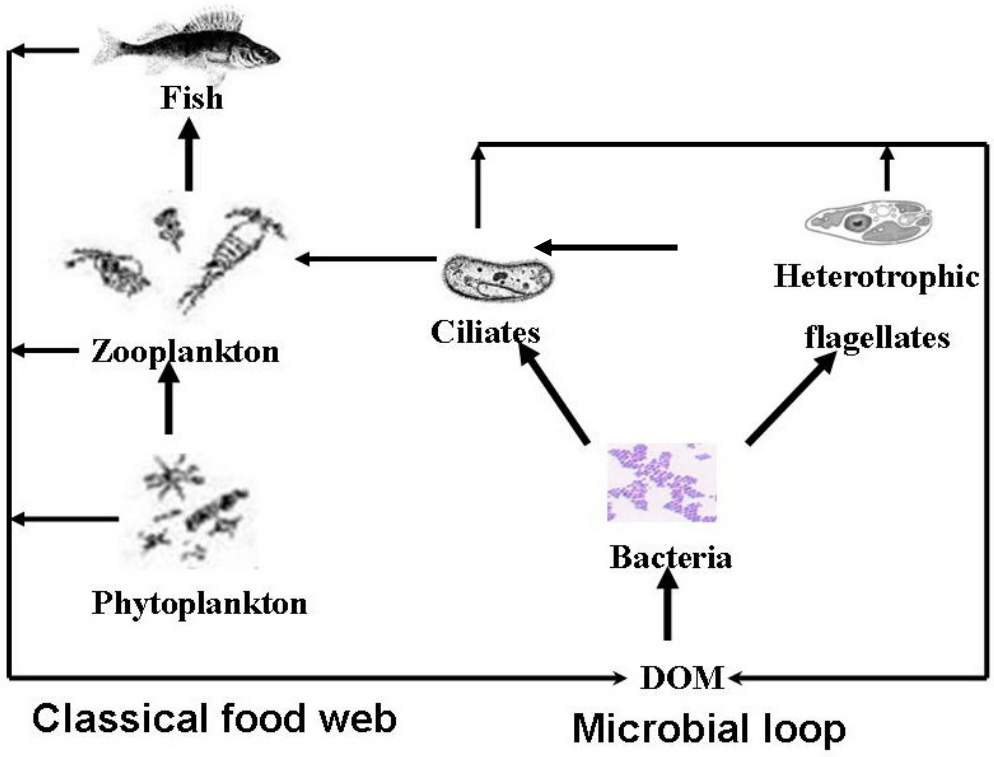


Figure 1.6 Schematic models of the different compartments occurring in pelagic food webs. Arrows represent the flux of matter and energy from one compartment to another. The left half transfers represent the classical food web, whereas the right transfers represent the microbial food web (Modified from Azam, 1983).

The importance of bacterial production and microplanktonic consumers to macrobial food webs is still poorly resolved, especially in freshwater environments (Neill,

1994). Many authors seem to agree that the high metabolic rates of bacteria dissipate almost all microbial production prior to consumption by macro-organisms, so that bacteria and their consumers serve mostly to remineralize inorganic nutrients subsequently used by primary producers (Currie, 1990). In this view, bacterial and protist biomasses may provide emergency rations or casual by-catch for suspension feeders, except under "unusual" conditions, such as in ecosystems driven by the input of external organic carbon. The opposing perspective suggests that major uncertainties still remain, particularly at small spatial and temporal scales (e.g. hotspots of production, mutualistic facilitation) and that productive freshwaters may be especially conductive to high fluxes of microbial production to higher trophic levels, despite apparent low efficiency (Neill, 1994).

1.4.2. Bottom-up, top-down and the trophic cascade in lakes

Plankton communities are structured by the simultaneous impact of bottom-up and top-down effects (Rothhaupt 2000, Begon et al., 2006). Top-down control refers to stimulations where the structure (abundance, distribution, and/or diversity) of lower trophic levels depends directly or indirectly on trophic activities of the higher trophic levels. And bottom-up control refers to direct or indirect dependence of community structure on factors producing variation at lower trophic levels or their resources. Discriminating between these two types of driving forces is important, as it determines which measures may or may not be successful when one wants to control biomass or species composition at a given trophic level. For example, if bottom-up factors are the main determinants of phytoplankton biomass, then the occurrence of phytoplankton blooms can only be controlled by reducing nutrient levels (e.g. Schindler, 1977; Wetzel, 2001). On the other hand, zooplankton grazing may control both algal biomass and species composition (top-down) (Timms & Moss, 1984, Carpenter et al., 1985, Carpenter & Kitchell, 1993, Scheffer et al., 1993). In

this situation, it may be possible to reduce the occurrence of algal blooms by changing zooplankton community structure through a manipulation of predation pressure on zooplankton by fish (Shapiro & Wright, 1984; Gulati, 1990; Scheffer et al., 1993; Scheffer, 1998, 1999).

The bottom-up hypothesis states that population densities and community structure of a given trophic level is determined by the availability of resources. In pelagic food webs, the importance of nutrients such as phosphorus and nitrogen for phytoplankton primary production is well-known (Schindler, 1977, Wetzel, 2001). They control the potential flux of carbon and energy that can be transferred to higher trophic levels. Several studies have shown a positive correlation between phosphorus concentrations and phytoplankton production and biomass (Currie 1990). However, residual variance in such relationships remains high. In the intermediate to high phosphorus range, phosphorus availability tends to mainly determine the maximal potential phytoplankton biomass, whereas the actual phytoplankton biomass is often much lower (Carpenter *et al*. 1985). This indicates that phytoplankton biomass cannot be explained solely by nutrient availability.

The top-down hypothesis postulates that population density and structure of the community at a given trophic level are controlled by the one-higher trophic level (Carpenter et al. 1985). In pelagic food webs, piscivorous fish control the densities of planktivorous fish and planktivorous fish control zooplankton densities. Grazing by zooplankton in turn will control phytoplankton density and community composition (Elser 1992).

The trophic cascade hypothesis (Carpenter et al. 1985) integrates both the bottom-up and top-down hypotheses. The top-down control of the highest trophic level on the second-highest one combined with the alternation of top-down / bottom-up control as one moves down along the food web is called a trophic cascade (Carpenter et al., 1985;

Rothhaupt, 2000). It argues that whereas the potential biomass and production of organisms at a given trophic level is controlled by resource availability, the actual biomass and dynamics of organisms at each trophic level is regulated by the adjacent higher trophic level via top-down control. Trophic cascades occur because predators in a food chain suppress the abundance of their prey, thereby releasing the next lower trophic level from predation (Carpenter et al. 1985, Carpenter & Kitchel 1993). Planktivorous fish can become very abundant when piscivorous fish are scarce, affecting the abundance and structure of zooplankton communities by selectively eliminating large-bodied zooplankton prey. This results in a decreased and more selective grazing pressure on phytoplankton, leading to increased phytoplankton biomass (Shapiro & Wright 1984, Vanni 1986, Carpenter & Kitchell 1993, Lampert & Sommer, 1997, Vanni & Layne 1997).

1.4.3. ***Fish, zooplankton and macrophyte interactions in eutrophic tropical shallow lakes***

Shallow lakes are characterized by the absence of stratification and as a consequence an intense sediment-water interaction. As shown for temperate shallow lakes, the food web structure in these lakes can basically show two contrasting stable states, the so-called clear-water and turbid states, each stabilized by their own positive feedback mechanisms (Scheffer, 1998). A clear water state is largely characterized by submerged plants and sufficient piscivorous fish biomass to exert strong control on planktivorous fish enabling large zooplankton (e.g., Daphnia) to control phytoplankton (Jeppesen et al., 1997a, Moss et al., 1997, Scheffer et al., 1997a). Submerged plants promote clear water state through several stabilizing buffer mechanisms namely: nutrient uptake, reduction of sediment resuspension, provision of refuge (especially for *Daphnia* against fish predation), enhancement of denitrification, and allelopathic effects on phytoplankton (Timms & Moss, 1984, Gumbricht, 1993, Hamilton & Mitchell, 1996). In the turbid water state, there are

few or no submerged plants, and there is total dominance of the fish community by planktivorous fish (Jeppesen et al., 1997a, Moss et al., 1997, Scheffer, 1997a). And the predation by planktivorous fish leads to changes in the zooplankton communities towards smaller body size. Bentivorous fish also promote phytoplankton growth by recycling nutrients (Attayde and Hansson, 2001). States dominated by floating macrophytes and filamentous cyanobacteria (see below) are also possible however (Scheffer and Van Nes 2007). Floating plants have primacy in competition for light but need high nutrient concentrations (Portielje & Roijackers, 1995). By contrast, rooted submerged macrophytes are susceptible to shading, but less dependent on nutrients in the water column as they may take up a large part of their nutrients from the sediment (Chambers et al., 1989). Still submerged plants can also use their shoots effectively for nutrient uptake from the water column (Robach et al., 1996) and by various mechanisms reduce nitrogen concentrations in the water column to below detection levels (Van Donk et al., 1993). This interaction may result in two alternative stable states: a floating plant dominated state in which invasion by submerged plants is prevented by shading, and a situation dominated by submerged plants in which invasion by free-floating plants is prevented by reduced nutrient availability (Scheffer et al., 2003). An alternative stable state for the phytoplankton community in shallow lakes may also result from the shade tolerance of filamentous cyanobacteria. Dominance by Oscillatoriaceae can be an alternative stable state of the algal community of shallow lakes because these shade-tolerant cyanobacteria are able to cause an increase in turbidity that favours their competitive advantage (Scheffer et al., 1997b). The relative inedibility of filaments to zooplankton may further enhance the stability of blue-green dominance. It has been reported that high flush rates reduce the probability of blue-green dominance because of their relatively slow growth rates (Scheffer et al., 1997b). But in less

turbid conditions, other groups of phytoplankton such as the green algae have a competitive advantage and can become dominant over blue green algae.

During the past centuries, increased nutrient loading on many shallow lakes has resulted in major changes in the biological structure and dynamics of lakes and often in a shift from a clear-water to a turbid state world-wide (Jeppesen et al, 2005). Food web-manipulation at higher trophic levels (using planktivorous and piscivorous fish) has become widely used practices to reverse the shift from turbid to a clear-water state in the temperate region (Carpenter et al., 1987, Jeppesen et al., 2005, Hart, 2006). However, several factors indicate that fish stock manipulations would not have the same positive effect on the environmental state in tropical lakes as in temperate lakes (e.g. Jeppesen et al., 2005): 1) the fish species richness is often higher in tropical and subtropical lakes and many of the fish species show partial niche overlap, which increases predator control of prey items (Lazzaro, 1997, Aguiaro & Caramaschi, 1998), 2) the fish stock in tropical and subtropical lakes is often dominated by omnivorous species that feed on zooplankton but also consume phytoplankton, periphyton, benthic invertebrates, and detritus (Lazzaro, 1997). The subtropical and tropical fish stock that has the potential of feeding on zooplankton may thus attain a higher carrying capacity than obligate zooplanktivores, which augments the potential control of the zooplankton; 3) top-down control by piscivores is most likely weaker in subtropical/tropical lakes than in temperate lakes. Only few large strictly piscivores and small-sized carnivores occur and sit-and-wait predators are often more frequent (Quirós, 1998), 4) fish density, but not necessarily biomass, is substantially higher in subtropical and tropical lakes than in comparable north temperate lakes (Scasso et al. 2001, Meerhoff et al., 2003).

Due to high predation by fish, the zooplankton communities in tropical and subtropical lakes are frequently dominated by small cladocerans (like *Diaphanosoma,*

Ceriodaphnia and *Bosmina*) and rotifers, and by juveniles and small copepodites among the copepods (Dumont, 1994, Garcia et al., 2002). When fish are absent, large *Daphnia* spp. may sometimes develop (Mazzeo et al., 2003). Omnivorous copepods usually dominate in terms of biomass in oligo-mesotrophic systems, whereas microzooplankton prevails in more eutrophic systems. The high temperatures, the daily fluctuations in physical and chemical conditions or sudden environmental changes due to heavy rains may add to the predominance of fast-recovering forms such as protozoans and rotifers in the zooplankton community. The classic control of phytoplankton by large zooplankton in temperate lakes is therefore not usually found in tropical lakes.

All but the smallest zooplanktivorous fish have a strong preference for large-bodied zooplankton because they are easier to detect visually (Zaret, 1980, Lampert, 1987a). A reduction in fish density will result in a shift in body size of the zooplankton towards larger sized individuals (Brooks & Dodson, 1965, Shapiro & Wright 1984, Lampert, 1987a). This is both because large zooplankton are stronger competitors than small zooplankton (Gliwicz, 1990) but also because most invertebrate predators, who are the main predators in the absence of fish, have a strong preference for small zooplankton due to gape limitation or problems to handle larger prey (Dodson, 1974, Zaret, 1980, Lair, 1990). Large-bodied zooplankton are much more efficient in grazing down algae than small- bodied zooplankton (Lampert, 1987b). As a result, the shift towards larger-bodied zooplankton upon release from fish predation pressure may strongly increase the top-down impact of zooplankton on phytoplankton. This shift in size structure is therefore a key element of the control of phytoplankton biomass and structure by manipulating fish communities (Shapiro & Wright, 1984), a management approach that has been applied successfully in quite some temperate lakes (e.g. Kairesalo et al., 1999).

Aquatic plants play a very important structuring role in most freshwater ecosystems, and especially in shallow lakes where they can potentially cover the whole lake bottom surface (Scheffer et al., 1993). In temperate nutrient-rich lakes, submerged plants often act as daytime refuges for zooplankton against fish predators (Timms & Moss, 1984, Lauridsen et al., 1996, Burks et al., 2002). At night, when the risk of predation is lower zooplankton migrate to the open water for feeding and thereby contribute to maintaining clear-water conditions in lakes with high macrophyte coverage (Jeppesen et al., 1997b). However, in the tropics and subtropics, the effects of macrophytes on trophic interactions are more complex. The few studies conducted so far in the subtropics and tropics indicate that fish, particularly the smallest species and individuals, aggregate in high numbers in the vegetation (Conrow et al., 1990, Meerhoff et al., 2003, Branco et al., 2007).One might therefore expect that the vegetation is a poor refuge for large-bodied zooplankton in warm lakes (Bachmann *et al.,* 2002, Meerhoff et al., 2003; Meerhoff, 2006), with as a consequence a less important structuring role of macrophytes in tropical than in temperate shallow lakes. On the other hand, macrophytes may perform better in tropical turbid lakes as they are not forced to regrow each spring from the bottom as in a temperate climate. Also, contrasting management approaches can alter the role of macrophytes in lake functioning and trophic interactions. While in temperate systems the introduction and development of aquatic plants are considered a key step in a restoration process (Moss et al., 1996), many aquatic plants in the tropics and subtropics are often considered as a nuisance and subject to severe eradication measures.

1.4.4. Zooplankton and cyanobacteria blooms

Cyanobacterial blooms are increasingly frequent in aquatic ecosystems around the world as a result of eutrophication (Chorus & Bartram, 1999; Huisman et al., 2005) and are likely to

increase in frequency with global warming. The study of the interactions between bloom-forming cyanobacteria and zooplankton has become important because of the potentials for top-down control of cyanobacterial blooms by zooplankton (Burns, 1987, Lampert, 1987b; Sommer, 1989). A large body of research has been directed at understanding the mechanisms by which zooplankton affect cyanobacteria and vice versa.

Cyanobacteria are characterized by several adaptations that may make them less sensitive to zooplankton grazing than other phytoplankton groups. Many cyanobacterial species form colonies that are often too large to be ingested by zooplankton (Hawkins & Lampert, 1989, Gliwicz, 1990, Gliwicz & Lampert, 1990). In addition, several species produce toxins (Eriksson *et al*., 1990, MacKintosh *et al*., 1990, DeMott & Dhawale, 1995, Ferreira et al., 2001, Pereira et al. 2000, Plumley, 1997), the effect of which ranges from a reduction in feeding activity (Haney et al., 1994) to immediate death (Reinikainen et al., 1994). For example, the filamentous morphology of certain cyanobacteria has been shown to negatively affect large cladocerans more than small cladocerans through reduced fecundity (Webster and Peters, 1978, Gilbert, 1990). Two major mechanisms have been proposed for the greater susceptibility of large cladocerans to colonial or filamentous blue-green algae (Webster & Peters 1978, Richman & Dodson, 1983, Porter & McDonough, 1984): 1) colonial or filamentous algae clog the filtering appendages of larger cladocerans, reducing their feeding rates on co-occurring nutritious food sources or increasing their respiration rates or both, 2) larger cladocerans ingest more readily colonial or filamentous blue-greens and are thereby more strongly affected by any toxic chemicals that these algae may possess. Finally, mucilaginous sheets may reduce digestibility, and many cyanobacteria are reported to be poor food in terms of biochemical composition (Porter & Orcutt, 1980, Lampert, 1987b; DeMott, 1989, Hawkins & Lampert, 1989; De Bernardi & Giussani, 1990). These characteristics suggest that cyanobacteria may be quite resistant to

zooplankton grazing, and this has indeed been reported by many studies (Sarnelle, 1993; DeMott, 1999). Yet, several studies report that the development of cyanobacteria can be suppressed by zooplankton grazing (Haney, 1987, Matveev et al., 1994).

A number of studies suggest that a dominance of *Daphnia* in the zooplankton community results in a reduced likelihood of cyanobacterial blooms (MacKay & Elser, 1998, Smith, 1983). This may at first sight seem counter-intuitive, because it has been shown that especially large-bodied cladocerans such as *Daphnia* suffer from clogging of their filtration apparatus by cyanobacteria filaments (Gliwicz, 1990). Yet, *Daphnia* are also the most efficient grazers of phytoplankton, and one likely scenario may be that grazing by *Daphnia* may prevent cyanobacteria to develop dense populations and produce filaments (Gliwicz, 1990). If so, timing of the presence of *Daphnia* can be important, because efficient grazing could then prevent bloom formation but not suppress an existing bloom. According to Elser (1999), *Daphnia* also reduces the importance of cyanobacteria in the phytoplankton because of its high need for phosphorus, which results in a relatively higher N:P ratio in the medium (Andersen & Hessen, 1991, Elser & Urabe, 1999; Sterner, 1990). As such, *Daphnia* would promote dominance of green algae over cyanobacteria indirectly, through a bottom-up effect.

Cyanobacteria species such as *Microcystis* have been shown to be polymorphic with respect to the production of toxins, with non-toxic and toxic strains often coexisting and showing seasonal changes in relative abundance (Kirk and Gilbert, 1992, Barreiro et al., 2007). There is a need for studies that systematically investigate the relationship between zooplankton, and especially *Daphnia*, and the occurrence of cyanobacteria in the phytoplankton community, their tendency to bloom, and the degree to which these blooms are toxic. It is currently insufficiently known whether grazing of zooplankton actually reduces or enhances cyanobacteria blooms and to what extent this is dependent on species

composition of the zooplankton community. The importance of cyanobacteria in many tropical lakes, for instance, is attributed to a lack of top-down control of phytoplankton by large zooplankton, due to the dominance of omnivorous copepods in oligo-mesotrophic systems and of microzooplankton in eutrophic systems (Jeppesen et al., 2005). Especially with respect to toxicity, this is a burning question: will intensive grazing by zooplankton prevent or enhance toxic blooms? If toxin production is a defence against zooplankton, one may expect that dense zooplankton populations may enhance cyanobacteria blooms to become toxic. On the other hand, if *Daphnia* can prevent bloom formation, they may perhaps also prevent the cyanobacteria populations to become toxic. It is therefore possible that the outcome of this interaction depends on initial conditions. Insight in this matter may be crucial in our efforts to reduce the occurrence of cyanobacteria blooms.

1.5. Study Area

Tigray, the northernmost Regional State of Ethiopia, lies between 12° N and 15° N Latitudes and 37° 10' E and 40° 10' E Longitudes (Fig. 6). The Tigray Regional State has an area of 50,078 km^2, out of which 19 per cent is cultivable (CSA, 2000). The Tigray region is drought-prone and suffered from high land degraded.

The annual rainfall pattern in North Ethiopia is uni-modal with a short rainy season and a prolonged dry season (Fig. 1.2). Rains are erosive as well as unreliable (Lemma, 1996; Nyssen *et al*., 2005). Some authors divide the year into three periods, with October to May being dry, the period from June to September as rainy but with moderately spaced heavy rains, and July and August as rainy with very intensive heavy rains (Daniel 1977; Nyssen et al., 2005). The climate is generally characterized as tropical semiarid (Virgo and Munro, 1978) with an annual rainfall range from 450 mm in the north, east and central zones to 980

mm in the southern and western parts of Tigray. In addition, within a given year, there is strong spatial variation in the amount of rainfall (Fig. 1.2).

1.5.1. ***General characteristics of the reservoirs in Tigray***

Dejenie et al. (2008) studied the limnological characteristics of a set of 32 reservoirs in Tigray (see Table 2 for the list of reservoirs; see also Tsehaye et al. 2007 for a description). The reservoirs are found at altitudes of 1833 to 2747 m.a.s.l., with most of the reservoirs (31 /32) located at an altitude $\geq$ 2000 m.a.s.l. The reservoirs vary in their abiotic characteristics. Generally they are small (1.8 to 45 ha. in surface area) and shallow. Depth varies among seasons, being reduced during the dry season, resulting from the use of water for irrigation (see Table 1.3 for a summary of the abiotic ecological characteristics of the reservoirs). A fraction of reservoirs dries up during the dry season. This fraction is higher than suggested in Table 1.3, as Dejenie et al. (2008) explicitly avoided sampling too many reservoirs that would fall dry during the dry season. The water in the reservoirs has a higher conductivity during the dry season due to evaporation. The reservoirs are alkaline, their pH ranging from 7.45 to 9.6. The trophic state of the reservoirs varies from mesotrophic to hypereutrophic. *Daphnia* is present in a majority of the reservoirs. Dejenie et al., (2008) identified a total of fifteen cladoceran species and the genus *Daphnia* was reported from all reservoirs as the most abundant.

The small riverine fish (*Garra sp.)* is the dominant fish in most reservoirs. Two introduced fish species, *Tilapia zillii* (Gervais, 1848) and *Oreochromis niloticus cancellatus* (Nichols, 1923), were reported from eight of the reservoirs (namely Dibla, Enda Gabriel, Haiba, Korir, Laelay Wukro, Mai Sessella, Shilenat IV and Tsinkanet).

Table 1.3 Geographic co-ordinates, altitude, surface area and depth of reservoirs (Dejenie et al., 2008)

Reservoir names	No.	Long.	Lat.	Altitude (m)	Area (ha)	Depth (m)	Year of construction
Adi Amharay	1	39.57	13.41	2,354	9.3	2.5	1996/1997
Adi Asme'e	2	38.96	13.65	1,833	2.1	4.5	1993/1994
Adi Gela	3	39.51	13.13	2,044	17.0	4.3	1997/1998
Adi Kenafiz	4	39.41	13.25	2,161	11.6	2.2	1997/1998
Betequa	5	39.34	13.32	2,260	6.0	4.0	1996/1997
Bokoro	6	39.57	14.20	2,673	3.8	3.5	1985/1986
Dibdibo	7	39.08	14.26	2,015	14.8	8.8	1998/1999
Dibla	8	39.49	14.23	2,446	1.8	0.5	1986/1987
Dur Anbesa	9	39.44	13.27	2,133	11.1	5.0	2000/2001
Enda Gabriel	10	39.58	14.18	2,673	3.8	3.3	1985/1986
Era Quihila	11	39.60	13.45	2,321	6.4	2.5	1996/1997
Gereb Awso	12	39.56	13.43	2,283	2.4	3.0	1997/1998
Gereb Beati	13	39.48	13.45	2,151	14.0	9.5	1999/2000
Gereb Mihiz	14	39.47	13.29	2,123	17.7	4.9	1997/1998
Gum Selasa	15	39.54	13.24	2,116	23.1	1.5	1994/1995
Haiba	16	39.28	13.28	2,263	45.4	3.5	1997/1998
Hashenghe	17	39.67	13.48	2,400	5.4	1.0	1996/1997
Hizaeti Wedi Cheber	18	39.54	13.36	2,245	32.6	2.5	1996/1997
Korir	19	39.61	13.75	2,022	13.7	4.0	1995/1996
Laelay Wukro	20	39.61	13.81	2,023	9.8	4.5	1997/1998
Mai Delle	21	39.52	13.22	2,105	8.0	2.1	1997/1998
Mai Gassa I	22	39.49	13.29	2,131	14.7	3.9	1996/1997
Mai Gassa II	23	39.49	13.29	2,130	9.1	1.3	1996/1997
Mai Leba	24	39.23	13.69	2,231	8.2	3.0	1997/1998
Mai Nigus	25	38.66	14.12	2,056	30.8	11.0	1996/1997
Mai Sessella	26	39.03	14.07	2,068	21.8	9.0	1999/2000
Mai Seye	27	38.80	14.06	2,007	15.9	10.0	2000/2001
Meala	28	39.35	13.29	2,370	14.5	4.5	1997/1998
Ruba Feleg	29	39.73	13.95	2,747	10.7	11.0	1995/1996
Shilanat IV	30	39.49	13.10	2,080	17.7	9.5	1997/1998
Tegh'ane	31	39.73	13.89	2,735	9.6	6.5	1996/1997
Tsinkanet	32	39.54	14.01	2,320	7.0	4.1	1993/1994

Table 1.4 Summary of some limnological variables of the reservoirs in Tigray for the wet and dry season of the year 2004/2005 (Dejenie et al. 2008)

	Wet season (2004)			Dry season (2005)		
Variable	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Altitude and morphometrics						
Altitude (m.a.s.l.)	2,235	1,833	2,747			
Mean depth (m)	3.4	0.5	10.3	1.5	0.2	7.9
Surface area (ha)	13.1	1.8	45.4			
Physicochemical variables						
pH	8.46	7.47	9.30	8.66	7.45	9.58
Conductivity (μScm^{-1})	203	75	471	283	135	824
Dissolved Oxygen (%)	109	82	162	153	84	304
Temperature (°C)	19.4	15.1	21.9	20.9	14.4	29.9
Secchi disc transparency (m)	0.46	0.05	1.50	0.44	0.12	2.00
Total phosphorus (μgl^{-1})	52	11	221			
Total nitrogen (μgl^{-1})	850	232	2,411			
Suspended matter ($mg\ l^{-1}$)	42	6	737	61	6	596
Biotic components						
Chlorophyll-a (μgl^{-1})	187	36	773	204	12	1,716
Cyanobacteria ($mg\ C\ l^{-1}$)	1,281	0	13,143	1,230	0	10,675
Zooplankton (μgl^{-1} dry weight)	295	9	1,603	722	8	4,137
Rotifers (μgl^{-1} dry weight)	16	0	135	177	0	4,127
Copepods (μgl^{-1} dry weight)	144	2	683	120	2	755
Cladocerans (μgl^{-1} dry weight)	135	1	1,128	308	1	2,065
Daphnia (μgl^{-1} dry weight)	18	0	254	117	0	1,597
Garra (CPUE; $kg\ net^{-1}\ day^{-1}$ fresh weight)	2.4	0	24.6			
Tilapia (CPUE)	0.6	0	5.9			
Vegetation cover (% cover)	6	0	50	9	0	50

1.6. Objectives and thesis outline

Reservoirs are important sources of water for agriculture and other domestic purposes. Although the direct use of the reservoirs is vivid, the occurrence of potentially toxic cyanobacteria blooms is a major threat to the use of water. Cyanobacteria are well

documented as being able to potentially synthesize an array of toxic substances. These toxins have been shown to have high toxicity to vertebrates including mammals. Although a large body of literature is available on bloom-forming cyanobacteria from temperate regions, research on these potentially toxic cyanobacteria in sub/tropical Africa is still limited. There is a need for studies on the occurrence of cyanobacteria blooms and the mechanisms that lead to bloom formation and toxin production. It is therefore the main aim of the present work to gain better understanding of the conditions that favor cyanobacteria bloom formation in the reservoirs in Tigray. To achieve this, we carried out field surveys on the occurrence and species composition of phytoplankton in 32 reservoirs in the region, with special emphasis on cyanobacteria. The descriptive information collected was supplemented with an experimental approach, designed to obtain insight in the relative importance of biotic (fish, zooplankton) and abiotic factors in determining the phytoplankton community composition. In addition, seasonal monitoring was carried out for eight selected reservoirs during a one-year period. We also explored bacterioplankton community structure in the reservoirs and to what extent they relate to environmental conditions and cyanobacterial blooms.

In the first part of this thesis (Chapter 2, 3 and 4), we focus on patterns of phytoplankton and bacterioplankton community structure as observed in the field. In a second part (Chapter 5 and 6), we present the results of experiments designed to test for specific mechanisms underlying the occurrence of cyanobacteria blooms in the reservoirs.

Chapter 2 deals with the phytoplankton community in 32 reservoirs with an emphasis on the dominant cyanobacteria of these communities. This study accompanies a large-scale survey in which the reservoirs were studied for a wide range of abiotic variables (Dejenie et al., 2008) and zooplankton. In an appendix to this chapter, we present the list of

phytoplankton genera that were observed in the study reservoirs, as there were no previous studies on the biota of these reservoirs.

Chapter 3 presents the seasonal changes in the phytoplankton community and their relations to changes in environmental variables of eight reservoirs selected to represent the range of environmental gradients in the region. This chapter presents data collected over a one year period (September 2005 - August 2006).

Chapter 4 describes the bacterioplankton community structure of the reservoirs in relation to abiotic and biotic environmental conditions in the reservoirs. More specifically, we also focused on whether the bacterioplankton taxon composition differed among reservoirs with and without *Microcystis* blooms.

Chapter 5 presents the results of two enclosure experiments designed to determine the impact of the presence of fish (*Garra*) on phytoplankton community composition and the occurrence and abundance of *Microcystis*. The experimental design allowed us to assess the relative importance of bottom-up and top-down control for phytoplankton species composition and cyanobacteria blooms.

Chapter 6 presents the results of a field experiment in which we manipulated zooplankton biomass so as to explore the extent to which phytoplankton community structure and the abundance of *Microcystis* can be influenced by top-down effects exerted by zooplankton.

In a final chapter (Chapter 7), we briefly discuss our research findings, and translate our observations to suggestions for future study and for management of the reservoirs with the aim of reducing the occurrence and toxicity of cyanobacterial blooms.

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Chapter 2

The phytoplankton communities of shallow reservoirs in a semi-arid subtropical region (Tigray, North Ethiopia), with an emphasis on the occurrence of bloom-forming cyanobacteria

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Abstract

Small shallow reservoirs are often constructed in tropical regions to ensure water availability for local communities. Knowledge of the aquatic food web in general and phytoplankton in particular, in such reservoirs is generally lacking, even though their shallowness and possible eutrophication implies a risk of cyanobacterial bloom development. Here, we describe the phytoplankton communities, with an emphasis on the occurrence of bloom-forming cyanobacteria, in 32 small reservoirs in a subtropical semiarid region (Tigray, North Ethiopia), which were sampled once in both the wet and dry season. Local phytoplankton richness was low with most reservoirs dominated by a single genus of cyanobacteria (mostly *Microcystis*), chlorophytes, euglenophytes, cryptophytes or dinophytes. Despite their recent construction, spatial configuration of the reservoirs didn't have a significant influence on phytoplankton community structure. In contrast, local reservoir characteristics were strongly associated with the phytoplankton communities. Environmental variables selected by redundancy analysis were zooplankton and pH. *Microcystis* was associated with a high pH, especially in the rainy season. In the dry season, the most important environmental factors in this exceptionally dry year were reservoir depth and conductivity, with deeper lower-conductivity reservoirs typically dominated by the dinophyte *Peridinium*. Despite their occurrence in several reservoirs, submerged macrophytes apparently didn't influence phytoplankton community structure, in contrast to the situation in temperate shallow water bodies. The apparent importance of zooplankton for phytoplankton community structure is surprising for (sub) tropical regions and may be related to the absence of fully developed fish communities in these reservoirs.

Keywords: Phytoplankton, shallow reservoir, semiarid subtropical climate, cyanobacteria, *Microcystis*, *Peridinium*

Introduction

Phytoplankton is among the main primary producers in aquatic environments. In the tropics, study of phytoplankton communities has focused on natural lakes and rivers (Hecky & Kling 1981, Talling 1986, Elizabeth & Amha 1994, Tudorancea *et al.,* 1999). However, in (semi-) arid regions, many small shallow reservoirs have been constructed to serve as a water source for irrigation and other purposes during the dry season. While the ecology and food web structure of shallow lakes in temperate regions is well established (Meijer et al., 1999, Scheffer & van Nes, 2007), such knowledge is scarce for the (sub)tropics (Tudorancea *et al.* 1999, Dejenie *et al.,* 2008). The study of these small reservoirs would contribute significantly to the understanding of tropical freshwater ecosystems and the impact of anthropogenic activities on them (Araoye, 2002), including the possible development of harmful algal blooms. In small meso- to eutrophic water bodies in temperate regions, phytoplankton communities are mainly influenced by the structure of the aquatic food web (Schriver et al., 1995, Scheffer, 1998, Muylaert et al., 2003, Vanormelingen et al., 2008), and by seasonal changes in abiotic conditions and the strength of food web interactions (Sommer et al., 1986, Pilkaityté & Razinkovas, 2007). However, important differences in the factors controlling phytoplankton community structure can be expected in a (sub)tropical climate characterized by a more constant high temperature. For instance, top-down control of phytoplankton by zooplankton rarely occurs in tropical lakes, most likely due to the high predation pressure of abundant and continuously reproducing fish on large zooplankton (Dumont, 1994, van Leeuwen et al., 2007), the association of fish with macrophyte beds preventing their use as zooplankton refuge (Meerhoff et al., 2003, 2005), and the higher contribution of, often inedible, cyanobacteria to the phytoplankton in warmer climates (Rondel et al., 2008).

The intensive use of small man-made reservoirs in a (semi-)arid climate often results in their eutrophication, making them especially vulnerable to the occurrence of cyanobacterial

blooms, which are increasingly frequent in aquatic ecosystems around the world (Chorus & Bartram, 1999, Van Ginkel, 2003, Huisman et al., 2005, Paerl and Huisman, 2008). The contribution of cyanobacteria to water quality deterioration is widely documented. Next to other negative aspects of phytoplankton bloom formation, a number of common cyanobacteria can produce a wide range of toxins, causing a risk to livestock and human health (Chorus & Bartram, 1999, Codd et al., 2005). Hypotheses to explain cyanobacterial dominance in eutrophic lakes are rather diverse and can be related to several factors: (1) An elevated water temperature, (2) Low photon irradiance, (3) a stable water column, (4) Zooplankton grazing, (5) low carbon dioxide/high pH, and (6) a low N/P ratio (Shapiro, 1990, Paerl & Huisman, 2008). Moreover such blooms are expected to only occur in the phytoplankton-dominated turbid state of shallow lakes, as opposed to the clear-water state dominated by submerged macrophytes (Scheffer, 1998). However, cyanobacterium identity is important; different cyanobacterial species have different physiological traits and requirements and are favored by different combinations of environmental variables (Kardinaal & Visser, 2005). Once established, a cyanobacterial bloom may itself influence (a)biotic conditions in such a way that positive feedback loops are established (increased internal phosphorus loading, low light conditions, high temperature, CO_2 depletion), which stabilize cyanobacterial dominance (Scheffer et al., 1997, Bicudo et al., 2007). This makes it difficult to disentangle cause and consequence of cyanobacterial bloom development.

