2009

Investigation of Factors Limiting Pelagic Phytoplankton Abundance and Composition in the Ancient Malili Lakes of Indonesia

Amy Snook
University of Windsor

INVESTIGATION OF FACTORS LIMITING PELAGIC PHYTOPLANKTON ABUNDANCE AND COMPOSITION IN THE ANCIENT MALILI LAKES OF INDONESIA

by

Amy Snook

A Thesis
Submitted to the Faculty of Graduate Studies
through Environmental Science
in partial fulfillment of the requirements for
the degree of Master of Science at the
University of Windsor

Windsor, Ontario, Canada

2009

INVESTIGATION OF FACTORS LIMITING PELAGIC PHYTOPLANKTON ABUNDANCE AND COMPOSITION
IN THE ANCIENT MALILI LAKES OF INDONESIA

by

Amy Snook

APPROVED BY:

_____

J. Gagnon
Department of Earth and Environmental Sciences

_____

M. Cristescu
Great Lakes Institute for Environmental Research

_____

G.D. Haffner, Advisor
Great Lakes Institute for Environmental Research

_____

A. Fisk, Chair of Defense
Great Lakes Institute for Environmental Research

May 21, 2009

AUTHOR'S DECLARATION OF ORIGINALITY

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication.

I certify that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

Factors regulating composition and relative abundance in phytoplankton communities were investigated in an ancient lake with a highly endemic food web. Lake Matano has genera diversity (21) similar to tropical lakes, but low phytoplankton biomass (0.0162 µg/L). Vertical mixing physically limits primary production (42% Z_{eu}:Z_m); phytoplankton are moved below the euphotic zone. Macronutrient concentrations (0.30 µmol/L N, 0.13 µmol/L P) are low. Nutrient bioassays used N and P (30-320 µmol/L individually, 60:5-1920:160 µmol/L N:P combined). After 16 days, no significant ($p > 0.05$) changes in relative abundance and no biologically significant changes in phytoplankton biomass occurred. CHU-10 growth media cultivation, however, significantly increased chl-*a*. Analysis identified Cr (0.14 µmol/L) above a threshold to restrict algal growth (0.12 µmol/L) and Mo (0.0005 µmol/L) below requirements by most aquatic life (0.008 µmol/L). Therefore, physical (mixing) and chemical (toxicity, micronutrient) factors are concluded to limit composition and relative abundance of phytoplankton in Matano.

DEDICATION

This thesis is dedicated to everyone who refuses bottled water.

ACKNOWLEDGEMENTS

I would like to acknowledge my supervisor, Doug Haffner, for inviting me into this limnological lineage, and asking all the tough questions. He was always there for guidance or reassurance or a laugh. Thanks to Joel and Melania for their participation in this project.

Thanks to the extended Indo lab, particularly Bramburger, for being both theoretical and down-to-earth, Lis Sabo for her previous work with the plankton in this system, and Ryan Walters for genetics and statistics advice. Olga Babanazarova and Paul Hamilton provided much-needed plankton identification help. I thank our Indonesian friends, without whom our work would not be possible. Special thanks to Sulung and Lili.

Mark Cook and J.C. Barrett provided technical assistance and humour. Moral support and friendship were plentiful with Robin Angell, Bailey McMeans, Jill Olin, Sandra Ellis, Christina Smeaton, Romina Bortoluzzi, Courtney Beneteau, and Pat Murray—who are all so smart and beautiful. I thank my family who never let me down. Sincerest thanks to Chris Formagin for his continuous support and unwavering encouragement.

Table of Contents

LIST OF TABLES

Table 3.1 Detection limits for ICP-MS elemental analysis and mean concentrations (μmol/L) for the upper waters of lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO). Standard errors are shown.

Table 3.2 Overview of production and diversity indices for three Malili Lakes and Lake Poso, March 2008. Standard errors for biomasses are listed when possible.

Table 3.3 Nutrient data (μmol/L) of nitrogen as NH_3 and NO_3/NO_2 and phosphorus as SRP and TP for lakes Matano, Mahalona, Towuti, and Poso. Means and standard errors for Lake Matano were calculated from samples collected in July 1999, January 2000, and August 2000; Lake Mahalona samples were collected in February 1997; Lake Towuti samples were collected in February 2002; Lake Poso samples were collected in March 2008.

Table 3.4 ICP-MS elemental concentrations (μmol/L) for lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO). Detection limits for each element and mean concentrations for the upper waters of each lake are listed in Table 3.1.

Table 3.5 Analysis of ICP-MS elemental data for lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO) among the upper waters (100 m for MAT, TOW, POSO and 30 m for MAH). Pairwise comparisons were performed (Mann-Whitney U-test) between lakes for those elements with significantly different concentrations (Kruskal-Wallis test, $p < 0.05$). Significant results and mean concentrations (μmol/L) for the upper waters of each lake are listed.

APPENDIX I

Table I Abundance of phytoplankton genera as number of cells per L for Lake Matano. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Table II Abundance of phytoplankton genera as number of cells per L for Lake Mahalona. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Table III Abundance of phytoplankton genera as number of cells per L for Lake Towuti. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Table IV Abundance of phytoplankton genera as number of cells per L for Lake Poso. Divisions are represented as Ch for chlorophytes, and Cy for cyanobacteria.

LIST OF FIGURES

Figure 3.7 Principal Coordinates Analysis (PCO) based on ICP-MS elemental analysis. Samples were collected in March 2008. 95% confidence level error bars are shown. PCO 1 accounts for 77% of the variation in the dataset and is negatively correlated with K and Mo and positively correlated with Cr. PCO 2 accounts for 17% of the variation and is negatively associated with Na and P.

Figure 3.8 Canonical Correlation Analysis was used to test for relationships between elemental concentrations and phytoplankton species abundance in the four lakes. As illustrated in Figure 3.1, the left hand quadrant including P, Zn, and Cu represents Lake Matano, and the right hand quadrant represents Lake Poso. The strongest relationships between phytoplankton species abundance and elemental concentrations exist in these two lakes.

LIST OF APPENDICES

Chapter 1: Ancient lakes as species libraries

1.1 Ancient lakes for endemism

Most inland water bodies are of Holocene age (< 18,000 years old) but a few dozen lakes in the world are over a million years old (Martens, 1997). These lakes have survived sediment influx, often as a result of tectonic plate shifts offsetting sedimentation (Schön and Martens, 2004). Ancient lakes are closed environments that provide a high potential for radiation events, especially for flora and fauna that invaded early in the development of the lake. The most notable examples of species radiation in ancient lakes are those described for the cichlids from the East African Rift valley lakes with hundreds of species radiating in the upper layers of lakes Tanganyika, Malawi, and Victoria (e.g. Meyer et al., 1990).

Ancient lakes have long been recognized as centres of endemism and aquatic biodiversity with highly unique biological communities. The high degree of endemicity in ancient lakes has led researchers to conclude that most taxa are neo-endemics—that they presently live and are restricted to the area they originated. If this is correct, this makes ancient lakes objects of special interest to evolutionary studies (Schön and Martens, 2004). Lake Baikal in Siberia may be 30 million years old with 75% endemism (Sherbakov, 1999) and Lake Tanganyika in East Africa is 9-12 million years old with approximately 56% endemism (Granina, 1997). In Lake Baikal, hundreds of species of gammaroids occupy a variety of adaptive spaces along ranges of bathymetry, trophy, lifestyle from mutualism to parasitism, and body size from dwarves to giants (Kozhova and Izmest'eva, 1998; Takhteev, 2000). Table 1.1

provides a comparison of key physical, chemical, and biological aspects of selected ancient lakes. The lakes listed offer a range of depths and volumes, primary production and productivity, and macronutrients, but all support very high levels of endemism.

For its remarkable biodiversity and endemism, Sulawesi Island has been recognized as one of 20 hotspots for global conservation priorities (Myers et al., 2000). The United Nations Environment Program (UNEP) cites Sulawesi Island as one of the single most important sites for aquatic biodiversity in Asia. High diversity is linked to system stability and for systems that may be vulnerable to pollution, invasion, or eutrophication, the more that is understood about the factors regulating composition and relative abundance of species, the better these systems can be managed and protected. Historically, the Malili lakes have been subjected to few anthropogenic stressors, and remain relatively pristine natural environments. However, the creation of a nickel mine in the area has great potential to disturb this system. It is for this reason, and the increased demand on the lakes as sources of freshwater and sinks for wastewater for a growing population, that these lakes have become the subject of more recent research.

Sulawesi Island, Indonesia, contains a 5-lake system, the Malili Lakes, with high levels of endemism (Hustedt, 1942; Kottelat, 1990a, b, c; Haffner et al., 2001; von Rintelen & Glaubrecht, 2003; Roy et al., 2004). Sulawesi Island may have the highest

percentage of endemic terrestrial biota in the world (99%), and is a remarkable hotspot for biodiversity and aquatic endemism (Hustedt, 1942; von Rintelen and Glaubrecht, 2003; Haffner et al., 2001). What drives speciation processes in these lakes is not entirely certain. For ancient lakes, three different circumstances can exist regarding the degree of isolation as a criterion (Martens et al., 1994): allopatric (speciation during physical separation of populations), sympatric (speciation while populations inhabit the same geographic region), and parapatric (speciation due to variations in mating habits of populations in the same area). It is likely that ancient lake radiations have originated under the influence of adaptive forces. In many cases, it seems sympatric speciation with adaptation by resource partitioning is a common mode for radiations (Roy et al., 2006; Brooks, 1950; Davis, 1982; von Rintelen, 2007). In the Malili Lakes, studies have illustrated the importance of extirpation, speciation and resource partitioning in the development of a unique flora (Bramburger et al., 2004, 2008).

To understand the mechanisms regulating the unique phytoplankton flora, a suite of factors must be considered. Community composition is a function of dispersal, habitat quality (physical, chemical, and biological factors are often interactive and interdependent) and biological interactions (Lewis, 1983). It is assumed that planktonic species can disperse to most lakes, but in-lake processes act as filters that determine the final assemblage (Dumont, 1998). The resolution of the specific factors that regulate and maintain diversity in the lower trophic levels of the Lake Matano is the focus of this thesis.

1.2 The interplay of diversity and production

An approach to protecting biodiversity, identifying hotspots, was developed by Mittermeier in 1988 as a way to prioritize conservation action. The focus was on areas that support intact natural ecosystems containing native species and communities and a high diversity of locally endemic species. The widespread threat of human-induced ecological impoverishment in nearly all ecosystems has prompted further research into developing a general theory of the determinants for biodiversity (Dodson et al., 2000).

There is a link between biodiversity and production in aquatic and terrestrial systems, and research has attempted to understand how community diversity relates to ecological properties such as system productivity and community stability (Yacchi and Loreau, 1999; Interlandi and Kilham, 2001). Ecology's central tenet, that biodiversity is a product of ecosystem function, is being challenged by a new paradigm: that biodiversity governs ecosystem function (Naeem, 2002). However, there are presently conflicting views in the literature on whether diversity results in an increase or a decrease in system production (Interlandi and Kilham, 2001; Hooper et al., 2005; Long et al., 2007). As biodiversity has two main components: the species (composition) and the number of species (species richness), it is unclear which component has the greatest influence on ecosystem functioning.

To resolve this question of how biodiversity relates to production, according to

Lewis (2000), research should be focused on the endemic species of ancient lakes, which can provide valuable insight. The relative isolation and long histories of these lakes make them excellent subjects for studies on speciation and biodiversity. This thesis addresses the mechanisms regulating diversity and production in the phytoplankton community of ancient Lake Matano and the Malili Lake system.

1.3 Lake Matano

The Malili lakes of Sulawesi Island consist of five lakes within the Larona River watershed: Matano, Mahalona, Towuti, Lawontoa and Masapi (see Figure 1.1). Lake Matano is located at 2 ˚S, 121 ˚E and Lake Mahalona is downstream, with a 70 m drop in elevation. Mahalona then drains into Lake Towuti. Lake Masapi is also within this watershed, but is not directly connected to the other lakes, whereas Lake Lawontoa drains into the Tomingaga River that connects Lakes Mahalona and Towuti.

Sulawesi Island is located at the junction of the Asian, Australian and Pacific plates. The island was formed approximately 8-14 million years ago by the collision of the East Asian plate and the Philippines Pacific plates (Hamilton, 1978; Villeneuve et al., 2002). Alfred Wallace first described the biogeographical area Wallacea in 1860. His expeditions to the area led to the delineation of "Wallace's line," that is the faunal break beginning south of the Philippines, curving down to separate the Indonesian islands of Sulawesi, Borneo, and Bali from Lombok. The west side of the line contains the Asian fauna with characteristics of similar species found in Eurasia. On

the other side of the line the Australian fauna Wallace expected the island of Sulawesi to contain Asian fauna, but instead it was distinctly different from that in either the Eurasian or Australian zones (Whitten et al., 1987).

Formed by volcanism and tectonic collisions, Sulawesi Island has had continuous geographical isolation, which contributes to its highly endemic, yet species-poor fauna (Whitten et al., 1987). Sulawesi's lakes represent the oldest island-based aquatic ecosystems in the world (Brooks, 1950; Haffner et al., 2001), and are unique among ancient lakes systems in that they form a hydrological continuum. Lake Matano supports a simple fish community with four endemic genera of less than 20 species (Kottelat, 1991). The lake supports very low standing crops of diatoms (Hustedt, 1942; Bramburger et al., 2004), zooplankton (Sabo, 2006), fish (Kottelat, 1991; Roy et al., 2004), and gastropods (Sarasin and Sarasin, 1895; von Rintelen and Glaubrecht, 2003), and top predators are rare (Roy, 2006).

Biodiversity represents the accumulation of species over time by a complex process of immigration, speciation and extinction (Martens, 1997). Generally, the species composition of tropical communities is a consequence of past and present interspecific competition, resulting in each species occupying the habitat or utilizing the resource for which it is the most effective competitor (Connell, 1978).

In addition to this species-poor fauna, the flora within the lakes has been reported as very low (Hustedt, 1942; Bramburger et al., 2004; Sabo, 2006). Attempts at

measuring biomass within the lake have revealed very low concentrations of phytoplankton: 1.30 x 10^1 µg/L (Sabo, 2006). Reynolds et al. (2000) determined that there is generally a linear relationship between primary production and latitude, with highest production near the equator (Lewis, 1996). Biomass in a large lake of a similar latitude as Lake Matano (2˚S) is 1.7 x 10^{10} µg/L for Tanganyika; a comparison of annual productivity and phytoplankton biomass in other ancient lakes is available in Table 1.1.

Understanding the causes of this low algal biomass is further complicated as there are also several taxonomic groups of phytoplankton (Chrysophytes, Cryptophytes, Centrales), deemed ubiquitous in most fresh waters, which have not been detected in the open waters of Lake Matano or the other Malili lakes (Bramburger, 2004; Sabo, 2006; Chapter 2, 3). Phytoplankton communities in temperate and tropical zones have a great deal of overlap with respect to composition (Lewis, 1974), which demonstrates the adaptability and ease of transport of phytoplankton. For example, Cryptophytes, though rarely dominant, are ubiquitous among freshwaters (Reynolds et al., 2000). Interestingly, some species of Centrales (e.g. *Aulosira spp.*) have been observed as common and abundant in Lake Poso (Bramburger, pers. comm.), a lake not connected to the Malili lakes but also located on Sulawesi Island. Endemism is generally not observed in phytoplankton due to ease of dispersal, but the absence of typically cosmopolitan, oligotrophic species in the Malili Lakes is curious. As described by Lodge (1993), community composition is a function of dispersal, habitat quality (physical, chemical, and biological factors are often interactive and

interdependent), and biological interactions. This thesis investigates factors contributing to the low phytoplankton production and unique composition.

Previous studies conducted in the Malili Lakes suggested that complex interactions between nutrient limitation and metal toxicity influence phytoplankton community dynamics within Lake Matano (Sabo et al., 2008). A major factor in maintaining the low concentration of nutrients in the euphotic zone is the sinking of nutrient rich particles below the mixed layer at approximately 100 m (Haffner et al., 2001). Vertical sinking of macronutrients from the euphotic zone has been noted as a limiting factor to the standing crop of phytoplankton in other systems (Kiefer et al., 1972). Also, many species of cyanobacteria are capable of fixing atmospheric nitrogen (Tilman, 1982), but in Lake Matano, for example, the cyanobacteria are dominated by non-nitrogen-fixing species (Sabo, 2006). Haffner et al. (2001) conclude that the primary production in Matano is possibly limited due to extensive vertical mixing which continuously moves phytoplankton out of the euphotic zone.

Studies by Sabo (2006) suggested that Lake Matano is P-limited. Aquatic systems with limited resources inevitably host stronger competitive interactions among species, so one species will eventually out-compete another, consistent with the competitive exclusion principle (Hutchinson, 1961). The potential for limitation by macronutrients N and P in the lake is addressed in Chapter 2.

The watershed of the Malili Lakes consists of iron-rich lateritic material, rich in metals. It is not known if the unique watershed chemistry plays an important role in determining phytoplankton composition and abundance. It has been speculated that elevated metal concentrations in Lake Matano's water column could inhibit the ability of cosmopolitan species to successfully colonize the lake. Crowe et al. (2007) reported relatively high levels of Cr (VI) in the epilimnion (1.8×10^{-1} µmol/L), and Fernando (1987) predicted that the lack of cladocerans in Lake Matano was a function of Cr toxicity. Experiments with Matano's phytoplankton assemblage reported an increase in phytoplankton biomass, although not statistically significant and with no significant changes in species composition, when soluble Fe, Mn, Cr and Ni were reduced in Lake Matano water (Sabo, 2006). In addition, Matano perturbation experiments, simulating an upwelling event, reported an increase in plankton abundance (Bramburger, unpubl. data). The potential for limitation by metals or nutrients other than N or P is addressed in Chapter 3.

In understanding the production base of the system, we require more information about what drives the system's aquatic foodweb. Food web assemblages can be regulated by predation (top-down) or physicochemical factors (bottom-up). In many cases, algal biomass and primary productivity are regulated by the trophic level above (Shapiro et al., 1975). There is evidence, however, that the food web of Lake Matano is regulated by bottom-up controls, such that resource partitioning plays a critical role in the evolution and maintenance of the endemic assemblages supported by the lake (Roy et al., 2007).

The epilimnia of lakes are variable in space and time, and therefore provide complex environments for phytoplankton to grow and multiply. Many ecological factors, including temperature, light, water movement, and abiotic factors are important in regulating organic production in aquatic ecosystems (Reynolds, 1994). To understand the factors that regulate primary production in Lake Matano, we must evaluate all available evidence that includes physical, biological, and chemical aspects. Chapter 2 examines the most common control of phytoplankton biomass, macronutrients nitrogen (N) and phosphorus (P). Both of these nutrients are present in very low concentrations (Haffner et al., 2001; Sabo, 2006). An additional set of experiments was conducted collaboratively with R. Sotero-Santos, and compared the growth dynamics of Matano phytoplankton and cultured chlorophytes and cyanobacteria with different nutrient treatments. In these experiments, CHU-10 growth media (rich in Ca, Fe, K, Mg, Na, and Si) was used to culture the phytoplankton. Growth media was serially diluted with Lake Matano water, which served to dilute the concentration of nutrients and introduce trace elements. The results of these experiments lead to the Chapter 3 analysis of Lake Matano water and a close analysis of the phytoplankton population of the three Malili lakes (Matano, Mahalona, Towuti) and Lake Poso. Chapter 4 discusses these findings in the realm of possible control factors in a tropical lake, and states the greater implications of these findings.

1.4 References

Bramburger, A.J. (2004) *Floristics and Taxonomy of the Endemic Diatom Flora of the Malili Lakes, Sulawesi Island, Indonesia.* Thesis, University of Windsor, Canada.

Bramburger, A.J.; Hamilton, P.B.; Hehanussa, P.E.; Haffner, G.D. (2008) Processes regulating the community composition and relative abundance of taxa in the diatom communities of the Malili Lakes, Sulawesi Island, Indonesia. *Hydrobiol*., **615**: 215-224.

Brooks, J.L. (1950) Speciation in ancient lakes (concluded). *Q. Rev. Biol.,* **25**, 131-176.

Connell, J.H. (1978) Diversity in Tropical Rain Forests and Coral Reefs. *Science,* **199,** 1302-1309.

Crowe, S.A.; O'Neill, A.H.; Katsev, S.; Hehanussa, P.; Haffner, G.D.; Sundby, B.; Mucci, A.; Fowle, D. (2007) The biogeochemistry of tropical lakes: a case study from Lake Matano, Indonesia. *Limnol. Oceanogr*., **53**, 319-331.

Davis, G.M. (1982) Historical and ecological factors in the evolution, adaptive radiation, and biogeography of freshwater mollusks. *Amer. Zoo.,* **22**, 375-395.

Dodson, S.I.; Arnott, S.E.; Cottingham, K.L. (2000) The relationship in lake communities between primary productivity and species richness. *Ecology*, **81**: 2662-2679.

Dumont, H.J. (1998) The Caspian Lake: history, biota, structure, and function. *Limnol. Oceanogr.,* **43**, 44-52.

Fernando, C.H. (1987) Tropical freshwater zooplankton with special reference to South East Asia (Oriental Region), *Rep. Nat. Geogr. Soc.,* **87,** 287-311.

Granina, L. (1997) The chemical budget of Lake Baikal: A review. *Limnol. Oceanogr*., **42**, 373-378.

Haffner, G.D.; Hehanussa, P.E. and Hartoto, D. (2001) "The biology and physical processes of large lakes of Indonesia" In: Munawar, M. and Hecky, R.E. (eds). *The Great Lakes of the World: Food-web, health, and integrity.* Backhuys Publishers, Leiden, the Netherlands, pp. 183-194.

Hamilton, W. (1978) Tectonics of the Indonesian Region. USGS Professional Papers #1078. Washingon, D.C. #20402. United States Government Printing Office, Washington, D.C.

Hooper, D.U.; Chapin, F.S.; Ewel, J.J.; Hector, A.; Inchausti, P.; Lavorel, S.; Lawton, J.H.; Lodge, D.M.; Loreau, M.; Naeem, S.; Schmid, B.; Setala, H.; Symstad, A.J.; Vandermeer,

J. and Wardle, D.A. (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol. Monog*., **75**, 3-35.

Hutchinson, G.E. (1961) The paradox of the plankton. *Amer. Nat*., **95**, 137-145.
Hustedt, F. (1942) Süßwasser-Diatomeen des indomalayischen Archipels und der Hawaii-Inseln. Interationale revue der Gesamten Hydrobiologie und Hydrographie. *J. Evol. Bio*., **17**, 1268-1276.

Interlandi, S.J. and Kilham, S.S. (2001). Limiting resources and the regulation of diversity in phytoplankton communities. *Ecology*, **82**, 1270-1282.

Kiefer, D.A.; Holm-Hansen, O.; Goldman, C.R.; Richards, R.; Berman, T. (1972) Phytoplankton in Lake Tahoe: deep-living populations. *Limnol. Oceanogr*., **17**, 418-422.

Kottelat, M. (1990a) Synopsis of the endangered Buntingi (Osteichthyes: Adrianichthydae and Oryziidae) of Lake Poso, Central Sulawesi, Indonesia, with a new reproductive guild and descriptions of three new species. *Ichthyo. Explor. Freshw.*, **1**, 49–67.

Kottelat, M. (1990b) The ricefishes (Oryziidae) of the Malili Lakes, Sulawesi, Indonesia, with description of a new species. *Ichthyo. Explor. Freshw.*, **1**, 321–344.

Kottelat, M. (1990c) Sailfin silversides (Pisces: Telmatherinidae) of Lakes Towuti, Mahalona, and Wawontoa (Sulawesi, Indonesia) with descriptions of two new species. *Ichthyo. Explor. Freshw.*, **1**, 35–54.

Kottellat, M. (1991) Sailfin silversides (Pisces: *Telmatherinidae*) of Lake Matano, Sulawesi, Indonesia, with descriptions of six new species. *Icthyol. Explor. Fresh.,***1**, 321-344.

Kozhova, O.M.; Izmest'eva, L.R. (eds) (1998) Lake Baikal—Evolution and biodiversity, second completely revised and enlarged ed. Backhuys Publishers.

Lewis, W.M. Jr. (1974) Primary production in the plankton community of a tropical lake. *Ecol. Monogr*., **44**, 377-409.

Lewis, W. (1996) Tropical lakes: how latitude makes a difference. *In* Schiemer, F. and Boland, K.T. (eds) *Perspectives in Tropical Limnology*. SPB Academic Publishers, Amsterdam, the Netherlands, pp. 43-64.

Lodge D.M. (1993) Species Invasions and Deletions: Community Effects and Responses to Climate and Habitat Change. In O.M. Kareiva, J.G. Kingsolver, and R.B. Huey (eds) *Biotic Interactions and Global Change*. Sinauer Associates, Sunderland, MA., pp. 367-387.

Long, Z.T.; Bruno, J.F. and Duffy, J.E. (2007) Biodiversity mediates productivity through different mechanisms at adjacent trophic levels. *Ecology*, **88**, 2821-2829.

Martens, K.; Coulter, G.; Goddeeris, B. eds. (1994) Speciation in ancient lakes, *Arch. Hydrobiol. Adv. Limnol. 44*.

