



Dynamics of phytoplankton and physicochemical features of the Ethiopian
soda lakes Chitu and Shala, and evaluation of the potential of their waters
for the production of *Arthrospira (Spirulina) fusiformis* (Cyanophyceae) in
laboratory cultures

Tadesse Ogato

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

Department of Zoological Sciences

Addis Ababa University

Addis Ababa, Ethiopia

June 2015

ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

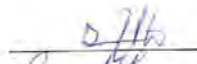
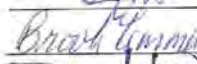
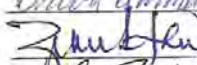
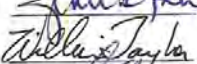
Dynamics of phytoplankton and physiochemical features of the Ethiopian soda lakes Chitu and Shala, and evaluation of the potential of their waters for the production of *Arthrospira (Spirulina) fusiformis* (Cyanophyceae) in laboratory cultures

By

Tadesse Ogato Gecho

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

Approved by Examining Board:

1. Dr. Demeke Kifle	(Advisor)		16	6	15
2. Prof. Brook Lemma	(Advisor)		16	06	2015
3. Dr. Zenebe Tadesse	(Examiner)		16	06	15
4. Prof. William David Taylor	(Examiner)		16	6	'15
5. Dr. Abebe Getahun	(Chairman)		16	06	2015

ABSTRACT

DYNAMICS OF PHYTOPLANKTON AND PHYSICOCHEMICAL FEATURES OF THE ETHIOPIAN SODA LAKES CHITU AND SHALA, AND EVALUATION OF THE POTENTIAL OF THEIR WATERS FOR THE PRODUCTION OF *ARTHROSPIRA* (*SPIRULINA*) *FUSIFORMIS* (CYANOPHYCEAE) IN LABORATORY CULTURES

Tadesse Ogato,

Addis Ababa University, 2015

Soda lakes provide great opportunity for the study and understanding of aquatic ecosystems, owing to their unique biotic assemblage of great ecological and economic values and high sensitivity to environmental changes. In addition, *Arthrospira* (*Spirulina*), an alga of high ecological values to soda lakes ecosystems and produced commercially using high-cost chemical medium as a nutritional supplement to humans, grows naturally in some of the soda lakes. In this study, physicochemical features and phytoplankton community of the soda lakes Chitu and Shala, morphology of *Arthrospira fusiformis* in its natural habitat and its production using waters of the soda lakes were investigated.

Measured parameters of underwater light climate of both lakes depicted light-limited conditions, which were associated with the accumulation of *Arthrospira* in Lake Chitu and non-biological turbidity in Lake Shala. The water column of Lake Chitu exhibited superficial thermal stratification with weak vertical mixing and considerable variability of dissolved oxygen (DO). Lake Shala showed superficial thermal stratification, but with turbulently mixing and well-oxygenated water column. Inorganic nitrogen sources in the euphotic zone of both lakes were often very low or undetectable while phosphorus sources were considerably high, possibly due to their phosphatic mineral-rich rock basins. NH_3 and soluble reactive phosphate (SRP) showed dramatic increase with depth in Lake Chitu due to internal loading. SiO_2 was remarkably low in both lakes, which was probably associated with biological uptake, some chemical processes within the lakes and organic matter accumulation.

The phytoplankton community of Lake Chitu, which was constituted by a few (15) species belonging to the algal classes Bacillariophyceae (60%), Cyanophyceae (26.7%) and Cryptophyceae (2.3%), was dominated by *A. fusiformis* throughout the annual cycle, accounting for up to 98% of total phytoplankton abundance. The observed high mean phytoplankton biomass (chlorophyll-*a*, $150 \mu\text{g chl-}a \text{ L}^{-1}$ and dry weight, 0.26 g L^{-1}) in Lake Chitu showed significant seasonality ($P < 0.05$) with higher levels occurring during the rainy season. The algal biomass and abundance were correlated strongly and positively with rainfall and negatively with alkalinity-salinity, probably suggesting that hydrological control of the salinity of the lake is the major driving force for algal dynamics in Lake Chitu. Phytoplankton community of Lake Shala, which was of relatively higher diversity (23 spp. belonging to Bacillariophyceae, Cryptophyceae,

Cyanophyceae and Dinophyceae), was persistently dominated by Bacillariophyceae and Cryptophyceae, respectively, accounting for about 57% and 22% of the total number of species, and 28% and 69% of total phytoplankton abundance. *Cryptomonas* spp. were the most abundant, contributing about 59 to 95% of total phytoplankton abundance of Lake Shala, while *Thalassiosira* sp. was the second most abundant (1–35%). Although seasonality was not large, the observed variations in the abundance of the dominant species in this lake were associated with water transparency, SiO₂, rainfall and its effects on salinity and pH. Mean algal biomass, which was generally low (17 µg chl-*a* L⁻¹), also showed small seasonal variations in Lake Shala, with strong but inverse relationship with water transparency. In general, the notable variations in phytoplankton and physicochemical parameters in Lake Chitu, probably suggest its sensitiveness to environmental perturbations and that any environmental changes including increased salinization may greatly affect the key alga (*A. fusiformis*) and the flamingos, with eventual impairment of its ecosystem services. On the other hand, the observed small variations in the physicochemical and phytoplankton variables in Lake Shala seem to suggest the lake's resilience to the current level of environmental perturbations, owing to its presumably high stress absorbing capacity associated with its large size and great depth.

Three distinct morphotypes of *A. fusiformis*, tightly coiled (H-type), spiral or loosely coiled (S-type) and intermediately coiled (C-type) were observed in Lake Chitu, with the H-type (50%) and S-type (40%) being the first and second most dominant morphotypes. The dominance of H-type and tightening of its helix pitch were strongly associated with NO₃⁻ and HCO₃⁻ deficiencies, and high levels of photosynthetically active radiation (PAR) and temperature of the lake, while the abundance of S-type was more strongly but negatively correlated with the high salinity of the lake. The variability in morphology seems to suggest that *A. fusiformis* undergoes morphological changes in response to environmental stresses in its natural habitat, resulting in the occurrence of various morphotypes of the same species.

Comparable growth rate (µ) and biomass (B) of *A. fusiformis* were observed in both Lake Chitu water-based media (CBM) and Lake Shala water-based media (SBM), with slightly higher values in the latter. Both CBM and SBM supplemented with the standard *Spirulina* medium (SM) (25% and 50%) supported considerably higher µ and B, which were probably associated with the reduction in pH and salinity, and provision of the limiting nutrient nitrogen in the supplemented media. The higher µ and B in SBM, probably due to some of their aggregate chemical parameters that were closer to those in the SM, and abundant SRP, seems to suggest that Lake Shala water is more conducive to *Arthrospira*. We contend that 25% and 50%-supplemented Lake Shala water can be preferably used to produce *Arthrospira*, thereby reducing the cost of nutrients by 75% and 50%, respectively.

ACKNOWLEDGEMENTS

God Almighty, I praise You! You are the only one who accomplishes everything alone and I know that nothing would have been possible without You.

I would like to thank Dr. Demeke Kifle, my advisor, for his unreserved and consistent mentorship throughout my study. His kindly assistance and encouragement helped me overcome all the academic and material challenges, bringing this study to success. I truly appreciate his easy personal quality, which gave me freedom to interact and discuss all matters without reservation. I am also grateful to Prof. Brook Lemma, who was also my advisor, for his valuable advices and comments during our personal discussion and at the thematic workshop, which contributed a lot to this study to attain its intended goals.

I also thank Department of Zoological Sciences of Addis Ababa University for the prompt facilitation and responses to my requests. My sincere thanks to Dr. Abebe Getahun (Chairman of the Department of Zoological Sciences), Dr. Seyoum Mengistou, Dr. Demeke Admasu and Dr. Tadesse Fetahi for their support and advice during course work and throughout the thesis work. They were ready and kind to provide any support that I requested for. I am also grateful to my colleagues, Dr. Girum Tamire, Dr. Getachew Beneberu, Abebe Tadesse, Akewak Geremew, Moges Beletie, Yeshimebet Major, Mulugeta Wakjira and Yezbie Kassa for their unreserved support and encouragement during the course of my study. I also thank Mathewos-field assistant and Getachew Taye-driver who assisted me in field works during data collection, and Kassahun Tessema and Neway Andarge for their support in the laboratory. My special thanks go to Dr. Lothar Krienitz for helping in phytoplankton identification and Dr. Avigad Vonshak for providing articles on *Spirulina*.

All my family, friends and church-fellows deserve special thanks for their support, encouragement and prayers, which offered me great strength to remain on the right track throughout my study.

This study was funded by Graduate Studies Program and Thematic Research Project (Ziway-Shala Basin Sub-thematic Research Group) of Addis Ababa University, which deserve special thanks. I acknowledge Debre Berhan University for giving me a study leave to pursue my study. I thank Ethiopian Wildlife Conservation Authority (EWCA) and Abijata-Shala Lakes National Park Administration for granting permission to conduct research in Lakes Chitu and Shala. I am also grateful to National Meteorology Agency of Ethiopia (NMA) for providing meteorological data of the study area.

TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGEMENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xii
SYMBOLS AND ABBREVIATIONS.....	xvi
CHAPTER 1 . GENERAL INTRODUCTION.....	1
1.1. BACKGROUND.....	1
1.2. RESEARCH OBJECTIVES	5
1.3. RESEARCH QUESTIONS	6
1.4. DESCRIPTION OF THE STUDY AREA	6
1.5. ORGANIZATION OF THE DISSERTATION.....	13
CHAPTER 2 . TEMPORAL AND VERTICAL VARIATIONS IN PHYSICOCHEMICAL PARAMETERS OF THE SODA LAKES CHITU AND SHALA, ETHIOPIA	16
2.1. INTRODUCTION.....	16
2.2. MATERIALS AND METHODS.....	17
2.2.1. Sampling protocol, <i>in situ</i> measurement and analysis of environmental parameters.....	17
2.2.2. Statistical analysis.....	19
2.3. RESULTS	19
2.3.1. Underwater light climate.....	19
2.3.2. Thermal regime: Water temperature and dissolved oxygen.....	22
2.3.3. Chemical characteristics.....	25
2.4. DISCUSSION	35
2.4.1. Underwater light climate.....	35

2.4.2. Thermal regime: Water temperature and dissolved oxygen.....	37
2.4.3. Aggregate chemical features (pH, alkalinity and conductivity)	41
2.4.4. Inorganic nutrients	45
CHAPTER 3 . PHYTOPLANKTON COMMUNITY COMPOSITION AND BIOMASS IN THE SODA LAKES CHITU AND SHALA, ETHIOPIA: TEMPORAL DYNAMICS IN RELATION TO ENVIRONMENTAL DRIVERS	
3.1. INTRODUCTION.....	51
3.2. MATERIALS AND METHODS	52
3.2.1. Sampling, <i>in situ</i> measurements and analysis of environmental parameters	52
3.2.2. Identification of phytoplankton and determination of their abundance and biomass	53
3.2.3. Estimation of zooplankton abundance	54
3.2.4. Statistical analysis.....	55
3.3. RESULTS	55
3.3.1. Phytoplankton composition and abundance	55
3.3.2. Phytoplankton biomass	60
3.3.3. Relation of abundance and biomass of phytoplankton to environmental variables	63
3.3.4. Redundancy analysis for species-environment relations in Lake Shala	67
3.4. DISCUSSION	69
3.4.1. Phytoplankton composition, abundance and biomass	69
3.4.2. Relations of abundance and biomass of phytoplankton to environmental variables	75
3.4.3. Redundancy analysis for species-environment relation in Lake Shala.....	79
CHAPTER 4 . MORPHOLOGICAL VARIABILITY OF <i>ARTHROSPIRA FUSIFORMIS</i> IN RELATION TO ENVIRONMENTAL VARIABLES IN THE TROPICAL SODA LAKE CHITU, ETHIOPIA	
	82

4.1. INTRODUCTION.....	82
4.2. MATERIALS AND METHODS.....	84
4.2.1. Description of the study area, sampling, measurement and analysis of physicochemical parameters	84
4.2.2. Determination of abundance and morphological parameters of <i>Arthrospira</i>	84
4.2.3. Statistical analyses	84
4.3. RESULTS	85
4.3.1. Morphological description of <i>Arthrospira</i>	85
4.3.2. Relationship between abundance and morphology of <i>Arthrospira</i> and environmental variables.....	89
4.4. DISCUSSION	93
4.4.1. Morphological descriptions	93
4.4.2. Relationships between abundance and morphology of <i>Arthrospira</i> and environmental variables.....	97
CHAPTER 5 . EVALUATION OF GROWTH AND BIOMASS PRODUCTION OF <i>ARTHROSPIRA FUSIFORMIS</i> IN LABORATORY CULTURES USING WATERS FROM THE ETHIOPIAN SODA LAKES CHITU AND SHALA.....	
5.1. INTRODUCTION.....	102
5.2. MATERIALS AND METHODS.....	104
5.2.1. Measurement of some physicochemical parameters of the soda lakes	104
5.2.2. Isolation of <i>Arthospira</i> and scaling-up of its seed cultures.....	105
5.2.3. Preparation of growth media for experimental cultures.....	106
5.2.4. Experimental design and growth conditions	107
5.2.5. Analytical methods	108
5.2.6. Statistical analysis.....	109
5.3. RESULTS	109

5.3.1. Some chemical features of the study lakes.....	109
5.3.2. Salinity and pH of the experimental culture.....	111
5.3.3. Growth and biomass production.....	113
5.4. DISCUSSION	116
5.4.1. Some chemical features of the study lakes.....	116
5.4.2. Salinity and pH of the experimental culture.....	118
5.4.3. Growth and biomass production.....	120
CHAPTER 6 . GENERAL CONCLUSIONS AND RECOMMENDATIONS	124
6.1. CONCLUSIONS.....	124
6.2. RECOMMENDATIONS.....	127
REFERENCES.....	131
APPENDICES.....	152

LIST OF TABLES

Table 1.1. Geographical and limnological features of Lake Chitu and Lake Shala (data source: Elizabeth Kebede <i>et al.</i> , 1994).....	9
Table 3.1. List of phytoplankton taxa identified in samples collected from Lakes Chitu and Shala during the study period.....	57
Table 3.2. Pearson correlation coefficients for abundance of <i>A. fusiformis</i> and phytoplankton biomass and some environmental variables in Lake Chitu.....	65
Table 3.3. Pearson correlations for biomass and total abundance of phytoplankton and some environmental variables in Lake Shala..	66
Table 3.4. Results of RDA of species-environmental variables relationships and correlations of environmental variables with the first two axes in Lake Shala.....	68
Table 4.1. Description of morphotypes of <i>Arthrospira fusiformis</i> on the basis of their morphological parameters. Ranges and means (in parentheses) of recorded values are given.	88
Table 4.2. Some environmental parameters of Lake Chitu measured in this study.....	90
Table 4.3. Summary of RDA results of morphotypes and morphology of <i>Arthrospira</i> , and selected environmental variables for the first two axes.	92
Table 5.1. Types and composition of growth media used for the experimental laboratory cultures of <i>Arthrospira fusiformis</i>	107
Table 5.2. Some chemical features of lakes Chitu and Shala waters and SM, which were used for experimental cultures.....	111
Table 5.3. The growth parameters of <i>Arthrospira fusiformis</i> cultured in various media for 18 days.....	115

LIST OF FIGURES

Fig. 1.1. Location map of the study lakes with sampling sites.	7
Fig. 1.2. Mean minimum and maximum air temperature (line plots) and total monthly rainfall (bar plot) of the study area during 2012 (a) and rainfall coefficient calculated using the rainfall data recorded over the years 2000 to 2012 (b).....	12
Fig. 2.1. Temporal variations among months (line plot) and between seasons (bar plot, mean $\pm$ SD, n = 6 or 5) of parameters of the underwater light climate in Lake Chitu.....	21
Fig. 2.2. Monthly (line plot) and seasonal (bar plot, mean $\pm$ SD, n = 6 or 5) variations in parameters of underwater light climate in Lake Shala.....	22
Fig. 2.3. Isopleths of depth profiles of temperature (upper) and dissolved oxygen (lower) in Lake Chitu during the study period.....	24
Fig. 2.4. Isopleths of depth profiles of temperature (upper) and dissolved oxygen (lower) in Lake Shala during the study period.....	25
Fig. 2.5. Temporal variations in pH, alkalinity and conductivity among months (line plots) and between seasons (bar plots, mean $\pm$ SD, n = 6 or 5) at the surface of Lake Chitu.	27
Fig. 2.6. Depth profiles of pH during the rainy (a) and dry (b) seasons and of conductivity during the dry season alone (c) in Lake Chitu (from February 2012–January 2013).	28
Fig. 2.7. Temporal variations in pH, alkalinity and conductivity among months (line plot) and between seasons (bar plot, mean $\pm$ SD, n = 6 or 5) in Lake Shala.	28
Fig. 2.8. Depth profiles of pH (a, rainy season and b, dry season) and conductivity (c) in Lake Shala.	29

Fig. 2.9. Temporal variations in NO_3^- , NH_3 , SRP, TP and SiO_2 among months (line plot) and between seasons (bar plot, mean $\pm$ SD, n = 6 or 5) in the trophogenic zone of Lake Chitu.	31
Fig. 2.10. Depth profiles of the inorganic nutrients NO_3^- , NH_3 , SRP and SiO_2 in Lake Chitu.	32
Fig. 2.11. Temporal variations in NO_3^- , NH_3 , SRP, TP, $\text{CO}_3^{2-}:\text{HCO}_3^-$ and SiO_2 among months (line plots) and between seasons (bar plots, mean $\pm$ SD, n = 6 or 5) in Lake Shala.	34
Fig. 2.12. Depth profiles of NO_3^- , NH_3 , SRP and SiO_2 in Lake Shala during the study period.....	35
Fig. 3.1. Percentage contribution to the total number of phytoplankton species (a) and relative abundance of algal classes (b), and seasonal variations in the percentage abundance of the dominant taxa (c) in Lake Chitu.	58
Fig. 3.2. Percentage contribution to the total number of phytoplankton species (a) and relative abundance (b) of phytoplankton taxa in Lake Shala.	58
Fig. 3.3. Temporal variations in the percentage contributions of the major classes to total phytoplankton abundance (a) and abundance (cells ml^{-1}) of dominant species (b) of the phytoplankton community in Lake Shala.	60
Fig. 3.4. Temporal variations in biomass (as chl- <i>a</i> and dry weight) and abundance of <i>A. fusiformis</i> among months (line plot) and between seasons (bar plot) in Lake Chitu.....	62
Fig. 3.5. Temporal variations in biomass (as chl- <i>a</i>) and abundance of phytoplankton among months (line plot) and between seasons (bar plot) in Lake Shala.	63

Fig. 3.6. Temporal variations in the three algal parameters in relation to the strongly correlated environmental variables in Lake Chitu.	65
Fig. 3.7. Temporal variations in phytoplankton biomass and abundance in relation to parameters of underwater light climate in Lake Shala.	66
Fig. 3.8. RDA ordination biplot of species-environment variables for the first two axes, axis 1(horizontal) and axis 2 (vertical), in Lake Shala.	69
Fig. 4.1. Plates of the morphotypes of <i>Arthrospira fusiformis</i> in Lake Chitu. a–c H-type, d–f S-type, and g–i C-type. <i>Scale bar</i> 50µm (at 200×) except a (100µm at 100×).	86
Fig. 4.2. Comparison of mean values (with standard deviation, $n = 11$) of abundance and morphological parameters of the three morphotypes of <i>Arthrospira fusiformis</i> in Lake Chitu. a helix pitch, b coils per trichome, c trichome length, d helix diameter, e trichome diameter, and f abundance.	89
Fig. 4.3. RDA ordination diagram of abundance of morphotypes and environmental variables for the first two axes. Axis 1 (<i>horizontal</i>) accounted for 77%, while Axis 2 (<i>vertical</i>) accounted for 8% of the variance in morphotypes.	92
Fig. 4.4. RDA ordination diagram of morphological parameters and environmental variables for the first two axes. Axis 1 (<i>horizontal</i>) accounting for 39% and Axis 2 (<i>vertical</i>) accounting for 22% of the variance in morphological parameters.	93
Fig. 5.1. Isolates of <i>Arthrospira fusiformis</i> from Lake Chitu used for experimental culture. <i>Scale bar</i> , 20 µm.	106
Fig. 5.2. Changes in pH (a and b) and salinity (c and d) with time during the cultivation of <i>Arthrospira fusiformis</i> . <i>a</i> and <i>c</i> for Lake Chitu water-based media, <i>b</i> and <i>d</i> for Lake Shala water-based media.	113

Fig. 5.3. Growth curves of <i>Arthrospira fusiformis</i> cultivated in various media at 35°C and photon flux density of 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the cultivation period. <i>a</i> for Lake Chitu water-based media, <i>b</i> for Lake Shala water-based media, the control (SM) in both <i>a</i> and <i>b</i>	114
Fig. 5.4. Growth rate (μ) and index of biomass (<i>B</i>) of <i>Arthrospira fusiformis</i> in relation to pH and salinity of the various media used for laboratory cultures.	116

SYMBOLS AND ABBREVIATIONS

B	=	biomass production
CBM	=	Lake Chitu water-based media
chl- a	=	chlorophyll- a
CM	=	Chitu medium
25CM	=	25% supplemented Chitu medium
50CM	=	50% supplemented Chitu medium
50CM ⁻	=	50% supplemented (with SM ⁻) Chitu medium
C-type	=	intermediately coiled
DO	=	dissolved oxygen
dt	=	doubling time
H-type	=	highly coiled
K_{algae}	=	light attenuation due to algae
K_{PAR}	=	vertical attenuation coefficient of PAR
μ	=	specific growth rate
PAR	=	photosynthetically active radiation
RDA	=	redundancy analysis
SBM	=	Lake Shala water-based media
SHM	=	Shala medium
25SHM	=	25% supplemented Shala medium
50SHM	=	50% supplemented Shala medium
50SHM ⁻	=	50% supplemented (with SM ⁻) Shala medium
SM	=	<i>Spirulina</i> medium
SM ⁻	=	SM lacking carbonate–bicarbonate components—Na ₂ CO ₃ , NaHCO ₃ and NaCl
SRP	=	soluble reactive phosphate
S-type	=	spiral or loosely coiled
TP	=	total phosphorus
Z_{eu}	=	euphotic depth
Z_{SD}	=	Secchi disk depth

CHAPTER 1 . GENERAL INTRODUCTION

1.1. BACKGROUND

Soda lakes are among the most productive water bodies in the world and are considered valuable natural resources with great ecological, economic and scientific values (Williams, 2002). They are suitable for the study of vital ecosystem processes owing to the unique composition and nature of their biota, their extreme environmental conditions and their being closed hydrological systems. These lakes are best known for their extreme environmental conditions associated with high pH, carbonate-bicarbonate alkalinity and salinity. Among the soda lakes, those in East Africa, for example, are highly productive due to the superabundant populations of *Arthrospira*, which support huge populations of flamingos (Talling and Lemoalle, 1998; Oduor and Schagerl, 2007a; Krienitz and Kotut, 2010), the characteristic birds of these soda lakes and one of the most fascinating wildlife scenes of the world (Mari and Collar, 2000). Although soda lakes are highly sensitive to changes in environmental conditions resulting from natural or human-induced disturbances, which have become a major threat to water resources around the world (Williams, 2002), there have been limited scientific studies and hence published information on the basic ecological processes and patterns of their temporal fluctuations (Talling and Lemoalle, 1998; Williams, 2002).

The few studies made on some African soda lakes have shown the variability of environmental factors with consequent changes in biological community structure (Wood and Talling, 1988; Oduor and Schagerl, 2007b; Krienitz and Kotut, 2010; Talling, 2010). Primarily, fluctuations in salinity and alkalinity, mainly due to seasonal and inter-annual

variations in precipitation and evaporative events, have caused shifts in phytoplankton assemblages and the consequent unpredictability of biomass of *Arthrospira*, the key phytoplankton of some soda lakes of East Africa (Melack, 1988; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Schagerl and Oduor, 2008; Kaggwa *et al.*, 2013a). Seasonal changes in such environmental variables as nutrients, stratification and mixing patterns, and underwater light climate have also been shown to affect phytoplankton composition and biomass in tropical African lakes (Talling and Lemoalle, 1998; Zinabu Gebre-Mariam, 2002; Oduor and Schagerl, 2007a). The functioning of soda lake ecosystems is generally linked to the seasonal alteration of hydrological and hydrographic events and the resulting physical, chemical and biological processes taking place within the lake and in the entire watershed. Natural processes of ecosystem change can be greatly influenced by human-induced disturbances on the watershed and inside the lakes. Diversions of surface inflows and salinization, for example, have been shown to exacerbate the natural changes with further degradation of values related to soda lakes (Williams, 2002).

Soda lakes are well represented in Ethiopia and are exemplified by Lakes Chitu and Shala, which are located in the Ethiopian Rift Valley, and are of high ecological and economic value. These lakes provide a preferred feeding and breeding ground for huge flocks of birds, mainly the flamingos and pelicans, which have made the lakes one of the well-known tourist attractions of the country. Lake Chitu also supports huge populations of *Arthrospira*, the main diet of the flamingos and the alga of high nutritional values for humans. However, these lakes are among the Ethiopian rift lakes, which are currently facing ecological degradation (Zinabu Gebre-Mariam, 2002; Tenalem Ayenew and

Dagnachew Legesse, 2007) though the lakes region is demarcated as a national park (Abijata-Shala Lakes National Park). Environmental degradation has already become prominent in the neighboring Lake Abijata (Tenalem Ayenew and Dagnachew Legesse, 2007). Given the ever increasing impacts of human interferences and the dynamic nature of physicochemical and biological variables of lakes, availability of up to date limnological data recorded over an extended period is deemed essential for sustainable management and utilization of the lakes.

Arthrospira (formerly and commercially known as *Spirulina*) is an edible photosynthetic cyanobacterium with a long recorded history of use. It has currently received world wide acceptance due to its high nutritional and pharmaceutical values (Amha Belay and Ota, 1993; Amha Belay *et al.*, 1996; Chen *et al.*, 1996). *Arthrospira* is used as a source of protein (50–70% protein by dry weight, surpassing all known standard plant proteins), rare unsaturated fatty acids (gamma-linolenic acid), vitamins (e.g. β -carotene and B₁₂), minerals (e.g. iron) and essential amino acids and other useful components. It has also therapeutic effects against various health problems (Amha Belay and Ota, 1993; Fox, 1996; Amha Belay, 2002). Moreover, the biomass of *Arthrospira* is the main diet for large populations of the Lesser Flamingos (*Phoeniconaias minor* Geoffroy) inhabiting soda lakes of East Africa (Harper *et al.*, 2003; Krienitz *et al.*, 2013). The seasonal ecological studies on *Arthrospira* in the natural ecosystem can provide useful information on the sustainability of the flamingos it supports and an insight into the feasibility of *Arthrospira* biomass production. Morphological aspects of *Arthrospira* are likewise very important as they affect behavior of grazers and metabolic rates of flamingos (Raven and Kubler, 2002), constitute taxonomic criteria used in the classification of *Arthrospira*

species (Lewin, 1980; Desikachary and Bai, 1996; Vonshak and Tomaselli, 2000) and are useful in the selection of high-quality strains (Amha Belay, 1997; Wu *et al.*, 2005) and efficient methods of harvesting (Kim *et al.*, 2007). Although data on the seasonal dynamics of biomass, trichome density and morphological characteristics of *Arthrospira* are of paramount importance, they are non-existent for the Ethiopian soda lakes.

Because of its invaluable benefits, mass production of *Arthrospira* has gained worldwide attention. Provision of appropriate physical and chemical growth conditions, however, is the main challenge of large-scale cultivation of *Arthrospira*. The cost of synthetic nutrient medium, for example, accounts for 15–25% of the total production cost of *Arthrospira* biomass (Amha Belay, 1997; Vonshak, 1997; Habib *et al.*, 2008), which makes the use of the standard synthetic medium unaffordable. Much effort has been made to come up with alternative methods geared towards lowering cost of production through biotechnological research and innovation (Vonshak, 1997). Promising results have been obtained using enriched seawater medium for the cultivation of *Arthrospira* (Materasi *et al.*, 1984; Tredici *et al.*, 1986). However, the potential of water from soda lakes for the cultivation of *Arthrospira* has not been evaluated so far. Lakes Chitu and Shala may be good candidates as potential sources of low-cost growth medium as they are rich in most chemical requirements of *Arthrospira*. Lake Chitu is a small cup-shaped crater renowned for its natural monoculture of *Arthrospira*. Lake Shala is a large and deep soda lake whose gross water chemistry is closely similar to that of the adjacent Lake Chitu (Wood and Talling, 1988; Elizabeth Kebede, 1996) though natural populations of *Arthrospira* have not been reported for reasons not known to date. An in-depth experimental testing

of these lake waters is, therefore, necessary to evaluate their potential for *Arthrospira* cultivation.

The present study, therefore, aimed at investigating the community structure of phytoplankton and physicochemical features of the soda lakes Chitu and Shala and evaluating the potential of water of the soda lakes for the production of *Arthrospira fusiformis* (Voronichin) Komárek et Lund in laboratory cultures.

1.2. RESEARCH OBJECTIVES

General objective

The purpose of this study was to investigate the community structure (species composition, abundance and biomass) of phytoplankton and physicochemical characteristics of the soda lakes Chitu and Shala and evaluate the production of *A. fusiformis* in laboratory cultures using growth media formed from the water of the soda lakes.

Specific objectives

- To study the temporal and spatial (vertical) variation in the physicochemical features of Lakes Chitu and Shala
- To investigate the temporal variations in community structure (species composition, cell density and biomass) of phytoplankton in relation to environmental factors in Lakes Chitu and Shala
- To characterize morphological variation of *Arthrospira* in response to environmental conditions in Lake Chitu

- To evaluate the potential of water from soda lakes (with or without supplementation) for the growth and biomass production of *A. fusiformis* in laboratory cultures, with the ultimate purpose of formulating a suitable low-cost growth medium.

1.3. RESEARCH QUESTIONS

- Do physicochemical variables in Lakes Chitu and Shala show temporal and spatial (vertical) variations?
- Do the phytoplankton communities of Lakes Chitu and Shala exhibit qualitative and quantitative changes over an annual cycle in response to environmental factors?
- Does *Arthrospira* exhibit morphological variability in response to environmental conditions in the natural ecosystem of Lake Chitu?
- Do the soda lake waters from Chitu and Shala (with or without supplementation) support good growth and biomass production of *A. fusiformis* in laboratory cultures?

1.4. DESCRIPTION OF THE STUDY AREA

Morphometric and physicochemical features

Lakes Chitu and Shala (Fig. 1.1), the present study lakes, are tropical soda lakes located within the Abijata-Shala Lakes National Park some 287 km south of Addis Ababa in the main Ethiopian Rift Valley. They are volcano-tectonic lakes (Le Turdu *et al.*, 1999) found in the hydrologically closed system of the Ziway-Shala basin that includes Lakes Ziway, Langano, Abijata and Shala. The lakes lie at the lowest terminal position, at an

altitude of 1558–1600 m above sea level, without any connection with other lakes within the basin. Although the two lakes share the salient properties of soda lakes, they differ greatly with regard to their morphometric and biological features. Lake Chitu, situated at about 1 km southwest of Lake Shala, is a small (0.8 km^2) relatively shallow (maximum depth 21 m) cup-shaped crater lake surrounded by a crater rim. On the other hand, Lake Shala is a very deep (max. 266 m) and large (329 km^2) caldera lake with vast catchment area (3920 km^2) and entirely exposed to wind action. Some geographical and limnological features of Lakes Chitu and Shala are given in Table 1.1.

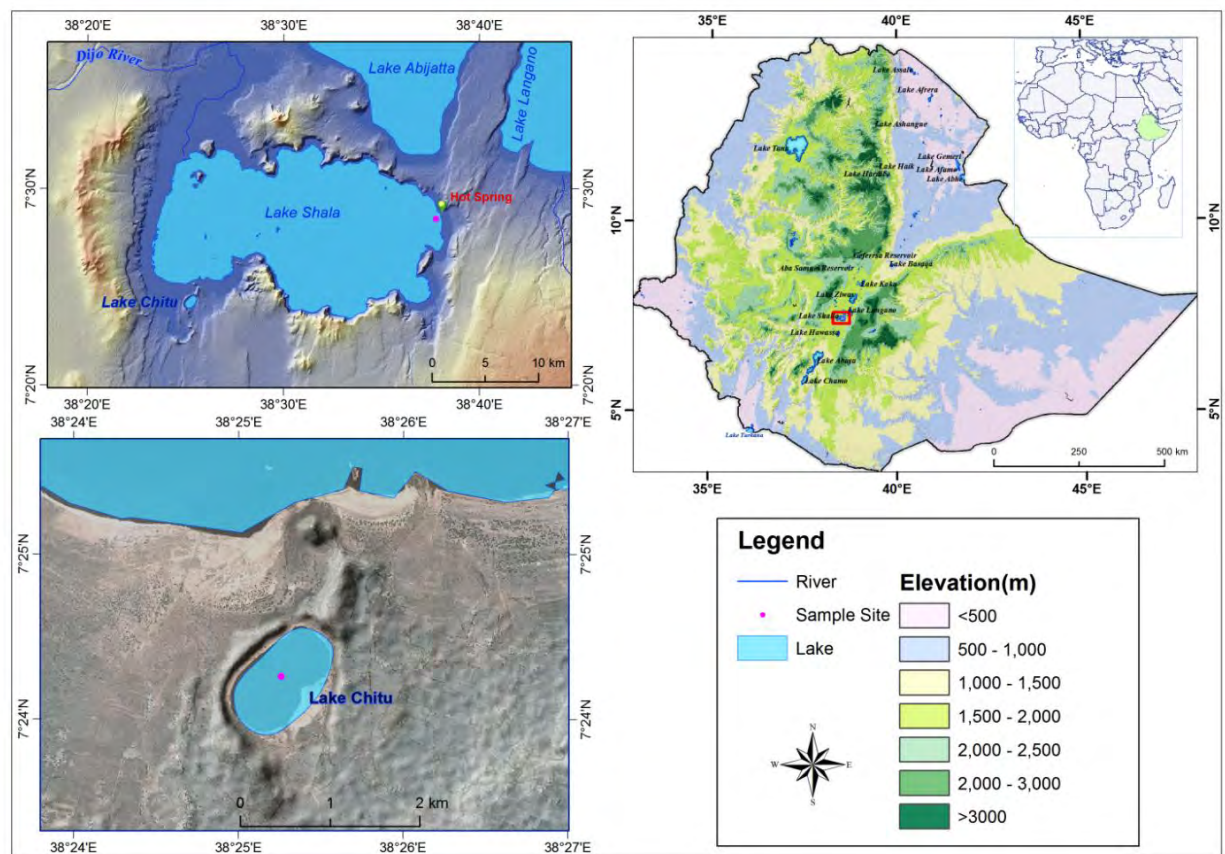


Fig. 1.1. Location map of the study lakes with sampling sites.

Lakes Chitu and Shala are fed mainly by direct precipitation and several hot springs but lack obvious surface outflow. Lake Shala also receives water mainly from two perennial

rivers (Dijo and Adabar) originating from the western and southeastern rift escarpments (Le Turdu *et al.*, 1999) while Lake Chitu does not have any surface influent. The surface water temperatures of Lakes Chitu and Shala range from 21 to 24 °C and from 22 to 26 °C, respectively. Many hot springs, varying in temperature, size and discharge rate, are found on the shores of both lakes and are used by a number of local people and visitors for sanitation and watering livestock. The largest and hottest spring (up to 90 °C) of all springs is the one located on the northeastern shore of Lake Shala (Fig. 1.1). Local inhabitants also collect dried soda ash (trona), which is sold to be used as cattle feed, from the shores of Lake Chitu.

The lakes are typical examples of African soda lakes characterized by high pH, salinity, $\text{HCO}_3^- + \text{CO}_3^{2-}$ alkalinity, Na^+ , Cl^- and phosphate, and with frequently limiting level of inorganic nitrogen compounds and low level of divalent cations (Ca^{2+} and Mg^{2+}). These lakes are among the soda lakes in which the concentrations of the major cations are in the order $\text{Na} > \text{K} > \text{Ca} > \text{Mg}$, which is in contrast to that in most temperate lakes, $\text{Ca} > \text{Mg} > \text{Na} > \text{K}$ (Tudorancea *et al.*, 1999). The high level of salinity and alkalinity of the lakes is related to the presence in large concentrations of the major cations and anions in the trachytic and rhyolitic rocks that formed the Ethiopian rift (Klemper and Cash, 2007) and the high evaporative concentration of ions in the lake waters (Wood and Talling, 1988; Tenalem Ayenew and Dagnachew Legesse, 2007).

Table 1.1. Geographical and limnological features of Lake Chitu and Lake Shala (data source: Elizabeth Kebede *et al.*, 1994).

Features	Lake Chitu	Lake Shala
Geographic position	7°24' N 38°25' E	7°28' N 38°30' E
Altitude (m)	1600	1558
Maximum depth (m)	21	266 (mean 87)
Surface area (km ²)	0.8	329
Catchment area (km ²)	-	3920
pH	10.15	9.65
Conductivity (K ₂₅ , µS cm ⁻¹)	49100	21940
Salinity (g L ⁻¹)	44.9	18.1
Na ⁺ (meq L ⁻¹)	864	272
K ⁺ (meq L ⁻¹)	31.20	4.56
Ca ²⁺ (meq L ⁻¹)	0.16	0.16
Mg ²⁺ (meq L ⁻¹)	0.05	0.07
HCO ₃ ⁻ + CO ₃ ²⁻ (meq L ⁻¹)	573	218
Cl ⁻ (meq L ⁻¹)	99	54.4
SO ₄ ²⁻ (meq L ⁻¹)	21.1	16.3
SRP (µg L ⁻¹)	1985	809
NO ₃ ⁻ +NO ₂ (µg L ⁻¹)	ND	ND
NH ₄ ⁺ (µg L ⁻¹)	ND	4.3
SiO ₂ (mg L ⁻¹)	222	56
Chlorophyll- <i>a</i> (chl- <i>a</i> , µg L ⁻¹)	224	15.8

ND, not detectable

Biological features (fauna and flora)

Lake Chitu is highly productive owing to the high photosynthetic biomass of *Arthrospira* (Table 1.1). Lake Shala, on the other hand, is known for its low phytoplankton productivity (Table 1.1) and is devoid of *Arthrospira* despite its saline-alkaline water that *Arthrospira* prefers to grow in. The algal community in Lake Shala was reported to be dominated by diatoms (Elizabeth Kebede *et al.*, 1994). Both lakes lack aquatic

macrophytes along their shorelines. The zooplankton community of the lakes comprises mainly the rotifers *Brachionus* and *Hexarthra*, with copepods (cylopoids and harpacticoids) also constituting the zooplankton assemblage in Lake Shala. These lakes and the park as a whole are known for their rich avifauna, providing home to over 400 species of resident and seasonal migratory birds, which accounts for about half of the total number of species recorded for the whole country (OEPO, 2005). The birds are the main reasons for the establishment of the park and are serving as its major tourist attractions (Stephenson, 1987). Lake Chitu is the home of huge flocks of flamingos, particularly Lesser Flamingos. Lake Shala supports a richly diverse avifauna, including pelicans and greater and lesser flamingos inhabiting the lake and its islands (Stephenson, 1987). There are several (about 8) volcanic islands in Lake Shala, some of which serve as the main breeding sites for pelicans (Stephenson, 1987). The pelican breeding islands in Lake Shala, “Pelican Islands”, are among the most important pelican breeding sites in Africa. There is no fishing activity at all in Lakes Chitu and Shala and only *Oreochromis niloticus* and a small-sized fish, *Aplocheilichthys* sp., were reported from the latter lake (Golubtsov *et al.*, 2002).

