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Characterization of the pelagic plankton assemblage of Lake Matano and determination of factors regulating primary and secondary production dynamics.

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CHARACTERIZATION OF THE PELAGIC PLANKTON ASSEMBLAGE OF LAKE MATANO AND DETERMINATION OF FACTORS REGULATING PRIMARY AND SECONDARY PRODUCTION DYNAMICS

by

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ABSTRACT

Lake Matano, Indonesia, is an ancient lake that provides an opportunity to examine the factors governing planktonic community structure. I determined that the phytoplankton community exhibits very low biomass relative to other lakes with similar assemblages and physicochemical characteristics. Phosphate additions made to algal cultures in lake water produced significant growth, suggesting that phosphorus availability limits production. To test for metal toxicity, phytoplankton were grown in water treated by flocculation. The enhanced growth in these cultures is consistent with the removal of a toxic constituent during flocculation. Ecological characterization of the secondary production revealed a depauperate community populated by the endemic calanoid *Eodiaptomus wolterecki*, and *Tropocyclops* species, in each year sampled (2000, 2002, 2004). Poor reproductive potential was suggested by the low proportion of egg-bearing individuals (less than 0.1%) in each year.

DEDICATION

This thesis is dedicated to my mother, Cathy Wilson.

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I would like acknowledge my supervisor, Doug Haffner, for his all help and teaching. Also, for making sure I had the opportunity to travel to the beautiful lakes that I had the joy of studying.

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STATEMENT OF ORIGINALITY

Intellectual contribution and execution of zooplankton sampling were provided by Denis Roy and G.D. Haffner for the years 2000 and 2002. In Chapter 4 and 5 of this thesis, intellectual contribution to experimental design was provided by G.D. Haffner, Paul Hamilton and Sean A. Crowe. The chemical profiles contained in Chapters 1, 3, 4 and 5 are drawn from sampling datasets collected by G.D. Haffner from 2000 to 2004.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

BBM – Bold's Basal Medium

1-D – 1-(diversity) index

DL – detection limit

ICP-MS – inductively coupled plasma mass spectrometry

MRW – metal removed water

MRNW – metal removed nutrient water

NA – not available

NR – not replicated

RCF – reduction coagulation filtration

Rpm – Rotations per minute

SD – Standard deviation

SE – Standard error of the mean

SNW – surface nutrient water

SRP – soluble reactive phosphorus

SW – surface water

TP – total phosphorus

CHAPTER 1 – GENERAL INTRODUCTION AND OVERVIEW OF LIMNOLOGY OF THE MALILI LAKES

1.0 -- General Introduction

1.1 -- Hotspot for biodiversity

In 2000, the biogeographical area of Wallacea was named one of 20 hotspots for global conservation priorities (Myers *et al.*, 2000). This designation was based on the criterion of exceptional levels of terrestrial biodiversity and high degree of threatened habitat.

The species composition unique to the biogeographical region of Wallacea was first described and delineated by Alfred Wallace in 1860. 'Wallace's line' represents the faunal break demarcated by an imaginary line that begins south of the Philippine Islands, curving down to separate the Indonesian islands of Sulawesi, Borneo and Bali from Lombok. Within the Indonesian islands on one side of the line, the Asian fauna reveals faunal characteristics found in Eurasia. On the other side of the line exists a unique Australian fauna. Wallace expected that the island of Sulawesi would maintain a rich Oriental fauna, but the fauna of this region was markedly different from that in either the Eurasian or Australian zones. Sulawesi exhibited a highly endemic, yet species-poor assemblage (Whitten *et al.*, 1987). About 14 million years ago, Sulawesi was formed by tectonic collisions associated with the Eurasian and Australasian plates (Hamilton, 1978), resulting in its geographical isolation. It is largely a result of this geographical isolation that the fauna of Sulawesi is so highly endemic.

1.2 -- Lake Matano and downstream Malili lakes

These high levels of endemism are also exemplified by the Malili Lakes system (Figure 1.1), found in central Sulawesi (formerly Celebes). Lake Matano, lies at S02°28.124, E121° 18.93. Downstream from Lake Matano, separated by a decline of 70 m, is Lake Mahalona, which drains into Lake Towuti. Lake Masapi also lies within the Malili Lakes watershed, but is not directly connected to the downstream lakes.

Lake Matano is ancient, estimated at 1-4 million years old (Haffner *et al.*, 2001). The lake harbours a highly endemic community, in which scientific interest has relatively recently peaked. High levels of endemism have been recorded in the diatom community (Hustedt, 1938, 1942; Bramburger *et al.*, 2004), gastropods (Sarasin and Sarasin, 1897; von Rintelen and Glaubrecht, 2003) and the fish (Kottelat, 1990a, b; c, 1991; Roy *et al.*, 2004).

To date, however, no detailed studies have focused on the community structure of the phytoplankton and zooplankton assemblages in this system. The first account of the zooplankton of Lake Matano stems from the Woltereck expedition of 1932. During this expedition, Brehm collected water samples containing a calanoid copepod which he described as *Eodiaptomus wolterecki*, determined to be endemic to the lake. The Woltereck expedition spanned the Philippines, Hawaii, Sulawesi (then Celebes), Flores, Bali and Java. However, the expedition aimed to be expansive not comprehensive. In 1950, John Langdon Brook described the patterns of endemism that had been observed in the Malili Lakes, citing *E.wolterecki* as the only recorded pelagic zooplankter. In 1987,

C.H. Fernando visited the Malili Lakes and noted the paucity of the zooplankton assemblage in Lake Matano (Fernando, 1987). Between 1991 and 1994, the Indo-Finnish Expedition Indodanau performed a survey of the basic water chemistry of the major lakes in Sumatra, Java, Bali, Lombok, Sulawesi and Irian Jaya, including Lake Matano (Lehmusluoto *et al.*, 1997). Whitten *et al.* (1987) described the ecology of Sulawesi and cited the unusual endemism characteristic of the lakes, but noted that too little was known about the biology of the lakes to sufficiently contribute to conservation efforts. C.S. Reynolds, in 2000, cited Lake Matano as one of the great lakes of the world, requiring further study.

Haffner *et al.* (2001) described Lake Matano as having a very simplified biological system, due to its highly oligotrophic nature. Examining the phytoplankton and zooplankton communities has the potential to yield important information about the factors governing the primary and secondary production base in this unique tropical freshwater system. Moreover, anthropogenic activity in and around Lake Matano has increased substantially over the last few decades. This development creates an immediate need to acquire information on processes regulating community dynamics in an ancient and relatively isolated lake system. Anthropogenic impact is predicted to pose a threat to the endemic biodiversity and heightens the need to evaluate biological properties such as composition and abundance.

1.3 -- Understanding community assemblage

Processes governing community assemblage and organization of the production base have long been a focal point of ecology. Hutchinson (1967) explored the 'paradox of the plankton', the co-existence of a diverse selection of plankton as a result of intermediate disturbance in an apparently homogeneous environment. Tilman (1996) demonstrated a unimodal trend in species diversity with increasing resources. Lewis (1980) demonstrated that the structure and complexity of zooplankton communities in the limnetic zone is more directly influenced by predation than by the resource gradient. Island biogeography theory (MacArthur and Wilson, 1967) predicts that species richness will increase with elevated dispersal rates in the absence of competition. The abiotic and biotic factors of a lake or region will produce variation in the success of this colonization after a species has arrived in a new environment (Dodson, 1992; Hobaek *et al.*, 2002). Habitat size plays a significant role in island biogeography. Lake area increases the likelihood of immigration, and with fewer restrictions on population size, lake volume lowers the potential for random extinction (Hobaek *et al.*, 2002). Furthermore, larger lakes offer greater potential for a diversity of microhabitats. For instance, large lakes offer more variation in substrate type or more refuges from predators than shallow lakes. Deep lakes have the potential for higher vertical microhabitat exploitation.

As modern research attempts to meet challenges such as climate change and eutrophication, it becomes increasingly obvious that understanding: 'what lives where and why' (Reynolds *et al.*, 2000) is as important now as it was during the early days of limnology. Historically, much of this knowledge has been generated from the study of

smaller lakes in temperate regions, making the extrapolation of information to larger scales and a wider variation in latitude difficult or incorrect. Phosphorus deficiency, for example, was originally an accepted paradigm of freshwater nutrient limitation (Schindler, 1977). Subsequent enlargement of the lake nutrient dataset revealed that, while phosphorus limitation was common in temperate lakes, nitrogen was commonly the limited nutrient in tropical lakes (Lewis, 2000).

1.4 -- Ancient lakes of the world

The vast majority of lakes are very young; few exceed the age of 10 000 years (Schon and Martens, 2004). Consequently, most lakes contain young assemblages. This restriction does not allow extensive insight into the influence of historical processes on shaping species, populations and communities. Ancient lakes are usually associated with major fault lines, prolonging the existence of these lakes, despite the continual sedimentation by which lakes disappear on geological timescales (Schon and Martens, 2004). Ancient lakes provide optimal model systems with which to elucidate selective processes operating to determine and regulate community structure.

Most ancient lakes are characterized by very high levels of diversity as well as very high levels of endemism, so extant community diversity may be linked to historical and current processes (Schon and Martens, 2004). Since extensive radiation of endemic species in a community takes time, the richness of the lacustrine community is influenced by the stability of an ancient lake throughout its existence. The presence of potentially exploitable habitats drives endemic radiation over long time periods. This concept is well

illustrated by the species flock of more than 350 amphipods in Lake Baikal, which accounts for 20% of all freshwater amphipod diversity (Verburg *et al.*, 2003). Several of these species have diversified enough to penetrate the deep water habitat with adaptations such as concentrated hemolymph (Zerbst-Boroffka *et al.*, 2000). Conversely, an ancient lake in which catastrophic events have occurred may contain a biologically young community (Schon and Martens, 2004). However, an ancient lake that has undergone intermediate fluctuations may have enhanced diversity. For instance, there is some evidence that the cichlid flock in Lake Tanganyika owes its extensive radiation to fluctuating water levels that may have separated populations through time (Livingstone, 1999).

While ancient lakes provide invaluable records of the fluxes and radiations of historical communities, through which extant assemblages may be better understood, the knowledge that may be gained from them is susceptible to loss. For instance, Lake Tanganyika, one of the world's oldest and deepest lakes, has not exhibited the ecological resistance to disturbance that might be predicted, based on the richly established, highly diversified nature of its community (Livingstone, 1999). According to Verburg *et al.* (2003), climate change has increased the resistance of Lake Tanganyika's thermal stratification to seasonal mixing. This lack of mixing has resulted in significant changes in lake chemistry such as reduction in the depth of oxygen penetration and concentrations of soluble reactive phosphorus (Verburg *et al.*, 2003). Within the past century, community effects have become evident such as reduction in phytoplankton biomass and diversity and disappearance of deepwater faunal populations (Verburg *et al.*, 2003;

Livingstone, 1999). Detailed recordings of ancient lake ecology are important for tracing the magnitude of an effect such as that of global climate change on Lake Tanganyika.

1.5 -- Large lakes of the world

Reynolds *et al.* (2000) provided a comprehensive study of the mechanisms of community organization in the phytoplankton communities of the large lakes of the world, correlating biological organization with important physicochemical characteristics. The lakes that Reynolds *et al.* selected for this survey drew from the morphometric data of Herdendorf (1982) and included 12 of the largest inland lakes by volume (>1500 km^3), the nine largest lakes in area (>80 000 km^2) and five of the deepest by depth (≥450 m). The study qualified the distribution of the dominant phytoplankton assemblages as a function of the properties of these lakes, including density stratification, thermal-bar formation, ice cover, the depth of the surface mixed layer, water clarity and parameters of basic water chemistry and phosphorus concentrations. Ultimately, Reynolds *et al.* provided a broad ecological overview of the status of some of the major freshwater bodies in the world, and his models provide a basis for future research. Reynolds *et al.* (2000) states that Lake Matano is not included in the models due to the paucity of studies available, representing an omission of some interest.

There is a need to better understand the processes regulating the biological production of Lake Matano, one of the relatively few ancient lakes worldwide as well as a large freshwater resource. The following discussion aims to investigate whether Lake Matano fits the model proposed by Reynolds *et al.* (2000). A comparison with

downstream lakes, Mahalona and Towuti, is also presented. These lakes (Table 1.1) provide a regional frame of reference for the plankton community assemblages of Lake Matano within its relatively isolated island lake system.

1.5.1 -- Morphology of Lake Matano, downstream lakes and other large lakes of the world

The morphology of the Malili Lakes has been previously described in Brooks (1950), Whitten *et al.* (1987) and Haffner *et al.* (2001). Morphological characteristics of these three lakes are summarized in Table 1.2. As can be seen, the depth of Lake Matano (590 m) clearly classifies it as one of the world's large lakes.

1.5.2 -- Seasonal stratification and mixing

Reynolds *et al.*'s list of the large lakes of the world was classified based on Lewis' mixing types (1983). For the most part, these lakes are deep and stratified for most of the year. The classifications of the Malili Lakes and lakes with similar classifications are presented in Table 1.3.

The data from Lake Matano (Figures 1.2-1.4) suggest that the lake is fully mixed to a depth of 80-90 m. At this depth, an oxycline begins, declining from >4 mg/L. A thermal structure occurs near a depth of 100 m, but is weak, suggesting that Lake Matano

is isothermal or nearly isothermal (Whitten *et al.*, 1987; Haffner *et al.*, 2001). Chemical characteristics such as concentrations of trace metals and nutrients also change at 100 m. This change in chemical characteristics indicates that the fully mixed depth of the water column does not extend beyond this depth. However, preliminary data suggest that trace quantities of oxygen may be detected below the mixed layer (Haffner *et al.*, 2001). This finding may be the result of density currents set up by the regular heavy rainfall events, which transport oxygen below this weakly stratified layer (Haffner *et al.*, 2001), combined with low anaerobic bacterial activity. In the tropics, the tendency towards anoxia will be amplified by the decline in oxygen solubility as well as the general lack of a full seasonal overturn and elevated microbial metabolic rates (Lewis, 2000). Therefore, the presence of even trace quantities of oxygen in deep, tropical lakes should be noted, as such lakes are subject to rapid anoxia (Lewis, 2000). Complete mixing refers to homogenization of the entire water column (Hutchinson and Loffler, 1956), while the available data suggest that Lake Matano is 'pseudo-meromictic' (Bramburger *et al.*, 2004). Lewis (1983) classified fully meromictic tropical lakes as warm monomictic, but suggested that they could remain meromictic for years, whereas Reynolds *et al.* (2000) makes the distinction between warm meromictic lakes and warm monomictic lakes.

Lakes Mahalona and Towuti (Figures 1.3 and 1.4) are classified as continuous warm polymictic lakes, along with lakes such as Tchad and Patos. 'Continuous, warm polymictic' refers to lakes with no seasonal ice cover, stratifying at most for a few hours at a time (Lewis, 1983). Such lakes rarely extend beyond 25 m in depth, are too shallow to sustain stable stratification and mix continuously, or at least nightly (Lewis, 1983).

Lake Mahalona is relatively small and shallow, with a maximum depth of 60 m so its near isothermal state is not unusual for a tropical lake. Compared with other warm polymictic lakes, however, Lake Towuti is exceptional in that it appears to be consistently isothermal over its depth of approximately 200 m and presents a challenge to the classification. In deeper, temperate lakes such as Crater Lake and possibly Lake Tahoe, full column overturn may occur once or twice a year as a result of significant seasonal temperature change. Such overturns occur frequently at higher latitudes, but are much rarer in the tropics due to the lack of seasonal variation (Lewis, 1983). Moreover, stratification is also more stable in the tropics because even slight changes in temperature produce definite density changes, since density at high temperatures responds more quickly to small changes in temperature (Lewis, 2000). It is not possible at this time to postulate what maintains the isothermal nature of Lake Towuti. The unusual occurrence of isothermal conditions is shared by another lake in the region, Lake Moat (Whitten *et al.*, 1987). As lakes Towuti, Matano and Moat all exist in tectonically active regions, it has been proposed that deep hot springs are present in these lakes (Whitten *et al.*, 1987; Haffner *et al.*, 2001). This hypothesis is supported by a study that reported an increase of 1°C in the deepest waters of Lake Towuti (Whitten *et al.*, 1987).

Other minor factors that contribute to mixing in this region include high rates of evaporation and heavy precipitation. Although pronounced seasonality does not exist in the region, periods of heavy precipitation do occur. Furthermore, lakes Matano and Towuti are fed by many small surface and groundwater streams that deliver cooler waters into the lake. The Malili Lakes, in common with all lakes at this latitude, will experience

higher and more consistent annual irradiance as well as more consistent irradiance (Lewis, 2000).

In contrast to other large tropical lakes, there is no evidence of a thermal bar in Lake Matano. The steeply sloping sides likely preclude the formation of a thermal bar and contribute to horizontal homogeneity. As a result, low horizontal heterogeneity in the plankton distribution should exist in Lake Matano. More information is required to assess the likelihood of thermal bar formation in Towuti.

1.5.3 -- Other physicochemical characteristics

Reynolds *et al.* (2000) presented the phytoplankton distributions of his lake database as a function of the following physicochemical characteristics: latitude, mean depth, Secchi depth, ratio of Secchi depth to mean depth, lake pH and nutrient status. Likewise, the physicochemical properties of Lake Matano and the downstream lakes are presented in Table 1.4 for comparison with Reynolds *et al.*'s dataset. Significant trends are discussed below.

In terms of depth, morphology and latitude, Lake Tanganyika compares most closely with Lake Matano. Other lakes located at comparable latitude to the Malili Lakes include lakes Victoria, Kivu and Toba. Lakes Toba, Tanganyika and Kivu are meromictic lakes; Lake Matano may be classified as near-meromictic for the purposes of this study. In contrast, lakes Towuti and Mahalona are classified as warm polymictic. As discussed

above, this classification is particularly unexpected for Lake Towuti, because the grouping consists of lakes Bangweolo, Eyre, Patos and Tchad, very shallow lakes with mean depths of 1.0, 2.0, 3.1 and 4.3 m, respectively. In addition, these lakes are mid-latitude lakes. Lake Matano is most comparable in depth to Lake Tanganyika and only surpassed by Lake Baikal, while Lake Towuti has similar depth to lakes Kivu and Toba.

The ultra-oligotrophic status of the Malili Lakes compares most closely with Lake Toba and Great Bear Lake. Low biomass in Lake Toba, located on Sumatra Island, Indonesia, may be attributed to low pH and high volcanic sulphur releases. In contrast, Great Bear Lake is a high latitude, arctic lake and, with the exception of Lake Toba and the Malili Lakes, all other ultra-oligotrophic lakes are also temperate to Arctic. Moreover, lakes Matano and Towuti are anomalies when water clarity is considered. Lake Matano ranks among the top four clearest lakes together with Lake Tahoe, Crater Lake and Great Bear Lake, with Secchi depths of 24-36, $\leq$34 and $\leq$29 m, respectively. Lakes Matano and Towuti are clearer than lakes Baikal (with Secchi depth 5-24 m), Malawi (with Secchi depth 13-23 m), Issyk-kul (with readings of 13-20 m) and Superior (with Secchi depth 10-17 m). With the exception of Lake Malawi, other tropical lakes have mid to very low water clarity. Secchi depths as low as 0.1 m are common (Reynolds *et al.*, 2000).

On the basis of these characteristics, Lake Matano is most closely aligned with the most unproductive, cold climate, Arctic type lakes. In Arctic lakes, high water clarity has been correlated with a combination of low nutrient levels and low temperature, which act

in synergy to create extremely low levels of primary production (Markager *et al.*, 1999). Lake surveys have verified a linear relationship between primary production and latitude, with primary production highest at the equator and decreasing towards the poles (Lewis, 1996), making Lake Matano a notable exception. The trend towards highest production at the equator is the result of greater quantities of consistent annual irradiation, sustained high temperatures and optimal nutrient cycling in the tropics (Lewis, 1996, 2000).

The ratio of Secchi depth to mixing depth is relatively high in Lake Matano and is similar to that found in lakes Malawi, Crater and Tahoe. Mixing to 100 m occurs in Lake Matano, with approximately 50 m of photic mixing and a deeper 50 m of aphotic mixing. With ratios of less than 0.1, much of the mixing in lakes Towuti and Mahalona will occur under light limitation.

1.6 -- Conclusions and further objectives

Based on the available data, Lake Matano is classified here as warm and pseudo-meromictic, but demonstrates similar physical processes to lakes Malawi, Crater and Tanganyika. Lakes Towuti and Mahalona are nearly isothermal, a characteristic of shallow lakes and thus not what might be predicted, especially for Lake Towuti. The ultra-oligotrophic status of Lake Matano and the downstream lakes is representative of clear, unproductive Arctic-type lakes.

The following chapters provide the first detailed recording of community composition of the plankton of Lake Matano. The purpose is to determine whether the

primary production of Lake Matano is limited and to explore possible factors regulating lake production. Firstly, the phytoplankton community of Lake Matano is compared with that of the downstream lakes Towuti and Mahalona, as well as that of Reynolds *et al.*'s (2000) other large lakes of the world. The second chapter evaluates whether nutrient limitation restricts growth in the Lake Matano phytoplankton community or, alternatively, toxicity limits growth (Chapter 3). The last chapter characterizes and describes the zooplankton community dynamics. In this chapter, the first null hypothesis is that no vertical structure exists in the zooplankton assemblage in the well-mixed upper waters of the lake. This hypothesis is examined using the following parameters: abundances, reproductive potential, size distributions, $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios and community composition of zooplankton with depth. Secondly, the hypothesis that predation effects regulate the secondary production is explored. This hypothesis is compared with the influence of bottom-up effects.

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Table 1.1 Locations for water chemistry sampling, Malili Lakes, 2004.

Lake	Sampling coordinates
Matano	S 02°28.124, E 121° 18.93
Towuti	S 02°41.902, E 121° 33.298
Mahalona	S 02°35.121, E 121° 29.129

Table 1.2 Physical and geographical characteristics of Lake Matano, Towuti and Mahalona (Brooks, 1950; Whitten *et al.*, 1987; Haffner *et al.*, 2001).