In Ethiopia, virtually no studies on the phytoplankton communities of small reservoirs and ponds exist, and the scientific information about these reservoirs is very limited (Melaku *et al.*, 1988, Tudorancea *et al.*, 1999). Independent studies on the occurrence and mechanisms of cyanobacterial blooms in small reservoirs are also almost non-existent except for some reports from the Ethiopian Rift Valley natural lakes. Cyanobacteria (including the genus *Microcystis*) were reported as dominant members of the phytoplankton community from these

Ethiopian natural lakes (Baxter & Wood, 1965, Wood & Talling, 1988, Elizabeth, *et al.,* 1992, Elizabeth & Amha, 1994, Elizabeth & Willen, 1996, Elizabeth, 1996). Here, we describe the phytoplankton communities of 32 small highland reservoirs in Tigray, North Ethiopia, and identify the main environmental factors correlated with phytoplankton community variation, with an emphasis on the importance of the aquatic food web structure. In addition, we tested specific hypotheses on the factors influencing the abundance of *Microcystis*, the main bloom-forming cyanobacterium in Tigray.

Material and methods

Reservoir characteristics

This study was carried out in 32 shallow semi-arid tropical reservoirs in the highlands of Tigray, the northernmost regional state of Ethiopia (figure 2.1). A detailed limnological description of the reservoirs is given in Dejenie et al. (2008). The sampled reservoirs are situated at altitudes between 1833 and 2747 meters above sea level and are all quite small (surface area ranged between 7.78 and 45.4 ha) and shallow (maximum depth in the wet season ranged between 1 and 11 meters). The water influx to the reservoirs is confined mainly to run-off rain water in the wet season (July to August). In the remaining months of the year there is no inflow to compensate for the strong drawdown caused by evaporation and water usage to supply agriculture. Mean lake depth was 1.5 m and 3.4 m in the dry and wet season respectively. In the dry season, three of the 32 reservoirs (Hashenge, Gum Selasa and Mai Leba) had dried out completely, whereas others became very shallow (Mai Delle, Adi Kenafiz, Mai Gassa II, Hizaeti Wedicheber, Adi Gela). Reservoir trophic state varied from mesotrophic to hypereutrophic with total phosphorus concentrations measured in the wet season varying between 11 and 221 µg P L^{-1}, whereas total nitrogen ranged between 230 and 2400 µg N L^{-1}.

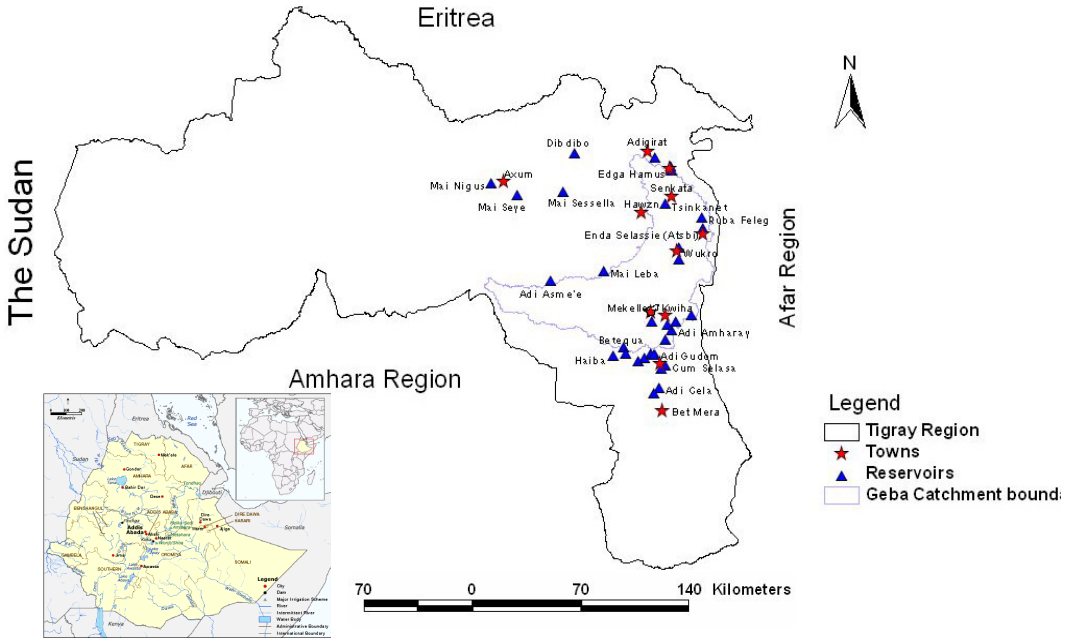

Figure 2.1 Map with the geographic location of the sampled reservoirs in Tigray, North Ethiopia.

Sampling methodology

The thirty two reservoirs were sampled twice; once at the end of the wet season (September 2004) and once near the end of the dry season (April 2005). The same sampling procedure was followed for all reservoirs (see Dejenie et al., 2008). Pelagic water samples were taken from a boat at a fixed location in the middle of the reservoir from three depths (surface, middle, and near to the bottom) using a 3-l Heart valve sampler and pooled. The pooled samples were transported to the shores immediately after their retrieval and 250 ml sub-samples were fixed with acid Lugol's solution to 0.1 % final concentration for phytoplankton counting. The fixed samples were kept cool during transport and stored in the dark till their analysis. Conductivity, dissolved oxygen, pH and temperature were measured *in situ* using a portable meter WTW Multi 340 I electrode. Conductivity was measured for the pooled samples, but dissolved oxygen, pH and temperature were recorded at the three depths. Depth was estimated with a pre-labeled rope and transparency was determined using a Snell's tube.

The concentration of chlorophyll a was measured in the field with a fluorometer (Turner Aquafluor; average of three measurements).

Zooplankton samples were collected with a Schindler-Patalas plankton trap (12L). Counting was done with a stereoscopic microscope (Dejenie et al., 2008). Individual counts were used to calculate densities (individuals per liter) and biomass. Suspended matter was determined as the dry weight of particles retained on a pre-weighed Whatman GF/C filters after filtration of depth-integrated water samples. Water samples for nutrient analysis were taken to the laboratory in a cool box with ice cubes and frozen at -18°C until further analysis. The concentrations of total phosphorus and total nitrogen were measured following the ascorbic acid method and the kjeldahl method, respectively (Anderson & Ingram, 1989). The average values and ranges for the environmental parameters are listed in Table 2.1.

Phytoplankton was enumerated using an inverted microscope (Lund, 1958, Wetzel & Likens, 2000). Bengal Rose B dye solution was added to facilitate distinction between detritus particles and living phytoplankton cells. The surface of the sedimentation chamber was scanned during enumeration and absolute phytoplankton abundance in the samples was calculated. Cells as well as colonies (filaments, coenobia) were considered as a single 'unit' during the counts. At least 200 units were counted for each sample analyzed at 200X magnification. Identification of the taxa was made to the genus level following standard keys (John, 2005, Komarek & Anagnostidis, 2000, 2005). For biovolume measurements, 15-50 units of each taxon were measured in each sample during the counts. If less than 15 units of a given species were encountered during the counts all units encountered were measured. Biomass estimates were obtained by using published biovolume calculations (Hillebrand et. al, 1999) and conversion of biovolumes to carbon biomass following Menden-Duer and Lessard (2000).

Table 2.1 List of environmental variables measured for the reservoirs and their abbreviations with the average value and range

		Wet season (2004)	Dry season (2005)
Variable	Code	Mean (range)	Mean (range)
Altitude (m.a.s.l.)	ALTITUDE	2235 (1833-2747)	
Mean depth (m)	DEPTH	3.4 (0.5-10.3)	1.5 (0.2-7.9)
Surface area (ha)	AREA	13.1 (1.8 - 45.4)	
pH	pH	8.46 (7.47-9.30)	8.66 (7.45-9.58)
Conductivity (µS cm^{-1})	COND	203 (75-471)	283 (135-824)
Dissolved Oxygen (%)	O2	109 (82 -162)	153 (84-304)
Temperature (°C)	TEMP	19.4 (15.1-21.9)	20.9 (14.4-29.9)
Snell's tube transparency (cm)	TRANSP	16.26 (3.00-40.00)	14.23 (3.00-40.00)
Total phosphorus (µg l^{-1})	TP	52 (11-221)	
Total nitrogen (µg l^{-1})	TN	850 (232-2411)	
Total nitrogen to total phosphorus ratio	TN/TP	24 (6-74)	
Suspended matter (mg l^{-1})	SM	42 (6-737)	61 (6-596)
Chlorophyll-*a* (µg l^{-1})	Chla	187 (36-773)	204 (12-1716)
Zooplankton (µg l^{-1} dry weight)	ZOOPB	295 (9-1603)	722 (8-4137)
Copepods (µg l^{-1} dry weight)	COPB	144 (2-683)	120 (2-755)
Daphnia (µg l^{-1} dry weight)	DAPHB	18 (0-254)	117 (0-1597)
Fish (CPUE; kg net^{-1} day^{-1} fresh weight)	FISH	2.4 (0-24.6)	
Fish eating birds	BIRD/PELIC	+/-	+/-
Cattle trampling (as derived from the number of cattle dung along the shoreline) (number m^{-2})	CATTLE	26.4 (6.4-79.2)	41.8 (5-104.8)
Submerged vegetation cover (% cover)	VEG	6 (0-50)	9 (0-50)

Statistical analysis

Multivariate statistical analyses were performed with CANOCO version 4.5 for Windows (Biometrics, Plant Research International, Wageningen, The Netherlands), except the forward selection of environmental variables, which was done using the package "packfor" in R (available from https://r-forge.r-project.org/R/?group_id=195 and http://www.r-project.org/ respectively). Absolute abundance data were used for the phytoplankton. Before multivariate analysis, phytoplankton genera occurring in a single pond were excluded and phytoplankton abundances and environmental variables (except pH, ZX1 and ZX2, see below) were log(x+1) transformed to reduce the effect of high values (ter Braak and Smilauer, 1998).

The variation in the phytoplankton communities was visualized with principal component analysis (PCA) for both the rainy and the dry season. Next, associations between the local environmental conditions (related to the aquatic food web structure and abiotic conditions) of the reservoirs and phytoplankton community structure were identified using Redundancy Analysis (RDA). In RDA, a forward selection procedure was used. To avoid inflation of the Type I error due to a large number of environmental variables, an *a priori* selection of environmental variables was made, and the significance of a global model including all variables checked before applying the forward selection procedure (Blanchet et al., 2008). We additionally checked whether the $R^2_{adjusted}$ of the forward selection model didn't exceed that of the full model. The following variables related to the aquatic food web structure were selected *a priori*; ZX1 and ZX2 (the two first axes of a PCA of the log(x+1)-transformed absolute biomasses of the different zooplankton taxa, representing the zooplankton communities, taken from Dejenie et al., 2008), FISH (total fish biomass), VEG (the percentage cover by submerged macrophytes), and TRANSP (water clarity). Also CATTLE (as derived from the number of cattle dung along the shoreline) was selected, as the presence of cattle can have a marked influence on the phytoplankton communities of small

water bodies (Villena-Alvarez et al. unpubl.). Potentially important abiotic variables included ALTITUDE (Altitude), DEPTH (average depth), TN (total nitrogen concentration), TP (total phosphorus), TN/TP, pH and COND (conductivity). Due to missing values for ZX1 and ZX2, the reservoirs ADIGEL, HIZWED and MAIGAS2 were excluded from the RDA in the dry season. The statistical significance in RDA was assessed by Monte-Carlo permutation tests (999 permutations).The contribution of the spatial configuration of the reservoirs to the phytoplankton community structure, and its (in)dependency of spatially correlated environmental variation, was also identified using RDA in a variation partitioning approach (Borcard et al., 1992). Spatial variables used for the RDA were obtained using the principal coordinates of neighboring matrix (PCNM) approach, in which principal coordinate analysis of a truncated Euclidean distance matrix is applied to construct eigenvectors, corresponding to positive Eigen values, which are then used as spatial descriptors (Borcard & Legendre, 2002, Borcard et al., 2004, Dray et al., 2006). The same procedure for the forward selection of variables was applied as described above for the environmental variables.

A simple Mantel test (Mantel, 1967; Mantel and Valand, 1970) was used to investigate the relation between the phytoplankton communities of the reservoirs in the wet season and the dry season. The test was carried out using the Zt software tool (Bonnet and Van de Peer, 2002) using the Bray-Curtis similarity matrices of the phytoplankton community data constructed in PRIMER).

Regressions and Pearson and Spearman Rank correlations were carried out with Statistica version 6.0 for Windows (StatSoft Inc., Tulsa, USA). A step-wise forward multiple regression approach was applied to identify environmental factors determining *Microcystis* biomass. Dry and rainy season data from the same reservoir were considered independent because of the fast turn-over of phytoplankton populations (e. g. Sommer et al., 1986, Chambouvet et al., 2008). Environmental variables included are related to (1) temperature

(TEMP, ALTITUDE), (2) nutrient level (TN, TP, TN/TP), (3) phytoplankton productivity (pH, Chla), (4) the clear-turbid alternative stable states in shallow lakes (Daph, TRANSP, VEG, FISH) and (5) water column stability (Season, DEPTH, COND). The same procedure for the forward selection of variables was applied as described above for the redundancy analyses (Blanchet et al., 2008).

Results

Phytoplankton community structure

27 out of 32 reservoirs in the wet season and 27 out of 29 reservoirs in the dry season were dominated by a single taxon (Fig. 2.2). Cyanobacteria dominated the phytoplankton biomass in 11 of the 32 reservoirs sampled in the wet season. *Microcystis* was the dominant taxon in ten of these reservoirs. Chlorophytes and Euglenophytes each dominated six reservoirs, and Dinoflagellates five. The dominance of the phytoplankton taxa shifted to dinoflagellates in the dry season; *Peridinium* dominated ten of the 29 reservoirs. Cyanobacteria were dominant in another ten reservoirs with *Microcystis* being the dominant genus in seven of them. No significant correlation was found between the phytoplankton community composition of the two season samples (simple Mantel test, $r = -0.021$, $p = 0.442$). This may be because of a high and inconsistent turn-over of phytoplankton communities in the reservoirs. The Shannon-Wiener diversity values of phytoplankton communities are depicted in Fig. 2.2. We observed a very low diversity of the phytoplankton communities with low values of Shannon-Wiener diversity index in both the wet (on average 0.829, range 0.026 – 1.9) and dry (0.529, 0.027 – 1.469) seasons.

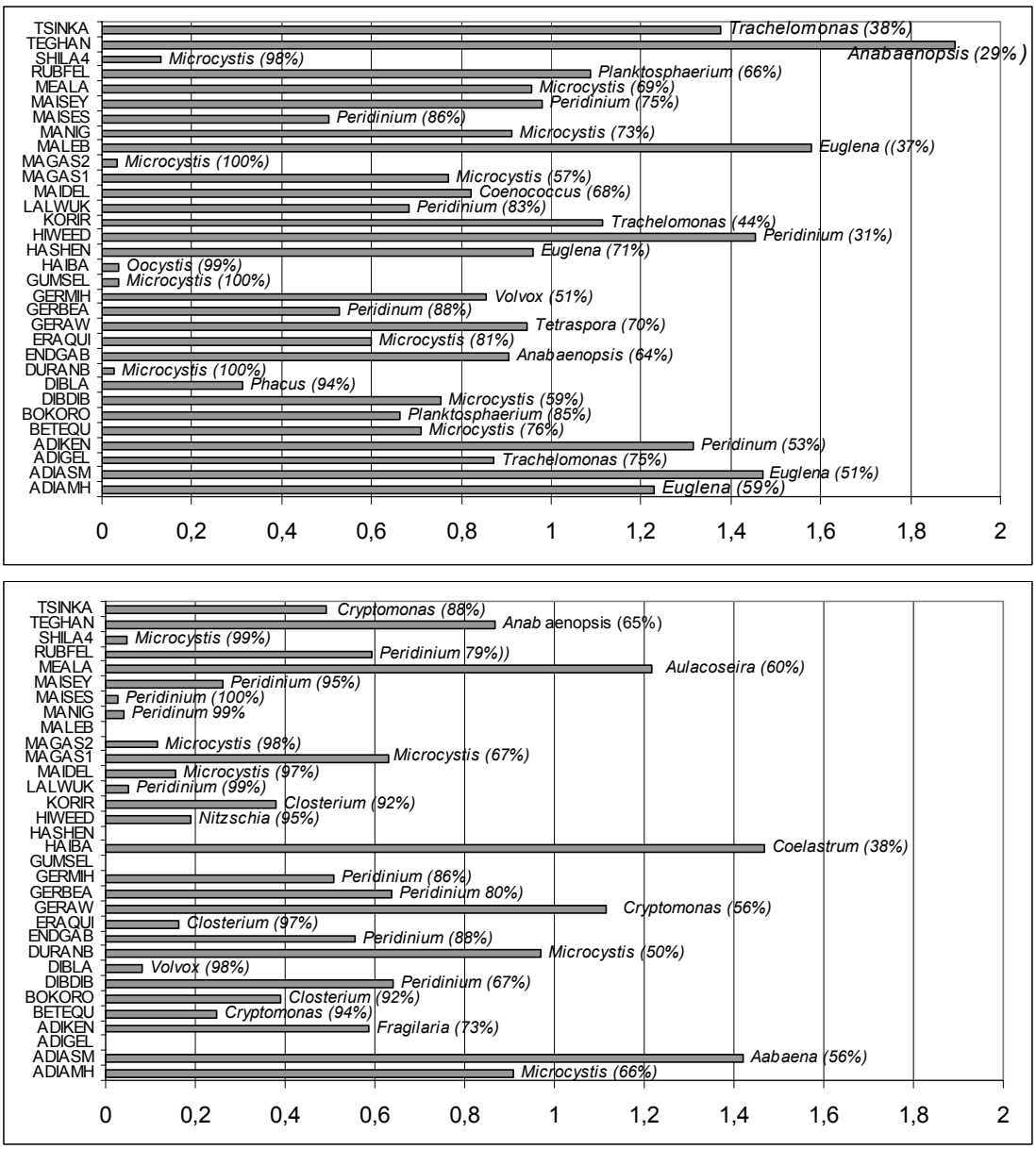

Fig.2.2 Shannon-Wiener diversity values of phytoplankton communities in the wet (a) and dry (b) seasons of the shallow reservoirs in a semi-arid subtropical region (Tigray, North Ethiopia). Genera behind bars are the dominant taxa and figures in parenthesis represent the % contribution to the total phytoplankton biomass in that particular reservoir and sampling.

The first axis of a PCA of the phytoplankton communities in the wet season explained 20% of the total variation and separated communities characterized by euglenophytes, most cyanobacterial, and some green algal genera (*Scenedesmus*, *Coelastrum*, *Pediastrum*) from the other phytoplankton communities (Fig. 2.3a). The second axis explained 15% and was mainly associated with *Microcystis* (Pearson correlation, $R^2 = 0.65$, $p < 0.0001$). In the dry

season, the first axis explained 25% and was mainly associated with *Peridinium*, several euglenophyte genera, and *Cryptomonas* (Fig. 2.3b). The second axis (explaining 17%) was, similar to the situation in the rainy season, mainly associated with *Microcystis* (R^2 = 0.62, p = 0.0003).

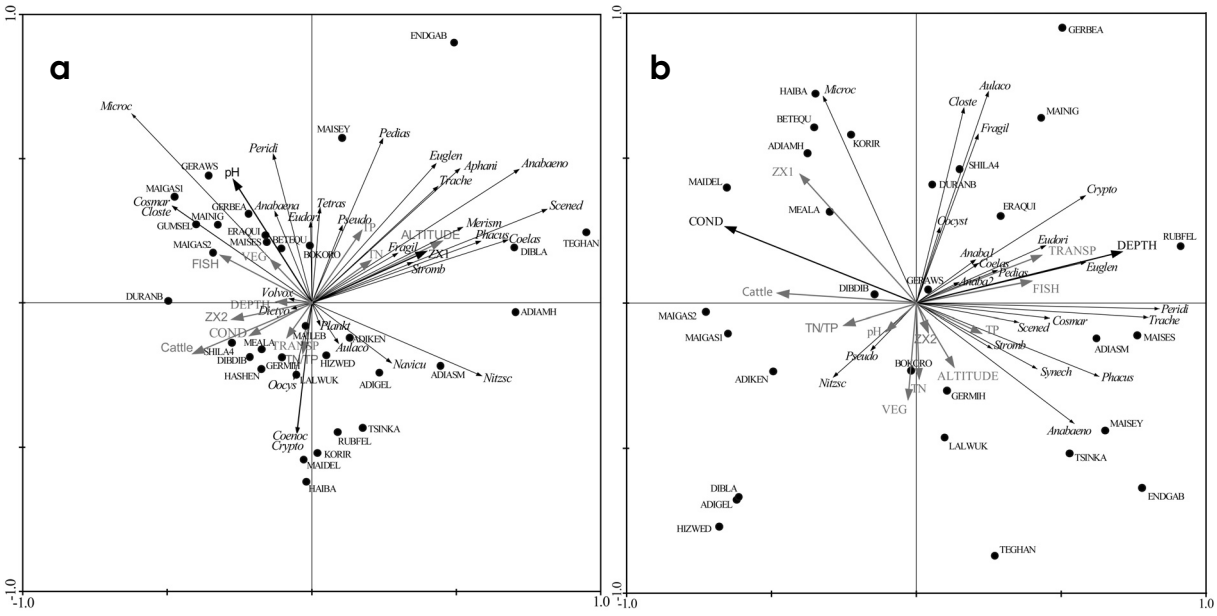

Figure 2.3 Biplot of PCA analysis performed on the phytoplankton communities in the wet (a) and dry (b) seasons. Environmental variables are plotted as supplementary variables. Variables significantly explaining phytoplankton variation as indicated by an RDA analysis are in black, other variables in gray. Filled circles represent the sampled reservoirs.

Structuring factors

The spatial configuration of the reservoirs was apparently unimportant for the phytoplankton community structure as the full RDA model including all PCNM eigenvectors was insignificant for both seasons (wet season, trace = 0.502, p = 0.092; dry season, trace = 0.191, p = 0.33). The full models remained insignificant when only the first five PCNM eigenvectors were selected *a priori*.

The full RDA model with all environmental variables included was highly significant for both seasons (Table 2.2), permitting a forward selection procedure to identify the most

important environmental variables. Environmental variables selected for the RDA in the wet season were zooplankton community structure (ZX1) and pH (Table 2.2, Fig. 2.3a). ZX1 was correlated with the first axis of the phytoplankton PCA (R^2 = 0.16, p = 0.025), while pH was correlated with the second axis (R^2 = 0.38, p = 0.0002), associated with *Microcystis*. A scatter plot of the correlation between *Microcystis* biomass and pH in the rainy season is shown in Fig. 2.5. In the dry season, average reservoir depth (DEPTH) and conductivity (COND) were selected (Table 2.2, Fig. 2.3b). Both variables were strongly correlated with the first axis of the phytoplankton PCA (R^2 = 0.67, p < 0.0001 and R^2 = -0.68, p < 0.0001 respectively), which is mainly associated with *Peridinium* (Fig. 2.3b). A scatter plot of the correlation between *Peridinium* and reservoir depth is shown in Fig. 2.4.

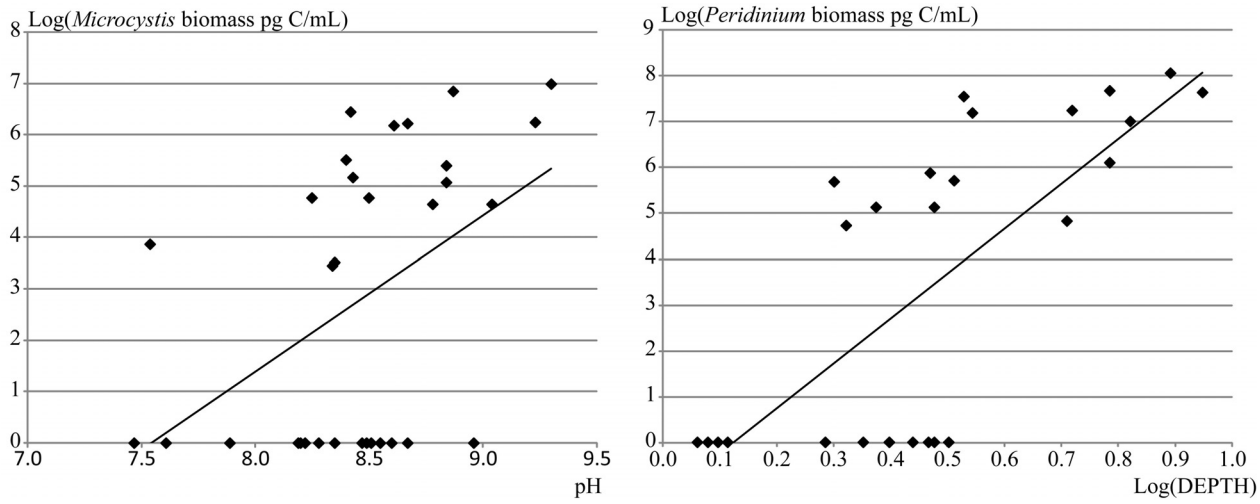

Figure 2.4 Scatter plots of pH versus *Microcystis* in the rainy season (left) and reservoir depth versus *Peridinium* in the dry season (right) in reservoirs in Tigray, Ethiopia. Both correlations are significant at p < 0.01 as shown by non-parametric Spearman Rank tests.

Table 2.2 Environmental variables significantly explaining variation in the phytoplankton communities in the rainy and dry season respectively, as identified by a forward selection Redundancy Analysis. "All" indicates the full model with all variables included, the other variables were selected by a forward selection procedure.

Season	Variable	R^2	(cumulative) adjusted R^2	F	*p*-value
Wet season	All	0.504	0.146	1.41	0.006
	ZX1	0.070	0.039	2.26	0.017
	pH	0.053	0.063	1.76	0.045
Dry season	All	0.593	0.152	1.46	0.009
	COND	0.120	0.083	3.26	0.001
	DEPTH	0.080	0.130	2.30	0.003

Microcystis

Microcystis was the single most dominant cyanobacterium in both the wet and dry season. Results of multiple regressions on the different classes of explanatory variables which can potentially influence *Microcystis* blooms only showed a positive association of *Microcystis* with phytoplankton productivity and more in particular pH (Table 2.3). The other factors tested, being temperature, nutrients, the clear-water versus turbid stable state, and water column stability appeared unimportant. Pearson correlation analysis also showed, apart from a highly significant correlation with pH (R^2 = 0.12, p = 0.0060), some trend for a negative

correlation of *Microcystis* with *Daphnia* biomass (R^2 = -0.065, p = 0.047), but this variable wasn't included in the multiple regression analysis due to an insignificant total clear-turbid model (Table 2.3).

Table 2.3 Multiple regression results for factors potentially influencing *Microcystis* biomass. In the selection model, only variables having a *P*-value < 0.05 after a forward stepwise selection procedure were included. (+) positive correlation, (-) negative correlation.

	Model	Variables included	R^2	R^2_{adj}	*P*
Temperature	All variables	TEMP, ALTITUDE	0.05	0.02	0.221
Nutrients	All variables	TN, TP, TN/TP	0.02	-0.03	0.785
Phytoplankton productivity	All variables	pH, Chla	0.14	0.11	0.011
	Selection	pH (+)	0.12	0.11	0.006
Clear/Turbid	All variables	Daph, TRANSP, VEG, FISH	0.12	0.06	0.11
Water column stability	All variables	SEASON, DEPTH, COND	0.04	0.00	0.470

Discussion

The phytoplankton assemblage of the study reservoirs was generally composed of taxa associated with meso- to hypertrophic freshwater systems. In eutrophic lakes cyanobacteria are favored relative to other phytoplankton (Tilzer, 1987, Reynolds, 1998). They are among

the phytoplankton assemblages that are considered eutrophic indicators. Several studies indicate perennial dominance of phytoplankton communities by cyanobacteria in eutrophic subtropical lakes and the formation of *Microcystis* water blooms over a range of temperatures in all seasons of the year in the tropics (Sommer et al., 1986, Zohary & Robarts, 1989, Sivonen & Jones, 1999, Akin-Oriola, 2003). Under conditions of nutrient enrichment, cyanobacteria are known to proliferate and form noxious blooms in freshwater environments (Reynolds, 1984). Overall, our observations show that colonial cyanobacteria (*Microcystis)* dominated in most of the cyanobacteria dominated lakes. This tends to defy the general hypothesis that colony forming species are more commonly dominant in deeper lakes with a stable water column and that long-term dominance by filamentous cyanobacteria is related to shallow lake depth (Schreurs, 1992, Scheffer, 1998). Other dominant phytoplankton groups, chlorophytes and euglenophytes are also associated with eutrophic conditions, and the last group more specifically with organically enriched shallow ponds and lakes (Reynolds, 1984). This agrees well with the previous characterization of the reservoirs as eutrophic based on nutrient concentrations (Dejenie et al. 2008). A few of the shallowest reservoirs in the dry season were dominated by the diatom *Nitzschia*, which probably has a tychoplanktonic existence in these extremely shallow waters.

An exception is the dominance of *Peridinium* in a large number of reservoirs, especially in the dry season. The RDA for the dry season samples showed a positive correlation of *Peridinium* with depth; while it was negatively correlated with conductivity. *Peridinium* tended to dominate relatively deep and clear waters. Reynolds (1984, 1998) has put *Peridinium* in the oligotrophic to mesotrophic phytoplankton assemblages. This indicates that the deeper reservoirs in Tigray might be less affected by eutrophication, probably due to a lower recruitment of nutrients from the sediment due to less mixing.

The local phytoplankton diversity of the reservoirs in Tigray is low with most reservoirs being dominated by a single genus of cyanobacteria (mostly *Microcystis*), chlorophytes, euglenophytes, cryptophytes or dinophytes. Low diversity environments normally correspond to Shannon-Wiener indices lower than 2.5 (Margalef, 1972, May, 1975). Lower species diversity and richness is associated with eutrophication of freshwater ecosystems (Huszar et al., 1998, Leibold, 1999, Dudgeon et al., 2006, Lo et al., 2008). The shallow reservoirs in Tigray are eutrophic in nature (Dejenie et al., 2008). A change of trophic state towards hypertrophy has been reported to lead to a reduction of species diversity (Huszar et al., 1998, Lo et al., 2008). Cyanobacteria are known to proliferate and form noxious blooms in freshwater environments under nutrient enriched conditions (Reynolds 1984).

The phytoplankton community data show no significant correlation between the data of the two seasons (correlation tested for the 29 reservoirs sampled twice). This may be due to the variation observed in lake depth (see Dejenie et al., 2008). Different reservoirs may respond differently to the environmental changes they face during the dry season. The difference in the pattern of the cyanobacterial blooms is an indication of the response of the reservoirs to the environmental changes. Seasonal monitoring of a subset of the 32 reservoirs studied here also showed the varied nature of the reservoirs (Tsehaye et al., unpublished, Chapter 3 of this thesis). The two high-altitude reservoirs (Ruba Feleg and Teghane) seem to be distinct from each other and from the remaining reservoirs. Whereas Teghane was dominated by cyanophytes (*Anabaenopsis*, *Aphanizomenon*, and *Merismopedium*), cryptomonads (mainly *Cryptomonas*), and green algae (like *Scenedesmus*), Ruba Feleg was mainly dominated by *Peridinium* and *Cosmarium*. The major determining factor for the observed difference may be the effect of depth as Ruba Feleg is much deeper than Teghane, a factor favouring *Peridinium*.

No effects of the spatial configuration of the reservoirs on phytoplankton communities were observed despite the recent construction of these reservoirs in a region with a historical low density of freshwater bodies; only local environmental variables significantly explain the phytoplankton community composition. Traditionally, studies on the phytoplankton communities of ponds and shallow lakes have emphasized the role of local environmental factors (Leibold, 1999; Dodson et al., 2000; Jeppesen *et al.,* 2000). Recently regional processes have been considered in studies of the phytoplankton metacommunities (Soininen et al., 2007, Vanormelingen et al., 2009). Structuring roles from local and regional factors on phytoplankton and zooplankton communities was reported by Soininen et al. (2007), while others report no structuring role (or only marginal impact) of spatial distance (Van der Gucht et al., 2007, Vanormelingen et al., 2008).

Top-down factors may be important in determining the phytoplankton community composition in the semi-arid subtropical reservoirs in Tigray, North Ethiopia. The phytoplankton community composition showed a correlation with the zooplankton community structure in the wet season. Zooplankton community structure in Tigray reservoirs is mainly associated with an altitudinal gradient in nutrient levels and chlorophyll a concentrations (Dejenie et al. 2008). While this productivity gradient may also directly influence phytoplankton community structure, the selection of zooplankton community structure in the RDA suggests that zooplankton causes changes in phytoplankton community structure with altitude. However, zooplankton might be unable to also control phytoplankton biomass, as suggested by the increasing chlorophyll a concentration with increasing nutrient concentrations (Dejenie et al., 2008). The impact of predators on freshwater plankton community structure and productivity has been reported in many studies (Carpenter, 1988, Arner et al., 1998). In many temperate lakes, top–down control of phytoplankton by large herbivorous zooplankton is reported from many studies to cause spring clear-water phase

(Lampert et al., 1986, Sarnelle, 1993, Hanson & Butler, 1994, Jurgens & Stolpe, 1995). In contrast, studies on tropical lakes indicate that chances for an effective top-down control over phytoplankton are lower than in temperate lakes (Fernando, 1994, Lazzaro, 1997). Reports of bottom-up regulation of phytoplankton biomass exist from both field observation and enclosure experiments (Havens, 2002). Zooplankton species in tropical lakes and ponds are generally much smaller than in temperate zones and large herbivorous zooplankton of the genus *Daphnia* seems to be rare or absent at lower latitudes (Hebert, 1978, Foran, 1986, Dumont, 1994b, Gillooly & Dodson, 2000, Brucet et al., 2005, Gyllstrom et al., 2005). Many tropical fish species reproduce throughout the year, and this can reduce the chances for top–down control of phytoplankton by *Daphnia*. Continuous reproduction leads to permanent presence of planktivorous young individuals, and thus a continuously high predation pressure on large zooplankton (Van Leeuwen et al., 2007). Our results, however, showed the presence of the large-bodied filter feeder, *Daphnia* especially in the dry season. This is surprising for a tropical region, and might be related to the absence of fully developed fish communities. The reservoirs in Tigray mainly host *Garra*, a small opportunistic and mainly benthivorous fish (Dejenie et al., 2008). Stomach analysis indicates that the fish do consume zooplankton but also seem to rely strongly on detritus (Mekonnen et al., in prep.). Garra may thus act as a nutrient pump through direct resuspension of sediments or the consumption of benthic resources which are then recycled in the water column, thus affecting water chemistry (Persson & Svensson, 2006). On the other hand, also bottom-up effects of phytoplankton on zooplankton are possible, and may (partly) explain the significant correlation between phytoplankton and zooplankton community structure. For instance, *Daphnia* is usually replaced by small-sized cladocerans and copepods when cyanobacterial blooms occur in eutrophic lakes (Jarvis, 1986, Paerl, 1988, Guo & Xie, 2006). We observed a similar shift in

zooplankton community composition in a separate enclosure experiment (Tsehaye et al., unpublished, Chapter 4 of this thesis).