Martens, K. (1997) Speciation in ancient lakes. *Tree*, **23**, 177-182.

Matzinger, A.; Schmid, M.; Veljanoska-Sarafiloska, E.; Patceva, S.; Guseska, D.; Wagner, B.; Müller, B.; Sturm, M.; Wüest, A. (2007) Eutrophication of ancient Lake Ohrid: global warming amplifies detrimental effects of increased nutrient inputs? *Limnol. Oceanogr.,* **52**, 338-353.

Mittermeier, R.A., Myers, N., Thomsen, J.B., da Fonseca, G.A.B., and Olivieri, S. (1988) Biodiversity hotspots and major tropical wilderness areas: approaches to setting conservation priorities. *Conserv. Biol.*, **12**, 516-520.

Myers, N.; Mittermeir, R.; da Fonseca, G. and Kent, J. (2000).“Biodiversity hotspots for conservation priorities”.*Nature*, **403**, 853-858.

Naeem, S.; Thompson, L.J.; Lawler, S.P.; Lawton, J.H. and Woodfin, R.M. (1994) Declining biodiversity can alter the performance of ecosystems. *Nature*, **368**, 734-737.

Naeem, S. (2002) Ecosystem consequences of biodiversity loss: the evolution of a paradigm. *Ecology*, **83**, 1537-1552.

Reynolds, C.S. (1994) The long, the short and the stalled: on the attributes of phytoplankton selected by physical mixing in lakes and rivers. *Hydrobiologia*, **289**, 9-21.

Reynolds, C.S.; Reynolds, S.N.; Munawar, I. and Munawar, M. (2000).The regulation of phytoplankton population dynamics in the world’s largest lakes. *Aquat. Ecosys. Health Manage*., **3,** 1-21.

Roy, D.; Docker, M.F.; Heath, D.D. and Haffner, G.D. (2004) Genetic and morphological data supporting the hypothesis of adaptive radiation in the endemic fish of Lake Matano. *J. Evol. Biol*., **17**, 1268-1276.

Roy, D. (2006). *The Evolutionary History and Ecology of Telmatherina in Lake Matano: an Example of Adaptive Radiation in an Ancient Lake.* Thesis, University of Windsor, Canada.

Sabo, E. (2006). *Characterization of the Pelagic Plankton Assemblage of Lake Matano and Determination of Factors Regulating Primary and Secondary Production Dynamics.* Thesis, University of Windsor, Windsor, Ontario.

Sabo, E., Roy, D.; Hamilton, P.B.; Hehanussa, P.E. McNeely, R.; Haffner, G.D. (2008) The plankton community of Lake Matano: factors regulating plankton composition and relative abundance in an ancient tropical lake of Indonesia. *Hydrobiol.,* **615**, 225-235.

Sarasin, P. and Sarasin, F. (1895). Reisebericht aus Celebes. IV. Reise durch central-Celebes vom Golf von Bonin ach den Golf von Tomieni. *Verh. Der Geschellschaft für Erdkunde.* Berlin, **30**, 312-352.

Schön, I.; Martens, K. (2004) Adaptive, pre-adaptive and non-adaptive components of radiations in ancient lakes: a review. *Org., Diver. & Evol*., **4**, 137-156.

Serruya, C. and Pollingher, U. (1983). *Lakes of the warm belt.* Cambridge University Press, Cambridge.

Shapiro, J.; Lamarra, V. and Lynch, M. (1975) Biomanipulation: an ecosystem approach to lake restoration. In Brezonik, P.L and Fox, J.L. (eds) *Water Quality Management Through Biological Control*. University of Florida, Gainesville, pp. 85-96.

Sherbakov, D.Y. (1999) Molecular phylogenetic studies on the origin of biodiversity in Lake Baikal. *Trend. Ecol. Evol.,* **14**, 92-95.

Takhteev, V.V. (2000) Trends in the evolution of Baikal amphipods and evolutionary parallels with some marine Malacostracan faunas. *Adv. Ecol. Res.,* **31**, 197–219.

Tilman, D. (1982) *Resource competition and community structure. Monographs in Population Biology.* Princeton University Press, Princeton. 296 p.

Villeneuve, M.; Gunawan, W.; Cornee, J.J. and Vidal, O. (2002) Geology of the central Sulawesi Belt (eastern Indonesia): constraints for geodynamic models. *Int. J. Earth Sci.,* **91**, 524-537.

von Rintelen, T. and Glaubrecht, M. (2003). New discoveries in old lakes: Three new species of Tylomelania Sarasin & Sarasin, 1879 (Gastropoda: Cerithoidea: Pachychilidae) from the Malili Lake system on Sulawesi, Indonesia. *J. Moll. Stud.,* **69,** 3-17.

von Rintelen, T.; Wilson, A.B.; Meyer, A. and Glaubrecht, M. (2004) Escalation and trophic specialization drive adaptive radiation of freshwater gastropods in ancient lakes on Sulawesi, Indonesia. *Proc. R. Soc. Lond. B.,* **271**, 2541-2549.

von Rintelen, T.; Bouchet, P.; Glaubrecht, M. (2007) Ancient lakes as hotspots of diversity: a morphological review of an endemic species flock of Tylomelania (Gastropoda: Cerithioidea: Pachychilidae) in the Malili lake system on Sulawesi,

Indonesia. *Hydrobiol*., **592**, 11-94.

Whitten, T., Mustafa, M. and Henderson, G.S. (1987) *The Ecology of Sulawesi*. Oey, E. (ed.) Gadjah Mada University Press, Singapore.

Yachi, S., and Loreau, M. (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proc. Nat. Acad. Sci.* (USA), **96**, 1463-1468.

Table 1.1 Key physical, chemical, and biological characteristics of ancient lakes; adapted from Schön and Martens, 2004; Martens, 1997; Matzinger et al., 2007

Lake	Age (million years)	Max. depth (m)	Surface (km^2)	Volume (km^3)	Species richness	Endemism, species level (%)	Annual Productivity ($gC/m^2/yr$)	Phyto-plankton Biomass (µg/L)	TP (µmol/L)
Baikal	25-30	1700	31 500	23 000	1825	75	3.9 x 10[12] [10]	n/a	0.26
Tanganyika	9-12	1470	32 600	18 880	1119	56	n/a	1.7 x 10[10] [7]	0.16
Malawi	4.5-8.6	785	30 800	8400	800[1]	99/100[1]	210	n/a	0.30
Caspian Sea	2-3	1025	384 400	78 700	1495	80[9]	n/a	1.0 x 10[1] - 5.0 x 10[3] [8]	0.07-0.11[8]
Victoria	0.75 or less[2]	70	70 000	2760	500[1]	99[1]	4000	n/a	3.5
Biwa	4	104	674	28	517 /88[5]	10	87.6	n/a	0.30
Titicaca	3	284	8448	8959	533	13	n/a	n/a	0.77
Ohrid	2-3	295	348	51	200[9]	90/60[11]	n/a	5.0 x 10[1] - 1.6 [9]	0.15
Matano	2-4[3]	590	164	n/a	33[6]/ 191[4]	80-99	200	1.3 x 10[1]	0.16
Poso	n/a	450	195	n/a	n/a	n/a	n/a	n/a	0.23

[1] cichlids (Snoeks, 2000), [2]Seehausen, 2002, [3]Haffner et al., 2001, [4]diatoms, Bramburger et al., 2006, [5]phytoplankton, Dodson et al., 2000, [6]snails, von Rintelen et al., 2004, [7]Hecky and Fee, 1981, [8]Dumont, 1998, [9]Matzinger et al., 2007, [10]Granina, 1997, [11]snails/crabs

Figure 1.1 Map of Sulawesi Island, indicating the Malili lakes: Matano, Mahalona, Towuti, Masapi and Wawantoa (Lawontoa), as well as Lake Poso.

Chapter 2: Nutrients—How does Matano grow?

2.1 Introduction

A considerable amount of lake research focuses on answering the following question: Is primary production in a lake nitrogen (N) limited or phosphorus (P) limited? The answer is often related to location; if the lake is temperate, it is typically P-limited (Lewis, 2000), if tropical, it is typically N-limited (Vollenweider, 1976; Schindler, 1977; Hecky and Kilham, 1988). Recent research has reported phytoplankton biomass increases from combined additions of N and P (Guildford et al., 2003; North et al., 2008). These observations are useful as guidelines, but due to wide differences among lakes, each lake must still be studied individually. As research seeks to answer questions related to nutrient limitation within lakes, several research methods have become standard: mesocosm and bioassay experiments. Experiments in this chapter utilize the bioassay approach for its simplicity and ease of use, and the opportunity to compare results to those of other studies.

Previous studies with Lake Matano water indicated that the phytoplankton were P-limited (Sabo, 2006), and this was concluded to be caused by the precipitation of soluble phosphorus by Fe (hydr)oxides (Crowe et al., 2007). Previous nutrient addition experiments with both N and P, however, did not significantly ($p > 0.05$) increase the algal biomass of Lake Matano (Sabo, 2006), but perturbation experiments and metals removal in the lake water did cause a slight increase in

plankton abundance (A. Bramburger, Windsor, unpubl. data; Sabo, 2006). Lake Matano is ultra-oligotrophic with the upper 100 m containing concentrations of nitrogen as NH_3 ranged from 0.29 ± 0.12 to 0.71 ± 0.35 µmol/L, and as NO_3/NO_2 from 0.04 ± 0.02 to 0.81 ± 0.66 µmol/L. Phosphorus as SRP ranged from 0.13 ± 0.03 to 0.48 ± 0.42 µmol/L and as TP from 0.13 ± 0.03 to 0.32 ± 0.16 µmol/L (see Chapter 3, Table 3.3).

To assess nutrient limitation as the cause of this lake's limited phytoplankton population, with respect to both species richness and biomass, two experiments were conducted. The aims of these experiments were to verify or reject the previous macronutrient limitation result, and to test if Lake Matano's water chemistry was limiting the growth and colonization potential of phytoplankton by means other than macronutrient limitation. The first experiment consisted of bioassay nutrient additions of N, P, and both N and P in various concentrations added to unfiltered Lake Matano water and spiked with a phytoplankton slurry concentrated from the surface water. Phytoplankton were identified and quantified for all samples. The hypothesis for this experiment was that significant changes in biomass or relative abundance in the phytoplankton assemblage would not be stimulated if N and P were not limiting factors. The questions we sought to answer were: (1) which nutrient addition treatments supported the highest total biomass, (2) which treatments gave the highest biomass for each of the three main divisions of phytoplankton, (3) was there a connection between biodiversity and production

(measured as Shannon Index of Diversity and biomass), and, more generally, (4) is this lake vulnerable to eutrophication as a result of increased nutrient loadings?

In the second experiment, conducted in collaboration with Dr. Rosana Sotero-Santos, Matano phytoplankton and cultured chlorophytes and cyanobacteria were grown separately in samples of CHU-10 media that was serially diluted with Lake Matano water. The species of cultured algae used are commonly deployed in toxicity tests and noted for their resilience to extreme conditions (Stein, 1973). Chlorophyll-*a* concentrations were analyzed for these samples over the duration of the experiment. The aim of this experiment was to assess whether the water chemistry of Lake Matano limits the growth and colonization potential of phytoplankton by means other than macronutrient limitation.

2.2 Methods

2.2.1 Nutrient Addition Study

A phytoplankton slurry was collected using vertical plankton hauls with a 64 μm mesh net from the open surface waters (0-30 m) of the central basin of Lake Matano. 200 mL of non-filtered surface lake water and 1 mL of the concentrated phytoplankton slurry were added to forty-five 250 mL clear plastic bottles. The algal biomass of the slurry was 1.145×10^{2} μg/L. The initial algal biomass in each bottle was approximately 5.725×10^{-1} μg/L, with a species richness of 10 (see Chapter 3).

Nutrient additions were made from serial dilution of 1 M KH_2PO_4 and NH_4NO_3 solutions. Concentrations of 20, 40, 80, 160, 320 µmol/L of N and P individually, and of 60:5, 240:20, 480:40, 960:80, 1920:160 µmol/L N:P (12:1) were added to three replicate samples. The relatively higher values were used to overcome any iron chelation effects on the phosphorus concentrations. The existing concentrations of nitrogen and phosphorus in the lake water (nitrogen as NH_3 ranged from 0.29 ± 0.12 to 0.71 ± 0.35 µmol/L, and as NO_3/NO_2 from 0.04 ± 0.02 to 0.81 ± 0.66 µmol/L. Phosphorus as SRP ranged from 0.13 ± 0.03 to 0.48 ± 0.42 µmol/L and as TP from 0.13 ± 0.03 to 0.32 ± 0.16 µmol/L) were very low and did not contribute significantly to the nutrient additions, thus existing concentrations were not factored into the calculations for the experimental concentrations. The bottles were randomly placed on a ledge under ambient light (about 50 m from the edge of the lake). The air temperature throughout the experiment was comparable to the lake water temperature (mean 27 °C). Daily inversions of the bottles were conducted to maintain well-mixed conditions, to prevent settling of phytoplankton, and to simulate natural water movements. The experiment was run for 16 days. An experimental duration of less than a week has previously been considered a "long-term" experiment (Dodds and Priscu, 1990; Børsheim et al., 2005), so the durations of this study and the growth potential experiment, 15-21 days, are longer than typically utilized in previous studies.

At days 2, 4, 8, and 16, 20 mL samples were removed from each bottle and stored in 25 mL scintillation vials. 1 mL of Lugol's/formalin solution was added to each

scintillation vial. 1 or 5 mL samples from each were transferred to sedimentation chambers (Utermöhl, 1958) and the cells were settled-out over five hours. Slides were viewed at 400 x magnification with an inverted light microscope and phytoplankton were identified and counted for a minimum of five fields of view for each sample. Identifications were made to the genus level, when possible, according to Wehr and Sheath (2003), Freshwater Algae of North America, and with assistance from Dr. Olga Babanazarova (Dept. Zoology and Ecology, Yaroslav State University, Yaroslavl, Russia) and Dr. Paul Hamilton (Museum of Nature, Ottawa, ON, Canada). Biovolume calculations were made according to Hillebrand et al. (1999); the cell biovolume method allowed for analysis on population characteristics such as changes in relative abundance and diversity. Algal biovolume is one of the better estimates of algal biomass, with the assumption that the mass of algal cytoplasm is the same among taxa; errors arise mainly when large vacuoles are not accounted for (Stevenson et al., 1996). Biomass, Shannon Diversity Index (range of 0-4.6), species richness, and evenness (range of 0-1), were used to assess changes in phytoplankton composition with respect to macronutrient additions. A Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows) was used to statistically evaluate relationships between biodiversity, production, and time; and between biomass, nutrient addition treatments, diversity, and time.

2.2.2 Growth Potential Study

Innocula of the chlorophytes *Scenedemus obliquus* (UTCC 5) and *Pseudokirchneriella subcapitata* (UTCC 37) and the cyanobacterium *Microcystis aeruginosa* (UTCC 124) were used to determine the growth capacity of Lake Matano water. Algae were

obtained from the University of Toronto Culture Collection (UTCC) of Algae and were maintained at the Great Lakes Institute for Environmental Research (University of Windsor) in culture media. CHU-10 media (Stein, 1973) was used with an adjusted pH of 6.4 for chlorophyta and 8.5 for cyanobacteria (J. Acreman, Toronto, pers. comm.), in a temperature controlled chamber at 23 ^{o}C with a 12:12 dark : light cycle.

CHU-10 growth media was serially diluted with Lake Matano water to make solutions of 0%, 12.5%, 25%, 50%, 75% and 100% Matano surface water. Table 2.1 contains the volumes of media and Matano water used, and the CHU-10 nutrient concentrations for each sample. Each culture tube contained 10 mL of solution and 0.1 mL of cultured algae. The growth potential tests were carried out for 15 days (chlorophytes) and 21 days (cyanobacteria). Culture tubes were stirred daily to maintain well-mixed conditions. After each experiment, 5 mL aliquots from each treatment were diluted in 5 mL of Milli-Q water. Chlorophyll-*a* (chl-*a*) was measured by filtering the diluted sample through a GF/C filter. Chl-*a* was extracted in 90% aqueous acetone solution (APHA, 1995) and its concentration determined spectrophotometrically (DU® 530, Beckman) following the method of Strickland (1968).

2.3 Results

2.3.1 Nutrient Addition Study

Phytoplankton genera observed

Table 2.2 provides a comprehensive list of the pelagic phytoplankton genera observed during Sabo's 2004 and this 2006 survey. In all samples of this experiment, 14 genera were identified from four divisions. From bacillariophyta: *Fragilaria*; from chlorophyta: *Chlamydomonas, Chlorococcus, Cosmarium, Staurastrum, Xanthidium, Tetraedron, Monoraphidium, Koliella;* from cyanobacteria: *Gloeocapsa, Merismopedia, Anabaena, Synechococcus;* and from pyrrophyta: *Peridinium*. Pryrrophytes were rare, and were observed only in the original inocula sample. Of these genera, five were observed in both this experiment and that of Sabo (2006): *Chlorococcus, Cosmarium, Staurastrum, Merismopedia,* and *Peridinium*. Taxa representative of nine genera previously not reported by Sabo in 2004 include *Fragilaria, Chlamydomonas, Xanthidium, Tetraedron, Monoraphidium, Koliella, Anabaena, Gloeocapsa,* and *Synechococcus*. Growth responses for the three dominant divisions, bacillariophyta, chlorophyta, and cyanobacteria, were examined in this study.

Changes in biomass with time

There was a trend of increasing total mean phytoplankton biomass with time, over the 16 days of the experiment, for all replicates. There was a statistically significant ($p = 0.001$, Kruskal-Wallis One-way Analysis of Variance; Systat 12 for Windows) increase in biomass between successive sampling days. Between days 2 and 4, there was a significant difference in the biomass change ($p = 0.001$; Mann-Whitney U-test

statistic (z) = 31). A significant difference in the biomass increase was also observed between days 4 and 8 ($p = 0.019$, $z = 56$); and between days 8 and 16 ($p = 0.001$ $z = 46$). The mean and standard error of phytoplankton biomass (μg/L) for each major division and total algal biomass at days 2, 4, 8 and 16 for each nutrient addition treatment are listed by sample day in Tables 2.3 to 2.6.

On day 2, the total biomass for nitrogen treatments ranged from 1.2 ± 0.1 to 21.9 ± 10.9 μg/L, phosphorus treatments from 2.8 ± 0.2 to 16.6 ± 1.6 μg/L, and N:P (12:1) treatments from 1.5 ± 0.33 to 4.2 ± 0.5 μg/L. Biomass concentrations ranged from 0.0 to 9.4 μg/L for bacillariophytes, 0.2 ± 0.1 to 21.8 ± 10.9 μg/L for chlorophytes, and 0.1 ± 0 to 1.0 ± 0.5 μg/L for cyanobacteria. There was a statistically significant difference ($p < 0.05$) between the highest biomass achieved for each of these divisions.

At day 16, the total biomass for nitrogen treatments ranged from 86.1 ± 37.7 to 816 ± 105 μg/L, phosphorus treatments from 91.5 ± 40.2 to 898 ± 577 μg/L, and N:P (12:1) treatments from 118 ± 46 to 1165 ± 851 μg/L. Biomass concentrations ranged from 0 to 831 ± 715 μg/L for bacillariophytes, 12.78 ± 5.4 to 708 ± 485 μg/L for chlorophytes, and 1.2 ± 0.1 to 24932 ± 24868 μg/L for cyanobacteria. There was no statistically significant difference ($p > 0.05$) between the highest biomass achieved for each of these divisions.

The change in biomass from day 2 to day 16 was 84.9 ± 37.7 to 25114 ± 24962 µg/L for nitrogen treatments, 88.7 ± 40.0 to 881 ± 575 µg/L for phosphorus treatments and 116 ± 45 to 1161 ± 851 µg/L for N:P treatments. These changes are illustrated in Figure 2.1. For both the lowest and highest changes with each nutrient addition treatment, standard error bars overlap, meaning there was no statistically significant difference ($p > 0.05$) between the nutrient addition treatments with respect to total biomass change.

The range of change in biomass for bacillariophytes from day 2 to day 16 was 0 to 821 ± 715 µg/L, 12.6 ± 5.2 to 686 ± 474 µg/L for chlorophytes and 1.1 ± 0.1 to 816 ± 105 µg/L for cyanobacteria. These changes are illustrated in Figure 2.2. As with the lowest and highest changes of total biomass, the standard error bars of the major divisions overlap, indicating no statistically significant difference ($p > 0.05$) in the low and high biomasses among the divisions.

The relationship between the change in biomass (day 2 to day 16) of the three main divisions and all nutrient treatments was evaluated using Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows). There were no significant differences for any of the three main divisions for total biomass change. For the change in chlorophyte biomass, $p = 0.3$ and $z = 16.3$; for cyanobacteria, $p = 0.3$ and $z = 15.5$; for bacillariophytes, $p = 0.3$ and $z = 15.7$; and for total biomass, $p = 0.3$ and $z = 16.0$.

Changes in diversity, species richness, evenness, and relative abundance

Diversity indices are summarized in Tables 2.7-2.10. The Shannon Index of the original injection was 1.39, similar to that of Lake Matano surface water, 1.57. Changes in diversity were measured over the 16-day experiment. There was a statistically significant increase in diversity between days 2 and 16 ($p = 0$ and $z = 30$), but no significant relationship between diversity and production at day 16 for all nutrient additions ($p = 0.9$ and $z = 5.8$). Mean species richness increased with time, ranging from 2.3-4.3 per sample and 9 total at day 2 and 4.67-10.67 per sample and 14 total at day 16. There was no trend in evenness with time.

Initial biomass was approximately 5.73×10^{-1} µg/L; chlorophytes comprised 87%, cyanobacteria comprised 0.00002%, and pyrrophytes comprised 12.99998%. Bacillariophytes were not detected in the original sample, but were observed in the treatment samples; pyrrophytes were only detected in one treatment sample at day 16. Although different from the inocula, the relative abundance of the three main divisions remained relatively constant in the treatments which supported the highest total biomass (40 µmol/L P, 160 µmol/L P, 60:5 µmol/L N:P, 240:20 µmol/L N:P, and 960:80 µmol/L N:P): chlorophytes occupied the greatest proportion (38-60%), followed by cyanobacteria (20-24%), and bacillariophytes (13-26%). There was no significant ($p > 0.05$) change in the relative abundance among the above treatments, but chlorophytes comprised a lower percentage, while cyanobacteria and bacillariophytes comprised a greater percentage of the total biomass than in the inoculate sample.

2.3.2. Growth Potential Study

Chlorophyll-*a* measurements of cultured phytoplankton (both chlorophytes and cyanobacteria, Figure 2.5) and Matano phytoplankton decreased as the proportion of Lake Matano water was increased (Figure 2.6). Chl-*a* measurements for *P. subcapitata* were consistent for 0, 12.5 and 25% Matano water, but decreased significantly ($p < 0.05$; by nearly 60%) at 50% Matano water. *S. obliquus* decreased by nearly 85% at 50% Matano water and no chl-*a* was detected in samples containing 100% Matano water. For *M. aeruginosa*, chl-*a* concentration decreased by nearly 70% at 50% Matano water, and by almost 93% at 100% Matano water. Matano's phytoplankton, both chlorophytes and cyanobacteria, exhibited significantly lower ($p < 0.05$) chl-*a* concentrations with increasing percentages of Matano water, however, these groups maintained higher chl-*a* concentrations than the cultured algae in the same treatment conditions. At 50% Matano water, Matano chlorophytes were present at 89 µg/L and cyanobacteria at 23 µg/L; chlorophytes *P. subcapitata* and *S. obliquus* were present at 33 and 45 µg/L and cyanobacteria *M. aeruginosa* at 9 µg/L.

As Matano water is higher in Mg (712 ± 3.7 µmol/L) than CHU-10 media (103 µmol/L), at 0, 12.5, and 25% Matano water, the concentration of Mg was below that required by some phytoplankton, according to Healey (1973). At 50% Matano water the concentrations of Fe and Si were below the mean concentrations required, at 75% Matano water the concentration of K was also below the mean concentration required by some phytoplankton, and at 100% Matano water Ca, Fe, K, Na, and Si

concentrations were all below the mean concentration required by some phytoplankton, as outlined in Table 2.1 (Healey, 1973).