Lake	Area (km^2)	Maximum depth (m)	Elevation (m)	Latitude	Longitude
Matano	164.08	590	396	2S	121E
Towuti	560	200	293	2S	121E
Mahalona	-	60	319	2S	121E

Table 1.3 Classification of mixing in Lake Matano and downstream lakes and comparison with other lakes of similar mixing category.
(** denotes a large lake described in Reynolds et al.'s model (2000)*

Malili Lakes & Reynolds' Lakes of Same Classification	Ice Cover	Thermal Stratification	Mixing Depths
		Meromictic lakes	
Tanganyika*	-	40-50m	100-250m
Malawi*	-	<40m	
Crater*	Rare	20-70m	Deepens to ~200m
Toba*	-	<300m all year	
Kivu*	-	<70m all year	Exposed to strong S.E. winds
		Near-meromictic lakes	
Matano	-	Presumed 100m all year	Deepens with rain-induced density currents, 100-120m
		Continuous warm polymictic	
Patos*			Typical shallow near continuous mixing
Tchad*			Typical shallow diel mixing
Eyre*			Presumed typical shallow near continuous mixing
Bangweolo*			Presumed typical shallow near continuous mixing
Towuti	-	Isothermal	Mixed to depth
Mahalona	-	Isothermal	Mixed to depth

Table 1.4 A comparison of the geomorphometric details of the Malili Lakes and the great lakes of the world (*table adapted from Reynolds et al., 2000. *Details of the lakes: Herdendorf, 1982, 1990; Beeton, 1984; cited in Reynolds, 2000*)

Lake	Latitude	Max. depth (m)	Secchi-disc depth (m)	Secchi-depth/mixed depth	SRP (µg/L)	TP (µg/L)
Great Bear*	66 N	446	≤29	≥0.4		≤0.1
Great Slave*	62 N	614	4-17	0.07-0.57		3-8
Onezhskoye*	62 N	120	3.6-7.5	0.13-0.38		5-10
Ladohskoyw*	61 N	230	1.5-5	0.03-0.20		13-40
Baikal*	53 N	1741	5-24	0.007-1.2	10-60	
Winnipeg*	53 N	18	0.3-2	0.02-0.13		2-8
Superior*	48 N	407	10-17	0.07-0.70		2-3
Balkash*	46 N	26.6	0.4-12	0.67-2		61
Huron*	45 N	229	1-8	0.02-0.32		4-5
Michigan*	44 N	282	9-13	0.11-0.52	≤20	10-30
Ontario*	44 N	245	2-6	0.02-0.27	≤20	≤30
Crater*	43 N	589	<=34	0.49-1.7	≤18	
Issyk-kul*	43 N	668	13-20	0.05-1.0		2-4
Erie*	42 N	64	5-9	0.06-0.41		9-25
Tahoe*	39 N	505	24-36	0.10-0.72	≤3	≤6
Dangting*	29 N	30.8	0.1-0.3	0.01-0.04		≤5
Tchad*	14 N	12	0.1-0.7	0.03-0.18		
Nicaragua*	12 N	70	≤5?	<0.3?		
Turkana*	4 N	73	1-4.5	0.03-0.16	≤786	2400
Toba*	3 N	529				0.2-0.6
Victoria*	1 S	84	0.2-2.5	0.01-0.06	≤6	≤12
Kivu*	2 S	480				≤55
Tanganyika*	6 S	1471	12-15	0.05-0.35		4-10
Bangweolo*	11 S	5				
Malawi*	12 S	706	13-23	0.16-0.60		1-10
Titicaca*	16 S	281	5-10	0.05-0.20	≤23	39-331
Eyre*	29 S	5.7			250-910	≤340
Patos*	31 S	5	0.1-2	0.05-1.0		≤1000
Matano	2 S	590	23	0.23	<2	<2
Mahalona	2 S	60	5.5	0.09	<2	<2
Towuti	2 S	200	19	0.11	<2	<2

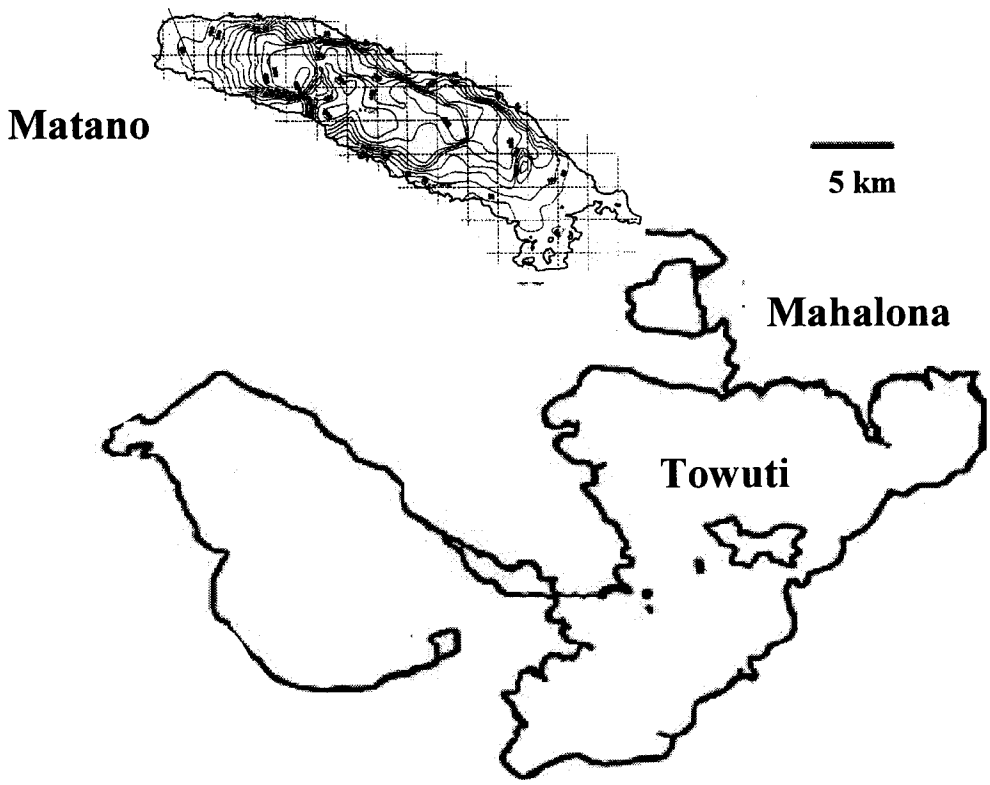

Figure 1.1 Map of the Malili Lakes, Sulawesi Island, Indonesia.

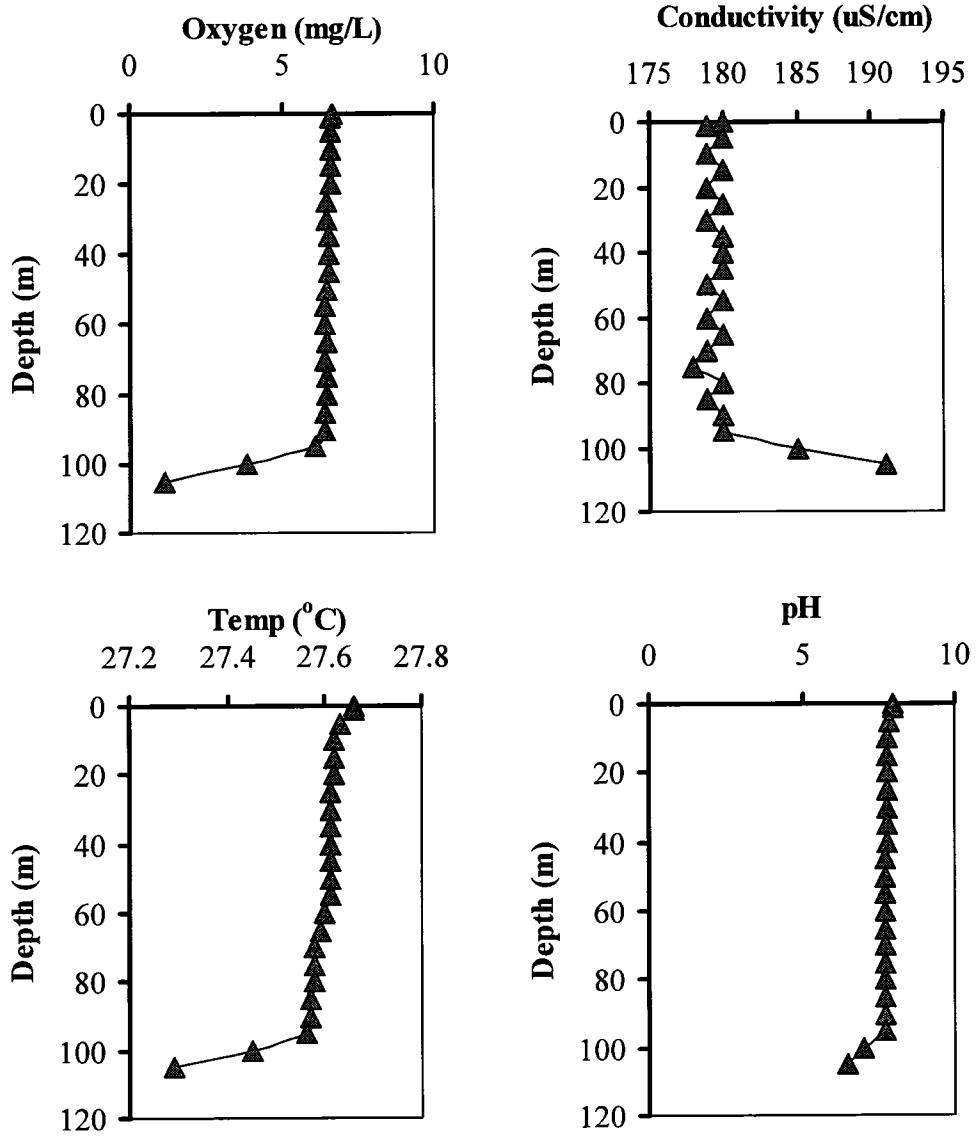

Figure 1.2 Oxygen, conductivity, temperature and pH profiles of the top 120 m of Lake Matano, (Hydrolab data) August, 2004.

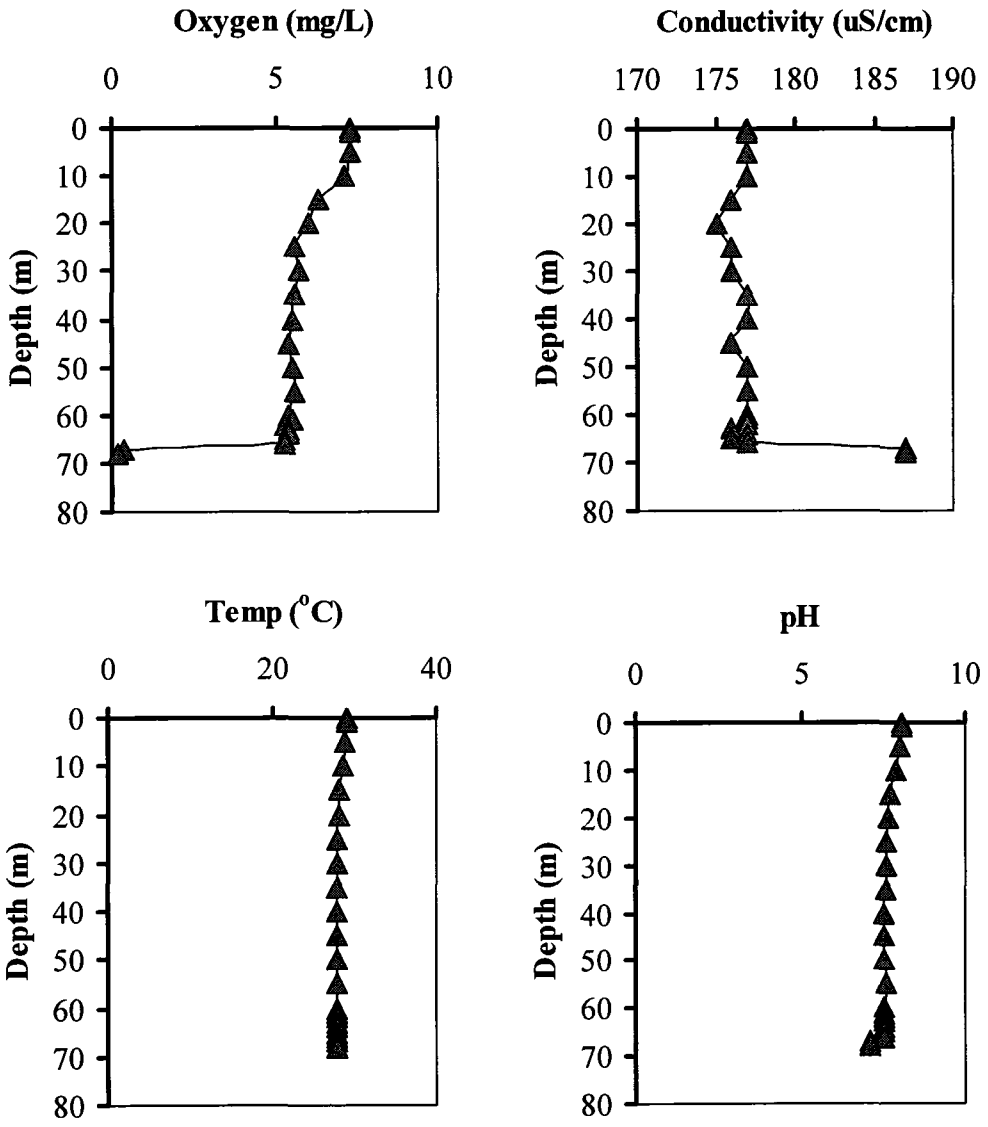

Figure 1.3 Oxygen, conductivity, temperature and pH profiles of the top 120 m of Lake Mahalona. (Hydrolab data) August, 2004.

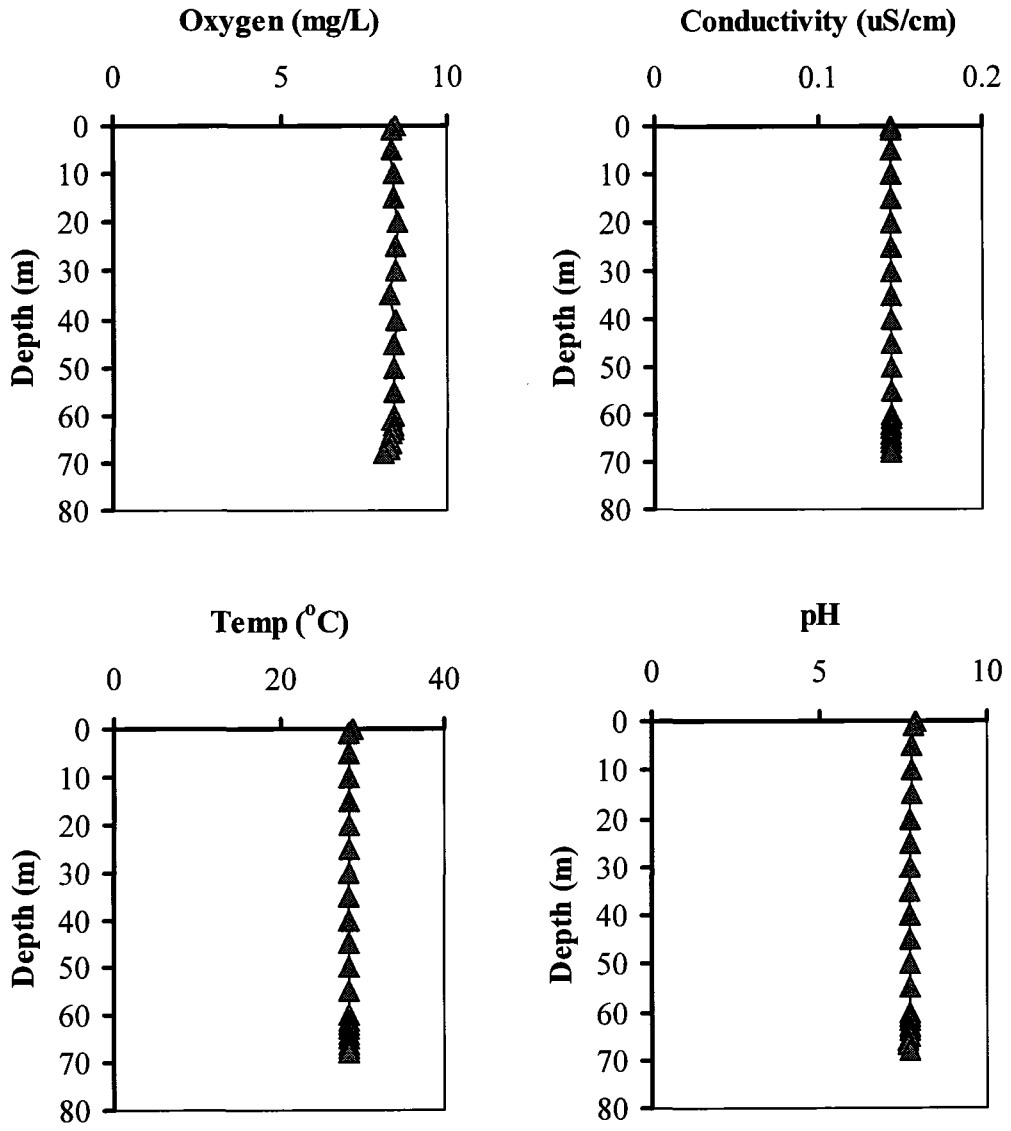

Figure 1.4 Oxygen, conductivity, temperature and pH profiles of the top 120 m of Lake Towuti. (Hydrolab data) August, 2004.

CHAPTER 2 – REGULATION OF THE PHYTOPLANKTON ASSEMBLAGES OF THE MALILI LAKES

2.1 -- Introduction

Ecology involves the study of the distributions of organisms and their interrelationships with their environment and with each other. The pelagic zone of lacustrine ecosystems has long inspired ecologists seeking to better understand the rules of community assemblage (Lund, 1965; Hutchinson, 1967; Lewis, 1990). The epilimnia of large lakes operate on variable spatial and temporal scales (Reynolds, 1994) that present a complex environment regulating the development of plankton populations and communities (Reynolds, 1994). With the increasing necessity to respond to contemporary freshwater conservation challenges, knowledge of these processes becomes increasingly important.

Therefore, there is a need for effective ecological tools of classification that will accurately identify significant functional and structural roles and adaptations. Phycology and plant ecology have met this need with systems for classifying vegetative associations (Reynolds, 2002). These associations are functional units that may or may not be phylogenetically related, as long as the species associations exhibit similar requirements and predominate under certain conditions. Classifications should provide a basic functional unit with which to characterize community processes in an aquatic ecosystem. Reynolds *et al.* (2000), proposed a qualitative model with which to classify dominant phytoplankton associations and to correlate these associations with important lake

physicochemical characteristics (see Chapter 1). This model has been used to compare the phytoplankton communities in the large lakes of the world (Reynolds *et al.*, 2000).

Insufficient information was available to include the pelagic plankton system of Lake Matano in the Reynolds *et al.* (2000) model (see Chapter 1). Lake Matano is one of the few ancient lakes of the world possessing a highly endemic community, yet relatively few studies have been conducted on the lake. The goal of this study was to determine whether the community assemblage of the limnetic phytoplankton of Lake Matano is comparable to Reynolds *et al.*'s qualitative model for large lakes of the world. In addition, the phytoplankton communities of the downstream lakes, Mahalona and Towuti, provide a regional comparison to understand factors regulating the phytoplankton assemblage at a local scale.

2.2 – Materials and Methods

The sampling site for collection of phytoplankton in each lake corresponded with the central location chosen for water sampling. Kemmerer bottle samples were collected at successive 10 m depth intervals, down to a depth of 110 m in lakes Matano and Towuti and to a depth of 60 m in Lake Mahalona. One sample of five hundred mL of water was collected at each of these depths and was preserved with Lugol's solution in sedimentation jars. These containers were refrigerated and phytoplankton sedimentation continued undisturbed for two weeks. After the completion of the sedimentation interval, the excess water above the sedimentation zone was gently siphoned from the jars and the concentrated plankton samples were stored for analysis. Samples of the supernatant were

also taken and examined to verify the efficacy of the sedimentation process. The primary production of the lakes was not directly quantified as a result of logistical difficulties resulting from the low standing biomass observed.

One mL aliquots from the phytoplankton samples were placed in 5 mL sedimentation chambers for 24 hours prior to analysis. Phytoplankton were microscopically analyzed under 200 to 600x magnification using a Leica® inverted microscope and identified according to Bramburger (2004) and Prescott (1978). Three subsamples were analyzed for each sample, which exceeded 100 counts of individuals of the most dominant species. The mean count for each species was used to generate a profile of composition and abundance as a function of depth. The volumetric dimensions of 30 individuals from each species were measured using Openlab® 3.15 image analysis software and the mean dimensions were used to calculate the biovolume for each species, in accordance with the methodology and formulation presented in Sun and Liu (2003). Biomass was also estimated with depth, based on volumetric relationships.

2.3 -- Results and Discussion

It is possible to qualitatively demonstrate the presence or absence of significant relationships between the nature of a lake and its phytoplankton composition (Reynolds *et al.*, 2000). Reynolds *et al.* (2000) detailed the distribution of such assemblages among his selected lakes, using primarily taxonomic categories of phytoplankton, proposed by Hutchinson in 1967. These assemblages are presented in Table 2.1. In addition, cryptomonads, picoplankton and nanoplankton may be prominent members of the phytoplankton community. However, less quantitative information is available regarding

their presence (Reynolds *et al.*, 2000). Figure 2.1 presents the distribution of the phytoplankton assemblages among Lake Matano and other large lakes and includes the downstream Malili Lakes. As latitude has been proposed as an important factor regulating the distribution of the phytoplankton assemblages, an axis of descending latitude is introduced here.

The abundances, compositions and representative phytoplankton assemblages specific to lakes Matano, Towuti and Mahalona are presented in Figures 2.3, 2.4 and 2.5, respectively. These assemblages are compared with the dominant assemblages of phytoplankton found in Reynolds *et al.*'s lakes (Figure 2.2). Relative to Reynolds *et al.*'s selection of lakes (Figure 2.6), which vary widely in characteristics such as depth, nutrient base and latitude, analysis of the phytoplankton communities for the Malili Lakes in August, 2004 reveals that the biomass of the primary producers in the three lakes are severely impoverished.

The phytoplankton community of Lake Matano was dominated by dinoflagellate plankton and non-nitrogen fixing cyanobacteria. Numbers of *Merismopedia* peak in abundance at the surface, with 3.1×10^3 (se=3.7×10^2) cells/L as the highest abundance of cells at this depth (Figure 2.3). According to the biomass profile of Lake Matano, the peak mean biomass of 1.3×10^{-2} (se= 7.0×10^{-3}) mg/L occurred in the upper waters (Figure 2.7). This biomass is comparable to that estimated from a brief pilot study on the lake, conducted in 2001, in which a biomass of 6.0×10^{-2} mg/L was calculated

(Hamilton, unpublished data). A general survey of Indonesian lakes undertaken by Lehmusluoto *et al.* (1997) reported a lower biomass of 2×10^{-3} mg/L. In comparison with the selection of other large lakes, the biomass of Lake Matano presents an extreme in the spectrum of biomass yield (Figure 2.6). This yield compared most closely with Great Bear Lake, for which the biomass peak has been recorded as 6.0×10^{-2} mg/L. Biomass throughout the upper waters of the lake was predominated by *Peridinium*, which peaked at the surface with 1.1×10^{-2} (se= 6.3×10^{-3}) mg/L, with 3.2×10^{2} cells/L (se= 1.9×10^{2}). Biomass declined until a second peak appeared at a depth of approximately 70 m. Below this depth, most species declined to rarity, although numbers of *Microspora* increased from 70 to 100 m, peaking at 100 m, with an abundance of 4.0×10^{2} (se= 115) cells/L. At this depth, some cells, consisting largely of *Microspora* and some diatom frustules, assumed a moribund condition, so the peak represented an accumulation of cells that had been mixed from the upper growth zones. In samples from 110 m, cell biomass and counts were negligible and any cells detected appeared moribund.

Phytoplankton biomass was lowest in Lake Mahalona (Figure 2.6), despite the small area and relatively shallow nature of the lake. The comparatively shallow Secchi disk depth (24 m) is not caused by standing crops of phytoplankton and the shading caused by the lake's turbidity may partially explain the paucity of phytoplankton. Like the community structure of Lake Matano, non-nitrogen fixing cyanobacteria dominated phytoplankton numbers (Table 2.1). *Aphanothece* represented the dominant species in the upper 30 m, reaching 3.2×10^{4} (se=3.0×10^{3}) cells/L at a depth of 10 m and *Snowella* and *Merismopedia* contributed largely to the remainder of the cell counts. *Microspora,* a

chlorophyte, was also detected and represented much of the biomass peak that occurs at 10 m, reaching 4.5×10^{-3} (se= 6.4×10^{-4}) mg/L. Total biomass at this depth was 5.3×10^{-3} (se=1.8×10^{-3}) mg/L and biomass declined thereafter, becoming barely detectable below 30 m.