The abundance of submerged macrophytes is a key variable in shallow lakes, affecting important functions like nutrient cycling, phytoplankton productivity and a wide range of food web interaction (Scheffer et al., 1993, Jeppensen et al., 1997, Scheffer, 1998, Bicudo et al., 2007). In both seasons we observed no important association of macrophytes in structuring the phytoplankton community composition. In shallow temperate lakes, submerged plants often provide refuge for pelagic zooplankton against fish predation, a mechanism with potential strong cascading effects on water transparency and on the entire ecosystem. In (sub)tropical lakes, however, the interaction between aquatic plants and predation may be more complex, particularly because fish density is high within the plant beds in such systems (Schriver et al, 1995, Meerhoff et al, 2003, Meerhoff et al., 2006).

Microcystis occurrence showed a significant correlation with pH. There is a controversy on the relationship of phytoplankton and pH; whether pH is a cause or a consequence of blooms. Shapiro (1997) supported the hypothesis forwarded by King (1972) which describes that blue-greens do well at high pH because that is when free CO_2 concentrations are sufficient for them but less sufficient for other groups. Shapiro (1997) also stated that cyanobacteria are the cause for low CO_2 concentrations by their photosynthetic use. Rapid withdrawal of CO_2 by algal growth leads to raised pH levels (Reynolds, 1998). The highest nutrient levels are encountered at higher altitudes, where *Microcystis* bloom occurrence might be less likely due to a lower temperature, although we failed to find significant altitude effects on *Microcystis* biomass. *Microcystis* could have taken the advantage of low water transparency as altitude was negatively correlated with water column transparency (see Dejenie et al., 2008). Success of *Microcystis* in aquatic systems is attributed to the passive mechanism of positive buoyancy which allows maximal exploitation of light

and gives a significant growth advantage in eutrophic waters (Humphries and Lyne, 1998, Dokulil & Teubner, 2000). The decrease of mean annual water temperature recorded with altitude might also decrease the chance of *Microcystis* bloom formation. *Microcystis* is reported to form blooms at higher temperatures (Robarts & Zohary, 1987). Effects of temperature and altitude on *Microcystis* abundance might be more pronounced during the winter months, when temperature is lowest, than in the months of sampling however. A seasonal study of the same reservoirs showed that high *Microcystis* biomasses were absent in the winter period, although little consistent temporal variation was observed in cyanobacterial abundance due to large differences among reservoirs (Tsehaye et al., unpublished; Chapter 3 of this dissertation).

Microcystis also showed a negative correlation with *Daphnia* biomass. A similar finding was reported by Dejenie et al. (2008) for total cyanobacterial biomass. Cladocerans, especially species of the genus *Daphnia,* are the most efficient grazers under eutrophic conditions (Lampert, 1987). Together with the trend for a positive correlation with fish biomass, the correlation of *Microcystis* with *Daphnia* may indicate a top-down control of *Microcystis* by *Daphnia*. Alternatively, the relationship may indicate the reverse, *Daphnia* populations suppressed by blooms of cyanobacteria (Gliwicz & Lampert, 1990, Deng, 2008). From our field observations it is not clear which mechanism is responsible, but results of an enclosure experiments with contrasting zooplankton treatments suggest *Microcystis* suppresses *Daphnia* (Tsehaye et al., unpublished, Chapter 6 of this thesis). This is in line with reports from both field and experimental studies (Deng et al., 2008), as seasonal and long-term changes in community structure of crustacean zooplankton in lakes have been reported to result from cyanobacterial blooms.

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Appendix 2.1 Taxa list

Chlorophyta, green algae

Chlamydomonas sp.

Closterium sp.

Coelastrum sp.

Coenococcus sp.

Coenocystis sp.

Cosmarium sp.

Dictyosphaerium sp.

Crucigenella sp.

Eudorina sp.

Keratococcus sp.

Nephrocystium sp.

Oocystis sp.

Pediastrum sp.

Planktosphaeria sp.

Scenedesmus sp.

Staurastaurum sp.

Tetraspora sp.

Volvox sp.

Cryptomonads

Cryptomonas sp.

Dinophyceae, dinoflagellates

Peridinium sp.

Cyanophyta, blue green

Anabaena sp.

Anabaena circinalis

Anabaenopsis sp.

Aphanizomenon sp.

Pseudoanabaena sp.

Merismopedia sp.

Microcystis aeruginos.

Diatomophyceae, diatoms

Aulacoseira sp.

Fragilaria sp.

Navicula sp.

Nitzschia sp.

Euglenophyceae, euglenoids

Euglena sp.

Phacus sp.

Strombomonassp.

Trachelomonas sp.

Chapter 3

Seasonal dynamics of environmental variables and associated phytoplankton community structure in tropical semi-arid highland reservoirs of Tigray, North Ethiopia

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Abstract

In semi-arid regions, many small man-made reservoirs have been constructed to ensure water availability for local communities, but a better knowledge of the aquatic food web in such reservoirs is necessary in order to maximize their benefits. We studied the temporal dynamics of the phytoplankton communities and associated environmental variables in eight shallow, semi-arid highland reservoirs in Tigray, North Ethiopia, during one year. Inter-annual variability was assessed by a comparison with a field survey carried out one year earlier. Statistical analysis using Friedman ANOVA and Kendall Coefficient of Concordance showed a significant temporal pattern for most environmental variables. Partial RDA showed that the main limnological changes were associated with seasonal differences in rainfall, while also water temperature differed strongly between winter and the rest of the year. Variables associated with the aquatic food web, i.e. chlorophyll a concentration, *Daphnia*, cladoceran and copepod biomass, and Secchi disc depth showed no or a less pronounced temporal variation, in sharp contrast to the situation in temperate water bodies. Phytoplankton biomass had two minima, one at the temperature minimum in winter and a more pronounced one during August, the month with the heaviest rainfall. Cyanobacteria seemed to have two main bloom periods, one in September-October and a more pronounced one in May-June. Seasonal variation in total phytoplankton and cyanobacterial biomass was, however, not significant, probably due to the large variation in total phytoplankton biomass among reservoirs and the near-absence of cyanobacteria in some reservoirs. Also for dinoflagellates and green algae, temporal variation was insignificant, while diatoms, green algae and cryptomonads had significantly higher biomasses in autumn and winter than in the remaining period. Despite the fact that seasonal differences in local environment and associated phytoplankton communities were significant, they were generally smaller than the more pronounced changes between the investigated reservoirs and year-to-year changes.

Introduction

In tropical (semi-)arid regions, the construction of small reservoirs is of vital importance to ensure water availability for irrigation and as drinking water for both people and their cattle. The main water source for such reservoirs is precipitation, which is seasonal in nature, as a consequence of a highly variable water input and associated water level fluctuations. Next to the dynamics of inflow and variable flushing rates, also seasonal changes in wind and temperature may alter environmental conditions. Due to their high turn-over rates, phytoplankton communities may respond rapidly to such environmental changes. Phytoplankton seasonality has been observed in several large natural lakes or reservoirs in the tropics and is usually driven by changes in hydrology, including water in- and out-put or water column structure and circulation due to seasonal changes in wind strength and direction (Talling, 1986 and references therein, Hooker and Hernandez, 1991). Despite the socio-economic importance of small reservoirs, their seasonal environmental dynamics and associated changes in the phytoplankton communities are largely unstudied, although they may differ substantially from larger water bodies.

The eutrophic nature of many (sub)tropical reservoirs and their constant high water temperature render them especially vulnerable to the occurrence of cyanobacterial blooms (Bouvy *et al.*, 2000), which are notorious for producing powerful toxins (Codd *et al*., 2005, Huisman *et al*., 2005). Other environmental conditions favoring the dominance of cyanobacteria over other phytoplankton include a high temperature and water column stability, which is enhanced by increased temperatures but also by reduced flushing rates (Harris and Baxter, 1996; Paerl and Huisman, 2008). Such conditions are often met in the dry season in (semi)arid tropical regions, but interannual variability in rainfall may be at least as important in determining cyanobacterial bloom occurrence (Harris and Baxter, 1996; Bouvy et al., 2000).

In Tigray, North Ethiopia, more than 70 small reservoirs were constructed to reduce problems of water scarcity in an area with a dry season lasting up to eight months. A previous field survey revealed that most reservoirs were in the eutrophic state and many were heavily impacted by cyanobacterial blooms, mainly of *Microcystis*, with chlorophytes and dinophytes as co-dominants (Dejenie et al., 2008; Tsehaye et al., unpublished, Chapter 2 of this thesis). The objective of the present study was to determine whether seasonal variation occurred in (1) limnological variables and (2) the phytoplankton communities of the highland reservoirs in Tigray, independent of among-reservoir differences. Inter-annual variability was assessed by a comparison with a field survey of phytoplankton communities conducted one year earlier (Dejenie et al., 2008, Tsehaye et al., unpublished, Chapter 2 of this thesis).

Material and methods

Study site

Tigray, the northernmost regional state of Ethiopia, is located between 12° and 15° N and 37°10' and 40°10'E (Figure 3.1). Eight reservoirs representing the main environmental gradients present in the region were selected for the present study from a set of 32 reservoirs characterized during a previous field survey (Dejenie *et al*., 2008; Tsehaye et al., unpublished, Chapter 2 of this thesis). The included reservoirs were permanent or temporary and varied in various ecological characteristics such as cyanobacterial bloom occurrence, macrophyte coverage, presence of fish, and zooplankton abundance (see Table 3.1. for an overview and Dejenie *et al*., 2008). Altitude, area, depth, turbidity and nutrient load were also considered in an effort to make the recorded seasonal variation representative for the large number of reservoirs in the region.

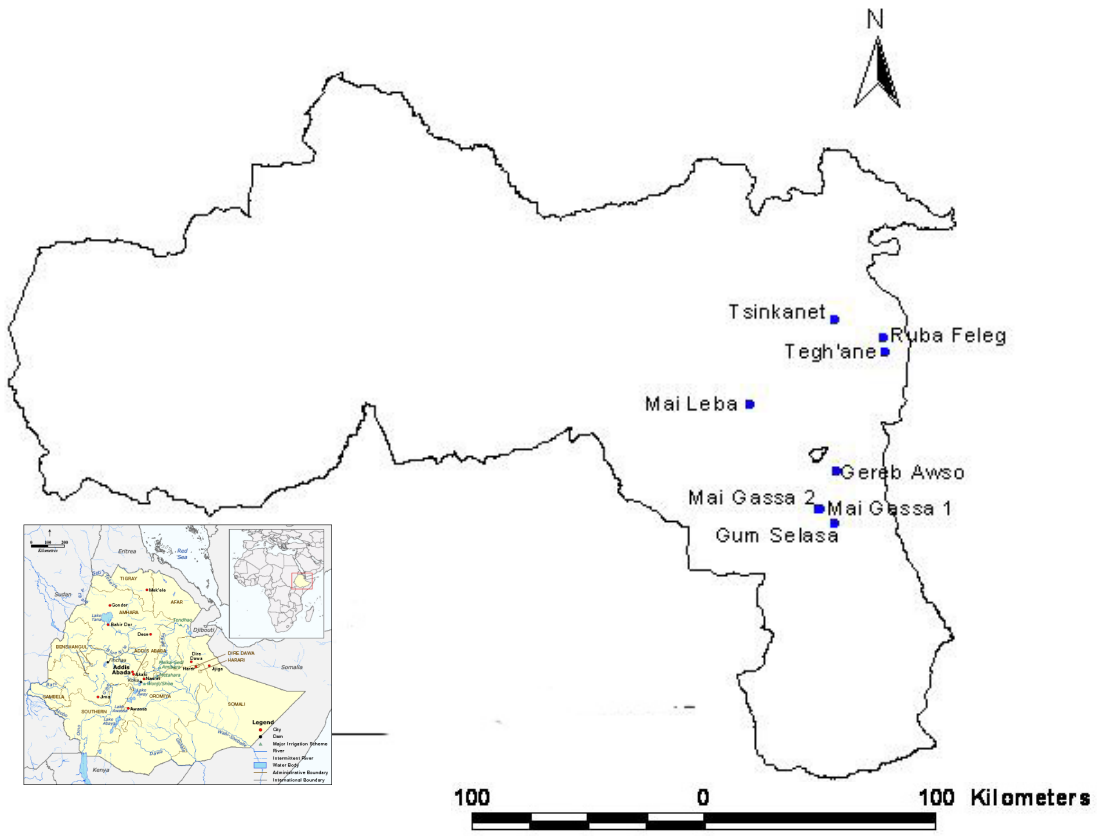

Figure 3.1 The geographic location of the eight reservoirs in Tigray, North Ethiopia, selected for the seasonal study of environmental variables and associated phytoplankton communities.

Sampling

Field samples from the 8 reservoirs were collected every month for an entire year, from September 2005 to August 2006. The sampling procedure was as described by Dejenie et al. (2008). Water samples, except for crustacean zooplankton, were taken at a fixed location from three depths: surface, middle (intermediate depth), and near to the bottom, using a 3 liter Heart valve water sampler and pooled. The pooled samples were transported to the shores immediately after retrieval and sub-samples were taken and fixed on the shores. At each site conductivity, dissolved oxygen, pH and temperature were measured with a portable meter WTW Multi 340 I electrode. Conductivity was measured for the pooled samples, but dissolved oxygen and pH were recorded at the three depths. Transparency was determined using a Secchi disc (diameter: 30 cm). Sub-samples for nutrient analysis were transported in a

cool box to the laboratory and stored at -18°C until analysis. Sub-samples for enumeration of phytoplankton were fixed with acid Lugol's solution to 0.1 % final concentration. Crustacean zooplankton were sampled from two pelagic sites and pooled. Twelve liters of water was taken from the surface down to near the bottom of the reservoir at an interval of one meter using a Schindler-Petalas trap (64 μm mesh size). Samples ware preserved with sugar saturated formalin. Wind speed data were obtained from the digital database of climatological and stream flow data of Geba catchment (MU – IUC, 2007).

Table 3.1 General characteristics of the eight reservoirs selected for assessing seasonal dynamics of environmental variables and associated phytoplankton communities.

Reservoir name	Altitude (m.a.s.l.)	Trophic state	Cyanobacteria bloom*	Fish	Macrophyte coverage
Gereb Awso	2283	Eutrophic	None	Present	0
Gum Selasa	2116	Eutrophic	Present	Present	25%
Mai Gassa I	2131	Mesotrophic	Present	Present	75%
Mai Gassa II	2130	Eutrophic	Present	Present	50%
Mai Leba	2231	Mesotrophic	None	Absent	0
Ruba Feleg	2747	Eutrophic	None	Present	< 25%
Tegh'ane	2735	Eutrophic	None	Absent	0
Tsinkanet	2320	Mesotrophic	None	Present	25%

* Bloom defined when cyanobacteria % biomass contribution is ≥ 65%

Sample analysis

Total nitrogen was analyzed with the kjeldahl method and total phosphorus was analyzed following the ascorbic acid method (Anderson & Ingram, 1989). Chlorophyll a was estimated *in situ* with a portable fluorometer (Turner Aquafluor; average of three measurements) from the pooled water samples. Zooplankton was counted with a stereoscopic microscope (Dejenie et al. 2008). Individual counts were used to calculate densities (individuals per liter) and biomass. Phytoplankton was enumerated using the inverted microscope method (Lund *et al*., 1958, Wetzel and Likens, 2000). Bengal Rose B dye solution was added to facilitate distinction between detritus particles and phytoplankton cells. Colonies (filaments, coenobia) were considered as a single 'unit' during the counts, and counting was continued until at least 300 units were counted. Biovolume calculations were made by measuring the linear dimensions of 50 units of each taxon in a sample and fitting the different taxa to geometric forms (Hillebrand et al., 1999). Phytoplankton biomass was estimated from cell biovolume measurements using previously published biovolume-to-carbon conversion data (Menden-Deuer & Lessard, 2000).

Data analysis

Temporal patterns were investigated for two sets of variables: (1) environmental variables and (2) phytoplankton taxa. The variables included in the first data set were rainfall, total nitrogen, total phosphorus, chlorophyll a, oxygen and suspended matter concentrations, Secchi disc depth, temperature, pH, conductivity, depth, *Daphnia* and copepod biomass. The second data set consisted of the biomass of the different recorded phytoplankton genera and higher taxon groupings. The relationships between temporal change and each set of variables, independent of differences between reservoirs, were investigated using partial redundancy analysis (partial RDA). Months were used as explanatory variables and reservoirs as co-variables to partial out

the effects attributable to differences between the reservoirs. Dummy variables were constructed for the months and reservoirs, and reservoirs were assigned as 'blocks' (Lepŝ and Ŝmilauer, 2003). Significance tests were based on 999 random Monte Carlo permutations, which were restricted to blocks to account for the dependency of data based on samples from the same reservoir. The partial RDA on the environmental data set was performed with standardized limnological variables.

To determine whether temporal changes for each separate environmental or phytoplankton variable were significant, we used Friedman ANOVA, a non-parametric alternative for one-way analysis of variance, and Kendall Coefficient of Concordance. The Kendall coefficient of concordance is similar to a non-parametric Spearman Rank correlation coefficient R, except that it expresses the relationships between more than two groups of data through time (Legendre, 2004). As wind speed was only measured for a single reservoir, temporal changes in wind speed were not tested statistically. To account for the large variability in phytoplankton community composition among reservoirs, we carried out the Friedman ANOVA analysis for reservoirs permanently dominated by cyanobacteria separately.

Inter-annual variation was assessed by comparing the data collected for this study in September 2005 and April 2006 with those obtained from a field survey in September 2004 and April 2005 (see Dejenie et al. 2008 and Tsehaye et al., unpublished, Chapter 2 of this thesis). Inter-annual (among year variation) and intra-annual (among months variation) of both abiotic and biotic variables were assessed using partial RDA as described above.

Prior to analysis, all abiotic and biotic variables were log(x+1) transformed. Univariate analyses were performed with the statistical program STATISTICA 7.1 (Stat Soft Inc., Tulsa, OK, U.S.A.) and multivariate analyses with CANOCO 4.5 (ter Braak & Smilauer, 1998; Lepŝ and Ŝmilauer, 2003).

Results

Environmental variation

A partial RDA shows that there was a significant temporal pattern in the environmental variation of Tigray reservoirs (Trace = 0.305, F = 7.444, p = 0.0001). Centroids of the different months displayed a marked trajectory on the RDA biplot, with centroids of the winter, summer, spring and autumn months in different quadrants of the biplot (Fig. 3.2). The first axis of the RDA explained 16.8% of the total environmental variation and was mainly associated with variation in rainfall. It was also associated with changes in temperature, total nitrogen and suspended matter concentrations. The second axis explained a much lower proportion of the environmental variation (5.6%) and was associated with changes in suspended matter and temperature. The following picture emerged: September to February are characterized by drought, low temperature (especially December and January), and low suspended matter and total nitrogen concentrations. The period March to August is associated with rainfall and high suspended matter concentrations (especially July and August) and high temperature, pH and conductivity (especially in spring). Friedman ANOVA of the limnological variables (Table 3.2) corroborated these findings as most variables displayed highly significant temporal variation. Chlorophyll a concentration (p = 0.037) and copepod (p = 0.015) and cladoceran biomass (p = 0.025) showed significant but rather weak temporal patterns, whereas we could not observe any temporal signal for Secchi disc depth (p = 0.13) and *Daphnia* biomass (p = 0.73). The low coefficients of concordance (Table 3.2) and low scores on the first two axes of the RDA (Fig. 3.2) also indicate that they fail to show a consistent temporal pattern.

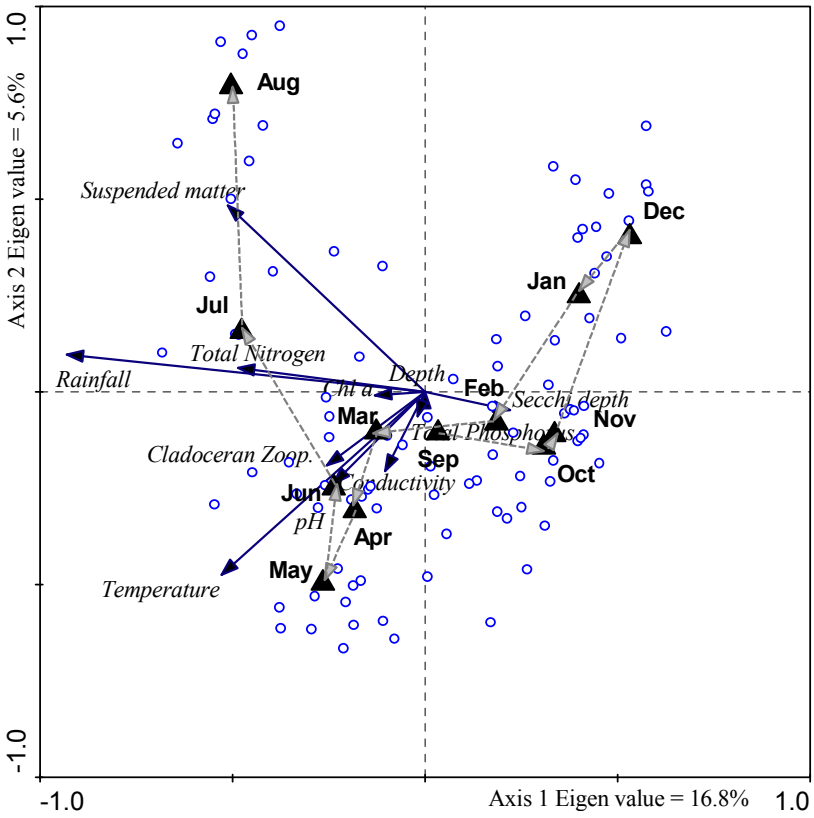

Figure 3.2 First and second axis of a partial RDA on the response of environmental variables in function of month of the year, corrected for the influence of reservoir by including reservoirs as co-variables. Environmental variables are indicated by arrows, months by up-triangles, and samples by empty circles. Broken arrows indicate the sequence of sample collection. The first and second axes explain 16.8 and 5.6% of the total environmental variation, respectively.

Table 3.2 Results of Friedman ANOVA and Kendall Coefficient of Concordance (W) calculations on the temporal dynamics of environmental variables recorded for eight reservoirs in Tigray, Ethiopia, between September 2005 and August 2006.

Variable	ANOVA Chi Sqr.	P	W
Physicochemical			
Total nitrogen	50.43	<0.001	0.57
Total phosphorus	74.09	<0.001	0.85
Secchi depth	16.17	0.14	0.18
Suspended matter	34.56	<0.001	0.39
Chlorophyll a	20.67	<0.04	0.24
pH	32.57	<0.001	0.37
Conductivity	67.51	<0.001	0.77
% O_2	25.41	0.007	0.29
Temperature	58.75	<0.001	0.67
Rainfall	78.52	<0.001	0.89
Depth	75.53	<0.001	0.86
Zooplankton			
Total	26.15	0.006	0.29
Daphnia	7.85	0.730	0.09
Cladocerans	21.96	0.025	0.25
Copepods	23.59	0.015	0.27

Month-to-month changes of the limnological variables are shown in Fig. 3.3(a-d). Rain fell between March and August with July and August showing a pronounced maximum. Associated with this is a gradual increase in conductivity from September to June and a steep decrease afterwards when the reservoirs are at full bank from the heavy rains of July and

August. Suspended matter concentrations increased in June and peaked in August. Water temperature ranged between 12.1°C and 22.4°C, was minimal in December and January and increased, first fast and then gradually, towards a broad maximum in spring and early summer (Figure 3d). pH peaked at the same time but declined fast afterwards. Total phosphorus levels were high until March but then dropped dramatically, stayed relatively low for the rest of the study period and increased again in August. High total nitrogen concentrations were associated with the rainy season but started to increase in winter before the first rainfall. Concentrations were somewhat lower in May-June but increased again in July-August. Dissolved oxygen was almost always relatively high and close to saturation (on average 91%). Secchi disc depth was on average low, usually not exceeding 1 m for most of the reservoirs, except Ruba Feleg.

Phytoplankton seasonality

A total of 28 phytoplankton genera were identified. The green algae were the most diverse group, accounting for 43 % of the total taxa identified. Six genera of cyanobacteria were counted, accounting for 21 % of the total number of genera. *Microcystis* was the single most abundant taxon and accounted for about 74% of the total phytoplankton biomass recorded over the year for all the reservoirs (Fig. 3.4). Other identified genera belonged to the diatoms, cryptomonads, dinoflagellates, and euglenophytes.

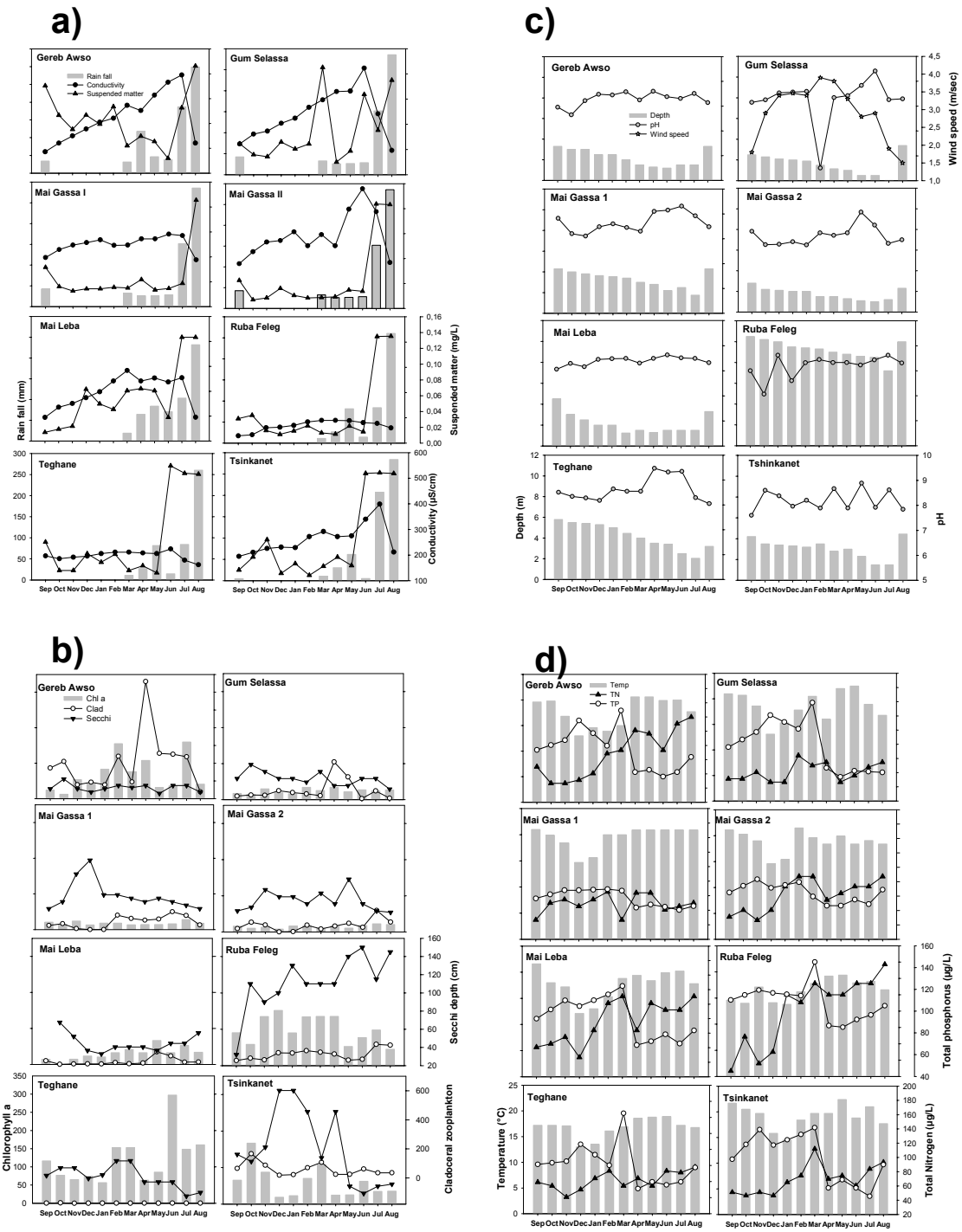

Figure 3.3 Changes in environmental variables during the course of one year in eight reservoirs in Tigray, North Ethiopia. a) Rain fall, conductivity and suspended matter; b) Chlorophyll a, cladoceran zooplankton biomass and Secchi depth; c) Depth, pH and wind speed; d) Temperature, total nitrogen and total phosphorus. Data for wind speed are available for only one station.

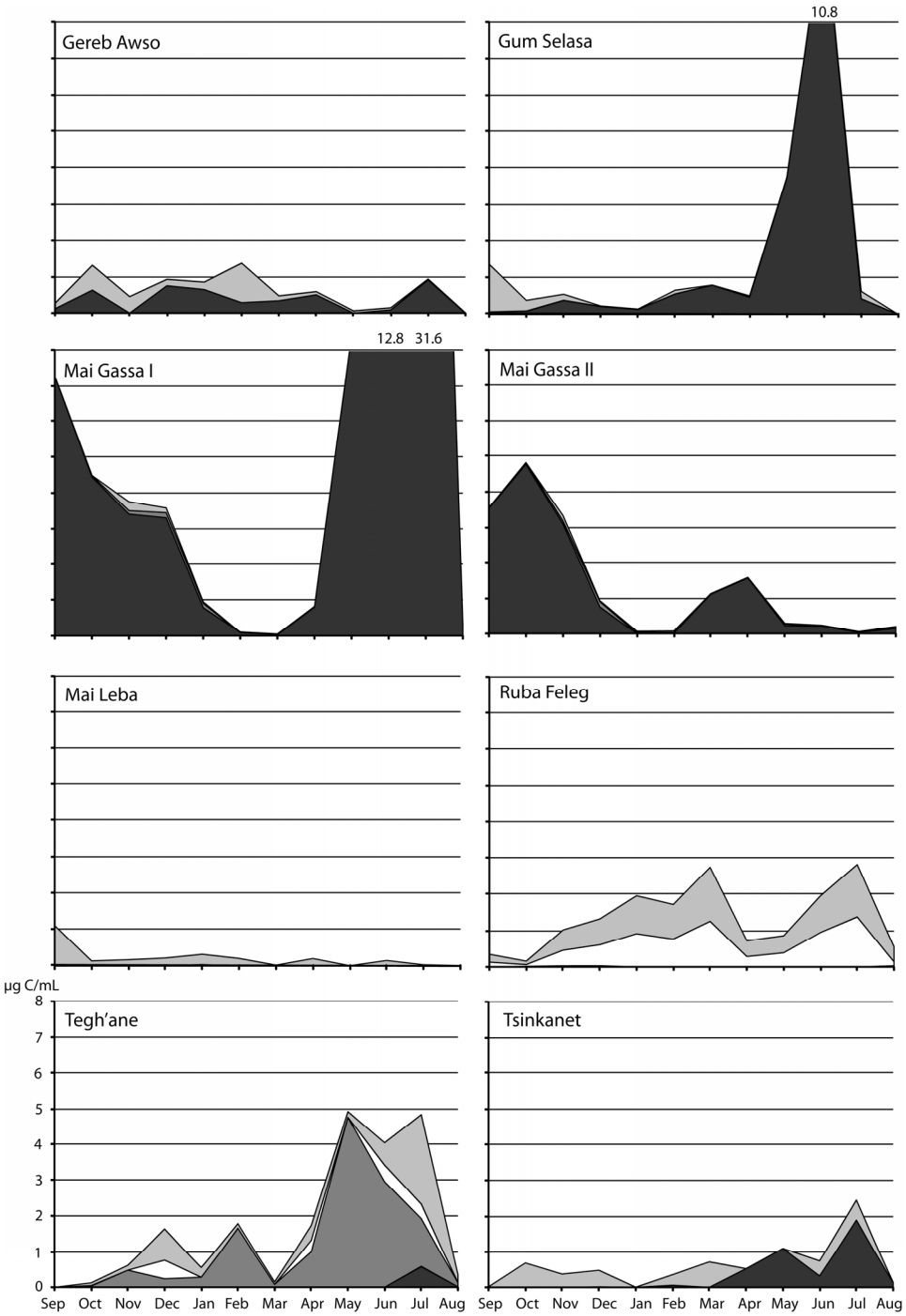

Figure 3.4 Changes in phytoplankton biomass during the course of one year in eight reservoirs in Tigray, North Ethiopia. Total phytoplankton biomass was divided into *Microcystis* (black), the single most dominant genus, the single dinoflagellate genus *Peridinium* (white), cyanobacteria other than *Microcystis* (dark grey), and biomass of all other phytoplankton (light grey).

A highly significant temporal turn-over in phytoplankton community composition was observed by partial RDA of the phytoplankton at the order level (Trace = 0.146, F = 1.846, *p*

= 0.0005). The first axis of the RDA explained 6.9% of the total variation and, similar to the partial RDA of the environmental variables, separated the period March-August from September-February (Fig. 3.5). A similar pattern emerged from a partial RDA in which the ten most abundant phytoplankton genera were used (Trace = 0.097, F = 1.517, p = 0.0020, biplot not shown). The main difference between both periods was the higher abundance of green algae, diatoms and cryptomonads in September-February. Euglenoids appeared to be mainly associated with the second axis, which explained 3.6% of the total variation, due to a peak in abundance of *Trachelomonas* in July. Cyanobacteria and dinophytes did not show seasonal variation, as seen by their low scores on the first two axes of the RDA. This pattern was confirmed by the Friedman ANOVAs showing significant temporal variation for diatoms, green algae, and cryptomonads, but not for cyanobacteria, dinoflagellates and euglenoids (Table 3.3). This may result from the difference in the occurrence of cyanobacterial blooms in the different reservoirs. Also partial RDA and Friedman ANOVAs run for reservoirs permanently dominated by cyanobacteria separately could not reveal temporal pattern of cyanobacteria. At the genus level, only *Cryptomonas sp.* showed a significant temporal variation. This may result from an inconsistent presence of some members of a genus in different reservoirs, the variation among reservoirs overwhelming the seasonal variation. But *Cryptomonas sp.* is the only recorded genus in our study reservoirs.