The vegetation type of the study area is open Acacia woodland, with sparse acacia trees. The catchment of the lakes area, which is highly overgrazed by livestock up to the lakes’ shores, is covered by pseudo-lateritic soils, the residual products of rock decay (EWNHS, 2009). Mixed agriculture-crop production and animal husbandry is the main means of livelihood for the local people.

Meteorological conditions

The lakes region has semi-arid to sub-humid type of climate and is characterized by high rate of evaporation that exceeds the mean annual rainfall, causing water deficit (Tenalem Ayenew and Dagnachew Legesse, 2007). Trends of some meteorological features of the study area are shown in Fig. 1. 2. Mean of the total annual precipitation of the study area recorded over the years 2000 to 2012 is 955 mm while means of the monthly minimum and maximum air temperature are about 8 and 25 °C, respectively. During 2012 (Fig. 1.2a), rainfall varied considerably with seasons, with high level of monthly rainfall, ranging from 133 to 200 mm during the period extending from May to September, while temperature exhibited small monthly variation, with minimum and maximum, respectively, ranging from 6 to 13 and 22 to 28 °C. An increase in air temperature, ranging from 25 to 28 °C, occurred in November to April (Fig. 1.2a). To distinguish between rainy and dry months, rainfall coefficient (the ratio of monthly rainfall to one-twelfth of the annual rainfall) was calculated according to Daniel Gamachu (1977) using the rainfall data of the 12 years. Months with rainfall coefficient of ≥ 0.6 were assigned to the rainy season, while those with < 0.6 were assigned to the dry season. Accordingly, the region is characterized by two seasons: rainy (March to September) and dry (October to February) seasons (Fig. 1.2b). The rainy season is characterized by two successive periods: minor rainy period extending from March to June and major rainy period spanning from July to September. This seasonal pattern generally characterizes the Ethiopian Rift Valley region (Daniel Gamachu, 1977; Tenalem Ayenew and Dagnachew Legesse, 2007).

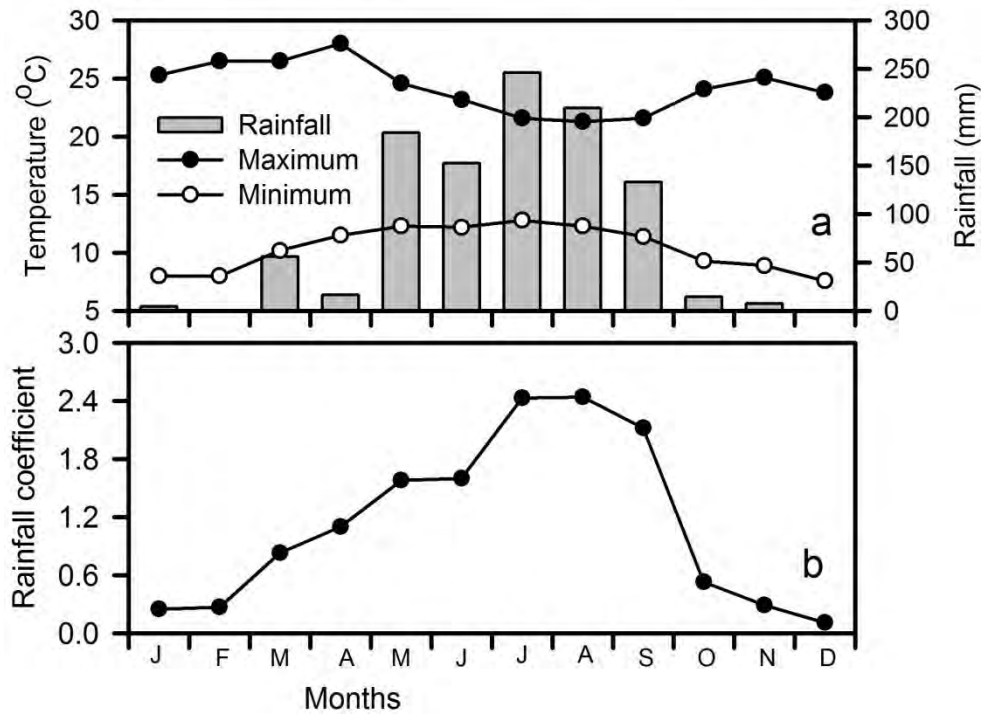


Fig. 1.2. Mean minimum and maximum air temperature (line plots) and total monthly rainfall (bar plot) of the study area during 2012 (a) and rainfall coefficient calculated using the rainfall data recorded over the years 2000 to 2012 (b). (Source: National Meteorological Agency of Ethiopia).

1.5. ORGANIZATION OF THE DISSERTATION

This dissertation is a compilation of results that emanated from studies made on physicochemical features and phytoplankton in soda lakes Chitu and Shala, morphological characterization of *A. fusiformis* in Lake Chitu and production of *A. fusiformis* in laboratory cultures using waters of soda lakes, which are presented in six chapters including the general introduction.

Chapter 1. General introduction

This chapter provides general introductory information, reveals the research gaps, lists the research questions and objectives, and describes the study area.

Chapter 2. Temporal and vertical variations in physicochemical characteristics of the soda lakes Chitu and Shala, Ethiopia

This chapter deals with physicochemical characteristics of the soda lakes Chitu and Shala, with particular focus on the underwater light climate, thermal and aggregate chemical features, and inorganic algal nutrients. Temporal and spatial (vertical) variations of the physicochemical parameters measured and their possible causes and implications for the lakes' ecosystem conditions are discussed. The following article has emanated from the data for Lake Chitu:

Underwater light climate, thermal and chemical characteristics of the tropical soda lake Chitu, Ethiopia: Spatio-temporal variations. *Limnologica* **52**:1–10.

Chapter 3. Phytoplankton community composition and biomass in the soda lakes Chitu and Shala, Ethiopia: temporal dynamics in relation to environmental drivers

This chapter discusses phytoplankton dynamics in the soda lakes Chitu and Shala, with emphasis on temporal variations in species composition, abundance and biomass of

phytoplankton in relation to the prevailing environmental conditions. It also describes the major phytoplankton species of key ecological importance and the environmental variables regulating their temporal dynamics. Data of this chapter constituted the following two manuscripts:

- Algal composition and biomass in the tropical soda lake Chitu with focus on seasonal variability of *Arthrospira fusiformis* (Cyanophyta). *Marine and Freshwater Research*. DOI: 10.1071/MF14426.
- Phytoplankton composition and biomass in the tropical soda lake Shala: seasonal changes in response to environmental drivers. Submitted to *Lakes and Reservoirs: Research and Management* (submission ID, LRE-14-061).

Chapter 4. Morphological variability of *Arthrospira fusiformis* in relation to environmental variables in the tropical soda lake Chitu, Ethiopia

It deals with morphological variability of *A. fusiformis* in Lake Chitu and describes the features of the morphotypes identified and relates their temporal dynamics to environmental variables in the natural ecosystem. The results presented in this chapter were published in the following article:

Morphological variability of *Arthrospira (Spirulina) fusiformis* (Cyanophyta) in relation to environmental variables in the tropical soda lake Chitu, Ethiopia. *Hydrobiologia* (2014): 738:21-33.

Chapter 5. Evaluation of growth and biomass production of *Arthrospira fusiformis* in laboratory cultures using waters from the Ethiopian soda lakes Chitu and Shala

This chapter reports the results of the laboratory investigation that evaluated the potential of soda lake waters of Chitu and Shala for large-scale production of *A. fusiformis*. This chapter presents data on growth rate and biomass production of the alga in standard chemical medium (*Spirulina* medium), and media formulated from the soda lake waters

by supplementing with *Spirulina* medium in various proportions. The physicochemical features of the lake waters were also determined with a view to identify factors of overriding importance in controlling its biomass production in soda lake waters-based media. The following published article emanated from the results described in this chapter:

Evaluation of growth and biomass production of *Arthrospira (Spirulina) fusiformis* in laboratory cultures using waters from the Ethiopian soda lakes Chitu and Shala. *Journal of Applied Phycology* (2014) 26: 2273–2282.

The last chapter outlines the conclusions drawn from the major findings of the study and recommends relevant further studies and workable management options for the study lakes, which are undergoing environmental degradation.

CHAPTER 2 . TEMPORAL AND VERTICAL VARIATIONS IN PHYSICOCHEMICAL PARAMETERS OF THE SODA LAKES CHITU AND SHALA, ETHIOPIA

2.1. INTRODUCTION

Variations in some environmental conditions have been shown to regulate biological community structure in African soda lakes (Wood and Talling, 1988; Schagerl and Oduor, 2008; Krienitz and Kotut, 2010; Talling, 2010). Fluctuation of salinity in soda lakes of East Africa, for example, has been demonstrated to cause changes in biological community, and large variability and crashes of biomass of the key soda lake phytoplankton *Arthrospira* (Wood and Talling, 1988; Schagerl and Oduor, 2008; Okoth *et al.*, 2009; Kaggwa *et al.*, 2013a). Increase in salinity is known to cause loss of biological diversity thereby leading to the dominance of organisms with high capacity to withstand osmotic stress (Hammer, 1986; Williams, 1998; Jones and Grant, 1999). Apart from salinity, seasonal changes in such environmental variables as nutrients, stratification and mixing patterns, and underwater light climate are also known to affect phytoplankton composition and biomass in tropical African lakes (Talling and Lemoalle, 1998; Zinabu Gebre-Mariam, 2002; Oduor and Schagerl, 2007a, b). As a result of their sensitivity to ecological disturbances, soda lakes are among the ecological systems for which continuous scientific investigations on their limnological variables should be given high priority.

Among the Ethiopian soda lakes, Lakes Chitu and Shala are known for their rich avifauna including the Lesser Flamingos and Great White Pelicans that made the lakes to be among the well-known tourist attractions of the country. However, these lakes are part of

the Ethiopian Rift Valley lakes, which are currently facing increased ecological degradation (Zinabu Gebre-Mariam, 2002; Tenalem Ayenew and Dagnachew Legesse, 2007). Human-induced disturbances along with the natural ecological changes may cause instability of their ecosystems, ultimately hampering the ecosystem services they render. Previous studies on these lakes investigated water chemistry and water column stability (Talling *et al.*, 1973; Baumann *et al.*, 1975; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Elizabeth Kebede, 1996; Zinabu Gebre-Mariam, 2002). These studies were not, however, systematic and involved only short-term observations. Given the increased human impacts and the dynamic nature of the physicochemical variables, availability of up to date limnological data recorded on long-term basis is necessary for sustainable management and utilization of the lakes. This study, therefore, aimed at investigating the temporal and vertical variation in physicochemical characteristics of the soda lakes Chitu and Shala using measurements made over a year.

2.2. MATERIALS AND METHODS

2.2.1. Sampling protocol, *in situ* measurement and analysis of environmental parameters

Detailed descriptions of the study area are given in chapter 1. Considering the closed nature of the lakes and the aim of our study (temporal dynamics), a single open water sampling station was selected in each lake and was marked with a colored buoy to be used for all sampling occasions. The sampling station in Lake Chitu was around the middle of the lake at a depth of 13 m, global position of 07°24'15.6" N 038°25'15.6" E at altitude of 1557 m, while that in Lake Shala was at a depth of 30 m, global position of 07°28'28.8" N 038°37'47.3" E at altitude of 1559 m. Water sample collection and *in situ*

measurements were done at these stations on eleven occasions over an annual cycle from February 2012 to January 2013. Composite samples were produced from samples taken from selected depths distributed within the euphotic zone (euphotic depths 0.4–1.0 m and 1–4 m for Lakes Chitu and Shala, respectively) using a bottle sampler (van Dorn, Horizontal model, 2 L capacity). The composite samples were used for the analysis of inorganic nutrients and alkalinities of the lakes. In addition, depth profiles of nutrients were determined using samples collected from 0, 1, 5, 10 and 13 m and 0, 5, 10, 20 and 30 m depths of Lakes Chitu and Shala, respectively.

Water transparency (Z_{SD}) was measured using a standard black and white Secchi disc (20 cm diameter). Photosynthetically active radiation (PAR) incident on the lake's surface and at different depths of the water column (0.25, 0.50, 0.75 and 1.0 m depths) was measured with LI-COR photometer (model SKP 200, Skye Instruments), and mean vertical attenuation coefficient (K_{PAR}) of down-welling irradiance was calculated according to Kirk (1994). Light extinction due to algae (K_{algae} , %) was calculated from K_{PAR} and chl-*a* using the empirically determined chl-*a* extinction coefficient of 0.016 (Dubinsky and Berman, 1976). Euphotic depth (Z_{eu}) was calculated from K_{PAR} as $Z_{eu} = 4.6/K_{PAR}$ (Kalff, 2002). Depth profiles of temperature and dissolved oxygen (DO) were determined using a DO-meter with an oxygen-temperature probe (model YSI-5000, YSI Incorporated). pH and conductivity were measured using digital pH meter (model HI 9024, Hanna Instruments) and conductivity meter (model CC-505, Elmetron), respectively. The conductivity measured *in situ* was adjusted to conductivity at 25°C using a temperature coefficient of 2.3% per °C (Talling and Talling, 1965). Alkalinity of unaltered samples was determined by titration with 1N HCl to pH 4.5 using a mixed

indicator (bromocresol green-methyl red) within a few hours of sample collection. Carbonate-bicarbonate alkalinities and concentrations of the constituent ions were calculated from total alkalinity and pH according to APHA *et al.* (1999). Using samples filtered through Whatman filter paper (GF/F), major algal nutrients NO_3^- , NH_3 , soluble reactive phosphate (SRP), and dissolved silica were determined by the sodium salicylate (APHA *et al.*, 1995), phenate, ascorbic acid and molybdosilicate methods, respectively (APHA *et al.*, 1999). Total phosphorus (TP), after persulfate digestion of unfiltered samples, was determined by the ascorbic acid method. The effect of high alkalinity of the lakes on the color reactions was not considered in nutrient analysis. Meteorological data (air temperature and rainfall) of the study area were obtained from the National Meteorological Agency of Ethiopia and rainfall coefficients were calculated to distinguish seasons (see chapter 1).

2.2.2. Statistical analysis

Independent sample t-test was used to analyze the seasonal variations in physicochemical parameters. Pearson correlation was used to determine the relationship of some parameters: dissolved oxygen and pH changes with chl-*a* and abundance of phytoplankton. All statistical analyses were done using the statistical program IBM SPSS (Version 20).

2.3. RESULTS

2.3.1. Underwater light climate

The temporal variations in Z_{SD} , Z_{eu} , K_{PAR} and K_{algae} of Lake Chitu are shown in Fig. 2.1. The Z_{SD} and Z_{eu} ranged from 0.21 to 0.43 m and 0.4 to 0.9 m, while K_{PAR} and K_{algae} varied from 5.4 to 11.3 m^{-1} and 20 to 47%, respectively. The mean values of Z_{SD}

(0.31 ± 0.06 m) and Z_{eu} (0.62 ± 0.14 m) were small, and those of K_{PAR} (7.8 ± 1.8 m⁻¹) and K_{algae} (31.5 ± 9.9 %) were large, showing low water transparency of the lake. Minimum Z_{SD} and Z_{eu} and maximum K_{PAR} and K_{algae} occurred during the months of peak rainfall, May to August. However, the differences between rainy and dry seasons were not statistically significant for all parameters except K_{algae} , which significantly ($t(9) = 2.3$, $P < 0.05$) increased during the rainy season.

As evident from the values of Z_{SD} , K_{PAR} and K_{algae} , underwater light climate in Lake Shala showed some temporal variations (Fig. 2.2). The Z_{SD} ranged from 0.52 to 1.80 (1.19 ± 0.40 m). Z_{eu} followed a seasonal trend similar to that of Z_{SD} and indicated slightly deeper euphotic zone, ranging from 1.0 to 4 (1.8 ± 0.9 m, mean $\pm$ SD). The K_{PAR} and K_{algae} varied from 1.1 to 4.5 (3.0 ± 1 m⁻¹) and 2.8 to 15.2 (8.6 ± 4.1 %, mean $\pm$ SD), respectively. The minimum values of Z_{SD} and Z_{eu} , and the maximum values of K_{PAR} were recorded in the rainy season, peaking in the months June, July and August. Increased values of K_{algae} were also observed in April during the minor rainy season and in January during the dry season though its mean value was low.

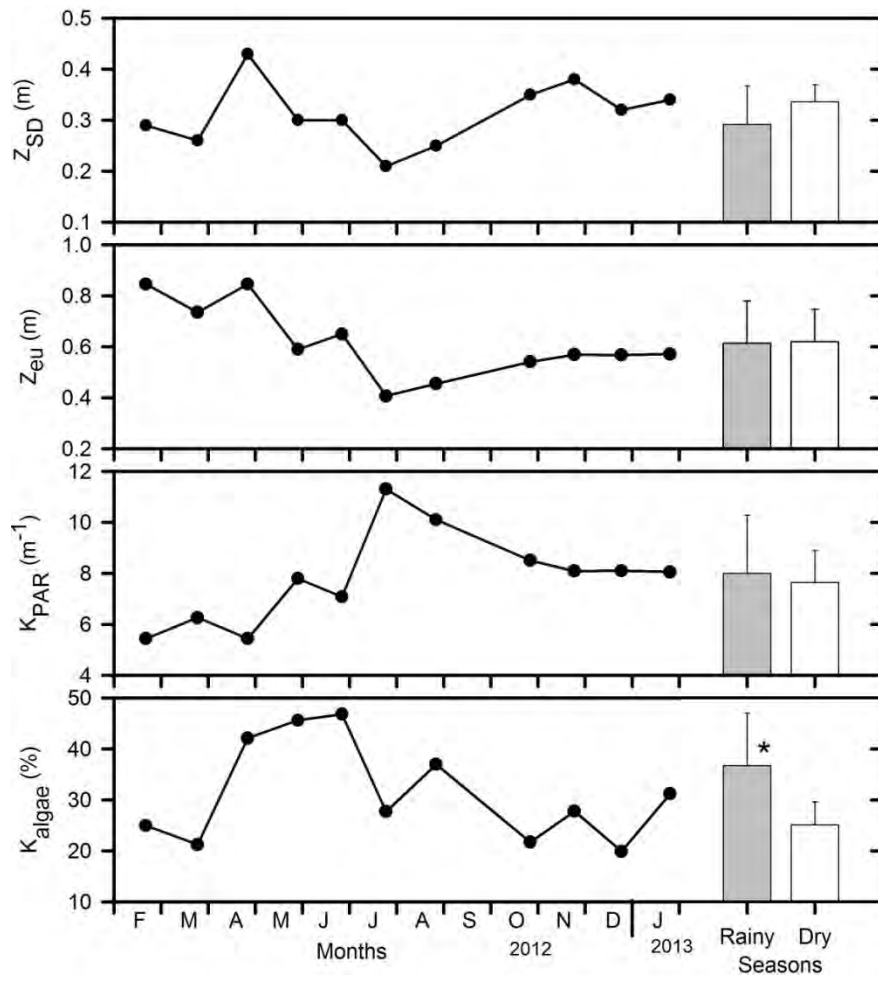


Fig. 2.1. Temporal variations among months (line plot) and between seasons (bar plot, mean $\pm$ SD, $n = 6$ or 5) of parameters of the underwater light climate in Lake Chitu.

*Significant at $p < 0.05$.

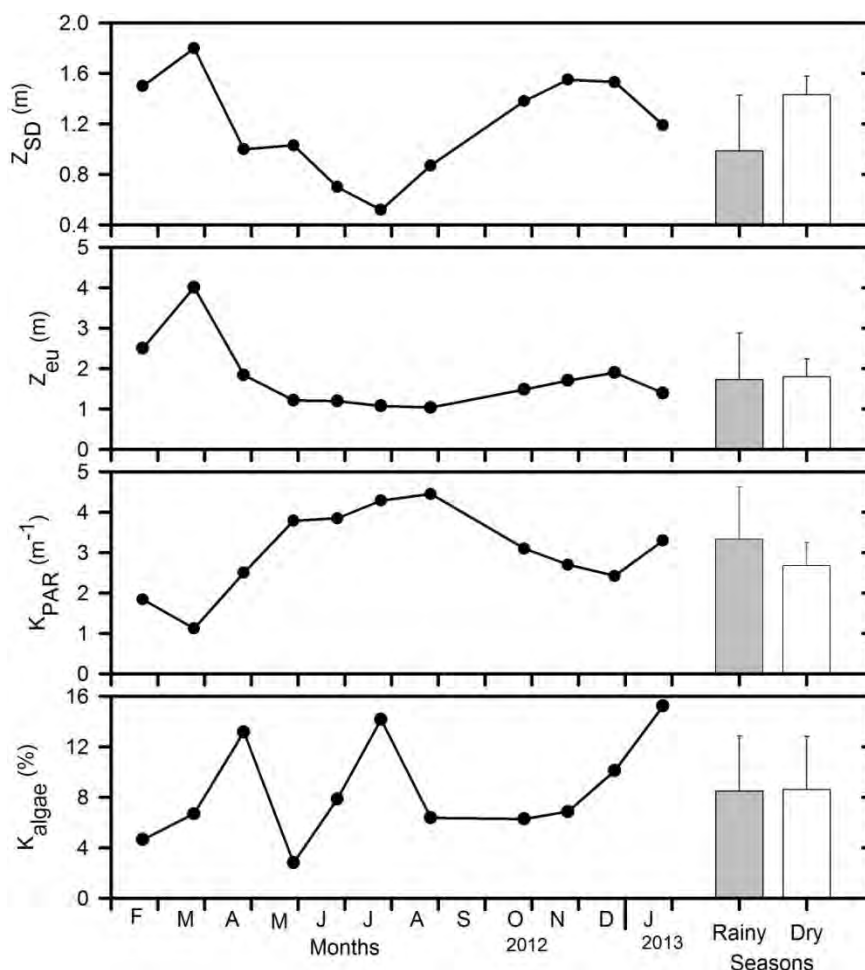


Fig. 2.2. Monthly (line plot) and seasonal (bar plot, mean $\pm$ SD, $n = 6$ or 5) variations in parameters of underwater light climate in Lake Shala.

2.3.2. Thermal regime: Water temperature and dissolved oxygen

Variations in surface water temperature in Lake Chitu among months and between seasons were low, ranging from 21.5°C in December to 24.4°C in April, with a mean of $22.8 \pm 1.0^{\circ}\text{C}$. In contrast, DO of surface water varied markedly among months though the difference between rainy and dry seasons was not significant. Unusually high values of about 18 mg L^{-1} ($> 200\%$ supersaturated) DO were recorded at the lake's surface in July, August and October while DO was depleted ($< 0.4\text{ mg L}^{-1}$) in most months of the dry season, and in March and May of the minor rainy season (Fig. 2.3). Depth profiles of

temperature and DO (Fig. 2.3) showed only superficial stratification, with weak temperature gradients occurring over the depth range of 0–3 m in most months of the dry season. Distinct temperature discontinuity between 2 and 3 m depths (about 1 °C drop) and between 0 and 1 m depths (1.24 °C drop) were observed in October and November of the dry season. In the rainy season, temperature differences between successive depths were indistinct and the temperature readings were uniform below 2 or 3 m depths throughout the water column in all months. The difference between the surface and bottom temperatures was often considerably small, averaging 0.9 °C and ranging from < 0.2 °C (near isothermy) in June, July and August to 1.9 °C in January. Dissolved oxygen showed subsurface-maxima and abrupt changes with discrete oxyclines between 0 and 3 m depths (Fig. 2.3) in most months of the rainy season and in October and February of the dry season. During the rest of the dry season and in March and May of the minor rainy season, DO was depleted (< 0.4 mg L⁻¹) immediately below the surface and almost the whole water column was anoxic. In all months, the water column below 3 m depth was nearly anoxic apart from the low level of DO (about 1.6 mg L⁻¹) recorded down to a depth of 8 m during the month of peak rainfall (July).

Surface water temperature and DO in Lake Shala showed some variability among months though the variations between the rainy and dry seasons were not significant. Temperature varied from 22.3 °C in March to 26.6 °C in May, averaging 24.8±1.2 °C. DO ranged from a minimum of 4.8 mg L⁻¹ in February to a maximum of 15.5 mg L⁻¹ in January, with a mean of 9.1±2.8 mg L⁻¹. Depth profiles of temperature and DO depicted superficial thermal stratification with small to somewhat steep temperature gradients (Fig. 2.4). Depth profiles of temperature revealed a sharp discontinuity in the near-

surface region, with a temperature gradient of 1.3 to 2.5 °C per meter between 0 and 3 m depths in May, June, August, October, January and February, and a temperature gradient of 1.1 to 1.12 °C per meter between 4 and 6 m depths in November and December. Below 3 to 6 m depths down to a depth of 17 m, there was no marked temperature discontinuity. The mean difference between the temperature at the surface water and that at 17 m depth was slightly larger (3.1 ± 0.9 °C). Dissolved oxygen exhibited a trend similar to that of temperature, with a clear discontinuity occurring between 0 and 3 m depths, and with gradual changes occurring below 4 m down to 17 m depths. Maximum DO occurred near the surface within the trophogenic zone.

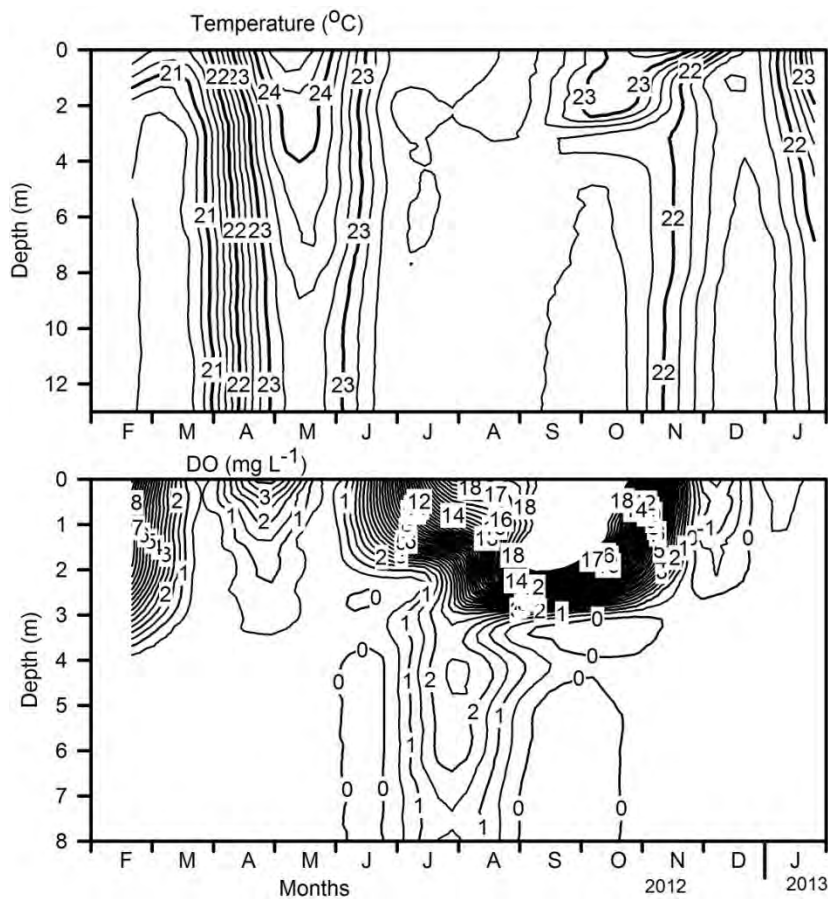


Fig. 2.3. Isopleths of depth profiles of temperature (upper) and dissolved oxygen (lower) in Lake Chitu during the study period.

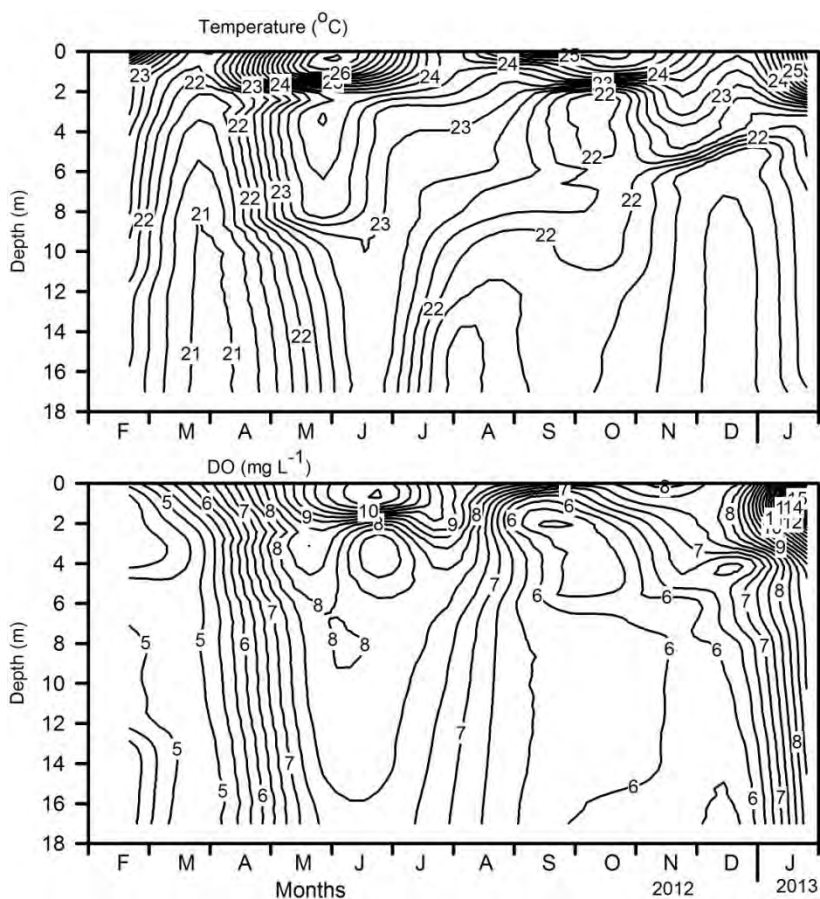


Fig. 2.4. Isopleths of depth profiles of temperature (upper) and dissolved oxygen (lower) in Lake Shala during the study period

2.3.3. Chemical characteristics

Aggregate chemical features (pH, alkalinity and conductivity)

The observed high levels of pH, $\text{CO}_3^{2-} + \text{HCO}_3^-$ -alkalinity and conductivity in Lake Chitu showed perceptible seasonal variations (Fig. 2.5). pH varied from 10.20 in August to 10.76 in July, with a mean of 10.45 ± 0.16 . Alkalinity and conductivity values ranged from 670.8 to 807.8 (737 ± 41 meq L⁻¹) and from 56.5 to 64.7 (61.9 ± 2.7 mS cm⁻¹), respectively. Salinity (40–46.4, mean 43.8 ± 2.2 g L⁻¹), which was calculated from conductivity according to UNESCO (1983), showed a trend similar to that of conductivity. Although their seasonal variations were not significant, pH showed an

increasing trend during the rainy season while alkalinity and conductivity decreased during this time, with their seasonal minima occurring during months of peak rainfall, July and August.

Depth profiles of pH and conductivity showed some variations with depth, exhibiting decreasing and increasing trends, respectively (Fig. 2.6). The pH, which was high near the surface, above 2 m depths in the trophogenic zone, decreased gradually with increasing depth during most of the study months. It showed a notable progressive decline in October and February while its levels were almost constant throughout the water column in December and January of the dry season.

pH, $\text{CO}_3^{2-} + \text{HCO}_3^-$ –alkalinity and conductivity in Lake Shala showed some seasonal variations (Fig. 2.7). The levels of pH (9.8–10.2, mean 9.96 ± 0.1), alkalinity (210–234, mean $224 \pm 6.6 \text{ meq L}^{-1}$) and conductivity (24.3–26.8, mean $25.8 \pm 0.6 \text{ mS cm}^{-1}$) were generally high, and are chemical features characteristic of soda lakes. Salinity varied from 14.7 to 16.4 g L^{-1} , with a mean of $15.7 \pm 0.4 \text{ g L}^{-1}$. These parameters increased slightly during the rainy season, with high pH in July and August and conductivity and alkalinity attaining maxima in April during minor rainy season.

Depth profiles of pH and conductivity determined for depths between 0 and 30 m depths in Lake Shala also indicated small variability with depth (Fig. 2.8). pH increased in the trophogenic zone, between 1 and 5 m depths (the region of thermal gradients), in most of the study months, and its reading was almost constant below 6 m depths. The increase in pH in the trophogenic zone was somewhat pronounced in the months from March to

May. Depth profiles of conductivity showed pronounced increasing trend immediately below the surface down to 30 m depths, particularly in February (Fig. 2.8).

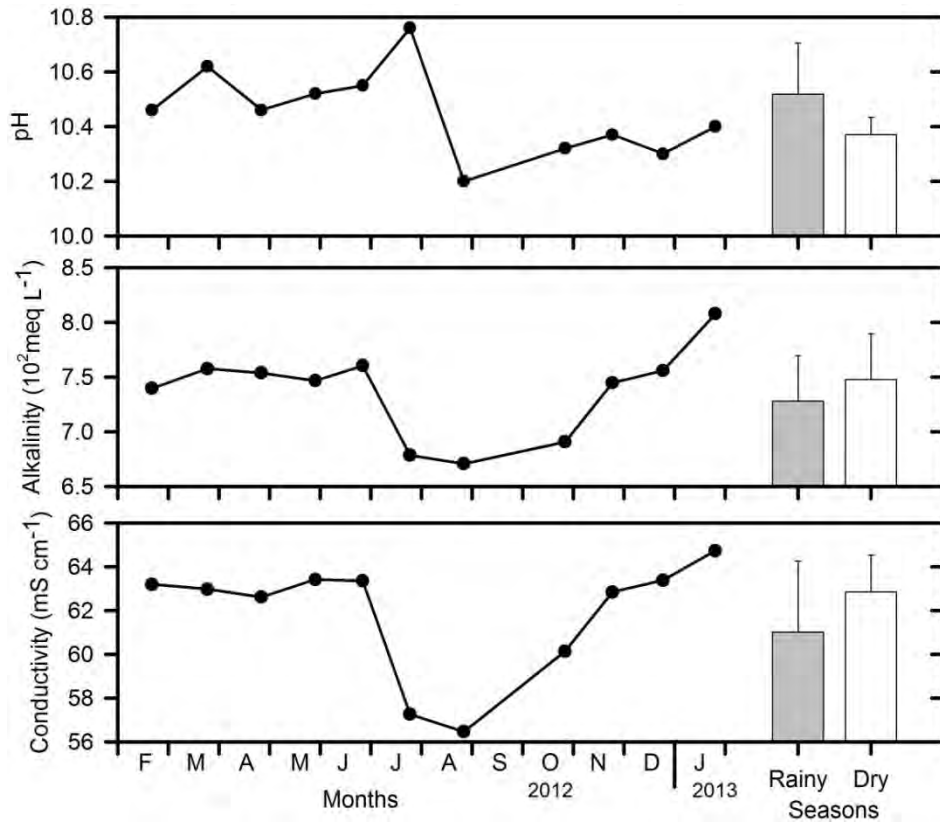


Fig. 2.5. Temporal variations in pH, alkalinity and conductivity among months (line plots) and between seasons (bar plots, mean $\pm$ SD, $n = 6$ or 5) at the surface of Lake Chitu.

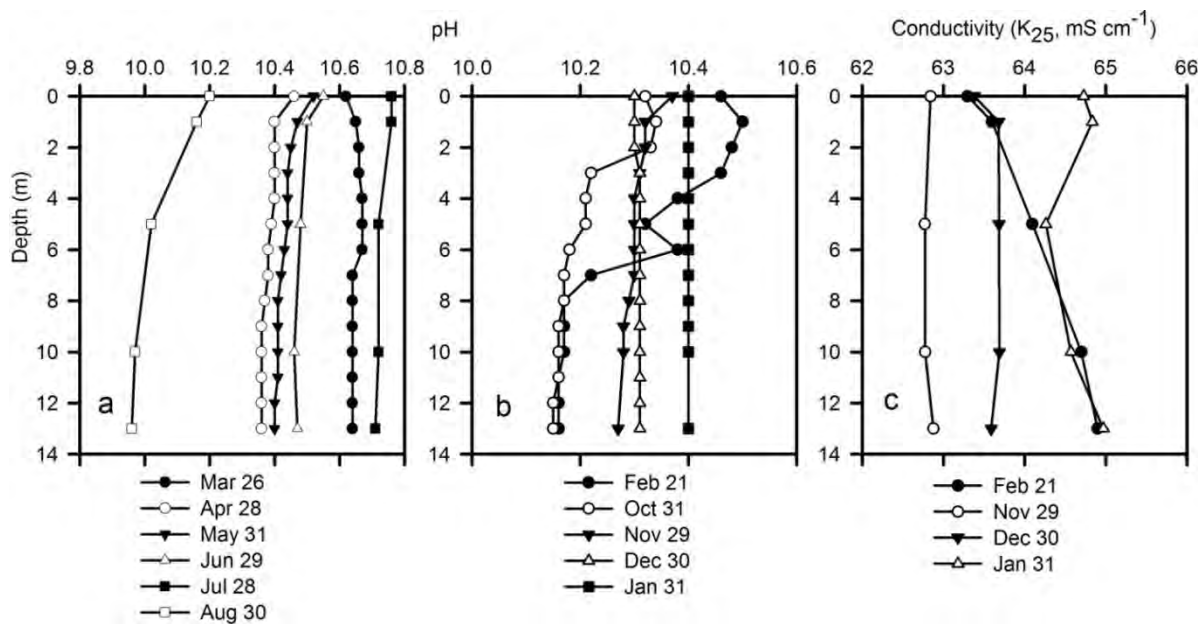


Fig. 2.6. Depth profiles of pH during the rainy (a) and dry (b) seasons and of conductivity during the dry season alone (c) in Lake Chitu (from February 2012–January 2013).

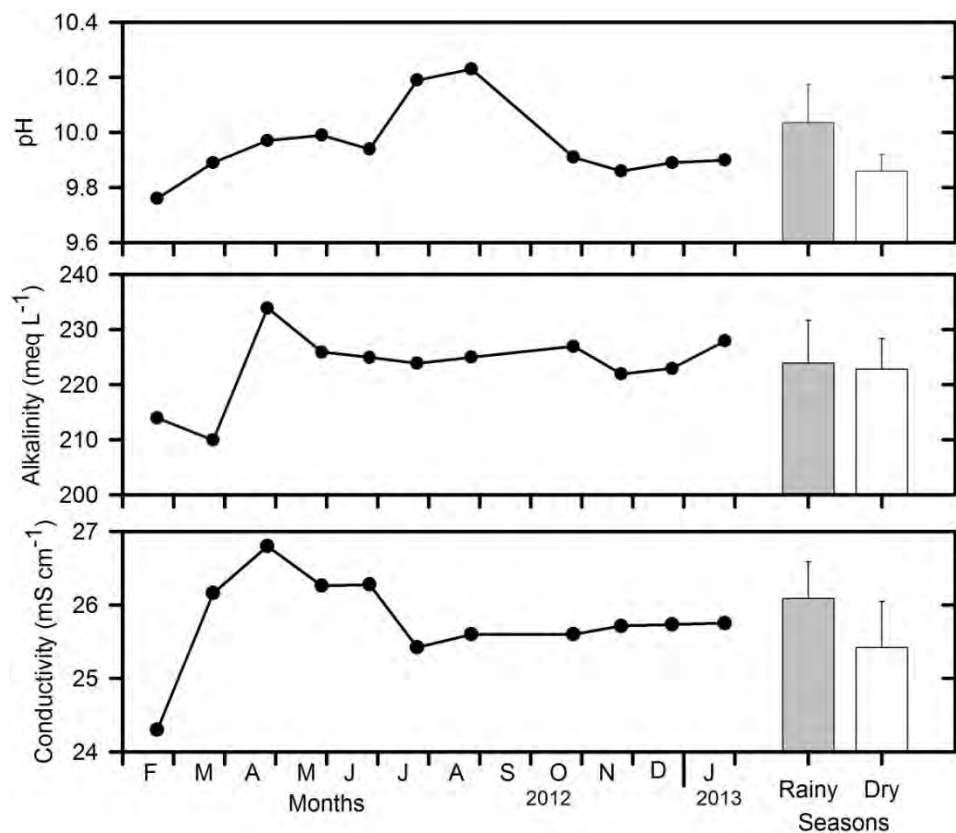


Fig. 2.7. Temporal variations in pH, alkalinity and conductivity among months (line plot) and between seasons (bar plot, mean $\pm$ SD, $n = 6$ or 5) at the surface of Lake Shala.