Biomass was also depressed in the surface waters of Lake Towuti, although the standing crop was greater than that observed in lakes Matano or Mahalona (Figure 2.4). Despite the well-mixed nature of Lake Towuti, a clear peak in biomass occurred at a depth of 60 m, with 9.0×10^{-2} (se=1.5×10^{-2}) mg/L. The existence of such a peak suggests that the phytoplankton distribution is regulated through the water column, although the regulating process cannot be determined at this time. Assuming no strong chemical gradient within the mixed waters, light attenuation with depth will provide a gradient that phytoplankton will track. In numbers, the lake was also dominated by cyanobacteria. *Snowella*, which reached a peak abundance of 2.0×10^{5} (se=1.4×10^{5}) cells/L, was followed by *Merismopedia* and *Aphanothece* in abundance (Figure 2.4). *Microspora* provided the highest biomass, reaching 7.6×10^{-2} mg/L (se=1.7×10^{-2}) at 60 m. *Snowella* and *Aphanothece* also contributed prevalently to the biomass throughout the water column. Lake Towuti exhibited higher production potential than Lake Matano and it is likely that this condition persists throughout the year. Contrary to Lake Matano, open water fisheries are common in Lake Towuti and the lake has been known to support top level predators, including crocodiles.

Representation by the other phytoplankton assemblages such as diatoms, chrysophyceans and nitrogen-fixing cyanobacteria occurred in very low numbers or not at all in the three lakes. Diatom representation in Reynolds *et al.*'s lakes is common and most of the assemblages are dominated by diatoms for a portion of the year (Reynolds *et al.*, 2000). *Cyclotella* figures largely in the open waters of these lakes, particularly deep, oligotrophic lakes with abundant light (Reynolds *et al*, 2000). No representatives of the group of centric diatoms were found in the open waters of any of the Malili Lakes and were also conspicuously absent from the periphyton of the littoral zone (Bramburger, 2004). Interestingly, the *Cyclotella* assemblage is represented in regionally proximal Lake Poso (Haffner, unpublished data). Diatom representation in the open waters of Lake Matano and the downstream lakes was predominantly limited to *Brachysira longirostris* and *Surirella wolterecki*. These species were present, but were limited in terms of numbers and biomass. It is interesting to note that neither *Brachysira longirostris* or *Surirella wolterecki* are considered typically planktonic species. Deteriorated frustules of *Surirella* species were also observed in low numbers throughout the samples from Lake Matano and, to a lesser degree, in Lake Towuti, but were not quantified.

Chrysophytes and cryptophytes were not found in any of the Malili lakes. *Pediastrum* and *Scenedesmus* represented the extent of the chlorophyte grouping within the Malili Lakes. These phytoplankton are common among the large lakes and are more often associated with eutrophic lakes such as lakes Bangweolo, Tchad, Victoria and Winnipeg (Reynolds *et al.*, 2000). No nitrogen-fixing bacteria were observed in the

samples from the three Malili lakes. Along with Lake Issyk-kul, the Malili Lakes are an exception to the affinity of *Merismopedia* for weakly acidic lakes.

Reynolds' qualitative model led to the proposal that the important physicochemical characteristics of a lake such as pH, Secchi depth, total phosphorus and maximum or mean depth are not sufficient to provide a cohesive trend by which to delimit the distribution of the major phytoplankton assemblages. Latitude appears to provide the best indication of the dominant phytoplankton assemblages inherent in a lake (Reynolds *et al.*, 2000). This finding may be mediated by water temperature, day length and annual irradiance, or even the availability of carbon dioxide (Reynolds *et al.*, 2000). Geographical proximity and the geological nature of the catchment may also play a role. Lake Matano and the downstream Malili Lakes exhibited dominant phytoplankton assemblages comparable to those found at similar latitude, where representation by cyanobacteria becomes more common with descending latitude (Figure 2.1). Non-N-fixing cyanobacteria (assemblage 10) are the dominant phytoplankton assemblage in the Malili Lakes and such non-N-fixing, as well as fixing (assemblage 9) cyanobacterial assemblages, also predominate in lakes Victoria, Kivu, Tanganyika and Malawi. These assemblages are rarely found above 14°N. However, in comparison with Reynolds *et al.*'s lakes along a spectrum of latitude, the diversity and species richness of the observed assemblages is poor in the Malili Lakes.

The distribution of such phytoplankton groupings may rely to some degree on a gradient of morphometric adaptations associated with a gradient of environmental conditions. Although the primary basis for delineation of the phytoplankton assemblages is strong co-occurrence rather than phylogenetic closeness, close morphometry is observed within the taxa. The functional groups of phytoplankton have similar morphometries that are quantifiable by descriptors such as biovolume (Reynolds, 2002). A plot of the size fractions of phytoplankton (in terms of biovolume) with descending latitude is presented in Figure 2.2. Dominant size fractions also follow a general latitudinal gradient, with a trend towards dominance by smaller biovolume classes towards the equator. The Malili Lakes are representative of this trend. The small phytoplankton size favored in the Malili Lakes presents several adaptive advantages for this dominant phytoplankton assemblage. The small sizes of the dominant cyanobacteria will be effective at reducing sinking rates and allowing the cells to remain suspended in the water column (Reynolds, 2002). This phenomenon will be particularly important in the Malili Lakes, where mixing and sinking out of the euphotic zone (see Chapter 1) presents a challenge to the resident phytoplankton.

This distribution of dominant phytoplankton assemblages also appears to correlate with a gradient in mixing patterns, from cold monomictic high latitude lakes and dimictic lakes, to meromictic and continuous warm polymictic lakes. The cyanobacterial assemblages dominate the meromictic and continuous warm polymictic lakes, in which the importance of resisting mixing provides a consistent explanation. Smaller sizes may

also offer a competitive advantage in environments where elevated light and temperatures up-regulate metabolic rates, particularly on a limited nutrient base.

2.4 -- Conclusions

This work provides the first detailed description of the phytoplankton community of Lake Matano. The Lake Matano phytoplankton community was compared with that of the other large lakes of the world to identify the likely factors regulating the primary production base. In addition, the analysis included the downstream Malili Lakes.

Differences in biomass occurred among the Malili Lakes. Lake Towuti supported a greater biomass than lakes Matano or Mahalona. The particular species in the cyanobacterial assemblage that dominated each of the lakes varied (*Merismopedia* in Lake Matano, *Snowella* in Lake Towuti and *Aphanothece* in Lake Mahalona), suggesting differences in community regulation among the lakes. The biomass profiles of the lakes, particularly in fully mixed Lake Towuti, showed vertical structure.

The Malili Lakes were each dominated by a phytoplankton assemblage that consisted of non-N-fixing cyanobacteria. The distribution of this assemblage is predicted by Reynolds' model, in which cyanobacteria and small size fractions become common at lower latitudes, despite variation in depth, mixing, trophic status and pH among the lakes. For instance, such an assemblage predominates in Lake Malawi (12°S), with a mean depth of 273 m, a Secchi depth of 13-23 m, a total phosphorus concentration of 1-10

μg/L. It also dominates Lake Patos (31°S), a continuous warm polymictic lake, with a mean depth of 2 m, a Secchi depth of 0.1 to 2 m and a total phosphorus concentration of up to 1000 μg/L. Meanwhile, the assemblage is rarely found dominating the northern latitudes. Factors correlated with latitude such as light, may play a role in effecting this distribution. The latitudinal gradient also correlates with a gradient in mixing types. The distribution of the small-celled cyanobacterial assemblages is nearly limited to lakes that are meromictic or continuous warm polymictic. The existence of deep partial or full continuous mixing makes small size an advantage because small cells are more resistant to mixing. This phenomenon is particularly important in the Lake Matano and the downstream lakes, in which deep mixing (see Chapter 1) presents a challenge to phytoplankton cells.

Although the tendency towards small biovolume and the proliferation of cyanobacteria is a common attribute of tropical lakes, Lake Matano and the downstream lakes exhibit low species richness in comparison with the suite of lakes chosen by Reynolds *et al.* (2000). Moreover, in comparison with the Reynolds *et al.*'s lakes, which offer a wide range in terms of nutrient status, latitude, morphology and mixing type, the biomass of the Malili Lakes is poor. Indeed, the yield of the phytoplankton standing crop is most characteristic of an unproductive Arctic-type lake.

2.5 – References

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Table 2.1 Summary of the phytoplankton assemblages presented by Reynolds (2000), in his comparison of the distribution of dominant assemblages among the large lakes of the world. Comparison of Lake Matano to other large lakes of the world references these phytoplankton assemblages.

Phytoplankton Assemblage	Phytoplankton Assemblage Assigned Number	Representative Genera in Assemblage
Diatoms:	1	*Cyclotella, Tabellaria*
	2	*Asterionella, Stephanodiscus*
	3	*Surirella, Nitzchia; chrysophyceans;*
	4	*Dinobryon*
Oligotrophic chlorococcals	5	*Sphaerocystis*
	6	*Botryococcus*
Eutrophic chlorophytes	7	*Pediastrum and Scenedesmus*
Dinoflagellates	8	*Peridinium, Ceratium*
Cyanobacteria	9	*N-fixers, Anabeana*
	10	*Microcystis, Merismopedia*
	11	*Oscillatoria*
	12	*Spirulina, Arthrospira*

Table 2.2 List of phytoplankton genera (and associated families) detected in the Malii Lakes, August, 2004.

Matano		**Mahalona**		**Towuti**	
Family	Genera	Family	Genera	Family	Genera
Peridiniaceae	*Peridinium*	Hydrodictyaceae	*Pediastrum*	Hydrodictyaceae	*Pediastrum*
Desmidiaceae	*Cosmarium*	Desmidiaceae	*Cosmarium*	Desmidiaceae	*Cosmariun*
Brachysiraceae	*Brachysira*	Brachysiraceae	*Brachysira*	Brachysiraceae	*Brachysira*
Merismopediaceae	*Merismopedia*	Desmidaceae	*Micrasterias*	Desmidaceae	*Staurastrum*
Merismopediaceae	*Snowella*	Merismopediaceae	*Merismopedia*	Merismopediaceae	*Merismopedia*
Synechococcaceae	*Aphanothece*	Merismopediaceae	*Snowella*	Scenedesmaceae	*Scenedesmus*
Surirellaceae	*Surirella*	Synechococcaceae	*Aphanothece*	Desmidaceae	*Staurastrum*
Microsporaceae	*Microspora*	Surirellaceae	*Surirella*	Merismopediaceae	*Snowella*
Desmidaceae	*Staurastrum*	Microsporaceae	*Microspora*	Synechococcaceae	*Aphanothece*
				Surirellaceae	*Surirella*
				Microsporaceae	*Microspora*
				Gomphonemateceae	*Gomphonema*

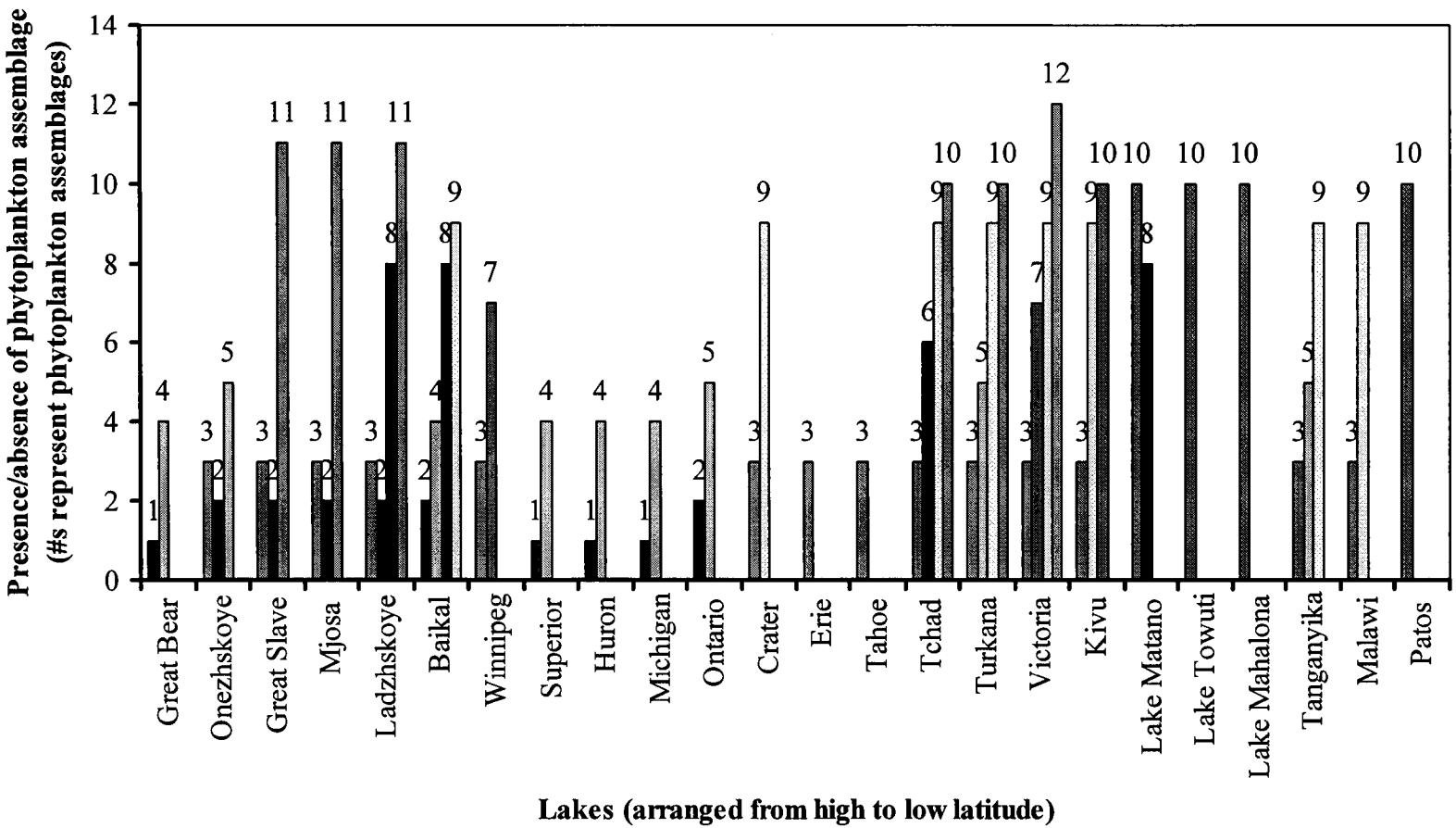

Figure 2.1 Presence/absence of dominant phytoplankton assemblages among the Malili Lakes and Reynold *et al.*'s (2000) great lakes of the world. Phytoplankton assemblages are represented by the designated numbers indicated in Table 2.1. From left to right, lakes are arranged from high latitude to near equatorial lakes, demonstrating the distribution of phytoplankton assemblages as a function of latitude. Cyanobacterial assemblages 9 and/or 10 dominate Lake Matano and other tropical lakes.

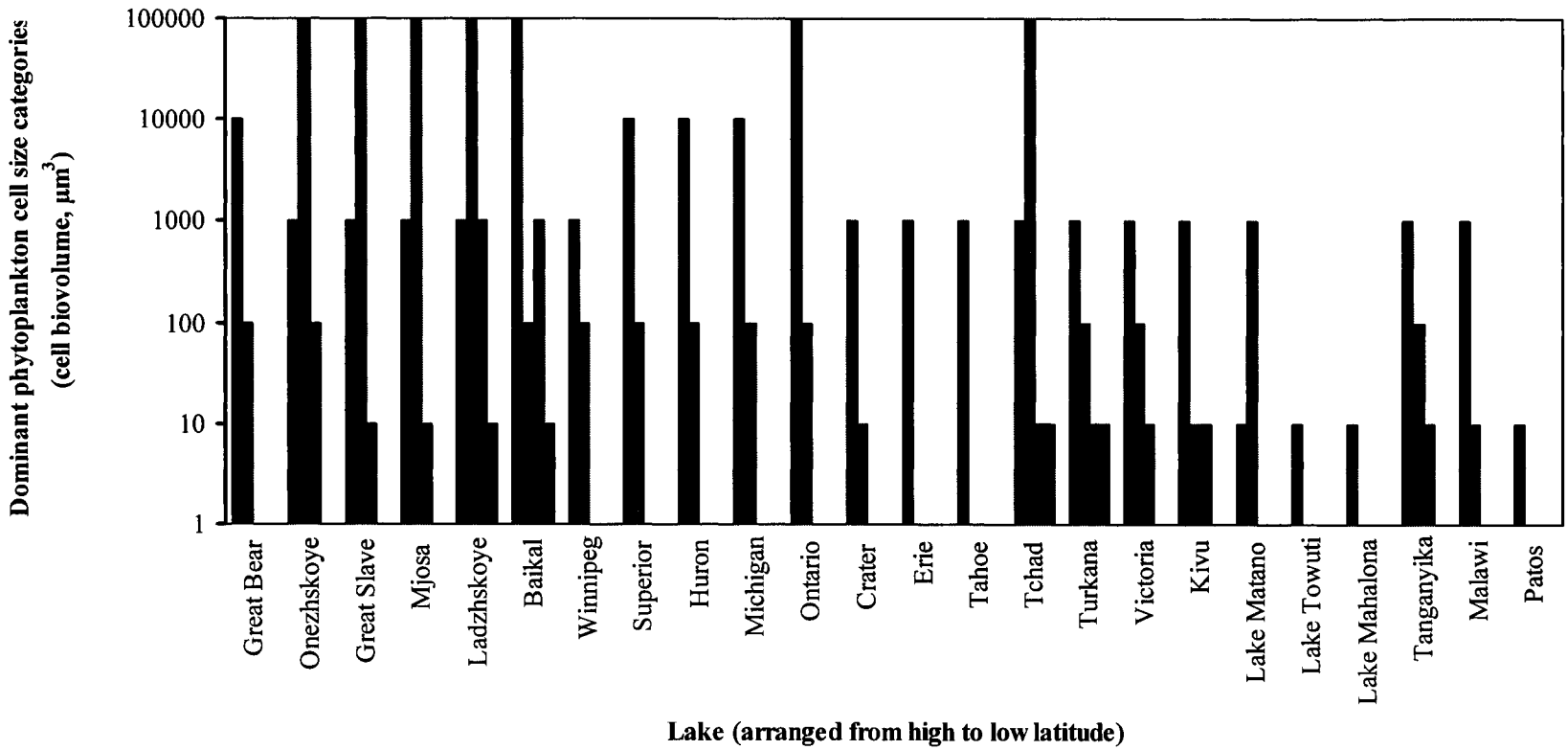

Figure 2.2 Presence/absence of dominant phytoplankton cell sizes, as biovolume, among the Malili Lakes and Reynold *et al.*'s (2000) great lakes of the world. From left to right, lakes are arranged from high latitude to near equatorial lakes, demonstrating the distribution of phytoplankton cell sizes as a function of latitude. Smaller cell sizes dominate Lake Matano and other tropical large lakes.

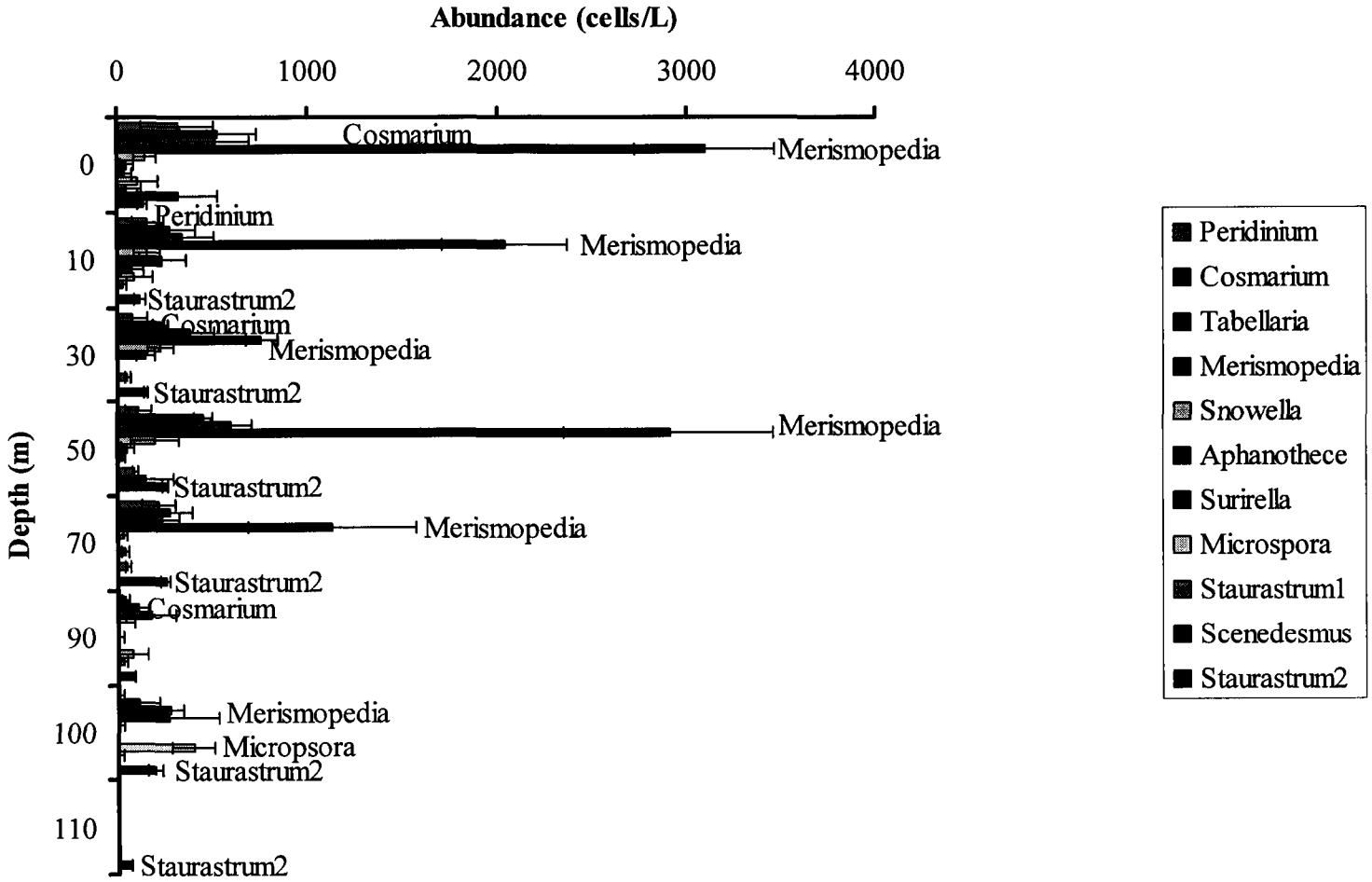

Figure 2.3 Abundances(± se) of phytoplankton with depth, in the upper 110 m of Lake Matano, 2004.