2.4 Discussion

Nutrient additions were able to increase the phytoplankton biomass with statistical significance over each sampling period of the 16-day experiment, although it is unlikely that the biomass produced by the macronutrient additions would have biological significance. While chlorophytes and P-treatments achieved the highest growth at day 2 and cyanobacteria and N-treatments achieved the highest growth at day 16, the N+P treatments supported the greatest total biomass change. Considering the large volumes of nutrients added, 20-320 µmol/L N and P individually and 60-1920 µmol/L and 5-160 µmol/L N:P in combination, massive algal blooms did not occur; the samples at day 16 still contained very small algal assemblages. The highest biomass achieved with nutrient additions at day 16 was $1.165 \times 10^3 \pm 851$ µg/L, which is still low compared to the phytoplankton biomass observed in other tropical lakes, such as Lake Tanganyika (1.7×10^{11} µg/L) and the Caspian Sea (1.0×10^1 to 5.0×10^3 µg/L) (see Table 1.1), and not significantly different from the phytoplankton biomass observed in Lake Matano by Sabo (2006), 1.3×10^1 µg/L. Comparatively, in large enclosures in the Amazon floodplain lake, Lake Calado, a 270% increase in chl-*a* was observed with N+P treatments less than 0.3 µmol/L in just 5 days (Setaro and Melak, 1984). In addition, the nutrient treatments with Lake Matano water did not produce any significant differences between the highest biomasses achieved by the taxonomic divisions at day 16, or

any significant differences in total biomass among nutrient treatments (N, P, N+P). Divisions responded proportionally to the nutrient inputs, maintaining relative abundances (as percentages) in the treatments which supported the highest total biomass (40 µmol/L P, 160 µmol/L P, 60:5 µmol/L N:P, 240:20 µmol/L N:P, and 960:80 µmol/L N:P) and suggest that macronutrients N and P do not regulate competition within or among the algal classes of Lake Matano.

The taxonomic richness of Lake Matano's phytoplankton is much greater than previously reported. Of the 14 genera observed, only five were common with the previous phytoplankton study (Sabo, 2006). This is likely a function of using a net to develop a phytoplankton innoculum slurry, where greater volumes of lake water were sampled. With both experiments, 21 genera have been documented for Lake Matano; previously reported as taxonomically-impoverished (Sabo, 2006), it contains a similar number of phytoplankton genera as in other tropical lakes in the Philippines, Indonesia, and Venezuela (Lewis, 1978). Species richness in the bioassay study increased over time, from 2-5 taxa per sample and 9 total at day 2 and 5-11 taxa and 14 total at day 16. Rare species such as chlorophytes *Staurastrum* and *Xanthidium*, and cyanobacteria *Merismopedia* and *Synechococcus* became more abundant and countable. This suggests that other phytoplankton taxa may be present in the lake, but at such low abundances that a more thorough sampling and counting regime would be required to detect them. There is potential for future microscopic analysis to add to the current taxonomic richness of the lake.

Standard biomass calculations are essential for comparing the relative abundance of different taxa in samples (Hillebrand et al., 1999). In mixed-species samples, large numbers of small-celled algae (such as *Chlorococcus*) may contribute a minor portion to the sample's biomass, whereas a few large-celled algae (such as *Peridinium*) may contribute a major portion to measured total biomass. For this reason, cell counts alone are not a good indicator of algal biomass. Chl-*a* measures do not allow for detailed population analysis, but do provide easily-obtained results that are comparable to algal biomass values.

The use of CHU-10 media in the growth potential studies was successful in significantly ($p < 0.05$) increasing the chl-*a* concentrations for Matano phytoplankton (e.g. 3.0×10^2 µg/L for chlorophytes at 0% dilution) above ambient biomass concentrations and those obtained in the nutrient addition study. A response indicative of a nutrient limitation threshold was demonstrated by the cultured phytoplankton to CHU-10 media diluted with Matano water, markedly at and above dilutions of 50% Matano water. Both the cultured phytoplankton and the Matano phytoplankton decreased in chl-*a* concentrations with higher percentages of Matano water in the growth solution. However, the decrease was a more linear decline for Matano phytoplankton, and a sharper decline for the cultured phytoplankton. Healey (1973) (see Table 2.1) compiled the values for the mean concentrations required by at least some phytoplankton (not all species require Na or Si): 217 µmol/L Ca, 106 µmol/L Fe, 443 µmol/L K, 231 µmol/L Mg, 265 µmol/L Na, and 1929 µmol/L Si. According to Healey (1973) and Hecky and Kilham (1988),

a relatively narrow range of elemental composition has evolved because all algal cells have to perform the same metabolic functions and have qualitatively similar structural requirements. Lake Matano water is naturally higher in Mg (712.0 ± 3.7 μmol/L) than in the CHU-10 media (103 μmol/L), so the concentration of Mg in 0, 12.5 and 25% Matano water samples was below that required by some phytoplankton, but this is not directly reflected in the chl-*a* concentrations of the phytoplankton, thus Mg is not considered a limiting nutrient. CHU-10 contains much higher concentrations of Ca (2500 μmol/L), Fe (143 μmol/L), K (1026 μmol/L), Na (10,000 μmol/L), and Si (2860 μmol/L) than Lake Matano, and in the case of Ca, K, Na, and Si, far exceed phytoplankton requirements. At 50% Matano water the sample's concentrations of Fe (72.3 ± 0 μmol/L), and Si (1572.4 μmol/L) were below the mean concentrations required. At 75% Matano water, the concentration of K (259.9 ± 0.2 μmol/L) was also below that required, and at 100% Matano water, the concentrations of Ca (233.0 ± 5.3 μmol/L), Fe (0.5 ± 0 μmol/L), K (3.8 ± 0.2 μmol/L), Na (53 ± 5.2 μmol/L), and Si (284.7 μmol/L) were below those required by some phytoplankton.

Phytoplankton require 20 or more elements for synthesis of protoplasm (Lewis, 2000) and the most probable macronutrient limitation is due to N, P, C, Si, C or Fe concentrations; each nutrient's potential to limit the phytoplankton community of Lake Matano is addressed.

Nutrient addition studies with Lake Matano water and a concentrated slurry of its

natural phytoplankton showed biologically insignificant growth responses of all dominant taxa to N and P additions. Carbon dioxide profiles show concentrations of 113.6 µmol/L in the upper 100 m, increasing to 1022.7 µmol/L at greater depths (Haffner, unpubl. data) and inorganic carbon limitation only occurs at very high rates of photosynthesis (Lewis, 2000). Lake Matano has concentrations of Si at 284.7 µmol/L (Haffner et al., 2001), and is unlikely to be limiting, as it is not required by all phytoplankton species and pelagic bacillariophyte abundance is very low in this lake. The catchment of the lake contains well-weathered lateritic soils; millions of years of soil leaching have made the lake very Fe-rich with concentrations of 25.1 µmol/L in surface waters and 110.0 µmol/L below 100 m (Crowe et al., 2007). Therefore, N, P, C, Si, and Fe are not primary limiting factors to the phytoplankton composition and abundance of Lake Matano.

Matano's natural phytoplankton assemblage responded more favourably than the cultured phytoplankton in the growth potential studies, as a higher chl-*a* concentration was maintained in samples with a greater portion of Matano water (i.e. 50, 75, 100% Matano water). This result suggests that Matano's phytoplankton are better adapted to the nutrient-limited waters of Lake Matano, and that nutrient and trace element limitation represent a barrier to new colonization in the lake—perhaps by such taxa as the Chrysophytes, Cryptophytes, and Centrales. The cultured chlorophytes achieved higher chl-*a* concentrations than cyanobacteria, whereas the Matano algae obtained higher chl-*a* values than the cultured algae, with Matano chlorophytes achieving the highest growth overall.

Limitation by trace metals has not received the attention that limitation by macronutrients N, P, and Si has. The availability of metals has been invoked as possibly growth-limiting in some freshwater situations (e.g. Hecky and Kilham, 1988). In testing a lake for nutrient limitation, approaches can be whole-lake enrichments, mesocosm experiments, or bioassay experiments. Each of these approaches has associated positive and negative aspects, and should be selected considering all factors and the desired outcome of the experiment. Whole-lake enrichment experiments are confined to experimental lakes, but provide the most reliable results of the effects of enrichment on the lake (Schindler, 1974). Mesocosm experiments can be quite costly and time-consuming in construction and operation, but can provide good approximations of natural ambient conditions, including temperature and irradiance (Martínez-Martínez et al., 2006), and have a higher degree of realism than bioassays (Egge, 1993). Bioassay or "bottle" experiments are very simple and affordable, the water can be filtered (Camacho et al., 2003), the experiment can be run in a temperature and light-controlled chamber (Downs et al., 2008), and bottles can be sealed to minimize atmospheric exchange of gases (Dzialowski et al., 2005). However, there are a number of potential problems associated with the use of this bioassay approach, including the absence of environmental heterogeneity and removal of natural sources of nutrient recycling and regeneration (Dzialowski et al., 2005). With all factors considered, the ease and affordability of the bioassay approach has made it one of the most commonly used methods to assess phytoplankton limitation in aquatic ecosystems (Elser et al.,

1990), and was the method selected for this experiment. The results of this experiment could be strengthened with phytoplankton sampling from different depths and locations in the lake, and with a more comprehensive inocula sample to test the effects of these treatments on more taxa present in the lake. Given the design of these experiments, results would not have been strengthened by the use of a mesocosm enclosure.

2.5 Conclusions

The addition of macronutrients N and P individually and in combination increased Matano phytoplankton biomass with statistical significance over each sampling interval in the experiment. However, the change in biomass observed does not constitute biological significance, as the highest biomass achieved at day 16 was not significantly different from the biomass reported for Lake Matano surface waters by Sabo (2006). This indicates that N and P are not limiting factors, and suggests that Lake Matano is not sensitive to the threat of cultural eutrophication from inputs of these macronutrients. This experiment also indicated that the taxonomic richness of the lake's phytoplankton is not impoverished, and is at minimum equal to that observed in other tropical lakes. Only 5 of the total 21 genera were common to this experiment and that of Sabo in 2004 (Sabo, 2006) and there was an increase in species richness from 9 at day 2 to 14 at day 16. This indication of species existing at very low abundances suggests there is great potential for further analysis to

increase the currently recorded taxonomic richness for Lake Matano, as

Growth potential studies showed significant growth for Matano phytoplankton in CHU-10 growth media. This media is rich in Ca, Fe, K, Mg, Na, and Si. The result of this experiment revealed Ca, Fe, K, Na, and Si may be limiting nutrients in the lake, as cultured phytoplankton and Matano phytoplankton showed significant decreases in chl-*a* concentrations as the concentration of these elements dropped below the threshold of concentrations required by some phytoplankton. Fe and Si may have been limiting in these cultures, but previous research has evaluated these concentrations in the lake and they are ample to sustain the phytoplankton population. Therefore, the results of this experiment demonstrate that Ca, K, and Na are limiting nutrients to the phytoplankton of Lake Matano.

Interspecific competition in the lake is not regulated by macronutrients N and P, as there was no significant difference among the highest biomass achieved by bacillariophytes, chlorophytes, or cyanobacteria. Competition among the dominant taxa, as well as new colonizers, may be related to nutrients and possible Cr (VI) toxicity.

Consistent with Reynolds et al.'s (2000) view of the world's largest lakes, it is unlikely that Lake Matano will ever support a high standing crop of phytoplankton. For those who live in the area, this means the lake will not be supporting a financially lucrative fishing operation in the near future, nor will these relatively

pristine waters suffer the typical outcomes of anthropogenic eutrophication. These experiments showed the natural phytoplankton's limited response to large nutrient inputs; with respect to maintaining the ecological integrity of the lake, this is a positive prediction.

Further analysis of this lake's chemical environment, an evaluation of its phytoplankton assemblage, and the potential for other limiting micronutrients are required to better understand the mechanisms driving the phytoplankton diversity and abundance in this system.

2.6 References

APHA, AWWA, WEF (1995). Standard methods for the examination of water and wastewater 19th ed USA: American Public Health Association, American Water Works Association and Water Environment Federation.

Børsheim, K.Y.; Vadstein, O.; Myklestad, S.M.; Reinertsen, H.; Kirkvold, S.; and Olsen, Y. (2005) Photosynthetic algal production, accumulation and release of phytoplankton storage carbohydrates and bacterial production in a gradient in daily nutrient supply. *J. Phyto. Res.*, **27**, 743-755.

Bramburger, A.J. (2004) Floristics and taxonomy of the endemic diatom flora of the ancient Malili Lakes, Sulawesi Island, Indonesia. M.Sc. thesis, University of Windsor, Canada.

Camacho, A.; Wurtsbaugh, W.; Miracle, M.; Armengol, X.; and Vicente, E. (2003) Nitrogen limitation of phytoplankton in a Spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment bioassay approach. *J. Plank. Res.,* **25**, 397-404.

Crowe, S.A., O'Neill, A.H., Katsev, S., Hehanussa, P., Haffner, G.D., Sundby, B., Mucci, A., Fowle, D. (2007). The biogeochemistry of tropical lakes: a case study from Lake Matano, Indonesia. *Limnol. Oceanogr*., **53**, 319-331.

Dodds, W.K. and Priscu, J.C. (1990) A comparison of methods for assessment of nutrient deficiency of phytoplankton in a large oligotrophic lake. *Can. J. Fish. Aquat. Sci*., **4**, 2328-2338.

Dzialowski, A.R.; Wang, S-H.; Lim, N-C.; Spotts, W.W.; and Huggins, D.G. (2005) Nutrient limitation of phytoplankton growth in central plains reservoirs, USA. J. Plank. Res., 27, 587-595.

Downs, T.M.; Schallenberg, M. and Burns, C.W. (2008) Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. *Aquat. Sci.,* **70**, 347-360.

Egge, J.K. (1993) Nutrient control of phytoplankton growth: Effects of macronutrient composition (N, P, Si) on species succession. PhD Thesis. University of Bergen, Norway.

Elser, J. J. , Marzolf, E. R., and Goldman, E. R. (1990) Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.,* **47**, 1468-1477.

Guildford, S.J., Hecky, R.E., Taylor, W.D., Mugidde, R., and Bootsma, H.A. (2003). Nutrient enrichment experiments in tropical great lakes Malawi/Nyasa and Victoria.

J. Great Lakes Res., **29**, 89-106.

Haffner, G.D., Hehanussa, P.E. and Hartoto, D. (2001) The biology and physical processes of large lakes of Indonesia. In Munawar, M. and Hecky, R.E. (eds) *The Great Lakes of the World: Food-Web Health and Integrity*, Backhuys, Leiden, pp. 183–194.

Healey, F.P. (1973) Inorganic nutrient update and deficiency in algae. *Crit. Rev. Microbiol.,* **3**, 69-113.

Hecky, R.E. and Fee, E.J. (1981) Primary Production and Rates of Algal Growth in Lake Tanganyika. *Am. Soc. Limnol. Oceanogr.,* **26**, 532-547.

Hecky, R.E. and Kilham, P. (1988) Nutrient Limitation of Phytoplankton in Freshwater and Marine Environments: A Review of Recent Evidence on the Effects of Enrichment**.** *Limnol. and Oceanogr*., **33,** 796-822.

Hillebrand, H.C-D.; Kurselen, C-D.; Kirschtel, D.; Pollingher, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol*., **35,** 403-424.

Lewis, W.M. Jr. (1978) A compositional, phytogeographical and elementary structural analysis of the phytoplankton in a tropical lake: Lake Lanao, Philippines. *J. Ecol.,* **66**, 213-226.

Lewis, W.M. Jr. (1983) A revised classification of lakes based on mixing. *J. Fish. Aquat. Sci.,* **40**, 1779-1787.

Lewis, W.M. Jr. (2000) Basis for the protection and management of tropical lakes. *Lakes Reserv.: Res. Manage*., **5**, 35-48.

Martinez-Martinez, J.; Norland, S.; Thingstad, T.F.; Schroeder, D.C.; Bratbak, G.; Wilson, W.H.; and Larsen, A. (2006) Variability in microbial population dynamics between similarly perturbed mesocosms. *J. Plank. Res.,* **28**, 783-791.

Meyer, A.; Kocher, T.D.; Basasibwaki, P.; Wilson, A.C. (1990) Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature*, **347**, 550-553.

North, R.L.; Guildford, S.J.; Smith, R.E.H.; Twiss, M.R.; Kling, H.J. (2008) Nitrogen, phosphorus, and iron co-limitation of phytoplankton communities in the nearshore and offshore regions of the African Great Lakes. *Ver. Int. Ver. Lim.,* **30**, 812-820.

Reynolds, C.S., Reynolds, S.N., Munawar, I., and Munawar, M. (2000) The regulation of phytoplankton population dynamics in the world's largest lakes. *Aquat. Ecosys. Health Manage.,* **3**, 1-21.

Sabo, E. (2006) Characterization of the pelagic plankton assemblage of Lake Matano

and determination of factors regulating primary and secondary production dynamics. M.Sc. thesis, University of Windsor, Canada.

Schindler, D.W. (1974) Eutrophication and recovery in experimental lakes: implications for lake management. *Science,* **184**, 897-899.

Schindler, D.W. (1977) The evolution of phosphorus limitation in lakes. *Science*, **195**, 260-262.

Seehausen, O. (2002) Patterns in fish radiation are compatible with Pleistocene desiccation of Lake Victoria and 14 600 year history for its cichlid species flock. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.,* **269**, 491-497.

Setaro, F.V. and Melack, J.M. (1984) Responses of phytoplankton to experimental nutrient enrichment in an Amazon floodplain lake. *Limnol. Oceanogr*., **29**, 972-984.

Snoeks, J. (2000) Systematics of the cichlids of the East African Lakes: from chaos to complexity. *Int. Symp. Gr. Lak. Wor*., abst., **22**.

Stein, J. (1973) Handbook of phycological methods. Culture methods and growth measurements. *Cambr. Univ. Press.* 448 pp.

Stevenson, R.J.; Bothwell, M.L.; Lowe, R.L. (eds) (1996) Algal ecology: freshwater benthic ecosystems. Academic Press, San Diego.

Strickland, J. D. H. and Parsons, T.R. (1968) A practical handbook of seawater analysis. *Bull. Fish. Res. Bd. Can*., **67**: 1–311.

Utermöhl, H. (1958) Zur Vervolkommnug der quantitative phytoplankton-methodik. *Limnologie*, **9**, 1-38.

Vollenweider, R.A. (1976) Advances in defining critical loading levels of phosphorus in lake eutrophication. *Mem. Ist. Ital. Idrobiol*., **33**, 53-83.

Wehr, J.D. and Sheath, R.G. (eds) (2003) Freshwater algae of North America; ecology and classification. Academic Press, Amsterdam.

Table 2.1 Treatments used for growth potential experiments. Elemental concentrations (µmol/L) were calculated from volumes of CHU-10 media and the mean concentrations for the upper 100 m of Lake Matano water (from ICP-MS analysis, Chapter 3 and unpubl. data); standard errors are shown when possible. Mean concentrations (µmol/L) of these elements required by some phytoplankton (Healey, 1973) are included below the elemental symbols.

Treatment				Elemental Concentration (µmol/L)					
Matano		CHU-10		Ca	Fe	K	Mg	Na	Si
				217	106	443	230	265	1929
%	Volume (mL)	%	Volume (mL)						
0	0	100	10	2500	143	1026	103	10,000	2860
12.5	1.25	87.5	8.75	2217.1 ± 0.7	125.1 ± 0	898.5 ± 0	179.0 ± 0.5	8756.6 ± 0.7	2538.6
25	2.5	75	7.5	1933.3 ± 1.3	107.1 ± 0	771.0 ± 0.1	255.0 ± 0.9	7513.3 ± 1.3	2216.2
50	5.0	50	5.0	1366.5 ± 2.7	72.3 ± 0	514.9 ± 0.1	408.0 ± 1.9	5026.5 ± 2.6	1572.4
75	7.5	25	2.5	799.8 ± 4.0	36.4 ± 0	259.9 ± 0.2	560.0 ± 2.8	2539.8 ± 3.9	928.5
100	10.0	0	0	233.0 ± 5.3	0.5 ± 0	3.8 ± 0.2	712.0 ± 3.7	53.0 ± 5.2	284.7

Table 2.2 List of pelagic phytoplankton genera observed during experiments on Lake Matano in 2004 (Sabo, 2006) and 2006. The five species observed in both experiments are indicated in bold.

Division	**Family**	**Genus**	**2004**	**2006**
Bacillariophyta	Brachysiraceae	*Brachysira*	Y	
	Fragilariaceae	*Fragilaria*		Y
	Surirellaceae	*Surirella*	Y	
	Fragilariaceae	*Tabellaria*	Y	
Chlorophyta	Chlamydomonadaceae	*Chlamydomonas*		Y
	Chlorococcacae	***Chlorococcus***	**Y**	**Y**
	Chlorococcacae	*Scenedesmus*	Y	
	Desmidiaceae	***Cosmarium***	**Y**	**Y**
	Desmidiaceae	***Staurastrum***	**Y**	**Y**
	Desmidiaceae	*Xanthidium*		Y
	Microsporaceae	*Microspora*	Y	
	Oocystaceae	*Tetraedron*		Y
	Oocystaceae	*Monoraphidium*		Y
	Trebouxiophyceae	*Koliella*		Y
Cyanobacteria	Chroococcales	*Gloeocapsa*		Y
	Merismopediaceae	***Merismopedia***	**Y**	**Y**
	Merismopediaceae	*Snowella*	Y	
	Nostocaceae	*Anabaena*		Y
	Synechococcaceae	*Aphanothece*	Y	
	Synechococcaceae	*Synechococcus*		Y
Pyrrophyta	**Peridiniaceae**	***Peridinium***	**Y**	**Y**
Total, each experiment			**12**	**14**
Total, both experiments				**21**

Table 2.3 Mean phytoplankton biomass (µg/L) of each major division at day 2 with each nutrient addition. Standard errors are shown when applicable.

	N, P [20 µmol/L] N:P [60:5 µmol/L]	N, P [40 µmol/L] N:P [240:20 µmol/L]	N, P [80 µmol/L] N:P [480:40 µmol/L]	N, P [160 µmol/L] N:P [960:80 µmol/L]	N, P [320 µmol/L] N:P [1920:160 µmol/L]
Bacillariophytes					
N	0	0	0	0	0
P	0	0	2.7	2.4	9.4
N:P (12:1)	0	0	1.2	0	0
Chlorophytes					
N	21.8 ± 10.9	2.3± 0.2	2.0 ± 0.4	1.0 ± 0.1	2.3 ± 0.4
P	15.9 ± 1.4	4.5 ± 1.5	4.2 ± 1.3	0.2 ± 0.1	1.7 ± 0.5
N:P (12:1)	1.5	2.8 ± 1.1	2.9 ± 0.4	2.4 ± 0.6	0.9 ± 0.1
Cyanobacteria					
N	0.1 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.21 ± 0.0	1.0 ± 0.4
P	0.7 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	0.20 ± 0.1	0.3 ± 0.1
N:P (12:1)	2.0 ± 0.6	1.0 ± 0.5	0.1 ± 0.0	1.02 ± 0.5	0.6 ± 0.2
Sum 3 Divisions					
N	21.9 ± 10.9	2.6 ± 0.3	2.3 ± 0.4	1.2 ± 0.1	3.3 ± 0.8
P	16.6 ± 1.6	4.7 ± 1.6	7.3 ± 1.4	2.8 ± 0.2	11.5 ± 0.4
N:P (12:1)	3.5 ± 0.6	3.8 ± 1.5	4.2 ± 0.5	3.4 ± 1.1	1.5 ± 0.3

Table 2.4 Mean phytoplankton biomass (µg/L) of each major division at day 4 with each nutrient addition. Standard errors are shown when applicable.

	N, P [20 µmol/L] N:P [60:5 µmol/L]	N, P [40 µmol/L] N:P [240:20 µmol/L]	N, P [80 µmol/L] N:P [480:40 µmol/L]	N, P [160 µmol/L] N:P [960:80 µmol/L]	N, P [320 µmol/L] N:P [1920:160 µmol/L]
Bacillariophytes					
N	2.7	0	0	0	0
P	0	0	0	0	10.6
N:P (12:1)	0	22.2	40.3	4.1	0
Chlorophytes					
N	1.2 ± 0.6	20.8 ± 10.7	21.4 ± 10.8	2.3 ± 0.2	39.9 ± 22.4
P	5.5 ± 0.3	24.1 ± 9.9	4.5 ± 1.5	21.3 ± 11.7	4.2 ± 2.0
N:P (12:1)	22.7 ± 11.9	2.6 ± 0.0	40.0 ± 10.4	0.7 ± 0.1	40.5 ± 22.1
Cyanobacteria					
N	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	1.2 ± 0.5	0.1 ± 0.0
P	0.5 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
N:P (12:1)	0.4 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
Sum 3 Divisions					
N	4.2± 0.7	21.0± 10.8	21.8± 11.0	3.5 ± 0.7	40.0 ± 22.4
P	6.0 ± 0.5	24.3 ± 9.9	4.6 ± 1.5	21.7 ± 11.7	15.0± 2.1
N:P (12:1)	23.1± 12.0	25.0 ± 0.1	80.5 ± 10.4	5.0 ± 0.1	40.6± 22.1

Table 2.5 Mean phytoplankton biomass (µg/L) of each major division at day 8 with each nutrient addition. Standard errors are shown when applicable.