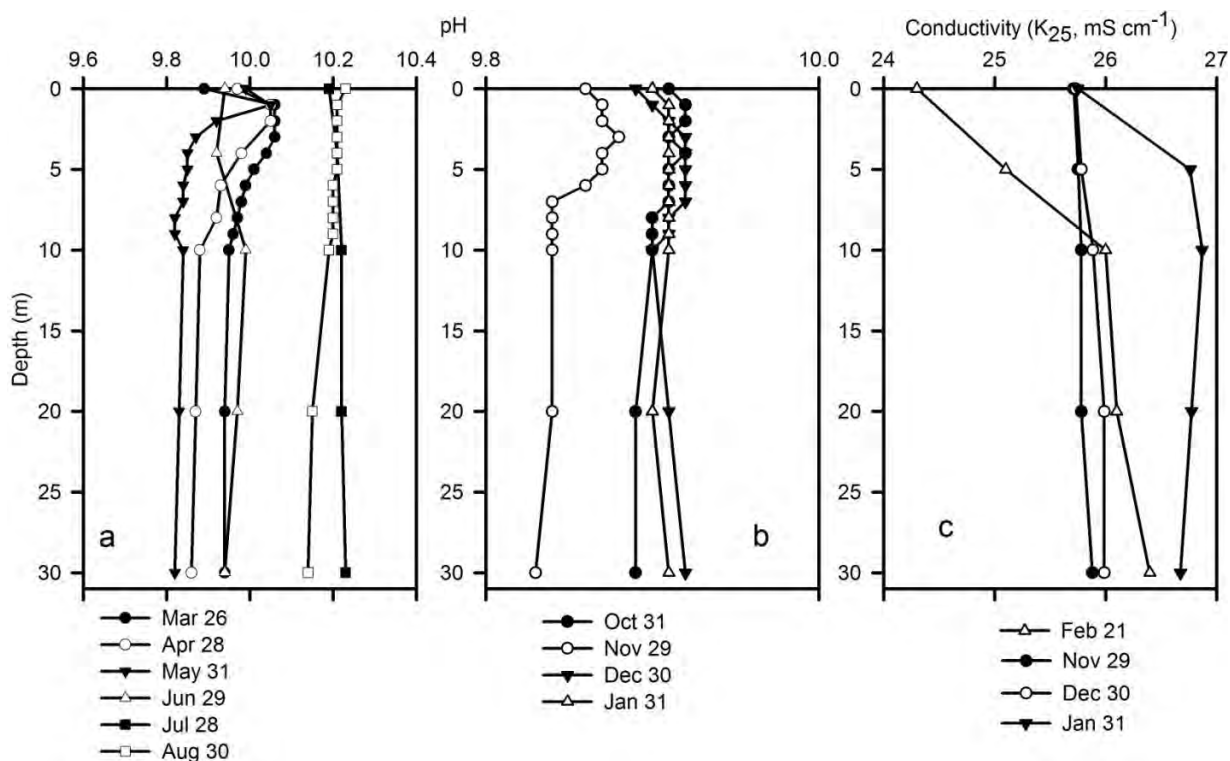


Fig. 2.8. Depth profiles of pH (a, rainy season and b, dry season) and conductivity (c) during dry season in Lake Shala.

Inorganic nutrients

Inorganic combined nitrogen forms (NO_3^- and NH_3), SRP, TP, ratio of inorganic carbon species ($\text{CO}_3^{2-}:\text{HCO}_3^-$) and SiO_2 in Lake Chitu showed variations among months (Fig. 2.9), as reflected in their large standard deviations, though their variations between the rainy and dry seasons were not significant. The concentration of NO_3^- in the trophogenic zone was almost undetectable throughout the study period while substantial NH_3 concentrations, ranging from 20.3–84 (mean $\pm$ SD, $46\pm30 \mu\text{g L}^{-1}$), were recorded only during four months, May, February, July and November. The concentrations of SRP and TP were large and varied, respectively, from 0.041 to 4.498 ($1.035\pm1.625 \text{ mg L}^{-1}$, mean $\pm$ SD) and from 1.909 to 4.529 ($2.726\pm0.816 \text{ mg L}^{-1}$, mean $\pm$ SD), with their maxima occurring in February. In most months (>64%) of the study period, the ratio of SRP:TP

was very low (< 0.1) although much larger values (about 0.98), indicative of availability of surplus SRP for algal uptake, were observed in February and August. The ratio $\text{CO}_3^{2-}:\text{HCO}_3^-$ was large (always greater than one) in most months, especially during the rainy season. However, low ratio of $\text{CO}_3^{2-}:\text{HCO}_3^-$, indicative of availability of abundant HCO_3^- , was recorded in August (0.7) and December (0.9). Dissolved SiO_2 varied within a relatively narrow range among months, from a minimum of 0.7 mg L^{-1} in October to a maximum of 2.3 mg L^{-1} in November ($1.4 \pm 0.5 \text{ mg L}^{-1}$, mean $\pm$ SD), with almost no differences between the means of the rainy and dry seasons.

Depth profiles of NH_3 , SRP and SiO_2 in this lake showed variation with depth (Fig. 2.10) whereas NO_3^- was undetectable or otherwise very small throughout the water column apart from the small increase observed at 10 m depth in January. From its low or undetectable surface values, NH_3 increased dramatically with increasing depth in almost all months, with remarkably high levels occurring between 1 and 10 m depths in October, November, January, February and August. Concentrations of SRP were low at the lake's surface and increased with depth in almost all months except February, with distinctly high concentrations somewhere in the mid-water column (between 1 and 5 m) in some months. However, low values of SRP were recorded at the bottom. SiO_2 increased with depth in all months, with more than four fold increase at the bottom in February.

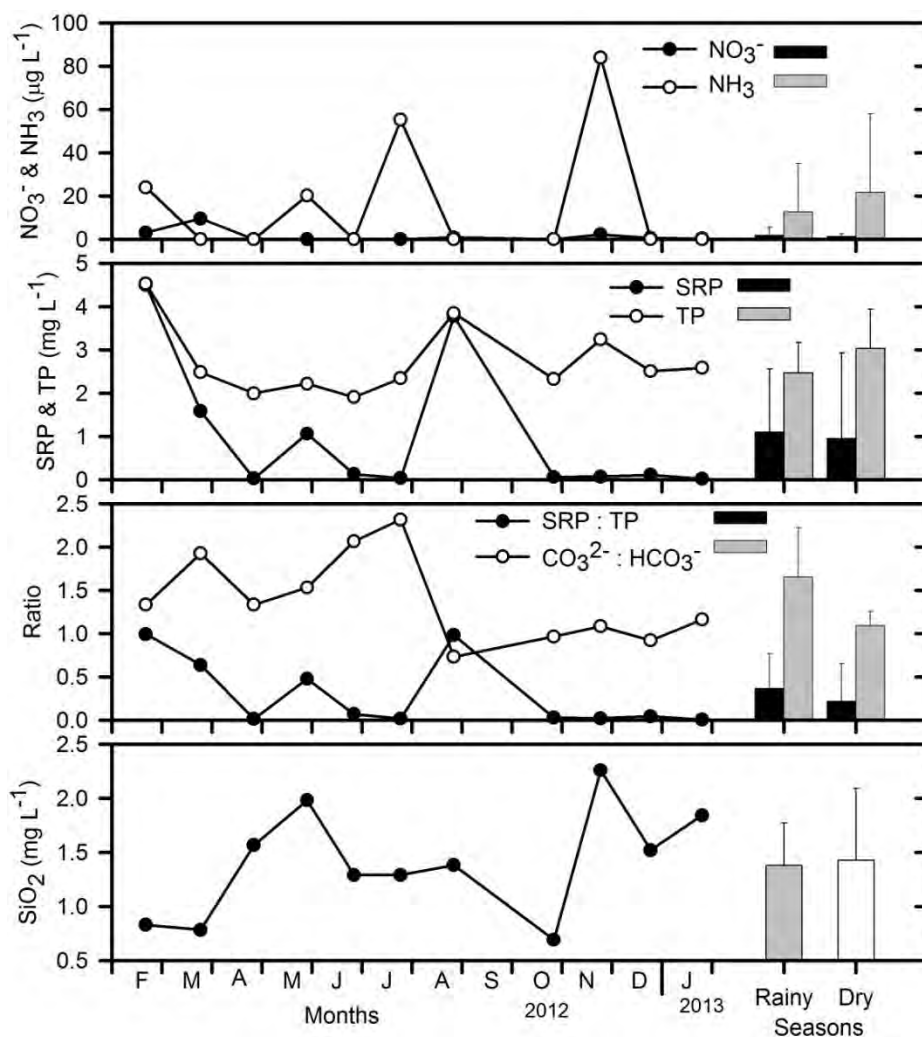


Fig. 2.9. Temporal variations in NO_3^- , NH_3 , SRP, TP and SiO_2 among months (line plot) and between seasons (bar plot, mean $\pm$ SD, n = 6 or 5) in the trophogenic zone of Lake Chitu.

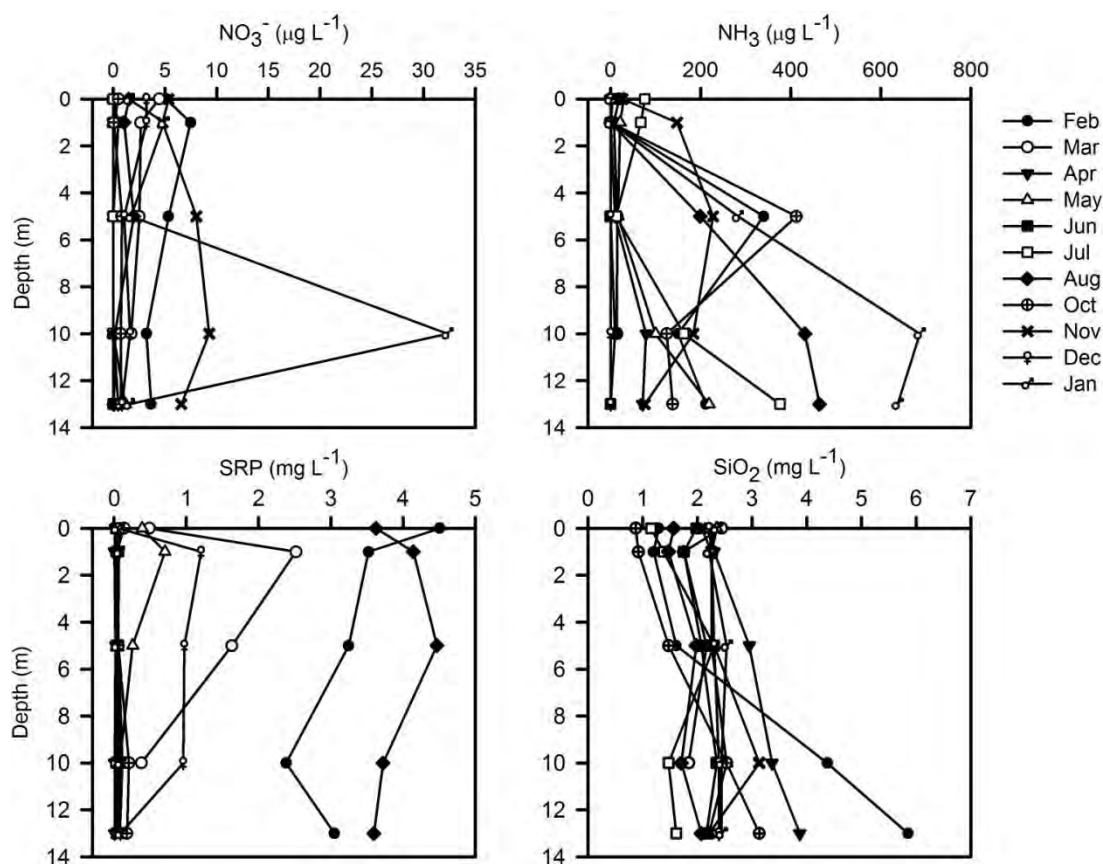


Fig. 2.10. Depth profiles of the inorganic nutrients NO_3^- , NH_3 , SRP and SiO_2 in Lake Chitu.

The nutrients NO_3^- , NH_3 , SRP, TP, SiO_2 and $\text{CO}_3^{2-}:\text{HCO}_3^-$ in the trophogenic zone of Lake Shala showed small temporal variations (Fig. 2.11). NO_3^- was almost undetectable throughout the study period while NH_3 of considerable levels, varying from 15.3 to 90.3 ($42 \pm 34 \mu\text{g L}^{-1}$, mean $\pm$ SD), was recorded only in November, August, May and October. The concentrations of SRP and TP were high, ranging from 0.906 to 1.161 ($0.961 \pm 0.075 \text{ mg L}^{-1}$, mean $\pm$ SD) and from 0.939 to 1.182 ($1.020 \pm 0.093 \text{ mg L}^{-1}$, mean $\pm$ SD), respectively, depicting the insignificant temporal variation observed throughout the study period. Both SRP and TP were at nearly comparable levels in all months, with their remarkably large ratio (SRP:TP) varying from 0.86 to 0.99 (0.94 ± 0.1 , mean $\pm$ SD). The

ratio $\text{CO}_3^{2-}:\text{HCO}_3^-$ varied narrowly from 0.3 to 0.7 (0.4 ± 0.1 , mean $\pm$ SD), indicating that the concentration of available HCO_3^- was higher than that of CO_3^{2-} in the lake. SiO_2 ranged from 0.37 to 1.3 (0.9 ± 0.3 mg L⁻¹, mean $\pm$ SD) in June and August, respectively. Unlike those of other nutrients, the variation of SiO_2 among months was somewhat large though its mean values recorded in the rainy and dry seasons were closely similar. Depth profiles of NO_3^- , NH_3 , SRP and SiO_2 in this lake depicted very small differences between 0 and 30 m depths (Fig. 2.12). The concentration of NO_3^- was less than 2 µg L⁻¹ at all depths of measurement, while NH_3 showed small increases between 0 and 5 m depths, with its levels reaching about 9 µg L⁻¹ in August and 30 µg L⁻¹ in May. SRP and SiO_2 exhibited gradual trends of increasing with depth in most months, with more perceptible increases in January and February, respectively.

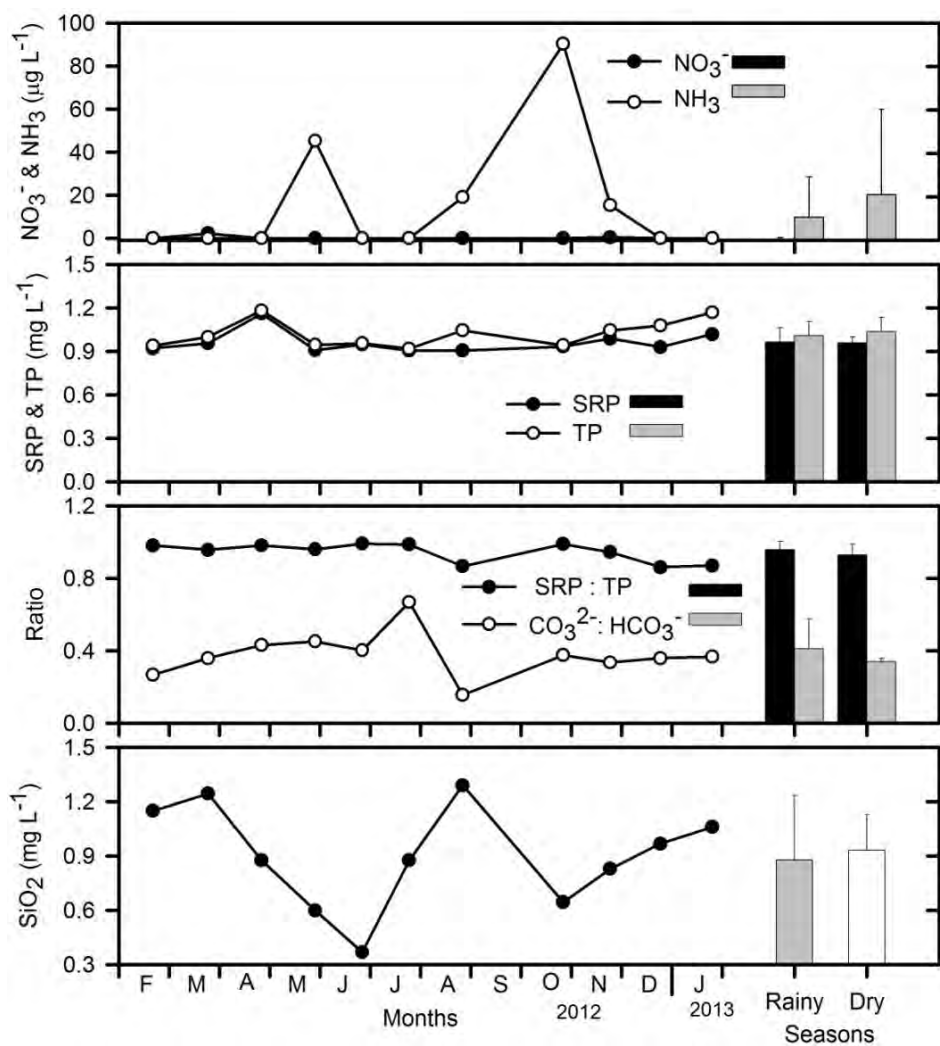


Fig. 2.11. Temporal variations in NO_3^- , NH_3 , SRP, TP, $\text{CO}_3^{2-}:\text{HCO}_3^{2-}$ and SiO_2 among months (line plots) and between seasons (bar plots, mean $\pm$ SD, n = 6 or 5) in Lake Shala.

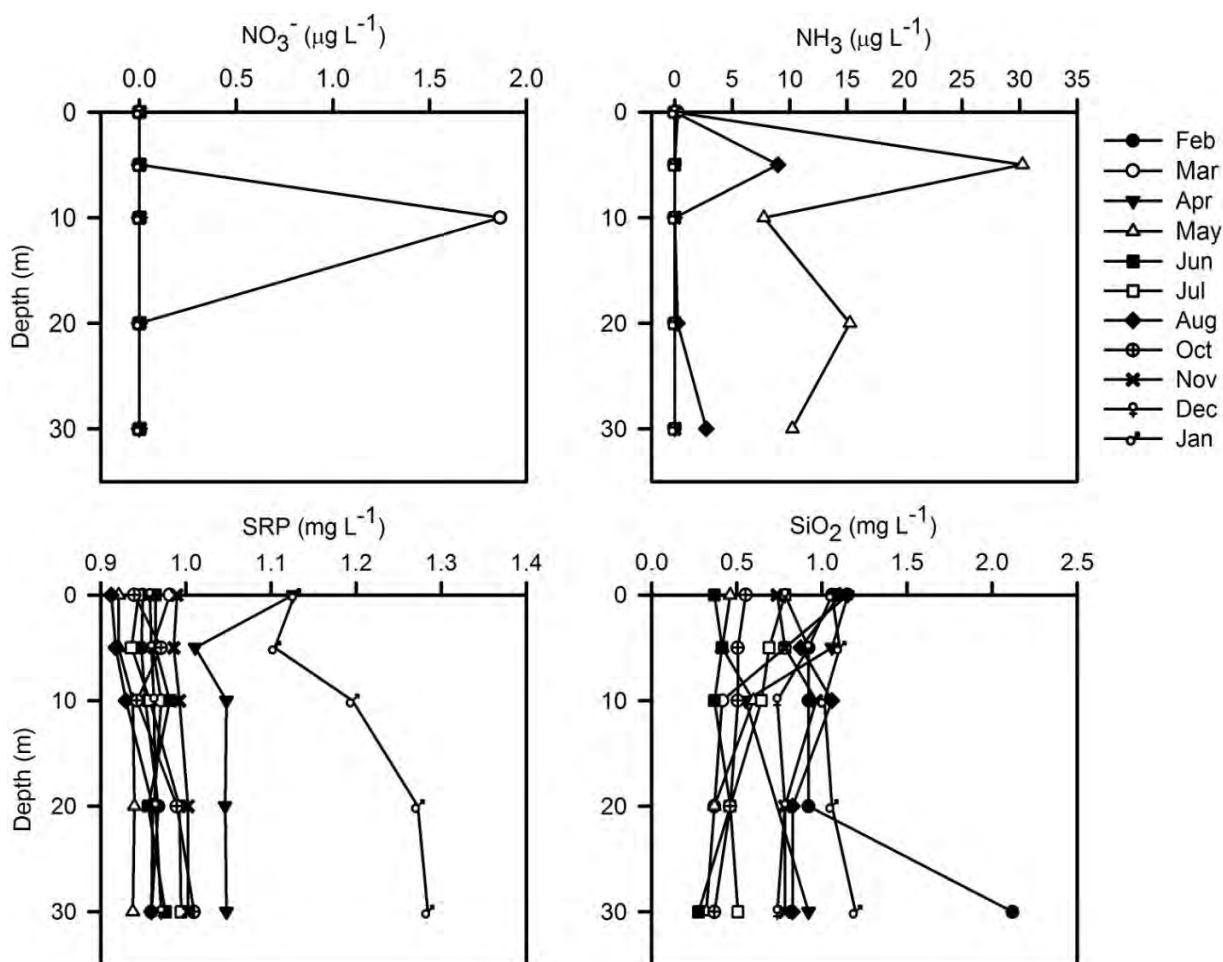


Fig. 2.12. Depth profiles of NO_3^- , NH_3 , SRP and SiO_2 in Lake Shala during the study period.

2.4. DISCUSSION

2.4.1. Underwater light climate

Regardless of the high irradiance incident at the lake's surface, light limitation within the water column is considered a major factor reducing productivity in tropical lakes (Talling, 2001). The observed parameters of underwater light climate generally indicate the low water transparency (high light attenuation) and the consequent vertically compressed trophogenic zone of Lake Chitu. This is expected for such highly productive water body supporting high biomass of the superabundant population of *A. fusiformis*.

throughout the study period. The poor underwater climate of the lake is the result of self-shading caused by the huge biomass of *A. fusiformis*, which was also evidenced by the observed high K_{algae} (mean $32 \pm 10\%$). This corroborates the algal biomass-associated light limitation reported earlier for the same lake (Elizabeth Kebede *et al.*, 1994). Similar phenomena of light limitation associated with strong attenuation of light by the hugely accumulated phytoplankton biomass within a narrow euphotic zone were also reported for several Kenyan soda lakes including Lakes Sonachi, Bogoria, Nakuru and Elementeita (Melack *et al.*, 1982; Oduor and Schagerl, 2007a).

Despite its low productivity, Lake Shala also had low light transparency (high light attenuation), but with considerably deeper trophogenic zone. The contribution of K_{algae} to total light attenuation in Lake Shala was low (mean $8.6 \pm 4.1\%$), which implies the presence of large non-algal (inorganic) turbidity contributing to the light attenuation in the lake. This is consistent with the finding of Wood *et al.* (1978) who also reported high light attenuation due to non-algal turbidity in Lake Shala and other lakes of the same drainage system (Langano and Ziway). Inorganic turbidity associated with re-suspension of sediments and precipitated CaCO_3 is known to cause high light attenuation in soda lakes (Kalff, 1983; Wood and Talling, 1988; Wetzel, 2001). In addition to turbidity, water column mixing also causes severe light limitation of phytoplankton production in lakes (Reynolds, 1987; Diehl *et al.*, 2002). Since Lake Shala had superficial type of stratification down to the depth of 17 m or so, mixing depth (Z_{mix}) is expected to be high. The recorded Z_{eu} of the lake was 1–4 m, which suggests that the ratio of Z_{mix} to Z_{eu} is greater than the critical values of 4–5 (Wood *et al.*, 1978). As a result, the high Z_{mix} may be the main factor determining phytoplankton production and composition through its

effect on light availability and loss by sedimentation in Lake Shala. In previous studies, this lake was also reported to be an optically deep and light-limited lake due to its deep mixing zone and relatively shallow euphotic zone (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Zinabu Gebre-Mariam, 2002).

The association of poorer underwater light climate during the rainy months with significant increase in K_{algae} in Lake Chitu seems to suggest that algal biomass was the primary cause of the low light condition of this time. External loading of materials from the small grazing land located on the shores of the lake (internal to the crater rim) also seems to have contributed to the low water transparency of the lake observed during the rainy season. However, external loading associated with rainfall, which has been identified as the major cause for the seasonal oscillation of limnological variables in tropical lakes (Melack, 1979; Wood and Talling, 1988), is not expected to have an impact of similar magnitude in Lake Chitu as it is a closed system situated in a cup-shaped crater topography. In Lake Shala, although the seasonal variations in all the parameters of underwater light climate were insignificant, a decline in water transparency was observed in the rainy months, which may be attributable to the increased hydrological events including introduction of runoff from its large catchment. However, marked seasonality in the underwater light climate may not be expected because of its voluminous nature, which would make the lake more resilient to some environmental changes, the salient feature of large lakes.

2.4.2. Thermal regime: Water temperature and dissolved oxygen

Baxter *et al.* (1965) contended that some Ethiopian lakes show two forms of thermal stratification, superficial and deep-seated, with the former type developing steep

temperature gradients during calm conditions. The depth profiles of temperature and DO in Lakes Chitu and Shala seem to show the prevalence of superficial type of stratification, with the former showing weak temperature gradients but with most parts of the water column being anoxic and the latter showing weak to somewhat strong temperature gradients, but with well oxygenated water column. Superficial thermal stratifications that usually affect the 0–3 m layer of water columns and, which are created by strong daily solar heating and destroyed by nocturnal cooling are common in East African lakes (Baxter *et al.*, 1965; Elizabeth Kebede *et al.*, 1994; Talling and Lemoalle, 1998; Melack, 2009).

Catchment topography of volcanic crater lakes with steep crater rims, which provide shelter from wind action, and depth are considered as important factors determining the occurrence of stratification in such lakes (Walker and Likens, 1975; Beadle, 1981). Although Lake Chitu is fairly deep (maximum depth 21 m) and sheltered from wind to some extent by its crater rim (altitude difference from the water surface to the tip of the crater rim ranges from 34 to 83 m), these physiographic features do not seem to have been strong enough to induce deep-seated and persistent thermal stratification apart from reducing the frequency and intensity of wind-mixing. The small size of the lake that creates very small temperature differentials may contribute to the observed weakness of thermal stratification. In superficially stratified lakes with maximum depths of less than 30 m, like Lake Chitu, complete mixing (holomixis) frequently occurs (Baxter *et al.*, 1965; Talling and Lemoalle, 1998). However, the persistent occurrence of anoxia in most parts of its water column seems to reflect the prevalence of a mixing process that was too

slow and weak in intensity to offset oxygen depletion in Lake Chitu, as reported for deep tropical lakes (Beadle, 1981; Verburg *et al.*, 2003).

Deep-seated thermal stratification with well marked thermoclines and considerable decrease in DO were not observed in the water column above 17 m depths in Lake Shala. Depth profiles of temperature, DO and some chemical constituents, determined by Baxter *et al.* (1965), did not also show the occurrence of such type of strong stratification between surface water and 100 m depth of Lake Shala. Baxter *et al.* (1965) also observed oxygen and water column mixing extending down to the greatest depth of measurement (100 m), which led them to suggest that complete or near-complete mixing probably occurs in a number of deep lakes including Lakes Shala and Langano. Deep-seated stratification with anoxic layer may be expected in the deeper parts of Lake Shala (max. 266 m) as depth is considered as an important factor for the occurrence of deep-seated and prolonged thermal stratification (Beadle, 1981). For the observed thermal regime of the water down to the depths covered in the present and previous studies on Lake Shala, wind-induced turbulence that occurs intensely in the afternoon seems to be the factor of overriding importance regardless of its great depth. The occurrence of wind-induced turbulent mixing in this lake is favored by its large wind fetch and absence of prominent crater walls and natural vegetation that can afford shelter from wind action. The occurrence of complete vertical mixing and the consequent well-oxygenated water column was linked to the degree of exposure of the water body to wind action in some Ethiopian crater lakes (Baxter *et al.*, 1965; Wood *et al.*, 1976) and some lakes of the neighboring Kenya (Melack, 1979; Vareschi, 1982). Seasonality of wind and rainfall are

also generally considered as the major factors determining water column mixing pattern in tropical lakes (Wood *et al.*, 1976).

The seasonal and vertical distribution patterns of DO in Lake Chitu were peculiar: supersaturation near the surface with small amount of DO down to a depth of 8 m during the peak rainy months, deoxygenation immediately below the surface in certain occasions and complete deoxygenation of most of the water column in most months. The occurrence of near-surface supersaturation and low DO concentration away from the surface are common in productive tropical African soda lakes (Vareschi, 1982; Oduor and Schagerl, 2007a). Rates of photosynthesis and respiration, oxidation-reduction reactions, and stratification and mixing patterns are considered to be the major factors governing the variability of DO in such productive soda lakes (Vareschi, 1982; Oduor and Schagerl, 2007a; Melack, 2009). The variability of DO in Lake Chitu seems to be controlled by the productivity, decomposition of alga biomass, and weakness of the mixing of the water column. Lake Chitu is one of the East African soda lakes known for their extremely high *Arthrospira* biomass and photosynthetic rates (Talling *et al.*, 1973). In this lake, DO showed positive significant correlation with *Arthrospira* abundance ($P < 0.01$) and positive but moderate correlation with chl-*a* (see chapter 3, Table 3.2), which significantly ($P < 0.05$) increased during the rainy season. This seems to suggest that the high *Arthrospira* biomass and hence the presumably high rate of photosynthetic oxygen-evolution caused the supersaturation of the trophogenic zone with DO, particularly in the rainy months. This is consistent with the observation that supersaturation of DO and high rate of gross photosynthesis occurred in the upper near-surface water layers during periods with abundant *A. fusiformis* in East African soda lakes including Lakes

Arenguade, Simbi, Bogoria and Nakuru (Talling *et al.*, 1973; Melack, 1979; Vareschi, 1982; Oduor and Schagerl, 2007a). The observed small amount of DO down to a depth of 8 m during the peak rainy months is probably associated with the occurrence of high DO production in the trophogenic zone.

It has been demonstrated that water column stratification together with algal biomass decomposition and high microbial activity could cause rapid depletion of DO in tropical soda lakes (Wood *et al.*, 1984; Melack, 2009). As Lake Chitu was characterized by very weak and superficial stratification with occasional near-isothermal conditions, the contribution of stratification to DO depletion seems to be less important. DO depletion in Lake Chitu seems to be attributable mainly to the decomposition of the dense algal biomass under the favorably high temperature by the abundant organotrophic bacteria that constitute the microbial community of the highly productive and *Arthrospira*-rich soda lakes of East Africa including Lake Chitu (Melack *et al.*, 1982; Jones and Grant, 1999; Lanzén *et al.*, 2013). Such type of persistent anoxia resulting from high oxygen demand for bacteria-mediated degradation of *Arthrospira* biomass was observed in the Kenyan soda lakes Sonachi, Bogoria and Elmenteita (Verschuren, 1999; Oduor and Schagerl, 2007a). In addition, the prevailing slow and weak mixing of the water column of Lake Chitu may have brought up anoxic water from the lower part of the water column, exacerbating DO depletion, particularly during the time of low photosynthetic oxygen production.

2.4.3. Aggregate chemical features (pH, alkalinity and conductivity)

The observed high pH, alkalinity and conductivity of Lakes Chitu and Shala are characteristic of most soda lakes of East Africa (Talling *et al.*, 1973; Melack *et al.*, 1982;

Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Talling and Lemoalle, 1998). The major driver for the saline-alkaline condition in these soda lakes is the high surface evaporation rate, which exceeds the water input (Jones *et al.*, 1994; Grant, 2006). Leaching of volcanic rock materials rich in Na (Klemper and Cash, 2007) and the low level of divalent cations (Ca^{2+} and Mg^{2+}), which enhances alkalinity due to the reduced precipitation of the divalent cations as carbonates (Zinabu Gebre-Mariam, 2002; Klemper and Cash, 2007), also contribute to the lakes' saline-alkaline properties.

Photosynthetic activities, respiration, buffering capacity and stratification of lakes can regulate pH (Melack, 2009; Talling, 2010). Although the present study lakes have high buffering capacity, some variability in pH was observed both temporally and along the vertical profile. Lake Chitu supports dense populations of *Arthrospira* and its high photosynthetic productivity (Talling *et al.*, 1973; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994) and the abundance of *Arthrospira* (see chapter 3) was positively correlated with pH ($r = 0.30$). Therefore, the increase in pH in the trophogenic zone of Lake Chitu likely resulted from the large algal (*Arthrospira*) biomass and the consequent increased photosynthetic removal of dissolved carbon dioxide (Talling, 2010). In a similarly productive soda lake of Ethiopia, Arenguade, Talling *et al.* (1973) also observed a rise in pH due to dense algal biomass and vigorous photosynthesis despite the high buffering capacity of the lake. In the much less productive lake Shala, pH was significantly positively correlated with rainfall ($P < 0.01$) and moderately and positively correlated with chl-*a* and cell density of phytoplankton (see chapter 3, Table 3.3). This seems to suggest that rainfall caused variation in pH to a certain extent through its effect on chemical constituents and algal photosynthesis in Lake Shala. The observed decrease

in pH with increasing depth in both lakes is the expected result of the decline with increasing depth in the amount of light, which drives photosynthesis. Increased respiration and decomposition that occur in the water column of productive soda lakes with high abundance of organotrophic bacteria such as Lake Chitu (Lanzén *et al.*, 2013) may also have contributed to the progressive decrease in pH in the deeper parts of Lake Chitu. However, large variation in pH may not be expected in both lakes because of their high alkalinity and superficial thermal stratification.

Although not large, the temporal variations in alkalinity and conductivity were perceptible for both lakes and showed contrasting seasonal trends; decreasing trend in Lake Chitu during the rainy season while the opposite was true for Lake Shala during this time. Hydrologically-controlled salinization is considered important in several rift valley lakes of Ethiopia and East Africa (Wood and Talling, 1988). The temporal variations in alkalinity and conductivity in Lake Chitu seem to be related to the seasonal influence of precipitation and evaporation; a decline during the rainy season due to increased precipitation and reduced evaporation rate, while concentration of ions occurs due to the opposite situation that prevails during the dry season. Bacterial sulfate reduction that commonly occurs in productive lakes with anoxic water column releases sulfide (H_2S) or sulfite (S^{2-}), resulting in generation of alkalinity (Kilham, 1984). Sulfate reduction is expected to be high, specially during anoxic period (dry season), in the productive and largely anoxic lake Chitu as the odor of H_2S was sensed in the water samples collected from 5 to 13 m depths and sometimes near the shores.

In Lake Shala, although evaporation loss is the prime route for the total annual water deficit of 246 million m^3 per year (Tenalem Ayenew and Dagnachew Legesse, 2007), it

appears to have low contribution during the rainy season as the measured variables increased during this time of the year. The alkalinity (46.3 meq L^{-1}), salinity (4.5 g L^{-1}) and conductivity (8.2 mS cm^{-1}) of inflowing hot springs around Lake Shala were very low compared to those of the lake water. It seems that some more saline inflows, probably through surface runoff from its degraded catchments, may have made considerable contributions to the increased salinity of Lake Shala during the rainy season. Similarly, Zinabu Gebre-Mariam (2002) observed an increase in salinity during the rainy season in some Ethiopian rift valley lakes including the neighboring soda lake Abijata, which was attributed to inflows (runoff) with high solute concentrations. Increased pH during the rainy season may also have contributed to the salinity increase. Modification of natural hydrological pattern through clearance of natural vegetations, overgrazing and other land-use activities within the catchments results in salinization of lakes, the major threat to water resources in semi-arid and arid regions of the world (Williams, 2002). This human-induced effect also seems to apply for Lake Shala as it is under similar ecological threats. However, the changes in the chemical features expected to accompany hydrological modification were not large in this lake owing probably to the lake's large water volume that may enhance its buffering capacity against marked changes in chemical features.

The observed somewhat progressive increasing trend of conductivity along the vertical profile in both lakes could be associated with the accumulation of solutes, a phenomenon described by Baumann *et al.* (1975) and Von Damm and Edmond (1984) who also observed small increases of salinity and major ions with depth in Lake Shala.

2.4.4. Inorganic nutrients

The measured algal nutrients of Lakes Chitu and Shala reflected the characteristic features of most soda lakes of East Africa: a large reserve of inorganic carbon sources and SRP, but limiting levels of nitrogen sources (Talling *et al.*, 1973; Melack *et al.*, 1982; Wood and Talling, 1988; Talling and Lemoalle, 1998). Inorganic sources of nitrogen or phosphorus or both were often suggested to be limiting to algal production in some African freshwaters (Melack *et al.*, 1982; Kalff, 1983; Talling and Lemoalle, 1998). The concentration of SRP and TP are quite high in the present study lakes in view of the levels recorded for most freshwaters. The present observations are consistent with the results of earlier studies made on the same lakes and the other soda lake Arenguede (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994). Such high concentrations of SRP are common in the soda lakes Bogoria, Sonachi and Simbi (Melack *et al.*, 1982; Vareschi, 1982; Oduor and Schagerl, 2007b) and are attributed mainly to the predominance of phosphatic mineral-rich rocks (Talling and Talling, 1965) and release from anoxic water column (Oduor and Schagerl, 2007b). However, the form of phosphorus existing in the water column may differ and affect algal productivity. Peters and MacInyre (1976) observed low SRP regeneration rates associated with the availability of a large amount of SRP in Lake Nakuru. The low SRP:TP ratio of the productive lake Chitu, in contrast to that of the unproductive lake Shala, indicates that most of the SRP is in the algal biomass or in particulate form.

Nitrogen sources, on the other hand, were almost always low or undetectable in the present study. Previous studies also reported similar levels of nitrogen sources in these and other soda lakes of Ethiopia (Wood and Talling, 1988; Elizabeth Kebede *et al.*,

1994). Nitrogen-limitation, which has been suggested for some other tropical African soda lakes (Melack *et al.*, 1982; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Talling and Lemoalle, 1998), seems to be more likely in these soda lakes. The high rate of denitrification by the abundant populations of denitrifiers, enhanced by the favorably high tropical temperature, is believed to contribute to the low nitrogen levels in soda lakes (Jones and Grant, 1999; Lewis, 2002). High diversity and abundance of denitrifiers were recently reported for Ethiopian soda lakes including Lakes Chitu and Shala (Lanzén *et al.*, 2013; Baye Sitotaw, 2014). Nevertheless, nitrogen sources other than NO_3^- seem to be important in Lake Chitu in alleviating nitrogen limitation of the dense non-nitrogen fixing *Arthrospira* population. The high concentrations of NH_3 observed in the lower parts of the water column may serve as a nitrogen source for *Arthrospira*, which is capable of assimilating multiple forms of nitrogen sources, and is able to utilize NH_3 at high pH due to its resistance to the toxicity of NH_3 (Boussiba, 1989). Organic nitrogen sources originating from feather degradation may also be important in Lake Chitu as keratin-degrading microbes and their effective degradation of keratin from the feathers of flamingos was reported for the same lake (Baye Sitotaw, 2014). High NH_3 ($207.3 \pm 129.3 \mu\text{g L}^{-1}$, $n = 11$, SD) was recorded in one of the hot springs found on the shore of Lake Shala and this may have contributed to the concentration of NH_3 occasionally observed in the lake water.