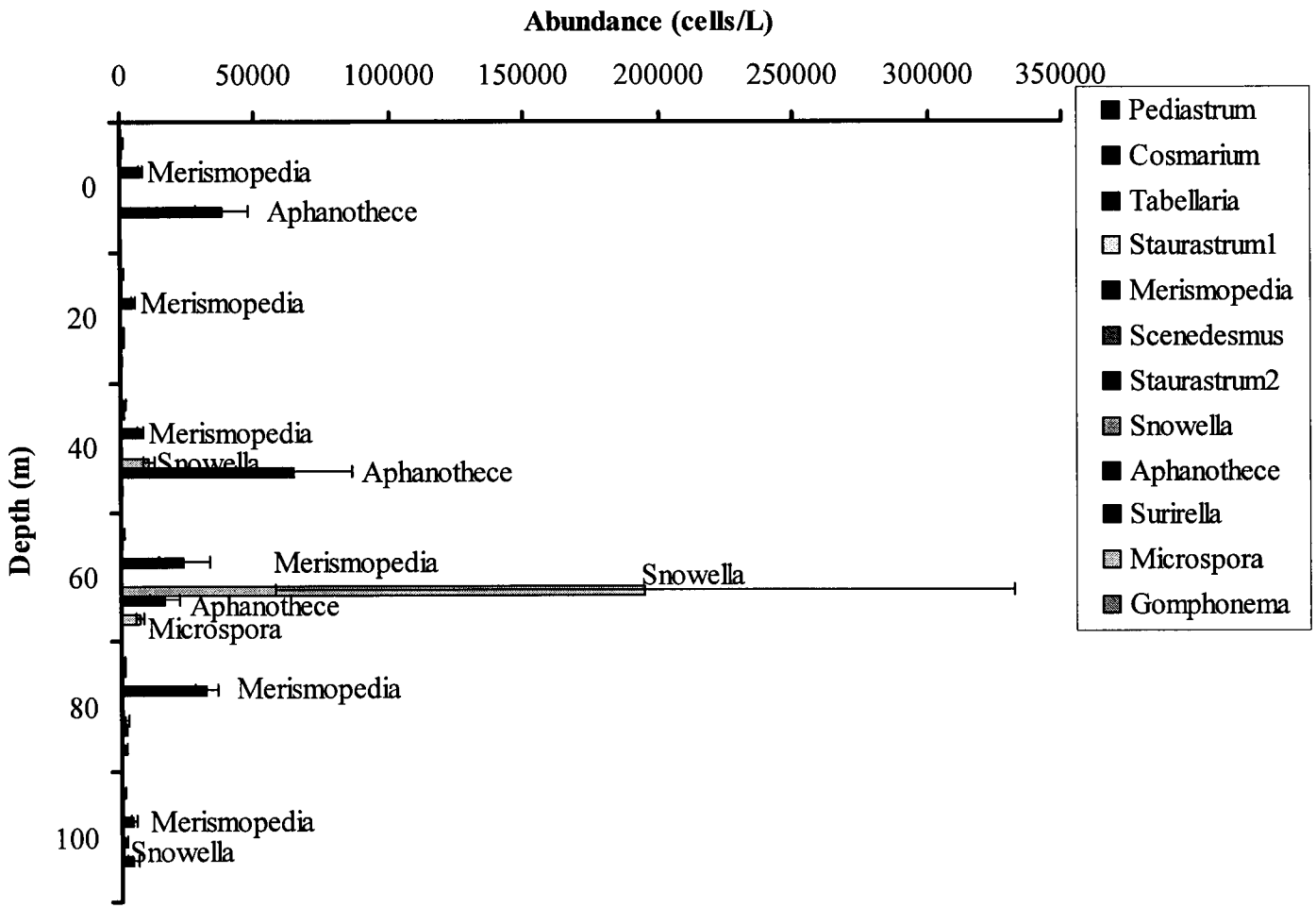

Figure. 2.4. Abundances (± se) of phytoplankton with depth, in the upper 110 m of Lake Towuti, 2004.

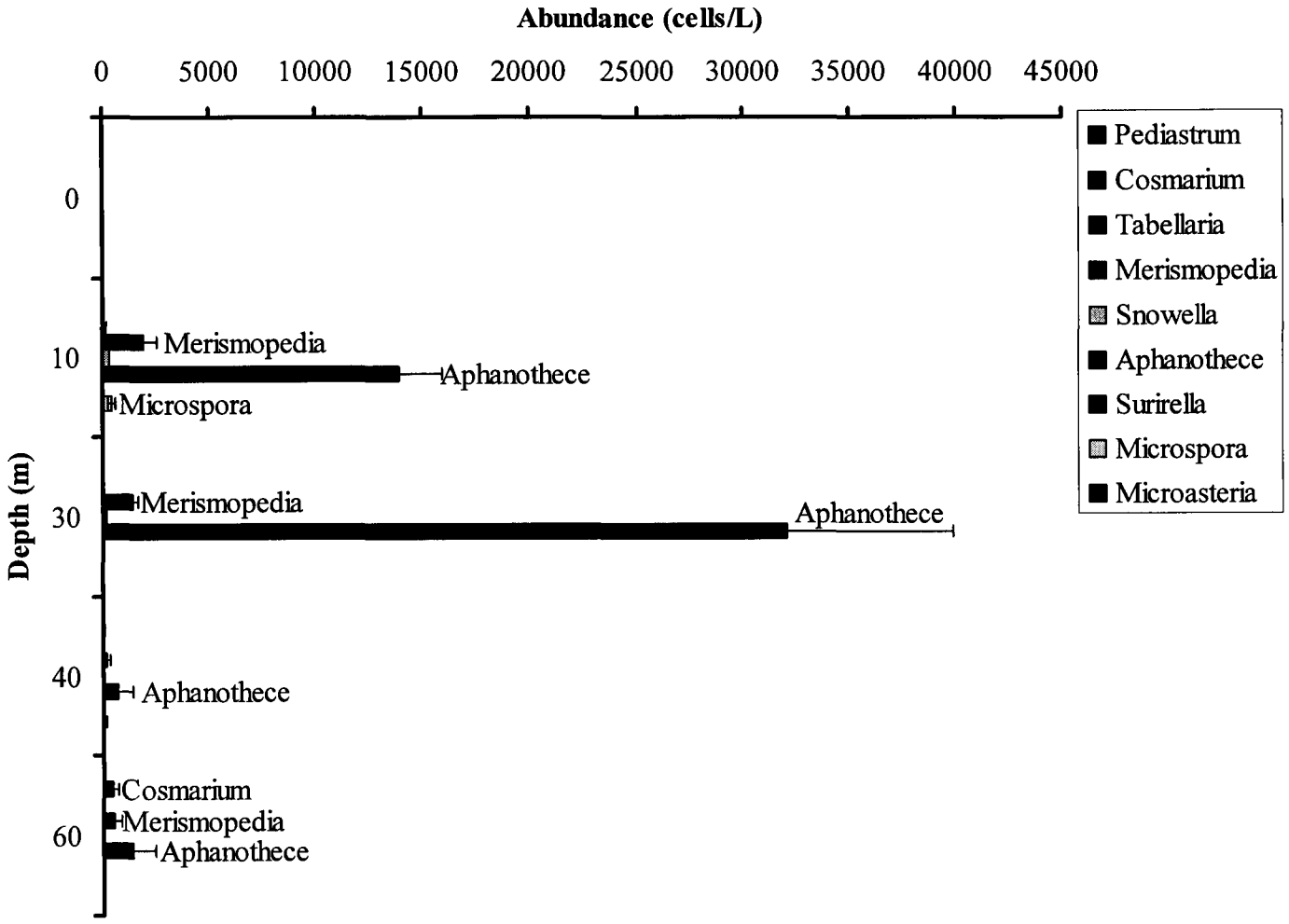

Figure 2.5 Abundances (± se) of phytoplankton with depth, in the upper 110 m of Lake Mahalona, 2004.

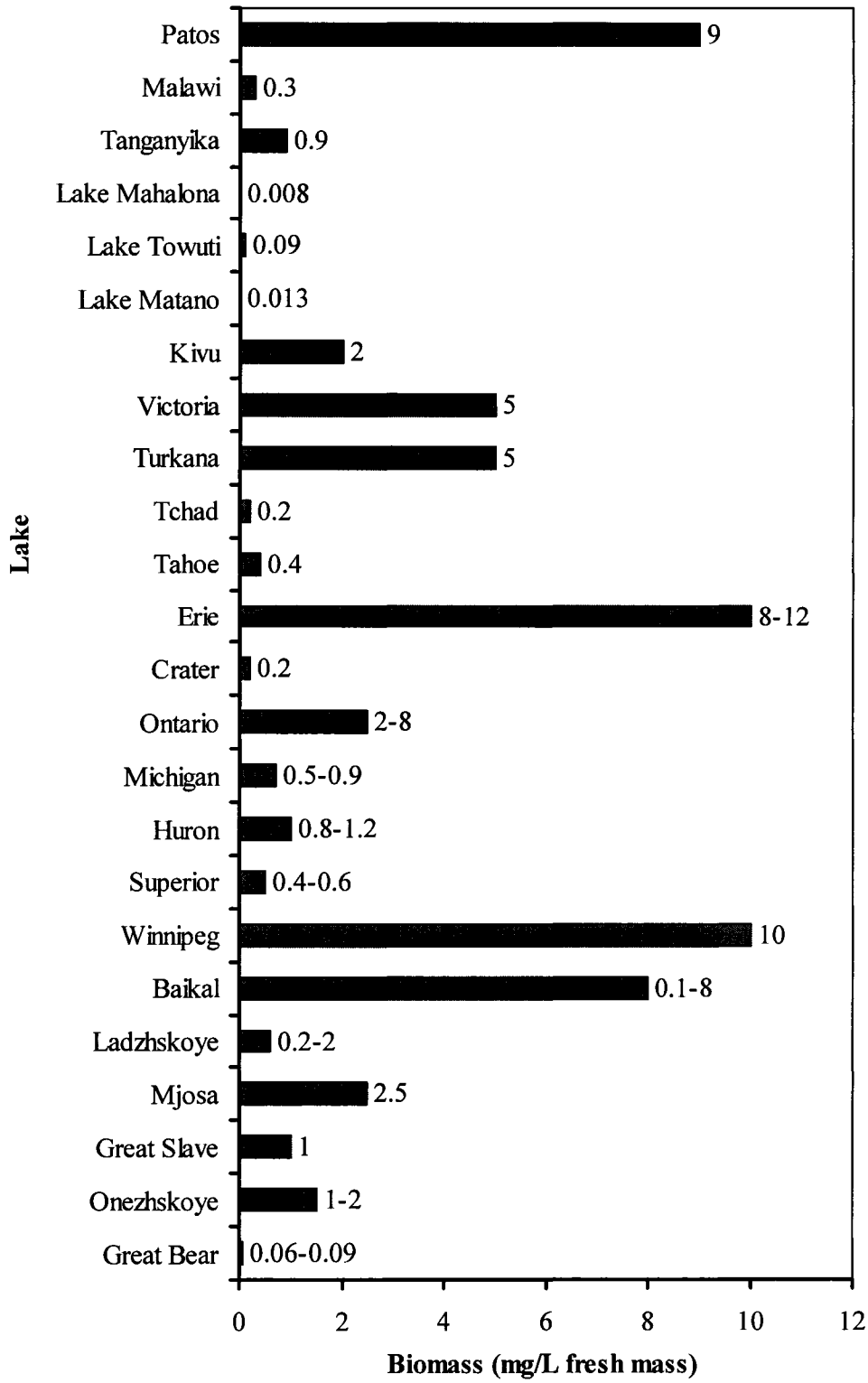

Figure 2.6 Phytoplankton biomasses in the Malili Lakes and Reynold *et al.*'s (2000) lakes.

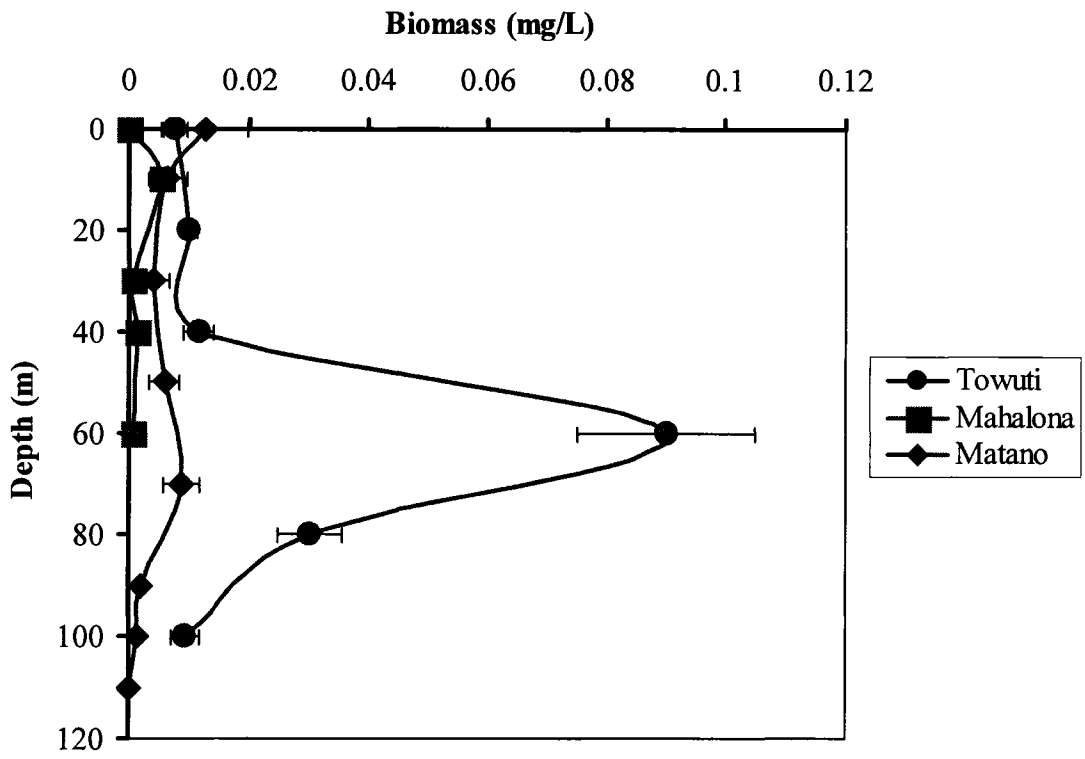

Figure 2.7 Phytoplankton biomass (± se) profiles of the Malili Lakes, with depth, 2004.

CHAPTER 3 – FACTORS LIMITING PRIMARY PRODUCTION IN LAKE MATANO: NUTRIENT LIMITATION

3.1 -- Introduction

The term 'nutrient limitation' describes at least three possible outcomes associated with the influence of nutrients on biological systems: the limitation of the growth of current populations, the limitation of net primary production, and the limitation of net ecosystem production (Beardall *et al.*, 2001; Howarth, 1988). In lakes, the first definition involves the ability of cells to maintain the stoichiometric ratio of C:N:P in the phytoplankton population. Redfield (1958) noted that a 16:1 ratio of nitrogen to phosphorus was statistically optimal for phytoplankton growth. A cellular N:P ratio near 16:1 indicates that conditions are sufficient to produce and maintain viable cells, even if the population inhabits oligotrophic waters (Goldman *et al.*, 1979). In a system where the ratio of N:P is not maintained, adding limiting nutrients may minimize nutrient stress in existing populations.

The second definition of nutrient limitation refers to a limitation of the potential rate of net primary production, whereby the addition of nutrients sustains the overall primary productivity of the system over a specific period of time. This condition may result from a change in community composition. For instance, increasingly eutrophic conditions favor dominance by previously suppressed cells. The third definition, a limitation of ecosystem production and structure, is difficult to quantify and less common (Beardall *et al.*, 2001). Nutrient limitation, as it pertains to net primary productivity, is the most often utilized concept in aquatic biology.

Phosphorus was traditionally considered to be the most important nutrient limiting factor in freshwater systems (Schindler, 1975). More recent investigations of tropical aquatic ecosystems have revealed that tropical lakes were most commonly nitrogen limited (Lewis, 1996). Lewis (2002) reviewed the widespread occurrence of nitrogen limitation on the growth of phytoplankton in tropical lakes, proposing that this trend resulted from either a low external supply of nitrogen to tropical lakes or a high internal loss. Lewis (2002) concluded that a high internal loss was most probable. Although partitioning of N from the atmospheric reservoir allows nitrogen concentrations to 'follow' phosphorus concentrations (Tyrell, 1999), consistently warmer temperatures decrease this effect for two reasons (Lewis, 2002). Firstly, metabolic rates are elevated so that new nitrogen is readily assimilated. Secondly, N_2 is less soluble in water at higher temperatures. Edmond *et al.* (1993) observed that upwelling events in Lake Tanganyika do not effectively regenerate the limited N supply, as NH_3 is readily oxidized to N_2 and lost to the atmosphere.

Lake Matano, an ancient (1-4 million years old) lake in south-central Sulawesi, represents a unique opportunity to provide insight into factors regulating primary production. Global interest in the lake relates to the high levels of endemism observed within the fish (Kottelat, 1990 a, b, c, 1991; Roy *et al.*, 2004), diatoms (Hustedt, 1938, 1942; Bramburger, 2004), and gastropods (Sarasin and Sarasin, 1897; von Rittelen and Glaubrecht, 2003). In spite of this endemism, the lake supports only a sparse phytoplankton community, and species numbers are low (see Chapter 2). The ultramafic catchment of the lake is nutrient-poor (Figures 3.1, 3.2) and the lake is ultra oligotrophic,

as nitrate and phosphate levels rarely exceed detection limits within the upper 100 m. As a result, the lake supports very low standing biomass of phytoplankton (see Chapter 2) and has an impoverished secondary production base (Fernando, 1987), with no top level predators (Haffner *et al.*, 2001). The lake is very deep (>590 m) and steep-sided, greatly restricting the breadth and extent of the littoral zone. There is evidence that mixing occurs to a depth of at least 100 m (see Chapter 1). Thus, a further loss of nutrients from the dissolved nutrient pool is expected as those cells contained within the standing crop are settled out from the euphotic zone. Meanwhile, effective regeneration of cells and their nutrients by physical mixing may be prevented by the depth of the lake and the persistent thermal structure.

Little investigation has been undertaken to elucidate the nature of the forces governing the primary production in Lake Matano. Increasing anthropogenic demands on the lake, as a source of freshwater and a wastewater disposal system, augment the need to clarify the nature of the regulatory forces at work within the lake. The goal of this study was to test the hypothesis that the phytoplankton community of the lake is nutrient limited.

3.2 – Materials and Methods

To investigate the potential role of phosphorus and nitrogen in limiting primary production, nutrient addition studies were made to determine the response of the natural phytoplankton assemblage to changes in the relative proportions of N and P.

3.2.1 Phytoplankton inoculum

The low standing biomass of phytoplankton in Lake Matano (<0.01 mg/L) (see Chapter 2), necessitated the use of a culture medium to yield an effective quantity of newly growing native cells. This medium was dilute (10%) Bold's Basal solution. Surface water was collected at mid-day from a central surface location in the open waters of Lake Matano (S02°28.124, E121°18.893). Ten millilitres of lake water were inoculated into the sterile culture media. The culture and an un-inoculated control were exposed to sun-lit conditions for two weeks until visible growth in the culture was ascertained by visual and microscopic observation. To ensure that the inoculum consisted of elevated densities of taxa from the natural assemblages of Lake Matano, 2 mL of the culture were inoculated into a second sterile tube of the same media and allowed a growth period of three days prior to inoculation at the start of the experiment.

3.2.2 -- Experimental water

Water from a depth of 1 m was obtained for culture water, using a Kemmerer sampler at a central lake location. This experimental water was filtered with an 80 μm mesh filter to remove interference by zooplankton and the larger colonial phytoplankton.

Nutrient enriched treatments and controls were performed in triplicate and consisted of 100 mL of the filtered Lake Matano surface water in transparent plastic jars. Nutrient additions consisted of either phosphorus or nitrogen, or both. Phosphorus growth limitation was tested using additions of KH_2PO_4, to provide enrichments of 0 (reference), 0.807, 1.61, 3.23 μM phosphorus (Table 3.1). Growth enrichments of 0 (reference), 1.42,

3.55, and 5.40 μM nitrogen were performed using additions of NH_4NO_3 (Table 3.1). Concentrations chosen for enrichment were based on literature values for algal cultures.

A set of replicate cultures containing a common bottled drinking water (Aqua brand) as a culture medium was also introduced. This treatment constituted an experimental out-group to control for the possibility of factors limiting nutrient bioavailability in Lake Matano. Analysis of the bottled drinking water was conducted using a Bran and Luebbe continuous flow autoanalyzer, which employs a hydrazine sulfate reduction method for nitrate/nitrite and automated ascorbic acid reduction method for phosphate (Eaton *et al.*, 1995). The water contained 139 μM nitrate and 4.26 μM total phosphorus.

Before inoculation, the fresh culture medium was centrifuged at 3000 rpm for 10 minutes. The plankton pellet was gently removed from the medium and placed in a second tube of a sterile medium, then mixed into 45 mL of lake water to wash the cells (repeated three times), before a final centrifugation. The water was poured off and the phytoplankton pellet was homogeneously mixed into 100 mL of lake water. One milliliter of this inoculum was added to each treatment, and each jar was placed in a partial sun location for the duration of 21 days. Throughout the course of the experiment, the cells were kept suspended by regular mixing. Culture jar openings were covered with several layers of Kim wipes held in place with elastic bands to permit gas exchange and to minimize contamination. Twenty mL sub-samples were removed just after inoculation and at the termination of the experiment (21 days). Samples were immediately preserved with Lugol's solution for cell counts and determination of composition. A loss of

replicates during transportation back to Canada precluded analysis of the full suite of treatments and treatments with only three intact replicates are presented in the analysis.

Cell counts and composition studies were conducted using a Neubauer cell chamber, observed with a Leica® inverted microscope at 400x magnification. Three replicate counts were performed on each sample. Phytoplankton in five of the large etched squares of the counting chamber were enumerated and abundance was calculated based on the volume counted. Biomass was calculated using the volumetric relationships established by Sun and Liu (2003).

The mean and standard errors of these counts were calculated to assure reasonable estimates of actual abundance. To test for initial even distribution of the inoculum, an ANOVA was performed on results from samples taken immediately after inoculation. To elucidate whether diversity was affected by treatment, phytoplankton were identified to genus and Simpson's Diversity (1-D) Index was calculated for each replicate. Individual numbers in representative genera were plotted for each replicate for presentation of relative community composition. A one-way ANOVA determined whether significant variation existed between biomass yields at the duration of the experiment.

3.3 – Results

Cell counts from samples taken immediately after inoculation did not show significant differences in either treatment or control. Mean biomass in the cultures, after inoculation, was determined as 2.5 mg/L (se=2.0).

In samples taken on day 21, the most pronounced effect was observed in the out-group treatment, which used bottled water as a growth medium (Figure 3.3). A highly significant difference ($p<0.01$) was observed in phytoplankton counts from cultures incubated in bottled water. At the end of the experiment, the mean biomass in the bottled water cultures was 97 mg/L (se=25), with a relative cell abundance of 1.1×10^7 cells/mL ($se=1.8 \times 10^5$). This finding represented a cell growth rate several orders of magnitude above that determined in the other treatments associated with Lake Matano water.

Differences also occured between the cultures of lake water (Figure 3.4). Visible growth became evident in the cultures with the highest additions of P and N, beginning near day 11. No obvious growth was observed in the cultures with intermediate nutrient enrichments of P and N. From cultures with an enrichment of 3.23 μM/L P, the final cell count was 1.3×10^6 cells/mL ($se=1.8 \times 10^5$), while an abundance of 3.3×10^5 cells/mL ($se=4.4 \times 10^4$) was determined in reference cultures. These abundances correlated with biomass estimates of 1.3 mg/L (se=0.69) and 0.14 mg/L (se=0.068), respectively. In comparison, the mean abundance in the cultures enriched with nitrogen was 4.7×10^5 cells/mL ($se=8.2 \times 10^4$), producing a mean biomass yield of 0.87 mg/L (se=0.66).

Simpson's Diversity Indices did not vary significantly among treatments (Table 3.2), although a slightly greater diversity was observed in the bottled water. In cultures of lake water, several changes occurred in the phytoplankton assemblages between initial and final treatments. The culture compositions, just after inoculation and on day 21 of the experiment, are presented in Figure 3.5. The distributions of the phytoplankton

immediately after inoculation represented heterogeneous cell mixtures, with no significant differences in the dominant species. After 21 days, the composition of the phosphorus enriched cultures exhibited higher abundances of most cells, with *Chlorella* being the dominant genera. In contrast, no species emerged as significantly dominant in the nitrogen enriched or reference cultures and only an increase in abundances from initial numbers occurred.