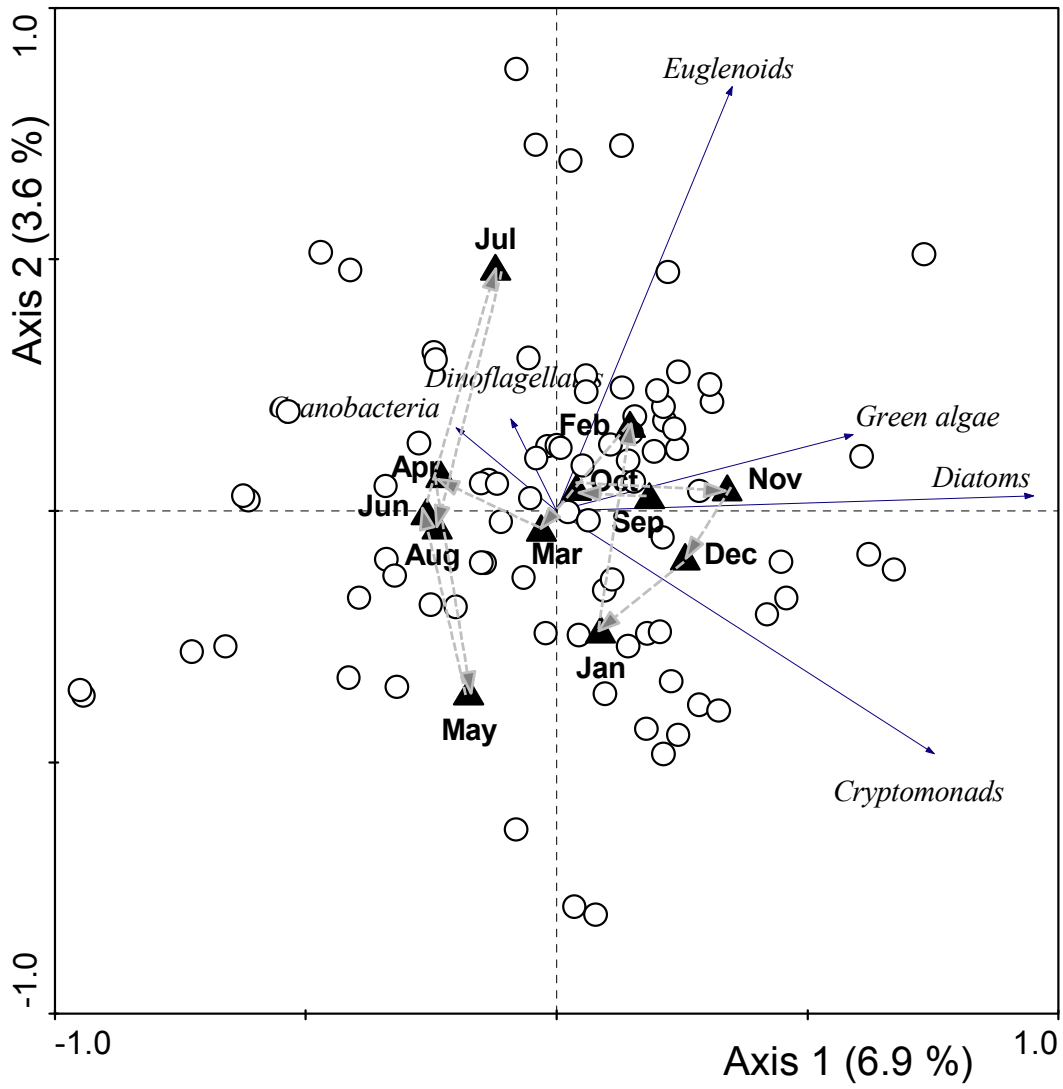

Figure 3.5 Partial RDA biplot on changes in phytoplankton taxon in function of time, corrected for the influence of reservoir by including reservoirs as co-variables. Phytoplankton taxa are indicated by arrows, months by up-triangles, and samples by empty circles. The first and second axes explain 6.9% and 3.9% of the total phytoplankton variation, respectively.

Table 3.3 Results of Friedman ANOVA and Kendall Coefficient of Concordance (W) calculations on the temporal dynamics of phytoplankton communities recorded for eight reservoirs in Tigray, Ethiopia, between September 2005 and August 2006.

Taxa	ANOVA Chi Sqr.	P	W
By division			
Diatoms	23.078	0.02	0.26
Green algae	19.84	0.047	0.23
Cryptomonads	27.79	0.004	0.32
Cyanobacteria	11.21	0.43	0.13
Dinoflagellates	12.99	0.29	0.15
Euglenoids	19.39	0.05	0.22
Total phytoplankton	15.77	0.15	0.18
By Genera			
Anabaena sp.	16.44	0.13	0.19
Aulacoseira sp.	16.08	0.14	0.18
Closterium sp.	8.28	0.69	0.09
Coelastrum sp.	16.43	0.13	0.19
Cryptomonas sp.	27.79	0.003	0.23
Euglena sp.	12.95	0.29	0.15
Fragilaria sp.	14.34	0.22	0.16
Microcystis	11.33	0.42	0.13
Peridinum sp.	12.99	0.29	0.15
Phacus sp.	10.88	0.45	0.12
Scenedesmus sp.	17.29	0.09	0.19
Trachelomonas sp.	18.34	0.07	0.21

In terms of total phytoplankton biomass, two maxima can be distinguished, the first at the beginning of the dry season (October – November) and the other at the end of the dry season (May – June) (Fig. 3.6). The two maxima are separated by two minima, one in January and the other, more pronounced one, in August. The lower value of average phytoplankton biomass in January coincides with a low water temperature, while in August it coincides with high rainfall and a high amount of suspended matter. A Friedman ANOVA failed to show significant variation in phytoplankton biomass, however (Table 3.3).

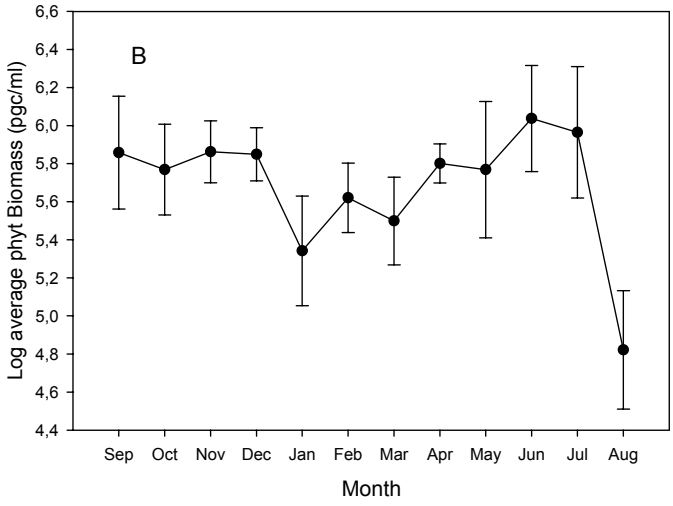

Figure 3.6 Average total phytoplankton biomass in Tigray reservoirs during the course of one year

The first axis of a partial RDA of the environmental variables (Trace = 0.311, F = 5.794, p = 0.002) and phytoplankton communities (Trace = 0.124, F = 1.63, p = 0.022) in the eight studied reservoirs in both sampling years (2004/2005 and 2005/2006) showed that the main variation in both data sets was associated with among-year differences (Fig. 3.7). Differences between seasons were associated with the second axis. Moreover, intra-annual variation in the phytoplankton communities was higher in the survey campaign 2004/2005 than in this study. The year 2004/2005 was exceptionally dry year, with lower than average spring rains, a late start of the main rainy season and low overall amount of rainfall during the

rainy season. Only August was with the typical amount of rainfall (MU – IUC, 2007).

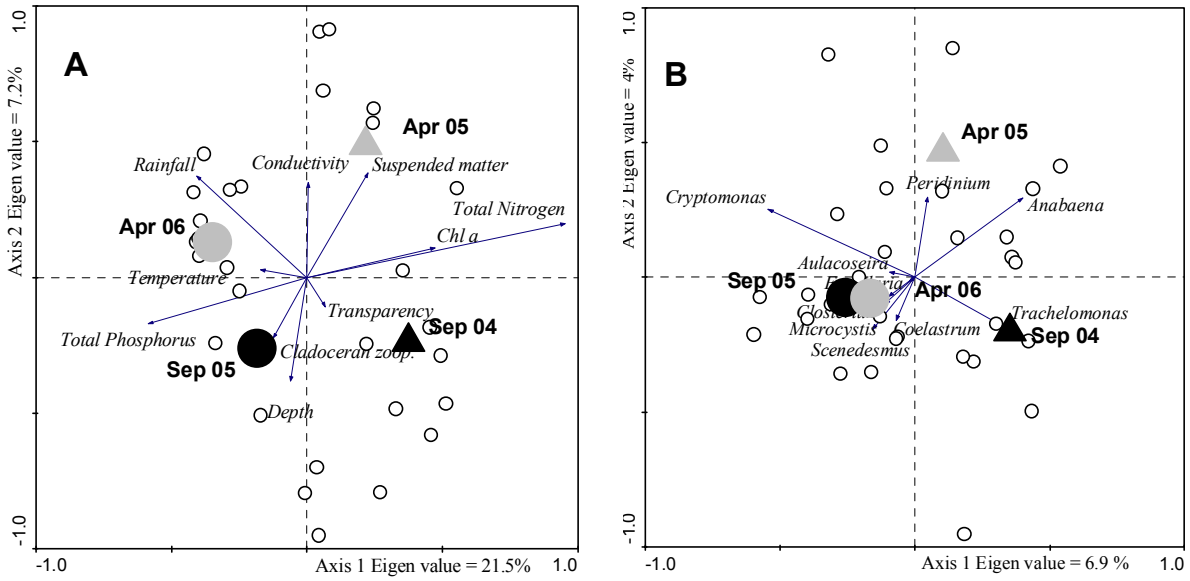

Figure 3.7 Standardized RDA plot on environmental variables (A) and phytoplankton community (B) in function of time (months), after correction for among reservoir differences. Circles and up-triangles represent centeroids of months and year. Year 2004/2005 is represented by up-triangle while 2005/2006 is represented by circles. Solid arrows represent response variables, and small circles represent individual samples.

Discussion

The abiotic variables measured in this study showed a clear temporal pattern associated with seasonal changes in rainfall and temperature. In tropical systems, marked variations in temperature and rainfall between seasons often influence the physico-chemical characteristics of water bodies (Adebisi, 1981, Chapman and Kramer, 1991). The conductivity of reservoirs and manmade lakes depends largely on that of inflowing rivers, turnover rates and the soil of the catchment area (Payne; 1986). In our study, the observed increasing trend for conductivity during the dry season (Fig. 3.3), peaking in June just before the main rain months, indicates the role of rain and the flood that enter the reservoirs and dilute the water in the reservoirs. The same pattern of conductivity has been reported for different lakes (Elizabeth & Amaha, 1994; Pointo-Coelho, 1998; Tamuka & Brain, 2007) which may be associated with the high

evaporation rates during the dry season. The draw-down of the reservoirs as water is used for irrigation during the dry season might have increased the impact of evaporation, consequently increasing conductivity. While the increase in pH in spring is probably mainly associated with the rise in conductivity, it might additionally be influenced by an increased photosynthetic productivity associated with the increasing temperature in spring. Photosynthesis removes CO_2 from the water, thereby decreasing the concentration of carbonic acid (Wetzel, 2001). Also associated with seasonal variation in rainfall is the concentration of suspended matter, showing a maximum in the main rain months July and August. Heavy rains cause a strong run-off from the catchment resulting in an accumulation of sediment particles in the reservoirs. Associated with this run-off might also be the increase in total phosphorus (TP), as TP is tightly coupled with sediment particles (Moss, 1998), and total nitrogen concentration (TN) in July and August. Total nitrogen (TN) concentration increased at the end of the winter period and remained high in spring, although there was a small decreasing. This could be due to an increased impact of trampling of the reservoirs by cattle as a result of clearing of crops from the farmlands. Average wind speed was maximal during the winter period and this may have contributed to the winter increase in nutrient concentrations, due to an increased recruitment of nutrients from the sediment in that period. This may especially be true for phosphorus concentration, which seems to be strongly correlated with wind speed. However, to better understand the nutrient cycle in these reservoirs, we recommend collecting data over several years and studying the trend in inter- and intra-annual variability of nutrients in relation to the phytoplankton community.

Despite the fact that there were highly significant seasonal changes in abiotic environmental variables, the variables indicative of aquatic food web structure (zooplankton biomass, chlorophyll a concentrations, water clarity) showed less significant or insignificant temporal variation. This is probably caused by the fact that the seasonal changes in the

limnology of these reservoirs were not large enough to cause large and consistent seasonal changes in the aquatic food web structure, contrary to the situation in temperate shallow water bodies (Kalff & Watson, 1986; Sommer et al., 1986). Phytoplankton seasonal studies on other reservoirs report minimum total biomass following the rainy seasons and heights near the end of the prolonged dry season (Kalff & Watson, 1986; Hooker & Hernandez, 1991). These studies, similar to ours and unlike reports from temperate regions, report consistent dominance by cyanobacteria. The sharp decline in phytoplankton biomass observed in August my result from the combined effect of dilution by floods coming into the reservoirs and the accompanied increased suspended matter which in-turn affects the light climate.

The taxon composition of the reservoirs in Tigray was dominated by the Chlorophyta. Reports from large tropical lakes also show a dominance of Chlorophytes in terms of diversity (Kalff & Watson, 1986; Elizabeth & Amaha, 1994). Cyanobacteria, however, dominate in terms of biomass. Blue green algae have a wide geographical distribution, although some species are specific to temperate or tropical regions. *Spirulina, Anabaenopsis and Cylidrospermopsis* species, for example, occur more frequently at lower latitudes (Gibson & Smith, 1982; Bouvy et al., 2000). Planktonic cyanobacteria, like *Microcystis*, are more commonly associated with eutrophic (Gibson & Smith, 1982) and alkaline waters (Wetzel, 1983). The reservoirs in Tigray are therefore not exceptional in having one fifth of the number of taxa and 75 % of the total phytoplankton biomass composed of cyanobacteria and *Microcystis*, respectively.

Comparing the partial RDA ordinations for the environmental and phytoplankton taxa, we can draw some conclusions with respect to the pattern of dominance of different phytoplankton taxa in relation to environmental variables. From September to February, with relatively low temperature and high wind speed, probably resulting in lower water column stability and high total phosphorus concentrations, the chlorophytes, cryptomonads, and

diatoms had a higher biomass, resulting in co-dominance with cyanobacteria. Cyanobacteria (dominated by *Microcystis*) and *Peridinum* (Dinoflagellates) tended to dominate from March to August, periods characterized by higher temperature, lower TP, and higher conductivity and pH. Large blooms of *Microcysis* came up in May in Mai Gassa I and Gum Selassa and a less pronounced maximum was observed in Tsinkanet during that same period. These blooms reached a maximum in June-July, right before they were diluted by the heavy rainfall in August. A similar pattern was observed for another cyanobacterium, *Anabaenopsis*, in Teghane – one of the high altitude reservoirs. A less pronounced *Microcystis* bloom was detected in early autumn in Mai Gassa I and II. Despite these strong *Microcystis* bloom dynamics, overall temporal patterns in cyanobacterial biomass were not significant, probably due to the fact that cyanobacterial blooms only developed in five of the eight reservoirs. In the other three reservoirs, mainly high altitude reservoirs, cyanobacteria blooms were not observed. Although we can see some associations between phytoplankton taxa and environmental variables, the phytoplankton assemblages could also have been influenced by more complex interactions of physical, chemical and biological factors (e.g., zooplankton biomass and community structure).

Seasonal variation in phytoplankton community structure is less important than variation among reservoirs (see Table 3.3). This may be the result of the large difference between the reservoirs in physicochemical variables like altitude and depth. Dejenie et al. (2008) reported that reservoirs in lowlands suffer more from cyanobacteria blooms compared with high altitude reservoirs.

The comparison of the April/September data set of this study with the field survey in the same months of the preceding year showed the lack of repeatability in temporal pattern among years. The limnological variables showed both inter-annual and intra-annual variation. The variation among seasons for the limnological variables and phytoplankton community

was more pronounced in 2004/2005 than in 2005/2006 (Fig. 3.7). This may be because the year 2004/5 was an exceptionally dry year. For example, of the 32 reservoirs surveyed in September 2004, eight were dry or very shallow in the subsequent dry season (April 2005; Dejenie et al., 2008). Our data show that the reservoirs are dynamic in their phytoplankton community assemblage. One of the major driving factors for this variation seems to be rainfall.

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Chapter 4

Bacterial community composition in semi-arid highland reservoirs of Tigray, North Ethiopia – impact of environmental factors and *Microcystis* blooms

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Abstract

We studied the taxon composition of bacterioplankton communities in semi-arid highland reservoirs in Tigray, northern Ethiopia using molecular fingerprinting (DGGE). A total of 26 reservoirs were sampled during both the wet and dry season. Abiotic factors as well as the composition and standing stock biomass of phytoplankton, zooplankton and fish were measured and were used to interpret variation in bacterial community composition. Multivariate analysis revealed that bacterial community composition was significantly explained by *Microcystis* and zooplankton (copepod) biomass in the wet season, and by nutrient concentrations, dissolved oxygen concentration, cattle and fish biomass in the dry season. Reservoirs characterised by a *Microcystis* bloom had a lower bacterial taxon richness as revealed by DGGE than reservoirs that did not harbour a *Microcystis* bloom.

Introduction

To date studies of bacterial communities in lakes and their relationship with biotic and abiotic environmental factors largely focused on temperate freshwater bodies (Eiler & Bertilsson, 2004; Allgair & Grossart, 2006; Shade et al., 2007; Van der Gucht et al., 2007; Dimitriu et al., 2008). A few studies in tropical and sub-tropical Africa focused on bacterioplankton ecology in relatively large lakes. Zenabu and Taylor (1989) reported vertical heterogeneity of heterotrophic bacterial activity during long stratification period from the Ethiopian rift-valley lake Awasa. De Wever *et al.* (2005) also studied the vertical and horizontal heterogeneity of bacterial community composition of Lake Tanganyika. Heterotrophic bacterioplankton production and mortality rates by grazing were also reported from East African lakes (Zenabu & Taylor, 1990; Pirlot et al., 2007). Bacterial grazing by predators (mainly protozoa and ciliates) was reported as the major cause of bacterial mortality in lakes Awassa and Tangnyka (Zenabu & Taylor, 1990; Pirlot et al., 2007). So far, however, there is little known on the bacterial communities inhabiting tropical and subtropical freshwater ecosystems, including the many artificial reservoirs that are constructed for irrigation in semi-arid areas.

Many freshwater lakes all over the world are affected by eutrophication. This is largely the result of high external nutrient loading associated with sewage influx and an intensification of agriculture. Especially shallow lakes are very sensitive to eutrophication, due to the intensive exchange of nutrients between the sediment and the water column (Moss, 1998; Scheffer, 1998). Because of eutrophication, blooms of cyanobacteria have become a common and recurrent phenomenon (Akin-Oriola, 2003; Chorus and Bartram, 1999; Huisman *et al*., 2005; Zurawell *et al*., 2005; Jayatissa *et al*.,

2006). Also in the man-made reservoirs in northern Ethiopia, cyanobacterial blooms are common. Tsehaye et al. (unpublished ms) detected dense *Miccrocystis* blooms in 10 out of 32 reservoirs in Tigray.

Cyanobacterial blooms might influence community composition of heterotrophic bacterial communities, as they are likely to affect the amount and quality of dissolved organic matter, the primary food source of bacterial communities. In addition, they often produce elevated levels of cyanotoxins and noxious allelopathic compounds which might have an inhibitory effect on the growth of bacteria (Gorham & Carmichael, 1988; Østensvik et al, 1998; Valdor & Aboal, 2007). Finally, there may also be direct competition for nutrients or trace elements between cyanobacteria and heterotrophic bacteria (Mure et al., 1999). The use of 16S rRNA based finger-printing methods has revealed shifts in bacterial community composition during the course of a phytoplankton bloom (Reimann & Winding, 2001) and also revealed dramatic changes in the bacterial community upon viral-induced termination of a cyanobacterial bloom (Van Hannen et al., 1999a). Aboal et al. (2000, 2002) showed that the production of microcystins was associated with a reduction in animal and algal community diversity, but did not study bacterial communities. Eiler & Bertilsson (2004), however, found that bacterial communities associated with massive blooms of cyanobacteria were very different in different habitats, suggesting that the cyanobacteria blooms did not result in a strong convergence in taxon composition of bacterial communities. They also found that bacterial communities accompanying cyanobacterial blooms are as diverse as non-bloom communities. Still, some clusters of bacterial sequences that may be characteristic for cyanobacterial blooms were detected.

The aim of the present study was to characterize the taxon composition of bacterial communities in reservoirs in the semi-arid highlands of northern Ethiopia in relation to environmental conditions, and with special emphasis on the occurrence of cyanobacteria (*Microcystis*) blooms. We investigated the taxon composition of bacterial communities in 26 eutrophic reservoirs using denaturing gradient gel electrophoresis (DGGE), and related this to environmental variables. The focus of this research was to investigate to what extent there is a causal relationship between the presence of a *Microcystis* bloom and the bacterial community composition. A comparison is made between bacterial community diversity in reservoirs with and without a *Microcystis* bloom.

Materials and Methods

Study sites and sample collection

Samples were collected from 32 shallow reservoirs in Tigray, the northernmost regional state of Ethiopia (between 12° and 15° N and between 37°10' and 40°10' E). Samples were taken once in September - October 2004 (end of the rainy season; "wet" season) and once in April - May 2005 (end of the dry season, "dry season") to explore seasonal differences. In addition to the sampling of bacterial communities a whole suite of morphometric, abiotic and biotic variables were quantified (Table 4.1). For details on sampling and analysis procedures of all variables, we refer to Dejenie *et al*. (2008). Fig. 4.1 shows the geographic location of the 32 reservoirs in Tigray. Only 26 reservoirs are included in this study because six reservoirs had dried/almost dried out at the moment of the second sampling, truncating the data-set for that season. Therefore, our study focused

on the characteristics of the permanent reservoirs in the region. To sample bacterial communities, water samples were taken at a fixed location from three depths: surface, middle , and near the bottom, using a 3 L Heart valve sampler. These samples were pooled for each reservoir and sampling date and the pooled sample was transported to the shore immediately after retrieval, where sub-samples were taken and fixed. For analysis of bacterial community composition (BCC), water was filtered over a 25mm 0.2 µm GSWP filter (Millipore) until the filter was clogged. The filter was folded twice and packed in sterile aluminium foil. Filters were transported on ice packs to the laboratory and stored at -20 °C until further processing.

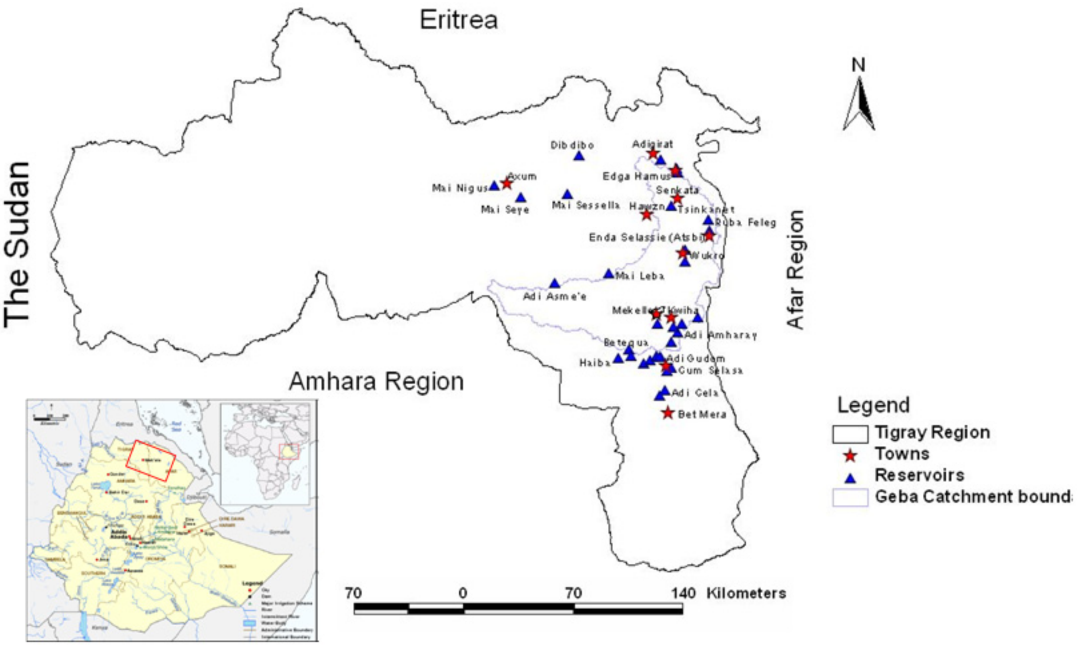

Fig. 4.1 Map with geographic location of the studied reservoirs in Tigray, North Ethiopia

DNA extraction and PCR

Genomic DNA from the natural bacterial communities was extracted following the protocol described by Zwart *et al.* (1998), which includes the bead-beating method concomitant with phenol extraction and ethanol precipitation. Bead beating is a method of disrupting cells using grinding small balls (200 micron) and a shaking homogenizer to smash, rip, and tear samples. After extraction, the DNA was purified on a Wizard column (Promega, Madison, WI) according to the manufacturer's recommendations. For DGGE analysis, a small rDNA fragment was amplified with primers 357F-GC-clamp (5'-CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCCCTACGGGAGGC AGCAG-3') and 518R (5'-ATTACCGCGGCTGCTGG-3'). Using these specific primers, our analysis is restricted to eunbacteria. The PCR was carried out in a Genius temperature cycler. After an incubation for 5 min at 94°C, a touchdown PCR was performed using 20 cycles consisting of denaturation at 94°C for 1 min, annealing at 65°C (the temperature was decreased by 0.5°C every cycle until the touchdown temperature of 56°C was reached) for 1 min and primer extension at 72°C for 1 min. Five additional cycles were carried out at an annealing temperature of 55°C. The tubes were then incubated for 10 min at 72°C. To determine the presence and concentration of PCR products, 5 µl of the product was put on 1% (wt/vol) agarose gels, stained with ethidium bromide, and compared to a molecular weight marker (Smartladder; Eurogentec).

Table 4.1 Summary statistics of limnological variables in the 32 studied reservoirs during the wet and dry season sampling campaign (2004 and 2005, respectively). Data on nutrient concentrations of the dry season were accidently lost; data of the wet season were used for all analyses. For more detailed information on the various variables, see Dejenie *et al*. (2008).

	Wet season (2004)			Dry season (2005)		
Variable	Mean	Min	Max	Mean	Min	Max
Abiotic variables						
pH	8.46	7.47	9.30	8.66	7.45	9.58
Conductivity (µS cm^{-1})	203	75	471	283	135	824
Secchi disc transparency (m)	0.46	0.05	1.50	0.44	0.12	2.00
Suspended matter (mg l^{-1})	42	6	737	61	6	596
Total phosphorus (µg l^{-1})	52	11	221			
Total nitrogen (µg l^{-1})	850	232	2411			
Phytoplankton (pgC l^{-1})	$20*10^5$	4000	$20*10^6$	$10*10^6$	9000	$10*10^7$
% *Microcystis*	34.8	0	99.7	5.1	0	98.2
Zooplankton (µg dry weight l^{-1})	295	9	1603	722	8	4137
Rotifers (µg dry weight l^{-1})	16	0	135	177	0	4127
Copepods (µg dry weight l^{-1})	144	2	683	120	2	755
Cladocerans (µg dry weight l^{-1})	135	1	1128	308	1	2065
Daphnia (µg dry weight l^{-1})	18	0	254	117	0	1597

DGGE analysis

Denaturing gradient gel electrophoresis (DGGE) is a molecular fingerprinting method that separates polymerase chain reaction (PCR)-generated DNA products (Muyzer et al., 1993). DGGE can separate PCR products based on sequence differences that results in differential denaturing characteristics of the DNA. During DGGE, PCR products encounter increasingly higher concentrations of chemical denaturant as they migrate through a polyacrylamide gel. Upon reaching a threshold denaturant concentration, the weaker melting domains of the double-stranded PCR product will begin to denature at which time migration slows dramatically. Differing sequences of DNA (from different bacteria) will denature at different denaturant concentrations resulting in a pattern of bands; each band theoretically representing a different bacterial population present in the community (referred and the Operational Taxonomic Unit, OTU).

Equal amounts of PCR products were analyzed on a 35 to 70% denaturant DGGE gel as described by Van der Gucht et al. (2001). DGGE gels were stained with ethidium bromide and photographed on a UV transillumination table (302 nm) with a CCD camera. As standard, a mixture of DNA from nine clones was used, which were obtained from a clone library of the 16S rRNA genes from a small eutrophic lake. On every gel, three or four standard lanes were analyzed in parallel to the samples. Since these bands are expected to form at the same denaturant concentration in the gel, their position was used to compare the patterns formed in different gels. Digitized DGGE images were analyzed using the software package Bionumerics 5.1 (Applied Maths BVBA, Kortrijk, Belgium).

The software performs a density profile through each lane, detects the bands and

calculates the relative contribution of each band to the total band signal in the lane after applying a rolling disk as background substraction. Bands occupying the same position in the different lanes of the gel were identified by visual inspection, and were subsequently analysed as operational taxonomic units (OTUs). Cyanobacteria were excluded during the analysis of the OTUs. Sequence information of a subset of the bands (see further) was used to check the grouping of bands into band classes. A matrix was compiled based upon the relative constribution of individual bands (representing OTUs) to the total band signal in each lane.

A number of bands with more than 40% relative band intensity in at least two samples were selected for sequencing. These bands were excised and sequenced after re-extraction and amplification. Sequencing was performed with an ABI-Prism sequencing kit (PE Biosystems) using primer Stef1Tex (5_- GCGTTCATCGTTGCGAG-3_) and an automated sequencer (ABI-Prism 377). A nucleotide BLAST search (http://www.ncbi.nlm.nih.gov/BLAST/) was performed in order to find the closest match and identity the taxa to which a sequence belongs.

Detection of toxin genes

Potentially toxic species of cyanobacteria were detected using PCR. We searched for two genes coding for different variants of the hepatotoxine microcystin: (1) *mcy A*, present in different toxin producing cyanobacteria such as *Microcystis*, *Anabaena* and *Planktothrix* (Hisbergues *et al.* 2003) and (2) *mcy E*, characteristic for *Microcystis* (Vaitooma *et al.* 2003).

Data analysis

DGGE banding data were used to determine bacterial community composition, using band intensity as a measure for the relative abundances of OTUs (see also Van der Gucht et al., 2007, Muylaert et al., 2003). We estimated bacterial richness by treating each band as an individual OTU and using the number of bands as an indicator of richness.

Multivariate statistical analysis on relative abundances of OTUs as determined by relative band intensity was used to explore relationships between BCC and environmental conditions as well as spatial variables. To evaluate the association between BCC and environmental variables, a principal component analysis PCA was performed followed by a redundancy analysis (RDA). Prior to the analyses, OTU's occurring in only one reservoir were omitted. All phytoplankton and environmental variables, except pH, were log(x+1) transformed. In RDA, a forward selection procedure was used to add significant explanatory variables to the model. They were added in the order of largest additional contribution to the total variance explained. To avoid inflation of the Type I error due to a large number environmental variables, an *a priori* selection of environmental variables was made, and the significance of a global model including all variables checked before applying the forward selection procedure (Blanchet et al., 2008). We additionally checked whether the R^2_{adjusted} of the forward selection model did not exceed that of the full model. The statistical significance in RDA was assessed by Monte-Carlo permutation tests (999 permutations). We also assessed the unique contribution of variables through partial RDA analysis. The computer program CANOCO version 4.5 for Windows (center for Biometry – Plant Research International, Wageningen; the Netherlands) was used to perform the PCA and RDA. Correlations were carried out with Statistica version 6.0 for

Windows (StatSoft Inc., Tulsa, USA). Data on environmental variables were taken from Dejenie et al. (2008).

To investigate the relationship between BCC and the occurrence of *Microcystis* blooms, the occurrence of a *Microcystis* bloom was identified from phytoplankton counts (Tsehaye et al., unpublished manuscript, chapter 2 of this thesis). Examination of the phytoplankton community in the reservoirs revealed three genera of cyanobacteria that are potentially able to produce toxins (see Paerl *et al*., 2001): *Anabaena, Aphanizomenon* and *Microcystis*. *Microcystis* was the most abundant cyanobacterium in both seasons. Reservoirs were considered to harbour a *Microcystis* bloom when *Microcystis* represented 65% or more of the total phytoplankton biomass. Bacterial OTU richness of the reservoirs in which a *Microcystis* bloom occurred and those without a *Microcystis* bloom were compared by unpaired Student's t test, using the software package Statistica 6.0 (Statsoft, Inc. 1995).

Results

Microcystis *blooms in relation to environmental variables*

Microcystis was observed to form blooms in four reservoirs in the dry season and seven reservoirs in the wet season (Table 4.2). *Microcystis* abundance in the 26 reservoirs was correlated over the two seasons (r = 0.156, p = 0.02). Microcystin synthetase genes (*mcyE and mcyA*) were detected in all reservoirs where *Microcystis* was detected. In the wet season, *Microcystis* was positively correlated with pH (r =0.4429, p=0.002) and fish (*Garra* species) biomass (r =0.467, p=0.016). In the dry season, *Microcystis* biomass was positively correlated with conductivity (r =0.5246, p=0.06) and rotifers (r =0.427, p=0.03), and negatively with *Daphnia* biomass (r =-0.4384, p=0.025) and altitude of the

reservoirs (r =-0.394, p=0.046). The biomass of *Daphnia* was significantly higher in the reservoirs without than with a *Microcystis* bloom (t-test p = 0.039).

Table 4.2 Occurrence of *Microcystis* blooms in highland reservoirs in Tigray during the wet and dry season. Only reservoirs are mentioned in which a *Microcystis* bloom was observed during at least one season. A bloom is defined by *Microcystis* accounting for 65% or more of total phytoplankton biomass.