Although the differences between rainy and dry seasons were small, NH_3 and SRP showed noticeable inter-monthly and vertical variations in Lake Chitu. External input of nutrients associated with seasonal hydrological events is expected to be minimal in Lake Chitu as it is a closed system lacking direct inflow and with a crater rim affording some

protection from runoff effects. Internal loading of nutrients, controlled mainly by biogenic and abiogenic transformations within the lakes, is regarded as a very important process regulating the ecological functioning of soda lakes (Kilham and Kilham, 1990; Carini and Joye, 2008). It has been reported that, in productive lakes such as Lake Chitu, over 90% of the total nitrogen is in the algal biomass and this is regenerated during decomposition of the organic materials (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994). The high diversity and abundance of microbes in Lake Chitu (Lanzén *et al.*, 2013) suggests that microbial processes favored by high tropical temperature, which have been shown to enhance internal regeneration of nutrients in productive soda lakes (Jones and Grant, 1999), may have played a crucial role in this lake. In addition, the presence of hydrogen sulfide in the anoxic lower layer favors NH_3 accumulation (Joye and Hollibaugh, 1995), and the anoxic nature of the water column and the resultant sulfate reduction (which reduces the potential of iron trapping SRP) promote the dissolution of metal precipitates with the consequent release of SRP to the water column (Grant, 2006). High rate of release of SRP ($7.5 \text{ mg m}^{-2} \text{ h}^{-1}$) from sediments with low oxygen levels at the sediment-water interface has also been reported for some soda lakes including Nakuru in Kenya (MacIntyre, 1981; Vareschi, 1982) and Mono Lake in USA (Jellison *et al.*, 1999).

Low concentrations of NH_3 and SRP occurred near the surface of Lake Chitu, which could be associated with biological consumption, oxygen-dependent metal precipitation of SRP and loss of NH_3 through nitrification and denitrification (Carini and Joye, 2008). The occurrence of SRP and TP maxima in February could be related to the seasonal minimum algal biomass of about $72 \text{ } \mu\text{g chl-}a \text{ L}^{-1}$ as nutrient loss to the sediment

decreases with decreased algal consumption in productive water bodies (Diehl *et al.*, 2002).

The concentrations of inorganic carbon sources (carbonate-bicarbonate) were large in both lakes, a salient feature of soda lakes (Talling *et al.*, 1973; Wood and Talling, 1988; Jones and Grant, 1999). The high level of carbonate-bicarbonate is important as a buffer system to maintain optimum alkaline pH and provides carbon source for the alkaliphilic cyanobacteria, e.g., *Arthrospira* (Richmond, 1990; Vonshak, 1997). Nevertheless, such benefits of carbonate-bicarbonates are highly dependent on their ratios. It has been shown that high ratio of $\text{CO}_3^{2-}:\text{HCO}_3^-$ at $\text{pH} > 10$ may suggest limitation of alkaliphilic cyanobacteria by HCO_3^- (preferred carbon source) and reduced buffering capacity against a rise in pH (Kaplan *et al.*, 1982). The ratio of CO_3^{2-} to HCO_3^- was often high in Lake Chitu, probably indicating the occurrence of HCO_3^- depletion and relatively reduced buffering capacity against rapid rise in pH caused by vigorous photosynthesis. The present high pH observed in Lake Chitu, coinciding with high algal biomass during the rainy season, and that observed in the productive soda lake Arenguade during periods of high rate of photosynthesis (Talling *et al.*, 1973), in spite of the lakes' high buffering capacity, support this conclusion. In contrast, the low $\text{CO}_3^{2-}:\text{HCO}_3^-$ ratio in Lake Shala suggests that its water is well buffered against a pH rise and surplus carbon source is present (abundant HCO_3^-).

Considering the high levels of SiO_2 commonly recorded for freshwater lakes, $>10 \text{ mg L}^{-1}$ (Talling and Talling, 1965; Talling, 1992), and that reported for Lakes Chitu and Shala previously, 222 and 56 mg L^{-1} , respectively (Elizabeth Kebede *et al.*, 1994), the SiO_2 concentration observed in the present study is remarkably low. The SiO_2 level of one of

the main hot springs located on the shore of Lake Shala, however, was high, $64.8 \pm 6.1 \text{ mg L}^{-1}$ ($n = 11$, SD), which is more than seventy fold higher than that recorded for the lake. These temporal and spatial variations of SiO_2 probably indicate the occurrence of some important processes within the lakes causing low SiO_2 level. Several studies made on tropical African lakes (e.g., Lemoalle, 1981; Hecky, 1993; Zinabu Gebre-Mariam, 2002) reported the association of SiO_2 depletion with the abundance of diatoms. Wood and Talling (1988) and Elizabeth Kebede *et al.* (1994) suggested that SiO_2 could be significantly removed from solution in Lake Shala, which was dominated by diatoms. In the present study, diatoms were found to be the persistently dominant taxa in terms of number of species (see chapter 3), although they were not the most abundant, in the turbulently mixing lake Shala and thus may be important removers of SiO_2 in the lake. This biological removal of SiO_2 is, however, unlikely in Lake Chitu as its phytoplankton community was dominated by *Arthrospira* with insignificant quantitative contribution of diatoms. Organic matter accumulation in the sediment, which inhibits dissolution rates of silicic acid from diatom frustules, as was reported for some alkaline lakes in East Africa (Hecky and Kilham, 1973), may have contributed to the low SiO_2 level in the productive lake Chitu. In addition, increased level of SiO_2 was recorded at the bottom in this study, supporting the proposal that reverse weathering, formation of aluminosilicate minerals in the sediment, removes SiO_2 and divalent cations (e.g. Ca^{2+}) from solutions in closed rift lakes of Ethiopia (Von Damm and Edmond, 1984). The very low or undetectable levels of Ca^{2+} and Mg^{2+} ions recorded in these lakes (Tadesse Ogato *et al.*, 2014) further support the likelihood of the phenomenon of reverse weathering.

The temporal and vertical variations of nutrients were generally very low in Lake Shala. Although Lake Shala has large catchments area, which imply high potential for external inputs, the low temporal and vertical variations in most nutrients could be related to the lake's large size and great depth, which make it less susceptible to environmental changes associated with hydrological events. As the lakes' region is characterized by rainfall deficit (Tenalem Ayenew and Dagnachew Legesse, 2007), the effect of hydrological events is also expected to be low. The depth profiles of temperature and chemical parameters generally suggest that Lakes Chitu and Shala are characterized by superficial thermal stratification, with small (in Lake Shala) to somewhat significant (in Lake Chitu) vertical differences in some chemical constituents, a pattern that was also previously reported by Baxter *et al.* (1965).

In general, most of the parameters of underwater climate, and thermal and chemical characteristics of Lake Chitu showed substantial temporal (on monthly basis) and vertical variability, which seem to be regulated by processes taking place within the lake. This shows that Lake Chitu can be very sensitive to changes in environmental conditions brought about by natural or human-induced processes. The observed environmental parameters of Lake Shala, on the other hand, showed small temporal and vertical variations, suggesting that Lake Shala could be more resilient to some environmental changes that may result from processes occurring in its large catchment area and within the lake.

CHAPTER 3 . PHYTOPLANKTON COMMUNITY COMPOSITION AND BIOMASS IN THE SODA LAKES CHITU AND SHALA, ETHIOPIA: TEMPORAL DYNAMICS IN RELATION TO ENVIRONMENTAL DRIVERS

3.1. INTRODUCTION

Phytoplankton are essential components of aquatic ecosystems as they are primary producers and often major ultimate energy sources for aquatic organisms of different trophic levels. The demand for phytoplankton is high in soda lakes, which are known to support grazer populations of high density. There is little growth of macrophytes and low allochthonous organic inputs. Some soda lakes, especially those in East Africa, are known for their high phytoplankton productivity and biomass due to superabundant populations of *Arthrospira* (*Spirulina*), and the associated high bird populations (Talling and Lemoalle, 1998; Oduor and Schagerl, 2007a; Okoth *et al.* 2009; Krienitz and Kotut, 2010) and thus provide great ecological, economic and scientific values. This alga is the main food source for the huge flocks of the Lesser Flamingos (*Phoeniconaias minor*) (Krienitz and Kotut, 2010). Although biological community structure in these African soda lakes has been demonstrated to fluctuate due to environmental modification resulting mainly from the seasonal alteration of hydrological conditions and human-induced disturbances (Wood and Talling, 1988; Oduor and Schagerl, 2007 b; Krienitz and Kotut, 2010; Talling, 2010), scientific studies on temporal patterns of phytoplankton are very limited (Talling and Lemoalle, 1998).

The soda lakes Chitu and Shala support large populations of avifauna including the Lesser and Greater Flamingos and Great White Pelicans. Lake Chitu is also known for its natural nearly monoalgal culture of *Arthrospira fusiformis* whose photosynthetic

productivity was previously reported to have reached the theoretical maximum of phytoplankton production for natural lakes (Talling *et al.*, 1973). Phytoplankton production is the basic energy source ensuring the perpetuation of aquatic biota and their services in these lakes. However, these lakes are among the Ethiopian Rift Valley lakes, which are currently facing increased ecological degradation (Zinabu Gebre-Mariam, 2002; Tenalem Ayenew and Dagnachew Legesse, 2007). The human-induced changes along with the natural ecological disturbances may have a profound impact on the biological components of their ecosystems, ultimately hampering all the ecosystem services they render.

Previous studies done on primary productivity and algal biomass of Lakes Chitu and Shala (Talling *et al.*, 1973; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Zinabu Gebre-Mariam, 2002) were sporadic and carried out for the purpose of comparison. Given the ever increasing human-induced impacts and the dynamic nature of biological variables, up to date data on various aspects of phytoplankton are among the important limnological information required for sustainable management and utilization of the lakes. The purpose of this study was, therefore, to investigate the seasonal variations in phytoplankton composition, abundance and biomass in relation to selected environmental parameters over an annual cycle in Lakes Chitu and Shala.

3.2. MATERIALS AND METHODS

3.2.1. Sampling, *in situ* measurements and analysis of environmental parameters

Detailed descriptions of the study lakes are given in chapter 1. Sampling procedures, sample preparations, and measurement of environmental parameters are described in

chapter 2. Composite samples were used for the identification of phytoplankton and estimation of their abundance and chlorophyll-*a* (chl-*a*) and dry weight biomass.

3.2.2. Identification of phytoplankton and determination of their abundance and biomass

For the identification of phytoplankton and estimation of their abundance, the composite samples were preserved with 4% formalin (recommended for highly alkaline lakes) in 125 ml (Lake Chitu sample) and 1 L (Lake Shala sample) bottles in triplicate. For the identification of diatoms, samples were cleaned with cold H₂O₂ cleaning technique (Taylor *et al.*, 2007). Phytoplankton taxa were identified to the genus or, when possible, to the species level using appropriate keys (e.g. Gasse, 1986; Komárek and Kling, 1991; Kelly, 2000; Komárek *et al.*, 2003; Bellinger and Sigee, 2010). Since Lake Chitu is highly productive, the composite sample was directly used for counting without concentration. But, for the unproductive Lake Shala, the preserved composite samples were concentrated in 1 L measuring cylinders for 48 hours in the dark to produce 10 times concentrated samples (concentration factor-10). Using aliquot samples, the cells of the major phytoplankton found in 40 randomly selected squares (grids) of the Sedgwick-Rafter chamber were counted in triplicates under an inverted microscope (Zeiss) at a magnification of 200× and 400×. For the filamentous form, *A. fusiformis*, filaments found in 100 fields were counted in triplicate at a magnification of 100×. The abundance of the major taxa was calculated using the equation in Hötzel and Croome (1999):

$$\text{Abundance (algal units ml}^{-1}\text{)} = \frac{N \times 1000 \text{ mm}^3}{A \times D \times F}; \text{ where,}$$

N = number of cells counted, A= area of field (mm²), D = depth of a field (mm) and F= number of fields counted.

Chlorophyll-*a* was determined spectrophotometrically using 90% acetone as an extraction solvent. A composite sample of appropriate volume (200–300 ml) was filtered through 47 mm diameter Whatman glass filter papers (GF/F) with the help of an electrically operated suction pump. To extract chl-*a*, the filters were left overnight in the dark after the addition of a small volume of 90% acetone. The filter was then ground using a tissue grinder and the extract was subsequently centrifuged at 3000 rpm for 10 minutes. The optical density (absorbance) of the extract was measured at 665 and 750 nm with UV-VIS spectrophotometer (model 6405, Jenway) and chl-*a* concentration, uncorrected for degradation products, was determined according to Talling and Driver (1963). For the determination of phytoplankton biomass as dry weight in Lake Chitu, composite samples of 200–300 ml were filtered in duplicate through pre-weighted Whatman filter paper (GF/F, 47 mm diameter), which were dried in an oven at 105 °C for 2 hours prior to filtration. The filter with algal seston was dried under the same conditions and weighted (Sartorius balance) again after cooling in a desiccator for 20 minutes. The algal biomass as dry weight (g L^{-1}) was calculated from the change in weight per volume of sample filtered.

3.2.3. Estimation of zooplankton abundance

Samples taken by vertical hauling from the euphotic depth using a plankton net (# 25, 64 μm) and preserved with 4% formalin solution were also used for the estimation of zooplankton abundance, which was used as an environmental variable for dynamics of phytoplankton in the euphotic zone. The major zooplankton taxa were randomly counted from aliquot samples in a Sedgwick-Rafter chamber and their abundance was estimated according to Edmondson and Winberg (1971).

3.2.4. Statistical analysis

Independent sample t-test was used to analyze the seasonal variations in abundance and biomass of phytoplankton. The relationships between algal and environmental variables were analyzed using Pearson correlation. Multiple regression analysis with stepwise procedure was applied to determine the environmental variables that best explain variations in algal biomass. These statistical tests were performed using the statistical program IBM SPSS Statistics (Version 20). The associations between species of phytoplankton and environmental variables were determined by direct gradient analysis using log-transformed species data. The lengths of the gradients were short (< 2) and thus the linear method, constrained redundancy analysis (RDA), was performed (Lepš and Šmilauer, 2003) using Canoco for Windows 4.5. The effect of multicollinearity was not differentiated in the RDA statistics.

3.3. RESULTS

3.3.1. Phytoplankton composition and abundance

The phytoplankton community in Lake Chitu was constituted by 9, 4 and 2 species of diatoms (Bacillariophyceae), cyanobacteria (Cyanophyceae) and cryptomonads (Cryptophyceae), respectively (Table 3.1), with the former two algal classes contributing about 60 and 27%, respectively, of the total number of species of the phytoplankton community in the lake (Fig. 3.1a). These algal classes were constituted largely by species that are capable of inhabiting saline water bodies. The cyanobacterium *A. fusiformis* occurred throughout the year while *Anabaenopsis abijatae* and other phytoplankton taxa were observed during the dry season, particularly when the biomass of the dominant species was reduced. The phytoplankton community of this lake was almost exclusively

constituted by *A. fusiformis*, with its high percentage contribution to total phytoplankton abundance (Fig. 3.1b) varying from 95 to 100% throughout the annual cycle (Fig. 3.1c). The quantitative importance of other taxa was almost negligible, with only very small contribution of diatoms (3–5%) occurring during the dry season (Fig. 3.1c). Abundance of *A. fusiformis* (trichomes ml⁻¹) exhibited seasonal variations, with large variations among months, ranging from 313 in March to 3167 in July (mean±SD, 1079±833), with the high levels occurring during the rainy months.

Phytoplankton community of Lake Shala comprised 23 species belonging to the algal classes Bacillariophyceae, Cryptophyceae, Cyanophyceae and Dinophyceae (Table 3.1). Bacillariophyceae, followed by Cryptophyceae, contributed the most to the number of species in the phytoplankton community, with 13 (56.4%) and 5 (21.7%) species, respectively (Fig. 3.2a). Among Bacillariophyceae, the genera *Cyclotella*, *Navicula*, *Nitzschia*, *Rhopalodia* and *Thalassiosira* spp. were dominant taxa, assemblage which are characteristic of alkaline-saline lakes. The genera *Cryptomonas* and *Glenodinium* solely represented the algal classes Cryptophyceae and Dinophyceae, respectively. The observed phytoplankton assemblage was persistent throughout the sampling period, with some changes in abundance. *Cryptomonas*, *Glenodinium*, *Nitzschia* and *Thalassiosira* spp. occurred as the most common taxa throughout the annual cycle. The phytoplankton community of the lake was generally dominated by Cryptophyceae, with percentage contributions to total phytoplankton abundance of about 69%, on the average, while the codominant Bacillariophyceae contributed only about 28% (Fig. 3.2b). The contribution of other classes to the total phytoplankton abundance in the lake was almost negligible.

Table 3.1. List of phytoplankton taxa identified in samples collected from Lakes Chitu and Shala during the study period.

Lake Chitu	Lake Shala
Class Cyanophyceae (blue-greens)	Class Cyanophyceae (blue-greens)
<i>Anabaenopsis abijatae</i> Kebede & Willén	<i>Anabaena cylindrica</i> Lemm.
<i>Arthrospira fusiformis</i> (Voronichin) Komárek et Lund	<i>Phormidium formosum</i> (Bory ex Gomont) Anagnostidis & Komárek
<i>Phormidium formosum</i> (Bory ex Gomont) Anagnostidis & Komárek	<i>Pseudanabaena</i> sp.
<i>Spirulina major</i> Kütz.	Class Bacillariophyceae (diatoms)
Class Bacillariophyceae (diatoms)	<i>Anomoeoneis sphaerophora</i> (Ehr.) Pfitz.
<i>Anomoeoneis sphaerophora</i> (Kütz.) Pfitzer	<i>Cyclotella iris</i> Brun et Heribaud
<i>Cyclotella meneghiniana</i> Kütz.	<i>Cyclotella meneghiniana</i> Kütz.
<i>Cyclotella</i> sp.	<i>Cyclotella</i> sp.
<i>Navicula</i> spp.	<i>Navicula</i> spp.
<i>Nitzschia</i> spp.	<i>Nitzschia</i> spp.
<i>Rhopalodia gibba</i> (Ehr.) O. Müller	<i>Rhopalodia gibba</i> (Ehr.) O. Müller
<i>Rhopalodia gibberula</i> (Ehr.) O. Müller	<i>Rhopalodia gibberula</i> (Ehr.) O. Müller
<i>Rhopalodia</i> sp. O. Müller	<i>Rhopalodia musculus</i> (Kütz.) O. Müller
<i>Synedra</i> sp.	<i>Rhopalodia vermicularis</i> O. Müller
Class Cryptophyceae (cryptomonads)	<i>Rhopalodia</i> sp. O. Müller
<i>Cryptomonas marssonii</i> Skuja	<i>Stephanodiscus</i> sp.
<i>Cryptomonas obovata</i> Skuja	<i>Thalassiosira</i> sp.
	Class Cryptophyceae (cryptomonads)
	<i>Cryptomonas marssonii</i> Skuja
	<i>Cryptomonas obovata</i> Skuja
	<i>Cryptomonas ovata</i> Ehr.
	<i>Cryptomonas reflexa</i> Skuja
	<i>Cryptomonas</i> sp.
	Class Chlorophyceae (green algae)
	<i>Oocystis</i> sp.
	Class Dinophyceae (dinoflagellates)
	<i>Glenodinium</i> sp.

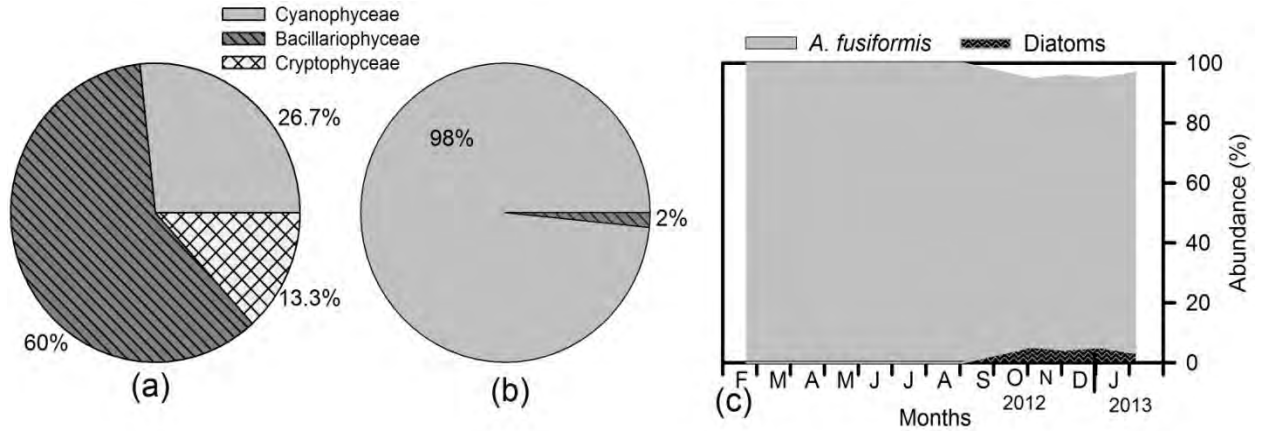


Fig. 3.1. Percentage contribution to the total number of phytoplankton taxa (a) and relative abundance of algal classes (b), and seasonal variations in the percentage abundance of the dominant taxa (c) in Lake Chitu.

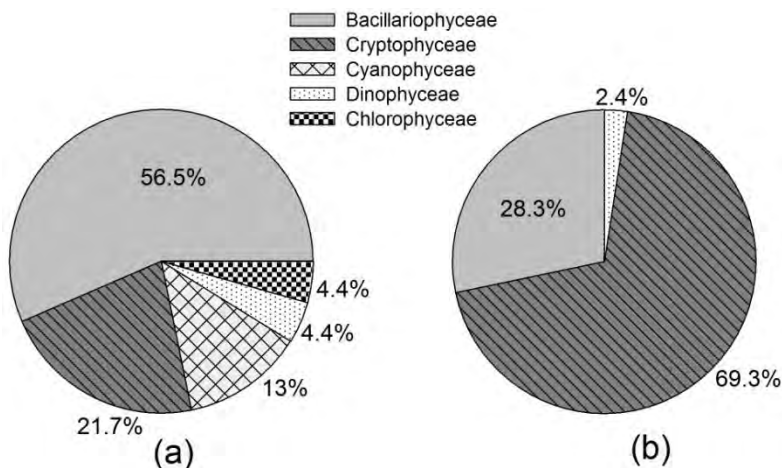


Fig. 3.2. Percentage contribution to the total number of phytoplankton species (a) and relative abundance (b) of phytoplankton taxa in Lake Shala.

In Lake Shala, Cryptophyceae was the most dominant taxon throughout the annual cycle, accounting for 59 to 95% of total phytoplankton abundance, except in January when its contribution was only 17% (Fig. 3.3a). The dominance of Cryptophyceae was due to *Cryptomonas* spp., whose abundance (cells ml^{-1}) ranged from a minimum of 1300 in January to a maximum of 8600 in April (Fig. 3.3b). Bacillariophyceae was the second most dominant taxon (3–39%) throughout the study period except in January of the dry

season, when its percentage abundance (83%) surpassed that of Cryptophyceae, with the small-sized planktonic centric diatom *Thalassiosira* sp. accounting for the largest proportion (82%) of its abundance. Abundance (cells ml⁻¹) of *Thalassiosira* sp. varied from 72 to 6534 with its maximum abundance occurring in January (Fig. 3.3b), while that of *Nitzschia* spp. increased from a minimum of 60 in January to a maximum of 521 in June of the rainy season (Fig. 3.3b) although its relative seasonal contribution was very small (1–14%). Although Dinophyceae, represented solely by *Glenodinium* sp., prevailed in all seasons with peak of abundance (430 cells ml⁻¹) occurring in July of the rainy season (Fig. 3.3b), its contribution to the total abundance of phytoplankton was the least (0.4–9%).

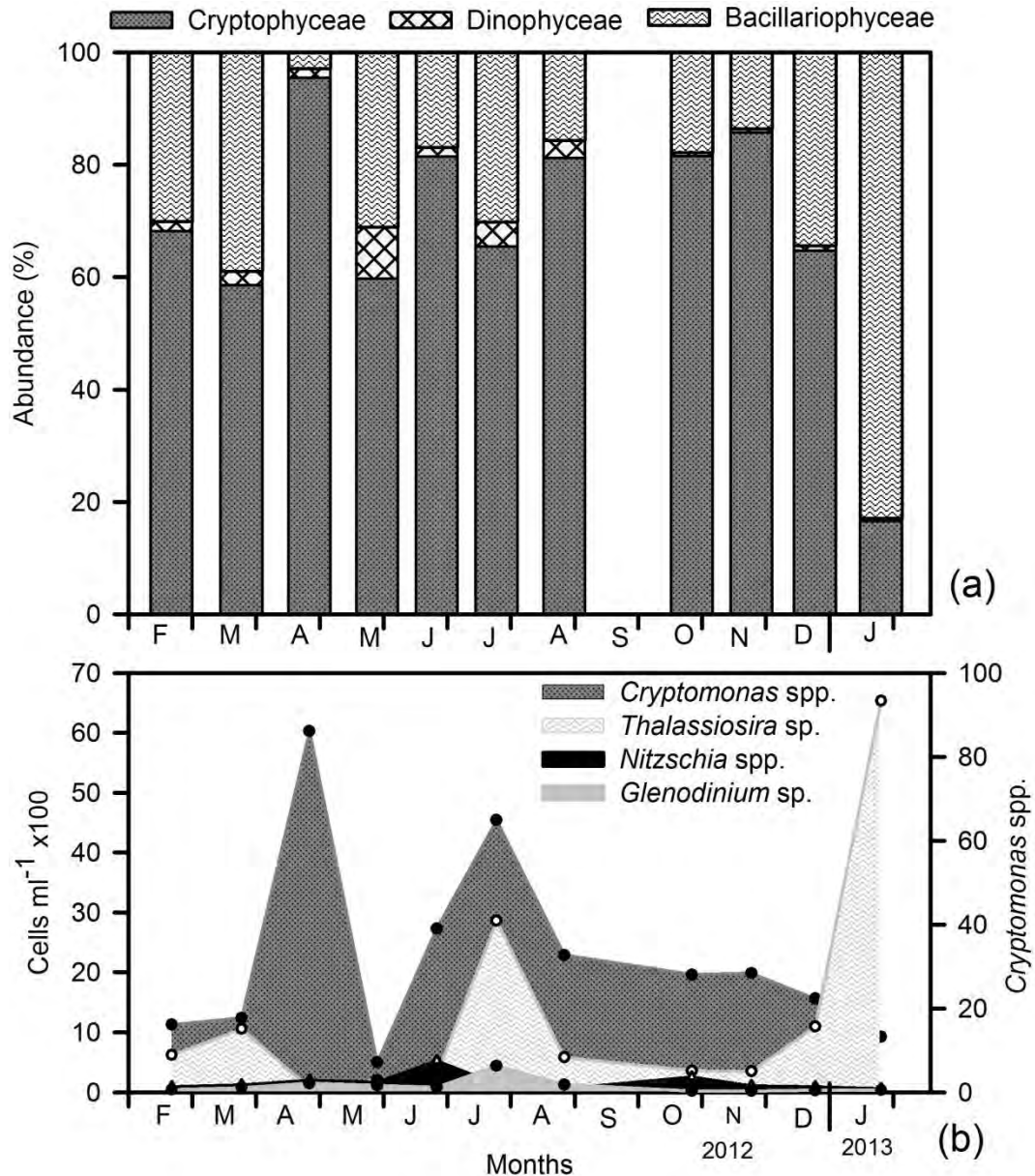


Fig. 3.3. Temporal variations in the percentage contributions of the major classes to total phytoplankton abundance (a) and abundance (cells ml⁻¹) of dominant species (b) of the phytoplankton community in Lake Shala.

3.3.2. Phytoplankton biomass

Biomass as chl-*a* and dry weight of phytoplankton in Lake Chitu showed seasonal variation (Fig. 3.4). Chl-*a* ranged from a minimum of about 72 µg L⁻¹ in February to a maximum of 234 µg L⁻¹ in August. Dry weight exhibited a seasonal trend similar to that

of chl-*a* biomass, with its lowest and highest values of 0.13 and 0.37 g L⁻¹ occurring in February of the dry period and August of the major rainy period, respectively. The differences in the levels of these parameters between the rainy and dry seasons were significant ($P < 0.01$ for dry weight and $P < 0.05$ for chl-*a*). The temporal changes in dry weight and chl-*a* biomass were broadly similar to those of the abundance of *A. fusiformis* (Fig. 3.4), with their peak values occurring during the rainy season when dense algal biomass was formed on the lake's surface. The correspondence of chl-*a* and dry weight to each other and to *Arthrospira* abundance is corroborated by their significant correlation with each other ($r = 0.76$, $P < 0.01$) and by their suggestive correlation ($r = 0.47$) with the abundance of *A. fusiformis*.

The level of algal biomass in Lake Shala was generally low and showed small seasonal variation with slight increasing trend occurring during the rainy season (Fig. 3.5). The total abundance of phytoplankton exhibited a seasonal pattern that was similar to that of biomass, a trend, which was also reflected in their strong significant correlation ($r = 0.90$, $P < 0.01$). Chl-*a* and abundance of phytoplankton ranged from 5.4 to 38.0 µg L⁻¹ (16.6 ± 10.6 , mean $\pm$ SD) and from 1190 to 9920 cells ml⁻¹ (4771 ± 2910 , mean $\pm$ SD), respectively. Both parameters were at relatively high levels during the major rainy period and reached their largest peak in July, but remained at low levels during the minor rainy and most dry months.

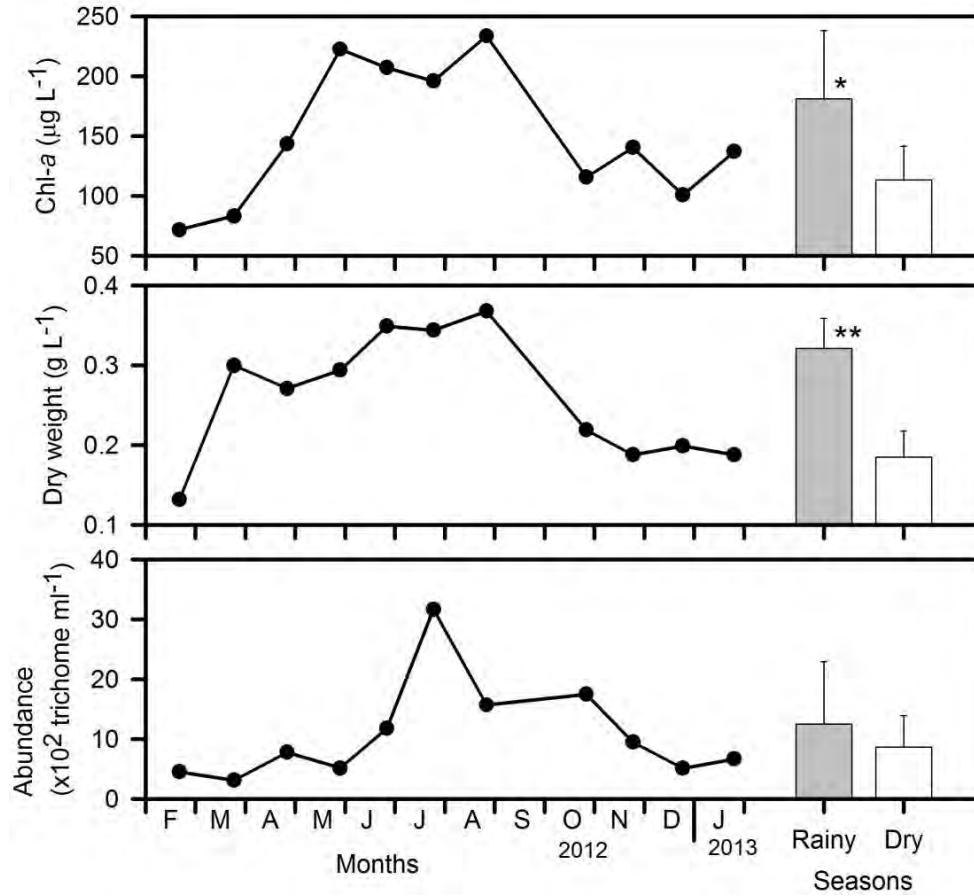


Fig. 3.4. Temporal variations in biomass (as chl-*a* and dry weight) and abundance of *A. fusiformis* among months (line plot) and between seasons (bar plot) in Lake Chitu. *significant at $P < 0.05$; ** significant at $P < 0.01$.

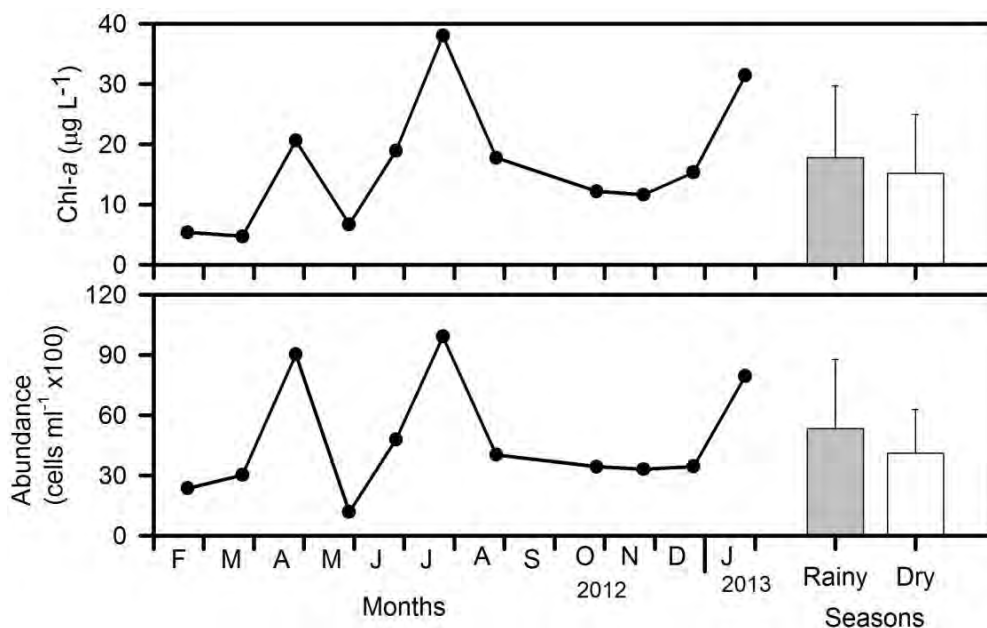


Fig. 3.5. Temporal variations in biomass (as chl-*a*) and abundance of phytoplankton among months (line plot) and between seasons (bar plot) in Lake Shala.

3.3.3. Relation of abundance and biomass of phytoplankton to environmental variables

Descriptions of physicochemical parameters of the lakes are given in chapter 2. As indicated in the Pearson correlation analyses for Lake Chitu (Table 3.2), all the three biological parameters (abundance, chl-*a* and dry weight) had strong to very strong correlations (r values ranging from 0.4 to 0.9) with such environmental variables as rainfall, alkalinity, salinity, K_{PAR} and rotifer abundance. The correlations of abundance, chl-*a* and dry weight with rainfall were significant ($P < 0.05$) and positive. Their correlations were positive with K_{PAR} and abundance of rotifers, but negative with salinity and alkalinity. Abundance of *A. fusiformis* was also significantly ($P < 0.01$) positively correlated with DO. The temporal variations in the biomass and abundance of *A. fusiformis* were strongly associated with the temporal trends of rainfall, alkalinity, salinity and K_{PAR} (Fig. 3.6). The pronounced increases in these algal parameters during

the rainy months corresponded to the notable decreases in alkalinity and salinity, and increases in rainfall and K_{PAR} during this period.

Biomass and total abundance of phytoplankton in Lake Shala showed strong correlations with some environmental variables (Table 3.3). Biomass was significantly ($P < 0.05$) negatively correlated with water transparency, but positively with surface water DO. Alkalinity and pH were also correlated strongly and positively with biomass though the correlations were not statistically significant. Total phytoplankton abundance also showed similarly strong correlations with these variables. Although its correlation was not significant ($r = -0.36$, $P > 0.05$), zooplankton density was correlated negatively with chl-*a*, indicating its potential influence on algal biomass in this lake. Stepwise multiple regression analysis of the environmental variables (variables entered in the model at $P < 0.05$) revealed that water transparency was the most important explanatory variable (47%, $P = 0.02$) for the variations in phytoplankton biomass. The seasonal changes in water transparency in Lake Shala corresponded well to the seasonal changes in algal biomass and abundance (Fig. 3.7), indicating its importance for the dynamics of these algal parameters in the lake.

Table 3.2. Pearson correlation coefficients for abundance of *A. fusiformis* and phytoplankton biomass and some environmental variables in Lake Chitu. The environmental variables with $r \geq \pm 0.40$, with at least one of the algal parameters, are indicated.

	Abundance	Chl- <i>a</i>	Dry weight	K _{PAR}	Rainfall	Rotifers	DO	Alkalinity
Chl- <i>a</i>	0.46							
Dry weight	0.47	0.76**						
K _{PAR}	0.80**	0.56	0.43					
Rainfall	0.61*	0.83**	0.92**	0.52				
Rotifers	0.49	0.40	0.40	0.56	0.52			
DO	0.79**	0.31	0.33	0.53	0.53	0.78**		
Alkalinity	-0.74**	-0.40	-0.43	-0.62*	-0.6*	-0.8**	-0.87**	
Salinity	-0.77**	-0.61*	-0.56	-0.68*	-0.72*	-0.75**	-0.76**	0.82**

*significant at 0.05, **significant at 0.001

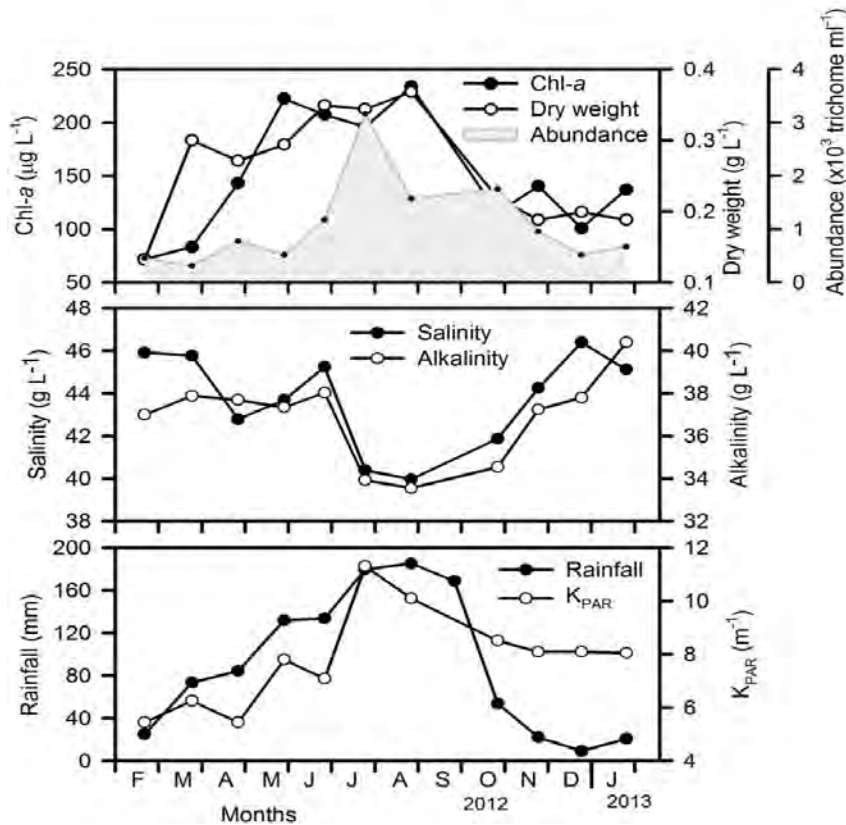


Fig. 3.6. Temporal variations in the three algal parameters in relation to the strongly correlated environmental variables in Lake Chitu.