3.4 – Discussion and Conclusions

The addition of 3.23 μM P significantly increased the biomass and abundance of phytoplankton relative to that of the control. Although not statistically significant, increases in biomass and abundance also occurred in the cultures enriched in 540 μM N and both N and P (3.55 and 1.61 μM, respectively).

Simpson's Diversity Indices showed no significant differences between the nutrient enriched cultures and the reference cultures. The increase in diversity did not necessarily follow the overall increase in productivity. Community composition did show varying trends among cultures at the end of the experiment. While changes in the dominant species did not occur in reference and nitrogen enriched cultures, the phytoplankton assemblage in the phosphorus enriched communities reflected a major shift in the dominant plankton. The predominance of *Chlorella* in the phosphorus enriched cultures suggests a modification from the dominant phytoplankton assemblages observed *in situ* (see Chapter 2), where non-N-fixing cyanobacteria dominate the water column. The results indicate that while nitrogen addition alleviates a growth restriction

on the current populations, increased availability of phosphorus may cause a shift in community composition and production potential.

The evidence for phosphorus limitation in Lake Matano contradicts the hypothesis that tropical lakes are fundamentally limited only by nitrogen. The significant growth response in the phosphorus enriched cultures suggests that phosphorus levels in Lake Matano are below Redfield levels, despite the depletion of nitrogen associated with tropical systems. Low external inputs of phosphorus from weathering of the metal-rich and nutrient-poor ultramafic catchment will maintain the oligotrophic status of the lake. In addition, high levels of iron in Lake Matano might prevent effective internal regeneration of P from the hypolimnion. Internal phosphorus cycling has been closely linked with that of iron, suggesting that determining phosphorus availability requires knowledge of the iron dynamics in a system (Lijklema, 1980). Soluble iron released from the sediment under anoxic conditions oxidizes at the oxic-anoxic boundary and forms colloidal interactions (Buffle *et al*, 1989). Upwelling phosphorus may be readily incorporated into the iron oxyhydroxy colloids formed in this process (Blomqvist and Gunnars, 1997). The colloids aggregate and sink out of the epilimnion, scavenging available phosphorus. The dissolved iron profile of Lake Matano (Figure 3.6) shows iron concentrations at detection level in the epilimnion, but peaking to 80 μM below 100 m. This profile is consistent with reduction of colloidal Fe III at the oxic-anoxic interface, producing a peak in soluble Fe II. The peak in Fe II also corresponds with the phosphorus peak, which would be predicted if reduction of iron was correlated with the release of bound phosphorus. It has been demonstrated that reduction in phosphorus regeneration is

significantly correlated to an Fe:P ratio of approximately 2:1 (Blomqvist, 2004). In Lake Matano the high potential for iron binding is signaled by an Fe:P ratio of nearly 50:1 at the oxic-anoxic boundary (Figures 3.1 and 3.6).

It is also noteworthy that noticeable growth response occurred only after a lag time of greater than 11 days. Furthermore, the effect of either phosphorus of nitrogen addition to the cultures of Lake Matano water was not as pronounced as might be predicted if the limiting nutrient were suddenly in supply at 5-20 times the ambient water concentration. The low ambient nutrient concentrations suggest that nutrient restriction may be an important factor limiting the standing biomass of phytoplankton.

3.5 -- References

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Table 3.1 Nutrient additions made to algal cultures of surface water from Lake Matano. Treatments lost during transport to Canada are labelled not available. Analysis considers only treatments with three treatments available.

# of Replicates	Enrichment (μM P)	Enrichment (μM N)	Replicates in Final Analysis
3	0	0	3
3	0	1.42	NA
3	0	3.55	NA
3	0	5.40	3
3	0.807	0	NA
3	1.61	0	NA
3	3.23	0	3
3	1.61	3.55	NA
3	0.807	3.55	NA
3	1.61	1.42	NA

Table 3.2 Simpson's Indices (1-D) observed in each treatment at the end of the experiment.

Treatment (μM)	Simpson's Diversity Index (1-D)
Control	0.58
3.23 P	0.56
5.40 N	0.56
Bottled water	0.68

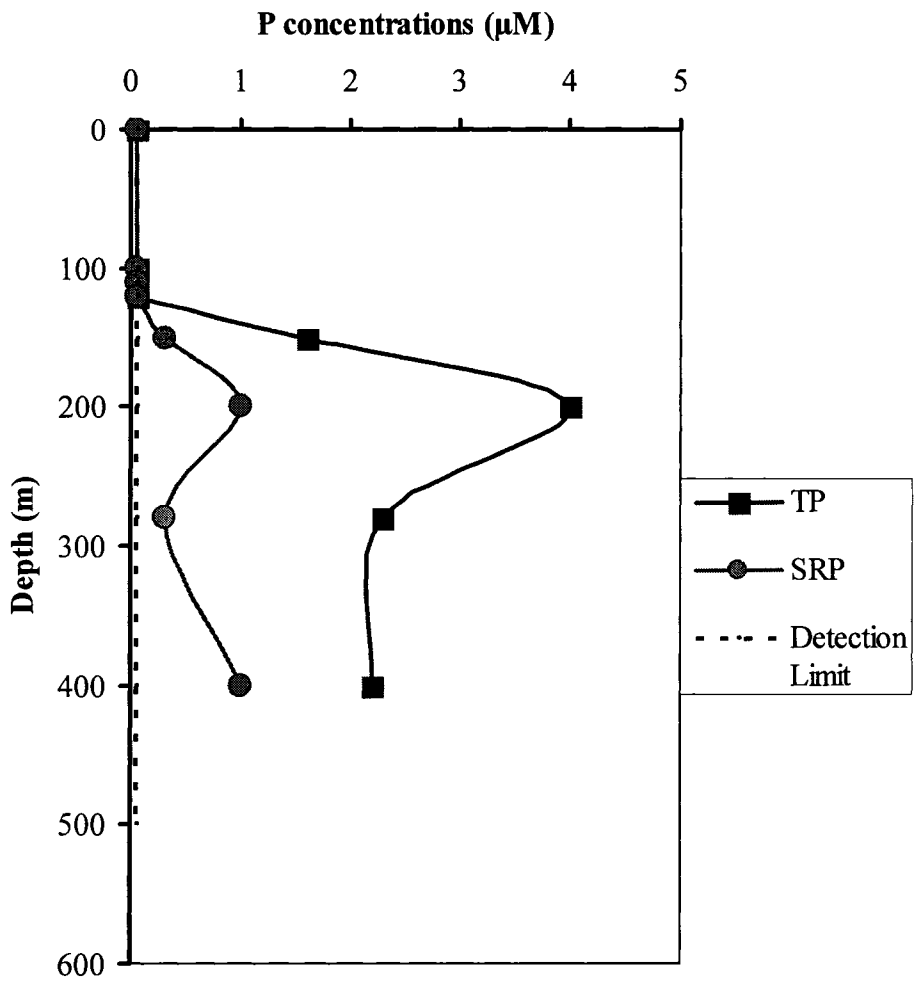

Figure 3.1 Total Phosphorus (TP) and soluble reactive phosphorus (SRP) measured with depth, Lake Matano, 2002. These nutrients are below detection level in the upper 100 m.

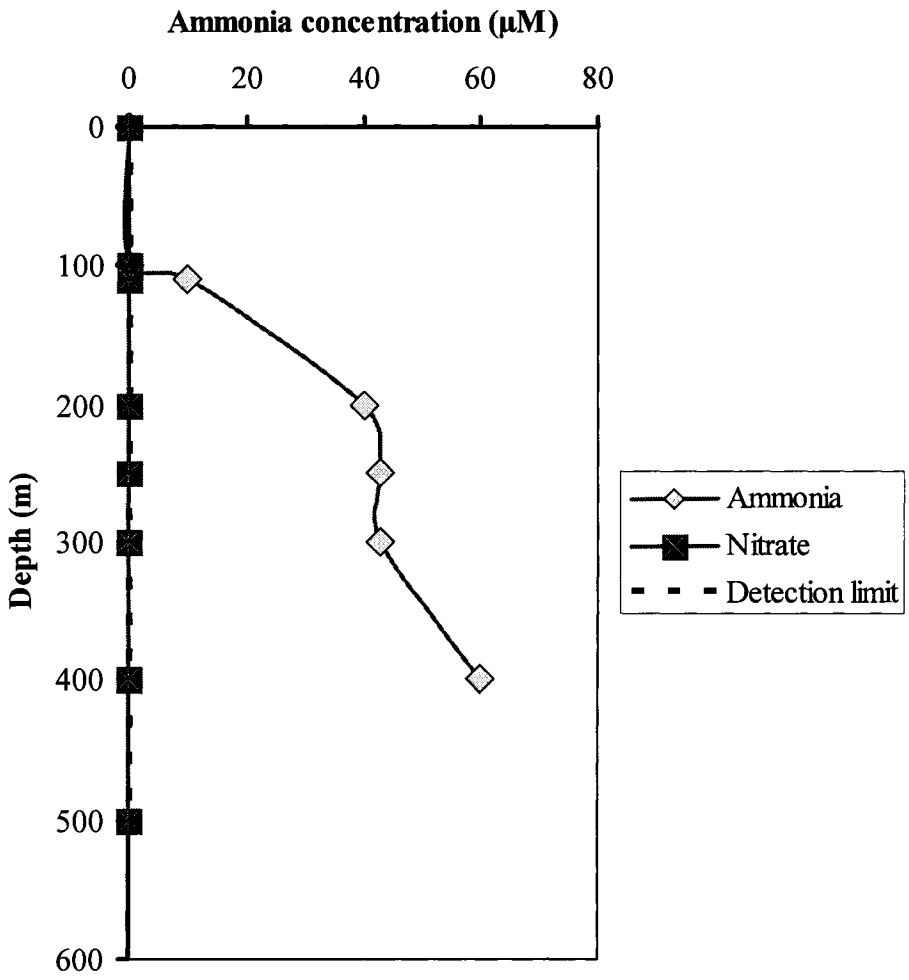

Figure 3.2 Ammonia and nitrate measured with depth, Lake Matano, 2002. These nutrients are below detection level in the upper 100 m.

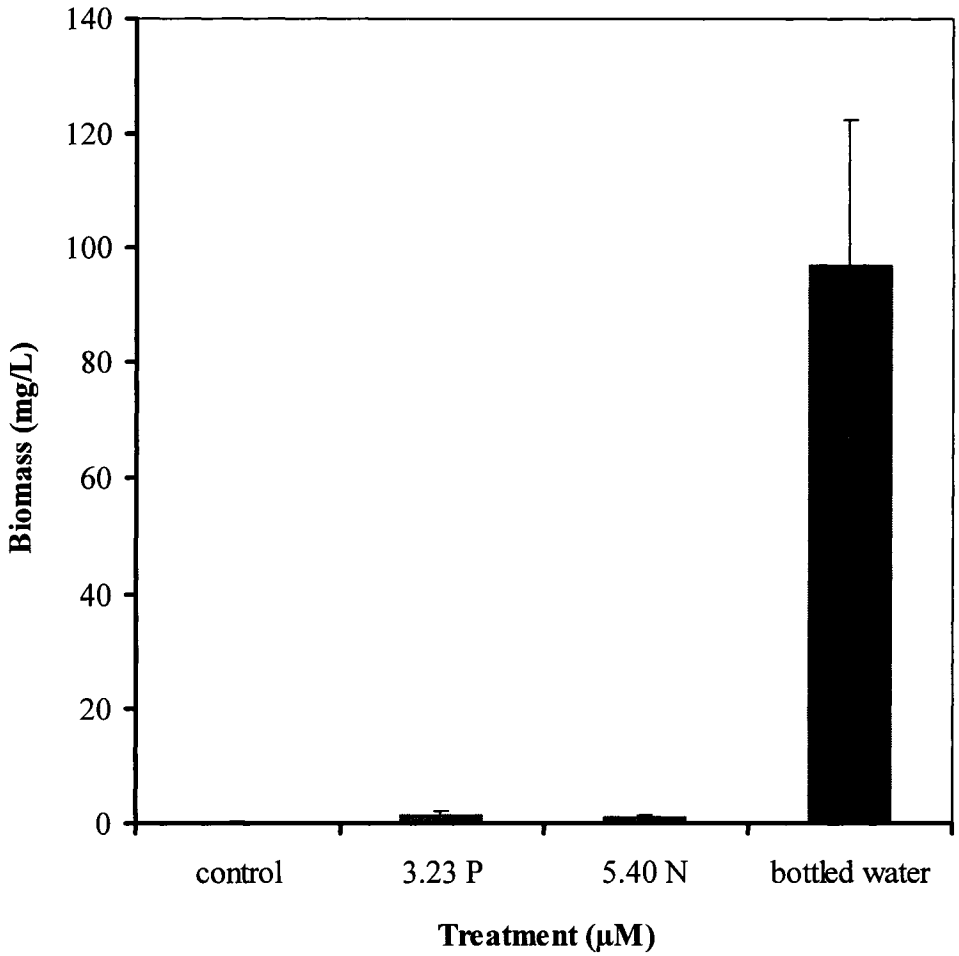

Figure 3.3 Total phytoplankton biomass (± se)observed in enrichment cultures and control after 21 day incubation, including bottled water outgroup. Growth is significantly ($p<0.01$) greater in bottled water outgroup.

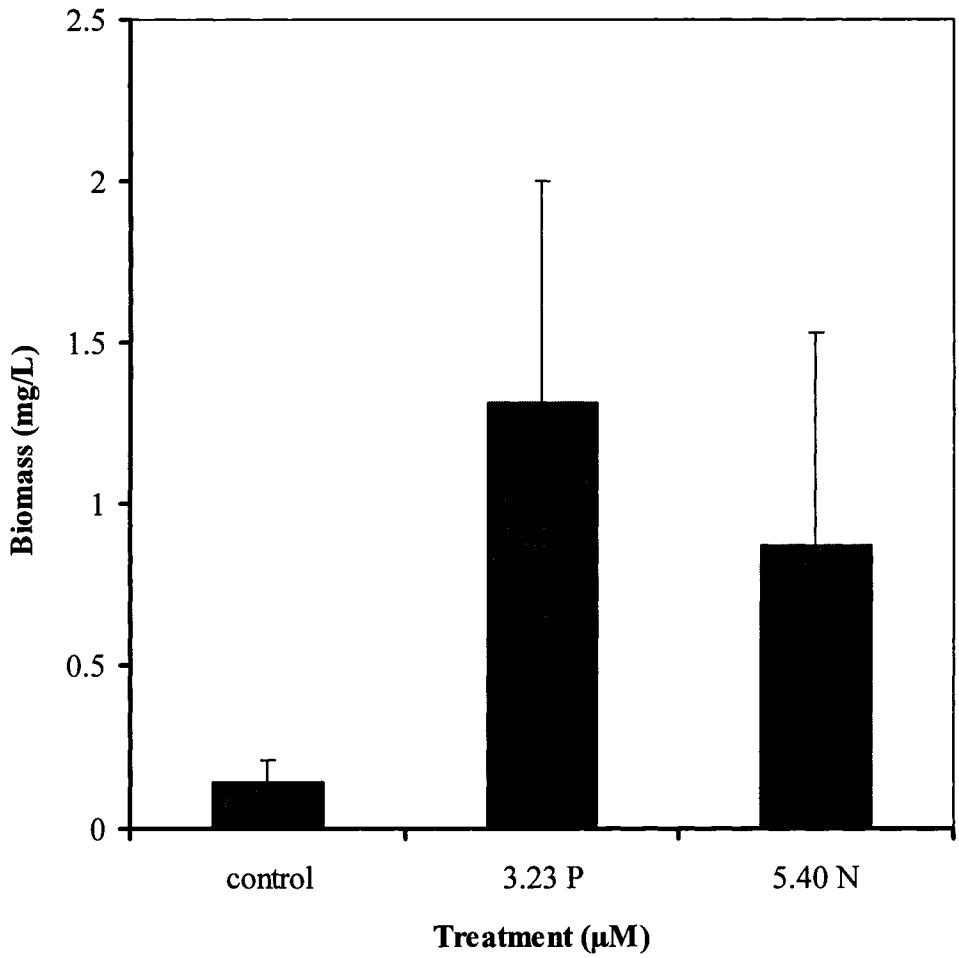

Figure 3.4 Total phytoplankton biomass (± se) observed in enrichment cultures and control after 21 day incubation, excluding bottled water outgroup. Growth is significantly ($p<0.05$) greater in cultures enriched with highest concentrations of phosphate.

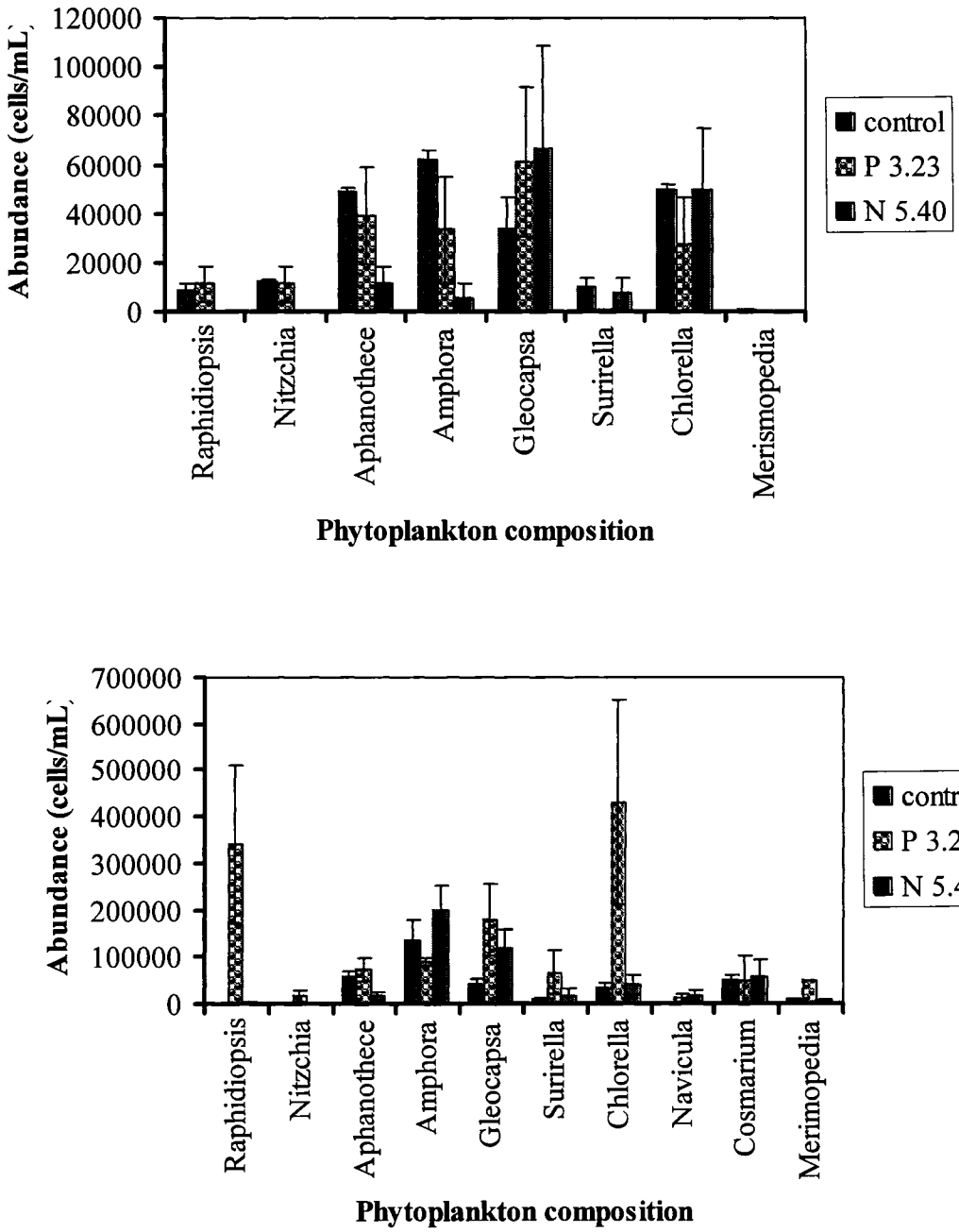

Figure 3.5 Abundance of genera (± se) in each treatment shows phytoplankton distribution among treatments (excluding outgroup). From top to bottom: immediately after inoculation genera were evenly distributed; after 21 days, a species shift occurred in phosphate enriched cultures.

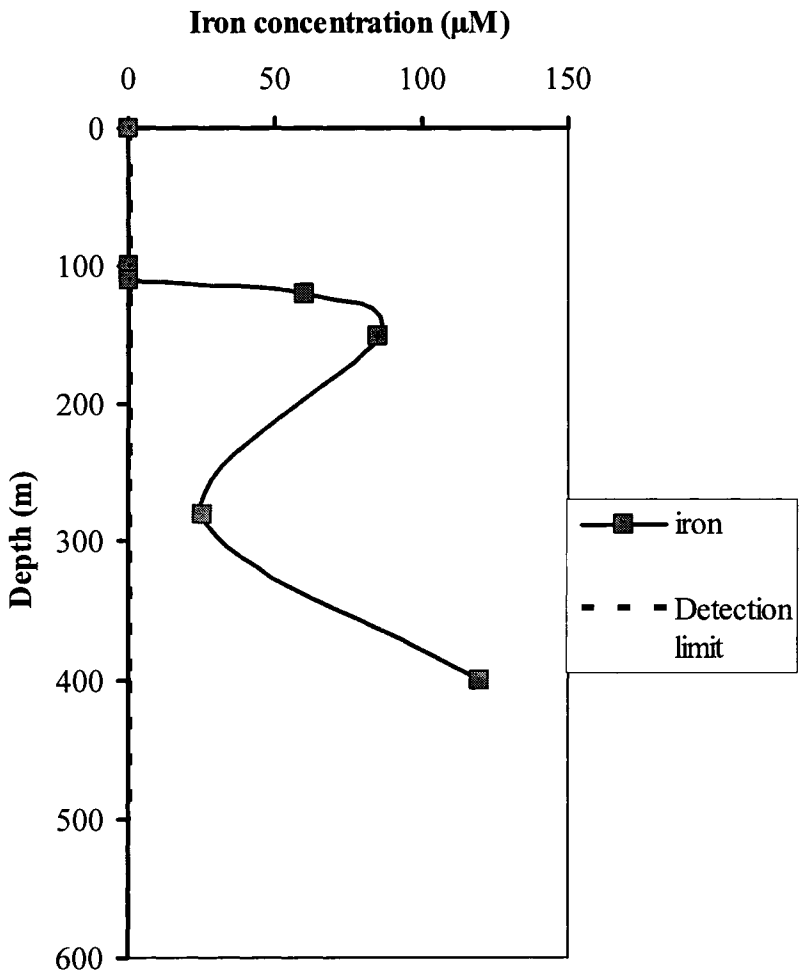

Figure 3.6 Dissolved Fe with depth, Lake Matano, 2002 (Haffner, unpublished data, analysis by ICP-MS).

CHAPTER 4 – FACTORS LIMITING PRIMARY PRODUCTION IN LAKE MATANO: METAL TOXICITY

4.1 – Introduction

In 2000, Myers *et al.* named Indonesia one of 25 global hotspots for conservation priorities, based on the twofold criteria of exceptional biodiversity and high habitat loss. Lake Matano, Sulawesi Island, Indonesia, is ancient (approximately 1-4 million years old) and harbours a biological assemblage in which endemic species comprise an estimated 80% of the total assemblage (Haffner *et al.*, 2001). Only in the past decade have detailed limnological investigations on the lake commenced. The importance of obtaining a better understanding of the factors regulating biological production in this unique system is amplified by recent increases in anthropogenic activity in and around the lake. The development of serious demands on the system, as a source of freshwater and wastewater for mining runoff disposal system, poses potential threats to the highly endemic communities of Lake Matano.