	N, P [20 µmol/L] N:P [60:5 µmol/L]	N, P [40 µmol/L] N:P [240:20 µmol/L]	N, P [80 µmol/L] N:P [480:40 µmol/L]	N, P [160 µmol/L] N:P [960:80 µmol/L]	N, P [320 µmol/L] N:P [1920:160 µmol/L]
Bacillariophytes					
N	2.7	0	2.7	5.4	5.4
P	0	0	2.7	1.2	2.7
N:P (12:1)	34.2	4.5 ± 0.7	7.5	8.2	2.7
Chlorophytes					
N	11.3 ± 4.4	22.7 ± 10.7	62.3 ± 19. 7	6.7 ± 0.4	49.8 ± 22.2
P	34.8 ± 11.0	50.8 ± 25.5	3.5 ± 0.7	45.7 ± 12.2	29.7 ± 13.3
N:P (12:1)	76.3 ± 19.3	64.6 ± 18.3	77.7 ± 11.0	22.9 ± 10.7	2.8 ± 0.7
Cyanobacteria					
N	4.6 ± 2.3	0.5 ± 0.1	0.2 ± 0.0	0.7 ± 0.1	7.1 ± 3.9
P	6.3 ± 3.1	4.7 ± 2.2	0.4 ± 0.1	0.9 ± 0.3	1.2 ± 0.2
N:P (12:1)	10.4 ± 5.3	1.6 ± 0.67	0.4 ± 0.0	0.9 ± 0.3	1.9 ± 0.5
Sum 3 Divisions					
N	18.6 ± 6.7	23.2 ± 10.8	65.3 ± 19.7	12.8 ± 0.5	62.3 ± 26.1
P	41.1 ± 14.1	55.6 ± 27.7	6.6 ± 0.8	47.7 ± 12.	33.6 ± 13.5
N:P (12:1)	120.8 ± 24.6	70.7 ± 19.7	85.6 ± 11.1	31.92± 11.0	7.4 ± 1.2

Table 2.6 Mean phytoplankton biomass (µg/L) of each major division at day 16 with each nutrient addition. Standard errors are shown when applicable.

	N, P [20 µmol/L] N:P [60:5 µmol/L]	N, P [40 µmol/L] N:P [240:20 µmol/L]	N, P [80 µmol/L] N:P [480:40 µmol/L]	N, P [160 µmol/L] N:P [960:80 µmol/L]	N, P [320 µmol/L] N:P [1920:160 µmol/L]
Bacillariophytes					
N	7.3 ± 3.7	5.6 ± 3.9	28.2 ± 26.1	8.1 ± 7.7	16.7 ± 14.4
P	4.5 ± 4.5	91.8 ± 81.6	0	24.5 ± 15.5	43.9 ± 32.2
N:P (12:1)	830 ± 715	167 ± 163	13.2 ± 6.9	26.2 ± 17.2	0
Chlorophytes					
N	75.9 ± 32.6	195 ± 91.0	75.9 ± 57.8	75.7 ± 28.5	188 ± 90.6
P	200 ± 100	708 ± 485	91.7 ± 30.7	95.5 ± 76.2	12.8 ± 5.4
N:P (12:1)	313 ± 132	301 ± 84.0	197 ± 172	111 ± 51.4	82.8 ± 35.2
Cyanobacteria					
N	4.7 ± 2.1	15.0 ± 9.8	1.2 ± 0.1	2.2 ± 1.5	24931 ± 24867
P	16.7 ± 4.7	21.4 ± 10.7	7.0 ± 5.2	103 ± 101	8.5 ± 2.6
N:P (12:1)	21.3 ± 4.0	98.2 ± 73.3	11.7 ± 2.5	11.7 ± 6.7	34.9 ± 10.5
Sum 3 Divisions					
N	87.9 ± 38.4	816 ± 105	105 ± 84.0	86.1 ± 37.7	25136 ± 24972
P	221 ± 109	898 ± 577	98.7 ± 35.9	222 ± 193	91.5 ± 40.2
N:P (12:1)	1165 ± 851	567 ± 320	223 ± 181	149 ± 75.3	117 ± 45.8

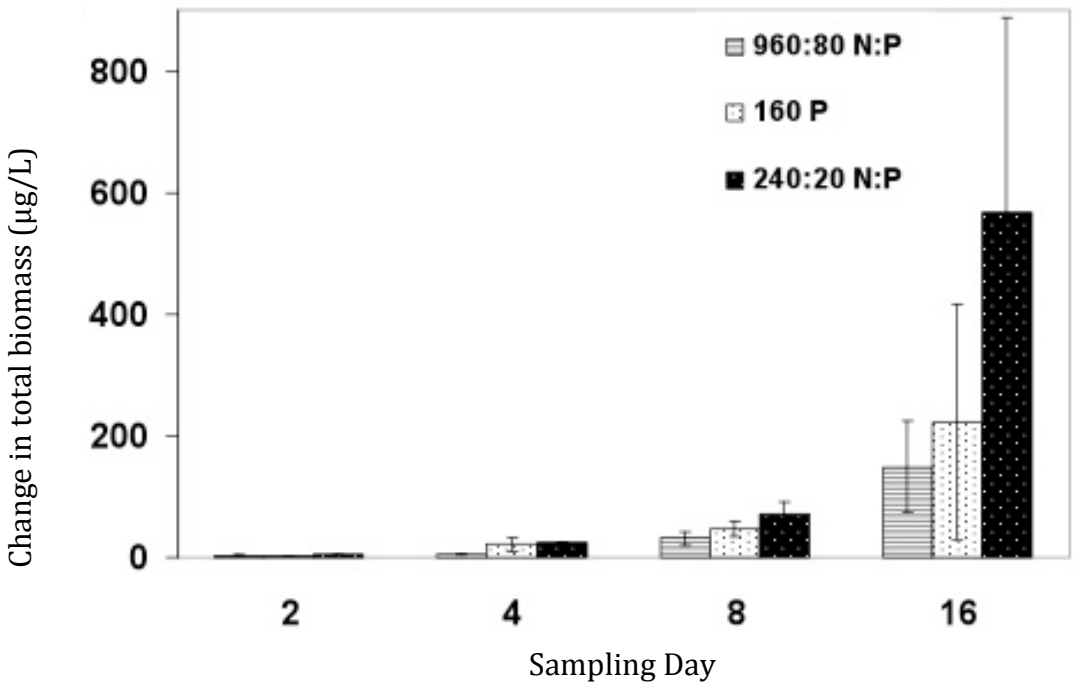

Figure 2.1 Change in biomass (µg/L) (total – inoculate) for the nutrient addition treatments that gave the highest change in biomass at day 16. Each sampling day with standard errors is shown.

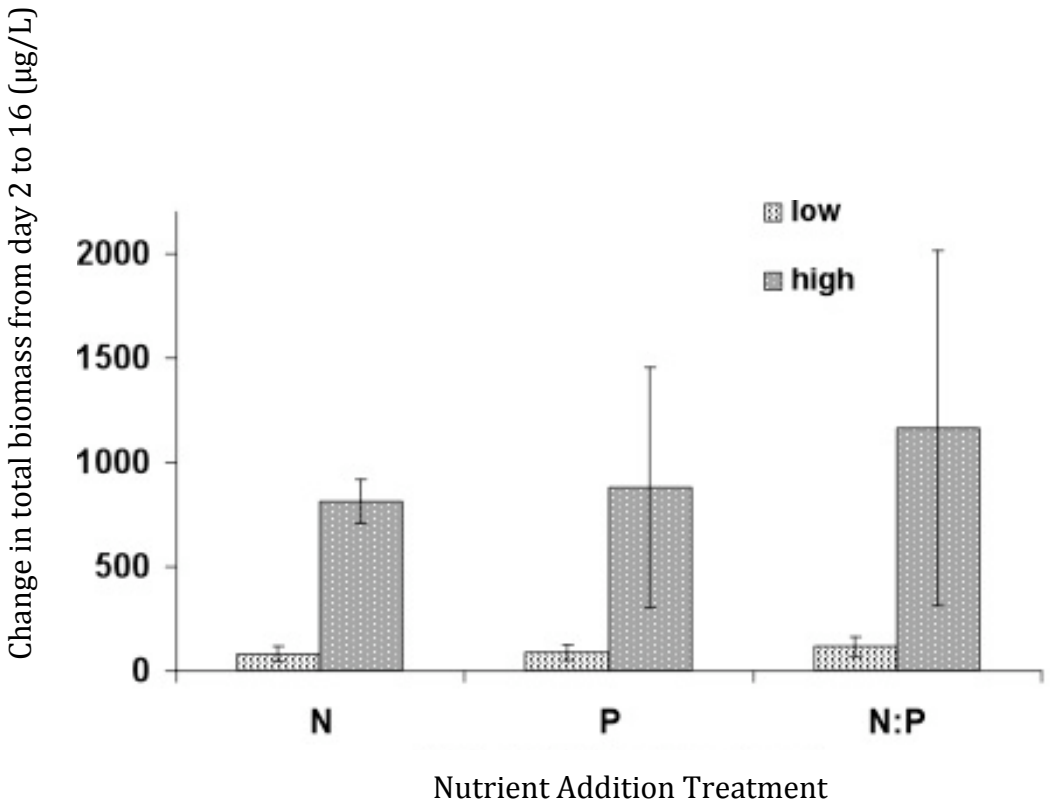

Figure 2.2 Change in total biomass (µg/L) (day 16 – day 2) with standard errors for the nutrient addition treatments. Low and high values for each set of treatments are shown.

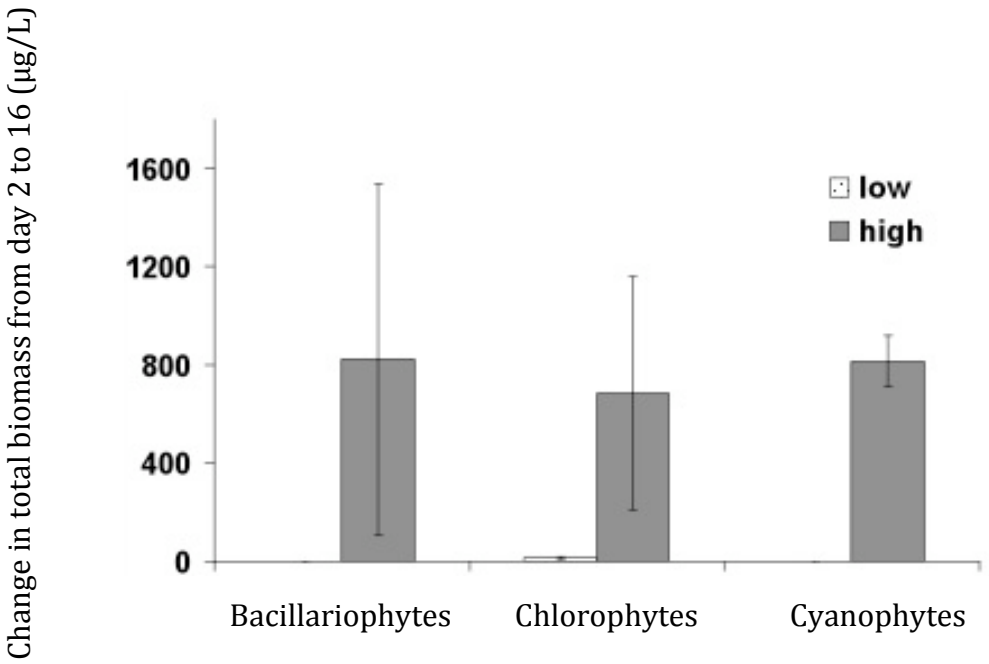

Figure 2.3 Change in total biomass (µg/L) (day 2 to 16) with standard errors for the three main phytoplankton divisions. Low and high values of biomass are shown for each.

Table 2.7 Diversity indices for nutrient addition samples at day 2. Mean biomass values are reported with standard errors when possible. Total species richness in all samples at day 2 was 9.

Nutrient	Concentration (µmol/L)	Mean Biomass (µg/L)	# Individuals/ mL	Shannon Index	Species Richness	Evenness
N	20	21.9 ± 10.9	64	0.5	3	0.4
	40	2.6 ± 0.3	39	0.6	3	0.5
	80	2.3 ± 0.4	58	0.5	4	0.4
	160	1.1 ± 0.1	33	0.4	3	0.3
	320	3.3 ± 0.8	43	0.4	3	0.4
P	20	16.6 ± 1.6	19	1.0	4	0.7
	40	4.7 ± 1.6	36	0.7	4	0.5
	80	7.4 ± 1.4	32	0.5	3	0.5
	160	2.8 ± 0.2	28	0.4	2	0.4
	320	11.5 ± 0.4	41	0.6	3	0.5
N:P	60:5	3.5 ± 0.6	51	0.2	2	0.3
	240:20	3.8 ± 1.5	32	0.7	4	0.5
	480:40	4.2 ± 0.5	23	1.1	5	0.7
	960:80	3.4 ± 1.1	23	0.6	3	0.5
	1920:160	1.5 ± 0.3	22	0.5	3	0.4

Table 2.8 Diversity indices for nutrient addition samples at day 4. Mean biomass values are reported with standard errors when possible. Total species richness in all samples at day 4 was 9.

Nutrient	Concentration (µmol/L)	Mean Biomass (µg/L)	# Individuals/ mL	Shannon Index	Species Richness	Evenness
N	20	4.2 ± 0.7	29	1.0	3	0.9
	40	21.0 ± 10.8	28	0.8	4	0.6
	80	21.8 ± 11.0	21	0.7	3	0.7
	160	3.5 ± 0.7	21	1.0	3	0.9
	320	40.0 ± 22.4	21	0.6	4	0.5
P	20	6.0 ± 0.5	33	0.6	4	0.5
	40	24.3 ± 9.9	30	1.0	4	0.7
	80	4.6 ± 1.5	25	0.7	4	0.6
	160	21. ± 11.7	42	0.8	4	0.6
	320	15.0 ± 2.1	16	1.0	4	0.7
N:P	60:5	23.1 ± 12.0	39	0.9	4	0.6
	240:20	25.0 ± 0.1	25	1.0	3	0.8
	480:40	80.5 ± 10.4	24	0.6	4	0.5
	960:80	5.0 ± 0.1	26	0.3	2	0.3
	1920:160	40.6 ± 22.1	26	0.6	4	0.4

Table 2.9 Diversity indices for nutrient addition samples at day 8. Mean biomass values are reported with standard errors when possible. Total species richness in all samples at day 8 was 10.

Nutrient	Concentration (µmol/L)	Mean Biomass (µg/L)	# Individuals/ mL	Shannon Index	Species Richness	Evenness
N	20	18.6 ± 6.7	131	1.0	5	0.6
	40	23.2 ± 10.8	137	1.0	5	0.6
	80	65.3 ± 19.7	55	0.7	3	0.6
	160	12.8 ± 0.5	124	0.7	5	0.4
	320	62.3 ± 26.1	165	0.6	5	0.3
P	20	41.1 ± 14.1	69	0.8	4	0.6
	40	55.6 ± 27.7	41	0.8	4	0.5
	80	6.6 ± 0.8	28	1.0	4	0.7
	160	47.7 ± 12.5	57	0.9	5	0.6
	320	33.6 ± 13.5	50	1.0	5	0.6
N:P	60:5	120.8 ± 24.6	162	0.9	6	0.5
	240:20	70.7 ± 19.7	80	0.8	7	0.4
	480:40	85.6 ± 11.1	50	0.5	4	0.3
	960:80	31.9 ± 11.0	245	0.4	4	0.3
	1920:160	7.4 ± 1.2	62	0.3	4	0.3

Table 2.10 Diversity indices for nutrient addition samples at day 16. Mean biomass values are reported with standard errors when possible. Total species richness in all samples at day 16 was 14.

Nutrient	Concentration (µmol/L)	Mean Biomass (µg/L)	# Individuals/ mL	Shannon Index	Species Richness	Evenness
N	20	87.9 ± 38.4	95	1.4	7	0.7
	40	815 ± 104	222	1.1	8	0.5
	80	105 ± 84.0	201	0.8	8	0.4
	160	86.1 ± 37.7	190	0.8	6	0.5
	320	25136 ± 24972	1191	0.6	9	0.3
P	20	221 ± 109	673	1.3	7	0.7
	40	898 ± 577	462	1.4	11	0.6
	80	98.7 ± 35.9	790	0.6	8	0.3
	160	222 ± 193	18418	0.2	5	0.1
	320	91.5 ± 40.2	408	0.5	6	0.3
N:P	60:5	1165 ± 851	2229	0.7	9	0.3
	240:20	567 ± 320	7941	0.8	10	0.4
	480:40	223 ± 181	1295	0.3	8	0.2
	960:80	149 ± 75.3	13795	0.3	7	0.1
	1920:160	117 ± 45.8	1838	0.4	6	0.2

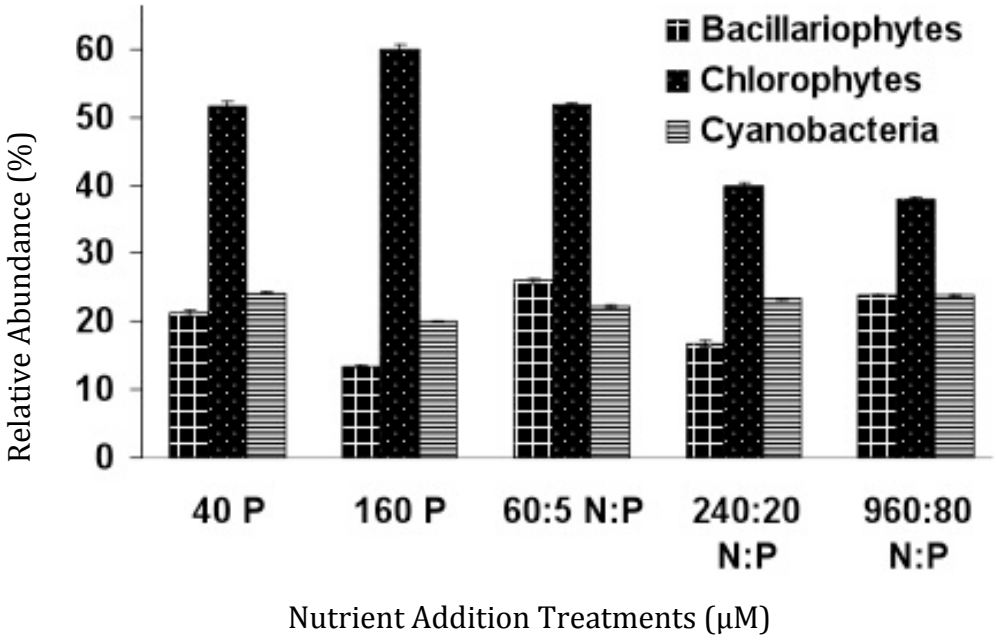

Figure 2.4 Day 16 relative abundances of the three main divisions for the nutrient addition treatments (µmol/L, 12:1 N:P) which gave the highest total biomass.

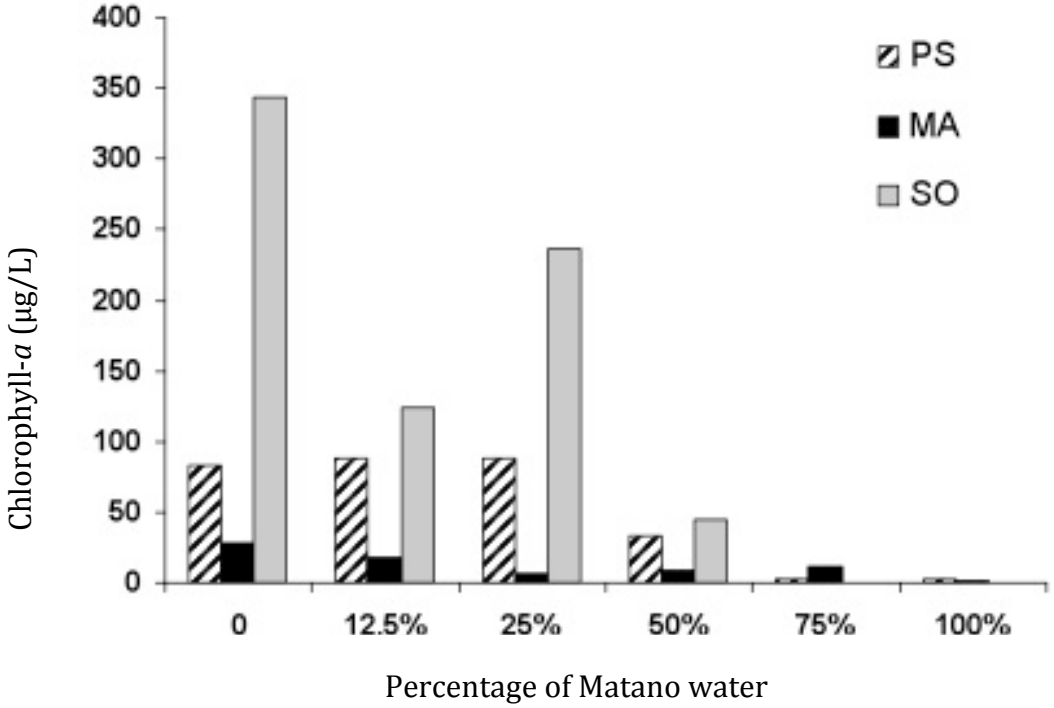

Figure 2.5 Concentration of Chlorophyll-*a* for chlorophytes *P. subcapitata* (PS) and *S. obliquus* (SO), and cyanobacteria *M. aeruginosa* (MA) cultivated in CHU-10 media, diluted with Lake Matano water.

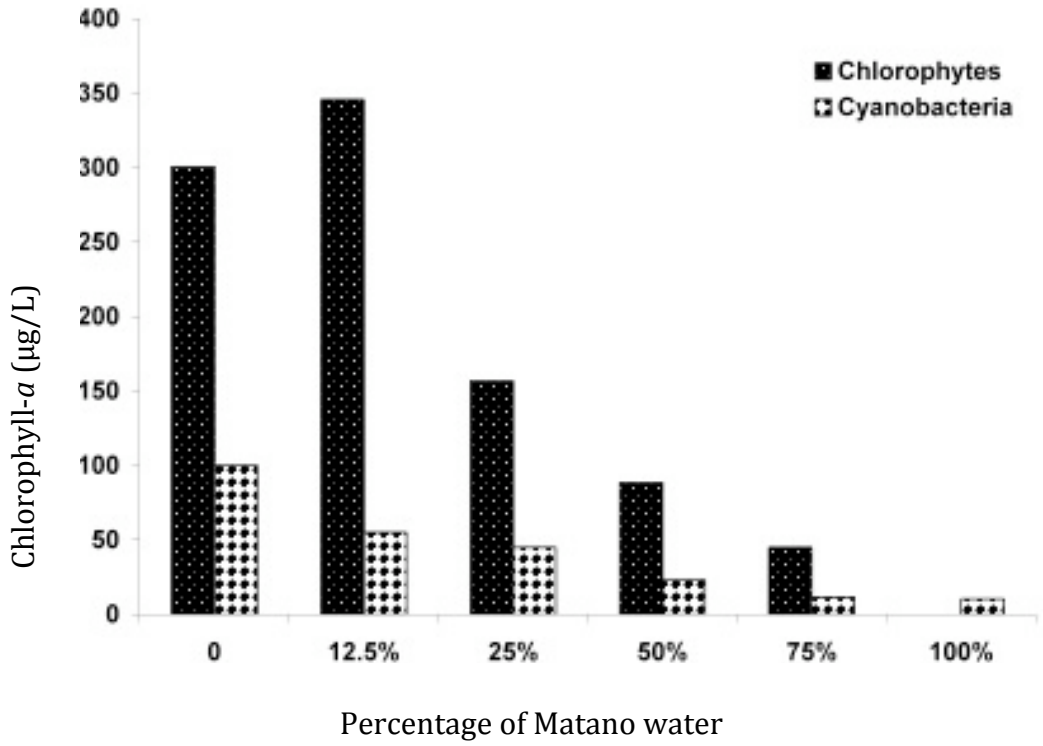

Figure 2.6 Concentration of Chlorophyll-*a* for phytoplankton of Lake Matano cultivated in CHU-10 media, diluted with Lake Matano water.

Chapter 3: Malili chemistry: metals and nutrients

3.1 Introduction

Biodiversity is related to system productivity (Tilman et al., 1982), therefore understanding the relationship between these two factors is important. From the combined results of two separate studies (Sabo, 2006; Chapter 2), it is evident that the number of phytoplankton genera in Lake Matano, 21, is at minimum equal to that of other lakes in Indonesia, Venezuela and the Philippines (Lewis, 1978). However, in the Malili lakes, entire taxa of phytoplankton (Cryptophyceae, Chrysophyceae, and Centrales), which typically have global distribution (Kugrens and Clay, 2003; Nicholls and Wujek, 2003), are absent, but have been observed in Lake Poso (Bramburger, pers. comm.), a lake also located on Sulawesi Island but within a different watershed. These missing taxa include species that are well adapted to ultra-oligotrophic conditions (Kugrens and Clay, 2003).

The Malili lakes are oligotrophic with very low concentrations of macronutrients N and P (e.g. 0.3 µmol/L NH_3 and 0.13 µmol/L TP) (Haffner, unpubl. data). The nutrient addition experiments of Chapter 2 indicated, however, that N and P are not sufficient factors to limit the observed phytoplankton growth of Lake Matano. However, even in instances where inorganic nutrients, including N and P, limit phytoplankton population dynamics, competition for a principal limiting nutrient does not necessarily affect phytoplankton diversity (Leibold, 1999).