Table 3.3. Pearson correlations for biomass and total abundance of phytoplankton and some environmental variables in Lake Shala. The environmental variables with r values $\geq \pm 0.40$, with at least one of the phytoplankton parameters, are indicated.

	Abundance	Chl- <i>a</i>	Z_{SD}	K_{PAR}	DO	pH	Alkalinity
Chl- <i>a</i>	0.90**						
Z_{SD}	-0.57	-0.68*					
K_{PAR}	0.30	0.58	-0.86**				
DO	0.55	0.65*	-0.48	0.48			
pH	0.45	0.54	-0.74**	0.77**	0.21		
Alkalinity	0.51	0.52	-0.58	0.58	0.69*	0.36	
SRP	0.52	0.18	0.03	-0.29	0.41	-0.20	0.49

*significant at 0.05; **significant at 0.01

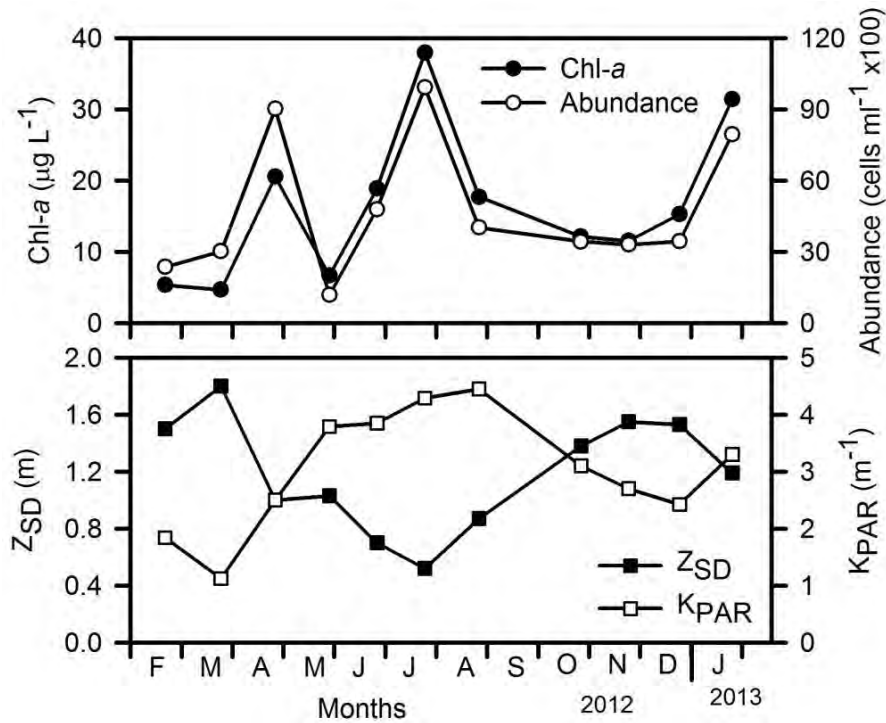


Fig. 3.7. Temporal variations in phytoplankton biomass and abundance in relation to parameters of underwater light climate in Lake Shala.

3.3.4. Redundancy analysis for species-environment relations in Lake Shala

The RDA showed that the first two axes (axis 1 and axis 2) sufficiently (82.3%) explained the cumulative percentage variance in the phytoplankton species-environmental variables relation in Lake Shala (Table 3.4). Axis 1 explained 52.4% of the variance, with which the environmental variables such as SiO_2 and salinity were strongly correlated, but contrasting with each other (Table 3.4, Fig. 3.8). The diatoms *Nitzschia* spp. and *Thalassiosira* sp. had strong but contrasting relationships with environmental gradients of SiO_2 and salinity along axis 1; the former occupied the gradient with low SiO_2 and increased salinity while the latter occupied the gradient with reversed conditions. *Nitzschia* spp. was also positively but moderately correlated with SRP. Axis 2 explained 29.9% of the variance, which was strongly but negatively correlated with water transparency (Z_{SD}) and strongly and positively correlated with pH and rainfall (Table 3.4; Fig. 3.8). Along axis 2, *Cryptomonas* spp. and *Glenodinium* sp. were strongly associated with low water transparency, and high rainfall and pH. Zooplankton, though it had negative relationship with *Thalassiosira* sp., exhibited low influence on the phytoplankton community in Lake Shala.

Table 3.4. Results of RDA of species-environmental variables relationships and correlations of environmental variables with the first two axes in Lake Shala.

Parameters	Axis 1	Axis 2
Eigenvalues	0.524	0.299
Cumulative % variance of species-environment relation	52.4	82.3
Z _{SD}	0.14	-0.81
K _{PAR}	-0.06	0.33
PAR	0.27	0.34
Rainfall	-0.39	0.67
Temperature	-0.10	-0.20
DO	0.07	0.23
pH	0.13	0.75
Salinity	-0.48	0.10
Alkalinity	-0.26	0.17
CO ₃ ²⁻ + HCO ₃ ⁻ ions	-0.12	-0.34
NO ₃ ⁻ + NH ₃	-0.24	-0.40
SRP	-0.32	0.04
SiO ₂	0.51	0.08
Zooplankton	-0.12	-0.26

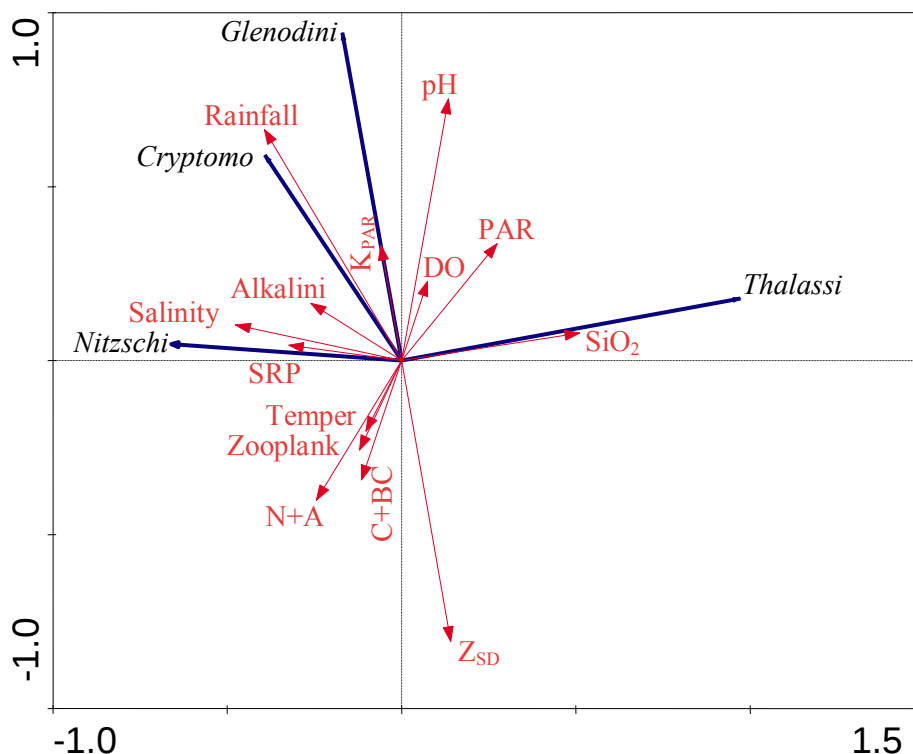


Fig. 3.8. RDA ordination biplot of species-environment variables for the first two axes, axis 1(horizontal) and axis 2 (vertical), in Lake Shala. Crptomo = *Cryptomonas* spp., Glenodini = *Glenodinium* sp., Thalassi = *Thalassiosira* sp., Nitzschi = *Nitzschia* spp., Z_{SD} = Secchi transparency, K_{PAR} = Vertical attenuation coefficient, PAR = Photosynthetically active radiation, Alkalini = alkalinity, SRP = soluble reactive phosphate, N+A = nitrate plus ammonia, C+BC = carbonate plus bicarbonate ions, Temper = water temperature, Zooplank = zooplankton.

3.4. DISCUSSION

3.4.1. Phytoplankton composition, abundance and biomass

The recorded low number of phytoplankton species and the exclusive overwhelming dominance of *A. fusiformis* in Lake Chitu indicate low algal species diversity. However, algal biomass was generally high in the lake. This suggests that low species diversity with superabundant populations of *A. fusiformis* and the resulting high biomass are characteristic of this soda lake, while reflecting the fact that its phytoplankton community

was subjected to stressful environmental conditions associated with its high salinity ($44 \pm 2.2 \text{ g L}^{-1}$, mean $\pm$ SD). Low phytoplankton diversity associated with predominance of *Arthrospira* and the resulting high phytoplankton biomass is a salient feature of several *Arthrospira*-rich soda lakes of East Africa including Lakes Nakuru and Bogoria in Kenya (Ballot *et al.*, 2004a; Okoth *et al.*, 2009; Kaggwa *et al.*, 2013a). In these soda lakes, the phytoplankton communities consisted of about 8 to 12 species with the dominance of *Arthrospira* in the overall algal biomass, contributing up to 98% (Okoth *et al.*, 2009; Kaggwa *et al.*, 2013a). The extreme environment of soda lakes, particularly the unusually high salinity, has been implicated as the main factor controlling community structure (Wood and Talling, 1988; Schagerl and Oduor, 2008), causing low diversity with dominance of organisms with high adaptive capacity to osmotic stress (Williams, 1998; Jones and Grant, 1999). These saline-alkaline conditions coupled with large reserves of inorganic carbon and phosphorus, and the favorable tropical climate lead to the dominance of *Arthrospira* spp. and the associated high algal biomass in most tropical soda lakes (Talling *et al.*, 1973; Melack *et al.*, 1982; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Jones and Grant, 1999).

The phytoplankton community of Lake Shala consisted of species characteristic of saline-alkaline lakes, although it lacked the species typically inhabiting tropical soda lakes-*Arthrospira*. The observed number of phytoplankton species of this lake is suggestive of the occurrence of a relatively diverse phytoplankton community as compared to those reported for the *Arthrospira*-dominated soda lakes of East Africa including Lakes Nakuru and Bogoria in Kenya (Ballot *et al.*, 2004a; Okoth *et al.*, 2009; Kaggwa *et al.*, 2013a) and Lake Chitu in Ethiopia. This could be due to the relatively low salinity of Lake Shala

($15.7 \pm 0.4 \text{ g L}^{-1}$), in comparison with the more saline soda lakes, as biological diversity generally decreases with an increase in salinity (Seaman *et al.*, 1991; Williams, 1998, 2002).

It is well established that seasonal successions in phytoplankton community are expected in water bodies with large and clear variability in environmental drivers including major algal nutrients, light availability, temperature, grazing pressure, water mixing regimes and changes in the hydrological pattern (Reynolds *et al.*, 2001; Schagerl and Oduor, 2008). Changes in phytoplankton composition and density, and occasional disappearance of *Arthrospira* with large and rapid variations in salinity have been documented for several soda lakes including Nakuru, Bogoria and Elmenteita (Vareschi, 1982; Melack, 1988; Shagerl and Oduor, 2008; Kaggwa *et al.*, 2013a). The species composition of phytoplankton in Lakes Chitu and Shala did not, however, exhibit distinct seasonal successions though some seasonal differences in the abundance of the dominant species were observed. The lack of defined seasonal successions is probably due to the muted seasonality in the major environmental drivers. Salinity, for example, exhibited very small seasonal variations ($40\text{--}46 \text{ g L}^{-1}$ in Lake Chitu and $14.7\text{--}16.4 \text{ g L}^{-1}$ in Lake Shala). The water column of Lake Shala was also continuously mixing, a major factor regulating the seasonal succession pattern of phytoplankton (Reynolds, 1996). Such a continuously mixing condition promotes the persistent dominance of species adapted to mixing such as diatoms, while hindering the success of taxa with less adaptability to mixing such as cyanobacteria (e.g. *Arthrospira*). Thus, the dominance of species adaptable to water column mixing in Lake Shala seems to suggest the overriding importance of mixing regime in determining phytoplankton composition in this lake.

Small-sized phytoplankton taxa such as cryptomonads and diatoms were dominant and persistent throughout the annual cycle in terms of both number of species and numerical abundance in Lake Shala. The dominance of these species could be due to their adaptations to stressful ambient conditions of the lake; low underwater light climate, low nitrogenous nutrients and turbulently mixing water column conditions (see chapter 2). The main adaptation that led to their success seems to be their small size and hence their large surface area to volume ratio, which enables them to efficiently utilize limiting resources thereby maintaining fast growth and high rate of photosynthesis (Finkel *et al.*, 2010; Allen and Polimene, 2011). The dominance of the flagellated taxa, cryptomonads, may have also resulted from their capability to migrate in the water column, enabling them to scavenge nutrients from the nutrient-rich deeper light-limited waters (Hansson *et al.*, 1994; Reynolds, 1996). In addition, some cryptomonads successfully grow at greater depths where light-limited conditions prevail because they possess the red accessory pigment phycoerythrin, which broadens the waveband within which they can harvest photons for their photosynthetic activity (Goodwin, 1974), and have the ability to grow mixotrophically (Caron *et al.*, 1993; Sinistro *et al.*, 2006). Moreover, increased mixing that assists in counteracting the problem of loss due to fast sinking (Reynolds, 1984; Estrada and Berdalet, 1997; Sullivan and Swift, 2003), high level of SRP (Reynolds, 1984) and the ability to utilize low level of nitrogenous nutrients, i.e., having lower half-saturation constant for nitrate and ammonium uptake (Thomas *et al.*, 1978) are among the conditions favoring growth of diatoms.

Previous studies in Lake Shala have reported the overall dominance of diatoms of the phytoplankton community (Gasse *et al.*, 1983; Elizabeth Kebede *et al.*, 1994). In the

present study, diatoms were the second most abundant phytoplankton although they contributed larger number of species. This is the first study to report the dominance of cryptomonads in Lake Shala, and is probably suggesting that the lake's phytoplankton community is in the transition from diatom-dominated to flagellate (cryptomonad)-dominated community. Such qualitative changes in phytoplankton community may be suggestive of changes in ecosystem conditions (Paerl *et al.*, 2007). SiO₂ concentration, the main limiting nutrient for diatoms, for example, was dramatically reduced from a previously reported high level (56 mg L⁻¹; Elizabeth Kebede *et al.*, 1994) to 0.9 mg L⁻¹ and may have resulted in the observed shift to dominance by cryptomonads of the phytoplankton community in Lake Shala. Diatoms are generally outcompeted by flagellates at low levels of SiO₂ (Egge and Aksnes, 1992), but the concentration of SiO₂ was still above the level known to be limiting for the growth of diatoms, 2 µM or 0.12 mg L⁻¹ (Egge and Aksnes, 1992). Thus, diatoms may remain highly competitive in the phytoplankton community of Lake Shala, as was observed in samples of January of the present study, provided the level of SiO₂ does not drop below the threshold concentration.

The diatoms assemblage in Lake Shala was constituted primarily by the haloalkalophilic species known to occur in soda lakes of East Africa with high alkalinity-salinity and pH (Hecky and Kilham, 1973; Gasse, 1986). Diatom species such as *Anomoeoneis sphaerophora* (Ehr.) Pfitz., *Cyclotella meneghiniana* Kütz., *Navicula elkab* O. Müller, *Nitzschia frustulum* (Kütz.) Grmlow, *Nitzschia sigma* (Kütz.) W. Smith and *Thalassiosira rudolfii* (Bachm.) Hasle are quantitatively important and achieve dominance in soda lakes of East Africa (Hecky and Kilham, 1973). Among the diatoms, *Thalassiosira* sp. was

numerically dominant throughout the present study in Lake Shala, outcompeting even *Cryptomonas* spp. on one occasion. *Thalassiosira rudolfii* was previously reported to grow optimally and achieve high percentage contribution in hyper-alkaline African lakes including Lake Shala (Gasse *et al.*, 1983; Gasse, 1986). *Thalassiosira* species are known for their adaptation to low light intensity (Sakshaug *et al.*, 1987; Popovich *et al.*, 2008) and ability to store nitrogen in cells (Dortch *et al.*, 1984), which might have favored its dominance in the light- and nitrogen-limited lake Shala. Although not comparable to *Thalassiosira* sp., the benthic diatoms *Nitzschia* spp., were also important in Lake Shala, probably because of their ability to attain planktonic life in mixing lakes (Hecky and Kilham, 1973). The dinoflagellate *Glenodinium* sp. also occurred throughout the study period in Lake Shala though its contribution to total phytoplankton abundance was very low. Dinoflagellates, in general, are more sensitive to turbulence than diatoms (Thomas *et al.*, 1995) and may not, therefore, be able to compete with diatoms in turbulently mixing lakes like Lake Shala.

The observed seasonal fluctuation in the abundance and biomass of phytoplankton in Lake Chitu is consistent with the results of the studies made on Kenyan soda lakes, which demonstrated the occurrence of large temporal fluctuations and crashes of *Arthrospira* spp. biomass (Schagerl and Oduor, 2008; Okoth *et al.*, 2009; Krienitz and Kotut, 2010; Kaggwa *et al.*, 2013a). These seasonal fluctuations were often attributed to the seasonal hydrological changes and the resulting effects on the chemical constituents, specifically salinity (Wood and Talling, 1988; Kalff, 2002; Schagerl and Oduor, 2008). The level of chl-*a* observed in Lake Shala is indicative of low algal biomass production, a finding consistent with the results reported previously for the same lake (about 15–18 µg chl-*a* L⁻¹

¹, Elizabeth Kebede *et al.*, 1994; Zinabu Gebre-Mariam, 2002). The small seasonal variation in abundance and biomass with slight increases during the rainy season in Lake Shala is probably linked to the muted seasonality of the environmental variables. The strong correlations among the three algal parameters (chl-*a*, dry weight and abundance of *A. fusiformis*) in Lake Chitu seem to indicate the insignificant contribution of non-algal components to the measured dry weight and the large contribution of *A. fusiformis* to the phytoplankton biomass in the lake. We, therefore, contend that chl-*a* and dry weight are good indices of *A. fusiformis* biomass in this lake though the contribution of the rare algal species and bacteria to the biomass estimates cannot be ruled out. The very strong correlation observed between chl-*a* and total abundance of phytoplankton in Lake Shala suggests that the dominant phytoplankton species could be representative of the total phytoplankton biomass in the lake.

3.4.2. Relations of abundance and biomass of phytoplankton to environmental variables

The environmental variables such as nutrients, salinity, light and seasonal hydrological events have been demonstrated to regulate biological community structure in African soda lakes (Wood and Talling, 1988; Oduor and Schagerl, 2007b; Krienitz and Kotut, 2010; Talling, 2010). The observed strong correlation of algal abundance and biomass with rainfall, alkalinity and salinity in Lake Chitu suggests the importance of these variables in controlling the algal abundance and biomass. Rainfall generally affects limnological properties and phytoplankton dynamics in lakes through its effect on nutrient loading and salinity (Kalff, 2002). The negative correlation of abundance of *A. fusiformis* and phytoplankton biomass with alkalinity and salinity indicates the

antagonistic effect of these variables on the algal parameters. The high level of salinity like that found in Lake Chitu may hamper the growth and biomass production of *A. fusiformis* despite the alga's capacity to adapt to a wide range of salinities (Vareschi, 1982; Vonshak *et al.*, 1988; Elizabeth Kebede, 1997). The considerable increase in the abundance and biomass observed in the rainy season, during the time salinity of the lake water was reduced mainly by rainfall-related dilution, seems to corroborate the probable effect of high salinity of the lake on algal abundance and biomass. This is also consistent with the finding that increased growth and biomass of *A. fusiformis* in laboratory cultures were achieved when the salinity of the soda lakes Chitu and Shala was reduced through dilution (Tadesse Ogato *et al.*, 2014). Considerable reduction in growth with an increase in salinity was also observed in several other laboratory experiments of *Arthrospira* spp. (Vonshak *et al.*, 1988; Elizabeth Kebede, 1997; Mussagy, 2006; Krienitz *et al.*, 2013). Zeng and Vonshak (1998) suggested that the reduction in growth at high salinity is associated with the decrease in photosystem II activities due to osmotic shock.

Regardless of the high incident radiation at lakes' surface, light-limitation within a water body is considered a major factor reducing productivity in tropical lakes (Talling, 2001) including the Ethiopian rift valley lakes (Zinabu Gebre-Mariam, 2002). It has been observed in outdoor cultures of *Arthrospira* spp. that solar radiation is highly absorbed in the upper layer of 30–40 cm depth at high cell densities, causing some 70% of the cells beneath the surface to be light-limited (Richomond *et al.*, 1980). Lake Chitu had high light attenuation coefficient (mean K_{PAR} , 8 m^{-1} and K_{algae} , 32%), which was caused by the accumulation of huge biomass of *A. fusiformis* in the near-surface zone, particularly during the rainy season. In a previous study, Elizabeth Kebede *et al.* (1994) also observed

poor water transparency, which could be limiting algal production in Lake Chitu. However, algal parameters measured in the present study showed strong positive correlation with water transparency. Schagerl and Oduor (2008) similarly observed positive relationship between light attenuation and *Arthrospira* biomass in some African soda lakes, with high light attenuation associated with algal biomass accumulation. This seems to suggest that low water transparency of *Arthrospira*-rich soda lakes is not a limiting factor for *Arthrospira* owing to its ability to maintain its position in the well-illuminated surface zone through buoyancy regulation and to the presence of accessory pigments that broaden the waveband within which light can be harvested by algal cells. Shallow mixing regime (chapter 2), which promotes cyanobacterial growth (Reynolds, 1987), and the consequent accumulation of *A. fusiformis* in the near-surface water of Lake Chitu probably resulted in light limitation that precluded the success of other phytoplankton taxa.

Similarly, the observed strong but inverse relationship between biomass and water transparency in Lake Shala suggests the importance of water transparency of the lake (chapter 2) in controlling phytoplankton biomass and its seasonal variations. The results of the present study contrast with the observations made previously for the same lake; decreased chl-*a* associated with more light-limitation (low water transparency) during the rainy season (Zinabu Gebre-Mariam, 2002). This is probably because of the shift in dominance to species adapted to low light condition, with consequent increase in the biomass of phytoplankton at times of low water transparency. Low water transparency, however, might have hindered the production of other phytoplankton species less adapted to low light conditions, resulting in low overall phytoplankton biomass in Lake Shala.

Light-limitation associated with background turbidity and deep mixing of a water column, a physical condition characteristic of Lake Shala, has been shown to reduce algal biomass in several lakes (Reynolds, 1987; Knowlton and Jones, 1996; Diehl *et al.*, 2002) and influence phytoplankton production and composition in tropical lakes (Talling, 2001).

Availability in adequate amounts of the major algal nutrients nitrogen and phosphorus enhances biomass production of algae (Melack *et al.*, 1982; Larned, 1998; Miller *et al.*, 1999). These nutrients did not, however, show notable relationship with algal biomass in both lakes, probably signifying absence of pronounced nutrient limitation despite the low levels of nitrogen sources in the euphotic zone. NH_3 was higher at deeper depths in Lake Chitu (chapter 2) and showed positive relationship with abundance of *A. fusiformis*, probably suggesting that release of NH_3 from the anoxic zone could be an important source of nitrogen for the massive growth of *A. fusiformis* in the lake. It has also been demonstrated in a laboratory study that *Arthrospira* spp. can preferentially take up nitrogen in the form of NH_3 at high pH (Boussiba, 1989).

Zooplankton grazing has commonly been reported to influence the seasonal variation in phytoplankton biomass in several lakes (Carpenter *et al.*, 1993; Stoyneva *et al.*, 2007) and seems to have the potential to do so in soda lakes (Vareschi and Vareschi, 1984). Although the correlation was not significant, the relationship between biomass and zooplankton density in Lake Shala suggests the potential of zooplankton grazing to exert top-down control of phytoplankton. Conversely, the abundance of rotifers in Lake Chitu was positively correlated with algal biomass and *Arthrospira* abundance, a finding that is consistent with some soda lakes in Kenya (e.g., Lakes Nakuru and Bogoria), where

Arthrospira can be an important control of the grazer density (Varesch, 1982; Ballot *et al.*, 2004a; Kaggwa *et al.*, 2013a). This is because the influence of zooplankton is minimal on *Arthrospira*-dominated phytoplankton community due to the low zooplankton density and the high phytoplankton biomass dominated by the filamentous cyanobacteria (Vareschi, 1982). The strong direct association of DO with phytoplankton biomass and abundance in Lake Shala and with the abundance of *A. fusiformis* in Lake Chitu, might have resulted from the increased DO production.

3.4.3. Redundancy analysis for species-environment relation in Lake Shala

Results of RDA for Lake Shala showed strong association of the observed phytoplankton species with water transparency, pH, rainfall, SiO₂ and salinity. *Cryptomonas* and *Glenodinium* spp. were associated with low water transparency, which could be due to their adaptations to low light conditions of the study lake (Finkel *et al.*, 2010; Allen and Polimene, 2011). Water-column transparency has been shown to influence algal community structure in soda lakes (Melack, 1988; Talling, 1992), promoting species which can grow in low light conditions. *Cryptomonas* and *Glenodinium* spp. were also associated with increased pH and rainfall. Rainfall was negatively correlated with water transparency ($r = -0.80$) and positively with pH ($r = 0.88$), probably suggesting that change in water transparency and pH of the lake by hydrological conditions could be an important regulator of the dynamics of flagellates in this lake. Since the success of most flagellates is associated with their adaptations to low light conditions (Finkel *et al.*, 2010; Allen and Polimene, 2011), water transparency probably overrides other variables in regulating the seasonal dynamics of the flagellates in this lake.

The diatoms *Thalassiosira* and *Nitzschia* spp. showed strong association with SiO₂ and salinity. The observed strong association of *Thalassiosira* sp. with increased SiO₂ at low salinity suggests that decreasing SiO₂ level may limit growth of *Thalassiosira* sp., creating favorable conditions for other algal competitors that do not require SiO₂. This was also evidenced by the dominance of *Thalassiosira* sp. in January coinciding with increased SiO₂ level. Wallace and Gobler (2014) also reported strong association of diatoms and SiO₂ in Jamaica Bay, in which *Thalassiosira* sp. was limited by the low level of SiO₂. Decreasing salinity might have favored SiO₂ uptake by *Thalassiosira* sp. as salinity affects uptake of SiO₂ (Olsen and Paasche, 1986). In contrast, the observed association of *Nitzschia* spp. with low SiO₂ concentration at increased salinity suggests that *Nitzschia* spp. may grow at low level of SiO₂ and increased salinity. This corroborates the contention that smaller diatoms have low SiO₂ requirement and may grow at low SiO₂ level ($< 0.005 \text{ mg L}^{-1}$) when SRP is high (Willén, 1991). The level of SRP was high in Lake Shala (low Si:P, 0.9) and *Nitzschia* spp. were positively correlated with it. *Nitzschia* spp. were probably favored by the increasing salinity owing to their ability to grow over a wide range of salinities (Zhang *et al.*, 1999; Blinn *et al.*, 2004). Diatoms, therefore, seem to have an important contribution to the removal of SiO₂ from Lake Shala water, as reported previously (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994). The importance of diatoms in effectively controlling SiO₂ concentration was also reported in several African soda lakes (Hecky and Kilham, 1973) and non-soda lakes (Lemoalle, 1981; Hecky, 1993).

In conclusion, the phytoplankton community of Lake Chitu was almost exclusively constituted by *A. fusiformis*, making this cyanobacterium the key biological component

of the lake. Algal biomass and abundance of *A. fusiformis* showed seasonality following the variability in some environmental drivers, mainly alkalinity and salinity. The relatively diverse phytoplankton community of Lake Shala lacked perceptible species succession and marked variability in its generally low biomass, probably due to the muted seasonality of environmental drivers. Cryptomonads and diatoms, which have special adaptive strategies for the prevailing low light and mixing conditions of the lake, persistently dominated the phytoplankton community throughout the annual cycle, suggesting the overriding importance of underwater light climate and hydrographic condition of the water column in controlling composition and biomass of phytoplankton in Lake Shala. The lack of considerable seasonality in the composition and biomass of phytoplankton of Lake Shala is probably because of the lake's insensitivity (i.e. high stress absorbing capacity) to the current level of changes in environmental drivers.

CHAPTER 4 . MORPHOLOGICAL VARIABILITY OF *ARTHROSPIRA FUSIFORMIS* IN RELATION TO ENVIRONMENTAL VARIABLES IN THE TROPICAL SODA LAKE CHITU, ETHIOPIA

4.1. INTRODUCTION

Arthrospira spp. are distinguished mainly by such distinctive morphological characteristics as regular helical coiling of their trichome, which is composed of shorter and broader cells with cross-walls visible under light microscopy. The morphological characteristics of *Arthrospira* have been used as taxonomic criteria in traditional phenotypic classification of this taxon (Lewin, 1980; Desikachary and Bai, 1996; Vonshak and Tomaselli, 2000), and in selecting high-quality strains and ranking of product quality (Amha Belay, 1997; Wu *et al.*, 2005). In addition, these parameters serve as important indicators of culture status for the maintenance of unialgal cultures (Amha Belay, 2008) and harvesting (Kim *et al.*, 2007). Furthermore, morphology determines filtration efficiency during harvesting and affects grazing behavior and metabolic rates of the aquatic organisms at higher trophic levels such as flamingos (Raven and Kubler, 2002). Therefore, it is of great importance to keenly observe and describe morphological aspects of *Arthrospira* species in their natural habitats and in culture systems.

The morphology of *Arthrospira* varies with the species and even with ecological races of the same species. It varies primarily from tightly coiled to straight forms, resulting in the occurrence of different morphotypes of the same species in nature and culture systems (Bai and Seshadri, 1980; Hindák, 1985; Li *et al.*, 2001; Ballot *et al.*, 2004b). Several studies involving laboratory and outdoor cultures have shown the association of morphological variability of *Arthrospira* species with environmental factors though

genetic mutations may occur occasionally leading to the formation of different strains (Wang and Zhao, 2005). Environmental factors including visible and UV light (Fox, 1996; Wu *et al.*, 2005; Ma and Gao, 2009), temperature and its interactions with light (Van Eykelenburg, 1979; Vonshak, 1997; Wu *et al.*, 2005; Li and Gao, 2008), nutrients and their interactions with light (Bai and Seshadri, 1980) and salinity (Elizabeth Kebede, 1997) have been shown to cause morphological changes in laboratory and outdoor cultures of *Arthrospira*, with the consequent occurrence of different morphotypes. The results of such studies suggested that morphological changes are adaptive responses of *Arthrospira* to different environmental conditions. However, studies on how *Arthrospira* species respond morphologically in their natural environmental conditions have received little consideration.

Arthrospira of Lake Chitu (Ethiopia) serves as the main food source for the large flocks of Lesser Flamingoes inhabiting the lake and has been used for various scientific studies and commercial cultures elsewhere (Li *et al.*, 2001). However, no studies have so far been conducted on the morphological aspects of *Arthrospira* and its taxonomic confusions resulting from morphological variations in the natural ecosystem of the lake. This study, therefore, investigated the morphotypes of *Arthrospira fusiformis* in relation to selected environmental variables in Lake Chitu. The results of such a study are crucial in understanding the morphological responses of *Arthrospira* to natural growth conditions and the consequent change in the feeding behavior of the flamingos. The results of the study may also help reduce taxonomic confusion and facilitate the selection of best strains of *Arthrospira* for large-scale production.

4.2. MATERIALS AND METHODS

4.2.1. Description of the study area, sampling, measurement and analysis of physicochemical parameters

Detailed description of the study area is given in chapter 1. Sampling procedures, sample preparation, and measurement and analysis of selected physicochemical parameters are provided in chapter 2.

4.2.2. Determination of abundance and morphological parameters of *Arthrospira*

For qualitative and quantitative analysis of *Arthrospira*, composite samples were preserved with 4% formalin in 125 ml brown bottles. The different morphotypes of *Arthrospira* in 100 grids of Sedgwick-Rafter chamber were counted in triplicate subsamples using a light microscope at a magnification of 100 $\times$. The abundance of the morphotypes, expressed in number of filaments ml⁻¹, was calculated using an equation given in Hötzel and Croome (1999). To characterize the different morphotypes, various morphological parameters often used for morphological description of *Arthrospira* (Bai and Seshadri, 1980) were qualitatively described and quantitatively measured or counted. These morphological parameters included number of coil (turns) per trichome, helix diameter, helix pitch (distance between coils), diameter, length, ends, color and abundance of trichomes. Average values of quantitative parameters were determined by counting and measuring of at least 40 trichomes of each morphotype, including large, medium and small-sized trichomes, during every sampling occasion.

4.2.3. Statistical analyses

The differences in the trichome abundance among the morphotypes and their morphological parameters were analyzed by one-way ANOVA and Tukey's least

significant difference (LSD) test for post-hoc multiple comparisons using the statistical program IBM SPSS (Version 20). The relationship between the morphotypes and morphological parameters (response variables), and the environmental variables (explanatory variables) were determined by direct gradient analysis, a method of multivariate analysis. Since the length of the longest gradient was < 3 (Lepš and Šmilauer, 2003), the linear method of gradient analysis, constrained redundancy analysis (RDA), was performed using the program Canoco for Windows 4.5. To reduce the effect of multicollinearity, some variables with high variance inflation factors (VIF) were removed from among the sets of strongly correlated variables until all the variables had VIF of < 20 (Ter Braak and Šmilauer, 1998). Alkalinity, pH and CO_3^{2-} were removed from the analysis because they were strongly correlated ($P < 0.05$) with some of the environmental variables such as PAR, salinity, conductivity and HCO_3^- . The environmental variables that best explain the variance in morphotype abundance and morphological parameters were selected and tested by RDA-associated forward selection and Monte Carlo permutation tests (unrestricted permutations 499, $P = 0.05$).

4.3. RESULTS

4.3.1. Morphological description of *Arthrospira*

Morphotypes of *Arthrospira* were characterized with regard to the degree of coiling of trichomes (helicity), coils per trichome, diameter and pitch of helix, color, shape of ends, diameter and length of trichome (Table 4.1). On the basis of these parameters, three distinct morphotypes (Fig. 4.1a–i), highly coiled (H-type), spiral or loosely coiled (S-type) and intermediately coiled (C-type) have been distinguished. Their trichomes were composed of short (cell height 4.5–5.4, mean $4.8 \pm 0.4 \mu\text{m}$, SD) and broader (cell diameter

8.1–10.8, mean $9.6 \pm 0.7 \mu\text{m}$, SD) cylindrical cells with visible cross-walls. Helix with left- and right-handed orientations occurred but their proportion was not estimated. The color of the trichomes varied slightly from deep blue-green to light blue-green. All the three types, however, differed in the degree of coiling, coils per trichome, shape of ends, abundance, diameter and length of trichome, pitch and diameter of helix (Fig. 4.2a–f).

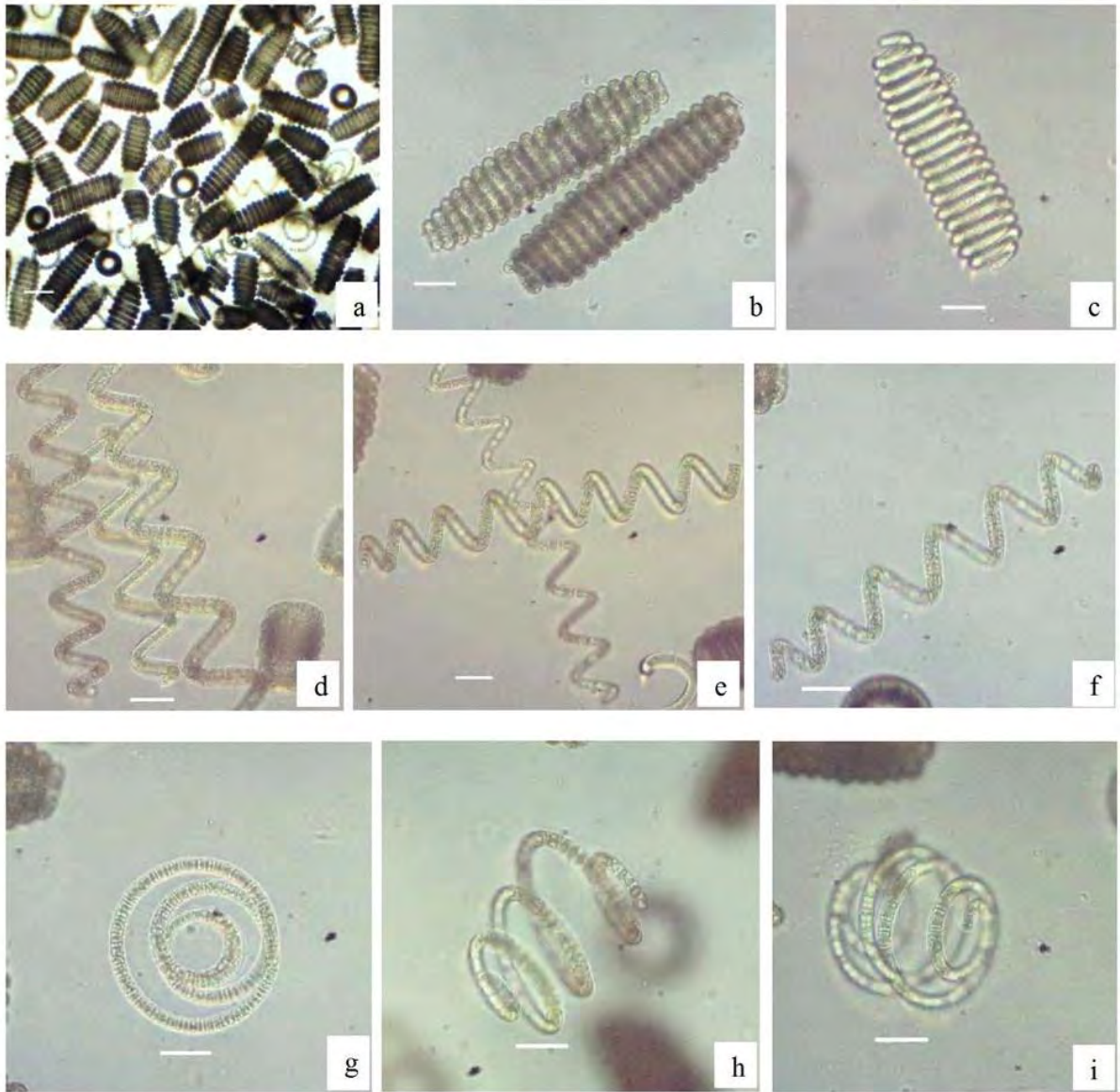


Fig. 4.1. Plates of the morphotypes of *Arthrospira fusiformis* in Lake Chitu. **a–c** H-type, **d–f** S-type, and **g–i** C-type. Scale bar 50 μm (at 200 $\times$) except **a** (100 μm at 100 $\times$).