Elevated endemism in Lake Matano does not correlate with either trophic complexity or rich speciation. The lake does not support a top piscivore trophic level (Roy *et al.*, 2004), nor is there a significant level of benthic production (Bramburger, 2004). Productivity in the lake is constrained, and biomasses of primary and secondary production have been consistently measured at very low levels (Lehmusluoto *et al.*, 1997; see Chapter 2 and 5). Attempts to enhance fish yield through aquaculture have met with limited success (Whitten *et al.*, 2001).

Proposed constraints on production in Lake Matano have included nutrient limitation (Haffner *et al.*, 2001; Chapters 1 & 2) and metal toxicity (Fernando, 1987; Lehmusluoto *et al.*, 1997; Haffner *et al.*, 2001). Lake Matano's ultramafic catchment and sediments contain a rich supply of heavy metals (Haffner *et al.*, 2001), particularly chromium, and are also very depleted in phosphorus and nitrogen. With respect to nutrient limitation, there is evidence that the oligotrophic status of the lake restricts primary production (Haffner *et al.*, 2001; Chapters 1& 2). Alternatively, toxicity might also limit production dynamics. In a review of the lakes of Southeast Asia, Fernando (1987) proposed that the plankton community was depauperate in significant taxa due to Lake Matano's having the highest levels of chromium recorded for the surveyed lakes of southeast Asia.

Chromium is a trace metal in most soils. It may also be introduced into the environment through industrial effluent (Ellis *et al.*, 2002). Chromium occurs naturally at very high levels in Lake Matano's bedrock. The concentration of total chromium in the oxygenated waters of Lake Matano has been measured at 293 nM (see Figure 4.1). Chromium exists most stably in the reduced, trivalent form or in the oxidized hexavalent form (Cotton and Wilkinson, 1988). The toxicity of chromium in the environment depends largely on its oxidation state, although research on the oxidation states of chromium is hindered by the difficulty of determining speciation. Unlike chromium (III), chromium (VI) forms anionic complexes and soluble compounds and is highly mobile and bio-reactive (Ellis *et al.*, 2002). It is associated with cytotoxic, genotoxic and mutagenic effects (Canivet *et al*, 2001) and may require industrial remediation if it

exceeds the drinking water guideline of 0.96 μM . Gorbi *et al.* (2004) found that *Daphia magna* experienced a significant reduction in both growth and survivorship when exposed to levels of chromium as low as 270 nM chromium (VI) over a period of several weeks. Cr VI was found to completely inhibit the growth of *Scenedesmus acutus* at concentrations of 19 μM (Gorbi and Corradi, 1993). Under subdued light at Cr VI concentrations from 19 to 192 μM, the algal cells survived longer than those exposed to normal light conditions. This finding was ascribed to decreased rates of Cr uptake in the cells under subdued light conditions, implicating Cr-uptake as an energy-dependent process (Gorbi *et al.*, 2004).

To date, no studies have been conducted to test the hypothesis that metal toxicity limits primary production in Lake Matano. This study utilizes a novel bioassay approach to determine whether reducing the ambient concentrations of a suite of metals, including chromium, in natural lake water, results in changes in the abundance and composition in the phytoplankton community of Lake Matano.

4.2 – Materials and Methods

Two experiments were implemented to test whether primary production was limited by metal toxicity (Experiments A and B). In addition, the effect of nutrient addition in conjunction with toxicity treatments was tested (Experiment B). Treatments for both experiments are summarized in Table 4.1.

4.2.1 Experiment A

The dissolved chromium profile for Lake Matano demonstrates that levels of chromium peak in the epilimnion, but reach detection level below the oxic-anoxic boundary. To obtain culture water from Lake Matano with significantly reduced levels of chromium, water from a depth of (approximately) 150 m was obtained using Kemmerer samples at a central lake location (S 02°28.124, E 121°18.893). Immediately after water collection, samples were taken from the Kemmerer and analyzed for Nitrate/Nitrite, orthophosphate and Fe (II)/Fe (III) concentrations with the Hach Ferrozine ® method, using 1,10 Phenanthroline as an indicator. Culture water was collected by overflowing water directly from the Kemmerer into glass jars, which were immediately sealed for transportation to the laboratory. Here, under minimal oxygen exposure, the water was filtered with 0.45 μm syringe filters to remove insoluble Cr (III) particles that could oxidize to Cr (VI). As Lake Matano contains high levels of other metals such as iron (see Chapter 3), the second stage of the metal removal process consisted of flocculation of these metals under oxygenated conditions. Moreover, aeration was intended to promote oxidation of Fe (II) to Fe (III) for coagulation with any remaining Cr (III). The filtrate was bubbled with oxygen for 60 hours. Finally, a second 0.45 μm filtration removed the resulting oxidized metal precipitates.

At the end of the metal removal process, a fresh phytoplankton collection was obtained from multiple surface sweeps at the central lake location, using a 64 μm-mesh plankton net. This plankton slurry was then passed through a 250μm mesh net to remove the larger zooplankton. Subsamples from both the experimental and surface waters were

collected and stored in triplicate 20mL vials and sealed for subsequent analysis of metal concentrations by ICP-MS.

The experimental set-up consisted of culture jars containing metal-removed water (MRWa) and surface water (SWa) in replicates of three. Jars were randomly distributed in a semi-sun location. The volume of fresh phytoplankton biomass was thoroughly yet gently mixed and distributed among the jars in equal allotments of 1mL.

Throughout the experiment, the cells were kept evenly suspended by regular mixing, and subsamples were removed just after inoculation, after seven days, and at 21 days, marking the termination of the experiment. The duration of the experiment was based on several pilot studies using surface water cultures, in which no growth occurred in cultures that had been maintained from three days to two weeks. Subsamples were preserved in Lugol's iodine for determination of phytoplankton abundance and composition.

4.2.2 -- Experiment B

The process for metal removal (in Experiment A) was repeated in Experiment B, with two modifications to experimental design. Firstly, to collect an adequate population of growing cells from the low standing biomass observed in Lake Matano, phytoplankton cells from Lake Matano were incubated in dilute Bold's Basal Media (10%) (see Chapter 3) for three weeks prior to initiation of this experiment. After dense growth was ascertained by visual and microscopic observation, 2mL of the culture were inoculated

into a second tube of the same medium. Incubation in this medium for a period of three days prior to the start of the experiment provided an inoculant of non-nutrient limited newly growing cells.

The second modification consisted of the addition of nutrients to replicate pairs of treatment and reference water. Nutrient enriched cultures received simultaneous additions of phosphate and nitrate in enrichments of 1.61 and 1.42 μM, respectively. These enriched cultures were compared with pairs of metal removed water and surface water that had not been enriched with nutrients. Therefore, this experiment tested pairs of replicates of surface water (SWb) and metal removed water (MRWb) as well as replicates of nutrient enriched metal removed (MRNW) and surface water (SNW). Table 4.1 presents the treatments and replicates in Experiment A and B.

Before inoculation, the fresh phytoplankton culture was centrifuged at 3000 rpm for 10 minutes. The plankton pellet was gently removed from the media and placed in a second sterile tube. The pellet was washed twice by mixing it into 45mL of lake water and centrifuging. The resulting plankton pellet was distributed within a small amount of surface lake water. One milliliter of this inoculant was added to each treatment. These cultures were maintained in part sun for the duration of seven days, a length of time that was determined by the appearance of a visible growth response. Subsamples were removed just after inoculation and at termination of the experiment.

4.2.3 – Analysis

Cell counts and composition studies were conducted using Neubauer cell chambers, observed with a Leica® inverted microscope at 400-600x magnification. Volumetric measurements, based on Sun and Liu (2003) were supported by Openlab® 3.15 image analysis software for conversion to biomass. Subsamples each received five separate counts. The mean of the counts was taken for treatment comparison. In experiment A, a t-test was used to test the null hypothesis that the phytoplankton biomasses in the SWa cultures were not different than the biomasses in the MRWa groups. In experiment B, an ANOVA was used to determine whether a difference existed in the biomasses obtained from the SWb, SNW, MRWb and MRNWb cultures.

4.3 -- Results and Discussion

The metal removal process consisted of three steps: immediate filtration under conditions of minimal oxygen exposure, flocculation during oxygenation of the filtrate, and a second filtration. Visible precipitation occurred during the second step. As shown in Table 4.2, the procedure decreased levels of the redox metals Cr, Ni, Fe and Mn, while levels of Na, K, Ca and Mg were conserved. Ferrous iron, present at 44 μM in the MW, declined to 0.1 μM after metal removal. Chromium concentrations declined from 184.1 nM in the 150 m water to 48.5 nM in this water after metal removal (MRW). In comparison, chromium occurred at 236.4 nM in the surface water of Lake Matano. Nickel concentrations were 61.4 nM at the surface and 42.6 nM in MRW.

In aqueous solution, Cr (VI) exists as CrO_4^{2-}, $HCrO_4^-$, $Cr_2O_2^{2-}$ (Richard and Bourg, 1991). Previous investigations have demonstrated that ferrous iron may reduce Cr (VI), resulting in precipitation or coagulation and removal from solution (Fendorf and Li, 1996; Sedlak and Chan, 1997). The metal removal process in this experiment is similar to methodology under development for remedial removal of Cr (VI) from drinking water (Qin *et al.*, 2005). Qin *et al.* constructed a reduction/coagulation/filtration (RCF) pilot system that decreased Cr (VI) concentrations of 1.92 μM to below detection level. The study showed that the ferrous sulfate was an effective reducing agent in the RCF system at neutral pH (Qin *et al.*, 2005). The efficacy of the filtration step was enhanced by factors such as low filtration rate and high Fe:Cr ratio (Qin *et al.*, 2005). Although ongoing research is necessary to elucidate speciation and the presence of reductants, a similar reduction mechanism predicted to occur near the oxic-anoxic zone in Lake Matano. In this experiment, collection below the oxic-anoxic boundary utilized the decline in soluble Cr observed in previous sampling. Further decrease in Cr levels resulted from deoxygenated filtration, aeration and oxygenated filtration.

Therefore, the experiment tested for the influence of metal removal on the growth potential of Lake Matano water. Some caution is necessary in experimental interpretation as the use of hypolimnetic water also increased the concentrations of alkaline and conservative cations relative to surface water. This phenomenon confounds the direct correlation of growth effects to metal removal. As well, analysis of the chromium profile with depth indicates that at 150 m, chromium is below detection level.

4.3.1 -- Experiment A

Mean relative phytoplankton abundance at day 0 ranged from 1.6×10^5 (se=2.2×10^4) cells/mL in the MRW to 1.8×10^5 (se=9.3×10^4) cells/mL in the SW, indicating that the inoculum was well distributed. The Simpson's Diversity Indices (1-D) revealed no difference in starting conditions between the SW and MRW (Table 4.3).

Moderate changes in cell abundance and biovolume occurred in the cultures from day 0 to day 7. Total abundance of cells in MRW cultures increased marginally to 1.1×10^6 (se=3.9×10^5) cells/mL, while total abundance in the SW was measured at 4.1×10^5 (se=1.7×10^4) cells/mL. On day 21 of the experiment, the abundance of phytoplankton grown in cultures with MRW was significantly greater ($p<0.05$) than that determined in cultures of SW (Figure 4.2). The mean abundance of cells in the MRW was 1.4×10^8 (se=1.7×10^6) cells/mL. The mean abundance of cells in the SW was determined at 5.2×10^6 (se=2.7×10^6) cells/mL. Final (21 day) biomasses demonstrated that the phytoplankton yield was significantly greater in the MRW cultures than in the SW. The mean final biomass in the MRW was 0.10 (se=0.014) mg/mL. In contrast, the mean final biomass in the SW was only 0.021 (se=0.0069) mg/mL.

Although production varied significantly between treatments, final diversity was similar between the SW and MRW cultures. This finding is demonstrated in the Simpson's Diversity Indices for each treatment (Table 4.3). The abundance of the phytoplankton genera present in each culture did not correlate with treatment. Diversity decreased in both SW and MRW from day 0 to day 21 as a result of predominance by

several taxa. In both cases, the predominant taxa in the final treatment was *Aphanothece,* followed by *Surirella* (Figure 4.3). *Aphanothece* is a dominant member of the pelagic phytoplankton community and *Surirella* is a common member of the periphyton, thus the treatment did not appear to provide toxicity release to specific genera.

The growth response of the phytoplankton in both SW and MRW was slower than predicted. Although a moderate increase in cell numbers was observed from the point of inoculation to the seven day subsamples, this effect was not significant.

4.3.2 -- Experiment B

Initial diversity values, presented in Table 4.4, were also comparable among cultures. Diversity in the surface cultures decreased, while diversity in the treated water increased. An increase in diversity was observed in the treated water without nutrient addition relative to enriched treated water and to SW, with and without nutrient enrichment. The higher abundance of cells overall in the MRW cultures would slightly elevate the diversity indices. However, in all cultures, the dominant phytoplankton assemblage remained consistent (Figure 4.5). *Aphanothece* and *Cosmarium* predominated throughout all treatments. These species correspond to dominant phytoplankton observed *in situ.*

Immediately following inoculation, mean cell counts in MRWb and MRNW were 1.2×10^6 (se=1.6×10^4) and 1.3×10^6 (se=8.9×10^4) cells/mL, respectively. Associated biomasses were 4.0×10^{-5} (se=1.9×10^{-5}) mg/L and 2.6×10^{-5} (se= 9.2×10^{-6}). Cell

abundance in the SNW and the enriched SW were 1.3×10^6 (se=1.6×10^4) and 1.9×10^6 (se=8.6×10^4) cells/mL, with biomasses 5.7×10^{-5} (se=3.7×10^{-5}) and 7.7×10^{-5} (se= 5.6×10^{-5}).

After the seven day period, a positive growth response had occurred in the MRW and MRNW cultures relative to the SW cultures, although this effect was not significant at the $p<0.05$ level (Figure 4.4). Although nutrient enrichment did not enhance growth at statistically significant levels, the greatest biomass was determined in the MRNW cultures. In the MNRW cultures, the mean cell abundance at the duration of the test was 3.8×10^7 (se=5.0×10^3) cells/mL, with biomass 2.4×10^{-3} (se=0.00038×10^{-4}) mg/L. Abundance and biomass in the MRW cultures were 2.7×10^7 (se=1.8×10^4) cells/mL and 2.4×10^{-3} (se=3.9×10^{-4}) mg/L. In the SNW cultures, mean cell abundance at the end of seven days was 2.0×10^7 (se=1.5×10^4) cells/mL, with a biomass of 1.5×10^{-3} (se=4.0×10^{-5}) mg/L. Abundance in the SW cultures was 2.4×10^7 (se=1.4×10^4) cells/mL.

Improvement in phytoplankton growth rates occurred in the cells cultured in experiment B relative to those observed in experiment A. The use of the BBM provided an inoculum for experiment B that contained a quantity of cells more than an order of magnitude greater than that contained in the inoculum for experiment A. Potentially, the BBM stimulated growth by relieving a nutrient limitation or by providing release from toxicity. The cells from this inoculum would then be healthier than those taken directly from Lake Matano. Therefore, using this inoculum may have dampened the magnitude of cellular response to treatment type between cultures during the seven day period.

4.4 -- Conclusions

Phytoplankton composition remained the same between the MRW and the surface water cultures in both experiments. The lack of any difference in the phytoplankton assemblages between the MRW cultures and the SW cultures in both experiments suggests that toxicity release was not operative in defining the community assemblage. Rather, the cells cultured represented the indigenous phytoplankton community, and growth rate of the dominant members of the community was enhanced under the conditions provided by the MRW.

In Experiment A, phytoplankton directly from the lake were inoculated into treatments of SW and MRW. The growth response of the phytoplankton in both SW and MRW was slow. Increase in cell numbers from the point of inoculation to the seven day subsamples was negligible and there was no significant difference between treatments at this time. By the conclusion of this experiment, a significant increase in cell numbers and biomass was determined in MW relative to SW.

Implementation of the dilute BBM to culture phytoplankton from the lake as an inoculum in Experiment B improved cellular growth rates and final yields in all treatments over the seven days. Biomass and cell numbers were elevated in the MRW and MRNW relative to the SW and SNW, although not at statistically significant levels. The dampened response may be a result of inoculation from the BBM. As well, the

shorter duration of the experiment may have influenced the magnitude of the difference in growth responses to the treatments.

The relative influences of potentially confounding factors such as changes in the status of trace nutrients, were not resolved in this experiment. However, these experiments demonstrate enhanced growth potential in Lake Matano water after metal removal. In addition, this study demonstrates relatively restrained growth in the surface water of Lake Matano. These results are consistent with toxicity release though lower levels of metals such as Cr, Fe and Ni in the MRW and substantiate the need for further research.

4.5 -- References

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Table 4.1 Treatments and corresponding abbreviations, days on which cultures were subsampled (for composition and biomass) and number of replicates in experiments A and B.

Experiment	Treatment	Abbreviation	Days subsampled	# Replicates
A	Surface water	SWa	0, 7, 21	3
A	Metal removed water	MRWa	0, 7, 21	3
B	Surface	SWb	0, 7	3
B	Surface + Nutrient	SNW	0, 7	3
B	Metal removed water	MRWb	0, 7	3
B	Treated + Nutrient	MRNW	0, 7	3

Table 4.2 Chemical concentrations measured in SW from Lake Matano, water from 150 m prior to metal removal, and MRW.

	SW	MW (Prior to removal)	MRW
(μM)			
PO_4^-	0.250 (DL)	0.25 (DL)	-
NO_3	32.0 (DL)	32.0 (DL)	-
Na	56.0	67.0	75.1
Mg	487	779	788
K	6.17	5.19	5.80
Ca	234	415	454
Fe	25.9	44.0	0.100
Mn	0.100	8.50	5.80
(nM)			
Cr	236	184	48.5
Ni	61.4	66.1	42.6

Table 4.3 Experiment A: Simpson's Diversity Index (1-D) for SW and MRW cultures immediately after inoculation, mid-experiment, and at the end of the experiment.

Treatment	Time (d)	Simpson's Diversity Index (1-D)
SWa	**0**	1.0
MRWa	**0**	1.0
SWa	**7**	1.0
MRWa	**7**	1.0
SWa	**21**	0.63
MRWa	**21**	0.61

Table 4.4 Experiment B: Simpson's Diversity Index (1-D), MRW and SW, with and without nutrient addition, immediately after inoculation and at the end of the experiment.

Treatment	Time (d)	Simpson's Diversity Index (1-D)
Surface	**0**	0.242
Surface + nutrient	**0**	0.288
MRW	**0**	0.166
MRW + nutrient	**0**	0.161
Surface	**7**	0.144
Surface + nutrient	**7**	0.191
MRW	**7**	0.260
MRW + nutrient	**7**	0.164

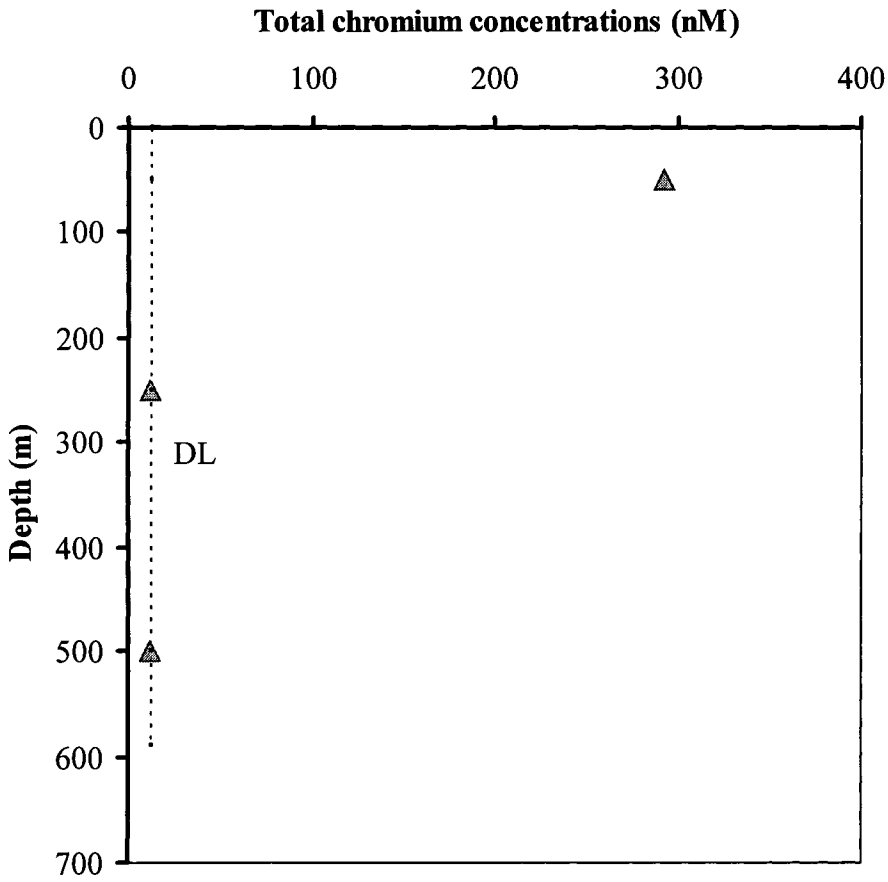

Figure 4.1 Chromium concentrations with depth, Lake Matano, 2002. (Haffner, unpublished data, analysis by ICP-MS DL: detection limit).

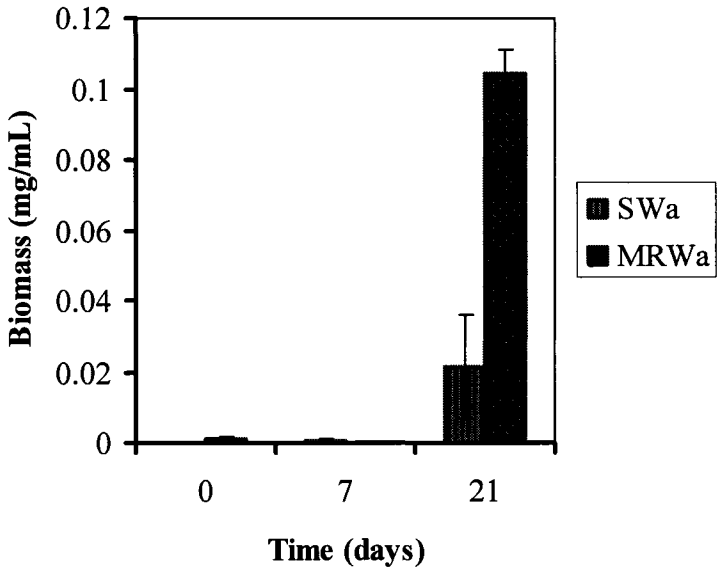

Figure 4.2 Experiment A: Mean biomass (± se), with time in SW and MRW.