Reservoir	% *Microcystis* biomass	
	Wet season	Dry season
Adi Amharay	0	65.5
Betequa	76.4	5.7
Dur Anbassa	99.7	49.8
Era Quihila	80.8	0
Mai Dele	0	96.2
Mai Gassa I	56.7	67.5
Mai Gassa II	99.6	98.2
Mai Nigus	73.1	0.1
Meala	69.3	4.5
Shilanat IV	97.6	0

Bacterial communities in relation to environmental variables

In total, 38 different bacterial OTUs were detected, of which 38 were observed in the wet season and 35 in the dry season. Bacterial richness for the individual reservoirs varied between 2 to 15 (average 7.2) and 3 to 13 (average 8.4) OTUs in the wet and dry season, respectively. OTU richness in the reservoirs was significantly different between the two seasons (Mann-Whitney U Test, $p < 0.05$, N = 26). We observed a positive correlation between bacterial richness and submerged vegetation (r = 0.4789, p = 0.013).

Mantel test (Zt, Bonnet and Van de Peer, 2002) revealed no correlation between the bacterial community composition (BCC) of the two seasons (r = 0.009; p = 0.450).

Redundancy analysis (RDA) with forward selection and unrestricted Monte Carlo permutation tests revealed that the percentage of *Microcystis* in the phytoplankton community (% *Microcystis*) and copepod biomass (CopB) significantly explain 18% of the variation in BCC of the wet season samples (Fig. 4.2a). The effect of *Microcystis* on the bacterial community diversity in the wet season remained significant after we controlled for the contribution of copepods in a variation partitioning analysis (Fig. 4.3). For the samples taken in the dry season, redundancy analysis (RDA) with forward selection and unrestricted Monte Carlo permutation tests revealed that TN and TP (both positively correlated with altitude), oxygen concentration (O_2; positively correlated to pH), the amount of cattle (as derived from the number of cattle dung along the shoreline, see Dejenie et al., 208) and fish biomass (fish B) significantly explain a total of 32% of the variation in BCC (Fig. 4.2b).

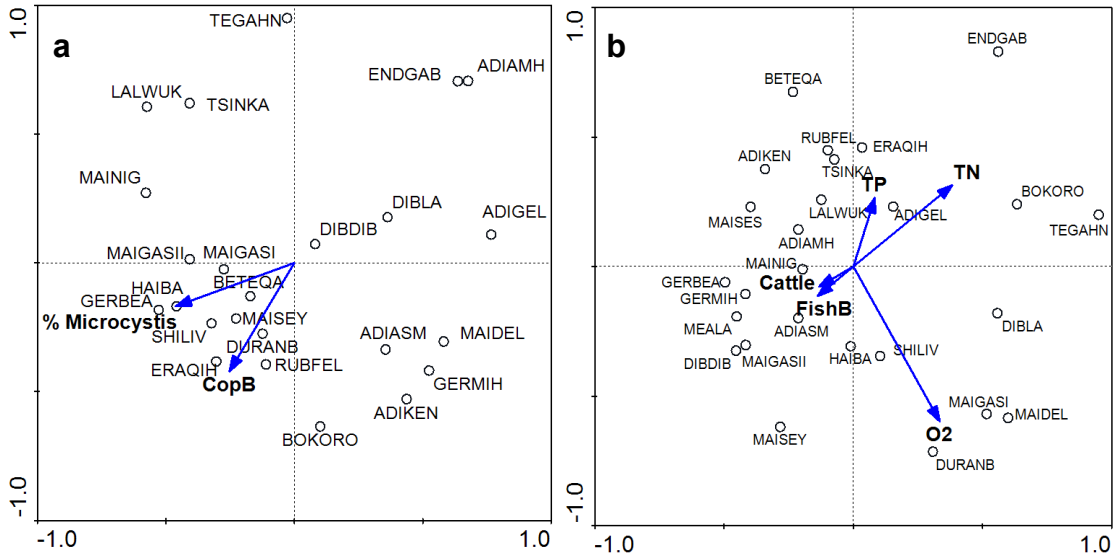

Fig. 4.2 Principal component analysis (PCA) of bacterial community composition (BCC) in the studied reservoirs in the wet (a) and dry (b) seasons, showing the structuring role of copepod biomass (CopB) and % Microcystis (accounting for 18% of the variation in the dataset of the wet season) and TN, TP, oxygen concentration (O2), cattle and fish biomass (fishB) (accounting for 32% of the variation in the dataset of the dry season)

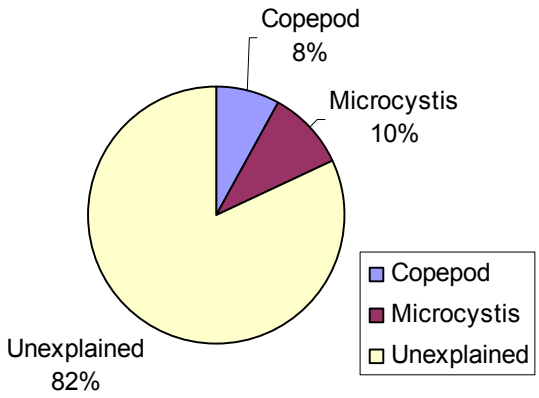

Fig. 4.3 Variance partitioning of the BCC in the wet season. Both % *Microcystis* and Copepod biomass explained variation undependently, there were no common effects.

Bacterioplankton richness in relation to the occurrence of Microcystis blooms

OTU richness in reservoirs with *Microcystis* bloom ranged from 2 to 9 (average 6.2), whereas it ranged from 3 to 14 (average 8.3) in reservoirs without a *Microcystis* bloom. This difference was statistically significant (*t*-test, $p = 0.042$). No overall correlation was, however, observed between OTU richness and *Microcystis* biomass or percentage contribution of *Microcystis* to total phytoplankton biomass.

Discussion

Bacterial taxon richness as detected by DGGE analysis in individual reservoirs ranged from 2 to 15, which is somewhat lower than the bacterial richness observed in most other lakes (Konopka et al., 1999; Van der Gucht et al. 2001, 2007). Van der Gucht et al. (2007) reported 11 to 37 bacterial OTUs from temperate lakes. Our results suggest that bacterial communities accompanying *Microcystis* blooms are characterised by lower

taxon richness than bacterial communities in reservoirs without *Microcystis* blooms. This is in contrast with the results of Eiler & Bertilsson (2004), who found no indications that the environmental conditions created by cyanobacterial blooms lowered the community richness or decreased the evenness of OTUs in a clone library study. We feel our observation should be considered with caution, because there is a possibility that the lower taxon richness observed in the reservoirs with a *Microcystis* bloom is the result of an artefact in the PCR reactions when there is a strong dominance of one taxon (Acinas et al., 2005).

Our data provide evidence that the relative abundance of *Microcystis* is significantly associated with taxon composition of the bacterial community, at least in the wet season. Cyanobacteria, including *Microcystis* strains, are known to produce secondary metabolites that have antimicrobial and toxic effects (Carmichael, 1992; Patterson *et al.* 1994). Valdor & Aboal (2007) demonstrated an inhibitory effect of both cyanobacterial extracts and pure microcystin on the growth of microalgae and bacteria. These inhibitory effects were even longer lasting on bacteria than on microalgae. Østensvik et al. (1998) reported antibacterial activity of secondary metabolites from cyanobacteria in a bacterial bioassay study of extracts from selected planktonic freshwater cyanobacteria, including *Microcystis aeruginosa*. Similarly, Casamatta & Wickstrom (2000) showed that *Microcystis* has toxic properties for some bacterioplankton strains.

In addition to the percentage of *Microcystis*, bacterial community composition in the reservoirs during the wet season was also significantly impacted by copepod biomass. Grazing by zooplankton, both protozoans as well as crustacean zooplankton, has been

identified as a force driving changes in bacterial community composition (Langenheder & Jürgens, 2001; van Hannen *et al.* 1999b). Jürgens et al. (1993) observed an association between copepod biomass and bacterial abundances in an enclosure study (Jürgens et al., 1993). Several studies have reported that the highest bacterial growth and abundances occur in the presence of copepods (Jürgens et al., 1993; Roman et al., 1998). This is suggested to result from a combined effect of cascading predation effects on protozoans that feed on bacterioplankton and copepod excretion stimulating the growth of bacteria.

In the dry season, the relationships of bacterial community composition with environmental factors are different from those observed in the wet season. We observed significant associations of bacterial community composition with total nitrogen (TN), total phosphorus (TP), oxygen, the number of cattle frequenting the reservoir, and fish biomass. Muylaert et al. (2002) reported significant associations of bacterial community composition of shallow lakes with both bottom-up factors (temperature, phytoplankton biomass, nitrogen concentration, phosphorus concentration, and pH) as well as top-down factors (biomass of oligotrich and non-oligotrich ciliates, *Daphnia* and *Ceriodaphnia*), but with a stronger impact of bottom-up factors in turbid lakes. Other studies too have reported a joint influence of bottom-up and top-down regulation of bacterioplankton (Pace & Cole, 1996), whereas Langenheder & Jürgens (2001) reported regulation of bacterial community composition by *Daphnia* grazing. Our data suggest that BCC in the studied reservoirs in the dry season were largely regulated by bottom-up factors, which is broadly in agreement with Muylaert et al. (2002), as the reservoirs in the dry season are characterized by higher conductivities, phytoplankton biomass and lower amounts of suspended matter than in the wet season. The amount of cattle frequenting the reservoirs

impacts bottom-up factors by influencing nutrient concentrations both directly by their excretion products as well as indirectly by trampling and thus enhancing sediment resuspension. Cattle may also change the quality of organic matter available for bacteria to grow upon. Although the impact of fish may reflect a top-down control, the biomass of fish may also strongly impact the ecosystem of the reservoirs through a bottom-up effect, both by excreting nutrients as well as by enhancing sediment resuspension.

We conclude that bacterioplankton community structure of Ethiopian highland reservoirs is significantly related to both bottom-up as well as top-down factors, but that the relative importance of these factors changed strongly among the wet and dry season in the study period. The relative abundance of the cyanobacterium *Microcystis* seems to have a significant influence on bacterial community composition during the wet season.

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Chapter 5

Impact of the fish Garra on the ecology of reservoirs and the occurrence of *Microcystis* blooms in semi-arid tropical highlands: an experimental assessment using enclosures*

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** Refer appendix 5.1 for the analysis of the phytoplankton community composition*

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Summary

Many man-made reservoirs in the semi-arid highlands of Northern Ethiopia (Tigray) are characterised by the occurrence of intensive blooms of cyanobacteria and a dominance of small riverine fishes belonging to the genus Garra. We carried out enclosure experiments to test for the effect of these small fish on abiotic characteristics, phytoplankton biomass and zooplankton community structure in the pelagic of two reservoirs (Gereb Awso and Tsinkanet). Two experiments were carried out in each of the reservoirs, one at the end of the rainy season (highest water level) and one at the end of the dry season (lowest water level). The presence of *Garra* in general increased the amount of suspended matter, nutrient concentrations (total nitrogen and total phosphorus), phytoplankton and *Microcystis* biomass (including the proportion of *Microcystis* in the phytoplankton community), and reduced water transparency. The positive effect of the presence of *Garra* on nutrient concentrations and phytoplankton productivity indicate that *Garra* has the potential to affect food web functioning indirectly through bottom-up effects, by enhancing nutrient concentrations through sediment resuspension and excretion of nutrients. Indeed, population densities of the cladoceran zooplankton taxa *Ceriodaphnia* and *Diaphanosoma* also showed an overall increase in enclosures with Garra. However, our data also provide some evidence for a potential of Garra to exert top-down control on large bodied daphnids (*Daphnia carinata, D. barbata*), although such effect varied among experiments. The limited capability of *Garra* to control zooplankton communities mainly reflects the low efficiency of these small, riverine and benthos-oriented fish in foraging on zooplankton and suggests the existence of an unoccupied niche for zooplanktivorous fish in the majority of the reservoirs. Although the main effects of *Garra* on the pelagic food web seemed to be mediated by bottom-up

mechanisms, our results also indicate that one of the key variables, the relative abundance of *Microcystis*, was impacted by *Daphnia*-mediated trophic cascade effects.

Introduction

Man-made reservoirs represent a considerable fraction of standing waters across the world (Cooke et al., 1993; Kalff, 2002). In semi-arid regions, reservoirs play an important role in providing access to water for irrigation and watering cattle. Often, water quality in such reservoirs is poor and is characterised by high nutrient levels, high turbidity and phytoplankton blooms. In the semi-arid highlands of Tigray, Northern Ethiopia, more than 70 small (c. 18 000–454 000 m^2) reservoirs have been constructed for the purpose of irrigation and watering livestock. These reservoirs were often constructed in valleys with rivulets, and many (c. 65%) became colonised by small riverine fishes of the genus *Garra*. Piscivorous fish are regionally absent from the network of rivulets in the Ethiopian highlands. Because of the high productivity of some of the reservoirs and the absence of predatory fish, *Garra* populations often reach high densities (Dejenie et al., 2008).

A survey of 32 reservoirs of Tigray revealed the presence of cyanobacteria in high relative (>20% of algal biomass) and absolute biomass in more than 65% of the studied reservoirs (Dejenie et al., 2008). The data from that survey also suggested a strong bottom-up effect, with the biomass of phytoplankton and fish (mainly *Garra*) being positively correlated with an altitude-related gradient of total phosphorus and with a positive correlation between the biomass of fish and cladocerans. Yet, the biomass of cyanobacteria was negatively related to the biomass of cladocerans (*Daphnia*), which may reflect the potential of daphnids to exert a top-down control on cyanobacteria. Overall, the survey suggested only limited top-down control by *Garra* on zooplankton. Representatives of the genus *Garra* are present in Asia and Africa (Getahun & Stiassny, 1998; Golubtsov, Degbuadze & Mina, 2002). About 60% of the African species are found in Ethiopia (Getahun & Stiassny, 1998) and many of them

are endemic to the region (Getahun, 2000). *Garra* are often found in large numbers in streams. The genus is not found higher than 3000 m a.s.l. (Getahun & Stiassny, 1998). Although very common in Tigray and some other regions of Eastern Africa, it is unclear to what extent this fish taxon can impact the pelagic food web of standing waters through planktivory. This study had two major objectives: (i) to assess experimentally the potential for top-down control of zooplankton by *Garra*, and (ii) to test for a causal relationship between the presence of *Garra* and the occurrence of cyanobacteria blooms. For this, we carried out in situ enclosure experiments in two ecologically contrasting reservoirs that are both characterised by the presence of *Garra*, albeit at different densities.

Methods

Study area and design of enclosure experiment

We performed two enclosure experiments in each of the reservoirs Gereb Awso (surface: 2.4 ha; depth: 3.5 m; 39°33'17"N; 13°25'59'E) and Tsinkanet (surface: 7 ha; depth: 4.3 m; 39°32'40"N; 14°54"E), two reservoirs located in the semi-arid highlands of Tigray, Northern Ethiopia (Dejenie et al., 2008). The two study reservoirs are ecologically quite distinct. Although they are both characterised by high nutrient concentrations (>0.5 mg TP L^{-1} and 50 mg TN L^{-1}), the turbidity is much higher in Gereb Awso (Secchi depth <20 cm; amount of suspended matter c. 40–130 mg L^{-1}) than in Tsinkanet (Secchi depth >50 cm in September; amount of suspended matter <20 mg L^{-1}). Population densities of *Garra* are much higher in Gereb Awso [catch per unit of effort (CPUE) of 922 individuals, with effort equalling two multi-mesh gill nets of 28 m total length during one night] than in Tsinkanet (CPUE of 65 individuals). Whereas Gereb Awso (GA) is located centrally in a small rural village,

Tsinkanet is located in farmland area. Probably related to this quieter location, Tsinkanet (TS) hosts a small group of *Pelecanus onocrotalus* L. (5–15 birds) and other fish-eating birds such as *Podiceps cristatus* L. and *Sterna albifrons* Pallas.

In each reservoir, we performed one enclosure experiment during September (GA1 and TS1, starting dates at 14 and 15 September 2005) and one enclosure experiment during April (GA2 and TS2, start at 26 and 27 April 2006). The month of September follows shortly after the rainy season, and in that period the reservoirs are filled to full capacity. The month of April is typically at the end of a prolonged dry period, and water levels tend to reach their annual minima.

For each experiment, we filled six floating enclosures (1000 L polypropylene tanks: diameter: 1 m, depth: 1.3 m) in the pelagic zone of the reservoirs with 64 μm mesh filtered water from the reservoir. Subsequently, we inoculated the enclosures with pelagic zooplankton of the reservoir. We collected this zooplankton by making vertical hauls with a conical zooplankton net (64 μm mesh), integrating the entire water column. The volume of lake water filtered by this method was equivalent to the volume of the enclosures. We applied this procedure to best represent the entire pelagic zooplankton community in the enclosures by accounting for the heterogeneity in the vertical distribution of the zooplankton.

We applied the same manipulation in all experiments. We added four equally sized *Garra* individuals (*Garra ignestii* Gianferrari; average length: 117 mm; average weight: 28 g) to three randomly selected enclosures (F), whereas the remaining enclosures were kept as control (no fish; NF). To prevent predation by birds, all enclosures were covered with nylon netting (mesh: 1 mm^2). We choose to use *Garra ignestii* for the fish treatment because *Garra* is the only dominant fish species found

in the reservoirs (Tsinkanet also hosts a small population of stocked *Oreochromis niloticus* L.).

Sampling and sample analysis

The enclosures were sampled at 4-day intervals during a period of 4 weeks. We measured dissolved oxygen, pH, temperature and conductivity in situ with a WTW Multi 340 I electrode. We collected depth integrated water samples from the central part of the enclosures with a tube sampler (diameter: 670 mm). These samples were taken for the quantification of phytoplankton, nutrients and suspended matter. The concentration of chlorophyll a was measured in the field with a fluorometer (Turner Aquafluor; average of three measurements). Phytoplankton samples were preserved with acid lugol solution. Suspended matter was determined by filtering 200 mL on a pre-weighed Whatman GF/C filter in the field and quantifying dry weight in the laboratory. The remainder of the samples were taken to the laboratory in a refrigerator box and frozen at -18 °C until further analysis. The concentrations of total phosphorus and total nitrogen were measured following the ascorbic acid method and the Kjeldahl method respectively (Anderson & Ingram, 1989). Unfortunately, the samples from the first enclosure experiments (GA1 and TS1) were lost in transport, so only the samples from GA2 and TS2 could be analysed for nutrient concentrations.

We collected zooplankton samples with a Schindler-Patalas plankton trap (12 L). We always took samples in the central zone of the enclosures, two at the surface and two at a depth of 1 m. Samples were fixed with sucrose-saturated formalin solution (4% final concentration) to prevent egg loss from cladoceran carapaces (Haney & Hall, 1973).

Phytoplankton was counted and identified to genus level and cyanobacteria to species level, following published manuals and identification guides (Whitford & Schumacher, 1973; Komarek & Anagnostidis, 2000, 2005; John, 2002). Biomass in terms of carbon of cyanobacteria was obtained through biovolume to biomass conversions following counting (Hillebrand et al., 1999; Menden-Deuer & Lessard, 2000). Zooplankton samples were counted using a stereomicroscope. Subsamples of known volumes were taken; for each sample at least 150 individuals of cladocerans and copepods were counted and identified. Cladocerans were determined to species level, except for *Diaphanosoma* and *Ceriodaphnia*. Copepods were determined to suborder level. Determinations of cladocerans were based on Benzie (2005) and Flossner (2000). In addition to the crustacean zooplankton, we also counted and measured larvae of the phantom midge *Chaoborus* (50 individuals per sample, if available).

Statistical analyses

We started the analyses with a multivariate approach to make an overall evaluation of the effect of *Garra* on pelagic communities and to avoid problems related to multiple testing. In these analyses, we distinguished between two groups of response variables: (i) abiotic variables (i.e. suspended matter, water transparency, pH, and oxygen saturation), chlorophyll a and the proportion of *Microcystis* biomass to the total biomass of the phytoplankton community, and (ii) the zooplankton density matrix. Prior to analysis, the response variables were averaged over time, log-transformed and abiotic variables, chlorophyll a and percentage *Microcystis* were also standardised to account for differences in units. We performed principal component analyses (PCA) to evaluate qualitative differences among experiments according to lake and season

and to visually assess the magnitude and direction of the shifts in response variables to the fish treatment. We performed redundancy analysis (RDA) to formally test the overall effect of fish on each of both sets of response variables for the four experiments simultaneously. Fish presence was here coded as a nominal predictor variable and the four experiments were specified as co-variables using dummy variables to account for variation among lakes and seasons. Significance testing for the effect of fish was done by 499 Monte Carlo permutations. Permutations were random but restricted to individual experiments (permutation restriction to blocks; Lepsˇ & Sˇmilauer, 2003). The RDA's were performed in CANOCO v4.5 (Ter Braak & Sˇmilauer, 2002). Significant fish effects were further explored in more detail by applying additional tests to each of the response variables separately. Finally, because species of the genus *Daphnia* are potential key players in trophic cascade interactions, we wanted to obtain a more detailed insight in their dynamics. We therefore executed repeated measures ANOVA on the logarithmically transformed density data of the most abundant *Daphnia* taxa (*Daphnia barbata* Weltner and *Daphnia carinata* King) for each of the enclosure experiments, separately. These analyses were performed with the software package STATISTICA 7.0.

Results

Effect of Garra on physicochemical characteristics and phytoplankton

Conditions in non-fish enclosures differed among seasons more than among lakes, with less chlorophyll a but a higher relative amount of *Microcystis* at the end than at the beginning of the dry season (Fig. 5.1a). In each experiment, enclosures with *Garra* exhibited a substantial shift in abiotic conditions compared to the non-fish enclosures, and these shifts occurred in a similar direction along PCA1 (Fig. 5.1a).

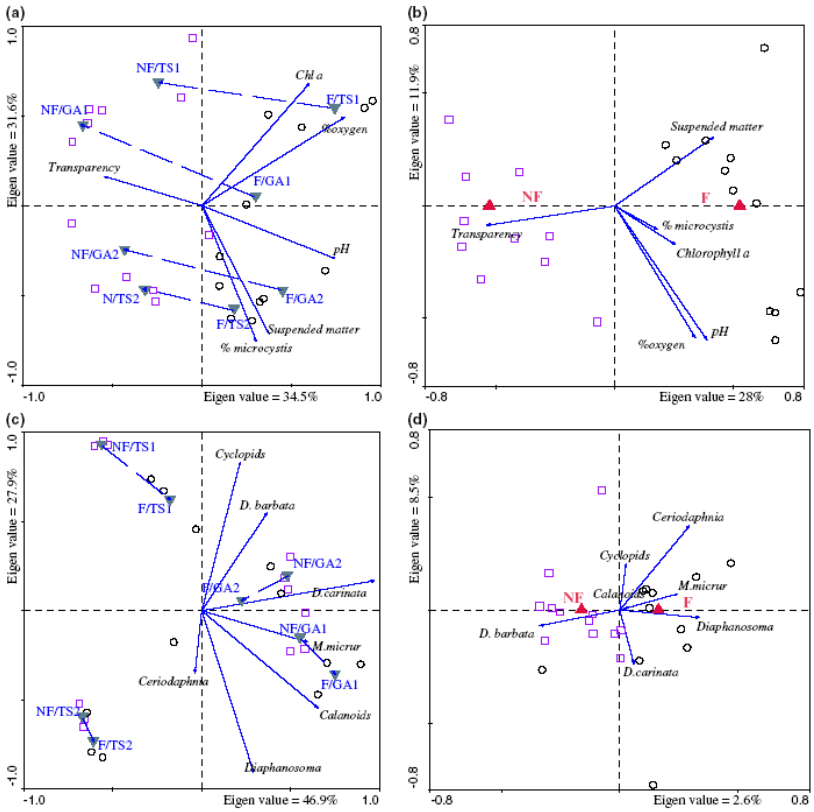

Fig. 5.1 PCA and RDA ordination plots, showing the response of limnological variables and zooplankton communities to the presence of fish (*Garra*) in enclosures: (a) PCA-plot of abiotic variables, chlorophyll a, and the relative biomass of *Microcystis* (percentage of total phytoplankton biomass), (b) RDA-plot of abiotic variables, chlorophyll a, and the relative biomass of *Microcystis* (percentage of total phytoplankton biomass), (c) PCA-plot of population densities of crustacean zooplankton taxa, (d) RDA-plot of population densities of crustacean zooplankton taxa. The analyses were performed on the entire dataset of the four enclosure experiments, but individual experiments were specified as co-variables in the RDA-analysis (see Methods for details). Squares, fishless enclosures; circles, enclosures with fish; filled triangles, centroids that show the average position of replicate enclosures for all the possible combinations of treatments, seasons and lakes (a and c) or for treatments (b and d); NF, no fish; F, with fish; GA1, Gereb Awso rainy season experiment; GA2, Gereb Awso dry season experiment; TS1, Tsinkanet rainy season experiment; TS2, Tsinkanet dry season experiment. Plot axes are scaled to sample distances.

Redundancy analysis on the entire dataset (four enclosure experiments, taking the individual experiments as co-variables) indeed indicated an overall significant effect of fish on this variable set [total fraction of explained variation (λ) = 0.28, F = 19.993, p = 0.002]. Analyses performed on individual variables indicated that Garra significantly decreased water transparency (λ = 0.59) and increased the amount of

suspended matter (λ = 0.35), nutrient concentrations (λ-values >0.43), pH (λ = 0.31) and the amount of dissolved oxygen (λ = 0.23, Figs 5.1b & 5.2). Overall, the presence of *Garra* also tended to increase phytoplankton chlorophyll a (λ = 0.115) and the relative proportion of Microcystis to total estimated phytoplankton biomass (λ = 0.068; Figs 5.1b & 5.2).

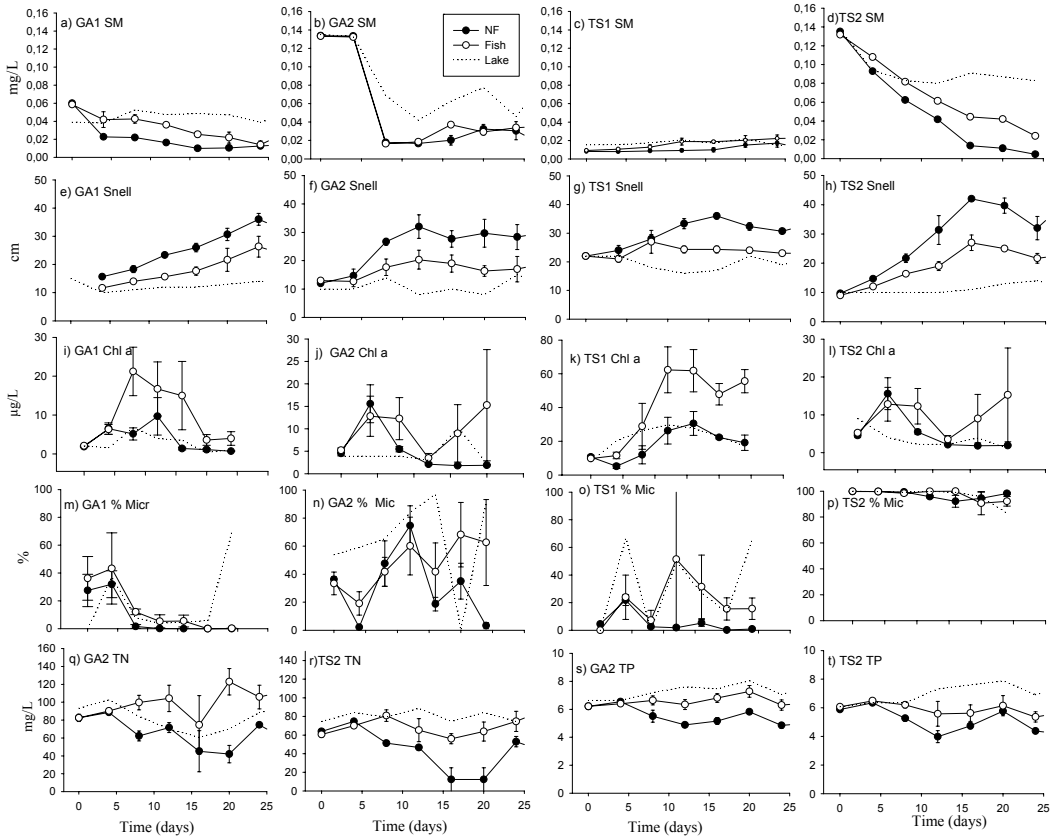

Fig. 5.2 The response of abiotic and phytoplankton variables to the presence of fish (*Garra*) in the enclosure experiments in Gereb Awso (GA1: rainy season; GA2: dry season) and Tsinkanet (TS1: rainy season; TS2: dry season): (a–d) suspended matter (SM), (e–h) water transparency (Snell's reading), (i–l) chlorophyll a; (m–p) percentage biomass of *Microcystis*; (q–r) total nitrogen (TN) and (s–t) total phosphorus (TP). F, enclosures with fish; NF, enclosures without fish; Lake, data from the pelagic zone of the lake. Note that no TN or TP data are available for the rainy season experiments (GA1 and TS1).

Effect of Garra on zooplankton communities

The species composition of the zooplankton communities differed considerably among lakes (Fig. 5.1c). The composition of the zooplankton communities in Gereb

Awso were relatively similar among experiments and were mainly composed of *D. carinata, Diaphanosoma, Ceriodaphnia* and cyclopoid copepods (Fig. 5.3). Zooplankton communities in the Tsinkanet enclosures showed pronounced differences among experiments. Tsinkanet zooplankton enclosure communities at the end of the rainy season were mainly dominated by *D. carinata* and *Ceriodaphnia*, whereas enclosure communities at the end of the dry season consisted mainly of *Diaphanosoma* and *Ceriodaphnia* (Figs 5.1c & 5.3).

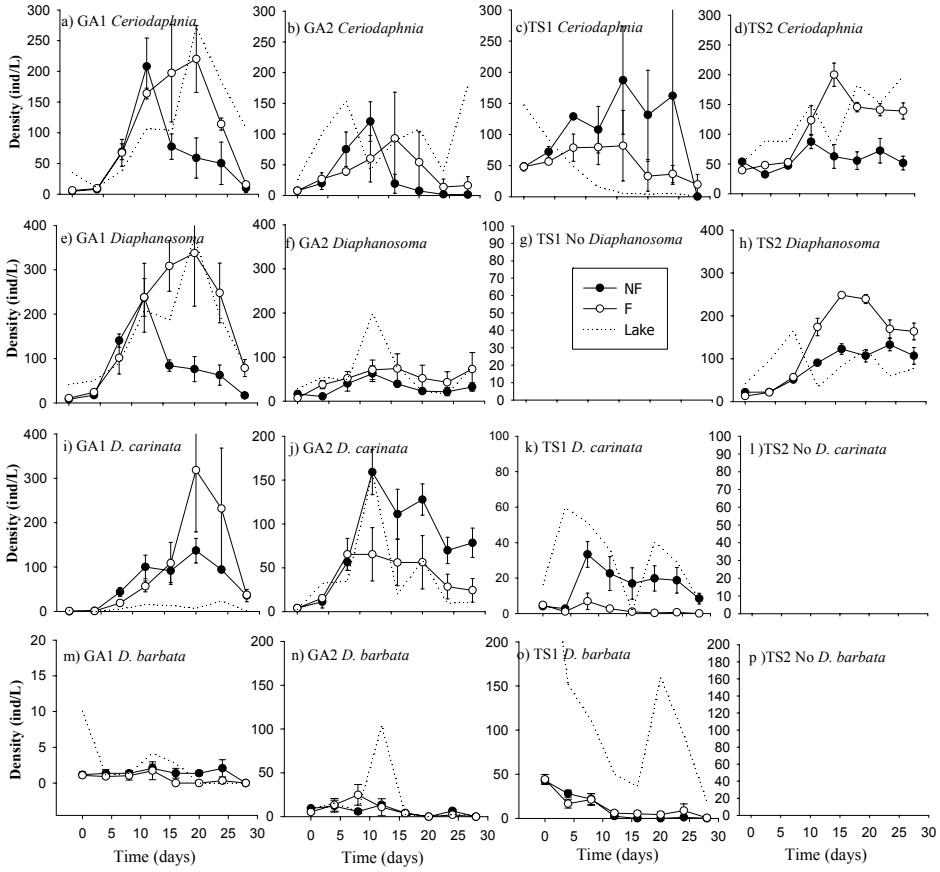

Fig. 5.3 Population densities of cladoceran taxa in response to the presence of fish (*Garra*) in the enclosure experiments in Gereb Awso (GA1: rainy season; GA2: dry season) and Tsinkanet (TS1: rainy season; TS2: dry season). F, enclosures with fish; NF, enclosures without fish; Lake, data from the pelagic zone of the lake. Note differences in abundance scale between panels.

There was an overall significant effect of *Garra* on cladoceran communities in the four enclosure experiments ($\lambda = 0.026$, $F = 2.629$, $P = 0.012$) (Fig. 5.1d).

According to analyses per individual zooplankton taxon, population densities of *Ceriodaphnia* ($\lambda = 0.138$, $p = 0.046$) and *Diaphanosoma* ($\lambda = 0.120$, $p = 0.002$) increased upon addition of fish in comparison to non-fish enclosures. Overall treatment effects were not significant for the *Daphnia* species. Apparently, this was because the strength of the response of these species to the fish treatments differed considerably among experiments. Repeated measures ANOVA on the density of *D. carinata* for each of the experiments, separately, revealed negative effects of *Garra* on this species during the rainy season experiment in Tsinkanet and the dry season experiment in Gereb Awso, but not in the rainy season experiment in Gereb Awso (Table 5.1, Fig. 5.3). Furthermore, *Garra* had a significant negative impact on *D. barbata* only in the rainy season experiment in Gereb Awso (Table 5.1).

Overall, there tended to be a negative association between densities of *D. carinata* and the proportion of *Microcystis* relative to total phytoplankton biomass, especially in the enclosures without fish (Fig. 5.4). During the rainy season experiment in Gereb Awso and Tsinkanet, the initial relative abundance of Microcystis dropped along with an increase in the densities of *D. carinata*, except for the enclosures with fish in Tsinkanet, where *D. carinata* densities remained low and *Microcystis* abundance remained relatively high during the entire experiment (averages ranging between 15% and 50% of total phytoplankton biomass). During the first half of the dry season experiment in Gereb Awso, both densities of *D. carinata* and the relative biomass of *Microcystis* increased concomitantly. However, during the second half of the experiment, the share of *Microcystis* in the total phytoplankton biomass declined to less than 5% in the enclosures without fish, whereas it remained at high levels in the enclosures with fish (ranging between 40% and 70%). During the

dry season experiment of Tsinkanet, *Microcystis* dominated the phytoplankton communities heavily in the absence of *D. carinata* (ranging between 95% and 99%).