The H-type (Fig. 4.1a–c) was so tightly coiled that the helix pitch was almost zero and coils per trichome significantly ($P < 0.01$) exceeded those of the other types (Fig. 4.2a, b). It had deep blue-green color and the largest trichome diameter of all types. The trichome length of this type is intermediate (Fig. 4.2c) between those of the other two variants; it is significantly lower than that of the S-type and higher than that of the C-type ($P < 0.01$). As can be seen from the helix diameter (Fig. 4.2d), this variant is averagely attenuated at the ends and the attenuation was higher than that of the S-type but lower than that of the C-type. It was the most dominant of all types accounting for about 50% of the total abundance throughout the study period (Fig. 4.2f). The S-type (Fig. 4.1d–f) was loosely coiled (spiral) and had the highest helix pitch with intermediate number of coils per trichome and the longest trichome (Fig. 4.2a–c), which was less than that of the H-type, but higher than that of the C-type. It had less deep blue-green color compared to the H-type. Its trichome diameter was larger than that of the C-type and had the lowest helix diameter and slight attenuation at the ends relative to other morphotypes. This morphotype was the second most dominant accounting for about 40% of the total abundance. The C-type (Fig. 4.1g–i) was coiled intermediately between the two morphotypes, with its helix pitch being closer than that of the S-type. It had light blue-green color and the shortest trichome length and the least number of coils per trichome (Fig. 4.2b). The trichome of this morphotype was much more attenuated and the diameter of its helix was much larger in the middle than at the ends (Fig. 4.2d), which was about 1/2 of the diameter at the middle. It was sometimes difficult to measure the trichome length of this morphotype because the trichome was lying upright on the flat surface at the bottom of the chamber. It was the least represented in *Arthrospira* populations of

Lake Chitu, with its contribution of about 10%, which was significantly ($P < 0.05$) low compared to those of the other morphotypes.

Table 4.1. Description of morphotypes of *Arthrospira fusiformis* on the basis of their morphological parameters. Ranges and means (in parentheses) of recorded values are given.

Morphological parameters	Morphotypes		
	H-type	S-type	C-type
Degree of coiling (helicity)	Highly (tightly) coiled	Loose helix (spiral)	Intermediate coil (closer helix than S-type)
Color of trichome	Deep blue-green	Blue-green	Light blue-green
Trichome end attenuation	More attenuated than S-type, 3/5	Indistinctly attenuated, 4/5	Highly attenuated, 1/2
Trichome diameter (μm)	9.6–11 (10.4)	9.1–11.5 (10.1)	8.6–9.6 (9.1)
Trichome length (μm)	155–209 (180.)	319–566 (421)	32–119 (78.1)
Helix diameter in the middle (μm)	51.2–59.2 (56.7)	36.4–54.5 (44.4)	72.8–118.3 (85.6)
Helix diameter at the ends (μm)	31.6–41 (36.1)	27.3–45.5 (34.6)	27.3–59.2 (44.1)
Helix pitch (μm)	≈ 0	32–54.6 (42.8)	11.1–45.5 (25.7)
Number of coils per trichome	10–18 (15)	7–13 (10)	3–5 (3.7)

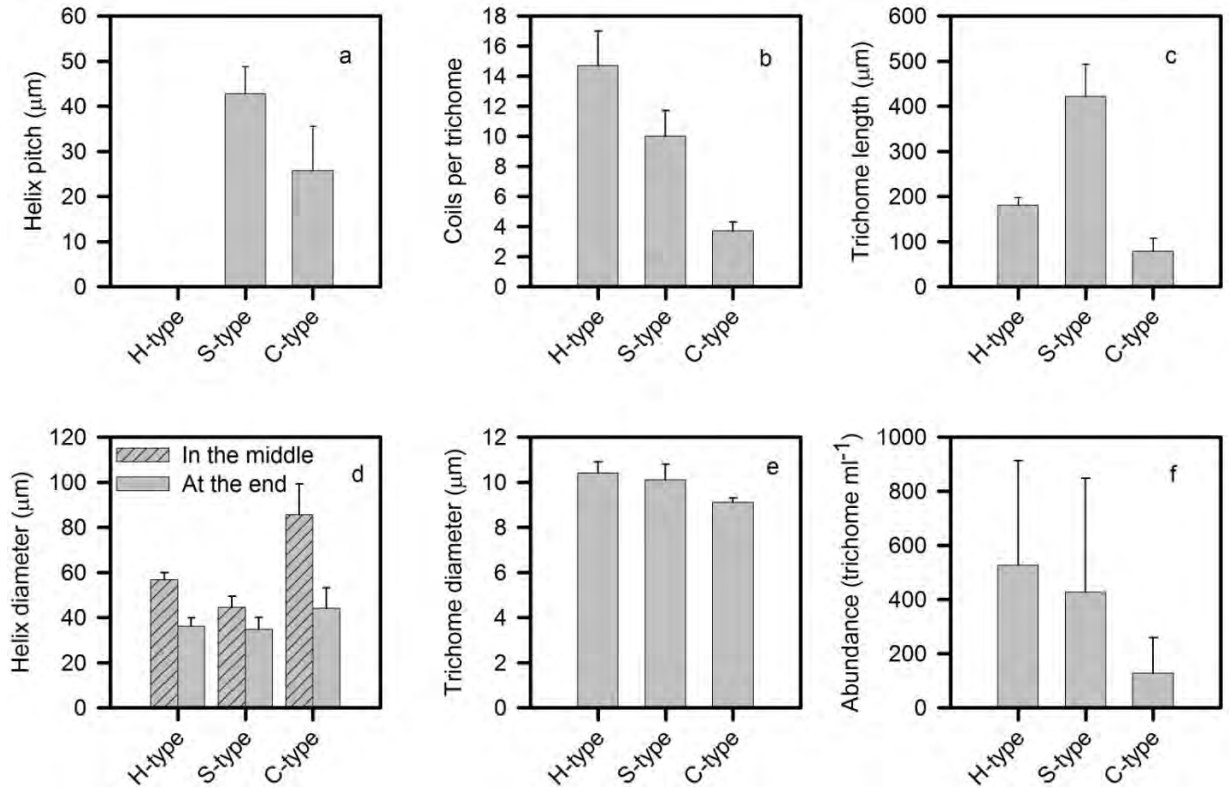


Fig. 4.2. Comparison of mean values (with standard deviation, $n = 11$) of abundance and morphological parameters of the three morphotypes of *Arthrospira fusiformis* in Lake Chitu. **a** helix pitch, **b** coils per trichome, **c** trichome length, **d** helix diameter, **e** trichome diameter, and **f** abundance.

4.3.2. Relationship between abundance and morphology of *Arthrospira* and environmental variables

Selected environmental variables, which are believed to have an effect on the abundance of morphotypes and morphological characteristics of *Arthrospira* species, are summarized in Table 4.2. Some parameters such as pH, alkalinity, conductivity and salinity were very high. The concentration of the major algal nutrient, soluble reactive phosphate (SRP), was also incredibly high. In contrast, the nitrogen sources (NO_3^- and NH_3) were very low and often undetectable in the lake. The concentrations of CO_3^{2-} and HCO_3^- were large; however, as can be seen from their ratio, the proportion of HCO_3^- , the

main carbon source for *Arthrospira* (Richmond, 1990; Binaghi *et al.*, 2003), was low because it is chemically reduced at high pH. Further explanations of these physicochemical parameters are provided in chapter 2.

Table 4.2. Some environmental parameters of Lake Chitu measured in this study.

Parameters	Range	Mean $\pm$ SD ($n = 11$)
Temperature ($^{\circ}\text{C}$)	21.5–24.4	22.8 $\pm$ 1.0
PAR ($\mu\text{ mol photon m}^{-2}\text{ S}^{-1}$)	1100–1999	1396.4 $\pm$ 300.2
pH	10.1–10.8	10.4 $\pm$ 0.2
Alkalinity ($\text{mg CaCO}_3\text{ L}^{-1}$)	3354–40387	36848.5 $\pm$ 2044.0
Conductivity ($\text{K}_{25}, \text{mS cm}^{-1}$)	56.5–64.7	61.8 $\pm$ 2.7
Salinity (g L^{-1})	40.0–46.4	43.8 $\pm$ 2.2
NO_3^- ($\mu\text{g L}^{-1}$)	0.0–9.5	1.5 $\pm$ 2.8
NH_3 ($\mu\text{g L}^{-1}$)	0.0–84.0	14.5 $\pm$ 28.6
SRP ($\mu\text{g L}^{-1}$)	17.7–3776.0	664.0 $\pm$ 1151.7
HCO_3^- (mg L^{-1})	7246.9–16435.6	12428.7 $\pm$ 3008.9
CO_3^{2-} (mg L^{-1})	12042.2–18106.8	15996.6 $\pm$ 1894.8
$\text{CO}_3^{2-}/\text{HCO}_3^-$	0.7–2.3	1.4 $\pm$ 0.5

As the results of RDA given in Table 4.3 show, the selected environmental variables sufficiently explained the variance in morphotype–environment relationships. About 90% of the variance of the morphotypes could be explained by the environmental variables. The first two axes of the RDA ordination (Fig. 4.3) accounted for 85% of the variance in the abundance of the morphotypes due to the environmental variables. The first axis is the most important explaining 77% of the variance, to which salinity was significantly ($P < 0.01$) negatively related (Fig. 4.3). This axis can, therefore, be described as the axis of the salinity, which accounted for 51% of the variance. The

second axis, which explained the least of the variance (8%), was negatively correlated with HCO_3^- . All the morphotypes were negatively associated with the salinity of the water. Of all morphotypes, the S-type was very strongly but negatively correlated with the axis of salinity, implying that this morphotype preferred low salinity more than others. The preference for low salinity increased in the order S-type > C-type > H-type. The dominance of H-type was associated with increasing levels of PAR as well as temperature (to some extent) and low concentrations of carbon (HCO_3^-) and nitrogen (NO_3^-) nutrients.

The RDA results for morphological parameters of *Arthrospira* show that they are well explained (87%) by the selected environmental variables (Table 4.3). The first two axes of RDA ordination (Fig. 4.4) explained about 61% of the variance in the morphological parameters of *Arthrospira*. Axis 1, accounting for 39% of the variance, was significantly but negatively correlated with temperature ($P < 0.05$) and strongly but positively correlated with HCO_3^- . Axis 2, which accounted for 22% of the variance, was positively related to NO_3^- and negatively to PAR. Trichome length was strongly and positively related to HCO_3^- , but negatively to temperature (Fig. 4.4), indicating that long trichomes occur in lake waters with low ambient temperature and rich carbon source (HCO_3^-). Increased coiling of trichomes was, however, related to low nitrogen source (NO_3^-) and increased PAR whereas increased helix pitch (loosening of helix) was associated with increased concentration of NO_3^- and low PAR. This means that tightly coiled trichomes with much more coils occur under conditions of increased PAR and NO_3^- deficiency. Helix diameter increased at low temperature.

Table 4.3. Summary of RDA results of morphotypes and morphology of *Arthrospira*, and selected environmental variables for the first two axes.

RDA statistics	Morphotypes		Morphological parameters	
	Axis1	Axis 2	Axis 1	Axis 2
Eigenvalues	0.77	0.08	0.39	0.22
Morphotype/morphology-environment correlations	0.97	0.82	0.89	0.93
Cumulative percentage variance of morphotype/morphology data	76.9	84.6	38.8	60.3
Cumulative percentage variance of morphotype/morphology-environment relation	85.4	94.0	50.0	77.7
Sum of all eigenvalues	1.0		1.0	
Sum of all canonical eigenvalues	0.90		0.87	

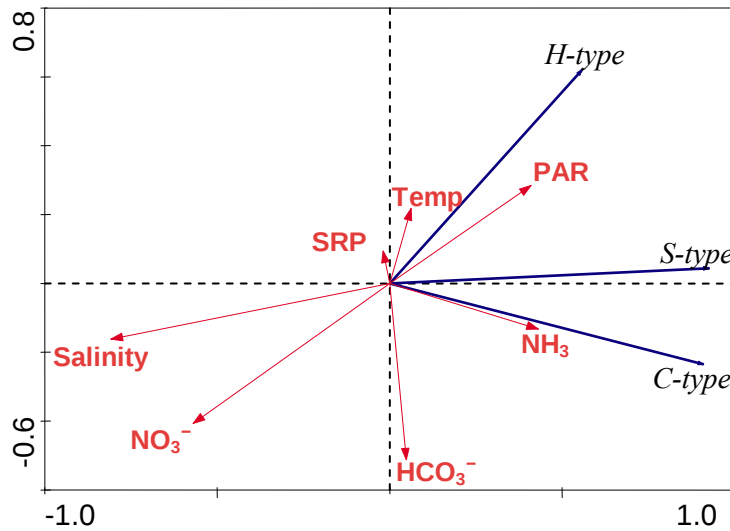


Fig. 4.3. RDA ordination diagram of abundance of morphotypes and environmental variables for the first two axes. Axis 1 (*horizontal*) accounted for 77%, while Axis 2 (*vertical*) accounted for 8% of the variance in morphotypes. The environmental variables are: *temp* temperature, *PAR* photosynthetically active radiation, salinity, *SRP* soluble reactive phosphate concentration, HCO_3^- bicarbonate (carbon source), NO_3^- and NH_3 nitrogen sources.

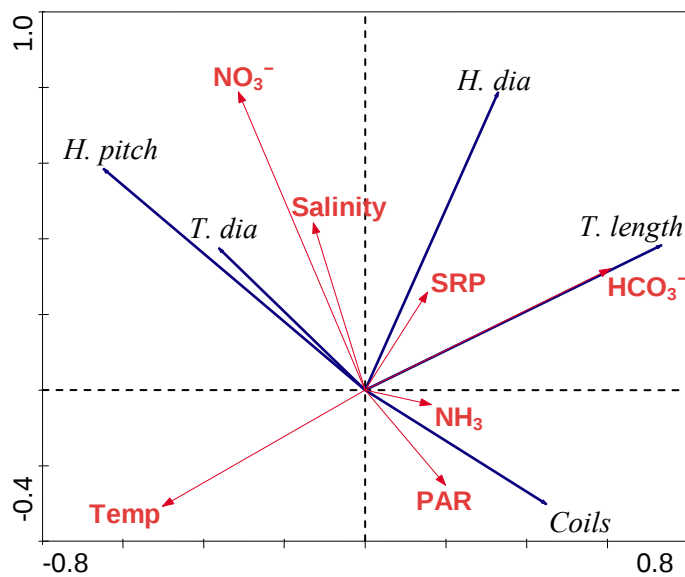


Fig. 4.4. RDA ordination diagram of morphological parameters and environmental variables for the first two axes. Axis 1 (*horizontal*) accounting for 39% and Axis 2 (*vertical*) accounting for 22% of the variance in morphological parameters. The morphological parameters are: *T. dia* trichome diameter, *H. pitch* helix pitch, *H. diam* helix diameter, *T. length* trichome length and *Coils* coils per trichome. The environmental variables are: *temp* temperature, *PAR* photosynthetically active radiation, *salinity*, *SRP* soluble reactive phosphate, HCO_3^- bicarbonate (carbon source), NO_3^- and NH_3 nitrogen sources.

4.4. DISCUSSION

4.4.1. Morphological descriptions

Variations in morphology occur in *Arthrospira* strains from the natural soda lakes and culture systems (Bai and Seshadri, 1980; Vareschi, 1982; Hindák, 1985; Krienitz and Kotut, 2010). From a sample of *Arthrospira* of Lake Chitu (Ethiopia), Thomasson (1960) identified two morphotypes, tightly coiled and loosely coiled forms. In a more recent study, Li *et al.* (2001) also observed two morphotypes with tightly and loosely coiled trichomes in samples and subcultures of *Arthrospira* from Lake Chitu. However, they did not report the intermediately coiled type, which was observed in samples collected over

an extended period of time of the present study. Several studies using *Arthrospira* isolates from Indian freshwaters (Bai and Seshadri, 1980) and saline–alkaline lakes of Kenya (Ballot *et al.*, 2004b) revealed three different morphotypes with tightly coiled, loosely coiled and intermediate types comparable to the morphotypes observed in this study. In their very recent study on two Kenyan soda lakes, Nakuru and Bogoria, Kaggwa *et al.* (2013b) identified three types of filament morphology: small, large-wide and large-narrow. With respect to their size and form of spirals, the S- and H-types are broadly similar to the large-wide and large-narrow filaments described by Kaggwa *et al.* (2013b), respectively. Each morphotype described in this study, however, differs considerably from the corresponding type of filament morphology reported by Kaggwa *et al.* (2013b) with regard to some of the parameters on which their distinction was based. The S-type has tighter helix pitch (42.8 μm) and large number of coils (10) compared to the large-wide filament morphology of Kaggwa *et al.* (2013b) (helix pitch 63.2 μm , coil per trichome 7.3). With its very tight helix pitch (≈ 0) and higher number of coils (15), the H-type differs markedly from the large-narrow of Kaggwa *et al.* (2013b) (helix pitch 12.6 μm , coil per trichome 8.6). The helix diameter (85.6 μm) and pitch (25.7 μm) of the C-type are considerably larger than those of the small type of filament described in Kaggwa *et al.* (2013b) (helix diameter 36.6 μm , helix pitch 11.1 μm) although both are small in size. In addition to the differences in filament morphology, the cells of the morphotypes of this study are much broader (cell diameter 8.1–10.8 μm) than those of the morphotypes described for *Arthrospira* of the Kenyan soda lakes (cell diameter 2–9 μm).

Among the morphological parameters, helix diameter is useful in describing the attenuation of a trichome, which is often used to distinguish the strains. Tomaselli (1997)

reported helix diameter ranging from 30 to 70 μm and helix pitch from 12 to 72 μm for *Arthrospira* isolates. Kaggwa *et al.* (2013b) also measured helix diameter of 36.6–60.5 μm and helix pitch of 11.1–63.2 μm for *Arthrospira* from Kenyan soda lakes. In this study, the helix pitch of S- and C-type was found to be within the reported range, while that of the H-type was very low. The helix diameter of the H- and S-types was also within the range reported by Tomaselli (1997) and Kaggwa *et al.* (2013b), while that of the C-type was considerably larger (52–84 μm). Most of the trichomes of the three morphotypes observed in this study were fusiform-shaped though the degree of attenuation varied among the morphotypes. Similar observations made by Desikachary and Bai (1996) showed the helix diameter to be fusiform or barrel-shaped in most strains of *Arthrospira*, with helix diameter that is almost uniform along the whole trichome being characteristic of only some strains. The size of *Arthrospira* in Lake Chitu (78–421 μm) is within the size range (40–800 μm) regarded suitable for efficient filtration by Lesser Flamingos (Jenkin, 1957), making this alga the main food source for the huge flocks of these birds inhabiting the lake.

Despite their variability with environmental conditions (Bai and Seshadri, 1980; Fox, 1996; Elizabeth Kebede, 1997; Wang and Zhao, 2005), morphological parameters are used as taxonomic criteria in the conventional taxonomic approach for distinguishing *Arthrospira* species (Desikachary and Bai, 1996; Vonshak and Tomaselli, 2000; Muhling *et al.*, 2006). In this approach, the tightly coiled and loosely coiled trichomes are grouped separately as *A. fusiformis* of the tropical region and *A. maxima* of the neotropical region, respectively (Komárek and Lund, 1990; Li *et al.*, 2001; Mussagy, 2006). For the tightly coiled and loosely coiled morphotypes observed from *Arthrospira* samples in Ethiopia,

Thomasson (1960) suggested that the two morphotypes probably represent two different taxa, *A. fusiformis* and *A. maxima*, respectively. The three morphotypes of *Arthrospira* described in this study share the morphological characteristics of *A. fusiformis* (a synonym of *A. platensis*) and *A. maxima* (Komárek and Lund, 1990; Tomaselli, 1997). This observation may tempt one to suggest, merely on the ground of the similarities in morphological parameters, that these two species of *Arthrospira* occur in Lake Chitu.

Genotypic analysis, which is currently considered as more reliable and preferred method for the identification of species of cyanobacteria (Turner, 1997; Oren, 2004; Hoffmann *et al.*, 2005), showed that the morphotypes are similar. In samples and subcultures of *Arthrospira* from Lake Chitu, Li *et al.* (2001) observed two morphotypes with tightly and loosely coiled trichomes. However, they found that the morphotypes belonged to the same species on the basis of 16S rRNA (100% similarity). For the three different morphotypes observed for isolates of Indian waters, Bai and Seshadri (1980) also concluded that the morphotypes belong to the same species because the difference in the morphology, particularly the degree of coiling, is not intrinsic. Likewise, in the phylogenic study of *Arthrospira* collected from Kenyan saline–alkaline lakes and Indian freshwater bodies, Ballot *et al.* (2004b) observed different morphotypes, but with 100% similarity in 16S rRNA. The more recent phylogenetic study made on *Arthrospira* from Africa, Asia and America did not show habitat-dependent phylogenetic differences among the strains either (Dadheech *et al.*, 2010). These and other related genotypic studies demonstrated that the different morphotypes of *Arthrospira* species show 99.7–100 % similarity in 16S rRNA gene sequences (Nelissen *et al.*, 1994; Li *et al.*, 2001; Ballot *et al.*, 2004b; Dadheech *et al.*, 2010), supporting the idea that different

morphotypes may be genetically the same species and vice versa. Consequently, on the basis of these genetic evidences, the various morphotypes of *Arthrospira* observed in this study probably belong to a single species, *A. fusiformis*, that shows morphology of the East African morphotypes.

Provided that the problem of cyanobacterial taxonomy could be resolved with the genetic approaches, the use of traditional morphological approach as a taxonomic tool for species identification could be less preferred though it has often been used in the identification of *Arthrospira* species.

4.4.2. Relationships between abundance and morphology of *Arthrospira* and environmental variables

The measured environmental parameters of Lake Chitu reflected the characteristic features of most soda lakes of East Africa (see chapter 2 for further discussion). The variation in the abundance of the morphotypes and morphology were strongly associated with such selected environmental variables of the lake as PAR, nutrients (NO_3^- and HCO_3^-), temperature and salinity. Various studies made using laboratory and outdoor cultures have shown that these environmental variables could cause changes in the morphology of *Arthrospira* species (Bai and Seshadri, 1980; Lewin, 1980; Bai, 1985; Fox, 1996; Elizabeth Kebede, 1997; Wang and Zhao, 2005). Nutrients, conductivity, temperature, zooplankton and wind speed were also found to affect morphology of *Arthrospira* in natural soda lakes (Kaggwa *et al.*, 2013b). Such changes in the morphology of *Arthrospira* species with changes in environmental factors are adaptive responses to stressful environmental conditions by having a shape that assists them to counteract the stresses (Wu *et al.*, 2005; Li and Gao, 2008).

The H-type was the most abundant variant in Lake Chitu. As shown in the RDA, the abundance of H-type was associated with high PAR and low concentrations of HCO_3^- and NO_3^- . In addition, the RDA of morphology–environmental variables shows strong association of increased helix pitch (loosening of helix) and decreased coiling with high NO_3^- and low PAR and of long trichomes with high concentration of HCO_3^- and low temperature. Therefore, the abundance of H-type with very close helix pitch may be related primarily to NO_3^- and HCO_3^- deficiencies, and high PAR and temperature of the lake. In East African soda lakes such as Lake Chitu, NO_3^- is frequently limiting (Elizabeth Kebede *et al.*, 1994; Talling and Lemoalle, 1998) and the concentration of HCO_3^- is reduced at high pH (>10) as it is also evident from the high ratio of CO_3^{2-} to HCO_3^- (1.4 ± 0.5) of the present study lake with a mean pH of 10.4 ± 0.2 .

At different levels of nutrients and light intensity, Bai and Seshadri (1980) observed three morphotypes of *Arthrospira*—tightly coiled, spiral and intermediate types. They observed that low levels of light and high nutrients favored trichome with loose helix (S-type), while high levels of light and low nutrients enhanced its conversion to that with very tight coil (H-type) with intermediately coiled type (C-type) occurring under high levels of light and nutrient concentrations. *Arthrospira* increases coiling of its trichomes under nutrient-deficient conditions because helical shape is a configuration that helps it distribute the stress equally over the whole cross-section (Bai and Seshadri, 1980). Various laboratory studies have also shown that increased levels of visible and UV radiations (Bai and Seshadri, 1980; Fox, 1996; Wu *et al.*, 2005; Ma and Gao, 2009) and temperature (Van Eykelenburg, 1979) cause *Arthrospira* strains to take tightly coiled shapes. Such tightening of the spirals of *Arthrospira* was considered to be associated with enhanced

expression of genes encoding for specific proteins at high light intensity (Wang and Zhao, 2005; Ma and Gao, 2009). These studies suggested that tightly coiled shape of *Arthrospira* enhances self-shading as a protective strategy (photoprotection) against high light intensity. At low light, however, *Arthrospira* assumes loose spiral shape, which enables the cells to harvest more light for photosynthesis (Ma and Gao, 2009). In contrast to the laboratory studies and this study, Kaggwa *et al.* (2013b) reported the association of large-narrow filament forms (corresponds to H-type) with increasing NO_3^- , and large-wide form (corresponds to S-type) with elevated temperature in Kenyan soda lakes.

Arthrospira has been shown to be affected by salinity of the medium though it can adapt to a wide range of salinities (Vonshak *et al.*, 1988; Elizabeth Kebede, 1997). As shown in RDA, abundance of all the morphotypes was negatively affected by the high salinity of the water (43 g L^{-1}), which is higher than the level of salinity required for best growth of *Arthrospira*. In laboratory cultures of *Arthrospira* isolated from Kenyan lakes, Krienitz *et al.* (2013) observed high biomass at a salinity of 8 g L^{-1} , with considerable reduction at higher (16 g L^{-1}) and lower (0.1 g L^{-1}) salinities. In similar growth experiments of *Arthrospira*, reduction in growth with an increase in salinity was also observed (Elizabeth Kebede, 1997; Vonshak, 1997). In a study of biomass production of *A. fusiformis* isolated from Lake Chitu, we also observed an increase in growth rate in the media prepared by dilution of water of the soda lakes Chitu and Shala (Tadesse Ogato *et al.*, 2014). In general, studies on African soda lakes have identified salinity as the main factor controlling biological diversity and community structure (Wood and Talling, 1988; Talling and Lemoalle, 1998).

The high salinity of Lake Chitu did not clearly separate the abundance of the morphotypes. However, the negative correlation between the abundance of S-type and salinity was somewhat stronger than that between abundance of other morphotypes and salinity. Besides, the abundance of S-type was lower than that of H-type. This may suggest that the high salinity of the lake is more suitable for H-type than for S-type. The present result seems to be consistent with the laboratory study made by Elizabeth Kebede (1997) who evaluated growth of *A. fusiformis* isolated from Lake Chitu at different salinity levels and observed the occurrence of unusually long trichomes at low salinity (13 g L^{-1}) and very short and closely coiled trichomes in chloride-rich and high salinity media ($55\text{--}88 \text{ g L}^{-1}$). There was a contrasting report from the natural soda lakes Nakuru and Bogoria (Kenya) that the dominance of large-wide morphotype (corresponds to S-type) was related to increased conductivity (Kaggwa *et al.*, 2013b). The change in morphology acts as an expression of a modification of physiological behavior in response to the increase in salinity (Elizabeth Kebede, 1997; Dhiab *et al.*, 2007). In the present study, salinity did not show clear effects on the morphological parameters. This may be attributed to the observed narrow range of salinity ($40.0\text{--}46.4 \text{ g L}^{-1}$) of the lake and the effects of other factors. Based on the difference in helicity in media with the same salinity but with different anions (SO_4^{2-} , HCO_3^- and Cl^-), Elizabeth Kebede (1997) argued that not only the salinity but also the anions involved can affect the degree of helicity. This suggests the probable difference in the morphological responses of *Arthrospira* to varying salinity levels in the presence of other interactive factors in the natural environment.

In conclusion, the variability in the abundance of morphotypes and their morphological parameters and their associations with some environmental variables seem to suggest that *Arthrospira* species could undergo morphological changes in response to environmental stresses in their natural habitats—soda lake ecosystems. The different morphotypes observed in Lake Chitu reflect the existence of different variants of the same species of *A. fusiformis*.

CHAPTER 5 . EVALUATION OF GROWTH AND BIOMASS PRODUCTION OF *ARTHROSPIRA FUSIFORMIS* IN LABORATORY CULTURES USING WATERS FROM THE ETHIOPIAN SODA LAKES CHITU AND SHALA

5.1. INTRODUCTION

Arthrospira is one of the microalgae that reproduce fast and has high productivity (Kilic *et al.*, 2006), and can be produced in small or large commercial scales using different types of production systems and growth media. Growth and biomass production of *Arthrospira* depends on many environmental factors, especially carbonate–bicarbonate alkalinity, pH, nutrient availability, temperature and light (Vonshak, 1997; Habib *et al.*, 2008). High biomass of the alga can be obtained when these environmental factors are optimized. It dominates in tropical and sub-tropical water bodies characterized by high carbonate–bicarbonate alkalinity and pH (Ciferri, 1983; Cogne *et al.*, 2001). However, optimization of growth conditions is the major challenge to biomass production of *Arthrospira* in cultivation systems and incurs high production cost. Out of the total production cost of *Arthrospira* biomass, 15–25% is accounted for by nutrients (mainly carbonate–bicarbonates), which form the second major cost item (Amha Belay, 1997; Vonshak, 1997; Habib *et al.*, 2008). As a result, production of *Arthrospira* biomass using standard carbonate medium is unaffordable.

Much effort has been made to investigate alternative methods of cultivation through biotechnological research and innovation in order to cut down the cost of production without compromising biomass productivity (Vonshak, 1997). Promising results have been found in experiments using seawater enriched with phosphate and urea as phosphorus and nitrogen sources, respectively. In such experiments, Materasi *et al.*

(1984) and Tredici *et al.* (1986), in laboratory and outdoor mass cultivations, respectively, obtained biomass yields which are slightly less than those obtained with the standard medium. Studies in Brazil by Costa *et al.* (2003) have also shown that comparable *Arthrospira* biomass could be obtained using lagoon water supplemented with bicarbonate and urea. Use of different organic sources of carbon, nitrogen and phosphorus has also resulted in increased biomass yield of *Arthrospira* (Neilson and Larsson, 1980; Baldia *et al.*, 1991; Habib *et al.*, 2008). However, the potential of water of soda lakes for biomass production of *Arthrospira* under laboratory or outdoor conditions has received little attention to date though these lakes are conducive for *Arthrospira*, with some of them supporting its abundant populations.

The Ethiopian alkaline soda lakes Chitu and Shala have several interesting features. Lake Chitu is a small shallow lake best known for its natural monoculture of *Arthrospira* (Talling *et al.*, 1973; Elizabeth Kebede, 1996), but harvesting *Arthrospira* biomass from the natural ecosystem of the small lakes like Chitu will not be sustainable ecologically and economically. Lake Shala is a large and deep soda lake whose gross water chemistry is closely similar to that of the adjacent Lake Chitu and which is suitable for *Arthrospira* production (Wood and Talling, 1988; Elizabeth Kebede, 1996). The lakes are situated in areas with high temperature and irradiance and nearly constant photoperiod, which seem to be ideal tropical climatic conditions favoring the high productivity of *Arthrospira* (Richmond and Grobbelaar, 1986; Vonshak, 1997; Talling and Lemoalle, 1998). These conditions presumably indicate potential of the large lake Shala water for *Arthrospira* production. However, natural populations of *Arthrospira* have not been reported for Lake Shala for reasons not known to date. This calls for an in-depth experimental testing of the

suitability of the lake water for *Arthrospira* production under laboratory and outdoor conditions. The purpose of this study was, therefore, to evaluate growth and biomass production of *Arthrospira fusiformis* in waters of the soda lakes Chitu and Shala with or without supplementation with standard *Spirulina* medium (SM) in the laboratory. The results that emanate from such investigations are crucial to efforts being made to develop suitable low-cost media for *Arthrospira* cultivation.

5.2. MATERIALS AND METHODS

5.2.1. Measurement of some physicochemical parameters of the soda lakes

Description of the study lakes is well provided in chapter 1. Before using for experimental culture, some chemical parameters of the lake waters were determined *in situ* or in the laboratory to see their variation under the two conditions. pH and conductivity were measured *in situ* and in laboratory using digital pH meter (model HI 9024, Hanna Instruments) and conductivity meter (model CC-505, Elmetron), respectively. Salinity (g L^{-1}) was calculated from conductivity measurements according to UNESCO (1983). Surface water samples, collected with 10 L plastic containers, were used for the analysis of some chemical features and preparation of growth media in laboratory. Alkalinity of unaltered water sample was determined by titration with 1 N HCl to pH 4.5 using a mixed indicator (bromocresol green-methyl red). Carbonate–bicarbonate alkalinities as CaCO_3 and their ions were calculated from total alkalinity and pH according to APHA *et al.* (1999). NO_3^- , NH_3 , soluble reactive phosphate (SRP) and SO_4^{2-} were determined, using samples filtered through Whatman filter paper (GF/F), by sodium salicylate (APHA *et al.*, 1995), phenate, ascorbic acid and turbidimetric methods, respectively (APHA *et al.*, 1999). Samples acidified to a pH of 2 with HNO_3 , were used

for the analysis of major ions and some micronutrients according to the standard analytical methods outlined in APHA *et al.* (1999): Na⁺ and K⁺ by flame photometric method, Ca²⁺ by direct nitrous oxide-acetylene flame method, Cl⁻ by argentometric method, Mg²⁺, Fe, Zn, Mn, Cu, and Co by direct air-acetylene flame method. Boron (B) was determined by azomethine H-colorimetric method (FAO, 2008).

5.2.2. Isolation of *Arthospira* and scaling-up of its seed cultures

Trichomes of *A. fusiformis* (Fig. 5.1) were isolated from Lake Chitu by the serial dilution technique (Andersen and Kawachi, 2005). An aliquot from a sample of the lake water was diluted with liquid SM, Zarrouk medium as modified by Aiba and Ogawa (1977), in a small test tube from which drops were transferred to multi-well plate using Pasteur micropipette and concentration of trichomes was checked under an inverted microscope. After a series of similar dilutions and observations, some trichomes were picked up with the micropipette and introduced into two small test tubes of 15 ml capacity containing about 4 ml of SM. The trichomes in the test tubes were allowed to grow at a photon flux density of about 20 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ (produced by two fluorescent lamps, 36 W each) and temperatures of 22–24 °C. Dense cultures of the microalga were diluted by the addition of the SM and scaled up to a large volume (125 ml). All these cultures, which served as sources of inocula, were mixed manually four to five times a day.



Fig. 5.1. Isolates of *Arthrospira fusiformis* from Lake Chitu used for experimental culture. Scale bar, 20 μm .

5.2.3. Preparation of growth media for experimental cultures

Soda lake water was first filtered through a plankton net (64 μm pore size) to remove large microorganisms and particulate materials, within a few hours of its collection. The filtered water was then sterilized by a chemical sterilization technique (bleaching) and neutralized with sodium thiosulfate solution following the procedure outlined in Kawachi and Noel (2005). To evaluate growth responses of *Arthrospira*, with the aim to reduce the cost of growth media by substituting standard medium with soda lake waters (by 50%, 75% or 100%), nine different types of media were prepared as indicated in Table 5.1. These media include SM, Lake Chitu water-based media (CBM) and Lake Shala water-based media (SBM). CBM and SBM consist of unsupplemented lake waters and lake waters supplemented in different proportions with SM and SM⁻ (SM lacking carbonate–bicarbonate components—Na₂CO₃, NaHCO₃ and NaCl). The idea of using SM⁻ was to determine if these chemicals (Na₂CO₃, NaHCO₃ and NaCl), which are required in large quantities in the standard SM, are replaceable by the carbonate- and bicarbonate-rich lake waters.

Table 5.1. Types and composition of growth media used for the experimental laboratory cultures of *Arthrospira fusiformis*.

Types of media	Designation	% (v/v) media composition
Standard: Spirulina Medium	SM	100 SM
CBM		
Chitu medium	CM	100 CM
25% supplemented Chitu medium	25CM	25 SM:75 CM
50 % supplemented Chitu medium	50CM	50 SM:50 CM
50 % supplemented Chitu medium (supplemented with SM ⁻)	50CM ⁻	50 SM ⁻ :50 CM
SBM		
Shala medium	SHM	100 SHM
25% supplemented Shala medium	25SHM	25 SM:75 SHM
50% supplemented Shala medium	50SHM	50 SM:50 SHM
50% supplemented Shala medium (supplemented with SM ⁻)	50SHM ⁻	50 SM ⁻ :50 SHM

SM⁻ is SM lacking Na₂CO₃, NaHCO₃ and NaCl; CBM Chitu water-based media; SBM Shala water-based media

5.2.4. Experimental design and growth conditions

The experiment was designed to test growth and biomass production of *Arthrospira* in a laboratory using media produced from soda lake waters. The independent variable to be optimized was the growth medium, keeping such growth conditions as temperature, light and mixing constant. The response variables analyzed were specific growth rate (μ), biomass production (B) and doubling time (dt).

The experiment was carried out in 300 ml Erlenmeyer flasks holding 150 ml culture media without replication. The culture flasks were inoculated with 15 ml aliquots, constituting 10% of the final culture volume, removed from exponentially growing cultures. The cultures were exposed to an artificial light source from fluorescent lamps

(three, 36 watt each), providing a photon flux density of about $50 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ on the surface of the culture. The experimental cultures were grown in a temperature-regulated water bath (model DKZ series) at 35°C in a light–dark cycle of 10:14 for 18 days. These light and temperature conditions were reported in several research articles as the optimal levels for *Arthrospira* growth in the laboratory (Vonshak, 1997; Oliveira *et al.*, 1999; Andersen and Kawachi, 2005). Mixing of the cultures was achieved manually by gentle shaking of the culture flasks four times a day.

5.2.5. Analytical methods

Measurement of pH and conductivity and estimation of chlorophyll-a and growth parameters

Before inoculation, initial value of the biomass index–chl-*a* of the inocula and the initial pH and conductivity of all culture media were measured. During the experimental period, similar measurements were made every 2 days except for the first set of measurements, which were taken after 24 h to check the occurrence of a lag phase. The measurement and analyses were not replicated for the variables.

Chl-*a* was determined spectrophotometrically from 5 ml samples filtered onto GF/F and extracted in 90% acetone. The absorbance of pigment extracts was measured at 665 and 750 nm with a UV–VIS Spectrophotometer (model 6405, Jenway) and chl-*a* concentration was estimated using the equation in Vonshak (1997). Biomass production (B , mg L^{-1}) was calculated as the change in biomass per volume of sample filtered (Colla *et al.*, 2007). μ and dt were calculated using the following equations used for batch culture of microalgae in the exponential growth phase (Guillard 1973; Vonshak 1997).

$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$, $dt = \frac{\ln 2}{\mu} \frac{0.6931}{\mu}$, where N_2 and N_1 are concentrations of the indicator of biomass (chl-*a*, mg L⁻¹) at the end and beginning of the time intervals, t_2 and t_1 , respectively.

5.2.6. Statistical analysis

The differences in growth parameters and some growth conditions of the various media were analyzed by one-way ANOVA, with Tukey's LSD post-hoc test for multiple comparisons. The variables which contributed to the observed variation in growth and biomass production of *Arthrospira* in various media were determined by multiple regression analysis. All statistical analyses were done using IBM SPSS statistical program (Version 20).