The biogeochemical cycling of micronutrients essential for phytoplankton growth, including Fe, Mn, Co, Zn, Mo, Cu, Na, B, Si, and V, is complex. Algal cells require elements in relatively fixed proportions, defined as the Redfield Ratio (C:N:P 106:16:1), in order to reproduce (Hecky and Kilham, 1988). Cell requirements are related to the bioavailability of various macro and micronutrients. Co, B, Si, Na, and V, however, are only required by some taxa (Cole, 1983). Generally, concentrations and availability of micronutrients in natural waters are adequate to sustain active populations of algae within the constraints of light, temperature, and macronutrient availability (Hecky and Kilham, 1988). Previous research often did not consider micronutrient limitation and it was relegated to extreme oligotrophic systems (granitic arctic, alpine, and volcanic areas) (Prescott et al., 1954). Recent research is discovering that micronutrient limitation might be more common than previously thought. Micronutrient limitation has now been reported in more than 40 lakes, and its prevalence is unrelated to lake size or trophic state (Downs et al., 2008). The potential for micronutrient limitation in Lake Matano was first examined in the growth potential study described in Chapter 2. When concentrations of Ca, K, and Na in the growth media diluted with Lake Matano water were below a threshold concentration reported by Healey (1973) as that required by some phytoplankton (217 µmol/L Ca, 443 µmol/L K, 265 µmol/L Na), chlorophytes and cyanobacteria displayed a sharp decrease in chl-*a* concentration, indicative of a nutrient limitation threshold. This decrease occurred at a dilution of 50% Matano water and 50% CHU-10 media, and continued as the concentration of Matano water increased. Water chemistry of the lakes, including analysis of elemental concentrations with the

potential to limit the phytoplankton composition and abundance, is addressed in this chapter.

Lake Matano, the best characterized of the Malili lakes (Lehmusluoto et al., 1997; Haffner et al., 2001; Crowe et al., 2007), combined with lakes Mahalona and Towuti provide an overview of water chemistry effects within the Malili Lakes system, and Lake Poso serves as a reference system external to the Malili Lakes (see Figure 1.1). Lake Poso and the Malili lakes are located on Sulawesi Island, but are situated in two different geological settings and have never been connected (Wilson and Moss, 1999). Lake Poso is more productive than the Malili lakes and supports a major eel fishery (Haffner, pers. comm.). Considering that these lakes are at similar latitudes, they are predicted to support similar primary production (Lewis, 2000) and algal composition (Reynolds, 2000).

Matano receives most of its water from surface runoff and groundwater with no large river systems entering the lake (Haffner et al., 2001). The lateritic soils surrounding the lake are rich in nickel and many elements have been leached out of these soils and drained into Lake Matano. According to Villeneuve et al. (2002), the catchment basin of the Malili lakes is primarily composed of ophiolite, a section of the Earth's oceanic crust and upper mantle that has been uplifted as a result of tectonic movement. The term 'ophiolite' describes a group of rocks, particularly serpentine, diabase, pillow lava, and cherts. The main elements contained in these rocks are Fe, Mn, Mg, Si, Cr, Co, and Ni (Villeneuve et al., 2002). Fe and Mn are

abundant in Matano and research has suggested that their redox cycling regulates chemical speciation and bioavailability in Lake Matano (Crowe et al., 2007), which in turn could effect primary production and species composition. Experiments conducted by Sabo (2006) reported a statistically significant ($p < 0.05$) increase in phytoplankton biomass, although no significant changes in species composition, when soluble Fe, Mn, Cr and Ni were reduced in Lake Matano water. Typically, natural watersheds impose only a minor influence on the phytoplankton of large lakes (Eccles, 1988), but previous research by Crowe et al. (2007) on Matano water suggested that the watershed runoff influences both the primary production and composition.

The absence of cosmopolitan planktonic groups, the lack of phytoplankton growth response or change in relative abundance with nutrient additions, and the nutrient-threshold response exhibited by cultured algae grown in Matano water (Chapter 2) form the basis of the hypothesis that the water chemistry of Lake Matano is playing a major role in the regulation of phytoplankton composition and relative abundance.

The phytoplankton abundance and composition for lakes Matano, Mahalona, Towuti, and Poso were evaluated using both diversity and similarity indices. Physical datasets, including temperature, oxygen, conductivity, pH, and Secchi depth, were collected and compared for the three Malili lakes and Lake Poso. Nutrient data for the lakes for N and P at various depths were analyzed for significant differences. Chemical analyses of the lakes' water columns were completed using a depth-

sampling regime. Samples were analyzed for nutrients and elements using spectrophotometic techniques and inductively coupled plasma-mass spectrometry (ICP-MS). This research examines the relationship between the lakes' phytoplankton assemblages and their physical and chemical characteristics in an attempt to identify the relative importance of physical/chemical factors in regulating the phytoplankton composition and biomass. It is hypothesized that lakes with similar physical and chemical conditions will have common assemblages with respect to composition and biomass.

3.2 Methods

3.2.1 Phytoplankton identification and counts

In 2008, phytoplankton from each of the four lakes was collected in 4 L surface water samples. Plankton was allowed to settle-out over two days. After this time, the supernatant was poured off and the remaining sample stored in 25 mL scintillation vials and preserved with 1 mL of Lugol's/formalin solution. 1 mL from each was transferred to a Gridded Sedgwick Rafter slide (model # 1801-G2) and ten squares (1 mm x 1 mm) were analyzed, with replicate samples for verification. Phytoplankton were identified, quantified, and evaluated for diversity, species richness, and evenness. The Shannon Diversity Index was used and takes into account both the number of species and evenness of the species present in a sample. The Sørensen Similarity Coefficient, a statistic used for comparing the similarity of two samples, was used here for pairwise comparisons of the four lakes'

compositions (based on genera) to express the percentage similarity of two lakes (Looman and Campbell, 1960). A Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows) was used to statistically evaluate relationships between the abundance and composition of the four lakes.

3.2.2 Temperature, Dissolved Oxygen, Conductivity, pH

Data for water column profiles of temperature, dissolved oxygen, conductivity and pH were collected using a Brancker XLT profiler that sampled multiple depths from surface to bottom for each lake. These data sets were combined with previous data sets collected by Haffner (unpubl. data).

3.2.3 Secchi Disk (SD) measurement

To obtain measurements, a disk with black and white quadrants was lowered over the shady side of the boat at mid-day. A weight was attached to keep the line vertical in the water. The disk was viewed using a scuba mask underwater to reduce the effect of surface light reflection. The disk was lowered until it disappeared from sight, lowered further, and then raised until it reappeared. The average of the depth of disappearance and reappearance was recorded as the Secchi depth. This depth is half the distance light travels to the disc and back up to the observer's eye (Cole, 1983). The minimum intensity of subsurface light that permits photosynthesis has been set at about 1.0% of incident surface light. Thus, the region from surface to the depth at which 99% of the surface light has disappeared is called the euphotic zone;

below this depth primary productivity is usually considered nil (Cole, 1983). The euphotic zone can be calculated as double the Secchi disc depth.

3.2.4 Nutrient data

In order to sample the deeper depths of the lakes as accurately as possible, sampling was performed on days with minimal wind to minimize drifting. Samples collected for total phosphorus (TP) analysis were preserved with sulfuric acid to pH 2. Concentrations were determined as per the Standard Methods Ascorbic Acid method for TP (Eaton et al., 1995). The basic principle of this method is ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid (phosphomolybdic acid) that is reduced by ascorbic acid to molybdenum blue. Absorbances were measured at 880 nm on a spectrophotometer and converted to concentration by use of linear regression created by the measurement of absorbances from six samples prepared by serial dilution of K_3PO_4. Soluble reactive phosphorus (SRP) was measured by reacting orthophosphorus with ammonium molybdate and potassium antimony followed by reduction with ascorbic acid. Nitrate-nitrite (NO_3/NO_2) was calculated by reducing nitrate to nitrite, diazotizing the nitrite with sulfanilamide and coupling with ethylenediamine dihydrochloride. Ammonium (NH_3) concentrations were determined using phenate-hypochloride colorimetry. A Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows) was used to statistically evaluate differences ($p < 0.05$) among the nutrient concentrations of the lakes. For significant

results, Mann-Whitney U-tests were conducted between every lake.

3.2.5 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis

Water samples were collected at various depths from each lake using a Kemmerer water sampler. Water was overflowed into 250 mL plastic sample bottles and preserved with 1 mL nitric acid. Samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to obtain elemental concentrations for Na, P, K, Cr, Mn, Fe, Co, Ni, Cu, Zn, and Mo. In this method, samples are atomized to positively-charged ions in a high temperature argon plasma and analyzed based on their mass-to-charge ratios. Four main processes occur: sample introduction and aerosol generation, ionization by an argon plasma source, mass discrimination, and the detection system. The spectra produced consist of a series of isotope peaks. A calibration curve based on an internal standard was used to compute quantitative measurements of the spectra (Skoog and Leary, 1992). Instrumental detection limits vary for each element and are listed in Table 3.1. Concentrations were corrected by subtracting the blank from the sample value.

Statistics

Generalized Discriminant Analysis (GDA) using Canonical Analysis of Principal Coordinates (PCO) (CAP: Anderson and Willis, 2003; Anderson and Robinson, 2003) was used to test for significant differences in phytoplankton species biomass in the four lakes and to test for significant differences in the elemental concentrations (determined by ICP-MS) in the four lakes, and for relationships between elemental

concentrations and phytoplankton species abundance in the four lakes. All CAP analyses were performed using the Bray-Curtis distance matrix and canonical test statistics were obtained via permutation tests (999 permutations). PCO is a useful tool for non-linear data that does not assume normal distribution. These multivariate analyses were used to identify key variables in the datasets. A Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows), Mann-Whitney U-tests were used to statistically evaluate relationships between lakes.

3.3 Results

3.3.1 Phytoplankton results

There were low numbers of phytoplankton species observed and consistently low biomass for all lakes. Using the Sørensen Similarity Coefficient, the phytoplankton compositions by genera were most similar between Matano and Mahalona (0.76), followed by Mahalona and Poso (0.71), Mahalona and Towuti (0.67), Matano and Poso (0.63), Towuti and Poso (0.60), and the least similar lakes were Matano and Towuti (0.47). Even in pairs of lakes with similar compositions, biomasses and relative abundances differed. A detailed summary of taxa and abundances (cells/L) is listed in Appendix I, Tables I-IV. Biomass (as the total volume of individuals per mL), diversity, species richness, and evenness are summarized in Table 3.2. Mean biomasses for Matano, Mahalona, Towuti, and Poso were $1.62 \times 10^{-2} \pm 8.39 \times 10^{-1}$ µg/L, $1.15 \times 10^{-3} \pm 5.08 \times 10^{-2}$ µg/L, $9.86 \times 10^{-4} \pm 5.32 \times 10^{-3}$ µg/L, and 1.28×10^{-1} µg/L, respectively.

With respect to diversity, the upper 50 m of Lake Matano contained the highest rating of the Shannon Diversity Index (1.58), highest species richness (10), and lowest evenness (0.68) per sample of the four lakes. Twelve genera were observed for all depths sampled (0, 50, 100 m): bacillariophyte *Fragilaria*; chlorophytes *Chlamydomonas*, *Chlorococcus*, *Cosmarium*, *Monoraphidium*, *Koliella*, *Staurastrum*, and *Tetraedron*; and cyanobacteria *Gloeocapsa*, *Merismopedia*, *Synechococcus* and *Anabaena*. Lake Mahalona contained the lowest diversity (0.87 at 0 m) and the highest evenness (0.95) at 30 m depth, as species from only 3 genera were observed: *Chlamydomonas*, *Chlorococcus*, and *Gloeocapsa*. For all depths, 8 genera were observed in Mahalona—6 chlorophytes and 2 cyanobacteria. Towuti, the lake furthest downstream, had the lowest phytoplankton biomass, contained the lowest species richness (e.g. 3 at 50 and 100 m), and had consistently high evenness (0.87 for 50-100 m), as individuals from only 4 chlorophyte genera (*Chlamydomonas*, *Chlorococcus*, *Cosmarium*, and *Tetraedron*) and one cyanobacteria genus (*Gloeocapsa*) were observed. Lake Poso had the highest phytoplankton biomass, one order greater than Matano, and three orders greater than Towuti. Six genera were observed and consisted of 5 genera of chlorophytes and one genus of cyanobacteria (for full list of genera, see Appendix I).

There was separation of the three Malili lakes and Poso based on phytoplankton composition, as illustrated in Figure 3.1. In this case, the x-axis (PCO 1) explained 70% of the variance in the dataset and was positively associated with the presence

of chlorophytes *Chlamydomonas*, *Staurastrum*, *and Chlorococcus* and cyanobacteria *Gloeocapsa*. The x-axis was negatively associated with the chlorophyte *Tetraedron*. The y-axis (PCO 2) explained 17% of the variance and was associated with the presence of chlorophytes *Chlamydomonas*, *Staurastrum, Monreaphidium*, and *Chlorococcus* and the cyanobacteria *Anabaena.* Error bars were based on 95% confidence intervals, but as only one sample was available from Lake Poso, confidence intervals could not be calculated for this lake. There was little overlap in the phytoplankton assemblage between Matano and Towuti along the x-axis and no overlap along the y-axis. Matano and Towuti had the most distinct clustering, remaining separated from Poso, while Mahalona had a broad distribution with overlap in both x- and y-axes for all lakes. Towuti clustered the furthest from Lake Poso. Lakes Mahalona, Towuti, and Poso did not contain any bacillariophytes, nor the cyanobacteria *Merismopedia* or *Synechococcus*. These genera were observed in Matano. The chlorophyte *Cosmarium* was observed in the Malili lakes, but not in Poso. Poso and Towuti did not contain the chlorophytes *Monoraphidium* and *Koliella*, seen in Matano and Mahalona. The chlorophyte *Staurastrum* was not observed in Mahalona or Towuti.

A Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows)
test was used to statistically evaluate relationships between all lakes and all depth samples. There were no significant relationships between lake and phytoplankton genera abundance; in all cases, $p = 0.1$ to 0.7. The differences in the taxa of these lakes are not statistically significant, but may be of biological significance.

3.3.2 Temperature profiles

Figure 3.2 compares the temperature of surface waters (upper 100 m for Matano, Towuti, and Poso and upper 30 m for Mahalona) of the four lakes. During May/June 2006, Lake Matano's average temperature was 26 °C. It was 29 °C in the epilimnion (upper 100 m), 29 to 27.5 °C in the thermocline (40-60 m) and 27.5 to 27 °C in the hypolimnion. Lake Mahalona had the highest surface water temperature of the four lakes at 30.5 to 29.5 °C in the epilimnion (upper 20 m), decreasing to 29.5 to 27.5 °C in the thermocline (20-35 m) and 27.5 °C in the hypolimnion. Lake Towuti was nearly 30 to 29.5 °C in the epilimnion (upper 40 m), 29.5 to 28.5 °C in the thermocline (40-60 m) and 28.5 °C in the hypolimnion. Lake Poso's epilimnion (upper 20 m) was 29 to 27.5 °C, 27 to 26.5 °C in the thermocline (20-50 m) and 26.5 °C in the hypolimnion. The epilimnion is the thoroughly mixed, uniform temperature region in the uppermost waters. It is highly irradiated, wind-stirred, and where primary productivity prevails (Cole, 1983). According to temperature-density relationships of pure water at one atmosphere and with density 1.0 at 4 °C, density increases are greatest between higher temperatures (Cole, 1983). Density change between the epilimnion and the hypolimnion was the greatest for lake Mahalona: from 30.5 to 27.5 °C the density decreased by 8.8×10^{-4} g/cm^3. For Poso, with a temperature change of 29 to 26.5 °C, the density decreased by 7.1×10^{-4} g/cm^3. For Matano, with a temperature change of 29 to 27 °C, the density decreased by 5.7×10^{-4} g/cm^3 and for Towuti, with a temperature change of 30 to 28.5 °C, the density decreased by 4.4×10^{-4} g/cm^3. A smaller density change between layers requires less energy to mix these waters, meaning Matano (mixing depth of 40 m) has the potential to mix more frequently than Poso (mixing depth of 20 m). Extensive

vertical mixing continuously moves phytoplankton out of the euphotic zone (Haffner et al., 2001), and contributes to Matano having lower phytoplankton biomass than lake Poso.

3.3.3 Dissolved oxygen and photosynthetic profiles

Figure 3.3 illustrates that the Malili Lakes had comparable dissolved oxygen concentrations of 7.8 mg/L at the surface, and decreased with depth. Matano and Towuti's concentrations remained consistent for the upper 40 m. At 100 m, Towuti's oxygen was 4.2 mg/L whereas Matano's oxygen was 0.2 mg/L. Mahalona's oxygen concentration decreased to 1.9 mg/L near the bottom, 60 m depth. Dissolved oxygen in Lake Poso surface waters was higher than that of the Malili Lakes, with a peak of 11 mg/L, decreasing to 7.8 mg/L at 40 m depth and 2.5 mg/L at 100 m depth. Figure 3.4 shows the profile of oxygen saturation in the upper waters of lakes Matano and Poso. Oxygen, a product of photosynthesis, is indicative of primary production in this layer. The surface waters (0-10 m) of Lake Poso were supersaturated, indicative of high rates of primary production, suggesting high rates of carbon turnover in this water column.

3.3.4 Conductivity profiles

The surface waters' conductivity in the four lakes was within a typical range for freshwater lakes, ranging between 100-200 µS/cm (O'Sullivan and Reynolds, 2004). Figure 3.5 illustrates that Lake Poso had the lowest conductivity, ranging from 114-124 µS/cm throughout its upper 100 m and suggests that the lake receives more runoff, which serves to dilute the ionic concentration. Towuti had the next lowest at 145-146 µS/cm, followed by Mahalona at 174-180 µS/cm. Matano, reflecting high

groundwater inputs, had the highest conductivity of the four lakes at 181-189 μS/cm. The River Continuum hypothesis, first proposed by Vannote in 1980, is a model for classifying and describing flowing water, and relates changes in lotic communities to the downstream gradient of abiotic factors from the headwaters to the mouth (Gordon et al., 2004). In the time water travels from the headwater and flows down the river, it has 'aged' from the effects of successive physical and chemical factors (Cole, 1983). For example, dissolved organic material is more homogenous downstream than upstream. This hypothesis predicts levels of dissolved and suspended solids and conductivity, for example, will increase downstream. In the Malili Lakes system, the conductivity was the highest in the headwater, Lake Matano, and decreased in the downstream lakes. This suggests that water is entering these lakes from different sources, as tributaries and impoundments, for example, can interrupt the hypothetical cline of the river continuum (Cole, 1983). This high conductivity in the headwater lake suggests underground water inputs are an important part of the water budget of Lake Matano, and may play a major role in determining the water chemistry of the lake (see section 3.3.8).

3.3.5 pH profiles

pH is a significant parameter in characterizing water quality (Wetzel, 2001). As seen in Figure 3.6, Lake Mahalona had the lowest surface water pH at 7.9 for the upper 35 m and declined to 7.6 at 55 m. Towuti had the next lowest surface pH at 8.2 for the upper 55 m of the lake. It decreased to 7.8 at 100 m. Lake Poso had a surface pH of

8.3, decreasing to 7.8 at 35 m, and reaching 7.1 at 100 m. Lake Matano had the highest surface pH of 8.5 for the upper 25 m, decreasing to 7.3 at 100 m. This range of pH values is considered typical for freshwater tropical lakes (Cole, 1983).

3.3.6 Secchi depths

Secchi depths for the lakes were 21 m for Matano, 11 m for Mahalona, 26 m for Towuti, and 9.5 m for Poso. This indicates euphotic zones of 42, 22, 52, and 19 m for each lake, respectively. The ratio of the euphotic depth, Z_{eu} (twice the Secchi depth), to the mixing depth, Z_m (epilimnion), can be an important controlling factor for lake productivity (Talling, 1971). As solar radiation passes downward from the surface of a lake, wind mixing of the upper layers of water distributes downward the heat that is absorbed in the surface strata, such that dense colder water lies beneath lighter warm layers. These regions are identified in the temperature profiles of the lakes (Figure 3.2). The epilimnions are approximately 100 m, 20 m, 40 m, and 20 m for Matano, Mahalona, Towuti, and Poso, respectively. $Z_{eu} : Z_m$ is 42% for Matano, 95% for Mahalona, 77% for Towuti, and 95% for Poso. Higher ratios, that is, more light relative to the volume of water (increased with a greater mixing depth), can support more photosynthetic activity (Talling, 1971; Cole, 1983).

3.3.7 Nutrient availability

Nutrient data for the lakes (Table 3.3) indicated both nitrogen and phosphorus were low in all four lakes. For the upper 100 m of Lake Matano, nitrogen as NH_3 ranged from 0.29 ± 0.12 to 0.71 ± 0.35 μmol/L, and as NO_3/NO_2 from 0.04 ± 0.02 to 0.81 ±

0.66 µmol/L. Phosphorus as SRP ranged from 0.13 ± 0.03 to 0.48 ± 0.42 µmol/L and as TP from 0.13 ± 0.03 to 0.32 ± 0.16 µmol/L. For Lake Mahalona, nitrogen as NH_3 was consistently 0.18 µmol/L, and as NO_3/NO_2 ranged from 0.18 to 5.29 µmol/L. Phosphorus as SRP was 0.06 µmol/L, and as TP ranged from 0.16 to 0.19 µmol/L. For the upper 100 m of Lake Towuti, nitrogen as NH_3 ranged from 0.18 to 1.65 µmol/L, and as NO_3/NO_2 ranged from 0.06 to 0.85 µmol/L. Towuti's concentration of phosphorus as SRP ranged from 0.13 to 0.10 µmol/L and as TP ranged from 0.16 to 0.19 µmol/L. For the upper 100 m of Lake Poso, there is currently no nitrogen data. Poso's phosphorus concentration as TP ranged from 0.19 to 0.87 µmol/L. Using pair-wise comparisons, there was a significant difference in NH_3 between Matano and Mahalona ($p = 0.015$, Mann-Whitney U Test Statistic (z) = 21.0). There was a trend of increasing NH_3 with depth (0-400 m) for Lake Matano, increasing from 0.29 ± 0.12 µmol/L at 0 m to 69.65 ± 27.64 µmol/L at 400 m. NO_3/NO_2 and SRP were not significantly different among the lakes ($p = 0.3$, $z = 3.0$). TP concentrations were the same in Mahalona and Towuti, and not significantly different from Matano or Poso ($p = 0.3$, $z = 16.2$). P concentrations were also measured in the ICP-MS analysis and are presented in section 3.3.8 below.

3.3.8 Major water column chemistry

The data sets collected in this analysis are summarized in Table 3.4. Canonical Analysis of Principal Coordinates (PCO) based on ICP-MS elemental concentrations from depth sampling of the four lakes is illustrated in Figure 3.7. With error bars calculated at a 95% confidence interval, there is overlap between Matano and

Mahalona, meaning these lakes are not significantly different with respect to the elements analyzed, and considering the strong advective flow from Matano to Mahalona, this similarity in water chemistry would be predicted. Towuti and Poso are significantly different from one another, and from Matano/Mahalona. PCO 1 (x-axis) accounted for 77% of the variance in the dataset. It was negatively correlated with K and Mo, and positively correlated with Cr. PCO 2 (y-axis) accounted for 17% of the variance in the dataset and was negatively associated with Na and P,

All elements analyzed via ICP-MS for the four lakes were tested for significant ($p < 0.05$) relationships between concentrations in the upper waters (100 m for MAT, TOW, POSO and 30 m for MAH) using Kruskal-Wallis One-way Analysis of Variance (Systat 12 for Windows). For elements with significant differences, pairwise comparisons were performed using the Mann-Whitney U-test. The significant pairwise comparisons, as well as the mean concentrations for each lake are listed in Table 3.5. The elements with significantly different concentrations between lakes were Na, P, K, Cr, Fe, Zn, Ni, and Mo.

Na concentrations for the four lakes ranged from $2.7 \times 10^{1} \pm 8.6 \times 10^{-1}$ µmol/L for Towuti and $5.3 \times 10^{1} \pm 5.2$ µmol/L for Matano. The four lakes are in a very similar range, and considering that some phytoplankton require Na at 256 µmol/L (Healey, 1973), it is possible that this nutrient is limiting in all four lakes.

The concentration of P in Lake Poso ($6.3 \times 10^{-2} \pm 8.7 \times 10^{-3}$ µmol/L) was two orders

lower than in Lake Matano (3.8 ± 8.8 x 10^{-1} µmol/L); Chapter 2 showed that the phytoplankton in Matano are not limited by P, and this further supports that conclusion, as Poso supports a higher phytoplankton biomass than Matano.

K concentrations were statistically significant between the lakes. Poso's concentration (1.4 x 10^{1} ± 1.0 x 10^{-1} µmol/L) was one order higher than in the Malili lakes (3.8 ± 1.7 x 10^{-1} µmol/L for Matano, 3.7 ± 6.6 x 10^{-2} µmol/L for Mahalona, 2.9 ± 4.2 x 10^{-2} µmol/L for Towuti), and still one order lower than that required by some phytoplankton (442.5 µmol/L; Healey, 1973). It is possible that this macronutrient is limiting in all four lakes.