Table 5.1 Results of repeated measures ANOVA testing for the effects of fish (F), time (T) and the interaction on densities of *Daphnia carinata* and *D. barbata* during experiments at the end of the rainy season (14 September 2005 to 15 October 2005) in Gereb Awso (GA) and Tsinkanet (TS) and the dry season (26 April 2006 to 25 May 2006) in Gereb Awso. No *D. carinata* or *D. barbata* were detected in Tsinkanet during the dry season

Daphnia carinata							*Daphnia barbata*					
	d.f.	MS effect	MS error	F	p		d.f.	MS effect	MS error	F	P	
Gereb Awso – rainy season												
F	1,4	13545	21282	0.64	0.470		1,4	7.05	0.822	8.58	0.43	F<NF
T	7,28	39379	6142	0.64	0.470		6,24	1.00	1.136	0.88	0.524	
F*T	7,28	9762	6142	1.59	0.180		6,24	0.65	1.13	0.57	0.746	
Gereb Awso – dry season												
F	1,4	17216	2773	6.20	0.067	F<NF	1,4	3.99	1.26	3.17	0.149	
T	7,28	8405	823	10.2	0.000		5,20	1.19	0.806	1.48	0.240	
F*T	7,28	2194	823	2.66	0.030	F<NF	5,20	0.49	0.806	0.60	0.694	
Tsinkanet – rainy season												
F	1,4	2228	246	9.05	0.040	F<NF	1,4	20.4	30.14	0.68	0.457	
T	7,28	198	41	4.76	0.001		7,28	1385	49.6	27.9	0.000	
F*T	7,28	135	41	3.25	0.012	F<NF	7,28	49.54	49.6	1	0.453	

F<NF refers to significant treatments or treatment x time interaction effects with fish affected population densities negatively.

Chaoborus were observed in most samples of the rainy season (average density in Tsinkanet and Gereb Awso enclosures: 2.7 and 0.6 individuals L^{-1}), but not during the dry season. One-way ANOVA indicated no significant differences in Chaoborus densities between treatments.

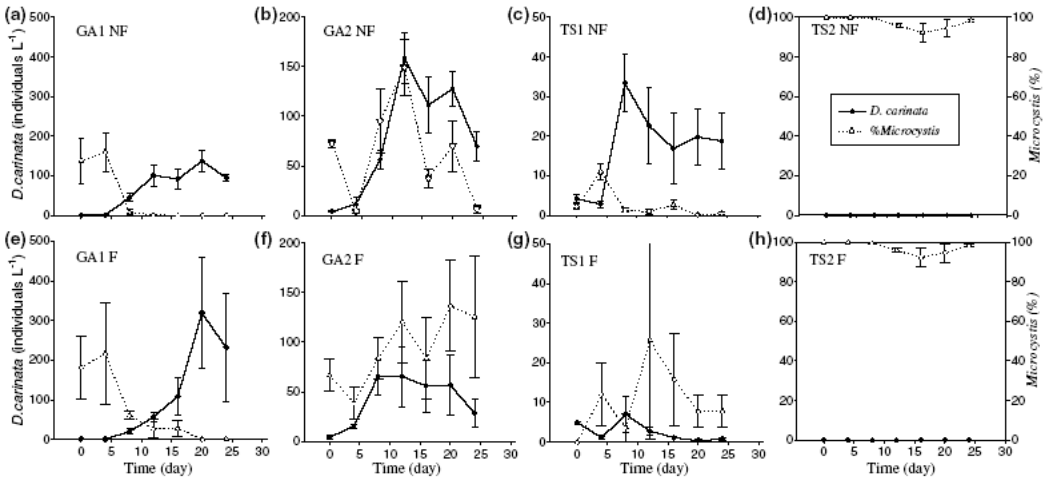

Fig. 5.4 The association between Daphnia carinata abundance and the relative biomass of Microcystis in the experiments in Gereb Awso (GA1: rainy season; GA2: dry season) and Tsinkanet (TS1: rainy season; TS2: dry season): (a–d) enclosures without fish, (e–h) enclosures with fish.

Discussion

Garra had a large impact on both biotic and abiotic water column variables in the enclosures. Higher concentrations of macronutrients (nitrogen and phosphorus) in the enclosures with *Garra* than in the fishless enclosures indicate an enhancement of nutrient recycling by fish (Vanni & Layne, 1997; Vanni, Layne & Arnott, 1997). Higher chlorophyll a concentrations and oxygen levels in the enclosures with *Garra* also indicate that higher nutrient availability had stimulated phytoplankton productivity, a response that was also associated with an increase in the relative abundance of the cyanobacterium *Microcystis*. Transparency was also lower in enclosures with *Garra* compared to fishless enclosures, due to the combination of enhanced phytoplankton growth and an increase in the amount of suspended matter, probably originating from the resuspension of sediment particulate matter by the foraging and swimming activity of the fish. Overall, the differences in abiotic conditions and phytoplankton biomass between enclosures with and without fish in our study indicate that *Garra* can potentially have an impact on important ecosystem

characteristics of the reservoirs (water transparency, nutrient availability, phytoplankton primary productivity).

Our results indicate that *Garra* have affected the density and composition of the zooplankton communities mainly indirectly through bottom-up mechanisms, by enhancing nutrient availability and stimulating phytoplankton growth, rather than through a direct top-down control. Abundant zooplankton species, such as *Diaphanosoma* and *Ceriodaphnia*, were promoted by the presence of *Garra* and the positive response of these species to *Garra* was probably the result of increased food availability. Similar results were obtained by Rejas et al. (2005), who reported increased zooplankton densities in enclosures with fish (*Moenkhausia dich*roura) along with increased levels of oxygen and phytoplankton biomass in a tropical varzea lake. In our experiments, the lack of a consistent, significant enhancement of *Daphnia* densities in the fish enclosures, despite increased phytoplankton productivity, may indicate a mild top-down control by *Garra*. This suggestion is consistent with our observations that brood sizes of *D. carinata* were higher in the presence than in the absence of fish (data not shown). Thus higher fecundity of this species may have been compensated by higher death rates, which would suggest an indirect bottom-up effect of *Garra* (increased fecundity because of higher food concentration) combined with a moderate top-down control.

In addition to indirect positive effects of fish on zooplankton via the enhancement of phytoplankton productivity, two other potential mechanisms may have contributed to the increased population densities of zooplankton in the fish enclosures: (i) reduced predation by macro-invertebrate predators (Sorano, Carpenter & He, 1993), and (ii) competitive release of competitively inferior taxa because of selective predation on strong competitors (cf. keystone predation). The only potential

macro-invertebrate predators we observed in the enclosures of Gereb Awso and Tsinkanet were larvae of the phantom midge *Chaoborus*. These larvae were only present during the rainy season experiment and there were no significant differences in their densities between enclosures with different fish treatments. Reduced predation by macro-invertebrate predators is therefore not a very probable explanation for the observed effects of *Garra*. The second mechanism may have played some role during the dry season in Gereb Awso, but there is no consistent evidence for this in the other experiments.

The higher nutrient concentrations in the enclosures with fish were not only related to higher phytoplankton biomass, but also to a shift in taxon composition within the phytoplankton towards more cyanobacteria (*Microcystis*). This is in agreement with other studies showing that increased nutrient concentrations are often associated with cyanobacteria blooms (Elser, 1999). The increase in relative importance of *Microcystis* in enclosures with fish may, however, also be partly related to a shift in zooplankton community towards smaller zooplankton at the expense of *Daphnia*. From our experimental results it is impossible to say whether the negative association between densities of *D. carinata* and the percentage of *Microcystis* in the phytoplankton in the reservoirs is due to a toxic effect of *Microcystis* on *Daphnia* or rather due to a top-down effect of *Daphnia* grazing on *Microcystis* (Reinikainen, Ketola & Walls, 1994). Close inspection of the timing of events, however, provides more support for a top-down than for a bottom-up impact. The observations in the dry season experiment in Gereb Awso (Fig. 5.4b,f), for instance, suggest a top-down effect, because both taxa first show an overall increase during the first 10 days of the experiment, indicating that *Daphnia* was able to grow to higher densities in the presence of an actively growing *Microcystis* population. Then, as *Daphnia* continued

to increase in densities, the *Microcystis* population started to crash, but only in the absence of fish (Fig. 4b). In the presence of fish (Fig. 5.4f), the growth of *Daphnia* levelled off at approximately 60 individuals L^{-1}, whereas *Microcystis* abundance remained high. In the other experiments, reductions in *Microcystis* dominance and increases in *Daphnia* densities occurred concomitantly, especially in the absence of fish. This is also more in agreement with a top-down control (grazing immediately impacting algae densities) than with a toxicity effect of *Microcystis* on *Daphnia*. If *Microcystis* toxins impacted *Daphnia* populations negatively by affecting their fertility and mortality rates, we would expect a recovery of *Daphnia* populations following a *Microcystis* decline only after some time lag.

The enclosures used in our experiment were self contained and did not allow interaction of the fish with lake sediments. This may have resulted both in an under- and overestimation of the effect of *Garra* on the pelagic food web. The absence of real lake sediments on the floor of the enclosures may have resulted in lower rates of sediment re-suspension and associated nutrient recycling by the foraging and swimming activity of fish compared to the fishless enclosures and the reservoirs. Despite this potential limitation, we were still able to show convincingly the effects of *Garra* on water column concentrations of nutrients, suspended matter and chlorophyll a. Conversely, the absence of lake sediments in the enclosures may have deprived the fish of access to benthic food resources, thereby forcing them to forage on zooplankton. This could have resulted in an overestimation of the top-down control of *D. carinata* and resulting trophic cascade effects on *Microcystis*. Our results are, nevertheless, overall very consistent with patterns that were detected during a survey study of 32 reservoirs (Dejenie et al., 2008). In that survey, we recorded a positive association between *Garra* biomass and total phosphorus, an association that is

undoubtedly due to the higher carrying capacity of phosphorus-rich reservoirs for *Garra* populations, but which may also reflect a higher recycling of phosphorus by high densities of *Garra* (see also this study). Furthermore, the survey yielded a positive association of *Garra* with the estimated biomass of cladocerans, which is in agreement with the observation of enhanced densities of *Diaphanosoma* and *Ceriodaphnia* in enclosures with *Garra*. The survey study also suggested a moderate top-down impact of *Garra* on cladocerans due to the lack of an association between phytoplankton and cladoceran biomass (see Dejenie et al., 2008), which is in line with the present findings of a subtle top-down effect of *Garra* on *Daphnia*. Furthermore, the field survey (Dejenie et al., 2008) also revealed a negative association between *Daphnia* and cyanobacteria biomass, suggesting a *Daphnia*-mediated trophic cascade effect.

The reason why the fish in our experiments and in the reservoirs exert only a limited top-down effect is likely related to the fact that they are opportunistic, largely benthivorous riverine fish that are not very well adapted to feed on zooplankton. This also indicates the existence of an unoccupied niche for zooplanktivorous fish in the majority of the reservoirs. Detailed studies on the diet of *Garra* and whole lake manipulation experiments are presently being undertaken to further broaden our knowledge on the ecology of *Garra* and its impact on reservoir food web structure and dynamics (Mekonen et al., unpubl. data).

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Appendix 5.1

The impact of fish on phytoplankton and cyanobacterial blooms in semi-arid tropical highland reservoirs in Tigray: analysis of phytoplankton community taxa

Introduction

Chapter 5 of this thesis deals with the results of the field enclosure experiments carried out to assess the impact of fish on the ecology of reservoirs and the occurrence of *Microcystis* blooms in semi-arid highland reservoirs in Tigray, Ethiopia. This manuscript is published online in the *Freshwater Biology*. In this appendix, we present part of the data and results of the enclosure experiments which were not included in this manuscript. We hear by present in this appendix the results of analysis for the phytoplankton community composition *Microcystis* biomass and genetic structure. We also present the methods for the DGGE profiling of the cyanobacteria. The experimental design, collection and analysis of samples, and statistical analysis are described in chapter 5. Finally we will also present some additional discussion on the effect of Garra on the phytoplankton composition communities.

Materials and methods

To study the *Microcystis* ITS rDNA population structure, water samples were manually filtered over a 25mm 0.2 μm GSWP filter (Millipore) until the filter was clogged. The filter was folded two times and packed with sterile aluminium foil. Filters were transported on ice packs to the laboratory and stored at -20 $^{\circ}$C until further processing. Genomic DNA from the GSWP filters containing field samples was extracted following the protocol described by Zwart et al. (1998), which includes beat-beating with phenol extraction and ethanol precipitation. After extraction, the DNA was purified on a Wizard column (Promega).

A specific nested-PCR protocol (Van Gremberghe et al. 2009) was used to specifically amplify only *Microcystis* ITS sequences from the water samples. In a first

PCR, a specific 16S rDNA primer for *Microcystis* (CH) described by Rudi *et al*. (1997) was used as forward primer and a general 23S rDNA primer (ULR) was used as reverse primer (Janse *et al*., 2003). For the second PCR the cyanobacterium-specific 16S rDNA primer (GC)-CSIF (with GC-clamp for DGGE-analysis) in combination with primer ULR (Janse *et al*., 2003) was used. For the composition of the reaction mixtures of the PCR's we refer to Van Gremberghe et al. (2009).

DGGE profiling

DGGE was essentially performed as described by Muyzer *et al*. (1993). Equal amounts of PCR products were loaded onto 8% (w/v) polyacrylamide gels (1 mm thick, in 1× TAE [20 mM Tris acetate (pH 7.4), 10 mM acetate, 0.5 mM disodium EDTA]). The denaturing gradient contained 35-40% denaturant [100% denaturant corresponded to 7 M urea and 40% (v/v) formamide]. Electrophoresis was performed for 16 h at 75 V and the temperature was set at 60°C. Finally, the gels were stained with ethidium bromide and photographed on a UV transillumination table with a CCD camera. Furthermore, a small piece of gel from the middle of the target band was excised from the DGGE gel for sequencing and incubated in 50 µl of sterile TE buffer (10 mM Tris, pH 7.6, 1 mM EDTA) for 24 h at 4 °C. The eluent was then re-amplified and purified on DGGE one or two times. The resulting PCR products were purified using a QiaQuick PCR purification kit (QiaGen). Finally, sequencing was performed with the ABI-Prism sequencing kit and the resulting sequencing reaction products were analysed on an automatic sequencer (ABI-Prism 3100).

On every gel, three standard lanes were analysed in parallel to the samples. These standard lanes are composed of several known bands positioned distinctly in the gel. As these bands should always be formed at the same denaturant concentration

in the gel, their position was used to compare the patterns formed in different gels. Digitalized DGGE images were analysed using the software package BioNumerics 4.5 (Applied Maths BVBA). The program detects the bands and groups the bands into band classes, based on their position in the gel. Sequence information of the bands was used to manually check the grouping of bands into band classes. Further, a matrix was compiled, based upon the band intensity or the presence or absence of bands in band classes. The band intensities were then converted into relative intensities (the relative contribution of each band to the total band signal in the lane).

Statistical analyses

We performed a Principal Component Analyses (PCA) and redundancy analysis (RDA) on the matrix with log transformed biomasses of the different phytoplankton taxa and the ITS types of *Microcystis* following the procedure described in methods of this chapter. For details of the statistical analysis, we refer to the methods section of this chapter (chapter 5). Finally, we executed repeated measures ANOVA in Statistica 7.0 to test for the effect of fish on the biomasses of the different taxa in all enclosure experiments separately.

Results

1. Effect of fish on phytoplankton community structure

The biomass of the different phytoplankton taxa in the two experiments carried out in Gereb Awso and Tsinkanet is shown in Fig. 5.5. The abundance of phytoplankton taxa differed considerably among lakes and among enclosure experiments (Fig. 5.6a). An overall increase in the biomass of phytoplankton in general and the biomass of *Microcystis* in particular was observed in the April experiment (Fig. 5.5).

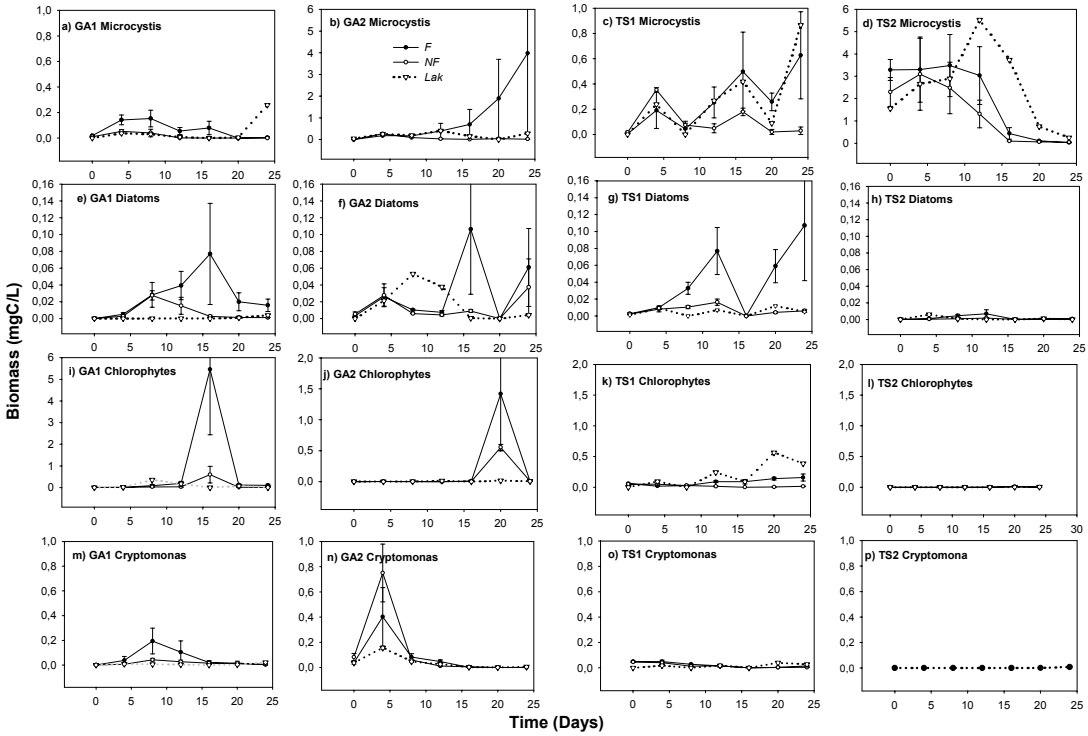

Fig. 5.5 Biomass of the different phytoplankton taxa in Gereb Awso (GA) and Tsinkanet (TS) experiments 1 and 2.

We observed an overall significant effect of *Garra* on the phytoplankton communities in the four enclosure experiments (Trace= 0.08, F= 4.3, p= 0.032) (Figure app. 5. 6b). Analyses performed on individual phytoplankton taxa indicated that *Garra* significantly increase diatom (T= 0.238, F = 14.778, p = 0.002), *Microcystis* (T = 0.249, F = 12.791, p = 0.002), and total phytoplankton biomass (T = 0.271, F = 11.229, p = 0.004). Fish treatment effects were not significant for Chlorophytes, Cryptomonas and Euglenoids. According to the repeated measures ANOVA there was a significant effect of *Garra* on *Microcystis* biomasses in experiments of the rainy season carried out in both Gereb Awso and Tsinkanet (Table 5.2). There was also a significant effect of fish on diatoms and total phytoplankton for the rainy season experiment in Tsinkanet.

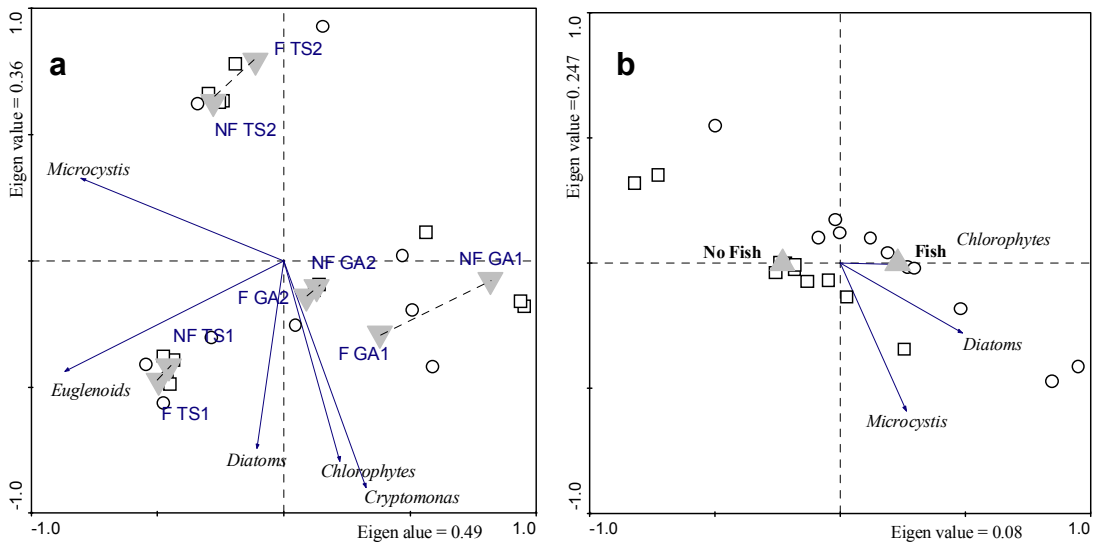

Fig. app. 5.6 (a)Triplot of PCA results performed on biomass of phytoplankton taxa in the four enclosure experiments. (b) Triplots of RDA results, showing the response to the presence of fish (Garra) of the biomass of phytoplankton taxa in the four enclosure experiments. Individual experiments were specified as co-variables. The nominal variables Fish and Non Fish in the RDA plot are represented by centroids. Circles represent enclosure with fish, squares represent fishless enclosures. F: with fish, NF: no fish; GA1and TS1 Gereb Awso and Tsinkanet September experiment respectively; GA2 and TS2: Gereb Awso and Tsinkanet April experiment respectively. Filled triangles represent centroids and show the average position of replicate enclosures in each treatment level.

Table 5.2 Results of repeated measures ANOVA on log transformed biomass date testing for the interaction effect of 'Garra' (Fish = F) and 'time' (T) on total phytoplankton biomass, biomass of Diatoms, and Microcystis during enclosure experiment 1 (September/October, 2005) and experiment 2 (April/May, 2006) in Gereb Awso (GA) and Tsinkanet (TS). F= fish, T= time and F*T= fish and time interaction.

Microcystis							***Diatoms***					
	d.f.	MS effect	MS error	F	p		d.f.	MS effect	MS error	F	p	
Gereb Awso – rainy season												
F	1,2	32.391	0.702	46.081	0.02	F>NF	1,4	5.734	1.431	4.007	0.11	
T	5	7.471		2.705	0.08		6	9.625		19.33	<0.01	
F*T	5,10	2.884	2.761	1.044	0.44		6,24	0.830	0.497	1.667	0.17	
Gereb Awso – dry season												
F	1,4	7.218	3.168	2.278	0.27		1,2	0.404	0.353	1.146	0.396	
T	6	2.443		1.753	0.19		5	0.548		4 .535	0.020	
F*T	6,24	0.761	1.393	0.546	0.76		5,10	0.140	0.121	1.163	0.391	
Tsinkanet – rainy season												
F	1,4	5.297	4.603	1.150	0.34		1,4	4.004	0.339	11.799	0.026	F>NF
T	6	7.729		2.521	0.04		6	12.01		47.529	<0.01	
F*T	6,24	10.85	3.065	3.540	0.01	F>NF	6,24	0.361	0.252	1.432	0.244	
Tsinkanet – dry season												
F	1,4	0.364	0.184	1.978	0.23		1,4	3.223	0.919	3.506	0.134	
T	6	3.934		71.057	<0.01		6	4.915		2.495	0.051	
F*T	6,24	0.104	0.055	1.874	0.13		6,24	2.014	1.969	1.023	0.434	
Total phytoplankton							***Total phytoplankton***					
Gereb Awso – rainy season							Tsinkanet – rainy season					
F	1,4	2.052	1.283	1.599	0.27		1,4	1.171	0.121	9.66	0.034	F>NF
T	6	2.278		2.522	0.05		6	1.890		59.71	<0.01	
F*T	6,24	0.937	0.903	1.037	0.43		6,24	0.295	0.032	9.32	<0.01	F>NF
Gereb Awso – dry season							Tsinkanet – dry season					
F	1,2	1.535	0793	1.934	0.30		1,4	0.355	0.197	1.801	0.25	
T	6	0.996		3.644	0.03		6	3.789		75.10	<0.01	
F*T	2,12	0.06	0.273	1.123	0.41		6,24	0.097	0.050	1.916	0.11	

F>NF refers to significant treatments or treatment x time interaction effects with fish affected population biomasses positively

2) *Effect of fish on Microcystis ITS types*

Twenty band classes (equivalent to different ITS types) were detected with the PCR amplified DGGE profiles of the 16S rDNA. Most of the band classes were, however encountered in one or two enclosures only once. We used, therefore, only the four band classes which were relatively abundant in all four enclosures. We used sequence information to verify whether the bands in a band class represent the same ITS

sequence. The ITS types tended to differ among lakes and among enclosures with no pattern in enclosures with fish and fishless enclosures showed among the September and April experiments (Fig. 5.7a). Overall the RDA (Fig. 5.7b) of the effect of *Garra* on the relative abundance of the ITS types was not significant (Trace = 0.058; F = 1.115; p = 0.338). This may result from the inconsistent presence of the different ITS types in the different enclosures and lakes. We further carried out RDA separately for the experiments in Gereb Awso and Tsinkanet. We observed an increase in the p-value (not significant) of the Tsinkanet experiment by 0.0198 (Trace 0.172, F 2.468, p 0.884). ITS type 1 seem to increase with the addition of fish. We also performed Mann-Whitney U tests for effects of *Garra* on the four different ITS types. We observed a significant effect of *Garra* on ITS type 1 (U=0.0000, Z=1.964, p=0.0496) in the dry season (April) experiment in Tsinkanet.

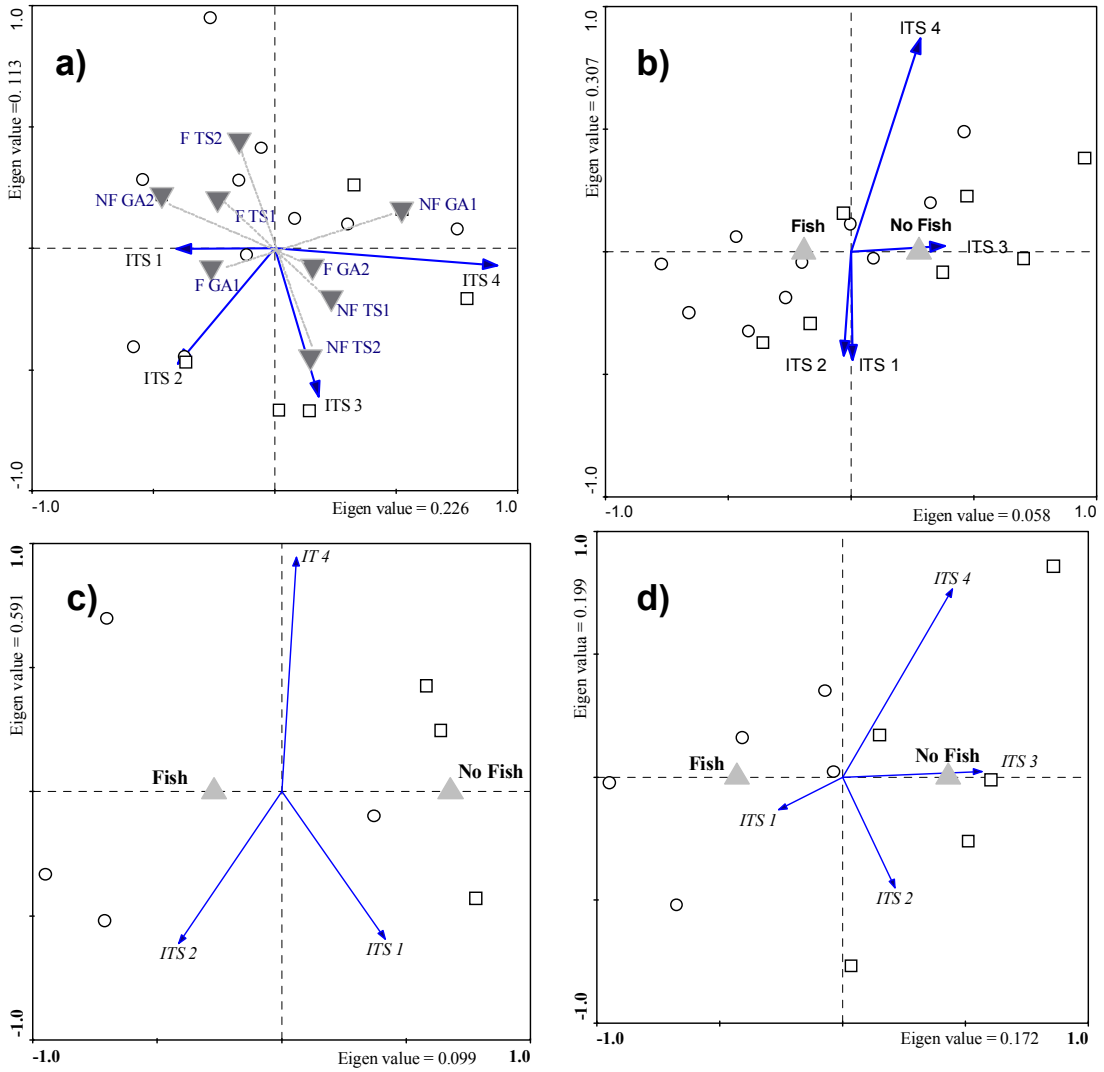

Fig. 5.7 Triplot of PCA results on the Microcystis ITS in the four enclosure experiments (GA1, GA2, TS1, and TS2) (a) and RDA triplots on Microcystis ITS (b-c): (b)the four enclosure experiments, (c) Gereb Awso experiments (GA1 and GA2) and (d) Tsinkanet experiments (TS1 and TS2). Circles represent enclosure with fish; squares represent fishless enclosures. F: with fish, NF: no fish; GA1and TS1 Gereb Awso and Tsinkanet rainy season experiment respectively; GA2 and TS2: Gereb Awso and Tsinkanet dry season experiment respectively. Filled triangles represent centroids and show the average position of replicate enclosures in each treatment level.

Discussion

Our results indicate that the presence of *Garra* markedly influenced phytoplankton biomass and *Microcystis biomass*. But no significant consistent influence was observed for the ITS types of *Microcystis.* We have reported (this chapter) an increase

in nutrient concentration and suspended matter and a decrease in transparency after addition of fish. Increased levels of dissolved oxygen and pH were also measured in the fish enclosures. This is an indication for a higher phytoplankton primary productivity in the presence of fish.

The increase in phytoplankton primary productivity associated with *Garra* is probably the result of increased nutrient concentrations (total phosphorus and total nitrogen) caused by fish. Reports indicate that phytoplankton community structure is frequently affected by the presence of fish (Vanni & Layne, 1997), resulting from a consumer mediated nutrient recycling. The higher nutrient concentrations in the fish compared to the fishless enclosures was not only related to a higher phytoplankton biomass, but also to a shift in taxon composition within the phytoplankton towards more *Microcystis* and diatoms. This is in agreement with other studies showing that increased nutrient concentrations are often associated with cyanobacterial blooms (Elser, 1999). *Microcystis* was the dominant phytoplankton taxon in the experiments, with an increase in biomass due to the addition of fish. The fish-zooplankton-*Microcystis* interactions are discussed in chapter 5.

We could not establish clearly the effect of fish on different strains of *Microcystis* represented by ITS types. It seemed that different strains (species) of *Microcystis* respond differently to the different treatments especially in Tsinkanet.

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Chapter 6

An experimental assessment of potential top-down effects of zooplankton on the phytoplankton communities, including toxic cyanobacteria, of eutrophic shallow reservoirs in Tigray, North Ethiopia

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(Unpublished manuscript)

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Abstract

An enclosure experiment was carried out to study the effect of zooplankton grazing on phytoplankton community structure, including the relative abundance of toxic cyanobacteria, in a semi-arid shallow reservoir in Tigray, North Ethiopia. Twelve 80 L buckets, suspended in the lake, were assigned to four treatments that represented factorial combinations of source water (34 μm filtered water from two reservoirs) and presence/absence of zooplankton. Plankton net concentrated phytoplankton was inoculated in equal densities to all buckets. Top-down regulation by zooplankton was observed for some of the phytoplankton taxa, including Anabaena, Euglenoids, Diatoms, Chlorophytes and Cryptomonads, whereas the impact of the presence of zooplankton on *Microcystis* and *Peridinum* biomass was limited. Microcystin was detected from all experimental units, and concentrations ranged from 0.082 to 1.268 μg L^{-1}. Higher microcystin concentrations were detected in the treatment with than without zooplankton. A positive association between zooplankton and microcystin concentration per unit biomass of *Microcystis* was observed. Overall, our results suggest that the presence of zooplankton stimulates toxin production.

Introduction

Efforts to understand the complex relationships between algae and herbivorous zooplankton have been a central focus of aquatic ecology. In recent years, the response of algal community to herbivorous zooplankton is considered to result from the net effect of ingestion by zooplankton and growth stimulation due to nutrient recycling (Carpenter & Kitchell, 1984, Lehman & Sandgren, 1985, Bergquist et al., 1986, Sterner, 1986). Zooplankton community composition is an important factor influencing algal grazing losses. The nature and intensity of grazing pressure on the phytoplankton community depend on numerous aspects of the zooplankton community. Elser et al. (1987) reported that algae respond more strongly to changes in zooplankton composition than to changes in zooplankton biomass. Different studies have demonstrated that zooplankton selective feeding can impose differential mortality on algal taxa (Bogdan and McNaught, 1975, Lampert and Taylor, 1985), and that algal taxa respond differentially to grazing in ways not strictly related to cell size (Porter, 1975, Havens and DeCosta, 1985, Bergquist *et al.*, 1986, Elser, 1999). Opposite shifts of phytoplankton size distribution were observed by Bergquist et al. (1985) from grazing by two zooplankton assemblages with different size distribution. Density of small phytoplankton taxa increase when grazed by small zooplankton, but decreased when grazed by large zooplankton. Conversely, large phytoplankton became less abundant in the presence of small zooplankton, but increased in density in the presence of large zooplankton. This is explanation for the contrasting size shifts of the phytoplankton are differences in grazing selectivity (Peters & Downing, 1984) and nutrient recycling rate (Peters, 1983). Consequently, the nature of the interaction between phytoplankton and their grazers is dependent on the composition

of the algal community as well as on overall nutrient availability and the composition of the zooplankton. *Daphnia* are automatic, relatively unselective filter feeding crustaceans (MacMahon & Reeve, 1965); but copepods feed discontinuously and show considerable discrimination when presented with a choice of several organisms. Selective feeding of copepods may be based on quality of food but they also apparently tend to pick large sized particles when given a choice (Mullin, 1963, Richman and Rogers, 1969). The difference in feeding behaviour between cladocerans and copepods is reported to cause complementary impact on phytoplankton (Sommer et al., 2001, 2003); cladocerans suppress small phytoplankton, while copepods suppress large phytoplankton. Nutrients released by grazing zooplankton are available for algal uptake (Lehman and Scavia, 1982) and, in some situations, can provide sufficient nutrients to satisfy a substantial proportion of the growth requirement of the algal community (Lehman, 1980, Axler *et al.,* 1981, Sterner, 1986, Elser, 1999). Nutrient recycling by herbivores is also size- and species-dependent (Lehman, 1980, Elser, 1999).