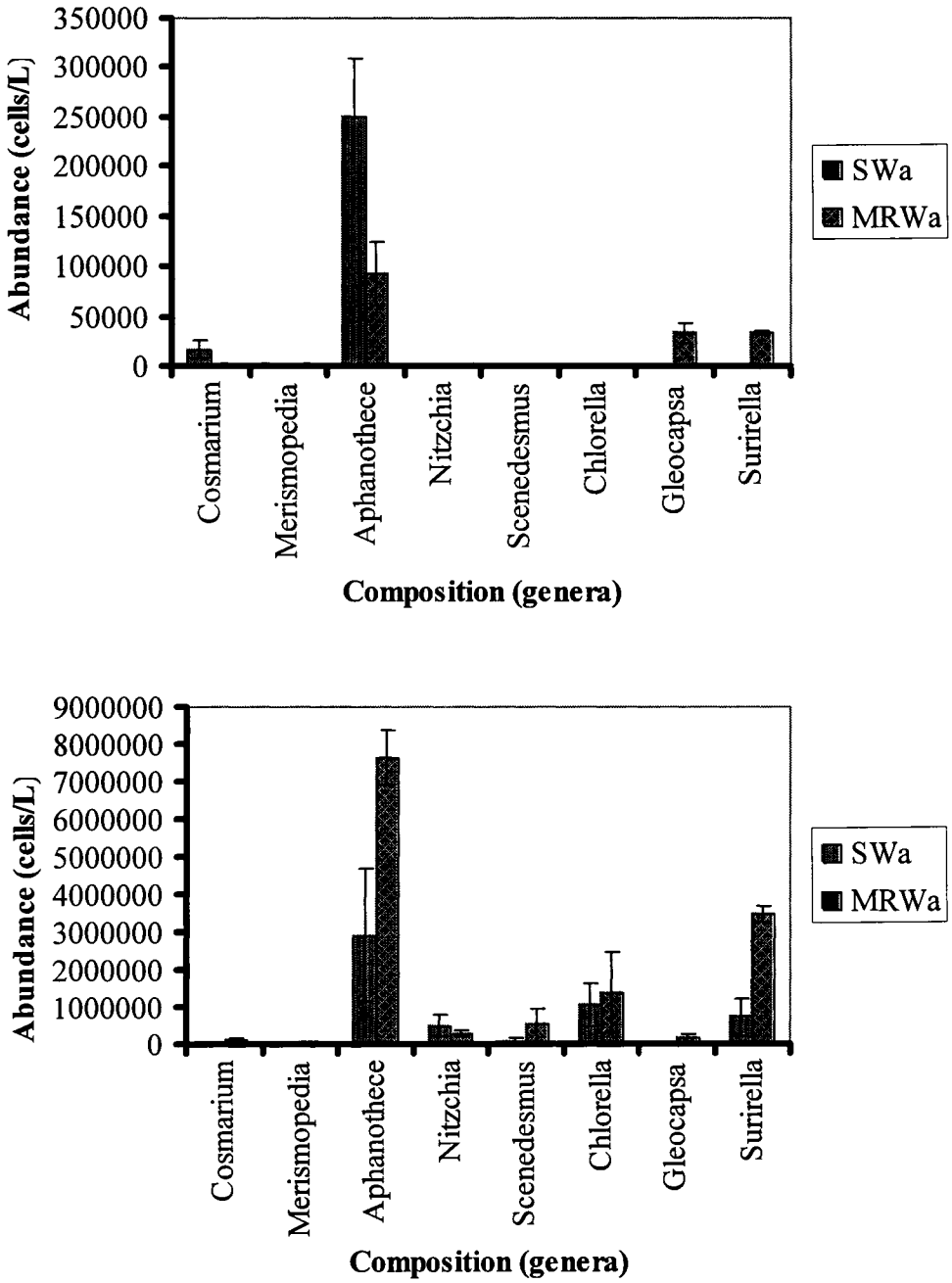

Figure 4.3 Experiment A: Abundance of genera (± se) in each treatment shows phytoplankton distribution among treatments. From top to bottom: immediately after inoculation genera were evenly distributed; after 21 days, no major species shift occurred in the cultures.

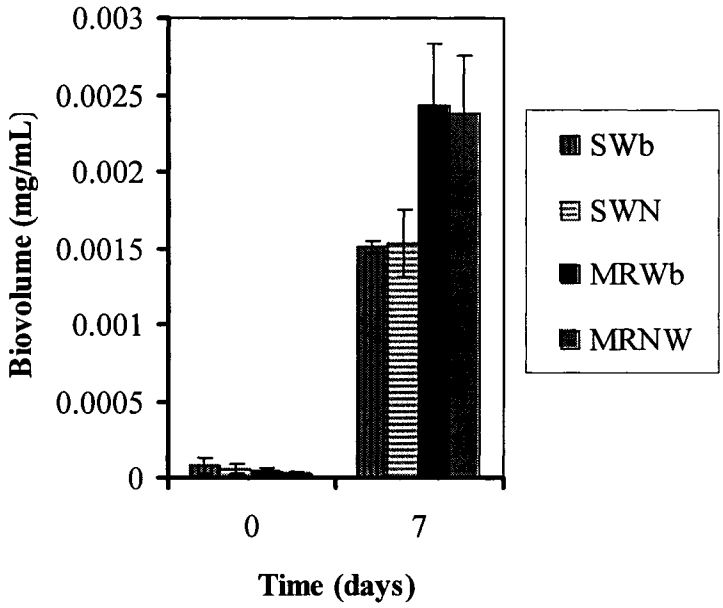

Figure 4.4 Experiment B: Biomass (± se) in SW and MRW, with and without nutrient additions.

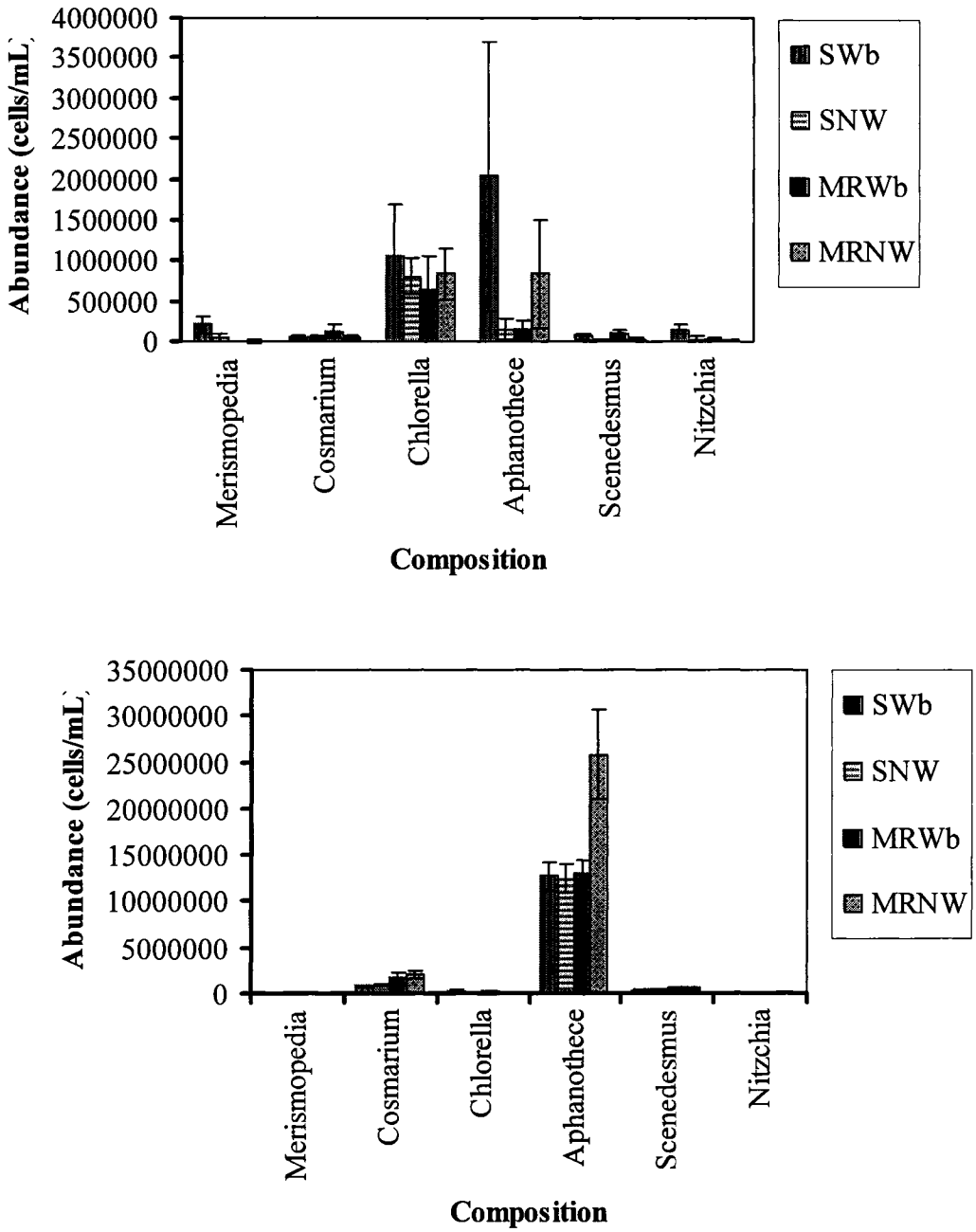

Figure 4.5 Experiment B. Abundance of genera (± se) in each treatment shows phytoplankton distribution among treatments. From top to bottom: immediately after inoculation genera were evenly distributed; after 7 days, no major species shift occurred in the cultures.

CHAPTER 5 – FACTORS REGULATING COMMUNITY STRUCTURE OF THE PELAGIC ZOOPLANKTON COMMUNITY

5.1 -- Introduction

The relationships between ecosystem composition, biodiversity and productivity have been extensively studied to understand the processes governing plankton community structure. The question: 'what lives where and why' (Reynolds, 1998) is a prevailing theme in studies of freshwater plankton ecology. This question has been classically addressed through exploration of the temporal and spatial distribution of the plankton (Talling, 1957; Hutchinson, 1967; Lewis, 1974).

The potential for high yields of the primary production base is a characteristic of tropical systems (Lewis, 2000). Given a sufficient nutrient supply, primary production in the tropics is expected to be at least twice that of higher latitudes (Lewis, 1996; Lewis, 2000). This production capacity is supported by efficient nutrient cycling, intra-seasonal deep mixing, higher temperatures, and by latitudinal trends in minimum annual irradiance (Lewis, 1996; Lewis, 2000). Tropical systems are supplied annually with a higher level of irradiance, which is distributed more evenly throughout the year. This phenomenon optimizes the interactions of other potentially limiting controls such as temperature, nutrients and photosynthesis (Lewis, 2000). Increases in the overall production base have a direct influence on community structure, species diversity and trophic interactions. For instance, trophic cascade models predict that an increase in primary production can

support a greater zooplankton biomass, until resource competition reduces zooplankton diversity. Higher productivity can also translate into greater predator densities, which can modify the composition of the prey community to favour species less vulnerable to predation.

Top-down control, in the form of predation, is also viewed as a dominant factor influencing the structure of freshwater zooplankton communities (McQueen and Post, 1989; Lewis, 1987; Fernando, 2002). This prediction (Pinto-Coelho *et al.*, 2005; Fernando, 2002) is substantiated by the increasing richness and diversity of planktivorous fish communities with decreasing latitudes. The relative lack of seasonality in breeding seasons for many tropical freshwater fish hinders the ability of zooplankton populations to decouple their life histories from those of their predators, a common defense strategy observed in temperate zooplankton species (Galbraith, 1967; Hall *et al.*, 1976; Zaret and Baskin, 1980; Fernando, 2002). Ample evidence supports the direct impact of predation pressures in structuring zooplankton communities, suggesting these pressures are a major factor in influencing zooplankton evolution and population structure (Hecky, 1991; Pinto-Coelho *et al.*, 2005). Year-round predation by sardine populations, for example, has virtually eliminated cladocerans from Lake Tanganyika (Hecky and Ogutuohwayo, 1991). Indeed, the typically larger size of cladocerans has effectively restricted both their overall sizes and their spatial distributions in many tropical systems (Fernando, 1994). A recent survey of 49 reservoirs on the Ivory Coast of Africa attributed the absence of *Daphnid* species to predatory influences from the introduced Nile *Tilapia* (Pinto-Coelho *et al.*, 2005). Low diversity of crustacean zooplankton in surveys of subtropical and

tropical lakes in Florida and Brazil have demonstrated strong predation forces (Pinto-Coelho *et al.*, 2005). It has also been shown that substantial predation pressure in the tropics has resulted in the development of smaller, less detectable copepods (Hall *et al.*, 1976). Consequently, predation pressure has become a substantial factor governing zooplankton community structure, species composition, size spectra and behaviours.

Thus, both bottom-up and top-down forces structure zooplankton communities in most systems, but quantifying the relative influences of each is difficult. Reynolds *et al.* (2000) identified the need to explore more thoroughly the extent to which communities at lower trophic levels are structured by competition and predation. Despite the presence of apparent paradigms, this question remains the subject of vigorous debate. This lack of knowledge is a result of the inherent difficulty in quantifying bottom-up forces and from the paucity of studies dealing with bottom-up forces in tropical freshwaters (Saunders and Lewis, 1988; Lewis, 1996).

Lake Matano, south-central Sulawesi, Indonesia, represents an optimal model system for determining the relative influence of bottom-up versus top-down forces structuring zooplankton communities. The depth (590 m) (Haffner *et al.*, 2001) and steeply sloping sides of this classical grabben lake (Brooks, 1950) minimize the influence of the littoral zone. Lake Matano is ancient, estimated at 1-4 million years old (Haffner *et al.*, 2001), and is characterized by endemism recorded notably in the diatoms (Hustedt, 1938, 1942; Bramburger, 2004) gastropods (Sarasin and Sarasin, 1897; von Rintelen and Glaubrecht, 2003) and the fish (Kottelat 1990a, b, c; 1991; Roy *et al.*, 2004).

To date, however, few detailed accounts have focused on the distinct community structure and zooplankton assemblages in this system. The earliest reports of zooplankton composition date to Brehm's account of the endemic calanoid *Eodiaptomus wolterecki* var. *matanensis,* described during the Woltereck Expedition to Indonesia in 1932 (Brehm, 1933b). The expedition covered Hawaii, the Philippines, Celebes, Flores, Bali and Java. It aimed to provide an extensive geographical comparison and not an understanding of zooplankton community dynamics. Brook (1950) reviewed the distribution of endemics in Lake Matano and downstream lakes and included the endemic calanoid as the only known pelagic zooplankter. Fernando (1987) visited the lake and mentioned an absence of cladocera, potentially as a response to naturally high levels of natural chromium.

Haffner *et al.* (2001) described Lake Matano as having a very simplified food web (i.e. no piscivore population) due largely to its highly oligotrophic nature. As such, examining the zooplankton community has the potential to yield important information about the factors governing the primary and secondary production base in this unique tropical freshwater system. Moreover, anthropogenic activity in and around Lake Matano has increased substantially within the last few decades. As global climate change and regional development have the potential to modify Lake Matano, it is essential to acquire information on community processes in this ancient and relatively isolated lake system, renowned for its biodiversity.

In this study, we quantified the composition, species diversity, abundances, size ranges, total biomass and the distribution of ovigerous females of the zooplankton community throughout the upper 110 m of Lake Matano in August 2000, January 2002 and August 2004. In addition, we determined the species diversity, abundances and carbon biomass distribution of the available standing phytoplankton community. Finally, the $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios were measured for the zooplankton and phytoplankton fractions throughout the epilimnion. We used these parameters to provide the first detailed characterization of the zooplankton community. The goals were to determine: 1) whether the zooplankton community revealed vertical structure in composition and abundance in Lake Matano and 2) the potential influence of top-down and bottom-up forces in regulating zooplankton community structure.

5. 2 – Materials and Methods

5.2.1 -- Zooplankton community analysis

The zooplankton sampling regime for the years 2000 and 2002 consisted of four approximately equidistant sampling locations along latitudinal transects across the lake, from 121°15'00 E to 121°24'00 E (Figure 5.1). In 2004, zooplankton sampling was implemented at only one of these the sampling sites (121°18'00). Sampling periods spanned two seasons, commencing in the 'dry season', mid-August (2000 and 2004), and in the 'wet season' of mid-January, 2002; sampling times ranged from early morning to afternoon throughout the sampling weeks. The same sampling procedure was applied to all collections. A vertical closing net with a 64 μm mesh was used to successively filter

10 m intervals of the water column, from 0-110 m. These vertical transects were designed to quantify the distribution of zooplankton in the water column across the lake and through the depth of the epilimnion. Filtered material was rinsed into plastic scintillation vials and immediately preserved in 95% ethanol. Vials were refrigerated at 4°C for storage purposes.

In the laboratory, each of the zooplankton samples from successive depths of the water column was subsampled for analysis of community composition and abundance. Sample vials were mixed thoroughly but gently, and subsamples of 1 millilitre each were removed using a wide bore pipette. Subsamples stood for 24 hours in sedimentation chambers before microscopic observation. After a detailed survey revealed that the community composition was consistent across the lake, replicated subsampling was conducted at locations 2 and 4 to produce vertical profiles of community composition and abundance. Counting and identification were performed using a Leica® inverted microscope at 50-600x magnification. To produce the vertical profile, counts of each species were replicated three times from each depth interval such that 100 individuals of the dominant species were enumerated within each sample. For composition and abundance data, post-naupliar copepods were identified to species level according to Reddy (1994), while naupliar stages of copepods were counted as a single group. The trophi of the adult rotifer were dissected out and rotifers were identified to species, according to Ruttner-Kolisko (1974). A lake-wide size profile of copepod body length measurements was obtained using Openlab® 3.15 image analysis software. Lengths were measured from the anterior end point of the head, between antennae, to the pre-setae edge

of the caudal rami. Standing biomasses of rotifers and copepods were calculated with depth interval, using standard formulae for dry weight estimates according to Dumont *et al.* (1975). In addition, the locations of ovigerous females were documented. Table 5.1 shows the combined sampling scheme for the three years.

5.2.2 -- $\delta^{13}C$ and $\delta^{15}N$ isotope analysis for the production base

Vertical net hauls were also conducted in August, 2004 to collect plankton biomass for stable $\delta^{13}C$ and $\delta^{15}N$ isotope analysis. To collect sufficient phytoplankton biomass, repeated net hauls were conducted with the 64 μm closing net at discrete depth intervals of 30 m from 0 to 90 m. The biomass portions collected were then separated through a 200 μm mesh filter, yielding a size fraction of biomass greater than 200 μm and a size fraction of biomass below 200 μm and above 64 μm for each depth interval. Subsequent microscopic analyses confirmed that these size fractions approximated a division of the microplankton into zooplankton and phytoplankton. Each fraction was placed in aluminum weighing dishes and dried for isotope analysis. Isotopic analysis was conducted at the Environmental Isotope Laboratory at the Centre for Research in Earth and Space at the University of Waterloo.

5.3 -- Results

5.3.1 -- Zooplankton

Analysis of the vertical net series revealed relatively low abundance of few taxa and exhibited a high degree of seasonal continuity. The zooplankton community in

August (the dry season) was comprised of the endemic calanoid, *Eodiaptomus wolterecki* and the tropical rotifer species *Horaella brehmi*. Samples from both January, 2002 (the wet season) and August, 2004 revealed a community composed of *Eodiaptomus wolterecki*, an endemic cyclopoid of the genus *Tropocyclops*, and *Horaella brehmi*.

Yearly abundance and biomass distributions of cyclopoids, calanoids and rotifers illustrate depth stratification (Figures 5.2-5.4). *E. wolterecki* numerically dominated the pelagic zone in all three sampling occasions. The highest density and biomass of *E.wolterecki*, at site 2 in 2000, occurred at the depth interval of 20-30 m, with a mean of 8.2×10^2 individuals/m^3 (se=62) and a biomass of 2.5 mg/L (se=0.190). The second highest abundance was observed at a depth of 10-20 m at site 4 and at a depth interval of 10-20 m at site 4, with 7.2×10^2 individuals/m^3 (se=89), 2.0 mg/L (se=0.032). In 2002, a mean high of 3.0×10^2 individuals/m^3 (se=21), biomass 5.3×10^2 µg/L (se=34), was found at depth interval 10-20 m at site 2. At the eastern end of the lake, site 4, a mean high of 8.1×10^2 individuals/m^3 (se=58) was found at a depth of 10-20 m. During the following sampling season (2004), the highest abundance was determined at the 20-30 m interval, with 4.1×10^2 individuals/m^3 (se=40) and 9.0×10^2 µg/L (se=1.0×10^2). Population numbers in all seasons declined sharply after a depth of approximately 50 m, decreasing to near 0 below 80 m. Likewise, rotifer densities were mostly detectable in the upper 50 m in each year and declined thereafter; they were virtually absent in samples below 80 m. In 2000, the highest abundance of *H. brehmi* occurred at a depth interval of 10-30 m from 2000-2004, with densities of 1.9×10^2 individuals/L (se=1.2×10^2) and 1.3×10^2 individuals/L (se=37), respectively. The cyclopoids were observed only in 2002 and

2004. In both years, the cyclopoids occurred exclusively below 80 m, where rotifers and calanoids were infrequently detected. The cyclopoid communities were not prolific, reaching densities of 91 individuals/m^3 (se=50) at 100-110 m in 2002 and 29 individuals/m^3 in 2004 (se=3.0).

Calanoid body lengths ranged from approximately 200 μm to 900μm in all three seasons sampled (Figure 5.5). In the years 2000, 2002 and 2004, respectively, the mean calanoid body lengths were 5.8 x 10^2 μm (se=28), 4.9 x 10^2 μm (se=32) and 5.8 x 10^2 μm (se=11.12). Cylopoid body lengths exhibited little seasonal variation, with a mean length of 3.9 x 10^2 μm (se=38) in 2002 and 3.2 x 10^2 μm (se=17) in 2004.

Ovigerous copepods were depauperate in all seasons (Table 5.3). For the year 2000, over the extent of the water column sampled (0 to 110 m), the mean abundance of ovigerous calanoids was 1.4 individuals/m^3 (se=0.41), with ovigerous individuals rarely observed in samples analyzed from depth intervals of 10-20 m, 20-30 m and more commonly observed between 90-100 m. In the wet season of 2002, ovigerous copepods (both calanoids and cyclopoids) were exclusively detected in samples from the 80-110 m depths of the lake. The mean abundance of ovigerous calanoids in the water column was 0.65 individuals/m^3 (se=0.33) and the mean RA of ovigerous cyclopoids throughout the water column was 1.2 individuals/m^3 (se=0.43). In 2004, however, ovigerous calanoids were not confined to the 80-110 m depths but were found in low numbers throughout the water column, with a mean abundance of 1.4 individuals/m^3 (se=0.41). The mean cylopoid abundance in 2004 was 0.20 individuals/m^3 (se=0.16).

5.3.2 -- δC and δN isotope analysis for the production base

Carbon and nitrogen isotopic analysis (Table 5.2) revealed that the zooplankton and phytoplankton were relatively enriched in the $\delta^{15}N$ isotope, particularly in the surface waters. The zooplankton fraction was depleted in the $\delta^{13}C$ isotope, with $\delta^{13}C$ of -29.2 °/oo (se=0.0300) in the upper waters. The $\delta^{13}C$ signature of the phytoplankton fraction becomes progressively less depleted, and more different from the zooplankton fraction with depth. The phytoplankton signatures range from -28.2 °/oo the surface waters to -26.5 °/oo from samples collected at 60-100 m. In the surface waters, zooplankton was enriched in $\delta^{15}N$ by 12.4 °/oo, while the phytoplankton fraction was enriched by 8.52 °/oo. Enrichment of the heavier $\delta^{15}N$ isotope decreases with depth. In samples collected from tows of 60-30 m depth, zooplankton had a $\delta^{13}N$ signature of 11.5, while the phytoplankton signatures were 5.55 °/oo.

5.4 – Discussion and Conclusions

Previous studies of the pelagic zooplankton in Lake Matano are few and prevent significant temporal comparisons beyond this study. Lakes at lower latitudes do not experience seasonal change to the extent of lakes at higher latitudes and it has been proposed that a single sampling series at any time of the year in the truly aseasonal tropics is sufficient to estimate total zooplankton richness (Dumont and Segers, 1996). In Lake Matano, the similarity in composition and vertical structure observed in each year sampled suggested that seasonality does not appear to be a significant factor structuring this zooplankton community. All sampling periods of this study revealed a simplified

plankton community, with few changes in composition and abundance. In all sampling periods, abundance data, size spectra and biomass estimates showed that, as a whole, water column production is very low.