5.3. RESULTS

5.3.1. Some chemical features of the study lakes

Certain physicochemical features of Lakes Chitu and Shala including those chemical features, which seem to be of overriding importance to the growth of *Arthrospira* are presented in Table 5.2. In laboratory and *in situ* measurement of some variables such as pH, conductivity and salinity did not show perceptible differences, and those physicochemical data measured in the laboratory were used in this study. The observed high levels of carbonate–bicarbonate alkalinity (as mg L⁻¹ CaCO₃), pH, salinity, conductivity, Na⁺, Cl⁻ and SRP in both lakes are characteristic of soda lakes. Most of the recorded chemical parameters were considerably higher in Lake Chitu than in Lake Shala and SM, with the levels in Lake Shala approaching those measured in the SM (Table 5.2). In both lakes, these parameters did not show considerable seasonal variations although

the variation was slightly higher in Lake Chitu due to its productivity and small size (see chapter 2). Na^+ , and $\text{HCO}_3^- + \text{CO}_3^{2-}$ and Cl^- ions accounted for large proportions of the total cations and anions, respectively, in both lakes. The concentrations of the divalent cations, Ca^{2+} and Mg^{2+} , are low compared to those of the dominant ions.

SRP, whose concentration in Lake Shala was about two times that in Lake Chitu, was quite high in both lakes considering its levels commonly recorded for freshwater lakes. In contrast, the concentration of nitrogen compounds NO_3^- and NH_3 , which were often low or undetectable, were rather higher in Lake Chitu than in Lake Shala. In both lakes, the concentration of these macronutrients was considerably low compared to that of SM.

Table 5.2. Some chemical features of lakes Chitu and Shala waters and SM, which were used for experimental cultures.

Parameters	Lake Chitu	Lake Shala	SM
pH	10.1	9.4	8.85
Salinity (g L ⁻¹)	37.50	15.74	13.53
Conductivity (μS cm ⁻¹)	56,330	25,800	22,460
HCO ₃ ⁻ + CO ₃ ²⁻ alkalinity	36,848.5	10,990.8	-
HCO ₃ ⁻	12,428.7	7,606.2	9,880
CO ₃ ²⁻	15,996.6 (1.3)	2,853.7 (0.4)	2,280 (0.23)
NO ₃ ⁻ (μg L ⁻¹)	20	ND	1,820,000
NH ₃ (μg L ⁻¹)	50	ND	-
SRP (μg L ⁻¹)	460	830	273,000
SO ₄ ²⁻	187.5	33.4	634
Na ⁺	20,500	8,000	6,550
K ⁺	1,200	260	672
Ca ²⁺	0.39	ND	11
Mg ²⁺	0.06	0.03	20
Cl ⁻	12,336.6	3,701	626
Fe	0.57	0.20	-
Zn	0.043	0.027	-
Mn	ND	ND	-
Cu	0.118	0.122	-
Co	ND	ND	-
B	16.23	11.5	-

Units are mg L⁻¹ unless otherwise indicated; data in *bracket* are CO₃²⁻ to HCO₃⁻ ratio; *ND* not detectable

5.3.2. Salinity and pH of the experimental culture

In all media, pH increased while salinity decreased progressively with time during the cultivation period (Fig. 5.2). The decrease in salinity and increase in pH was gradual during early phase of the growth period, but became abrupt towards the end starting from

days 11 to 12 in all media. pH increased more slowly in lake waters-based media than in the SM.

The mean pH values of all CBM and SBM were higher than that of SM (9.52). Compared to CBM, SBM had relatively low pH and supplementation with SM reduced it further to values approaching the SM. Statistically significant differences in pH ($F(8, 81) = 2.45$, $P < 0.05$) were observed among all media, with significantly ($P < 0.05$) higher values in all CBM except 50CM. In contrast, the pH of all SBM (SHM, 25SHM, 50SHM and 50SHM⁻) were not significantly different from that of SM despite the decreased pH levels in 25SHM and 50SHM. Mean salinity also showed significant differences ($F(8, 63) = 40.61$, $P < 0.01$) among the media, with significantly ($P < 0.01$) higher salinity being in all CBM than those of other media. The salinities of 25CM, 50CM and 50CM⁻ were significantly lower ($P < 0.05$) than that of CM although they were still higher than that of SM. Among SBM, those supplemented with SM showed some reduction in salinity (SHM > 25SHM > 50SHM), particularly that of 25SHM and 50SHM was closely approaching the salinity of SM (12.88 g L⁻¹), but that supplemented with SM⁻ (50SHM⁻) was significantly reduced ($P < 0.05$).

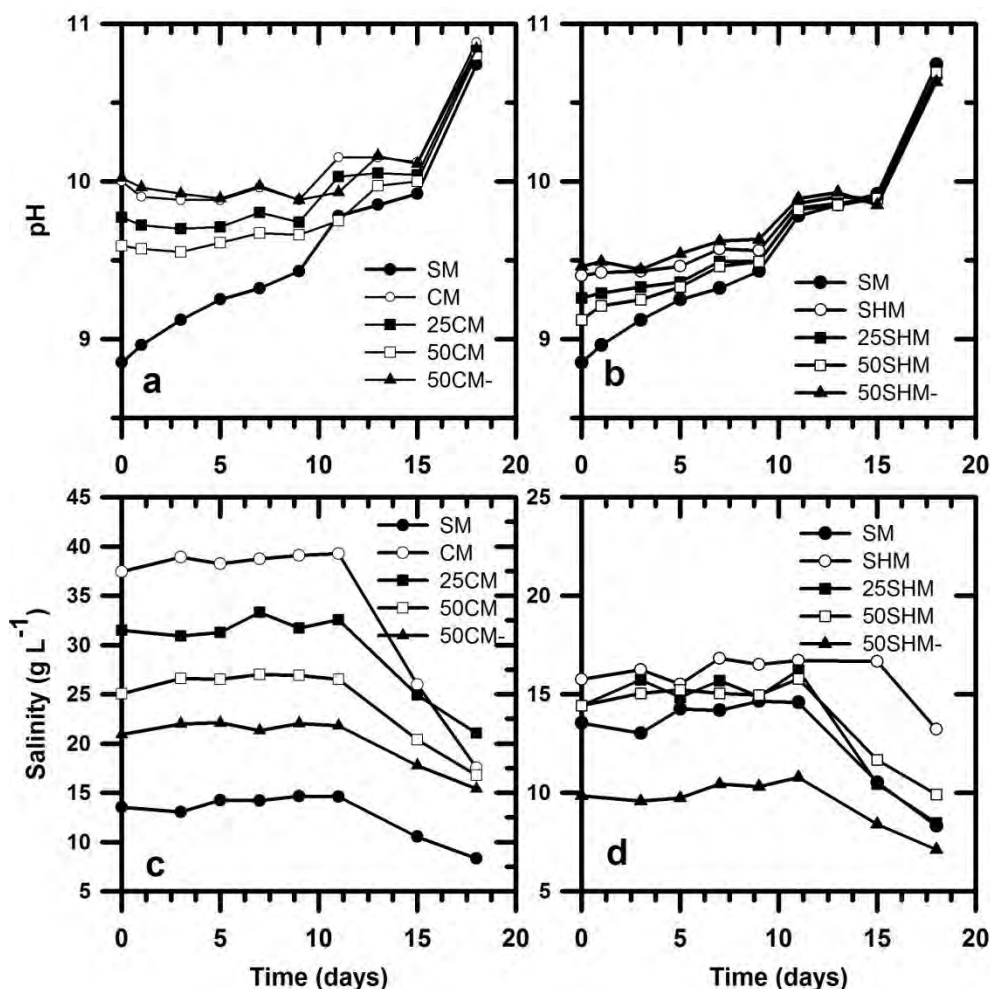


Fig. 5.2. Changes in pH (a and b) and salinity (c and d) with time during the cultivation of *Arthrospira fusiformis*. a and c for Lake Chitu water-based media, b and d for Lake Shala water-based media.

5.3.3. Growth and biomass production

Figure 5.3 shows growth curves of *Arthrospira* cultured in different complex media in comparison with that in SM. The growth curves show the typical pattern of exponential growth of microalgae without distinct lag phase. High growth of *Arthrospira* was observed in the SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM although better growth was exhibited by the cultures in the SBM. Low growth was observed in the CM, SHM, 50CM⁻ and 50SHM⁻.

Responses of *Arthrospira* reflected in such growth parameters as specific growth rate (μ), biomass production (B) and doubling time (dt) determined from chl-*a* measurements are given in Table 5.3. Higher μ and B and the shortest dt were achieved in SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM, with higher values comparable to that of SM in the SBM. The least values of μ and B and the largest dt were observed in CM and 50CM⁻. The μ and B in both 50CM⁻ and 50SHM⁻ showed a slight increase compared to SHM and CM. Analysis of variance (ANOVA) of the growth parameter B did not, however, show significant differences among the various media.

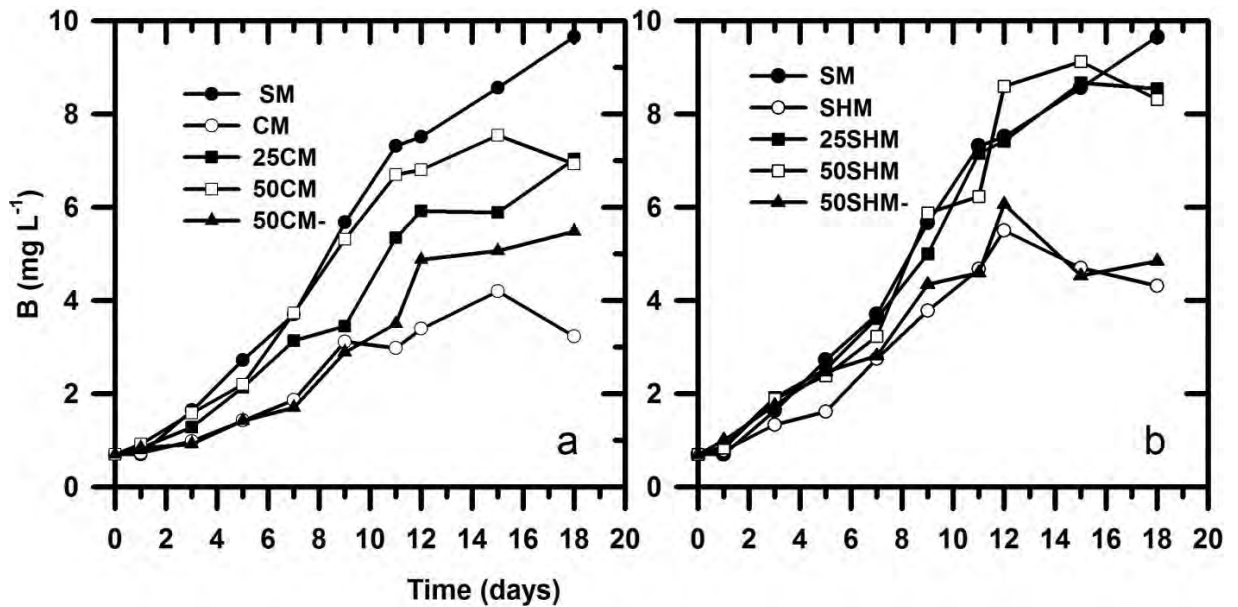


Fig. 5.3. Growth curves of *Arthrospira fusiformis* cultivated in various media at 35°C and photon flux density of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ during the cultivation period. *a* for Lake Chitu water-based media, *b* for Lake Shala water-based media, the control (SM) in both *a* and *b*.

Table 5.3. The growth parameters of *Arthrospira fusiformis* cultured in various media for 18 days.

Types of culture media	Growth parameters			
	$B_{\max}$	Mean B	μ	dt
SM	9.65	4.82±2.3	0.20	3.47
CM	4.20	2.26±0.8	0.13	5.22
25CM	7.03	3.57±1.3	0.18	3.85
50CM	8.54	4.15±1.9	0.19	3.62
50CM ⁻	5.48	2.74±1.1	0.16	4.24
SHM	5.50	3.01±1.0	0.17	3.99
25SHM	8.67	4.64±2.1	0.20	3.48
50SHM	9.12	4.72±2.3	0.21	3.28
50SHM ⁻	6.06	3.31±1.1	0.18	3.81

Maximum ($B_{\max}$) and mean of index of biomass (mg chl-*a* L⁻¹, $n=10$), μ (/day) and dt (days). The parameters μ and dt are calculated from the exponential growth phase (12 days)

The results of multiple regression analysis of the causal relationship between the response variable (B) and the chemical factors—pH and salinity indicated the statistically significant ($F(2,69) = 21.52$, $P < 0.01$) effect the two factors had on the biomass production of *Arthrospira*. pH was the main factor accounting for more than 52% of the variations in biomass. The relationships of μ and B with mean pH and salinity in different media of the cultures (Fig. 5.4) show that the increased μ and B in SM, 25CM and 50CM of CBM and 25SHM and 50SHM of SBM coincided with decreased mean pH and salinity while the lowest values of μ and B in CM and 50CM⁻ corresponded to the highest pH.

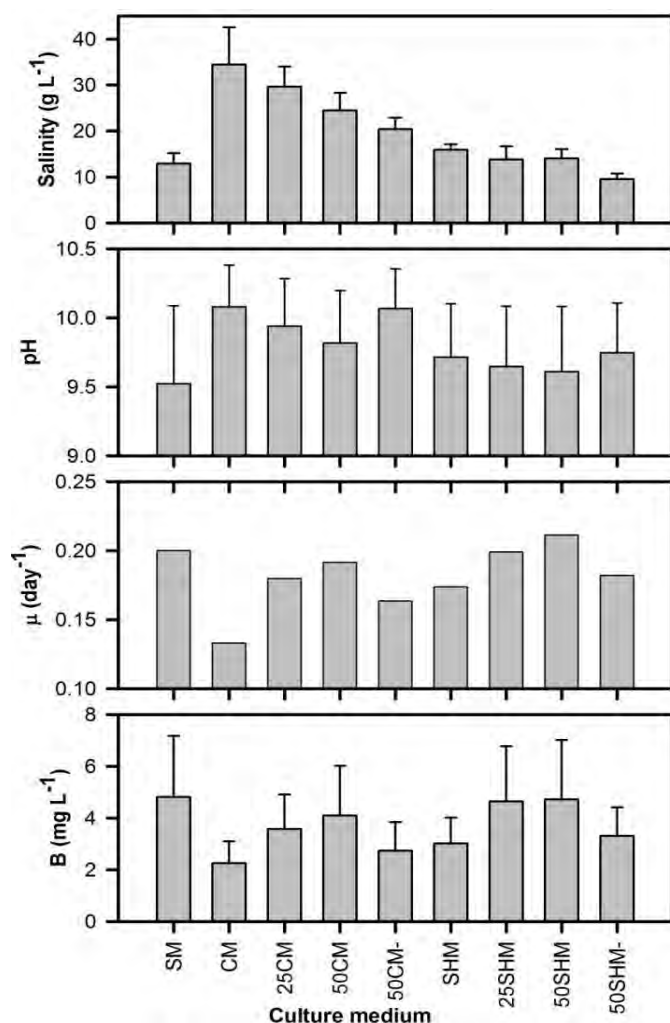


Fig. 5.4. Growth rate (μ) and index of biomass (B) of *Arthrospira fusiformis* in relation to pH and salinity of the various media used for laboratory cultures.

5.4. DISCUSSION

5.4.1. Some chemical features of the study lakes

The present study lakes are characterized by saline-alkaline features with high Na^+ , $\text{HCO}_3^- + \text{CO}_3^{2-}$ and Cl^- ions and low level of divalent cations (Ca^{2+} and Mg^{2+}). The main mechanisms for the formation of these ions are leaching of rock materials rich in Na from volcanic rocks of the catchment areas and evaporative concentration enhanced by the dry and warm climate (Klemper and Cash, 2007). In most saline lakes of arid regions,

these dominant ions are responsible for the high pH, alkalinity, conductivity and salinity of their waters (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994). The low level of the divalent cations is a salient feature of tropical saline–alkaline lakes (Elizabeth Kebede *et al.*, 1994; Zinabu Gebre-Mariam, 2002; Klemper and Cash, 2007) as these cations are precipitated from solution as their respective carbonates at high alkaline pH.

High pH and salinity and a large reserve of inorganic carbon sources and SRP are characteristic of some of the East African soda lakes, which favor dense populations of microalgae (Talling *et al.*, 1973; Melack *et al.*, 1982; Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Jones and Grant, 1999). Further explanation on these parameters is given in chapter 2. The pH and salinity of a medium affect different physiological processes such as growth, photosynthesis and chemical production of *Arthrospira* (Guterman *et al.*, 1989; Vonshak *et al.*, 1996; Elizabeth Kebede, 1997). The high level of carbonate–bicarbonate is important as a buffer system to maintain optimum alkaline pH and provides carbon source for aquatic autotrophs such as alkaliphilic cyanobacteria (Richmond, 1990; Vonshak, 1997). It has been suggested that high level of CO_3^{2-} to HCO_3^- ratio at pH >10 may indicate a low level of HCO_3^- , which is the principal and preferred carbon source for *Arthrospira* (Binaghi *et al.*, 2003) and buffer against a pH rise. The low CO_3^{2-} to HCO_3^- ratio (or high HCO_3^-) in Lake Shala compared to Lake Chitu may indicate that its water is well buffered against a pH rise and presence of surplus carbon source for growth of *Arthrospira*.

Growth and biomass production of algae are enhanced when nitrogen and phosphate sources are sufficiently available (Melack *et al.*, 1982; Larned, 1998; Miller *et al.*, 1999). In some African freshwaters, inorganic sources of nitrogen and phosphorus were often

suggested to be limiting to algal production (Talling and Talling, 1965; Melack *et al.*, 1982). Very high concentration of SRP was, however, recorded in the present study lakes, supporting the previous reports (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994). Thus, SRP may not be limiting algal growth in these soda lakes but its concentration may not be adequate to support optimal growth of *Arthrospira* from the production point of view. Nitrogen sources were low or undetectable in both lakes in the present study, corroborating the contention that nitrogen limitation of algal production is more likely in these soda lakes (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Talling and Lemoalle, 1998). Further information of these nutrients is provided in chapter 2. The unusually low level of nitrogen sources in Lake Shala may be attributed to the low concentration of decomposable organic matter and reduced rate of recycling owing to the great depth of the lake.

5.4.2. Salinity and pH of the experimental culture

The observed changes in pH of the experimental culture during the growth period can result from the chemical reactions among the carbonate systems and photosynthetic activities by dense algal biomass. According to Talling *et al.* (1973), dense algal biomass and vigorous photosynthesis in Lake Arenguade caused a rise in pH more than one pH unit despite the high buffering capacity associated with the lake's large carbonate–bicarbonate alkalinity. During growth, *Arthrospira* preferably consumes bicarbonate and releases carbonate into the medium and this carbonate/bicarbonate imbalance causes a progressive pH rise. The association of carbonate with proton (H^+) to produce bicarbonate, which is rapidly consumed by the alga, and dissolution of bicarbonate to CO_2 causes a rapid increase in pH (Richmond and Grobberlaar, 1986; Richmond, 2002).

The slower increase in pH in lake waters-based media than in the SM may be attributable to the high buffering capacity of the lake waters owing to their high carbonate–bicarbonate contents. The rapid decline of salinity observed after exponential phase may have resulted from the chemical transformation of carbonate–bicarbonate species, the major salinity components, and dilution of the culture through growth (assimilation of HCO_3^-).

The pH of Lake Chitu water was close to the upper limit of the optimal range for the growth of *Arthrospira* (8–10) (Richmond, 1990; De Oliveira *et al.*, 1999). Supplementation of the lake water with SM, however, led to significant pH reduction, which was pronounced in 50CM. The pH of Lake Shala water was lower than that of Lake Chitu water and supplementation caused its further reduction, though not significant, close to that of SM. The reduction in pH of the lake waters shortly after supplementation with SM (pH: 8.9) may be due to addition of carbonate–bicarbonates from SM, as the dissociation of bicarbonate to carbonate releases proton (H^+) in the system causing a short term pH decline. The change in pH due to supplementation was not large in Lake Shala because it had a low ratio of CO_3^{2-} to HCO_3^- , which probably made it more resistant to pH change. On the other hand, supplementation with SM^- (pH: 9.87) did not cause changes in pH (almost similar to the lake water), which may be due to the absence of the carbonate–bicarbonates in the supplement.

Lake Chitu water had relatively high salinity but supplementation with SM (salinity: 13.53 g L^{-1}) and SM^- (salinity: 5.23 g L^{-1}) significantly reduced its salinity closer to that of SM. The salinity of Lake Shala water was relatively low and supplementation reduced it further very close to that in the SM. The reduction in salinity following

supplementation could result from the dilution effect of the SM and SM^- , which had low salinity compared to both CBM and SBM. The large reduction in salinity of CBM and SBM when supplemented with SM^- seems to have resulted from the greater dilution effect of SM^- as it was more dilute than SM due to the lack of the major ions (Na^+ , Cl^- and $\text{CO}_3^{2-} + \text{HCO}_3^-$). In general, supplementation of the lake waters with SM lowered pH and salinity to values approaching that of the standard SM while supplementation with SM^- resulted in a large decline in salinity but not pH. Supplementation of the soda lake waters, by 25% and 50% with SM is, therefore, necessary to adjust pH and salinity to the optimal levels and also to provide deficient nutrients including nitrogen and phosphorus.

5.4.3. Growth and biomass production

Saline–alkaline conditions and availability of nutrients in the soda lakes are ideal conditions for the growth and production of *Arthrospira*. Better growth and biomass production, comparable to those observed in the SM, occurred in both CBM and SBM supplemented with SM by 25% and 50%, which had relatively low pH and salinity. This seems to have resulted from the reduction in pH and salinity by supplementation to levels, which were closer to those in the standard SM, due to dilution and addition of bicarbonate–carbonates. In contrast, the relatively low growth rates and biomass production observed in the CM and 50CM^- may be attributable to the high pH of the media. The pH determines the formation of carbonate–bicarbonate species and the solubility of minerals in the lake waters thereby influencing the metabolism of algae (Guterman *et al.*, 1989). Supplementation with SM adds more carbonate–bicarbonates that maintain alkaline pH and prevents carbon depletion, which is an important condition for the optimal growth of *Arthrospira* (Richmond, 1990; Vonshak, 1997). In an

experiment carried out using lagoon water supplemented with sodium bicarbonate and urea, Costa *et al.* (2003) also obtained the highest biomass production with the addition of 2.88 g L⁻¹ sodium bicarbonate. Salinity of a medium affects different physiological processes such as growth, photosynthesis and chemical production of *Arthrospira* (Vonshak *et al.*, 1996; Elizabeth Kebede, 1997). Several studies made on the growth and production of *A. fusiformis* in media with different salinity levels have demonstrated the association of higher growth rate and production of the alga with media of relatively low salinity (Elizabeth Kebede, 1997; Vonshak, 1997; Mussagy, 2006). Exposure of *Arthrospira* culture to high salinities was found to result in the reduction and eventual cessation of growth (Vonshak, 1997; Mussagy, 2006), which was associated with substantial decrease in photosystem II activities due to osmotic shock (Zeng and Vonshak, 1998). In addition, availability of limiting nutrients such as nitrogen and phosphorus enhances growth and biomass production of algae (Larned, 1998; Miller *et al.*, 1999). Supplementation may have provided these nutrients, particularly nitrogen, which is limiting in these soda lakes. The results of experiments carried out using seawater enriched with phosphate and urea, in both laboratory and outdoor mass cultivation of *Arthrospira*, showed high biomass yield, which was slightly less than that in the standard medium (Tredici *et al.*, 1986; Materasi *et al.*, 1984).

The higher growth rate and biomass production with shorter doubling time observed in SBM seems to be associated with some chemical features of Lake Shala. Primarily, pH, salinity, conductivity, CO₃²⁻ + HCO₃⁻ and Na⁺ of this lake were closer to those of the SM, indicating suitable conditions and huge carbon source (HCO₃⁻) for the alga. Secondly, the abundant SRP level in Lake Shala, which was about twice higher than that

in Lake Chitu, could favor better growth and higher biomass production of the species under consideration. Furthermore, Lake Shala was deficient in nitrogen sources compared to that of Lake Chitu, and hence supplementation would provide this nutrient thereby enhancing the production of the alga.

The large reserve of inorganic carbon, which prevails even at high pH values, is a salient feature of soda lakes that favor dense populations of *A. fusiformis* (Talling *et al.*, 1973; Wood and Talling, 1988). In the natural ecosystem, Lake Chitu supports superabundant populations of *Arthrospira* while the exceptionally deep adjacent Lake Shala, which has broadly similar gross chemical features, is devoid of *Arthrospira* and much less productive. On the basis of the high light extinction coefficient and optical depth, and undetectable concentration of nitrogen compounds in Lake Shala, light and nitrogen were previously identified as the major factors associated with its low phytoplankton productivity (Wood and Talling, 1988; Elizabeth Kebede *et al.*, 1994; Zinabu Gebre-Mariam, 2002). As the results of the present study show, Lake Shala water supported better growth of *Arthrospira* with provision of light and nutrients, and its aggregate chemical features except nitrogen were in the range required for *Arthrospira* growth. Light and nitrogen nutrients together with other hydrographic and morphometric variables, therefore, probably have contributed to the low productivity and absence of *Arthrospira* in the natural ecosystem of Lake Shala. Pilot-scale experimental testing of *Arthrospira* production in shallow ponds using Lake Shala water may enable one to provide further plausible explanations for the low productivity of the lake.

In conclusion, comparable growth and biomass production of *Arthrospira* was achieved using water of the soda lakes Chitu and Shala. Supplementation of the waters of the soda

lakes with SM by 25% and 50% further enhanced the growth and biomass production of *Arthrospira*. Supplementation with SM reduced pH and supported better growth and more production than SM⁻, indicating the importance of addition of carbonate–bicarbonates even to the lake waters rich in these compounds. Lake Shala-based media supported better growth and higher biomass of *Arthrospira* than Lake Chitu-based media, indicating that some of the chemical features of Lake Shala are more conducive for *Arthrospira* production. Therefore, *Arthrospira* can be produced using 25% and 50%-supplemented soda lake waters, preferably the large lake Shala water, thereby reducing the cost of nutrient media by 75% and 50%, respectively, which is a rough estimation based on the proportion of lakes water that could be combined with SM to produce a culture medium.

CHAPTER 6 . GENERAL CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

This study addressed the physicochemical characteristics and phytoplankton community in the soda lakes Chitu and Shala, morphology of *Arthrospira* in the natural ecosystem, and production of *Arthrospira* using water from the soda lakes. In this chapter, the main findings of the study are summarized and some recommendations are forwarded.

The first study investigated temporal and spatial (vertical) variations in physicochemical characteristics of Lakes Chitu and Shala. Lake Chitu showed poor underwater light climate, superficial thermal stratification with weak temperature gradients, slow water column mixing, anoxic water column, and high SRP with low or undetectable level of nitrogen nutrients in the euphotic zone. Most of the physicochemical parameters measured for Lake Chitu showed substantial temporal (on monthly basis) and vertical variability, which seemed to be regulated by processes basically taking place within the lake, associated most importantly with algal productivity, microbial activity, anoxic water column and hydrological changes. This shows that Lake Chitu can be very sensitive to changes in environmental conditions brought about by natural or human-induced processes and these alterations may drastically affect its ecological integrity and the key phytoplankton, *Arthrospira* and its ecological values. The observed environmental parameters of Lake Shala also indicated the occurrence of poor underwater light climate, superficial thermal stratification with strong temperature gradients, and high SRP with low or undetectable levels of nitrogen sources in the euphotic zone. This lake had turbulently mixing and well oxygenated water column, with the development of weak chemical stratification. The measured parameters of Lake Shala, however, showed small

temporal and vertical variations, suggesting that Lake Shala could be more resilient to the current level of environmental changes that may have resulted from processes that occur in its large catchment area and within it, possibly because of its large size and great depth. In both lakes, variations of most of the environmental parameters measured with seasons were small although the variations on month to month basis were notable, particularly for the small productive lake Chitu.

The second component of the study investigated the qualitative and quantitative variations of phytoplankton over an annual cycle in Lakes Chitu and Shala, in relation to environmental factors. The phytoplankton community of Lake Chitu was dominated exclusively by *A. fusiformis* throughout the annual cycle, making it the key ecological component of the lake. Biomass (chl-*a* and dry weight) and abundance of *A. fusiformis* were shown to undergo considerable seasonal variations following variability in some environmental drivers, mainly alkalinity-salinity, associated with seasonal hydrological changes. This suggests that any pronounced changes in environmental variables, particularly salinity, may greatly affect *A. fusiformis* biomass production thereby causing instability and starvation of the flamingos populations with eventual impairment of the ecosystem values of Lake Chitu. The relatively diverse phytoplankton community of Lake Shala lacked perceptible species succession and marked variability in its generally low biomass. Cryptomonads and diatoms, which have special adaptations to the prevailing low light and mixing conditions of the lake, persistently dominated the phytoplankton community throughout the annual cycle while exhibiting some seasonal variations in abundance. The strong association of the abundance of the most dominant taxa and total phytoplankton biomass with low water transparency, and the continual

dominance of species adapted to low light and mixing suggest the overriding importance of low underwater light and water column mixing in controlling composition and biomass of phytoplankton in Lake Shala. It seems that this physical condition has hindered the growth of such other algal species as *Arthrospira*, which are less adaptable to mixing conditions, with consequent low overall biomass productivity in Lake Shala. The results of the study on Lake Shala show that the composition and biomass of phytoplankton of the lake did not exhibit considerable seasonal changes, probably because of the muted seasonality of physicochemical variables owing to the lake's insensitiveness (i.e. high stress absorbing capacity) to the current level of changes in environmental factors.

The third component of the study determined morphological variability of *Arthrospira* in response to environmental conditions in the natural ecosystem of Lake Chitu. Three different morphotypes of *Arthrospira*, varying typically in their helicity, trichome features (dimensions, numbers and end-shape), and abundance, occurred in the lake. Such variations were mainly associated with variations in salinity, light intensity, temperature and nutrient concentrations in the lake. The observed variability in the abundance and morphology of morphotypes and their associations with some environmental variables seem to suggest that *Arthrospira* species could undergo morphological changes in response to environmental stresses in their natural habitats—soda lake ecosystems. The different morphotypes observed in Lake Chitu reflect the existence of different variants of the same species of *A. fusiformis*. The measured size of all the morphotypes was within the size range considered to be suitable for efficient filtration by the flamingos, suggesting that the alga is a suitable food source for these birds.

The last study evaluated the potential of soda lake waters from Chitu and Shala for biomass production of *A. fusiformis* in laboratory cultures. Comparable growth and biomass production of *A. fusiformis* was achieved using water of the soda lakes Chitu and Shala. Supplementation of the waters of the soda lakes with standard *Spirulina* medium by 25% and 50% further enhanced the growth and biomass production of *A. fusiformis*. pH and salinity played considerable regulatory roles in the growth and biomass production of *Arthrospira* and supplementation with the standard medium adjusted the pH and salinity close to the optimal range by dilution and addition of carbonate–bicarbonates. Lake Shala-based media supported better growth and higher biomass of *A. fusiformis* than Lake Chitu-based media, suggesting that some of the chemical features of Lake Shala are more conducive for *Arthrospira* production. This was the desired result as the main goal was to show the use of the large volume of Lake Shala water for mass production. Thus, *Arthrospira* can be produced using 25% and 50%-supplemented soda lake waters, preferably Lake Shala water, thereby reducing the cost of nutrient media by 75% and 50%, respectively.

6.2. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are given:

- ❖ Owing to the sensitive nature of Lake Chitu as reflected in the observed environmental parameters, it is more likely that a small impact emanating from anthropogenic activities on its catchment (inner side of the crater wall) or at lakeshore may bring drastic ecological changes. Therefore, it is high time that we establish regulatory and management systems to mitigate the potential effect of anthropogenic disturbances on the lake ecosystem and to ensure sustainable use of

the lake resources. The observed small temporal and vertical variations in most of the measured parameters in Lake Shala were not because of the absence of environmental degradation, which has already been identified as a threat, but due mainly to the lake's high buffering capacity. As it is evident from its poor underwater light climate caused by non-algal turbidity, high impact of runoff from the highly degraded and deforested catchment of Lake Shala is likely to ensue in the not-to-far future, affecting the composition and productivity of phytoplankton, and causing siltation problems in the lake. This suggests the need for immediate catchment management actions to be carried out, with emphasis on and priority being given to the more sensitive and dynamic lake Chitu. Possible general measures may include soil and vegetation management, and implementing proper park demarcation and management schemes and strategies. Reforestation of the degraded crater wall and catchment of the lakes, prohibiting clearing of trees and shrubs for the purpose of producing fuel wood and charcoal, and reducing agricultural activities and grazing pressure emanating from the huge populations of livestock commonly seen on the catchment areas and at lakeshores could be among the crucial specific measures to be taken. Other anthropogenic activities in the lakes and on their catchments without appropriate management and regulatory actions on the existing degradation problems may aggravate the problem.

- ❖ In both lakes, the observed notable monthly variations in most of the environmental parameters, particularly in the small productive lake Chitu, suggest the need to investigate short-term (at least weekly) changes that our longer (monthly) sampling intervals might have missed out. Further investigations on

short-term intervals, on daily or weekly basis, may be important to show a clear picture of changes occurring in the lakes, particularly in Lake Chitu. In addition, the depth profiles determined in this study for the deep lake Shala were insufficient. Further studies covering the entire water column may clearly demonstrate the patterns of thermal and chemical stratification in its water column. Further studies are also needed to determine the spatial (horizontal) variability in environmental parameters in the large lake Shala.

- ❖ *A. fusiformis* was found to be the major phytoplankton controlling the ecological integrity in Lake Chitu and its significant seasonal changes were highly associated with salinity. Any natural or human-induced processes that enhance salinization should, therefore, be controlled to reduce its negative effects. Catchment management schemes described before in this chapter could be among the crucial measures that should be taken. There should also be long-term studies on algal dynamics to closely monitor and predict the potential ecological changes and their effects on flamingos in the dynamic lake Chitu.
- ❖ Although the observed morphotypes showed the existence of different variants of the same species of *Arthrospira*, further molecular studies using high-resolution molecular markers may be of high importance to make a clear distinction among the observed morphological variants. The measured size of *Arthrospira* suggests that the alga could be the main food source for the flamingos. However, since the species has been shown to undergo morphological changes in response to environmental variables in its natural habitat, environmental modifications that enhance compacting of the filaments (reduce spiral forms) may affect the feeding

behavior of the flamingos by clogging their filtering systems. Thus, avoiding such environmental modifications as salinization deserve particular attention.

- ❖ The evaluation of soda lake waters showed that *Arthrospira* can be produced using 25% and 50%-supplemented soda lake waters, preferably Lake Shala water. However, in order to make use of soda lake water as a low-cost medium for *Arthrospira* production, evaluations in outdoor conditions and further optimization studies with environmental variables including nutrients are essential. Furthermore, analysis of the nutritional profile of the algal biomass produced using soda lake water is valuable.

REFERENCES

- Aiba, S. and Ogawa, T. (1977). Assessment of growth yield of a blue-green alga *Spirulina platensis* in axenic and continuous culture. *J. Gen. Microbiol.* **102**:179–182.
- Allen, J.I. and Polimene, L. (2011). Linking physiology to ecology: towards a new generation of plankton models. *J. Plankton Res.* **33**:989–997.
- American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF) (1999). *Standard methods for the examination of water and Waste water*, 20th ed. APHA, Washington, DC.
- American Public Health Association (APHA) American Water Works Association (AWWA) and Water Environment Federation (WEF) (1995). *Standard methods*, 19th ed. APHA, Washington, DC.
- Amha Belay (2008). *Spirulina (Arthrospira)*: production and quality assurance. **In**: *Spirulina* in human nutrition and health, pp.1–26, (Gershwin, M.E. and Amha Belay, eds). CRC Press, Boca Raton.
- Amha Belay (2002). The potential application of *Spirulina (Arthrospira)* as a nutritional and therapeutic supplement. *JANA* **5**:27–48.
- Amha Belay (1997). Mass culture of *Spirulina* outdoors—the earthrise farms experience. **In**: *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology, pp. 131-158, (Vonshak, A., ed.). Taylor and Francis, London.
- Amha Belay, Kato, T. and Ota, Y. (1996). *Spirulina (Arthrospira)*: potential application as an animal feed supplement. *J. Appl. Phycol.* **8**:303-311.

- Amha Belay and Ota, Y. (1993). Current knowledge on potential health benefits of *Spirulina*. *J. Appl. Physiol.* **5**:235-241.
- Andersen, R.A. and Kawachi, M. (2005). Traditional microalgae isolation techniques. **In:** *Algal culturing techniques*, pp. 83–100, (Andersen, R.A., ed.). Elsevier, London.
- Ayala, F., Vargas, T. and Cardenas, A. (1988). Chilean experiences on microalgae culture. **In:** *Algal biotechnology, proceedings of the 4th international meeting of the SAA*, pp. 229–236, (Stadler, T., Mollion, J., Verdus, M.C., Karamanos, Y., Morvan, H. and Christiasen, D., eds). Elsevier, London.
- Bai, J.N. (1985). Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. *Arch. Hydrobiol. Suppl.* **71**:191-199.
- Bai, J. N. and Seshadri, V. C. (1980). On coiling and uncoiling of trichomes in the genus *Spirulina*. *Arch. Hydrobiol.* **60**:32–47.
- Baldia, S.F., Nishijima, T. and Hata, Y. (1991). Effects of physicochemical factors and nutrients on the growth of *Spirulina platensis* isolated from Lake Kojima, Japan. *Nippon Suisan Gakka.* **57**(3):481–490
- Ballot, A., Krienitz, L., Kotut, K., Wiegand, C., Metcalf, S.J., Codd, A.G. and Pflugmacher, S. (2004a). Cyanobacteria and cyanobacterial toxins in three alkaline rift valley lakes of Kenya-Lakes Bogoria, Nakuru and Elmenteita. *J. Plankton Res.* **26**:925-935.
- Ballot, A., Dadheech, P.K. and Krienitz, L. (2004b). Phylogenetic relationship of *Arthrospira*, *Phormidium* and *Spirulina* strains from Kenyan and Indian water bodies. *Algol. Stud.* **113**:37–56.

- Baumann, A., Forstner, U. and Rohde, R. (1975). Lake Shala: water chemistry, mineralogy and geochemistry of sediments in an Ethiopian Rift Lake. *Geol. Rdsch.* **64**:593-609.
- Baxter, R.M., Prosser, M.V., Talling, J.F. and Wood, R. B. (1965). Stratification in tropical African lakes at moderate altitudes (1500-2000). *Limnol. Oceanogr.* **10**:510-520.
- Baye Sitotaw (2014). *Microbial diversity of two Ethiopian soda lakes having contrasting physicochemical features*. PhD Thesis, Addis Ababa University, Addis Ababa. 204 pp.
- Beadle, L.C. (1981). *The inland waters of tropical Africa: an introduction to tropical limnology*. Longman, New York. 475 pp.
- Bellinger, E.G. and Sigeo, D.C. (2010). *Freshwater algae: identification and use as bioindicators*. John Wiley and Sons, Ltd, Chichester. 118 pp.
- Binaghi, L., Borghi, A.D., Lodi, A., Converti, A. and Borghi, M.D. (2003). Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. *Process Biochem.* **38**:1341–1346.
- Blinn, D.W., Halse, S.A., Pinder, A.M., Shiel, R.J. and McRae, J.M. (2004). Diatom and micro-invertebrate communities and environmental determinants in the western Australian wheatbelt: a response to salinization. *Hydrobiologia* **528**:229–248.
- Boussiba, S. (1989). Ammonia uptake in the alkalophilic cyanobactrium *Spirulina platensis*. *Plant cell Physiol.* **30**:303–308.