Cyanobacterial blooms are increasingly frequent in aquatic ecosystems around the world as a result of eutrophication (Chorus & Bartram, 1999, Huisman et al., 2005) and are likely to increase with global warming. Factors such as nutrient concentrations (nitrogen and phosphorus), temperature, light, pH and alkalinity, buoyancy, selective zooplankton grazing, hydrologic and meteorological conditions, and the morphology of the impoundment have all been implicated (Shapiro, 1984, Chorus & Bartram, 1999) as factors contributing to cyanobacterial bloom formation. Interactions between bloom-forming cyanobacteria and zooplankton are important to study considering the potential of top-down control of cyanobacterial blooms (Burns, 1987, Lampert, 1987, Sommer, 1989). Consequently, a large body of research has

been directed at understanding the mechanisms by which cyanobacteria affect zooplankton and vice versa. This information has important implications for understanding planktonic community structure and function, as well as water quality in lakes. For example, the filamentous morphology of certain cyanobacteria has been shown to negatively affect large cladocerans more than small cladocerans through reduced fecundity (Webster and Peters, 1978, Gilbert, 1990), a mechanism that may help to explain declines in the dominance of large cladocerans with lake eutrophication (Jeppesen *et al*., 2000). Two major mechanisms have been proposed for the greater susceptibility of large cladocerans to colonial or filamentous blue-green algae (Gliwicz, 1977, Webster & Peters 1978, Richman & Dodson, 1983, Porter & McDonough, 1984). First, colonial or filamentous algae clog the filtering appendages of larger cladocerans, reducing their feeding rates on co-occurring nutritious food sources or increasing their respiration rates or both. And second, larger cladocerans more readily ingest colonial or filamentous blue-greens and thereby are more strongly affected by any toxic chemicals that these algae may possess.

Bloom-forming cyanobacteria are in general considered a poor food source for herbivorous zooplankton (Porter and Orcutt, 1980). Three properties of cyanobacteria have been proposed to account for this poor quality: filamentous/colonial morphologies, production of intracellular secondary metabolites with toxic properties, and deficiencies in essential nutrients (Porter & Orcutt, 1980, Lampert, 1987, DeMott, 1989). Of these, morphology and toxins have attracted most interest (Lampert, 1982, Fulton & Paerl, 1987b, Lürling, 2003). In addition, a number of cyanobacterial genera produce a wide range of toxins, causing a potential risk to livestock and human health (Resson et al., 1994, Chorus & Bartram, 1999, Carmichael et al., 2001). Several reports suggest that cyanobacterial toxins act as a defence against

zooplankton grazing by reducing the feeding rate of zooplankton (Lampert, 1981, Nizan *et al*., 1986, Fulton & Paerl, 1987a, DeMott, 1999). An in increase toxin production by *Microcystis aeruginosa* in response to direct and indirect exposure to herbivorous zooplankton of several species has been reported by Jang et al. (2003); in support to the hypothesis that this response is an induced defence mediated by the release of chemicals called "infochemicals" from zooplankton. These are chemical signals released by predators that can induce defences in aquatic prey (Bronmark & Hansson, 2000). Defence mechanisms such as colony formation, regulation of growth rate and toxin production could be induced in *M. aeruginosa* by one or more infochemicals released by zooplankton (Larsson & Dodson, 1993, Jang et al., 2003, 2008).

Different strains of the same phytoplankton species may coexist in the phytoplankton community (Kirk and Gilbert, 1992; Barreiro et al., 2007). These strains may differ in their toxic properties, a case documented for toxic cyanobacteria (Laamanen et al., 2001). It is also reported that some zooplankton organisms are able to select between algae differing in toxicity (Huntley et al., 1986, DeMott, 1989, Gilbert, 1990, DeMott and Moxter, 1991, Kirk and Gilbert, 1992, Teegarden, 1999). The ability of grazers to select non-toxic food is a mechanism which favours toxic algae against non-toxic algae, and is possibly one of the mechanisms favouring bloom initiation in toxic phytoplankton species (Gilbert, 1990, Guisande et al., 2002). However, zooplankton grazers could be less able to select between coexisting toxic and non toxic strains of the same species. In this aspect, the benefits of producing toxic compounds in order to avoid grazing may be minimal.

In this study, a short-term enclosure experiment was carried out to study the effect of zooplankton on the phytoplankton community, including toxic cyanobacteria, in a semi-arid shallow reservoir in Tigray, North Ethiopia. The central question addressed was to what extent a top-down effect of zooplankton grazing on cyanobacteria can regulate bloom formation and to what extent zooplankton influences microcystin production. In addition, we wondered if the reservoir origin of the water used in the experiment has implications for the phytoplankton community composition and how this interacts with the presence of zooplankton. Therefore, phytoplankton was collected from two different reservoirs, mixed, and grown in the two water sources of which phytoplankton was collected, in the presence or absence of zooplankton.

Materials and methods

Experimental design

An enclosure experiment was carried out in April 2007 in Mai Gassa I reservoir, (13° 17' 13.5" N, and 39° 29' 25" E, Tigray, North Ethiopia), a shallow reservoir characterized by both the presence of algal blooms and submerged vegetation (see Dejenie et al., 2008). The enclosures consisted of synthetic polyethylene plastic cone shaped buckets (top diameter: 40 cm, bottom diameter: 30 cm, depth: 80 cm, volume: 80 L) filled with plankton net (34 μm) filtered lake water. The enclosure buckets were reinforced, fixed in a wooden frame, and positioned at the centre of the lake with anchors. Before filling, the twelve bucket enclosures were randomly assigned to four treatments (two source waters and presence/absence of zooplankton) according to a factorial design: 1) three enclosures received filtered Mai Gassa I water and zooplankton (MGZ+), 2) three received filtered Mai Gassa I water without

zooplankton (MGZ-), 3) three received filtered water from a second reservoir Era Quihila water and zooplankton (EQZ+), and 4) three received filtered Era Quihila water without zooplankton (EQZ-). The enclosures were set up on April 16, 2007. The experiment lasted for 16 days. The two source reservoirs for filtered water, phytoplankton and zooplankton, Mai Gassa I and Era Quihila, are both characterized by cyanobacterial blooms, but the blooms are more pronounced in Era Quihila than in Mai Gassa I. Mai Gassa I is also characterized by the presence of submerged vegetation. All buckets were inoculated with a mixture of phytoplankton collected with a plankton net (34 μm mesh size) from Mai Gassa I and Era Quihila reservoirs. Equal amounts of phytoplankton from the reservoirs were added to the mixture prepared as inocula by measuring chlorophyll a with a fluorometer (Turner Aquafluor) set on the chlorophyll mode. For the treatments with zooplankton, *Daphnia* from a third reservoir, Adi Amharay, were collected with a zooplankton net and introduced into the zooplankton treatments. Adi Amharay was selected as a source of the zooplankton inocula because *Daphnia,* the zooplankton grazer with potentially the strongest top-down influence on phytoplankton communities was exceptionally dominant in this reservoir. These *Daphnia* represent enrichment to the zooplankton that was introduced in the buckets together with the phytoplankton inoculum. The zooplankton inoculum was equal across all experimental units of the zooplankton treatment, and represented a mixture of zooplankton of Mai Gassa I and Era Quihila supplemented with *Daphnia* from Adi Amharay reservoir.

Sampling and analysis of samples

Phytoplankton, zooplankton, and water samples for quantification of toxin concentrations were collected from the enclosures on days 0, 2, 4, 8 and 16. Samples

were collected with a tube sampler. Water samples (250 ml) for phytoplankton counting were preserved with acid lugol's solution, while zooplankton samples (2 L filtered over 34 μm) were immediately fixed with a formalin-sucrose solution (4% final concentration) to prevent egg loss from cladoceran carapaces (Haney & Hall 1973). Phytoplankton biomass was estimated from cell bio-volume measurements and previously published biovolume-to-carbon conversion data (Menden-Deuer & Lessard, 2000). Zooplankton was counted at the start of the experiment (d 0) and on days 4 and 8. The counts were used to calculate densities (individuals per litre) and biomass. Biomass estimates were obtained using published length-weight regressions (Bottrell et al., 1976).

Detection of toxins

The total microcystin concentration (μg ml^{-1}) in the samples was determined by ELISA (Enzyme Linked Immunosorbent Assay). For the extraction of microcystins, the cells were boiled for 20 minutes (van der Oost, 2007), and then centrifuged for 10 minutes at 14,000 rpm after which the supernatant was used for ELISA. The ELISA-test was done according to the manufacturer's instructions (SDI - EnviroGard Microcystin ELISA plate kit). The microcystin concentration was recalculated into concentration per biomass (μg microcystin per mg C *Microcystis* biomass). Due to accidental loss of part of the samples, toxin concentrations could only be measured on day 4 of the experiment.

Data analysis

Prior to statistical analysis, all variables were Log (X+1) transformed. Multivariate analyses were used to investigate the relation between phytoplankton taxon

composition, with emphasis on potentially toxic cyanobacteria, and experimental treatment (source water and zooplankton). We performed redundancy analysis (RDA) to test the overall effect of zooplankton and source water on the phytoplankton community composition. The RDA was performed in CANOCO version 4.5 for Windows (Ter Braak & S'milauer, 2003). Treatments effects were further explored in more detail by applying additional tests to each of the phytoplankton genera separately. We executed two way analysis of variance (ANOVA) on log transformed biomasses of different phytoplankton taxa for those we observed significant effect by the regression analysis. Finally, spearman rank correlation coefficients were calculated between the biomass of different phytoplankton taxa and the different zooplankton species to check for specific associations. The two way ANOVA was also used to investigate the significance of the differences in microcystin concentration (mg/L) between the zooplankton and water source treatments. All univariate analyses were carried out with the software Statistica version 6.0 for Windows (StatSoft Inc., Tulsa, USA).

Results

The filtered water that was used to fill the enclosures was not completely free from phytoplankton ($61x10^3$ pg C ml^{-1} in Mai Gassa I filtrate and $246x10^3$ pg C ml^{-1} in Era Quihila filtrate), but the filtration procedure enabled us to start with comparable phytoplankton biomass and taxon composition in the two treatments, as the inoculum that was added to all experimental units was much more dense than the remaining phytoplankton in the filtrate. Total phytoplankton biomass was similar in the two source reservoirs, but the taxon composition differed. Fig. 6.1 shows the taxon composition of the experimental units on day 0. The communities were dominated by

Microcystis (derived from both Mai Gassa I and Era Quihila at a ratio of approximately 1:2) and *Peridinium* (mainly derived from Mai Gassa I); some diatoms, chlorophytes, cryptomonads and *Anabaena* were also encountered, mainly in Era Quihila water.

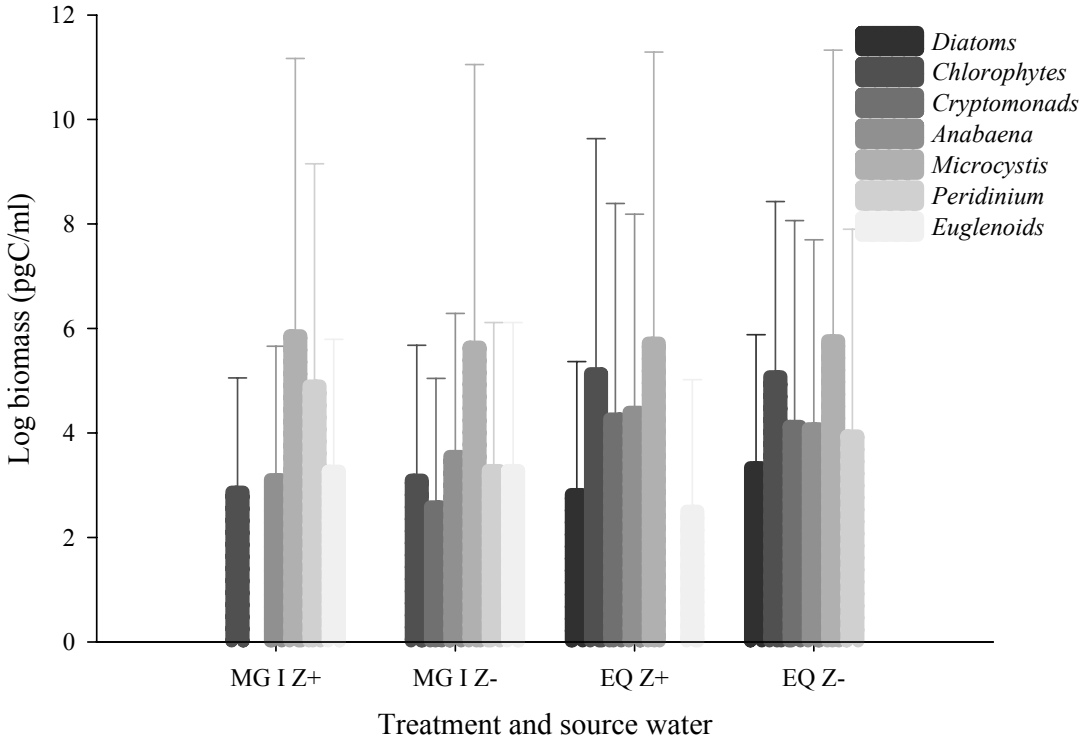

Fig. 6.1 Biomass of major phytoplankton taxa in the experimental units on the starting day (day 0) of the experiment. Error bars represent one standard error. MG I, Mai Gassa I; EQ, Era Quihila; Z+, Zooplankton treatment; Z-, non-zooplankton treatment.

Overall, the algal biomass tended to increase during the course of the experiment in all treatments (Fig. 6.2a). In contrast, we observed a major decline in zooplankton biomass in the zooplankton treatment (Fig. 6.3). The biomass of *Diaphanosoma* and *Daphnia* rapidly declined and became similar across all treatments from day 4 onwards. The biomass of copepods, although declining, remained higher in the zooplankton than in the non-zooplankton treatments.

Most phytoplankton groups tended to show lower biomasses in the presence

than in the absence of zooplankton in our experiment, but the extent by which phytoplankton growth was reduced differed among taxa (Fig. 6.2). Of the cyanobacteria, *Anabaena* was strongly suppressed in the presence of zooplankton, whereas the impact of zooplankton on *Microcystis* was much less pronounced. *Microcystis* reached higher biomass in Mai Gassa I water both in the absence and presence of zooplankton compared to the respective treatments with Era Quihila water (Fig. 6.2). Chlorophytes seem to be strongly suppressed by zooplankton except in the Mai Gassa I treatment at the end of the experiment. *Peridinium* biomass tends to be reduced in the presence of zooplankton, although only to a small extent.

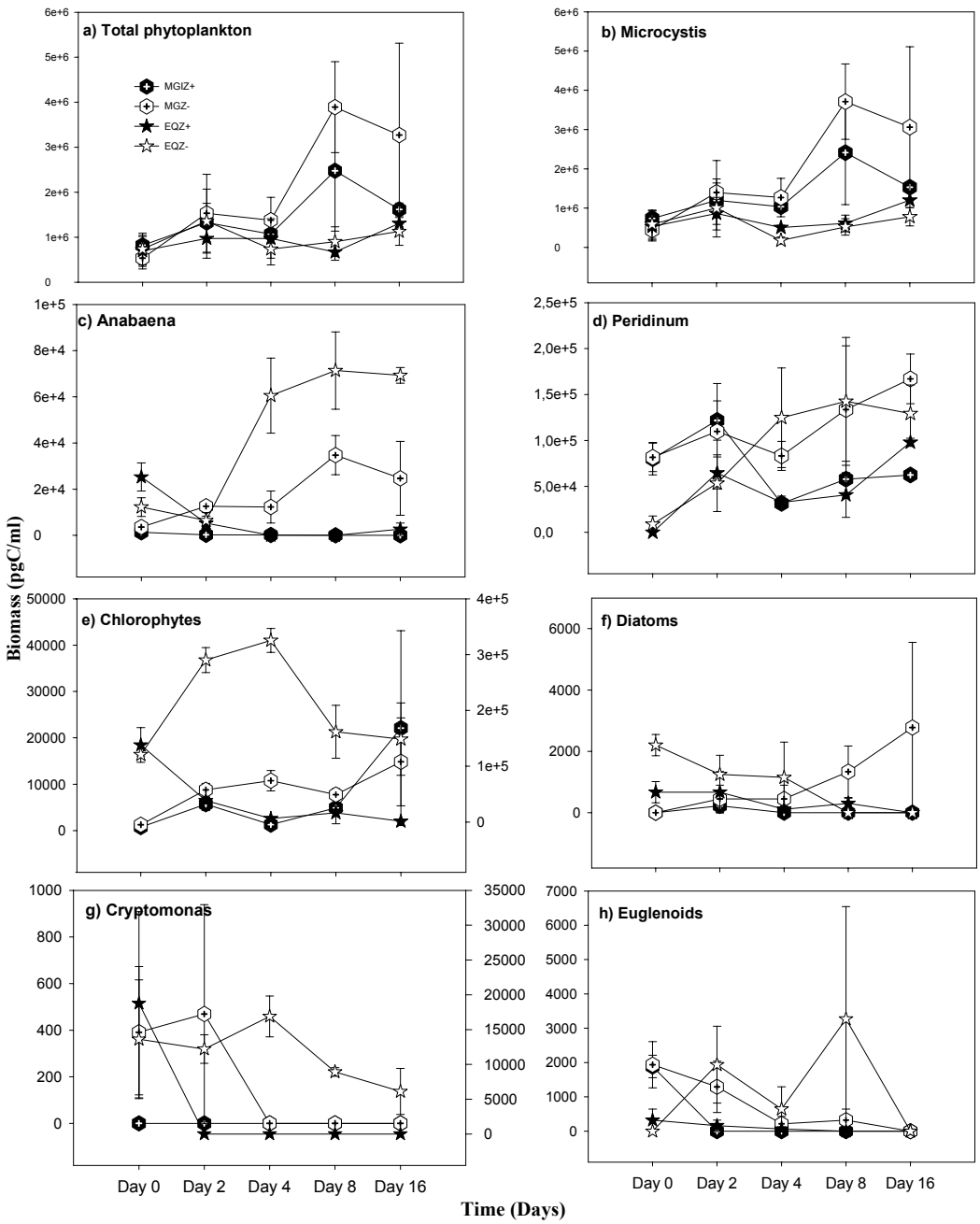

Fig.6.2 Changes in total biomass (pg C/ml; panel a) and biomass of the major phytoplankton taxa (panels b-h) in the different treatments during the course of the experiment.

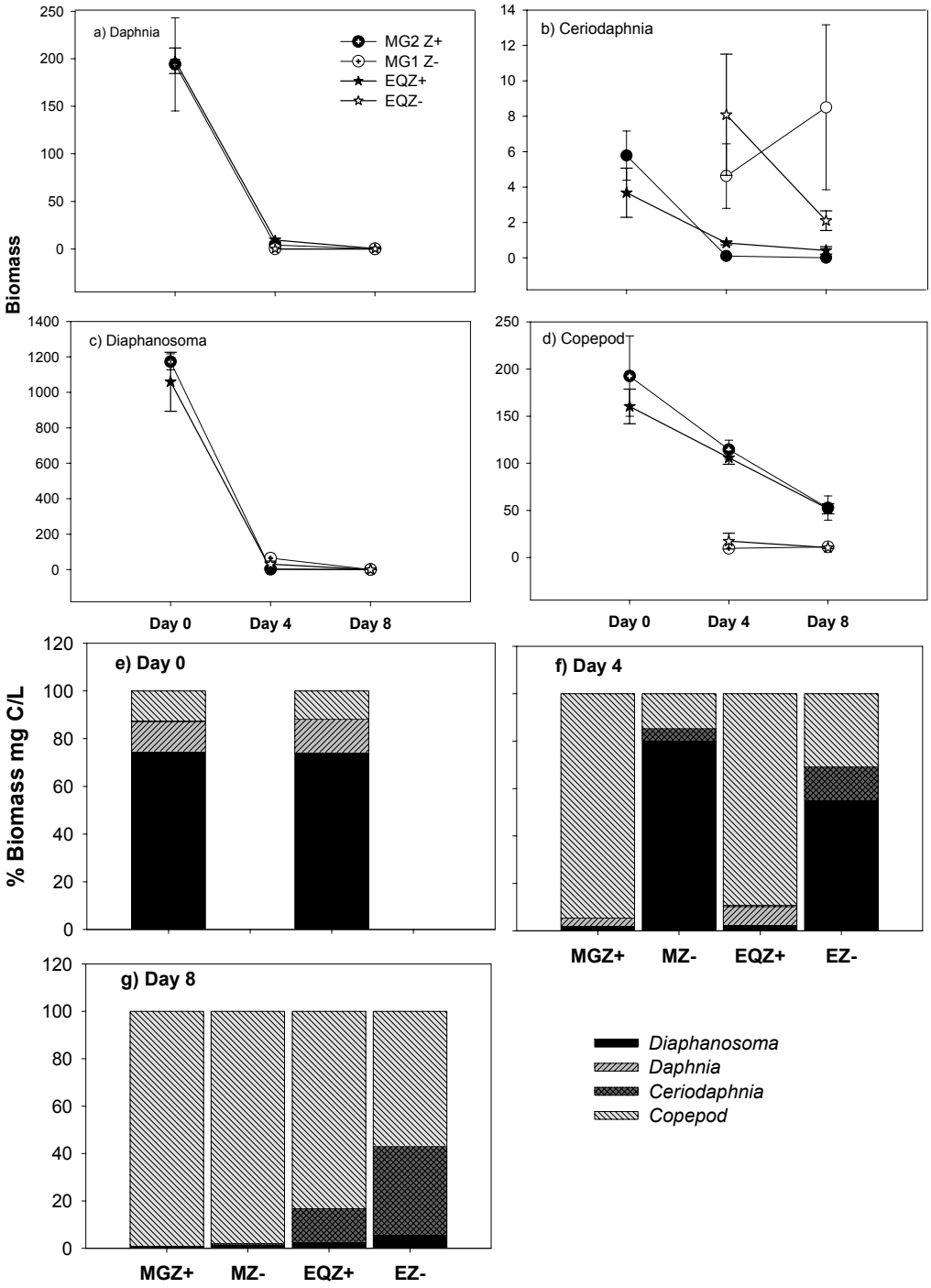

Fig. 6.3 Changes in biomass (mg C/L) of the major zooplankton taxa during the course of the enclosure experiment. (a – d, biomass of each genera over the course of time; e – g, % contribution of each genera at each day and treatment).

Partial RDA analysis was performed to investigate the pattern of phytoplankton taxon composition in function of source water and the presence/absence of zooplankton, correcting for the impact of time (Fig. 6.4a). It appears that the differences between the presence and absence of zooplankton (along first axis, with Eigen value of 28.5%) (Trace = 0.363, F = 12.261, p = 0.002) were far more important than the variation caused by source water (Mai Gassa I or Era Quhila water; second axis, with Eigen value of 6.6%). To zoom in on the response of phytoplankton to the treatments, we ran the RDA for the different sources of water separately (Fig. 6.4b & c). We observed similar pattern of impact of the zooplankton on *Anabaena,* chlorophytes, cryptomonads and to a lesser extent on diatoms, euglenoids, *Peridinium* and *Microcystis* for Mai Gassa I (Trace = 0.441, F = 22.267, p = 0.0001) and Era Quihila (Trace = 0.252, F = 13.138, p = 0.002). These analyses suggest an overall tendency for biomass of most phytoplankton groups to be higher in the treatment without than in the treatment with zooplankton, but the impact differs among taxa. According to analyses per individual phytoplankton taxon, significant effect of zooplankton and source water was observed for chlorophytes, cryptomonads, euglenoids, *Anabaena, and Microcystis* (Table 6.1). Overall treatment effects were not significant for *Peridinium* and diatoms. Repeated measures analysis of variance (ANOVA) confirmed these results, with a significant effect of zooplankton on the biomass of chlorophytes, cryptomonads, and *Anabaena* and chlorophytes but not on *Microcystis* and euglenoids (Table 6.2). A significant effect of source water was observed for chlorophytes, cryptomonads, *Anabaena* and *Microcystis,* but not for euglenoids. The Spearman rank correlation coefficient calculations also showed significant associations (negative) of *Anabaena* with several zooplankton taxa (*Diaphanosoma sp., R*=0.684, p =0.0141; *Daphnia carinata, R* = -0.861, p = 0.0003;

Ceriodaphnia cornuta, R=0.998, p =0.0018; and the calanoid copepods, R=-0.853, p =0.00042).

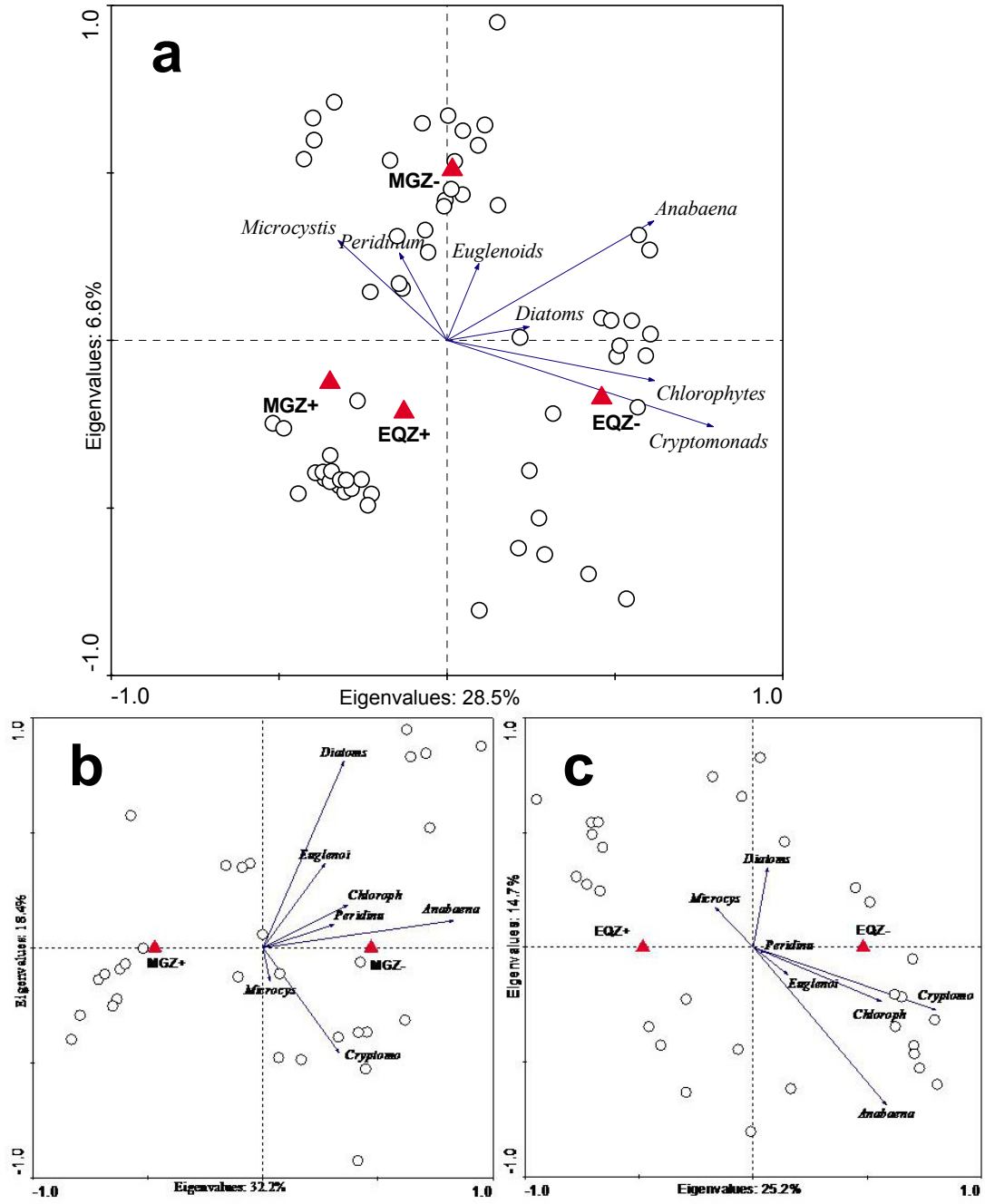

Fig. 6.4. Partial RDA biplot showing the response of phytoplankton taxon composition to the source water and zooplankton treatments, correcting for impact of time (time represented as co-variable). (a) Mai Gassa I and Era Quihila waters together, (b) Mai Gassa I water, (c) Era Quihila water. Arrows represent phytoplankton taxa; upwards pointing triangles are centeroids of the treatments, and empty circles represent samples.

Table 6.1 Results of regression analysis testing for the effect of zooplankton and source water on phytoplankton taxa biomass in the enclosure experiment (April 2007)

Taxon	Trace	F	*p*
Diatoms	0.358	4.452	0.0500
Chlorophytes	0.697	41.698	0.0020
Cryptomonads	0.540	11.787	0.0040
Euglenoids	0.504	7.567	0.0100
Anabaena	0.394	15.894	0.0020
Microcystis	0.214	6.134	0.0020
Peridinium	0.137	2.864	0.0540

Table 6.2 Results of two way ANOVAs testing for the effect of zooplankton, water source and their interaction on log transformed biomasses of different phytoplankton taxa in the enclosure experiment (April 2007). Z = zooplankton treatment, W = water source, Z*W = interaction between both treatments.

Chlorophytes			*Anabaena*	
Source	F	*p*	F	*p*
Z	11.38	0.009	112.43	<.001
W	74.06	<0.001	50.48	<0.001
Z*W	4.25	0.073	13.74	<0.005
Cryptomonads			*Microcystis*	
Z	6.59	0.033	0.01	0.93
W	49.87	<0.001	10.75	0.01
Z*W	0.77	0.41	0.74	0.41
Euglenoids				
Z	1.26	0.29		
W	3.18	0.11		
Z*W	0.54	0.48		

To investigate changes in the dominance of phytoplankton taxon composition during the course of the experiment, we performed partial RDA controlling for differences among treatments (Fig. 6.5). We detected a significant overall temporal variation in the phytoplankton taxa (Trace = 0.124; F = 2.998; $p < 0.002$). Centeroids of days displayed a circular trajectory on the RDA-plot (Fig. 6.5), indicating that the

dominant phytoplankton taxa shifted with time. *Anabaena,* cryptomonads, euglenoids, diatoms and chlorophytes tended to dominate early in the experiment, while *Microcystis* and *Peridinum* dominated later in the experiment.

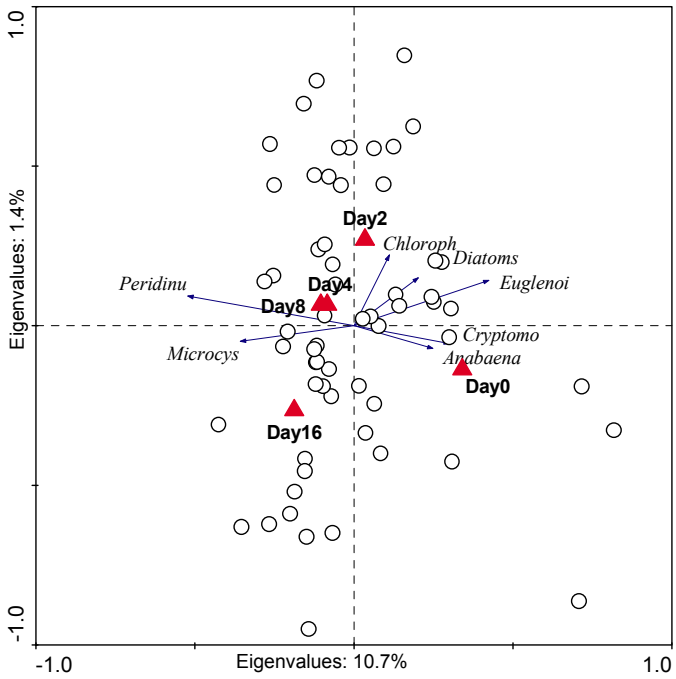

Fig. 6.5 Partial RDA biplot on the response of phytoplankton taxon composition in function of time, correcting for among treatment differences (treatments specified as co-variables). Arrows represent phytoplankton taxa; upwards pointing triangles are centeroids (days), and empty circles are samples (enclosures).

Microcystin was detected in all the enclosures, and concentrations ranged from 0.082 to 1.268 µg L^{-1}. We observed significantly higher microcystin production in the treatments with zooplankton (range: 0.22 - 1.27 µg L^{-1}) than the treatments without zooplankton (range: 0.08 – 0.13 µg L^{-1}) ($F = 7.76$, $p = 0.014$) (Fig. 6.6). The microcystin concentration per unit biomass of *Microcystis* (micricystin, µg/mgC) was also significantly higher in the zooplankton treatments ($f = 7.33$, $p = 0.027$). But no significant effect of water source on microcystin production was observed ($F = 0.876$, $p = 0.377$).

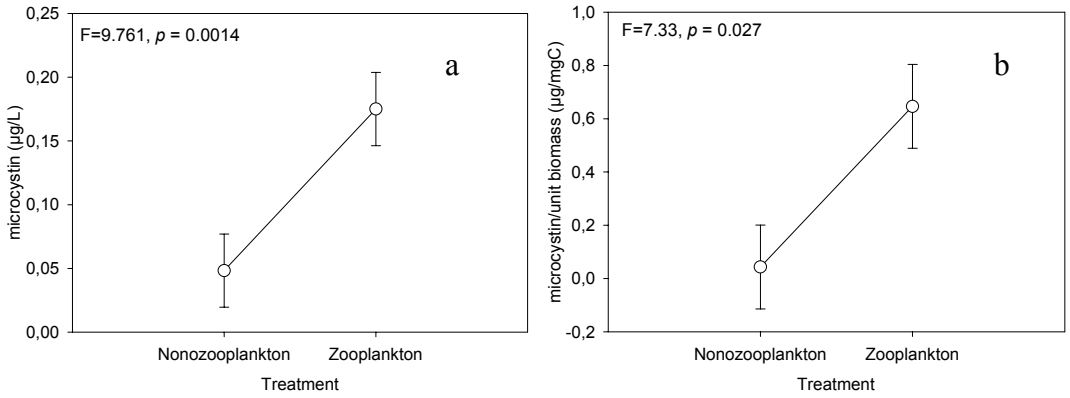

Fig. 6.6 Plots representing associations between microcystin production and zooplankton treatments in the enclosure experiments. a) microcystin concentration (µg/L) and b) microcystin concentration per unit biomass of *Microcystis* (µg/mgC).