The chemical speciation of Cr cannot be ascertained from ICP-MS analysis, but hexavalent Cr (IV) is the dominant form in natural waters (Cranston and Murray, 1978; Riedel, 1984) and it is the soluble salts of Cr (IV) that can induce a toxic response (Pawlisz et al., 1997). High concentrations of Cr are toxic, and reductions in algal growth can occur if concentrations exceed 1.2 x 10^{-1} µmol/L; water guidelines range from 1.9 x 10^{-2} to 3.2 µmol/L (Pawlisz et al., 1997). The ranges measured in these lakes (1.0 x 10^{-1} ± 1.1 x 10^{-2} µmol/L for Matano, 1.0 x 10^{-1} ± 4.3 x 10^{-3} µmol/L for Mahalona, 8.9 x 10^{-2} ± 5.7 x 10^{-3} µmol/L for Towuti, 2.3 x 10^{-2} ± 2.3 x 10^{-3} µmol/L for Poso) may pose toxic stress on the phytoplankton.

The range of Fe concentrations in the Malili lakes (1.3 x 10^{-1} ± 1.5 x 10^{-3} µmol/L for Towuti to 4.9 x 10^{-1} ± 1.8 x 10^{-2} µmol/L for Matano), when compared to

concentrations required by algae (105.7 µmol/L; Healey, 1973), appear limiting. However, the geological setting of these lakes, that is, the well-weathered lateritic soils that surrounds them inputs large quantities of Fe into this system (e.g. 2.4×10^{1} µmol/L at 200 m in Lake Matano). Fe is not statistically different between the Malili lakes and Lake Poso (e.g. $p = 0.077$ for pairwise comparison of Poso and Towuti), and among the Malili lakes this difference is not biologically significant. Fe is abundant in this system and is not a limiting nutrient.

Ni is not required by phytoplankton and the concentrations in these lakes are low ($4.2 \times 10^{-2} \pm 9.5 \times 10^{-4}$ µmol/L for Towuti to $4.3 \times 10^{-2} \pm 6.9 \times 10^{-3}$ µmol/L for Matano) and not biologically restrictive.

The concentration of Zn was statistically different ($p = 0.001, 0.005$) between Matano ($1.1 \times 10^{-1} \pm 2.4 \times 10^{-2}$ µmol/L) and Mahalona ($2.8 \times 10^{-2} \pm 1.7 \times 10^{-3}$ µmol/L), and Matano and Towuti ($2.5 \times 10^{-2} \pm 1.1 \times 10^{-3}$ µmol/L), but not between Matano and Poso ($2.9 \times 10^{-2} \pm 2.0 \times 10^{-4}$ µmol/L; $p = 0.866$). These relationships do not support the trend in biomass data for these lakes, thus Zn is not perceived to be a limiting nutrient. In addition, Zn is rarely in short supply as rain carries from 3.8 to 183 µmol/L (Cole, 1983).

Mo concentrations were statistically significant between Matano and Towuti ($p = 0.022$), Matano and Poso ($p = 0.049$), Mahalona and Towuti ($p = 0.022$), Mahalona and Poso ($p = 0.037$), and Towuti and Poso ($p = 0.028$), but not between Matano and

Mahalona ($p = 0.788$). According to Healey (1973), phytoplankton require Mo at a mean concentration of 8.0×10^{-3} µmol/L. The concentrations of Mo in the lakes were one to two orders of magnitude lower than this concentration ($5.0 \times 10^{-4} \pm 3.3 \times 10^{-5}$ µmol/L in Matano, $4.0 \times 10^{-4} \pm 0$ µmol/L in Mahalona, $2.7 \times 10^{-4} \pm 1.9 \times 10^{-5}$ µmol/L in Towuti, $8.5 \times 10^{-4} \pm 3.5 \times 10^{-5}$ µmol/L in Poso) and it is very likely that Mo is limiting in all four lakes.

Canonical Correlation Analysis, used to test for relationships between elemental concentrations and phytoplankton species abundance (Figure 3.8), combined the phytoplankton data with the ICP-MS data collected at the same depths (Matano 0, 50, 100 m; Mahalona 0, 30, 60 m; Towuti 0, 50, 100 m; Poso 0 m). This analysis showed significant relationships ($p < 0.05$) between the phytoplankton composition and elemental concentrations. The strongest relationships existed in Lake Matano (left quadrant, based on lake clusters from ICP-MS plot, Figure 3.1) with Cu for *Tetraedron*, with Zn and P for *Koliella*, and with Mo for *Merismopedia*. In Lake Poso (right quadrant), K is positively associated with the presence of *Chlamydomonas* and *Staurastrum*.

3.4 Discussion

There is evidence to support the hypothesis that lakes with similar physical and chemical conditions will support similar phytoplankton assemblages, with respect to biomass and composition. In the case of Matano and Mahalona, the use of the Sørensen Similarity Coefficient for pairwise comparisons of the lakes' phytoplankton

compositions demonstrated that they were the most similar (0.76), and phytoplankton counts showed the most similar biomasses between these two lakes ($1.62 \times 10^{-2} \pm 8.39 \times 10^{-1}$ µg/L for Matano, $1.15 \times 10^{-3} \pm 5.08 \times 10^{-2}$ µg/L for Mahalona). In addition, these two lakes showed no statistically significant difference ($p > 0.05$) in the PCO constructed from the lakes' elemental data. There is strong advective flow between these two lakes, and it is apparent that they share similar aquatic environments and support similar phytoplankton assemblages, thus factors regulating the composition and abundance in Matano are likely having the same effects in Mahalona.

There were no statistically significant differences ($p > 0.05$) among the phytoplankton compositions of lakes Matano, Mahalona, Towuti, or Poso. All lakes contained a prevalence of chlorophytes *Chlamydomonas*, *Chlorococcus*, and *Gloeocapsa,* with *Chlorococcus* being the most dominant genus with respect to biomass (e.g. 2.4×10^{-3} µg/L in Matano). Lake Matano contained the highest diversity (1.58, Shannon's Diversity) and species richness (12 genera) in these samples, yet much lower biomass than lake Poso, which contained the highest biomass of the four lakes ($1.62 \times 10^{-2} \pm 8.39 \times 10^{-3}$ µg/L for Matano, 1.28×10^{-1} µg/L for Poso). However, only one Poso sample was available for counting, while three samples were available for each of the Malili lakes. This one sample for Poso may under-represent the abundance and species richness of the lake. It is known that individuals from the order Centrales (centric diatoms), which are absent from the Malili Lakes, have been observed in Poso (Bramburger, pers. comm.) and this lake is

productive enough to support an eel fishery (Haffner, pers. comm.). Although the biomass value reported for Poso may be low relative to in-lake concentrations, photosynthetic profiles showed Poso's surface waters (0-10 m) supersaturated with oxygen, and Secchi depth (9.5 m) was the lowest of the four lakes. This supports the conclusion that Poso is more productive than the Malili lakes, and there is evidence to support physical and chemical factors as regulating this difference in biomass and composition.

Mixing processes in Lake Matano constitute a physical factor that likely contributes to the low phytoplankton biomass observed, and may also play a role in regulating the species composition. Temperature profiles were constructed to understand mixing depths in the four lakes. It is known that temperature has only an indirect effect on composition via regulating water column structure and stability, as well as seasonality (Reynolds et al., 2000). In Lake Matano, temperature may be affecting the abundance of phytoplankton, as the density change between the epilimnion and hypolimnion of Lake Matano was lower (5.7×10^{-4} g/cm^3) than that of Lake Poso (7.1×10^{-4} g/cm^3), meaning Matano is more likely to mix (in the upper 40 m). Such vertical mixing has been previously reported as a factor limiting autotrophic production in this system (Haffner et al., 2001). This mixing process continuously moves phytoplankton out of the euphotic zone, thus limiting photosynthesis and biomass, and has the potential to affect composition; for example, N-fixing cyanobacteria that are absent from Lake Matano (Sabo, 2006) prefer a stable water column (Cole, 1983). Matano's ratio of Z_{eu} : Z_m (euphotic to mixing depth) is 42%,

compared with 95% in Lake Poso; this lower ratio in Matano indicates less photosynthetic activity can be supported as such it relates to lower phytoplankton biomass (Talling, 1971; Cole, 1983). In addition, Matano is a meromictic lake, so no mixing occurs below 100 m. Nutrients or phytoplankton that settle below this layer are not re-introduced to the surface waters. Given these conditions, mixing processes in Lake Matano somewhat affect the composition and abundance in the phytoplankton community.

The pattern of decreasing conductivity in downstream Malili lakes (opposite of the River Continuum hypothesis), supports previous reports that these lakes receive water from different sources (Haffner et al., 2001); Lake Matano receives surface runoff as well as underground water inputs which contribute to its higher ionic content (~180 μS/cm), while the other lakes predominantly receive surface runoff. Lake Poso's conductivity (~120 μS/cm) was lower than that of Towuti (~145 μS/cm), suggesting this lake also predominantly receives surface runoff. Inputs of water from different sources may contribute to the differences in elemental concentrations between Matano and Poso, and in turn, relate to the differences in phytoplankton biomasses and abundances.

A Kruskal-Wallis One-way Analysis of Variance test (Systat 12 for Windows) identified P, Na, K, Cr, Fe, Ni, Zn, and Mo as significantly ($p < 0.05$) different among the lakes. Considering the mean concentrations, the geological setting, and biological vs statistical significant, it is highly unlikely that Fe, Ni, or Zn play a role in regulating

the phytoplankton composition in the Malili Lakes. Arguments for P, Na, K, Cr, and Mo are discussed.

As indicated by the nutrient addition study in Chapter 2, P is not a major limiting nutrient for the phytoplankton community of Lake Matano. This conclusion was further supported by a higher concentration of P, analyzed by ICP-MS, in Matano (3.8 μmol/L) than in Poso (6.3×10^{-2} μmol/L). A limitation of elemental analysis by ICP-MS is that chemical speciation is not known, so the ICP-MS results may be contradictory to the statistical analysis that identified no significant difference ($p = 0.3$) between TP concentrations of the two lakes (0.19 ± 0.07 μmol/L for Matano, 0.27 μmol/L for Poso). However, given that Poso is more productive than Matano, one would expect that if the macronutrient P were limiting, the concentration would be significantly ($p < 0.05$) lower in Matano. Both sets of results strengthen the conclusions of Chapter 2 and provide further evidence that P is not directly limiting phytoplankton growth and abundance in Lake Matano.

Na concentrations were within a close range for all four lakes (from $2.7 \times 10^{1} \pm 8.6 \times 10^{-1}$ μmol/L for Towuti to $5.3 \times 10^{1} \pm 5.2$ μmol/L for Matano). Only some algae require Na, and when so, approximately 265 μmol/L is required (Healey, 1973); it is not clear which of the genera extant in these lakes require this micronutrient. Concentrations are such that the Malili lakes may be Na-limited, but given that Na is typically an abundant metal in lakes, is very soluble and tends to remain in solution when leached from rocks (Cole, 1983), Na is not deemed to be a major factor

regulating the phytoplankton community.

Concentrations of K were significantly higher in Poso ($1.4 \times 10^{1} \pm 1.0 \times 10^{-1}$ µmol/L) than in Matano ($3.8 \pm 1.7 \times 10^{-1}$ µmol/L; $p = 0.011$) and Towuti ($2.9 \pm 4.2 \times 10^{-1}$ µmol/L; $p = 0.034$). Poso was one order higher in K concentration than these lakes, and still lower than what is required by phytoplankton, according to Healey, 442.5 µmol/L (Healey, 1973). In addition, K was an element that separated the lakes in the PCO constructed from the linking of datasets for chemical differences and phytoplankton composition differences. K concentration was strongly related to the abundance of *Chlamydomonas,* the second most abundant genera, and *Staurastrum,* a relatively large-celled genus, in Lake Poso. This evidence suggests K as a possible limiting macronutrient in Lake Matano. However, K is usually the fourth ranking cation in lake water (Cole, 1983), and its potential to affect composition is limited, since it is required by all genera of phytoplankton. K may be present in limiting concentrations, but it is not a primary driver regulating the composition and abundance in Lake Matano.

Canadian Water Quality Guidelines indicate that reductions in algal growth can occur at concentrations of Cr above 1.2×10^{-1} µmol/L (Pawlisz et al., 1997), and Matano at 0 m contained a greater Cr concentration, 1.4×10^{-1} µmol/L. This measurement is consistent with previous reports of Lake Matano's epilimnion containing high levels of Cr (VI), at 1.8×10^{-1} µmol/L (Crowe et al., 2007), and logical since Cr is one of the main elements contained in the ophiolite rocks in the Malili

Lakes' catchment basin (Villeneuve et al., 2002). Research has shown concentrations as low as 2.0×10^{-2} μmol/L Cr (VI) can significantly inhibit the growth of phytoplankton (Wallen, 1996), and attempts to reduce Cr in Matano water caused higher biomasses and changes in relative abundance (Sabo, 2006; Bramburger, unpubl. data). There was a statistically significant difference between Cr concentrations in Matano and Poso ($p = 0.028$), and in order of decreasing Cr concentration: Matano > Mahalona > Towuti > Poso. Cr toxicity may affect the phytoplankton composition in these lakes by preventing colonization by cosmopolitan groups, and reducing the abundance of bacillariophytes, such as Centrales (centric diatoms). These diatoms have been observed in Lake Poso but not in the Malili Lakes (Bramburger, pers. comm.), and there is research to support that Cr tolerance varies among species with bacillariophyte biomass having the greatest decline with Cr additions (Wallen, 1996). This also supports previous conclusions that Cr toxicity affects composition at higher trophic levels, such as the absence of cladocerans from Lake Matano (Fernando, 1987). This evidence strongly supports the argument that Cr concentrations are inhibiting the composition and abundance of phytoplankton in the Malili Lakes system, and supports the conclusion by Crowe et al. (2007) that watershed runoff influences the primary production and composition in Lake Matano.

In recent research on micronutrient-limited lakes, Mo is frequently listed as an important co-factor in enzymes crucial to N-fixation, photosynthesis and respiration (Morel et al., 1994; Goldman, 1972; Axler et al., 1980; Cole, 1983; Twiss et al., 2000).

The Mo concentrations in the four lakes were significantly different ($p = 0.022$ to 0.049): in order of decreasing concentrations, Poso > Matano > Mahalona > Towuti. Although these concentrations were low ($5.0 \times 10^{-4} \pm 3.3 \times 10^{-5}$ µmol/L for Matano, $8.5 \times 10^{-4} \pm 3.5 \times 10^{-5}$ µmol/L for Poso), they are different enough to elicit a micronutrient limitation in Matano. Comparing the mean Mo concentrations in the upper waters, Poso contained a 60% higher Mo concentration than Matano. One experiment of individual additions of Mo at 10 times the ambient concentration increased primary productivity by 38% ($p < 0.001$) over the controls (Downs et al., 2008). Considering this example, the difference in Mo concentrations between lakes Poso and Matano is both statistically and biologically significant. Nutrient limitation by Mo could prevent colonization by cosmopolitan species, and species adapted to the low Mo concentration may be suffering limitations to their growth and abundance.

3.5 Conclusions

The phytoplankton composition and abundance in the Malili Lakes is primarily driven by the concentrations of Cr and Mo in the lakes. Concentrations of Cr were significantly higher in Lake Matano than in Lake Poso, and are above the Canadian Water Quality Guidelines to reduce algal growth (Pawlisz et al., 1997). This is consistent with previous Cr measurements in Matano (Crowe et al., 2007). Cr toxicity has the potential to regulate both phytoplankton composition and abundance as it reduces the growth potential of species with Cr tolerance and inhibits the colonization of species that are not tolerant to high Cr concentrations.

Mo concentrations were significantly different among the four lakes and although low in each lake, the concentration difference between Matano and Poso is enough to pose a nutrient limitation in Lake Matano. Mo is required by all phytoplankton and has the potential to limit both composition and abundance. As a limiting micronutrient, it suppresses the growth of species tolerant to low Mo concentrations, and it prevents colonization by cosmopolitan species that are not adapted to such concentrations.

Na and K may be other limiting nutrients, but their effect is not as strong as that of Cr and Mo. The measured Na and K concentrations in the Malili Lakes were significantly ($p < 0.05$) different among the lakes and lower than those required by some phytoplankton (Healey, 1973). However, given that these nutrients are typically very abundant in freshwaters (Cole, 1983), it is not likely that they are the primary driving factors limiting the phytoplankton community in this system.

These differences in elemental concentrations may be due to the lakes receiving water inputs from different sources. The conductivity profiles suggest Lake Matano primarily receives underground water inputs, while the other lakes receive surface runoff.

The low phytoplankton abundance observed in Lake Matano is due, in part, to the extensive vertical mixing in the upper waters. This process limits photosynthesis by moving phytoplankton from the euphotic zone. Mixing may also affect composition

by providing unfavourable water conditions for N-fixing cyanobacteria (Cole, 1983), which were not observed in Lake Matano in this or previous studies (Sabo, 2006).

Water column elemental data, as well as phytoplankton biomass and composition data for lakes Matano and Mahalona support the hypothesis that lakes with similar physical and chemical conditions will support similar phytoplankton assemblages.

3.6 References

Anderson, M.J. and Robinson, J. (2003) Generalized discriminant analysis based on distances. *Austral. New Zeal. J. Stat.*, **45**, 301-318.

Anderson, M.J. and T.J. Willis (2003) Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. *Ecol.*, **84**, 511-525.

Axler, R.P.; Gersberg, R.M.; and Goldman, C.R. (1980) Stimulation of nitrate uptake and photosynthesis by molybdenum in Castle Lake, California. *Can. J. Fish. Aquat. Sci.,* **37**, 707-712.

Cole, G. (1983) Textbook of limnology, 3rd ed. Waveland Press, Illinois. 4-332.

Cranston, R.E.; Murray, R.W. (1978) The determination of chromium species in natural seawater. *Anal. Chim. Acta.,* **99**, 275-282.

Crowe, S.A.; Pannalal, S.J.; Fowle, D.A.; Cioppa, M.T.; Symons, D.T.A.; Haffner, G.D.; Fryer, B.J.; McNeely, R.; Sundby, B.; Hehanussa, P.E. (2004) Biogeochemical cycling in Fe-rich sediments from Lake Matano, Indonesia, p. 1185-1189, 11th International symposium on water-rock interaction. A.A. Balkema.

Crowe, S.A.; O'Neill, A.H.; Katsev, S.; Hehanussa, P.; Haffner, G.D.; Sundby, B.; Mucci, A.; Fowle, D. (2007) The biogeochemistry of tropical lakes: a case study from Lake Matano, Indonesia. *Limnol. Oceanogr.*, **53**, 319-331.

Downs, T.M.; Schallenberg, M. and Burns, C.W. (2008) Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. *Aquat. Sci.,* **70**, 347-360.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E. (eds.) (1995) 'Ascorbic Acid Method for TP Analysis' In: Standard Methods for the Examination of Water and Wastewater, 19th ed., 4.113-4.114.

Eccles, D.H. (1988) A quarter century of great lakes research in Africa. *J. Limnol. Soc. S. Africa,* **14**, 41-48.

Fernando, C.H. (1987) Tropical freshwater zooplankton with special reference to South East Asia (Oriental Region) *Rep. Nat. Geogr. Soc.,* **87,** 287-311.

Goldman, C.R. (1972) The role of minor nutrients in limiting the productivity of aquatic ecosystems. In: G.E. Likens (ed.), Proceedings of the symposium on nutrients and eutrophication: the limiting-nutrient controversy. *Am. Soc. Lim. Oceanogr.*, 21-23.

Goldman, C.R. (1960) Molybdenum as a factor limiting primary productivity in Castle Lake, California. *Science*, **132**, 1016-1017.

Golightly, J.P. (1981) Nickeliferous laterite deposits. *Econ. Geol.,* **75th Anniv. Vol**., 710-735.

Gordon D.N., T.A. McMahon, B.L. Finlayson, C.J. Gippel, and R.J. Nathan (2004) Stream Hydrology - An Introduction for Ecologiste. John Wiley & Sons, Chichester, W Suss.

Haffner, G.D.; Hehanussa, P.E. and Hartoto, D. (2001) "The biology and physical processes of large lakes of Indonesia" In: Munawar, M. and Hecky, R.E. (eds). *The Great Lakes of the World: Food-web, health, and integrity.* Backhuys Publishers, Leiden, the Netherlands, 183-194.

Healey, F.P. (1973) Inorganic nutrient update and deficiency in algae. *Crit. Rev. Microbiol.,* **3**, 69-113.

Hecky, R.E. and Kilham, P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. *Lim. and Ocean*., **33**, 796-822.

Kugrens, P. and Clay, B.L. (2003) Cryptomonads. In Wehr, J.D. and Sheath, R.G. (eds), Freshwater Algae of North American Ecology and Classification. Academic Press, Amsterdam, 715-755.

Lehmusluoto, P.; Machbub, B.; Terangna, N.; Achmad, F.; Boer, L.; Sembiring, S.; Setiadji, B.; Priadie, B.; Herawan, K.; and Goeltenboth, F. (1997) "Limnology in Indonesia: From the legacy of the past to the prospects for the future" In: Wetzel, R. and Gopal, B (eds.) *Limnology in Developing Countries*, Vol. 2. International Scientific Publication, New Delhi, India, pp. 119-234.

Leibold, M.A. (1999) Biodiversity and nutrient enrichment in pond plankton communities. Evolutionary Ecology Research. **1**: 73-95.

Lewis, W.M. Jr. (1978) A compositional, phytogeographical and elementary structural analysis of the phytoplankton in a tropical lake: Lake Lanao, Philippines. *J. Ecol.,* **66**, 213-226.

Lewis, W.M. Jr. (2000) Basis for the protection and management of tropical lakes. *Lakes Reserv.: Res. Manage*., **5**, 35-48.

Looman, J. and Campbell, J.B. (1960) Adaptation of Sørensen's K for estimating unit affinities in prairie vegetation. *Ecology*, **41**, 409-416.

Morel, F.M.M.; Reinfelder, J.R.; Roberts, S.B.; Chamberlain, C.P.; Lee, J.G.; and Yee, D. (1994) Zinc and carbon co-limitation of marine phytoplankton. *Nature*, **369**, 740-742.

Nicholls, K.H. and Wujek, D.E. (2003) Crysophycean algae. In Wehr, J.D. and Sheath, R.G. (eds), Freshwater Algae of North American Ecology and Classification. Academic Press, Amsterdam, 471-521.

O'Sullivan, P.E. and Reynolds, C.S. (eds) (2004) *The Lakes Handbook*. Vol.1. Blackwell Publishing, Oxford.

Pawlisz, A.V.; Kent, R.A.; Schneider, U.A.; Jefferson, C. (1997) Canadian water quality guidelines for chromium. *Enviro. Tox. Wat. Qual.,* **12**, 123-183.

Prescott, G.W.; Bamrick, J.; Cawley, E.T.; Jaques, G. (1954) *How to know the freshwater algae*. W.C. Brown Company, Dubuque, Iowa. pp. 298-312.

Riedel, G.T. (1984) Influence of salinity and sulphate on the toxicity of chromium (VI) to the estuarine diatom *Thalassiosira pseudonana*. *J. Phycol.,* **20**, 496-500.

Reynolds, C.S., Reynolds, S.N., Munawar, I., and Munawar, M. (2000) The regulation of phytoplankton population dynamics in the world's largest lakes. *Aquat. Ecosys. Health Manage.,* **3**, 1-21.

Sabo, E. (2006) *Characterization of the Pelagic Plankton Assemblage of Lake Matano and Determination of Factors Regulating Primary and Secondary Production Dynamics.* Thesis, University of Windsor, Windsor, Ontario.

Skoog, D.A. and Leary, J. (1994) *Principles of Instrumental Analysis.*4th ed. Saunders College Publishing, Fort Worth.

Talling, J.F. (1971) The underwater light climate as a controlling factor in the production ecology of freshwater phytoplankton. *Mitt. Int. Ver. Theor. Angew. Limno*. **19**, 214-243.

Tilman, D., Kilham, S.S., and Kilham, P. (1982) Phytoplankton community ecology: the role of limiting nutrients. *Annu. Rev. Ecol. Syst.,* **13**,349-372.

Twiss, M.R.; Auclair, J.-C.; and Charlton, M.N. (2000) An investigation into iron-stimulated phytoplankton productivity in epipelagic Lake Erie during thermal stratification using trace metal clean techniques. *Can. J. Fish. Aquat. Sci.,* **57**, 86-95.

Villeneuve, M.; Gunawan, W.; Cornee, J.J. and Vidal, O. (2002) Geology of the central Sulawesi Belt (eastern Indonesia): constraints for geodynamic models. *Int. J. Earth Sci*., **91**, 524-537.

Wallen, D.G. (1996) Adaptation of the growth of the diatom *Fragilaria crotonensis* (Kitton) and the phytoplankton assemblage of Lake Erie to chromium toxicity. *J. Great Lakes. Res*., **22**, 55-62.

Wetzel, R.G. (2001) Limnology, 3rd ed. American Press, San Diego.

Wilson, M.E.J. and Moss, S.J. (1999) Cenozoic paleogeographic evolution of Sulawesi and Borneo. *Paleogeogr. Paleoclimatol. Paleoecol.* **145**, 303-337.

Table 3.1 Detection limits for ICP-MS elemental analysis and mean concentrations for the upper waters of lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO) in µmol/L. Standard errors are shown.