- Carini, S.A. and Joye, S.B. (2008). Nitrification in Mono Lake, California: activity and community composition during contrasting hydrological regimes. *Limnol. Oceanogr.* **53**(6): 2546–2557.
- Caron, D.A., Sanders, R.W., Lim, E.L., Marrase, C., Amaral, L.A., Whitney, S., Aoki, R.B. and Porter, K.G. (1993). Light-dependent phagotrophy in the freshwater mixotrophic chrysophyte *Dinobryon cylindricum*. *Microbe Ecol.* **25**:93–111.
- Carpenter, S.R., Lathrop, R.C. and Muñoz-del-Rio, A. (1993). Comparison of dynamic models for edible phytoplankton. *Can. J. Fish Aquat. Sci.* **50**:1757–1767.
- Chen, F., Zhang, Y. and Guo, S. (1996). Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnol. Lett.* **18**(5):603-608.
- Ciferri, O. (1983). *Spirulina*, the edible micro-organism. *Microbiol. Rev.* **47**(4):551–578.
- Cogne, G., Lasseur, C., Cornet, J.F., Dussap, C.G. and Gros, J.B. (2001). Growth physiology of microorganism (*S. platensis*) by pressure measurement. *Biotechnol. Lett.* **23**:1309–1314.
- Colla, L.M., Reinehr, C.O., Reichert, C.J. and Costa, A.V. (2007). Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresour. Technol.* **98**:1489–1493.
- Costa, J.A.V., Colla, L.M. and Filho, F.D. (2003). *Spirulina platensis* growth in open raceway ponds using freshwater supplemented with carbon nitrogen and metal ions. *Z. Naturforsch.* **58** (c):76–80.
- Dadheech, P.K., Ballot, A., Casper, P., Kotut, K., Novelo, E., Lemma, B., Pröschold, T. and Krienitz, L. (2010). Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Cyanobacteria) of African, Asian and American origin

- deduced by 16S–23S ITS and phycocyanin operon sequences. *Phycologia* **49**:361–372.
- Daniel Gamachu (1977). *Aspects of climate and water budget in Ethiopia*. Addis Ababa University Press, Addis Ababa. 71 pp.
- Desikachary, T.V. and Bai, N. J. (1996). Taxonomic studies in *Spirulina*. II. The identification of *Arthrospira* (*Spirulina*) strains and natural samples of different geographical regions. *Algol. Stud.* **83**:163–178.
- Dhiab, R.B., Ouada, H.B., Boussetta, H., Franck, F., Elabed, A. and Brouers, M. (2007). Growth, fluorescence, photosynthetic O₂ production and pigment content of salt adapted cultures of *Arthrospira* (*Spirulina*) *platensis*. *J. Appl. Phycol.* **19**:293-301.
- Diehl, S., Berger, S., Ptacnik, R. and Wild, A. (2002). Phytoplankton, light and nutrients in a gradient of mixing depths: field experiments. *Ecology* **83**:399–411.
- Dortch, Q., Clayton, J.R., Thoresen, S.S. and Ahmed, S.I. (1984). Species differences in accumulation of nitrogen pools in phytoplankton. *Mar. Biol.* **81**:237–250.
- Dubinsky, Z. and Berman, T. (1976). Light utilization efficiencies of phytoplankton in Lake Kinneret (Sea of Galilee). *Limnol. Oceanogr.* **21**:226–230.
- Edmondson, W.T and Winberg, G.C. (1971). *A manual on methods for the assessment of secondary productivity in fresh waters, IBP hand book 17*. Oxford black well Scientific Publications. 358 pp.
- Egge, J.K. and Aksnes, D.L. (1992). Silicate as a regulating nutrient in phytoplankton competition. *Mar. Ecol. Prog. Ser.* **83**:281–289.

- Elizabeth Kebede (1997). Response of *Spirulina platensis* (*Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. *J. Appl. Phycol.* **9**:551-558.
- Elizabeth Kebede (1996). *Phytoplankton in a salinity-alkalinity series of lakes in the Ethiopian Rift Valley*. PhD Thesis, Uppsala University, Uppsala.
- Elizabeth Kebede, Zinabu Gebre-Mariam and Ahlgren, I. (1994). The Ethiopian Rift Valley lakes: chemical characteristics of a salinity–alkalinity series. *Hydrobiologia* **288**:1–12.
- Estrada, M. and Berdalet, E. (1997). Phytoplankton in a turbulent world. *Sci. Mar.* **61**:125–140.
- Eugster, H.P. (1970). *Chemistry and origin of the brines of Lake Magadi, Kenya*. Mineralogical Society of America Special Paper 3, pp. 213–235.
- EWNHS (2009). *Settlement expansion and natural resource management problems in the Abijata-Shala lakes National Park*. Walia No. 26, Ethiopian Wildlife and Natural History Society (EWNHS), Addis Ababa. 56 pp.
- Finkel, Z.V., Beardall, J., Flynn, K.J., Quigg, A., Rees, T.A.V. and Raven, J.A. (2010). Phytoplankton in a changing world: cell size and elemental stoichiometry. *J. Plankton Res.* **32**:119–137.
- Food and Agriculture Organization of the United Nations (FAO) (2008). Guide to laboratory establishment for plant nutrient analysis. *FAO Fertil. Plant Nutr. Bull.* 19.
- Fox, R.D. (1996). *Spirulina: production and potential*. Edisud, Aix en Provence, France. 231 pp.

- Gasse, F. (1986). *East African diatoms taxonomy and ecological distributions*. J. Cramer, Stuttgart. 201 pp.
- Gasse, F., Talling, J.F. and Kilham, P. (1983). Diatom assemblages in East Africa: classification, distribution and ecology. *Rev. Hydrobiol. Trop.* **16**:3–34.
- Golubtsov, A.S., Dgebuadze, Y.Y. and Mina, M.V. (2002). Fishes of the Ethiopian Rift Valley. **In**: *Ethiopian Rift Valley Lakes*, pp. 167–258, (Tudorancea, C. and Taylor, W.D., eds). Backhuys, Leiden.
- Goodwin, T. W. (1974). Carotenoids and biliproteins. **In**: *Algal physiology and biochemistry*, pp. 176–205, (Stewart, W. D. P., ed.). University of California Press, Berkeley.
- Grant, W.D. (2006). Alkaline environments and biodiversity. **In**: *Extremophiles* (Gerday, E.C. and Glansdorff, N., eds). UNESCO, EOLSS Publishers (<http://www.eolss.net>), Oxford, UK.
- Guillard, R.R.L. (1973). Culture methods and growth measurements division rates **In**: *Handbook of phycological methods*, pp. 289–311, (Stein, J.R., ed.). Cambridge University Press, Cambridge.
- Guterman, H., Vonshak, A. and Ben-Yaakov, S. (1989). Automatic online growth estimation method for outdoor algal biomass production. *Biotechnol. Bioeng.* **34**:143–152.
- Habib, M.A.B., Parvin, M., Huntington, T.C. and Hasan, M.R. (2008). *A review on culture production and use of Spirulina as food for humans and feeds for domestic animals and fish*. FAO Fisheries and Aquaculture Circular No 1034, Rome. 33 pp.

- Hammer, U.T. (1986). *Saline lake ecosystems of the world*. Junk publishers, Dordrecht. 616 pp.
- Hansson, L.A., Rudstam, L.G., Johnson, T.B., Soranno, P. and Allen, Y. (1994). Patterns in algal recruitment from sediment to water in a dimictic, eutrophic lake. *Can. J. Fish Aquat. Sci.* **51**:2825-2833.
- Harper, D.M., Childress, R.B, Harper, M.M., Boar, R.R., Hickley, P., Mills, S.C., Otieno, N., Drane, T., Vareschi, E., Nasirwa, O., Mwatha, W.E., Darlington, J.P.E.C. and Escute-Gasulla, X. (2003). Aquatic biodiversity and saline lakes: Lake Bogoria National Reserve, Kenya. *Hydrobiologia* **500**:259–276.
- Hecky, R.E. (1993). The eutrophication of Lake Victoria. *Mitt. Internat. Verein. Limnol.* **25**:39–48.
- Hecky, R.E. and Kilham, P. (1973). Diatoms in alkaline saline lakes: ecology and geochemical implications. *Limnol. Oceanogr.* **18**:53-71.
- Hindák, F. (1985). Morphology of trichomes in *Spirulina fusiformis* Voronichin from Lake Bogoria, Kenya. *Arch. Hydrobiol.* **38/39**:201–218.
- Hoffmann, L., Komárek, J. and Kastovsky, J. (2005). System of cyanoprokaryotes (cyanobacteria)-state in 2004. *Algol. Stud.* **117**:95–115.
- Hötzl, G. and Croome, R. (1999). *A phytoplankton methods manual for Australian freshwaters*. Occasional papers 22/99, Land and Water Resource Research Development Corporation, Canberra. 51 pp.
- Jellison, R., MacIntyre, S. and Millero F.J. (1999). Density and conductivity properties of Na-CO₃-Cl-SO₄ brine from Mono Lake, California. *Int. J. Salt Lake Res.* **8**:41–53.

- Jenkin, P.M. (1957). The filter feeding and food of flamingos (Phoenicopteri). *Philos. Trans. R. Soc. London, Ser. B* **240**:401– 493.
- Jones, B.E. and Grant, W.D. (1999). Microbial diversity and ecology of the Soda Lakes of East Africa. **In:** *Microbial biosystems: new frontiers: proceedings of the 8th international symposium for microbial ecology*, pp. 681–687, (Bell, C.R., Brylinsky, M. and Johnson-Green, J.P., eds). Antic Canada Society for Microbial Ecology, Halifax.
- Jones, B.E., Grant, W.D., Collins, N.C. and Mwatha, W.C. (1994). Alkaliphiles: diversity and identification. **In:** *Bacterial diversity and systematic*, pp.195–230, (Priest, F.G., Ramos-Cormenzana, A. and Tindall, B.J., eds). Plenum Press, New York.
- Jourdan, J.P. (1993). Solarium *Spirulina* farm in the Atacama Desert (North Chile). *Spiruline algue de vie Bull. Inst. Oceanogr. Monaco Mus. Oceanogr.* **12**:191–194.
- Joye, S.B. and Hollibaugh, J.T. (1995). Influence of sulfide inhibition of nitrification on nitrogen regeneration in sediments. *Science* **270**(5236):623-625.
- Kaggwa, M.N., Gruber, M., Oduor, S.M. and Schagerl, M. (2013a). Detailed time series assessment of the diet of Lesser Flamingos: further explanation for their itinerant behavior. *Hydrobiologia* **710**:83–93.
- Kaggwa, M.N., Burian, A., Oduor, S.M. and Schagerl, M. (2013b). Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. *Microbiology open* **2**:881– 891.
- Kalff, J. (2002). *Limnology: Inland water ecosystems*. Prentice-Hall Inc., New Jersey. 592 pp.

- Kalff, J. (1983). Phosphorus limitation in some tropical African lakes. *Hydrobiologia* **100**:101–112.
- Kaplan, A., Zenvirth, D., Reinhold, L. and Berry, J.A. (1982). Involvement of a primary electrogenic pump in the mechanism of HCO_3^- uptake by *Anabaena variabilis*. *Plant Physiol.* **69**:978–982.
- Kawachi, M. and Noel, M.H. (2005). Sterilization and sterile techniques. **In:** *Algal culturing techniques*, pp. 65–81, (Andersen, R.A., ed.). Elsevier, London.
- Kelly, M. (2000). Identification of common benthic diatoms in rivers. *Field Studies* **9**:583–700.
- Kilham, P. (1984). Sulfate in African inland waters: sulfate to chloride ratios. *Verh. Int. Ver. Limnol.* **22**:296–302.
- Kilham, P. and Kilham, S.S. (1990). Endless summer: Internal loading processes dominate nutrient cycling in tropical lakes. *Freshwater Biol.* **23**:379–389.
- Kilic, C., Goksan, T., Ak, I. and Gokpinar, S. (2006). İki farkli *Spirulina paltensis* supunun buyume ozelliklerinin karsilastirilmesi. *E.Ü. Su Ürünleri. Dergisi.* **23** (1-2):189–192.
- Kim, C.J, Jung, Y.H. and Oh, H.M. (2007). Factors indicating culture status during cultivation of *Spirulina (Arthrospira) platensis*. *J. Microbiol.* **45**:122–127.
- Kirk, J.T. (1994). *Light and photosynthesis in aquatic ecosystems*. Cambridge Uni. Press, Cambridge. 357 pp.
- Klemper, S.L. and Cash, M.D. (2007). Temporal geochemical variation in Ethiopian Lakes Shala, Arenguade, Awasa, and Beseka: Possible environmental impacts from underwater and borehole detonations. *J. Afr. Earth Sci.* **48**:174–198.

- Komárek, J. and Kling, H.J. (1991). Variation in six planktonic cyanophyte genera in Lake Victoria (East Africa). *Algol. Stud.* **61**:21- 45.
- Komárek, J., Kling, H.J. and Komárková, J. (2003). Filamentous cyanobacteria. In: *Freshwater algae of North America, ecology and classification*, pp. 117–191, (Wehr, D.J. and Sheath R.G., eds). Academic Press, Massachusetts.
- Komárek, J. and Lund, J.W.G. (1990). What is „*Spirulina platensis*’ in fact? *Algol. Stud.* **58**:1–13.
- Krienitz, L., Dadheech, P.K. and Kotut, K. (2013). Mass development of a small sized ecotype of *Arthrospira fusiformis* in Lake Oloidien, Kenya, a new feeding ground for Lesser Flamingos in East Africa. *Fottea* **13**(2):215–225.
- Krienitz, L. and Kotut, K. (2010). Fluctuating algal food populations and the occurrence of Lesser Flamingos (*Phoeniconaias minor*) in three Kenyan rift valley lakes. *J. Phycol.* **46**:1088–1096.
- Lanzén, A., Simachew, A., Gessesse, A., Chmolarska, D., Jonassen, I. and Øvreås, L. (2013). Surprising prokaryotic and eukaryotic diversity, community structure and biogeography of Ethiopian soda lakes. *PLoS ONE* **8**(8):e72577.
- Larned, S.T. (1998). Nitrogen-versus phosphorus-limited growth and sources of nutrients for coral reef algae. *Mar. Biol.* **132**:409–421.
- Lemoalle, J. (1981). Photosynthetic production and phytoplankton in the euphotic zone of some African and temperate lakes. *Rev. Hydrobiol. Trop.* **14**:31–37.
- Lepš, J. and Šmilauer, P. (2003). *Multivariate analysis of ecological data using Canoco*. Cambridge Univ. Press, Cambridge. 283 pp.

- Le Turdu, C., Tiercelin, J.J., Gibert, E., Travi, Y., Lezzar, K.E., Richert, J.P., Massault, M., Gasse, F., Bonnefille, R., Decobert, M., Gensous, B., Jeudy, V., Tamrat, E., Mohammed, M.U., Martens, K., Atnafu, B., Chernet, T., Williamson, D. and Taieb, M. (1999). The Ziway-Shala lake basin system, Main Ethiopian Rift: influence of volcanism, tectonics and climatic forcing on basin formation and sedimentation. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **150**:135–177.
- Lewin, R.A. (1980). Uncoiled variants of *Spirulina platensis* (Cyanophyceae). *Algol. Stud.* **26**:48–52.
- Lewis, W.M. Jr. (2002). Causes for high frequency of nitrogen limitation in tropical lake. *Verh. Int. Verein. Theor. Angew. Limnol.* **28**:210–231.
- Li, R.H., Debella, H.J. and Carmichael, W.W. (2001). Isolates identifiable as *Arthrospira maxima* and *Arthrospira fusiformis* (Cyanobacteria) appear identical on the basis of a morphological study in culture and 16S rRNA gene sequences. *Phycologia* **40**:367–371.
- Li, P. and Gao, K. (2008). Effects of solar UV and visible radiations on the spiral structure and orientation of *Arthrospira platensis* (Cyanophyta). *Phycologia* **47**:573–579.
- Ma, Z. and Gao, K. (2009). Photoregulation of morphological structure and its physiological relevance in the cyanobacterium *Arthrospira (Spirulina) platensis*. *Planta* **230**:329–337.
- MacIntyre, S. (1981). *Stratification and mixing in shallow tropical African lakes*. PhD Thesis, Duke University, Durham, NC. 338 pp.
- Mari, C. and Collar, N. (2000). *Pink Africa*. Harvill, London. 207 pp.

- Materassi, R., Tredici, M. and Balloni, W. (1984). *Spirulina* culture in seawater. *Appl. Microbiol. Biotechnol.* **19**:384–386.
- Melack, J. M. (2009). Diel variability and community metabolism in African soda lakes. *Natural Resources and Environmental Issues: saline lakes around the world*, vol. 15, article 28.
- Melack, J.M. (1988). Primary producer dynamics associated with evaporative concentration in a shallow, equatorial soda lake (Lake Elmenteita, Kenya). *Hydrobiologia* **158**:1–14.
- Melack, J.M. (1979). Temporal variability of phytoplankton in tropical lakes. *Oecologia* **44**:1–7.
- Melack, J.M., Kilham, M.P. and Fisher, T.R. (1982). Responses of phytoplankton to experimental fertilization with ammonium and phosphate in an African soda lake. *Oecologia* **55**:1-6.
- Miller, M.W., Hay, M.E., Miller, S.L., Sotka, E. and Szmant, A.M. (1999). Effects of nutrients versus herbivores on reef algae: a new method for manipulating nutrients on coral reefs. *Limnol. Oceanogr.* **44**:1847–1861.
- Muhling, M., Somerfield, P.J., Harris, N., Belay, A. and Whitton, B.A. (2006). Phenotypic analysis of *Arthrospira* (*Spirulina*) strains (Cyanobacteria). *Phycologia* **45**:148–157.
- Mussagy, A. (2006). *The cyanophyte Arthrospira fusiformis in African waters-ecophysiology and potential use in tropical aquaculture*. PhD Thesis, Lund University, Lund. 118 pp.

- Neilson, A.H. and Larsson, T. (1980). The utilization of organic nitrogen for growth of algae: physiological aspects. *Physiol. Plant* **48**(4):542–553.
- Nelissen, B., Wilmotte, A., Neefs, J.M. and De Wachter, R. (1994). Phylogenetic relationships among filamentous helical cyanobacteria investigated on the basis of 16S ribosomal-RNA gene sequence analysis. *Syst. Appl. Microbiol.* **17**:206–210.
- Oduor, S.O. and Schagerl, M. (2007a). Phytoplankton primary productivity characteristics in response to photosynthetically active radiation in three Kenyan rift valley saline-alkaline lakes. *J. Plankton Res.* **29**(12):1041–1050.
- Oduor, S.O. and Schagerl, M. (2007b). Temporal trend of ion contents and nutrients in three Kenyan rift valley saline-alkaline lakes and their influence on phytoplankton biomass. *Hydrobiologia* **584**:59–68.
- OEPO (2005). Review of the current status and an outline of a future management plan for Lakes Abijata and Ziway. Oromia Environmental Protection Office. Unpublished Technical Report, Addis Ababa. 80 pp.
- Okoth, O.E., Mucai, M., Shivoga, W.A., Miller, S.N., Rasowo, J. and Ngugi, C.C. (2009). Spatial and seasonal variations in phytoplankton community structure in alkaline-saline lake Nakuru, Kenya. *Lakes Reserv. Res. Manag.* **14**:57–69.
- Oliveira, M.A.C.L., Monteiro, M.P.C., Robbs, P.G. and Leite, S.G.F. (1999). Growth and chemical composition of *Spirulina maxima* and *S. platensis* biomass at different temperatures. *Aquac. Int.* **7**:261-275.
- Olsen, S. and Paasche, E. (1986). Variable kinetics of silicon-limited growth in *Thalassiosira pseudonanna* (Bacillariophyceae) in response to changed chemical composition of the growth medium. *J. Phycol.* **21**:183–190.

- Oren, A. (2004). A proposal for further integration of the cyanobacteria under the bacteriological code. *Int. J. Syst. Evol. Microbiol.* **54**:1895–1902.
- Paerl, H.W., Valdes-Weaver, L.M., Joyner, A.R. and Winkelmann, V. (2007). Phytoplankton indicators of ecological change in the eutrophying Pamlico Sound system, North Carolina. *Ecol. Appl.* **17**:S88–S101.
- Peters, R. H., MacIntyre, S. (1976). Orthophosphate turnover in an East African lake. *Oecologia* **25**: 313-319.
- Popovich, C.A., Spetter, C.V., Marcovecchio, J.E. and Freije, R.H. (2008). Dissolved nutrient availability during winter diatom bloom in a turbid and shallow estuary (Bahía Blanca, Argentina). *J. Coast. Res.* **24**: 95–102.
- Raven, J. A. and Kubler, J. E. (2002). New light on the scaling of metabolic rate with the size of algae. *J. Phycol.* **38**:11–16.
- Reynolds, C.S. (1996). The plant life of the pelagic. *Verh. Int. Ver. Theor. Angew. Limnol.* **26**:79–113.
- Reynolds, C. E. (1987). Cyanobacterial water blooms. **In**: *Advances in Botanical Research*, pp. 67-143, (Callow, J., ed.). Academic Press, London.
- Reynolds, C. S. (1984). Phytoplankton periodicity: interactions of form, function and environmental variability. *Freshwat. Biol.* **14**:111–142.
- Reynolds, C. S., Irish, A. E. and Eliot, J. A. (2001). The ecological basis for stimulating phytoplankton responses to environmental change (PROTECH). *Ecol. Model.* **140**: 271–91.
- Richmond, A. (2002). Microalgal biotechnology at the turn of the millennium: a personal view. *J. Appl. Phycol.* **12**:441–451.

- Richmond, A. (1990). Large scale microalgal culture and applications. **In:** *Progress in phycological research*, pp. 269–330, (Round, F.E. and Champman, D.J., eds). Biopress, Bristol.
- Richmond, A. and Grobbelaar, J.U. (1986). Factors affecting the output rate of *Spirulina platensis* with reference to mass cultivation. *Biomass* **10**:253–64.
- Richmond, A., Vonshak, A. and Arad, S. (1980). Environmental limitations in outdoor production of algal biomass. **In:** *Algae biomass*, pp. 65–72, (Shelef, G. and Soeder, C.J., eds). Elsevier/North Holland Biomedical Press, Amsterdam.
- Sakshaug, E., Demers, S. and Yentsch, C.M. (1987). *Thalassiosira oceanica* and *Thalassiosira pseudonana*: two different photoadaptational responses. *Mar. Ecol. Prog. Ser.* **41**: 275–282.
- Schagerl, M. and Oduor, S.O. (2008). Phytoplankton community relationship to environmental variables in three Kenyan Rift Valley saline–alkaline lakes. *Mar. Freshwater Res.* **59**:125–136.
- Seaman, M. T., Ashton, P. J. and Williams, W. D. (1991). Inland salt waters of southern Africa. *Hydrobiologia* **210**:75-91.
- Sinistro, R., Izaguirre, I. and Asikian, V. (2006). Experimental study on the microbial plankton community in a South American wetland (Lower Parana ´ River Basin) and the effect of the light deficiency due to the floating macrophytes. *J. Plankton Res.* **28**:753–768.
- Stephenson, J.G. (1978). An appraisal of the conservation of nature in the Lakes Abijata and Shala locality with recommendations. Ethiopian Wildlife Conservation Organization (EWCO), Addis Ababa.

- Stoyneva, P.M., Descy, J-P. and Vyverman, W. (2007). Green algae in Lake Tanganyika: is morphological variation a response to seasonal changes? *Hydrobiologia* **578**:7–16.
- Sullivan, J. M. and Swift, E. (2003). Effects of small-scale turbulence on net growth rate and size of ten species of marine dinoflagellates. *J. Phycol.* **94**:83–94.
- Tadesse Ogato, Demeke Kifle, Tadesse Fetahi and Baye Sitotaw (2014). Evaluation of growth and biomass production of *Arthrospira (Spirulina) fusiformis* in laboratory cultures using waters from the Ethiopian soda lakes Chitu and Shala. *J. Appl. Phycol.* **26**:2273–2282.
- Talling, J.F. (2010). pH, the CO₂ system and freshwater science. *Freshwater Rev.* **3**:133–146.
- Talling, J.F. (2001). Environmental controls on the functioning of shallow tropical lakes. *Hydrobiologia* **458**:1–8.
- Talling, J. F. (1992). Environmental regulation in African shallow lakes and wetlands. *Rev. Hydrobiol. Trop.* **25**: 87-144.
- Talling, J. F. and Driver, D. (1963). Some problems in the estimation of chlorophyll-*a* in phytoplankton. Proceedings of a conference on primary productivity measurements, marine and freshwater, pp.142-146. U.S. Atomic Energy Comm. TID-7633.
- Talling, J.F. and Lemoalle, J. (1998). *Ecological dynamics of tropical inland waters*. Cambridge Univ. Press, Cambridge. 441 pp.
- Talling, J.F. and Talling, I.B. (1965). The chemical composition of African lake waters. *Int. Rev. Gesamten. Hydrobiol.* **50**:421–463.

- Talling, J.F., Wood, R.B., Prosser, M.V. and Baxter, R.M. (1973). The upper limit of photosynthetic productivity by phytoplankton: evidence from Ethiopian soda lakes. *Freshwater Biol.* **3**:53–76.
- Taylor, J.C., Harding, W.R. and Archibald, C.G.M. (2007). *A methods manual for the collection, preparation and analysis of diatom samples, version 1*. Water research commission, South Africa.
- Tenalem Ayenew and Dagnachew Legesse (2007). The changing face of the Ethiopian rift lakes and their environs: call of the time. *Lakes Reserv. Res. Manag.* **12**:149–165.
- Ter Braak, C.J.F. and P. Smilauer, 1998. *CANOCO reference manual and user's guide to Canoco for Windows*. Microcomputer Power, Ithaca, USA. 352 pp.
- Thomas, W.A., Dodson, A.N. and Reid, F.M.H. (1978). Diatom productivity compared to other algae in natural marine phytoplankton assemblages. *J. Phycol.* **14**: 250-253.
- Thomas, W.H., Vernet, P.M. and Gibson, C.H. (1995). Effects of small-scale turbulence on photosynthesis, pigmentation, cell division, and cell size in the marine dinoflagellate *Gonyaulax polyedra* (Dinophyceae). *J. Phycol.* **31**:50–59.
- Thomasson, K. (1960). Ett fall av tropisk vattenblomning. *Botaniska Notiser* **113**:592–601.
- Tomaselli, L. (1997). Morphology, ultrastructure and taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira* (*Spirulina*) *platensis*. **In**: *Spirulina platensis* (*Arthrospira*): physiology, cell biology and biotechnology, pp.1-15, (Vonshak, A., ed.). Taylor and Francis, London.

- Tredici, M., Papuzzo, T. and Tomaselli, L. (1986). Outdoor mass culture of *Spirulina maxima* in seawater. *Appl. Microbiol. Biotechnol.* **24**:47–50.
- Tudorancea, C., Zinabu Gebre-Mariam and Elias Dadebo (1999). Limnology in Ethiopia. **In: *Limnology in developing countries***, pp. 63–118, (Wetzel R.G. and Gopal, B., eds). International Scientific Publications, New Delhi.
- Turner, S. (1997). Molecular systematics of oxygenic photosynthetic bacteria. *Plant Syst. Evol. Suppl.* **11**:13–52.
- UNESCO (1983). *Algorithms for computation of fundamental properties of seawater*. UNESCO technical papers in marine science 44. 58 pp.
- Van Eykelenburg, C. (1979). The ultrastructure of *Spirulina platensis* in relation to temperature and light intensity. *A. Van Leeuw.* **45**:369–390.
- Vareschi, E. (1982). The ecology of Lake Nakuru (Kenya): III. Abiotic factors and primary production. *Oecologia* **55**:81–101.
- Vareschi, E. and Vareschi, A. (1984). Ecology of Lake Nakuru (Kenya). IV. Biomass and distribution of consumer organisms. *Oecologia* **61**:70–82.
- Verburg, P., Hecky, R. E. and Kling, H. J. (2003). Ecological consequences of a century of warming in Lake Tanganyika. *Science* **301**:505–507.
- Verschuren, D. (1999). Influence of depth and mixing regime on sedimentation in a small, fluctuating tropical soda lake. *Limnol. Oceanogr.* **44**:1103–1113.
- Von Damm, K.L. and Edmond, J.M. (1984). Reverse weathering in the closed-basin lakes of the Ethiopian Rift and Lake Turkana. *Amer. J. Sci.* **284**:835–862.

- Vonshak, A. (1997). Outdoor mass production of *Spirulina*: the basic concept. **In:** *Spirulina platensis (Arthrospira): Physiology, cell biology and biotechnology*, pp.79–99, (Vonshak, A., ed.). Taylor and Francis, London.
- Vonshak, A., Guy, R. and Guy, M. (1988). The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch. Microbiol.* **150**:417–420.
- Vonshak, A. and Tomaselli, L. (2000). *Arthrospira (Spirulina)*: systematics and ecophysiology. **In:** *The Ecology of Cyanobacteria*, pp. 505–522, (Whitton, B.A. and Potts, M., eds). Kluwer, Dordrecht, the Netherlands.
- Vonshak, A., Torzillo, G., Accolla, P. and Tomaselli, L. (1996). Light and oxygen stress in *Spirulina platensis* (cyanobacteria) grown outdoors in tubular reactors. *Physiol. Plant* **97**: 175–179.
- Walker, K.F., Likens, G.E. (1975). Meromixis and a reconsidered typology of lake circulation patterns. *Verh. Int. Verein. Theor. Angew. Limnol.* **19**:442–458.
- Wallace, R.B. and Gobler, C.J. (2014). Factors controlling blooms of microalgae and macroalgae (*Ulva rigida*) in a Eutrophic, Urban Estuary, Jamaica Bay, New York. *Estuar. Coast.*: DOI 10.1007/s12237-014-9818-1.
- Wang, Z.P. and Zhao, Y. (2005). Morphological reversion of *Spirulina (Arthrospira) platensis* (Cyanophyta): from linear to helical. *J. Phycol.* **41**:622–628.
- Wetzel, R.G. (2001). *Limnology: lake and river ecosystems*. Academic Press, San Diego. 1006 pp.
- Willén, E. (1991). Planktonic diatoms-an ecological review. *Algol. Stud.* **62**:69–106.
- Williams, W.D. (2002). Environmental threats to salt lakes and the likely status of inland saline ecosystems in 2025. *Environ. Conserv.* **29**(2):154–167.

- Williams, W.D. (1998). Salinity as a determinant of the structure of biological communities in salt lakes. *Hydrobiologia* **381**:191–201.
- Wood, R.B., Baxter, R.M., Prosser, M.V. (1984). Seasonal and comparative aspects of chemical stratification in some tropical crater lakes, Ethiopia. *Freshwater Biol.* **14**:551–573.
- Wood, R.B., Prosser, M.V. and Baxter, R.M. (1978). Optical characteristics of the Rift Valley Lakes, Ethiopia. *SINET: Ethiop. J. Sci.* **1**:73–85.
- Wood, R.B., Prosser, M.V. and Baxter, R.M. (1976). The seasonal pattern of thermal characteristics of four of the Bishoftu crater lakes, Ethiopia. *Freshwater Biol.* **6**:519–530.
- Wood, R.B. and Talling, J.F. (1988). Chemical and algal relationships in a salinity series of Ethiopian inland waters. *Hydrobiologia* **158**:29-67.
- Wu, H., Gao, K., Villafane, V., Watanabe, T. and Helbling, E.W. (2005). Effects of solar UV radiation on morphology and photosynthesis of the filamentous cyanobacterium *Arthrospira platensis*. *Appl. Environ. Microbiol.* **71**:5004–5013.
- Zeng, M.T. and Vonshak, A. (1998). Adaptation of *Spirulina platensis* to salinity-stress. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **120/121**:113-118.
- Zhang, Q., Gradinger, R. and Spindler, M. (1999). Experimental study on the effect of salinity on growth rates of Arctic-sea-ice algae from the Greenland Sea. *Boreal Env. Res.* **4**:1–8.
- Zinabu Gebre-Mariam (2002). The effect of wet and dry seasons on the concentrations of solutes and phytoplankton biomass in seven Ethiopian Rift Valley lakes. *Limnologica* **32**:169–179.

APPENDICES

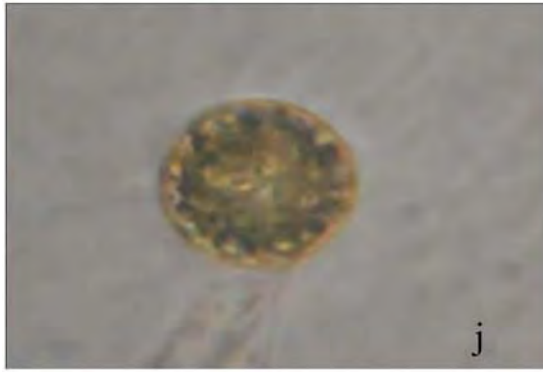
Appendix 1. Some phytoplankton species identified in samples collected from Lake

Chitu: a–c *Arthrospira fusiformis*; d–f *Anabaenopsis abijatae*; g *Phormidium formosum*;

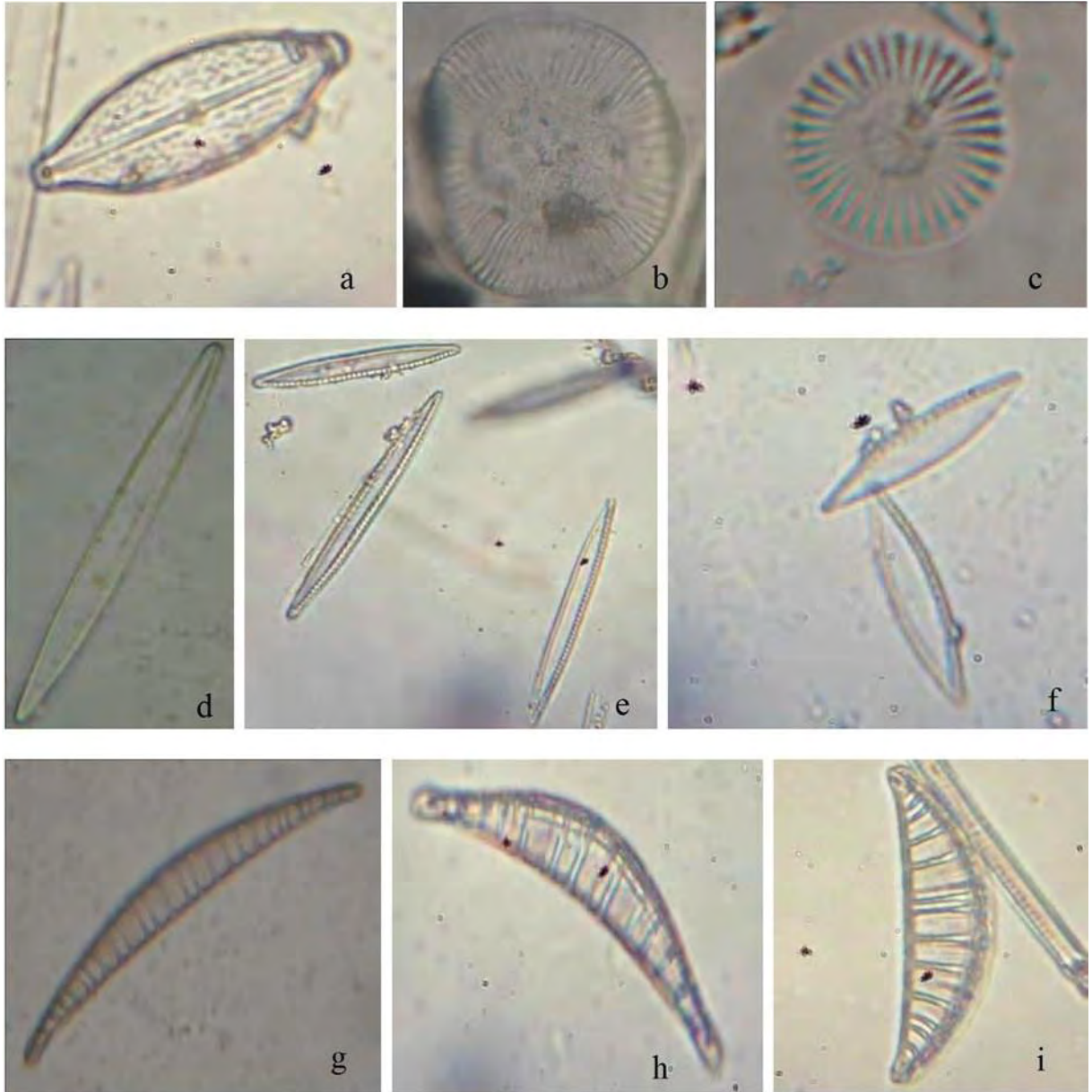
h *Spirulina major*; i *Anomoeoneis spaerophora*; j *Cyclotella* sp.; k *Navicula* sp.; l–n

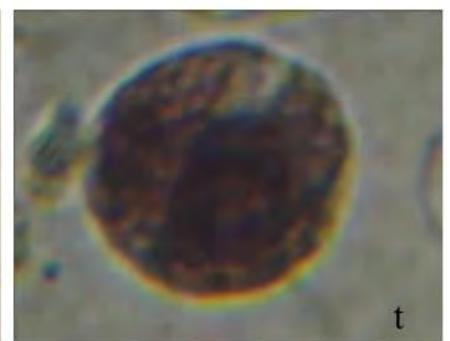
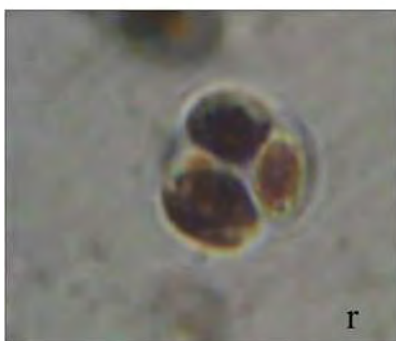
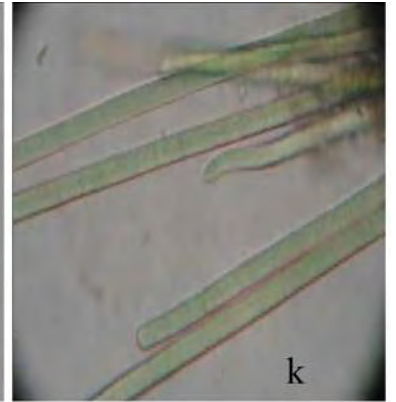
Nitzschia spp.; o *Rhopalodia gibberula*; p *Rhopalodia* sp.; q *Cryptomonas marssonii*.





Appendix 2. Some phytoplankton species identified in samples collected from Lake Shala: a *Anomoeoneis sphaerophora*; b *Cyclotella iris*; c *Cyclotella meneghiniana*; d–f *Nitzschia* spp.; g–f *Rhopalodia gibberula*; i *Rhopalodia musculus*; j *Rhopalodia gibba*; k *Phormidium formosum*; l–n *Thalassiosira* sp.; o–p *Cryptomonas marssonii*; q *Cryptomonas obovata*; r *Oocystis* sp.; s–t *Glenodinium* spp.

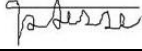




DECLARATION

I, the undersigned, declare that this dissertation is my original work and that all sources of materials used for the dissertation have been duly acknowledged.

Name: Tadesse Ogato

Signature: 

Date: June 25, 2015