Discussion

The main observations from our experimental study on the effect of zooplankton on phytoplankton biomass and taxon composition in a highland reservoir in semi-arid Northern Ethiopia are that (1) zooplankton exerts a top-down control on phytoplankton; (2) but this top-down effect is taxon-specific; (3) the toxic cyanobacterium M*icrocystis* is not significantly affected by zooplankton in our experiment; (4) rather, our results indicate that survival of zooplankton in our experiment was quite low, which might be related to the presence of toxic cyanobacteria; and (5) the concentration of the toxin microcystin was significantly higher in the presence of zooplankton. In the following paragraphs, we discuss these observations.

Our results indicate that the presence of zooplankton has a more important impact on phytoplankton composition than water source. Multivariate analysis shows that the presence/absence of zooplankton results in a stronger differentiation of phytoplankton community structure than whether medium of Mai Gassa I or Era Quihila was used (Fig. 4a). This plot also suggests that the presence of zooplankton

tends to make the taxon composition more similar across waters. In both media, the impact of zooplankton was similar, as is shown in Fig. 4 a & b. Overall, zooplankton tends to suppress the abundance of phytoplankton in our experimental units, but the impact differs strongly depending on the taxon considered. The top-down impact of zooplankton was strong for *Anabaena*, chlorophytes, and cryptomonads, but was not significant for *Microcystis* and *Peridinum.* It is well known that phytoplankton taxa are differentially grazed upon by zooplankton (Burns, 1987, Hartmann, 1985, Bern, 1994), and our results are generally in line with expectations, except for *Anabaena*. No changes in abundance of large cyanobacteria (*Anabaena affinis, A. flos-aquae*) were reported in another short-term enclosure experiments (Porter, 1973, Lehman & Sandgren 1985), while the response of *Anabaena circinalis* in yet another experiment was equivocal (Bergquist & Carpenter, 1986). Geller (1984) reported a high grazing rate of the zooplankton community on *Anabaena flos-aquae*. This difference may probably be a result of difference in both the cyanobacteria and the grazer zooplankton. Fore example, calanoid copepods are reported to be adapted to graze on large cyanobacteria (Haney, 1987). Haney and Traut (1985) also reported the preference of adult calanoid copepods to feed on large food dominated by filamentous cyanobacteria. Large dinoflagellates like *Peridinum* species are known not to be grazed efficiently by filter feeding zooplankton (Sandgren, 1992). Similarly, *Microcystis* is known to be well-protected against zooplankton grazing, by both the formation of colonies as well as by producing toxins (Lampert, 1981, Nizan *et al*., 1986, Fulton & Paerl, 1987b, Kirk & Gilbert, 1992, DeMott, 1999).

We did not observe top-down regulation of *Microcystis* by zooplankton in our experiment, even though the inoculated densities of zooplankton were quite high. We observed a massive decline of most zooplankton species during the first days of the

experiment, including a complete collapse of the *Daphnia*. The only exceptions were the calanoid copepods and the small-bodied cladoceran *Ceriodaphnia*. This likely reflects *Microcystis* impacting zooplankton rather than the reverse. We observed a higher concentration of the toxin microcystin per unit biomass of *Microcystis* in the zooplankton treatments, which suggests that increased toxin production by the *Microcystis* may have resulted in the collapse of zooplankton. The increased levels of toxin may either reflect an induced change or selection favouring more toxic strains. Jang *et al.* (2003) reported that several strains of *Microcystis aeruginosa* increased toxin production in response to direct and indirect exposure to herbivorous zooplankton. Toxin production by different species of cyanobacteria has indeed been considered a defence mechanism against zooplankton grazing (Lampert, 1981, Lampert, 1987, Ghadouani *et al. al,* 2003, Jang *et al.,* 2003). Laboratory experiments showed that *Daphnia* died considerably faster than starving controls when offered pure cultures of toxic strains of *Microcystis* (Lampert, 1981, Nizan *et al.* 1986). Reduction of population growth rate and reproduction abilities of cladoceran zooplankton by *Microcystis strains* has been reported from many freshwater systems in tropical and temperate regions (DeMott, 1999, Ferrao-Filho *et al.,* 2000, 2003). Many studies have, however, also reported that cladoceran zooplankton can develop tolerance to cyanobacterial toxins upon exposure (Fulton and Paerl, 1987a, Gustafsson and Hansson, 2004). *Daphnia magna* populations that were repeatedly exposed to toxic cyanobacteria in their natural habitat were indeed less affected by the toxin than populations lacking exposure (Gustafsson & Hansson, 2004). In addition, it has been shown that *Daphnia* show genetic differences in tolerance to microcystin and Hairston et al. (1999) have reported evolutionary adaptation of a local *Daphnia* population to increased tolerance of *Microcystis*. The *Daphnia* we used in our

experiment were obtained from a reservoir where the genes for microcystin synthesis were not detected (Tsehaye Asmelash, unpubl. data). The animals we used in our experiment were thus not acclimatized to the presence of microcystin, nor were they derived from a population that had been exposed to selection by microcystins. Although not tested and thus speculative, it is possible that our observations would have been different if we would have used *Daphnia* that had been derived from a habitat that is characterized by the regular occurrence of toxic cyanobacteria blooms. In an earlier enclosure experiment, we observed that resident *Daphnia* in two other reservoirs (Tsinkanet and Gereb Awso) were able to reduce dominance of *Microcystis* (Dejenie et al., 2009). Dejenie et al. (2008) also reported a negative association between *Microcystis* and *Daphnia* from a survey of reservoirs in Tigray, North Ethiopia.

As mentioned, there was a major decline in zooplankton biomass during the first days of our experiment. This collapse was pronounced for all cladoceran species except for *Ceriodaphnia*, a species that, however, occurred in low frequencies. In contrast to the cladoceran zooplankton, we recorded higher copepod biomass in the treatments with than without zooplankton. Copepods are selective feeders and have been reported not to be directly affected by toxins of cyanobacteria as long as an alternative food source is available (Kozlowsky-Suzuki et al, 2003). Fulton and Paerl (1988) reported from a laboratory competition experiment that blooms of *M. aeruginosa* can alter zooplankton competitive relations, favouring small-bodied cladocerans and copepods at the expense of large-bodied cladocerans. Small-sized cladocerans generally are characterized by stronger tolerance against *Microcystis* than large-sized species, which may in part be related to the fact that they are too small for

the filaments to interfere with their feeding (Jarvis, 1986, Fulton and Paerl, 1987a, Guo and Xie, 2006).

Overall, the decline of the zooplankton community is remarkable, but our experiment does not allow us to give a definitive conclusion on the causes for the decline because we did not have controls with out *Microcystis.* It could be the *Microcystis,* but could also be something else as well. We suggest repeating similar experiments in the future where zooplankton presence/absence is crossed with *Microcystis* presence/absence.

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Chapter 7

General discussion

The Ethiopian highlands support about 88% of the population of the country (MNRDEP, 1994; Mohamed-Saleem, 1995), more than 70% of the livestock population, and 90% of the economic activities of the country (Constable, 1984; FAO, 1986). The Northern parts of the highlands are semi-arid and are among the most ancient settled areas in the world (Huffnagel, 1961; McCann, 1995). The region suffered from a long history of land degradation and loss of vegetation (El-Swaify and Hurni, 1996) which resulted in episodes of recurrent droughts (Haile, 1988). Currently, there is a trend to collect rain water in reservoirs for irrigation based agriculture, electric power generation, and drinking water production. To this end, numerous small reservoirs have been constructed in the Tigray Regional State, North Ethiopia, in the last two decades (Hurni, 1993; De Wit, 2003; Nigussie *et al.*, 2006; Tsehaye *et al*. 2007). The reservoirs suffer from excessive nutrient loading associated with sediment influx (Nigussie *et al*. 2006) which resulted from massive erosion linked to land degradation (Nyssen *et al.*, 2005). Many of the reservoirs, therefore, suffer from phytoplankton blooms, including cyanobacterial blooms (Dejenie *et al*. 2008).

This study was initiated with the aim of getting a better understanding of the conditions that favor the formation of cyanobacterial blooms in the reservoirs in Tigray, North Ethiopia. We studied spatial and temporal patterns in phytoplankton community composition in a set of reservoirs in the region, with an emphasis on potentially toxic cyanobacteria and the conditions and processes that may lead to bloom formation. In addition, the bacterial community composition and its

relationship with abiotic and biotic components of the ecosystem were investigated. Finally, we also tested the impact of fish and zooplankton on phytoplankton community composition in enclosure experiments.

In the following, we discuss our major results with respect to the different objectives outlined in the introduction. Finally, we formulate some recommendations for the management of the reservoirs.

7.1. Phytoplankton community composition of the reservoirs

While the limnological characteristics of the reservoirs studied in this thesis have been discussed by Dejenie et al. (2008), we here describe the phytoplankton species composition, with an emphasis on the occurrence of bloom-forming cyanobacteria in 32 small reservoirs which were sampled twice, once in the wet season and once in the dry season. Overall, phytoplankton richness was low, with most reservoirs being dominated by a single genus of cyanobacteria (mostly *Microcystis*), chlorophytes, euglenophytes, cryptophytes or dinophytes. We report low phytoplankton richness compared to reports from sub-tropical and tropical lakes and reservoirs (Elizabeth and Amha, 1994, Ariyadej et al., 2004; Nabout et al., 2007). This probably results from the eutrophic nature of the system (Huszar et al., 1998, Leibold, 1999, Dudgeon et al., 2006, Lo et al., 2008). Year round dominance of cyanobacteria (mainly *Microcystis)* is common in eutrophic lakes (Sommer et al., 1986, Tilzer, 1987, Zohary & Robarts, 1989; Reynolds 1998, Sivonen & Jones, 1999; Akin-Oriola, 2003).

The phytoplankton community using the biomass data showed associations only with local environmental variables; despite the importance of regional variables in explaining the spatial configuration of communities (e. eg. Soininen et al., 2007,

Vanormelingen et al., 2009). We observed dynamic phytoplankton community and environmental variables in the reservoirs in Tigray. The phytoplankton community compositions of the two seasons were not correlated to each other. This may result from the variations between the reservoirs and the high turnover of the phytoplankton communities. Different reservoirs respond differently to the environmental changes they face during the dry season (Dejenie et al., 2008). We documented a tendency of shift of dominance from cyanobacteria (mainly *Microcystis)* to dinoflagellates (mainly *Peridinium)* in the dry season. Environmental factors explaining the phytoplankton community composition differ depending on season. Zooplankton community structure, and pH showed significant association with phytoplankton community composition in the wet season, whereas the variations in the phytoplankton community composition was associated with reservoir depth and conductivity in the dry season. We reported significant temporal variation in phytoplankton community assemblages (chapter 3). We were able to draw some pattern of dominance of different phytoplankton taxa corresponding to the prevailing environmental variables. From September to February, the chlorophytes, cryptomonads, and diatoms co-dominate with cyanobacteria. Cyanobacteria (dominated by *Microcystis*) and *Peridinum* (Dinoflagellates) dominate from March to August. But, no apparent seasonal variation was reported for cyanobacteria, dinophytes and euglenoids. We observed higher inter-annual variation (variation among years) in the abiotic variables compared with the intra-annual variation (variation among different months). We also observed temporal turn-over in the phytoplankton and zooplankton community composition. The variability in the abiotic and biotic components of the reservoirs revealed the dynamic nature of the systems and absence of repeatability. The intra-annual variation was mainly associated with

variation in rainfall and temperature. In tropical systems, marked variations in temperature and rainfall between seasons often influence the physico-chemical characteristics of water bodies (Adebisis, 1981, Chapman & Kramer, 1991). Seasonal changes in phytoplankton community structure associated with droughts and water level declines and periods of inflow have been reported from other sub-tropical reservoirs (Horris et al., 1996).

7.2. The bacterial communities of the reservoirs

In chapter 4 we reported bacterial community composition using DGGE band intensity as a measure of relative abundance of operational taxonomic units (OTUs). We estimated bacterial richness by treating each band as an individual OTU and using the number of bands as an indicator of richness. We reported low bacterial richness (range between 2 and 15) compared to reports from other lakes (Konopka et al., 1999, Van der Gucht et al. 2001, 2007). In contrast to other reports (Eiler & Bertilsson, 2004), we also reported a lower bacterial taxon richness in reservoirs accompanying *Microcystis* blooms than bacterial communities in reservoirs without *Microcystis* blooms. Our data can not explain this difference in taxon richness, but we can speculate it is caused by the toxicity of *Microcystis.*

Similar to the response of the phytoplankton communities in the seasonal survey (chapter 2), different environmental factors explained the bacterial community composition in different seasons. Percentage contribution of *Microcystis* to the total phytoplankton biomass and copepod biomass showed significant association with the bacterial community composition in the wet season whereas variation in bacterial community composition was associated with total nitrogen (TN), total phosphorus (TP), oxygen, the number of cattle frequenting the reservoir, and fish biomass in the

dry season. Both top-down and bottom-up factors are reported to regulate bacterial communities (Pace & Cole, 1996; Langenheder & Jürgens, 2001, Muylaert et al., 2002). In most situations bacteria are limited primarily by resources (Pace & Cole, 1996). Predation by zooplankton has also been reported to be an important structuring factor for planktonic bacterial communities (Pace & Cole, 1996, Langenheder & Jürgens, 2001). Seasonality of bacterial community structure in eutrophic lakes has been reported to depend on substrate sources as well as on the food web structure (Muylaert et al., 2002). *Microcystis* may also impact the bacterial community with their secondary metabolites which are reported to have antimicrobial and toxic effects (Carmichael, 1992, Patterson *et al.* 1994, Casamatta & Wickstrom, 2000, Valdor & Aboal, 2007).

7.3. Structuring factors

In addition to the field work, we carried out two different experiments with an aim to 1) directly assess the impact of fish on the abiotic conditions and the different trophic levels in the reservoirs and 2) asses the potential top-down effects of zooplankton on the phytoplankton communities, including toxic cyanobacteria.

In the following, we try to present our observations of the surveys and the two experiments in an effort to explain the relationships of phytoplankton communities in the reservoirs with the abiotic and biotic components of the system. We present our explanations in the framework of bottom-up and top-down control and we also try to elaborate the role of cyanobacteria in the trophic structure.

7.3.1. *Bottom-up control as a major structuring factor*

The regulation of the bacterioplankton by environmental variables has been discussed in section 7.2 of this chapter. Both bottoms-up and top-down factors are important as regulators of the bacterioplankton in our study system. We have also reported the importance of *Microcystis* to structure the bacterioplankton. We report lower richness of the bacterial community in reservoirs with *Microcystis blooms.*

Strong bottom-up regulation of the phytoplankton community was not observed from our survey study based on the phytoplankton biomass estimates. We observed, however, an indirect bottom-up influence of *Garra* on the phytoplankton community by positively influencing the nutrient (chapter 5) concentrations (total phosphorus and total nitrogen) in the water column. Our results are consistent with patterns that were detected during a survey study of 32 reservoirs (Dejenie et al., 2008). In that survey, a positive association between *Garra* biomass and total phosphorus was recorded. The association of fish with bacterial community composition (chapter 4) may also reflect increased nutrient availability in the water column either through direct excretion and recycling of benthic detritus or via sediment re-suspension. Positive influences by fish on nitrogen and phosphorus have been reported from other enclosure experiments and whole lake manipulations (Northcote, 1988; Vanni et al., 1997, Vanni & Layne, 1997, Scheffer *et al.*, 2003, Persson & Sevensson, 2006). Overall our results of the enclosure experiment (chapter 5) indicate that the zooplankton community composition has been affected by *Garra* through indirect bottom-up mechanisms. Abundant zooplankton species, such as *Diaphanosoma and Ceriodaphnia,* were promoted by the presence of *Garra.* Our observations could not, however, clearly demonstrate whether the effect on the larger bodied zooplankton (mainly *Daphnia)* is from direct top-down regulation of

zooplankton by fish or an indirect effect of fish by affecting the phytoplankton community composition. Two alternative explanations are possible 1) *Garra,* with a preference for larger zooplankton, causes the observed shift to the small-sized zooplankton by selective feeding on the large zooplankton or; 2) *Garra* by influencing the nutrient concentration in the water column indirectly influence the phytoplankton community and toxins form cyanobacteria (mainly *Microcystis)* regulate zooplankton. Cyanobacteria are known to produce a wide range of toxins which act as defence against zooplankton (Lampert, 1981, Nizan *et al*., 1986, Fulton & Paerl, 1987, DeMott, 1999), resulting in reduced population growth rate and reproduction of cladoceran zooplankton (Gliwicz & Lampert, 1990, DeMott, 1999, Ferrao-Filho *et al.,* 2000, 2003, Deng, 2008). Furthermore, we observed a shift in zooplankton community composition towards smaller bodied cladocerans and copepods in our second enclosure experiment (chapter 6), with no apparent effect of zooplankton on *Microcystis.* Jang et al. (2003) reported an increase in toxin production by *Microcystis* in response to direct and indirect exposure to herbivorous zooplankton of several species. We could not, however, reach a firm conclusion on the mechanisms of *Garra* – zooplankton (mainly *Daphnia*) – *Microcystis* interactions. Our data could not clearly demonstrate whether the effect on *Daphnia* is from top-down regulation by fish or an indirect effect of fish by affecting the phytoplankton community composition. We could report a tendency for *Daphnia* to regulate *Microcystis* from one of our experiment (chapter 5), but *Daphnia* rapidly declined in the second experiment (chapter 5) which may result from toxic effect of *Microcystis.*

The main purpose of the reservoirs in Tigray is to supply water for irrigation, but they are also used as sources of water for domestic animals and different household purposed. Our report on the presence of toxic strains of *Microcystis*

(detection of the genes responsible for microcystin production and the toxins), therefore, is of public health concern. Cyanobacterial toxins are known to affect animals including humans (Lampert, 1981, Nizan *et al*., 1986, Fulton & Paerl, 1987, Resson et al., 1994, Chorus & Bartram, 1999, DeMott, 1999, Carmichael et al., 2001). Incidents of animal deaths and human illnesses attributed to toxic cyanobacteria are reported (Kuiper-Goodman et al., 1999). The World Health Organization (WHO) has set a provisional guidline value for microcystin-LR in drinking water (0.001 mg L^{-1} for total microcystin-LR, free plus cell-bound) (Chorus & Bartram, 1999, WHO, 2006).

7.3.2. Top-down control as a major structuring factor

In our seasonal survey (chapter 2) we reported association of phytoplankton community with zooplankton (top-down regulators). The chances for top-down regulation of phytoplankton are lower in tropical lakes (Fernando, 1994; Lazzaro, 1997) because *Daphnia* seem to be rare or absent at lower latitudes (Hebert, 1978, Foran, 1986, Dumont, 1994b, Gillooly and Dodson, 2000, Brucet et al., 2005; Gyllstrom et al., 2005). In tropical lakes, fish species reproduce throughout the year, and this can reduce the chances for top–down control of phytoplankton by *Daphnia*. Our study reservoirs mainly harbour the riverine fish, *Garra.* Dejenie et al. (2008) observed a positive association for specific taxa of zooplankton, such as *Daphnia barbata,* small sized cladocera with *Garra*. This, together with our results of the enclosure experiment (chapter 5) indicates that the ability of *Garra* to control zooplankton is limited. This may be due to the fact that *Garra* is a riverine fish with a ventrally oriented mouth that presumably feeds mainly on detritus, benthos or larger invertebrates (Mekonen et al., unpublished).

We were able to see an apparent top-down regulation of phytoplankton by the zooplankton community in the second experiment (chapter 6). We observed top-down regulation by zooplankton on some of the phytoplankton taxa including *Anabaena*, chlorophytes, Cryptomonads, but the impact on *Microcystis* and *Peridinum* was limited. Reports on differential grazing by zooplankton on different sizes of phytoplankton support this observation (Burns, 1987, Hartmann, 1985, Bern, 1994). The difference in response to grazing zooplankton by the two dominant taxa of cyanobacteria in the reservoirs, *Microcysytis* and *Anabaena,* is interesting. *Anabaena* tended to be negatively affected, while *Microcystis* increased in the presence of zooplankton. Contrasting reports on the ability of zooplankton to regulate *Anabaena* exist (Geller, 1984, Haney and Traut, 1985, Lehman & Sandgren 1985, Bergquist & Carpenter, 1986). This probably resulted from differences in the sizes of different *Anabaena* taxa and the grazer zooplankton. Calanoid copepods are adapted to utilize large cyanobacteria (Haney, 1987). We reported a negative correlation of *Anabaena* with calanoid copepods and *D. carinata*. Preferential feeding of adult calanoid copepods on large food dominated by filamentous cyanobacteria has been reported by Haney and Traut (1985).

We reported a negative association of *Microcystis* with *Daphnia* and a tendency for a positive relationship with fish in chapter 2. But from the enclosure experiment (chapter 6) we reported lower ability of zooplankton to regulate *Microcystis;* and an over-all effect of fish on *Microcystis* is reported in chapter 5. We also reported a negative effect of *Microcystis* on the bacterial community richness in the reservoirs. All these results show the importance of *Microcystis* in our system and its relationship with the biotic components is central. These results also indicate an inter-play between bottom-up (possibility of *Microcystis* affecting the zooplankton

composition) and top-down (zooplankton grazing) regulation. We have detected toxins from our experimental units in one of the experiments we carried out and higher levels of toxins were produced in the presence of zooplankton than in the absence. These observations support the hypothesis that *Microcystis* regulate zooplankton. On the other hand, negative association of *Microcystis* with *Daphnia* has been suggested mainly from the field survey. Dejenie et al. (2008) also reported negative association of the percentage contribution of *Microcystis* to the total phytoplankton with *Daphnia.* From our field observations and the experiments, we can conclude that the zooplankton grazing can not regulate *Microcystis* blooms in these reservoirs and that zooplankton can increase the microcystin concentration of *Microcystis* blooms. But, the possibility remains, and is in fact quite realistic, that *Daphnia* may control *Microcystis* at lower biomasses until it reaches a certain biomass and *Microcystis* "escapes" potential control by zooplankton after which it will poison all zooplankton due to the high densities.

7.4. The impact of fish on water quality

In the reservoirs of Tigray, the predominant fish belongs to the genus *Garra,* an opportunistic benthivorous fish. It strongly depends on detritus, but it can also feed on cladocerans in addition to benthic invertebrates (Mekonen et al., in prep.). We reported from our enclosure experiment that *Garra* influences major environmental variables including total nitrogen, total phosphorus, water column transparency and suspended matter. Thus, it favoured increased phytoplankton productivity and increased biomass accumulation mediated by a bottom-up effect, through enhanced nutrient availability in the water column. Previous studies on other taxa of fish have observed that the presence of fish results in an increase in both phytoplankton

biomass and zooplankton densities (Rejas et al., 2005) and positive effects on nutrient (phosphorus and nitrogen) (Persson and Svensson, 2006).

7.5. General suggestions and recommendations

Based on the collected data on the environment gradients and the biota and their relationships in space and time in semi-arid highland reservoirs in Tigray, we can put some general suggestions and recommendations for sustainable utilization while maintaining the ecological integrity of the reservoirs. One major aspect we aim to recommend is the issue of water quality. We have reported the stocking of few of the reservoirs with commercial fish. But fish stocking in the northern part of Ethiopia as part of the farming activity has not been fully explored, although it can contribute to the economic use of the reservoirs and help to alleviate the chronic food insecurity in the region. But before engaging in stocking, some issues like the capacity of the reservoirs to sustain fish production, social aspects of fish consumption in the region and fish marketing, and the impact of fish on water quality need to be understood. Based on the data collected and the major finding we document, hereunder we forward some general suggestions and recommendations for sustainable management of the reservoirs:

1) *Setting up of cyanobacteria monitoring and survey for hazardous effects*: cyanobacterial blooms are already a prominent characteristic of many if not most of the reservoirs in the highlands of Tigray. Given that most reservoirs harbour toxic strains and people use the reservoirs for watering cattle and other household purposes, it is quite logic that potential hazards are in place. Awareness about the hazards of toxic cyanobacteria is minimal in Ethiopia. We, therefore, recommend the following measures to be implemented:

a. Awareness creation to responsible authorities on the potential hazards of cyanobacteria and familiarization of local people with the appearance and emergence of cyanobacterial blooms. In contrast to several other waterborne microbial and toxicant health hazards, cyanobacteria are often readily apparent to the human eye and as such easy to monitor.

b. Survey of animal and human health problems related to cyanobacterial blooms in localities with reservoirs infested with cyanobacteria. Whether the toxic effects of the existing blooms of cyanobacteria (mainly *Microcystis*) have already been translated into human and/or animal health problems in the vicinity of the reservoirs is not known. We believe it is quite important to undertake a survey and document the animal and/or human health.

c. Installing mechanisms of monitoring blooms and emergence of toxic strains. We recommend this to be routinely introduced as *hazard ala*rming system beyond an academic interest. This, we believe, will help to take measures before heavy damage occurs.

2) *Reduce nutrient loading and sediment input*: the reservoirs are eutrophic and our survey indicates that they are impacted with phytoplankton blooms. It is imperative that nutrient loading is reduced. We can only make a very general recommendation as this issue requires a concerted effort of various stake holders, including local communities, the government and non government bodies. Catchment restoration is a global recommendation. Currently the major source of nutrient to the reservoirs is erosion losses and transport of sediment. It is, therefore, important to restore the terrestrial vegetation and reforestation of the valleys in which the reservoirs are situated. The activities of creating exclosures in some areas of the region are encouraging, and such activities along the hills can

allow vegetation re-growth and reduce erosion. In addition, we think it might be important to take measures to allow vegetation to develop in the immediate neighbourhood of the reservoirs, i.e. in a buffer area around the shore.

3) *Reduce the impact of cattle*: quite a large number of cattle visit freely the reservoirs. Cattle trampling and nutrient input by large amounts of livestock is likely contributing to the deteriorated water quality in the reservoirs. Complete prevention of access to livestock seems impractical. Restricting access to certain part of the reservoirs and to drink animals from canals can reduce the impact of trampling enormously. Restricting cattle to certain area of the reservoir may also serve another function: prevent or minimize the risk of intoxication of animals by toxic blooms of cyanobacteria if selection of site for watering animals is assisted by experts. Establishing cattle free zones is crucial to allow littoral vegetation development and for the establishment of reed land that may strongly reduce nutrient input in the reservoirs. The construction of drinking tubs or small ponds that are fed by water of upstream reservoirs would even be more efficient.

   Cattle production in Ethiopia is not efficient, traditionally communities measure wealth by the number of cattle not by the quality of cattle they have. And this leads to higher density of cattle, with as a consequence negative impact on the environment. To recommend overall reduction of the cattle stock seems impractical in the current situation, and it is far beyond the scope of this study. We, however, recommend for policies to guide and pave the way towards a more limited number of cattle.

4) *Reduce the impact of fish*: our results indicate that the presence of fish, mainly *Garra*, has a negative impact on water quality by influencing turbidity, nutrient concentration and thereby abundance of cyanobacteria. Our results suggest that

fish contribute to the likelihood of cyanobacteria blooms. We see three potential ways of reducing the impact of *Garra* on the system, each with their own practical limitations, risks and potential benefits:

a. *Removal of Garra by fishing or other appropriate methods*: this is one way to reduce the impact of *Garra.* Mechanical removal of the small fish may be practically very cumbersome and inefficient, but it would be a safe way to try to reduce cyanobacteria blooms. Advocating activities can be coupled with the removal to use the fish as animal (chicken) feed.

b. *Introduction of predatory fish*: This might be a very efficient way to reduce densities of *Garra* to reasonable levels, but it is associated with some risks. Introducing predatory fish always entails a risk for damage either locally or in connected systems. Introduction of predatory fish should be considered as the last alternative to avoid the associated risks. It should be noted here that if introduction of predatory fish is considered, we strongly recommend indigenous fish to be used in order to avoid the unpredictable impact of exotic species on local biodiversity.

c. *Fish culture in the reservoirs*: we know that quite a number of the reservoirs can support fish production. Introduction of commercial fish (thereby replacing *Garra*) like the well adapted *Tilapia* can bring an added value to the reservoirs. With respect to water quality, it requires an investigation to determine the density of *Tilapia* the reservoirs can support without a compromise to water quality. Some research results show that the presence of filter feeder fish may help in reducing cyanobacteria blooms. Stocking *Tilapia* has been recommended by Yirgaw et al (2000) from southern Ethiopia for management of a turbid lake. *Tilapia* can feed by filtering cyanobacteria. High

survival under low dissolved oxygen and high suspended matter is reported for this fish; thus it is a good candidate for stocking and evaluation of the trophic state of our systems.

Each option forwarded for the reduction of *Garra* has its own pros and cons. Thus, it is of paramount importance that whole lake manipulations with biological means of control need to be well thought and studied. Before widespread application of an option, we recommend thorough investigation and evaluation on selected study reservoirs. Finally we recommend for accumulation of knowledge and expertise on fisheries and fish to consciously implement corrective measures on the potential hazards of cyanobacterial blooms.

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Summary

Ethiopia is the third largest country in Africa with an area of over one million km^2. It is one of the most populous countries in Africa (probably second), with more than 80 million inhabitants. The Ethiopian highlands represent about 43% of the country but support about 88% of the population. The highlands account for 95% of the regularly cropped land, more that 70% of the livestock population, and 90% of the economic activities of the country. They are considered to be amongst the most degraded lands in Africa by some authors. Rain fed agriculture is the main stay for most farmers. The frequent rainfall anomalies suggest that there are recurrent periods of drought every 3-5 years in the northern parts of Ethiopia and every 6-8 years over the whole country. Part of the variability in the seasonal and annual rainfall across time and space is known to be associated with the El Nino-Southern Oscillation (ENSO) phenomenon. The conditions in Tigray are worse. Extreme spatial and temporal variation in rainfall is characteristic for this region. To tackle the problem associated with the rain fall pattern, several small reservoirs have been constructed over the last two decades. Given the population intensity and long history of agriculture in the highlands of Ethiopia, massive erosion linked to land degradation is a prominent problem. This is expected to bring excessive nutrient to the reservoirs. And many of the reservoirs are expected to be characterized by high nutrient loads and phytoplankton blooms, including cyanobacteria blooms. This has indeed observed in a field survey of reservoirs, most of them suffer from heavy blooms of cyanobacteria.

In this study we started with a field survey of a set of 32 shallow semi-arid sub-tropical reservoirs in the highlands of Tigray, Ethiopia. This survey was carried out in both the wet and dry season to capture seasonal variations of phytoplankton communities and associated environmental variables. We assessed seasonal variation

in more details by monitoring eight selected reservoirs (sub sets of the 32 reservoirs) on a monthly basis during a whole year. We also carried out field enclosure experiments in an effort to better understand the trophic structure of the reservoirs and identify mechanisms that potentially lead to cyanobacterial blooms. First we tested the impact of fish on abiotic conditions in the water column as well as the dynamics of phytoplankton species composition and cyanobacteria biomass. In the second experiment we assessed the potential top-down effects of zooplankton on the phytoplankton communities including the toxic cyanobacteria.

The studied reservoirs were characterized by high nutrient concentrations and high turbidity. Most of the reservoirs harbor the riverine fish *Garra*. Overall, the local phytoplankton richness was low with most reservoirs dominated by a single genus of cyanobacteria (mostly *Microcystis*), chlorophytes, euglenophytes, cryptophytes or dinophytes. Similarly the bacterial community richness in the studied reservoirs was also low. Lower bacterial taxon richness was encountered in reservoirs with *Microcystis* blooms than bacterial communities in reservoirs without blooms. High altitude reservoirs were more nutrient-rich and associated with high abundances of green algae, euglenophytes or cyanobacteria other than *Microcystis*. *Microcystis* was associated with high pH in the rainy and high conductivity in the dry season. Additional factors correlated with *Microcystis* biomass were *Daphnia* biomass and possibly altitude and fish biomass. Environmental factors explained the bacterial community composition differently among season. Percentage contribution of *Microcystis* to the total phytoplankton biomass and copepod biomass showed significant association with the bacterial community composition in the wet season whereas variation in bacterial community composition was associated with total nitrogen (TN), total phosphorus (TP), oxygen, the number of cattle frequenting the

reservoir, and fish biomass in the dry season.

Pronounced temporal variation was observed for both biotic and abiotic variables in our study systems. This variation involved both the intra-annual and inter-annual variations. For the intra-annual variation, the main limnological changes were associated with seasonal differences in rainfall, while also water temperature differed strongly between winter (sub-tropics) and the rest of the year. We observe two minima for phytoplankton biomass: one in winter and a more pronounced one during August. We also observed two main bloom periods for cyanobacteria: one in September-October and a more pronounced one in May-June. Seasonal variation in total phytoplankton and cyanobacterial biomass was, however, not significant.

The first field enclosure experiment was the experiment with fish. The results of this experiment showed that the presence of *Garra* in general increased the amount of suspended matter, nutrient concentrations (total nitrogen and total phosphorus), phytoplankton and to some extent also *Microcystis* biomass (including the proportion of *Microcystis* in the phytoplankton community*)*, and reduced water transparency. The second experiment was carried out to study the effect of zooplankton grazing on phytoplankton community structure, including the relative abundance of toxic cyanobacteria. From this experiment top-down regulation by zooplankton was observed for some of the phytoplankton taxa, including *Anabaena*, Euglenoids, Chlorophytes and Cryptomonads, whereas the impact of the presence of zooplankton on *Microcystis* and *Peridinum* biomass was limited. From the same experiment we observed negative correlation between *Anabaena* and calanoid copepods and *Daphnia carinata.* We also detected microcystin from all experimental units in the second experiment; and higher concentrations were detected in the treatment with than without zooplankton.

We draw some important associations from the field observations and field enclosure experiments for *Microcystis* in our system. The results indicate an inter-play between bottom-up (possibility of *Microcystis* affecting the zooplankton composition) and top-down (zooplankton grazing) regulating the *Microcystis*. Negative association between *Microcystis* and *Daphnia* has been observed mainly from the field survey and to some extent the enclosure experiments with fish and fishless treatment demonstrated a top-down regulation. From these results we can conclude that the zooplankton grazing can not fully regulate *Microcystis.* But, the possibility remains, and is in fact quite realistic, that *Daphnia* also control *Microcystis,* mainly at lower biomasses of *Microcystis* until it reaches a certain biomass and *Microcystis* "escapes" potential control by zooplankton after which it may poison the major grazer zooplankton due to the high densities.

Based on our results of the present study, we put some general suggestions and recommendations for sustainable utilization, maintaining the ecological integrity of the reservoirs and protecting water quality deterioration. The recommendations follow in the following statements. 1) Cyanobacterial monitoring and survey for hazardous effects to assess if the toxins of the organisms are translated into problems of animal or human health should be set-up. 2) Reduction of nutrient loading and sediment input to the reservoirs to curb the eutrophication of the reservoirs. Catchment treatment with reforestation and setting up of exclosures can serve the purpose. 3) Reduction of cattle trampling by restricting cattle access to the reservoirs at selected sites of the reservoir. 4) Reduction of fish (mainly *Garra)*. Here we recommend the use of methods to reduce the riverine fish with a high level care to protect the reservoirs from unpredictable consequences like the introduction of exotic fish.

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