Element	Detection Limit (µmol/L)	Mean Concentration (µmol/L) for upper waters (100 m MAT, TOW, POSO); 60 m MAH)			
		MAT	MAH	TOW	POSO
Na	5.1×10^{-1}	$5.3 \times 10^{1} \pm 5.2$	$4.5 \times 10^{1} \pm 6.2 \times 10^{-1}$	$2.7 \times 10^{1} \pm 8.6 \times 10^{-1}$	$3.1 \times 10^{1} \pm 4.3 \times 10^{-2}$
P	8.7×10^{-2}	$3.8 \pm 8.8 \times 10^{-1}$	$5.5 \times 10^{-1} \pm 4.8 \times 10^{-3}$	$5.2 \times 10^{-1} \pm 2.1 \times 10^{-2}$	$6.3 \times 10^{-2} \pm 8.7 \times 10^{-3}$
K	3.6×10^{-2}	$3.8 \pm 1.7 \times 10^{-1}$	$3.7 \pm 6.6 \times 10^{-2}$	$2.9 \pm 4.2 \times 10^{-2}$	$1.4 \times 10^{1} \pm 1.0 \times 10^{-1}$
Cr	1.9×10^{-3}	$1.0 \times 10^{-1} \pm 1.1 \times 10^{-2}$	$1.0 \times 10^{-1} \pm 4.3 \times 10^{-3}$	$8.9 \times 10^{-2} \pm 5.7 \times 10^{-3}$	$2.3 \times 10^{-2} \pm 2.3 \times 10^{-3}$
Mn	2.0×10^{-4}	$7.7 \times 10^{-2} \pm 7.1 \times 10^{-3}$	$9.5 \times 10^{-2} \pm 1.9 \times 10^{-3}$	$1.6 \times 10^{-1} \pm 7.2 \times 10^{-2}$	$6.9 \times 10^{-2} \pm 4.8 \times 10^{-3}$
Fe	2.0×10^{-4}	$4.9 \times 10^{-1} \pm 1.8 \times 10^{-2}$	$3.7 \times 10^{-1} \pm 1.8 \times 10^{-4}$	$1.3 \times 10^{-1} \pm 1.5 \times 10^{-3}$	$8.8 \times 10^{-1} \pm 1.4 \times 10^{-1}$
Co	2.0×10^{-4}	$1.8 \times 10^{-3} \pm 3.1 \times 10^{-4}$	$9.0 \times 10^{-4} \pm 7.1 \times 10^{-5}$	$5.7 \times 10^{-4} \pm 3.8 \times 10^{-5}$	$9.9 \times 10^{-3} \pm 7.0 \times 10^{-3}$
Ni	1.7×10^{-3}	$4.3 \times 10^{-2} \pm 6.9 \times 10^{-3}$	$8.3 \times 10^{-2} \pm 3.2 \times 10^{-3}$	$4.2 \times 10^{-2} \pm 9.5 \times 10^{-4}$	$6.1 \times 10^{-2} \pm 3.7 \times 10^{-2}$
Cu	8.0×10^{-4}	$9.7 \times 10^{-3} \pm 8.9 \times 10^{-4}$	$4.1 \times 10^{-3} \pm 2.1 \times 10^{-4}$	$4.1 \times 10^{-3} \pm 2.6 \times 10^{-4}$	$7.2 \times 10^{-3} \pm 6.8 \times 10^{-4}$
Zn	1.5×10^{-2}	$1.1 \times 10^{-1} \pm 2.4 \times 10^{-2}$	$2.8 \times 10^{-2} \pm 1.7 \times 10^{-3}$	$2.5 \times 10^{-2} \pm 1.1 \times 10^{-3}$	$2.9 \times 10^{-2} \pm 2.0 \times 10^{-4}$
Mo	1.0×10^{-5}	$5.0 \times 10^{-4} \pm 3.3 \times 10^{-5}$	$4.0 \times 10^{-4} \pm 0$	$2.7 \times 10^{-4} \pm 1.9 \times 10^{-5}$	$8.5 \times 10^{-4} \pm 3.5 \times 10^{-5}$

Table 3.2 Overview of production and diversity indices for three Malili Lakes and Lake Poso, March 2008. Standard errors for biomasses are listed when possible.

Sample	Biomass (µg/L)	# Individuals / mL	Shannon Diversity Index (0-4.6)	Species Richness	Evenness (0-1)
Matano					
0 m	4.52×10^4	36	1.58	10	0.68
50 m	3.24×10^3	33	1.56	10	0.68
100m	1.07×10^2	21	0.92	6	0.51
Mean	$1.62 \times 10^4 \pm 8.39 \times 10^3$	30	1.35	9	0.62
Mahalona					
0m	4.47×10^2	45	0.87	5	0.54
30m	1.05×10^2	9	1.32	3	0.95
60m	2.9×10^3	15	1.00	6	0.56
Mean	$1.15 \times 10^4 \pm 5.08 \times 10^3$	23	1.06	5	0.68
Towuti					
0m	2.83×10^3	9	0.96	4	0.69
50m	8.36×10^1	7	0.96	3	0.87
100m	4.31×10^1	5	0.94	3	0.87
Mean	$9.86 \times 10^2 \pm 5.32 \times 10^2$	7	0.95	3	0.81
Poso					
0 m	1.28×10^5	61	1.09	6	0.56

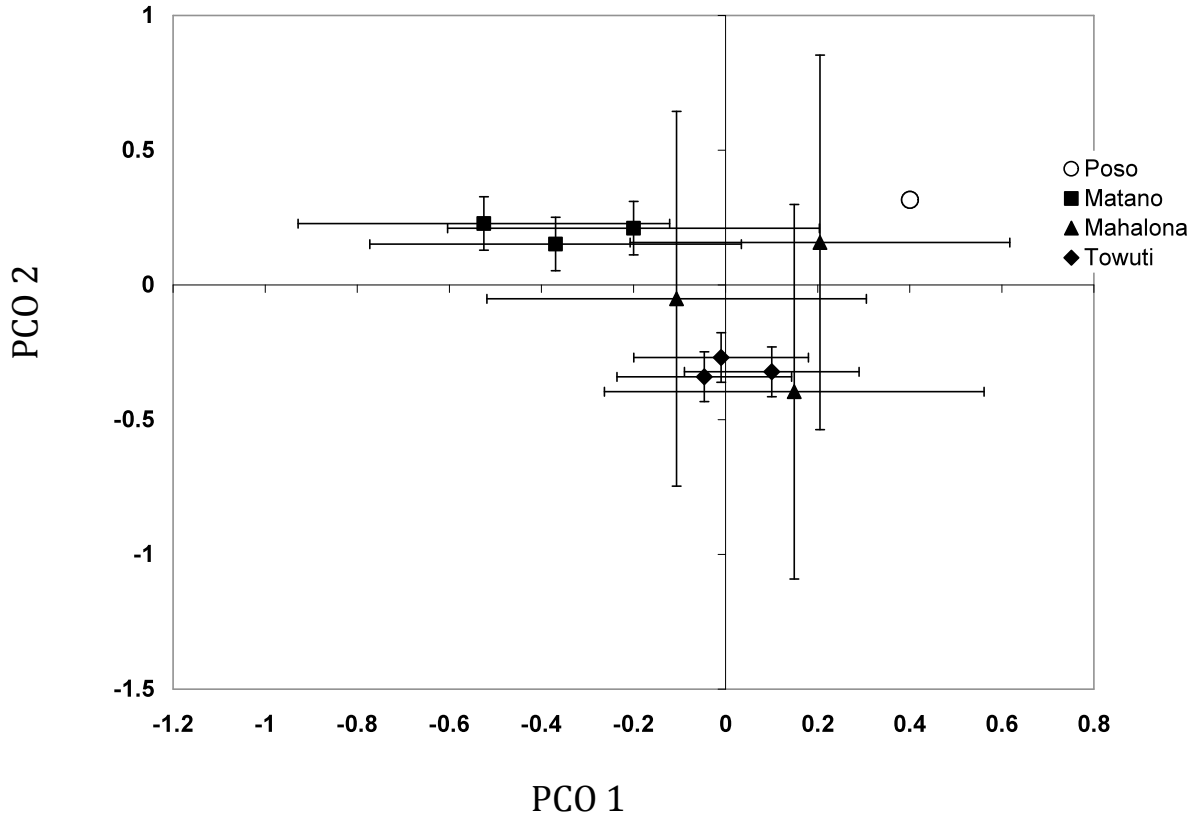

Figure 3.1 Principal Coordinates Analysis (PCO) of the four lakes based on phytoplankton composition. X and Y error bars show 95% confidence intervals. Only one sample was available for Lake Poso, thus confidence intervals could not be calculated. This figure shows the separation of the three Malili lakes and Poso based on phytoplankton composition.

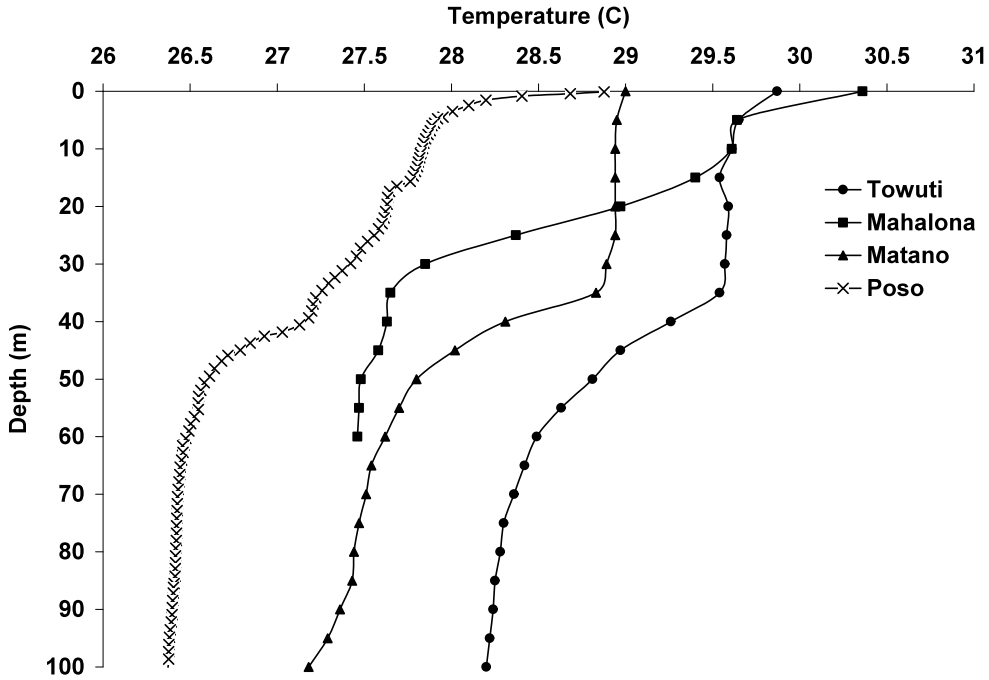

Figure 3.2 Temperature profiles for lakes Matano, Mahalona, Towuti, and Poso

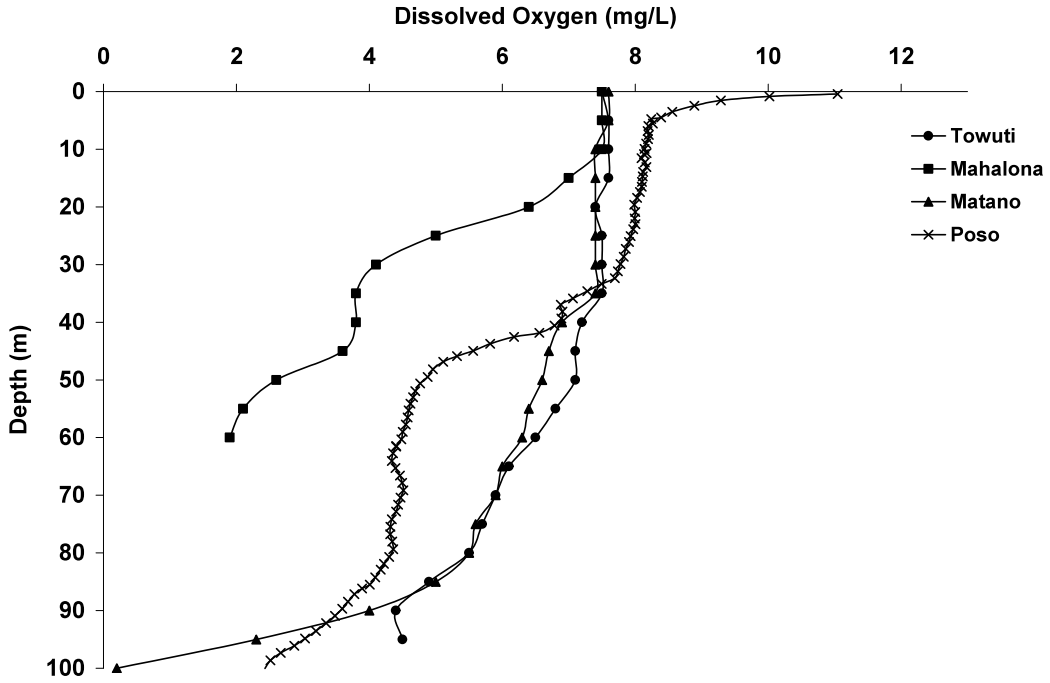

Figure 3.3 Dissolved oxygen profiles for lakes Matano, Mahalona, Towuti, and Poso

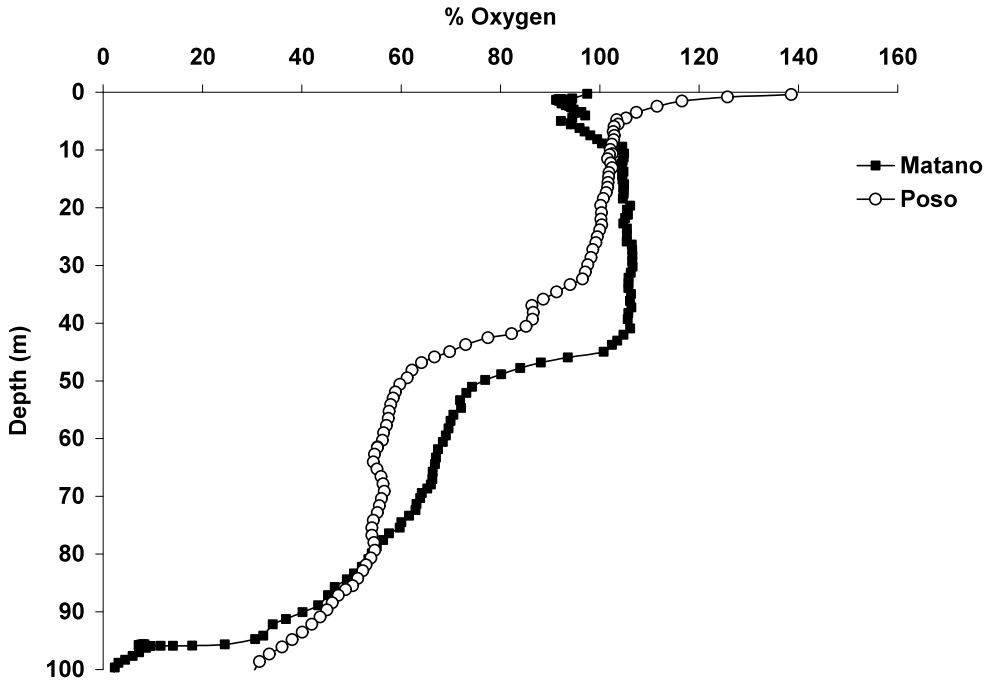

Figure 3.4 Photosynthetic oxygen profiles for lakes Matano and Poso

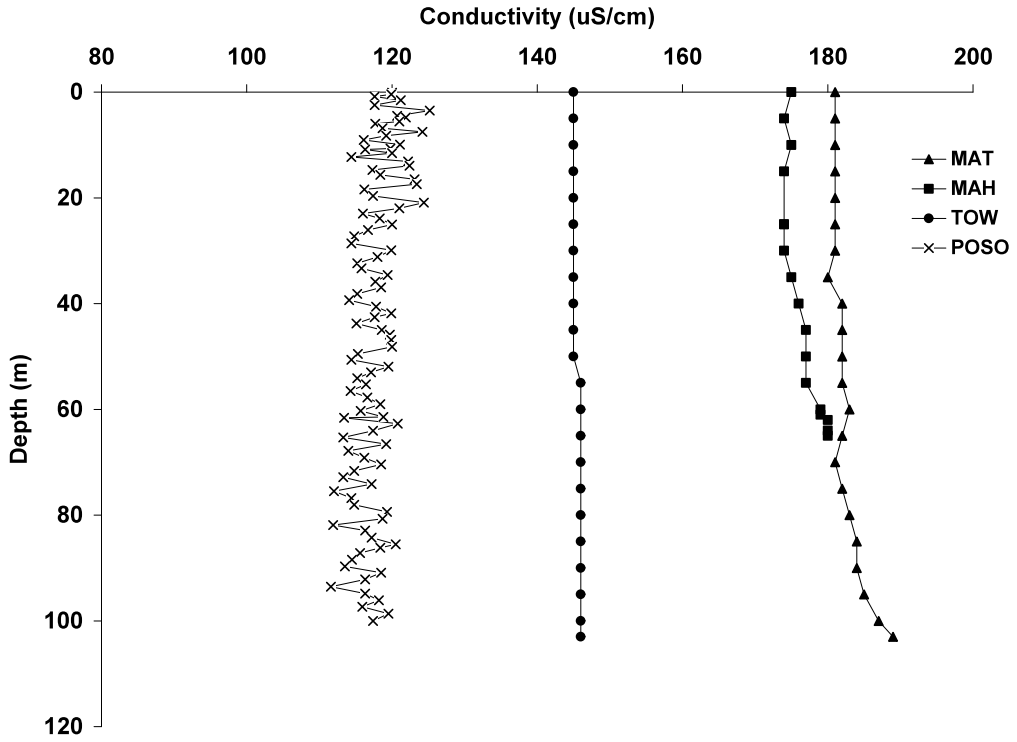

Figure 3.5 Conductivity profiles for lakes Matano, Mahalona, Towuti, and Poso

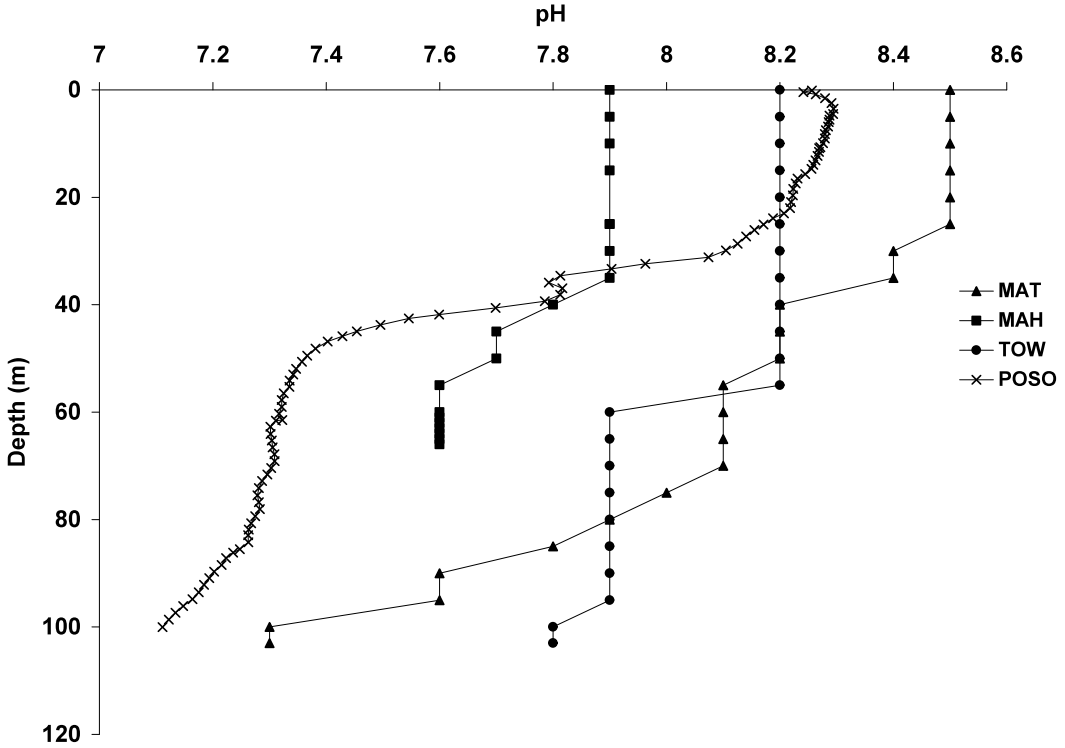

Figure 3.6 pH profiles for lakes Matano, Mahalona, Towuti, and Poso

Table 3.3 Nutrient data (µmol/L) of nitrogen as NH_3 and NO_3/NO_2 and phosphorus as SRP and TP for lakes Matano, Mahalona, Towuti, and Poso. Means and standard errors for Lake Matano were calculated from samples collected in July 1999, January 2000, and August 2000; Lake Mahalona samples were collected in February 1997; Lake Towuti samples were collected in February 2002; Lake Poso samples were collected in March 2008.

Lake	Depth (m)	NH_3 (µmol/L)	NO_3/NO_2 (µmol/L)	SRP (µmol/L)	TP (µmol/L)
Matano	0	0.29 ± 0.11	0.53 ± 0.30	0.16 ± 0.06	0.13 ± 0.03
	50	0.71 ± 0.35	0.11 ± 0.02	0.48 ± 0.41	0.32 ± 0.16
	100	0.47 ± 0.23	0.81 ± 0.66	0.12 ± 0.03	0.13 ± 0.03
	150	12.71 ± 5.06	0.19 ± 0.12	0.26 ± 0.06	0.19 ± 0.10
	200	60.27 ± 23.95	1.09 ± 0.96	1.55 ± 0.52	2.23 ± 1.00
	300	44.79 ± 9.89	0.11 ± 0.04	2.29 ± 1.22	1.26 ± 0.55
	400	69.69 ± 26.66	0.11 ± 0.04	1.32 ± 0.68	1.81 ± 0.74
Mahalona	0	0.18	0.21	0.06	0.19
	30	0.18	1.93	0.06	0.16
	55	0.18	1.28	0.06	0.16
Towuti	0	1.65	0.06	0.06	0.19
	50	0.59	0.06	0.06	0.16
	100	0.18	0.86	0.10	0.16
	190	0.18	1.50	0.39	0.16
Poso	0	na	na	na	0.19
	100	na	na	na	0.35
	300	na	na	na	0.87

Table 3.4 ICP-MS elemental concentrations (µmol/L) for lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO). Detection limits for each element and mean concentrations for the upper waters of each lake are listed in Table 3.1.

Lake	Depth (m)	Na	P	K	Cr	Mn	Fe	Co	Ni	Cu	Zn	Mo
MAT	0	7.1×10^{1}	6.8	4.3	1.4×10^{-1}	1.0×10^{-1}	5.5×10^{-1}	2.9×10^{-3}	6.7×10^{-2}	1.2×10^{-2}	2.0×10^{-1}	6.0×10^{-4}
	50	3.6×10^{1}	2.2	3.5	9.0×10^{-2}	6.8×10^{-2}	4.7×10^{-1}	1.2×10^{-3}	3.3×10^{-2}	6.8×10^{-3}	5.9×10^{-2}	5.0×10^{-4}
	100	4.4×10^{1}	2.4	3.4	7.9×10^{-2}	6.1×10^{-2}	4.5×10^{-1}	1.4×10^{-3}	3.0×10^{-2}	1.0×10^{-2}	8.2×10^{-2}	4.0×10^{-4}
	105	4.4×10^{1}	1.8	3.7	8.9×10^{-2}	8.2×10^{-2}	4.7×10^{-1}	7.0×10^{-4}	3.2×10^{-2}	3.9×10^{-3}	7.3×10^{-2}	4.0×10^{-4}
	110	4.2×10^{1}	1.8	3.5	8.4×10^{-2}	8.3×10^{-2}	4.5×10^{-1}	7.0×10^{-4}	3.5×10^{-2}	5.0×10^{-3}	4.5×10^{-2}	4.0×10^{-4}
	150	6.6×10^{1}	4.6	5.2	4.2×10^{-2}	1.5×10^{1}	1.6×10^{1}	2.7×10^{-2}	2.6×10^{-2}	4.6×10^{-3}	1.3×10^{-1}	3.0×10^{-4}
	200	8.1×10^{1}	6.8	6.1	2.9×10^{-2}	1.4×10^{1}	2.4×10^{1}	4.3×10^{-2}	2.5×10^{-2}	4.7×10^{-3}	1.5×10^{-1}	7.0×10^{-4}
	300	4.2×10^{1}	1.6	3.7	6.9×10^{-2}	7.7×10^{-1}	1.4	2.0×10^{-3}	2.7×10^{-2}	2.5×10^{-3}	4.9×10^{-2}	3.0×10^{-4}
	400	8.7×10^{1}	8.8	6.6	2.5×10^{-2}	1.2×10^{1}	2.9×10^{1}	3.5×10^{-2}	2.9×10^{-2}	5.5×10^{3}	1.7×10^{-1}	5.0×10^{-4}
	500	8.4×10^{1}	8.1	9.2	2.3×10^{-2}	1.3×10^{1}	2.5×10^{1}	4.3×10^{-2}	2.7×10^{-2}	5.2×10^{-3}	1.6×10^{-1}	3.0×10^{-4}
MAH	0	4.4×10^{1}	5.5×10^{-1}	3.6	9.8×10^{-2}	9.3×10^{-2}	3.7×10^{-1}	1.0×10^{-3}	8.8×10^{-2}	3.8×10^{-3}	2.6×10^{-2}	4.0×10^{-4}
	30	4.6×10^{1}	5.4×10^{-1}	3.8	1.1×10^{-1}	9.8×10^{-2}	3.7×10^{-1}	8.0×10^{-4}	7.9×10^{-2}	4.4×10^{-3}	3.1×10^{-2}	4.0×10^{-4}
	60	4.4×10^{1}	5.8×10^{-1}	3.7	1.2×10^{-1}	8.0×10^{-2}	3.5×10^{-1}	5.0×10^{-4}	6.6×10^{-2}	4.6×10^{-3}	3.4×10^{-2}	4.0×10^{-4}
TOW	0	2.7×10^{1}	4.6×10^{-1}	2.9	7.3×10^{-2}	3.2×10^{-2}	1.4×10^{-1}	7.0×10^{-4}	4.4×10^{-2}	3.5×10^{-3}	2.2×10^{-2}	3.0×10^{-4}
	50	2.6×10^{1}	5.3×10^{-1}	2.8	8.6×10^{-2}	3.0×10^{-2}	1.3×10^{-1}	5.0×10^{-4}	4.2×10^{-2}	3.8×10^{3}	2.8×10^{-2}	2.0×10^{-4}
	100	2.7×10^{1}	5.8×10^{-1}	3.0	1.1×10^{-1}	4.0×10^{-1}	1.3×10^{-1}	5.0×10^{-4}	3.8×10^{-2}	5.0×10^{-3}	2.5×10^{-2}	3.0×10^{-4}
	200	2.7×10^{1}	6.2×10^{-1}	2.8	1.8×10^{-1}	4.8×10^{-1}	7.4×10^{-1}	6.1×10^{-3}	1.3×10^{-1}	7.1×10^{-3}	2.7×10^{-2}	3.0×10^{-4}
POSO	0	3.1×10^{1}	6.2×10^{-1}	1.4×10^{1}	1.9×10^{-2}	6.2×10^{-2}	6.9×10^{-1}	0.0	8.9×10^{-3}	8.5×10^{-3}	2.9×10^{-2}	9.0×10^{-4}
	100	3.2×10^{1}	6.4×10^{-1}	1.4×10^{1}	2.6×10^{-2}	7.6×10^{-2}	1.1	2.0×10^{-2}	1.1×10^{-1}	5.8×10^{-3}	2.8×10^{-2}	8.0×10^{-4}
	300	3.2×10^{1}	3.3	1.4×10^{1}	1.6×10^{-2}	8.5	5.9	4.8×10^{-3}	1.4×10^{-2}	2.2×10^{-2}	1.0×10^{-1}	7.0×10^{-4}

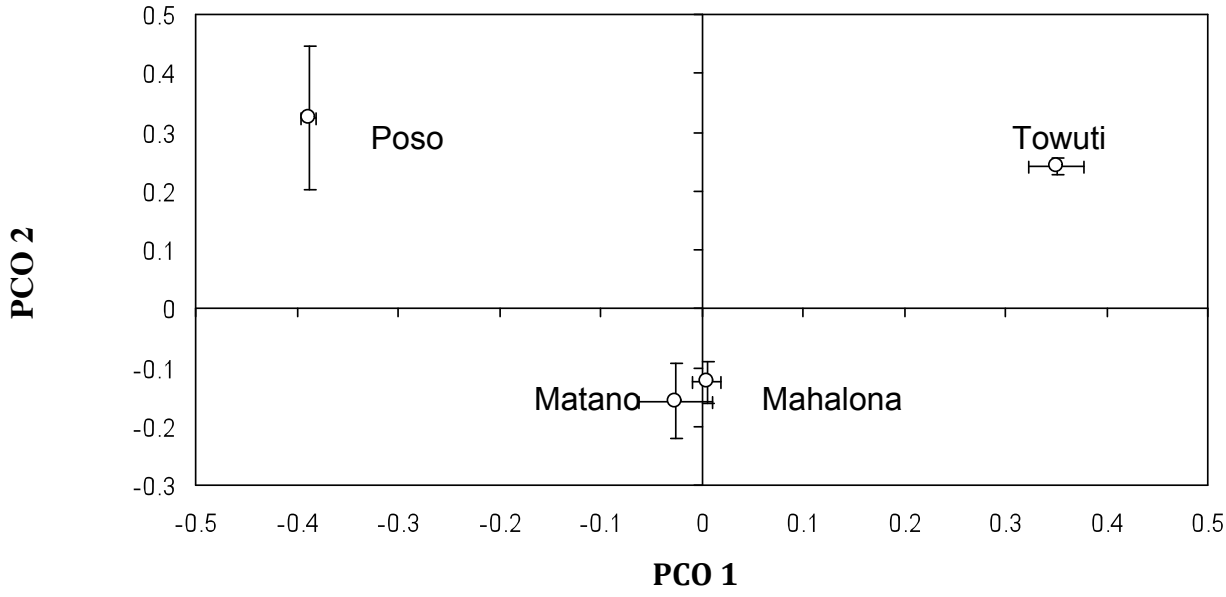

Figure 3.7 Principal Coordinates Analysis (PCO) based on ICP-MS elemental analysis. Samples were collected in March 2008. 95% confidence level error bars are shown. PCO 1 accounts for 77% of the variation in the dataset and is negatively correlated with K and Mo and positively correlated with Cr. PCO 2 accounts for 17% of the variation and is negatively associated with Na and P.

Table 3.5 Analysis of ICP-MS elemental data for lakes Matano (MAT), Mahalona (MAH), Towuti (TOW), and Poso (POSO) among the upper waters (100 m for MAT, TOW, POSO and 30 m for MAH). Pairwise comparisons were performed (Mann-Whitney U-test) between lakes for those elements with significantly different concentrations (Kruskal-Wallis test, $p < 0.05$). Significant results and mean concentrations (µmol/L) for the upper waters of each lake are listed.

Element	Lakes		p-value	Mann-Whitney U-test statistic (z)
Na	MAT ($5.3 \times 10^{1} \pm 5.2$)	TOW ($2.7 \times 10^{1} \pm 8.6 \times 10^{-1}$)	0.005	12.0
	MAT	POSO ($3.1 \times 10^{1} \pm 4.3 \times 10^{-2}$)	0.011	30.0
	TOW	POSO	0.034	12.0
	MAH ($4.5 \times 10^{1} \pm 6.2 \times 10^{-1}$)	TOW	0.034	12.0
P	MAT ($3.8 \pm 8.8 \times 10^{-1}$)	MAH ($5.5 \times 10^{-1} \pm 4.8 \times 10^{-3}$)	0.011	0.0
	MAT	TOW ($5.2 \times 10^{-1} \pm 2.1 \times 10^{-2}$)	0.005	40.0
	TOW	POSO ($6.3 \times 10^{-2} \pm 8.7 \times 10^{-3}$)	0.034	12.0
K	MAT ($3.8 \pm 1.7 \times 10^{-1}$)	TOW ($2.9 \pm 4.2 \times 10^{-2}$)	0.005	40.0
	MAT	POSO ($1.4 \times 10^{1} \pm 1.0 \times 10^{-1}$)	0.011	0.0
	MAH ($3.7 \pm 6.6 \times 10^{-2}$)	TOW	0.034	12.0
	TOW	POSO	0.034	12.0
Cr	MAT ($1.0 \times 10^{-1} \pm 1.1 \times 10^{-2}$)	MAH ($1.0 \times 10^{-1} \pm 4.3 \times 10^{-3}$)	0.043	27.0
	MAT	POSO ($2.3 \times 10^{-2} \pm 2.3 \times 10^{-3}$)	0.028	28.0
	TOW ($8.9 \times 10^{-2} \pm 5.7 \times 10^{-3}$)	POSO	0.034	0.0
Fe	MAT ($4.9 \times 10^{-1} \pm 1.8 \times 10^{-2}$)	MAH ($3.7 \times 10^{-1} \pm 1.8 \times 10^{-4}$)	0.011	0.0
	MAT	TOW ($1.3 \times 10^{-1} \pm 1.5 \times 10^{-3}$)	0.034	35.0
Ni	MAT ($4.3 \times 10^{-2} \pm 6.9 \times 10^{-3}$)	MAH ($8.3 \times 10^{-2} \pm 3.2 \times 10^{-3}$)	0.018	29.0
	MAT	TOW ($4.2 \times 10^{-2} \pm 9.5 \times 10^{-4}$)	0.016	3.0
Zn	MAT ($1.1 \times 10^{-1} \pm 2.4 \times 10^{-2}$)	MAH ($2.8 \times 10^{-2} \pm 1.7 \times 10^{-3}$)	0.011	0.0
	MAT	TOW ($2.5 \times 10^{-2} \pm 1.1 \times 10^{-3}$)	0.005	40.0
	TOW	POSO ($2.9 \times 10^{-2} \pm 2.0 \times 10^{-4}$)	0.034	12.0
Mo	MAT ($5.0 \times 10^{-4} \pm 3.3 \times 10^{-5}$)	TOW ($2.7 \times 10^{-4} \pm 1.9 \times 10^{-5}$)	0.022	35.5
	MAT	POSO ($8.5 \times 10^{-4} \pm 3.5 \times 10^{-5}$)	0.049	3.5
	MAH ($4.0 \times 10^{-4} \pm 0$)	TOW	0.022	12.0
	MAH	POSO	0.037	0.0
	TOW	POSO	0.028	12.0

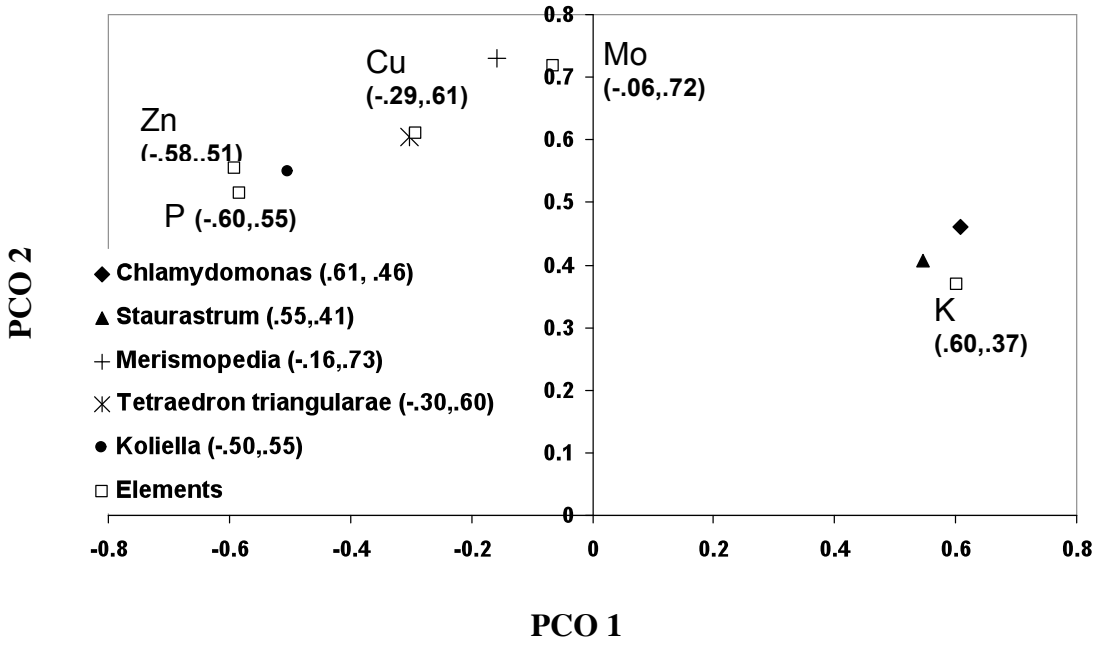

Figure 3.8 Canonical Correlation Analysis was used to test for relationships between elemental concentrations and phytoplankton species abundance in the four lakes. As illustrated in Figure 3.1, the left hand quadrant including P, Zn, and Cu represents Lake Matano, and the right hand quadrant represents Lake Poso. The strongest relationships between phytoplankton species abundance and elemental concentrations exist in these two lakes.

Chapter 4: Conclusions

The objective of this thesis was to identify and better understand the mechanisms responsible for the phytoplankton abundance and composition in the Malili Lakes. Understanding the mechanisms regulating phytoplankton growth and composition is important for lake management and protection, and for the greater understanding of the interplay of biodiversity and production in aquatic ecosystems and factors that govern community dynamics and assembly. In an attempt to understand the factors regulating biomass and composition, biological (phytoplankton analysis), physical (e.g. temperature, Secchi depth), and chemical factors (e.g. nutrient and elemental analysis) of the three main Malili Lakes and Lake Poso were analyzed.

Lake Matano has low biomass compared to other lakes at similar latitudes and with similar histories and is lacking several common taxa in its phytoplankton community. Phytoplankton studies from 2004 (Sabo, 2006) and 2006 (Chapter 2) indicated this lake contains a similar number of phytoplankton genera compared to that of other tropical lakes, but the low phytoplankton biomass counts were consistent with earlier research on these lakes. Previous studies have suggested macronutrient limitation and metal toxicity as the factors responsible for these impoverished community characteristics (Sabo, 2006; Bramburger, unpubl. data). The results of this thesis concluded that physical and chemical factors were important in regulating phytoplankton composition and biomass.

Physical factors that regulate the phytoplankton community are the ratio of Z_{eu} : Z_m in the lakes. Matano's resultant mixing continuously moves phytoplankton out of the euphotic zone, thus limiting photosynthesis and biomass. This is consistent with Haffner et al.'s reports that the autotrophic production is limited due to extensive vertical mixing (Haffner et al., 2001). From the pattern of decreasing conductivity downstream, contrary to the River Continuum hypothesis (Gordon et al., 2004), and the differences in elemental concentrations between connected lakes Mahalona and Towuti, and differences in elemental concentrations at different depths in Matano, it is evident that these lakes receive water from different sources. Matano receives water from underground water inputs as well as surface runoff, while the other lakes are dominated by inputs from surface runoff. Differences between lake water chemistry of Matano and Poso reflect different water sources, which may in turn be responsible for the difference in phytoplankton production capacity observed with respect to algal standing crops.

The nutrient addition experiments in Chapter 2 provided strong evidence that N and P, unlike in so many lakes, are not the primary factors driving the phytoplankton growth and composition. Additions of N and P individually and in combination, at various concentrations, did not produce significant changes in relative abundances or biologically significant changes in biomass. The highest biomass after 16 days of cultivation ($1.165 \times 10^3 \pm 8.51 \times 10^2$ µg/L) was still comparable to that in ambient waters (1.3×10^1 µg/L), as reported by Sabo (2006), and in other tropical lakes (i.e. 1.0×10^1 to 5.0×10^3 µg/L in the Caspian Sea) (Dumont, 1998). There were also no

significant differences ($p > 0.05$) in the relative abundances of the three dominant taxa for any nutrient treatments. Nutrient analysis in Chapter 3 indentified no significant difference between TP concentrations in Matano and Poso. The macronutrients N and P are not limiting the observed phytoplankton abundance and diversity in Lake Matano, and Matano is likely not subject to the threat of anthropogenic eutrophication as increased N and P inputs are predicted to have little effect in stimulating biological production.

Growth potential studies using CHU-10 growth media diluted with Matano water showed significant ($p < 0.05$) increases in chl-*a* concentrations for Matano phytoplankton. This indicates a nutrient limitation in the lake water that was alleviated with the addition of growth media. In addition, cultured chlorophyte and cyanobacteria chl-*a* concentrations showed a sharp decline for treatments containing dilutions of 50% or more Matano water, indicative of a nutrient limitation threshold or a chemical limitation. According to Healey's concentrations of elements required by some phytoplankton (Healey, 1973), this experiment indicated Fe, Si, K, Ca and Na were below what is required by some phytoplankton and these nutrients may be limiting.

Considering all available evidence from statistical differences, the geological setting, previous research, and biological significance, low Na and K concentrations may be minor contributors to the regulation of phytoplankton growth, but Cr and Mo are the most influential metals causing limitation to growth and abundance in Lake Matano.

Crowe et al. (2007) have reported relatively high levels of toxic Cr (VI) (1.8 x 10-3 µmol/L). The analysis conducted for this thesis measured the Cr concentration at 0-50 m for Lake Matano (1.4×10^{-1} µmol/L) above that required for a reduction in algal growth, 1.2×10^{-1} µmol/L, according to the Canadian Water Quality Guidelines (Pawlisz et al., 1997), and within a range of concentrations that caused inhibition of growth and changes in relative abundance (Wallen, 1996). These findings support previous research, in example Sabo showed an increase in phytoplankton biomass with reductions of Cr (Sabo, 2006), and Bramburger showed an increase in biomass and relative abundances of diatoms with simulation of an upwelling event, which caused reductions of Cr (Bramburger, unpubl. data). The lack of cladocerans in Lake Matano has been concluded to be a function of Cr toxicity (Fernando, 1987). This is strong evidence to support the limitation of phytoplankton abundance and composition by Cr toxicity.

Mo was not used for the Growth Potential Tests, but the ability of Mo to cause limitation in fresh water has been reported in multiple studies (Goldman, 1960; Downs et al., 2008). Experiments showed an addition of ten times the ambient Mo concentration (2.6×10^{-2} µmol/L) could stimulate a near 40% increase in primary productivity ($p < 0.001$) over the controls (Downs et al., 2008). Lake Poso had a Mo concentration 60% higher than that of Matano, and a phytoplankton biomass ten times higher than that in Matano. Mo concentrations were very low for all lakes (e.g. 5.0×10^{-4} µmol/L for Matano, 8.5×10^{-4} µmol/L for Poso), but this difference

between Poso and Matano is enough to limit phytoplankton growth and abundance, and limit new colonization by species not adapted to low Mo concentrations.

In conclusion, it is physical factors (mixing regime) and chemical factors (elevated concentrations of Cr and limiting concentrations of Mo) that are driving the phytoplankton community with respect to composition and abundance. Further investigations into the specific requirements of and physiological uptake by the 'missing' phytoplankton (Chrysophytes, Cryptophytes, Centrales), as well as that of many common cosmopolitan species, may provide greater insight into the regulatory aspects of the chemical environments of the Malili lakes. Chemical modeling would provide a useful picture of chemical speciation and reactions occurring at various depths within this lake. For now, there is an optimistic future for a lake resistant to eutrophication or invasion.

4.1 References

Crowe, S.A.; O'Neill, A.H.; Katsev, S.; Hehanussa, P.; Haffner, G.D.; Sundby, B.; Mucci, A.; Fowle, D. (2007) The biogeochemistry of tropical lakes: a case study from Lake Matano, Indonesia. *Limnol. Oceanogr*., **53**, 319-331.

Downs, T.M.; Schallenberg, M. and Burns, C.W. (2008) Responses of lake phytoplankton to micronutrient enrichment: a study in two New Zealand lakes and an analysis of published data. *Aquat. Sci.,* **70**, 347-360.

Dumont, H.J. (1998) The Caspian Lake: history, biota, structure, and function. *Limnol. Oceanogr.,* **43**, 44-52.

Fernando, C.H. (1987) Tropical freshwater zooplankton with special reference to South East Asia (Oriental Region) *Rep. Nat. Geogr. Soc.,* **87,** 287-311.

Goldman, C.R. (1960) Molybdenum as a factor limiting primary productivity in Castle Lake, California. *Science*, **132**, 1016-1017.

Gordon D.N., T.A. McMahon, B.L. Finlayson, C.J. Gippel, and R.J. Nathan (2004) Stream Hydrology - An Introduction for Ecologiste. John Wiley & Sons, Chichester, W Suss.

Haffner, G.D.; Hehanussa, P.E. and Hartoto, D. (2001) "The biology and physical processes of large lakes of Indonesia" In: Munawar, M. and Hecky, R.E. (eds). *The Great Lakes of the World: Food-web, health, and integrity.*Backhuys Publishers, Leiden, the Netherlands, 183-194.

Healey, F.P. (1973) Inorganic nutrient update and deficiency in algae. *Crit. Rev. Microbiol.,* **3**, 69-113.

Pawlisz, A.V.; Kent, R.A.; Schneider, U.A.; Jefferson, C. (1997) Canadian water quality guidelines for chromium. *Enviro. Tox. Wat. Qual.,* **12**, 123-183.

Sabo, E. (2006) *Characterization of the Pelagic Plankton Assemblage of Lake Matano and Determination of Factors Regulating Primary and Secondary Production Dynamics.*Thesis, University of Windsor, Canada.

Wallen, D.G. (1996) Adaptation of the growth of the diatom *Fragilaria crotonensis* (Kitton) and the phytoplankton assemblage of Lake Erie to chromium toxicity. *J. Great Lakes. Res*., **22**, 55-62.

APPENDIX I: Detailed phytoplankton counts from lakes Matano (Table I), Mahalona (Table II), Towuti, (Table III) and Poso (Table IV)

Table I Abundance of phytoplankton genera as number of cells per L for Lake Matano. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Sample	Division	Genus	Abundance (cells/L)
Matano 0m	B	*Fragilaria*	500
	Ch	*Chlamydomonas*	5500
	Ch	*Chlorococcus*	19000
	Ch	*Cosmarium*	500
	Ch	*Monoraphidium*	1000
	Ch	*Koliella*	500
	Cy	*Merismopedia*	16000
	Cy	*Anabaena*	17500
	Cy	*Gloeocapsa*	6500
		Total	**67 000**
Matano 50 m	B	*Fragilaria*	1000
	Ch	*Chlamydomonas*	3500
	Ch	*Chlorococcus*	17500
	Ch	*Cosmarium*	500
	Ch	*Staurastrum*	500
	Ch	*Tetraedron*	4000
	Ch	*Monoraphidium*	500
	Ch	*Koliella*	500
	Cy	*Merismopedia*	32000
	Cy	*Synechococcus*	2500
		Total	**62 500**
Matano 100 m	Ch	*Chlamydomonas*	1500
	Ch	*Chlorococcus*	15500
	Ch	*Tetraedron*	1500
	Ch	*Monoraphidium*	1500
	Cy	*Anabaena*	10000
	Cy	*Gloeocapsa*	20000
		Total	**50 000**
		Mean	**59 833 ± 2936**

Table II Abundance of phytoplankton genera as number of cells per L for Lake Mahalona. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Sample	Division	Genus	Abundance (cells/L)
Mahalona 0m	Ch	*Chlamydomonas*	5000
	Ch	*Chlorococcus*	34000
	Ch	*Cosmarium*	500
	Ch	*Monoraphidium*	32000
	Ch	*Koliella*	500
	Cy	*Anabaena*	500
	Cy	*Gloeocapsa*	37000
		Total	**109 500**
Mahalona 30 m	Ch	*Chlamydomonas*	2000
	Ch	*Chlorococcus*	3500
	Cy	*Gloeocapsa*	22500
		Total	**28 000**
Mahalona60 m	Ch	*Chlamydomonas*	1500
	Ch	*Chlorococcus*	8500
	Ch	*Cosmarium*	500
	Ch	*Monoraphidium*	1000
	Ch	*Tetraedron*	2500
	Cy	*Gloeocapsa*	25000
		Total	**39 000**
		Mean	**58 833 ± 14 741**

Table III Abundance of phytoplankton genera as number of cells per L for Lake Towuti. Divisions are represented as B for bacillariophytes, Ch for chlorophytes, and Cy for cyanobacteria. Mean cell abundance per liter at sampling depths is ± standard error.

Sample	Division	Genus	Abundance (cells/L)
Towuti 0m	Ch	*Chlamydomonas*	2000
	Ch	*Chlorococcus*	5500
	Ch	*Cosmarium*	500
	Ch	*Tetraedron*	500
		Total	**8500**
Towuti 50 m	Ch	*Chlamydomonas*	2000
	Ch	*Chlorococcus*	4000
	Cy	*Gloeocapsa*	20000
		Total	**26 000**
Towuti 100 m	Ch	*Chlamydomonas*	1500
	Ch	*Chlorococcus*	2500
	Cy	*Gloeocapsa*	6500
		Total	**10 500**
		Mean	**15 000 ± 3193**

Table IV Abundance of phytoplankton genera as number of cells per L for Lake Poso. Divisions are represented as Ch for chlorophytes, and Cy for cyanobacteria.

Sample	Division	Genus	Abundance (cells/L)
Poso 0m	Ch	*Chlamydomonas*	21500
	Ch	*Chlorococcus*	33000
	Ch	*Staurastrum*	500
	Ch	*Tetraedron*	1500
	Cy	*Anabaena*	12500
	Cy	*Gloeocapsa*	11000
		Total	**80 000**

VITA AUCTORIS

Name: Amy Jill Snook

Place of Birth: Corner Brook, Newfoundland, Canada

Date of Birth: August 2, 1984

Undergraduate Degree: Honours Environmental Science (Chemistry), Sir Wilfred Grenfell College/Memorial University of Newfoundland