The zooplankton community of Lake Matano is dominated in biomass and numbers by the endemic calanoid copepod, *Eodiaptomus wolterecki.* This finding correlates to more than half the total pelagic plankton community in biomass and numbers in each year sampled. The rotifer *Horaella brehmi* occurs in all samples, representing less than a quarter of the pelagic zooplankton. The cyclopoid community was found only in samples from the years 2002 and 2004, representing less than 10% of the zooplankton community.

In conjunction with low abundances of each species, the small body sizes in Lake Matano translate into very low biomass of secondary production. Copepods, in the range of 1-2 mm, prevail in tropical systems and are considered small relative to size ranges found in temperate systems (Fernando, 2002). The mean size ranges of the Lake Matano copepods are barely half this norm for tropical systems.

Egg production and recruitment capability of a population has been established as an endpoint signifying the concentration and quality of food available to adults and nauplii (Poulet *et al.*, 1995). This finding is likely due to copepod egg production following the structural weight concept (Carlotti *et al.*, 1993), in which energy is allotted to reproduction only after the female has attained a critical weight and nutritional

fulfillment (Hirche and Carlotti, 1997). Therefore, greater resource quality and quantity will yield faster maturation times and higher fecundity. With fecundity represented by the number of ovigerous individuals, very low fecundity was observed throughout the study period, with ovigerous individuals comprising less than 1% of the calanoid population. Due to the dependence of clutch size in copepods on their habitat quality (Fernando, 2002), this yearly low population fecundity reflects the resource limitations imposed on the zooplankton of Lake Matano. Total copepodite and nauplii contribute less than 20% of the total copepod counts in all years, reflecting very low biomass turnover in the copepod community. The proportion of total copepodite and nauplii is quite high relative to ovigerous copepods. This observation is consistent with habitats that present a food limitation to adults but not to nauplii, so that egg production is stilted while naupliar growth occurs at a regular rate (Poulet *et al.*, 1995). However, further work to elucidate naupliar development time in Lake Matano is merited.

The lack of diversity in the pelagic zone agrees with previous accounts of simple zooplankton assemblages in the tropics (Lewis, 1996). Explanations of the poor speciose nature of pelagial communities in tropical systems have tended towards a mechanism of top-down control exerted by diverse and abundant fish communities. This top-down control may decrease the number of realized species in the secondary production base, in spite of conditions favorable to primary production such as constant irradiance and efficient nutrient cycling (Lewis, 1996). Moreover, it is widely documented in tropical systems that the tendency towards smaller zooplankton at lower latitudes is strongly selected for by fish predation.

Such a predation mechanism does not appear to drive the small sizes of the zooplankton in Lake Matano. Unlike downstream lakes in the vicinity, Lake Matano supports no top-level predators (Roy *et al.*, 2004, Haffner *et al.*, 2001). The fish community, though endemic, is simple and not abundant (Roy *et al.*, 2004), consisting mainly of very small fish (<10 cm) (Haffner *et al.*, 2001). Furthermore, the fish community is primarily confined to the littoral zone (Roy, personal communication). Indeed, within the last 40-50 years, the low fish production of the lake has driven the implementation of several aquaculture schemes. These attempts utilized introduced species, since it was considered that the endemic community was restricted in its use of the lake's resources (Whitten *et al.*, 1987), but met with little success. Recent efforts use small, culture nets moored just at the shoreline that are fed by hand. Thus, predation does not play a significant role in reinforcing small sizes and depauperate community composition in Lake Matano.

The size structure, composition and biomass of the plankton community are important indications of food quality and quantity (Hall *et al.*, 1976). Relatively high total zooplankton densities are common in tropical lakes, but, as expected, biomass varies with nutrient status (Pinto-Coelho *et al.*, 2005; Dodson *et al.*, 2000). In lakes of high total phosphorus (TP) and chlorophyll such as eutrophic lakes and reservoirs, densities of total zooplankton greater than 10^3 individuals$\cdot L^{-1}$ are common (Pinto-Coelho *et al.*, 2005). In Lake Matano, above the euphotic depth of 50 m, the peak phytoplankton biomass is 0.013 mg L^{-1}, indicating an impoverished production base that will have ramifications for

the zooplankton community (see Chapter 2). According to Reynolds *et al.*'s (2000) classification of lakes, Lake Matano ought to exhibit production in keeping with its tropical nature. In contrast to other warm meromictic lakes such as Lake Tanganyika, with a biomass of 0.9 mg/L, rating Matano's primary production using Reynolds *et al.*'s (2000) puts it in the range of the most unproductive Arctic type lake. The very low primary production in Lake Matano (see Chapter 2) substantiates the importance of resource-base control in this system. Specifically, the critical range of phytoplankton required to support obligate filter feeders such as *Daphnia* is 0.1 to 0.4 mg C/L (Reynolds *et al.*, 2000), several orders of magnitude above phytoplankton biomass in Lake Matano. Moreover, the selection of phytoplankton found in Lake Matano is not high and biomass is largely contributed by unpalatable species such as *Microspora,* and *Peridinium.* Although zooplankton communities will also avail themselves of microbial populations and detritus, Lake Matano does not have the capacity to sustain an expanded zooplankton community.

Carbon and nitrogen stable isotope analysis was performed to further elucidate trophic structure and energy flow throughout the pelagic plankton community (Peterson *et al.*, 1987) of the Lake Matano pelagic plankton community. Two limitations on the analysis were presented by the paucity of standing biomass. Firstly, trophic levels were necessarily approximated, based on size fractionation. Secondly, the portion of the food web that passed through the 64 μm net was excluded. However, several important trends emerge from the analysis.

The zooplankton fraction was more depleted in $\delta^{13}C$ than the phytoplankton fraction and this disparity increases with depth. The $\delta^{13}C$ enrichment of a consumer tends to mirror the signature of the food it consumes, while variation in $\delta^{13}C$ signatures between food webs is introduced at the level of primary production. This finding suggests that in Lake Matano, the microphytoplankton sampled do not provide the only source of energy/carbon to the zooplankton community, particularly with increased depth. This finding is not surprising, given the range in size and type of phytoplankton sampled in this size fraction. Larger, inedible phytoplankton such as *Staurastrum* and *Peridinium* dominated this size fraction and are too large to be edible by the bulk of the zooplankton community, given the small sizes of the calanoid, cyclopoids and rotifer populations. The disparity between $\delta^{13}C$ in the zooplankton and phytoplankton populations suggests that a partial dependence on alternate foodwebs, likely detrital or microbial, may exist for the primary consumers in this system.

Enrichment of the $\delta^{15}N$ isotope is also very high in the phytoplankton and zooplankton, particularly in the surface waters. The $\delta^{15}N$ enrichment of the zooplankton fraction is on the upper extreme of $\delta^{15}N$ observed for primary consumers. These values are highly lake specific and range from 1-13 ‰ (Cabana and Rasmussen, 1994). While highly enriched $\delta^{15}N$ levels are frequently associated with elevated trophic level, this is not likely the case in Lake Matano, particularly since the primary production base is also highly enriched in $\delta^{15}N$. This enrichment varies with depth, suggesting that migration of the zooplankton within the mixed upper waters is limited. The high nitrogen values of the phytoplankton fraction are representative of the non nitrogen fixing community that

appears to dominate the microphytoplankton. In aqueous systems with ample available nitrogen, non-nitrogen fixers preferentially take up the lighter isotope (Adams *et al.*, 2004). In systems with chronic nutrient limitation, the primary production base becomes progressively enriched in $\delta^{15}N$ (Vander Zanden *et al.*, 2005). The enrichment in $\delta^{15}N$ of the phytoplankton fraction in Lake Matano supports that nitrogen is not readily available in the surrounding media, creating a baseline of $\delta^{15}N$ enrichment. Substantial enrichment in $\delta^{15}N$ at the primary consumer level, as observed in this analysis, has also been demonstrated to result from limitations in available nitrogen (Zanden *et al.*, 2005). For instance, a study investigating the change in N enrichment within a single species (*Daphnia magna*) under conditions of nutritional depletion resulted in a $\delta^{15}N$ that might have been indicative of a change in trophic position (Adams *et al.*, 2004).

It is concluded in this study that bottom-up forces dominate secondary production dynamics in Lake Matano. Lake Matano consists of a single endemic grazer (a calanoid) and a limited selection of predatory species. This assembly agrees with the community structure that Dumont and Segers (1996) argued was common to the pelagic zone of ancient lakes, attributing this structure to the lack of potential niche diversification. While the age and volume of the lake are factors that will promote diversification, this diversity will be strongest in the fish and benthos (Hobaek *et al.*, 2002), while the pelagic zone is frequently too homogeneous to sustain high levels of zooplankton diversity (Schon and Martens, 2004). Given the poor nutrient base in Lake Matano, it is supposed that the potential for niche diversification of zooplankters and planktivorous communities is very low, in spite of the age of the lake. However, there is some suggestion that this low niche

diversification may drive the zooplankton community in Lake Matano to be structured according to the degree of spatial heterogeneity along the vertical gradient. In both years, cyclopoids were observed almost exclusively below 80 m, whereas calanoids and rotifers predominated above, indicating community depth selection within the epilimnion. The region of the water column below 80 m represented a change in the physical and chemical properties of the water column, as oxygen declined and a weak thermal structure begins. This will be a region where nutrient exchange can occur between the deeper waters and the epilimnion. Our dataset showed this depth selection in both years but the stability of this community stratification during a 24-hour cycle was not determined. However, extreme deviation from this depth selection through diel vertical migration seems unlikely, based on the consistency between both wet season (January, 2000) and dry season (August, 2004) dynamics, and the range in sampling times from early morning to late afternoon throughout successive sampling days. It is expected that, based on the size of individuals in the zooplankton community, populations would not traverse great distances through the water column on a daily basis. Furthermore, daily vertical migration is an energetically costly behaviour primarily performed as a mechanism for predation avoidance. The lack of predators in the water column will not reinforce this defense.

The importance of Lake Matano today as a freshwater resource, being targeted for increased production and development, augments the necessity of understanding its historical and current status. Of particular significance are the community compositions in the production base of the system. The compositional, size spectra, fecundity and

abundance data from this investigation of the zooplankton community reflect production constraints. This study provides the first temporally and spatially detailed determination of the pelagic zooplankton community composition and proposes that bottom-up forces are most influential in regulating zooplankton community structure.

5.5 -- References

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Table 5.1 Sampling scheme for 2000, 2002, 2004.

Year	Sites sampled	Depths sampled (10m intervals)	# Replicates per depth
2000	1	0-110	1
	2	0-110	3
	3	0-110	1
	4	0-110	3
2002	1	0-110	1
	2	0-110	3
	3	0-110	1
	4	0-110	3
2004	2	0-120	3

Table 5.2 δC and δN isotope analysis (±se) for the production base. (NA=not available, NR=replication not possible due to biomass considerations).

Plankton Fraction (μm)	Major Composition	Depth (m)	δC13	δN15	SE, δC13	SE, δN15
>250	**Zooplankton**	**30-0**	-29.20	12.42	0.03	0.27
64>, <250	**Phytoplankton**	**30-0**	-28.19	8.52	NR	NR
>250	**Zooplankton**	**60-30**	-30.68	11.49	NR	NR
64>, <250	**Phytoplankton**	**60-30**	-26.20	5.55	NR	NR
>250	**Zooplankton**	**90-60**	NA	NA	NA	NA
64>, <250	**Phytoplankton**	**90-60**	-26.50	4.04	0.11	0.07

Table 5.3 Mean abundances of ovigerous copepods, Lake Matano.

Depth	2000		2002		2004	
	Calanoid	Cyclopoid	Calanoid	Cyclopoid	Calanoid	Cyclopoid
0	0	NA	0	0	4.71809	0
10	0	NA	0	0	2.35905	0
20	0	NA	0	0	2.35905	0
30	0	NA	0	0	1.17952	0
40	2.47223	NA	0	0	0	0
50	3.65652	NA	1.79288	0	2.35905	0
60	1.88724	NA	0	0	0	0
70	1.2541	NA	0	0	0	2.35905
80	1.9698	NA	0	0	0	0
90	0	NA	4.93041	2.52418	0	0
100	0	NA	5.80326	0	0	0

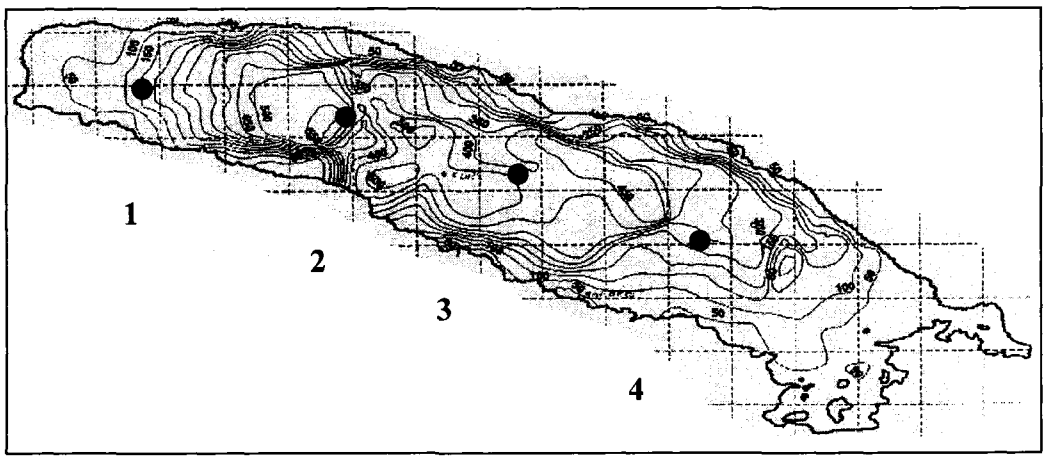

Figure 5.1 Lake Matano sampling stations. Stations 1-4: were sampled in the years 2000, 2002; Station 2: sampled in 2004.

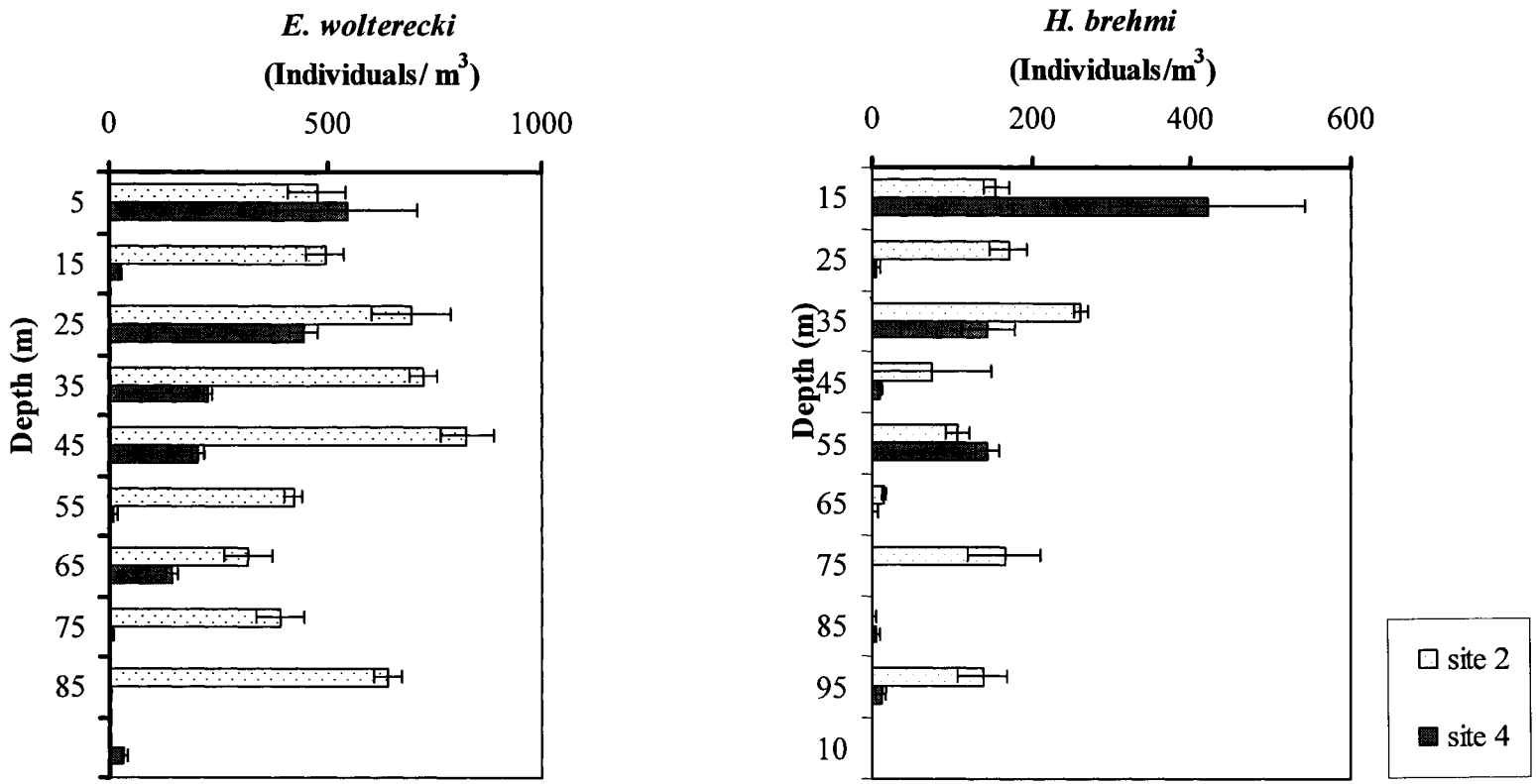

Figure 5.2 Abundances of zooplankton species (± se), with depth, at sampling sites 2 and 4, August, 2000. Upper bar: Site 2, Lower bar: Site 4. From left to right: ***Eodiaptomus wolterecki, Horaella brehmi and copepod nauplii***. Note the lack of ***Tropocyclops* sp.**

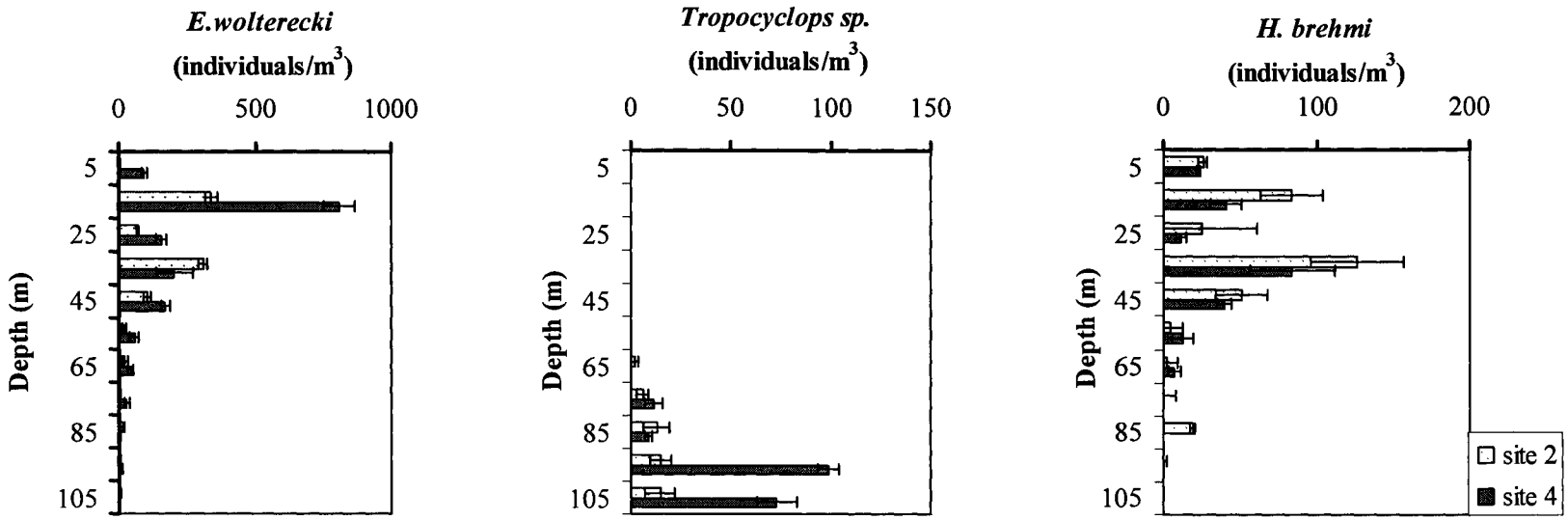

Figure 5.3 Abundances of zooplankton species (± se), with depth, at sampling sites 2 and 4, January, 2002. Upper bar: Site 2, Lower bar: Site 4. From left to right: ***Eodiaptomus wolterecki, Tropocyclops* sp., *Horaella brehmi.***

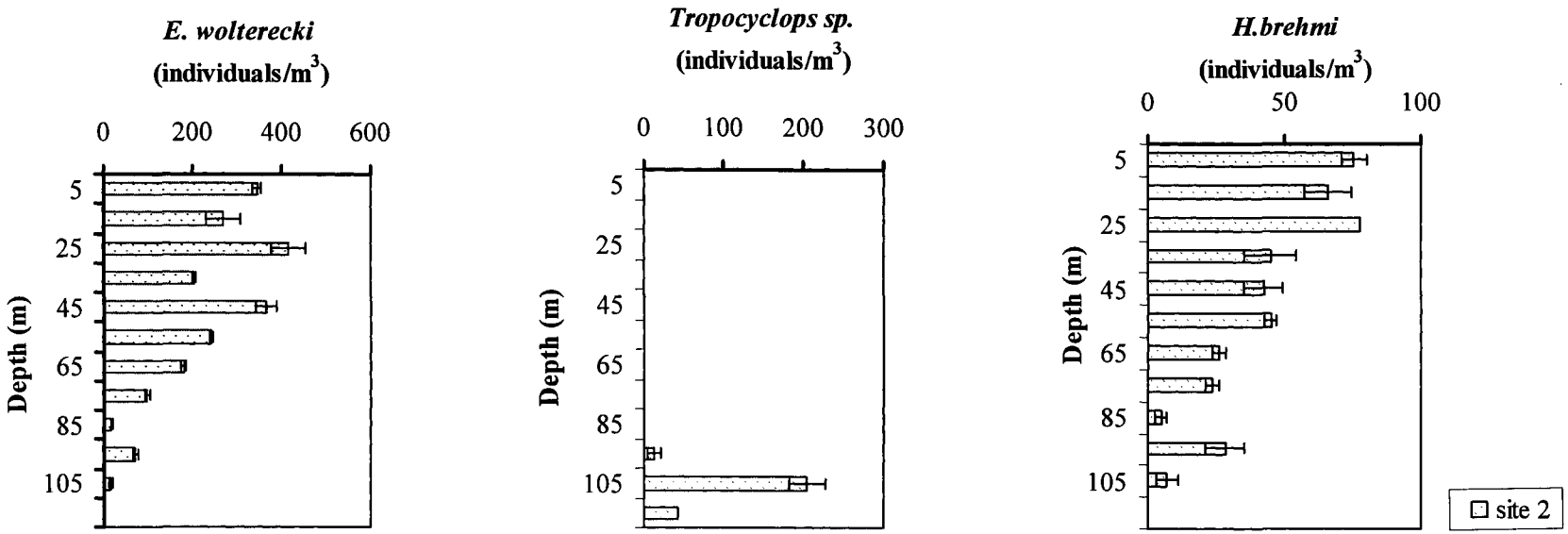

Figure 5.4 Abundances of zooplankton community (± se), with depth, at site 2, August, 2004. From left to right: ***Eodiaptomus wolterecki, Tropocyclops* sp., *Horaella brehmi.***

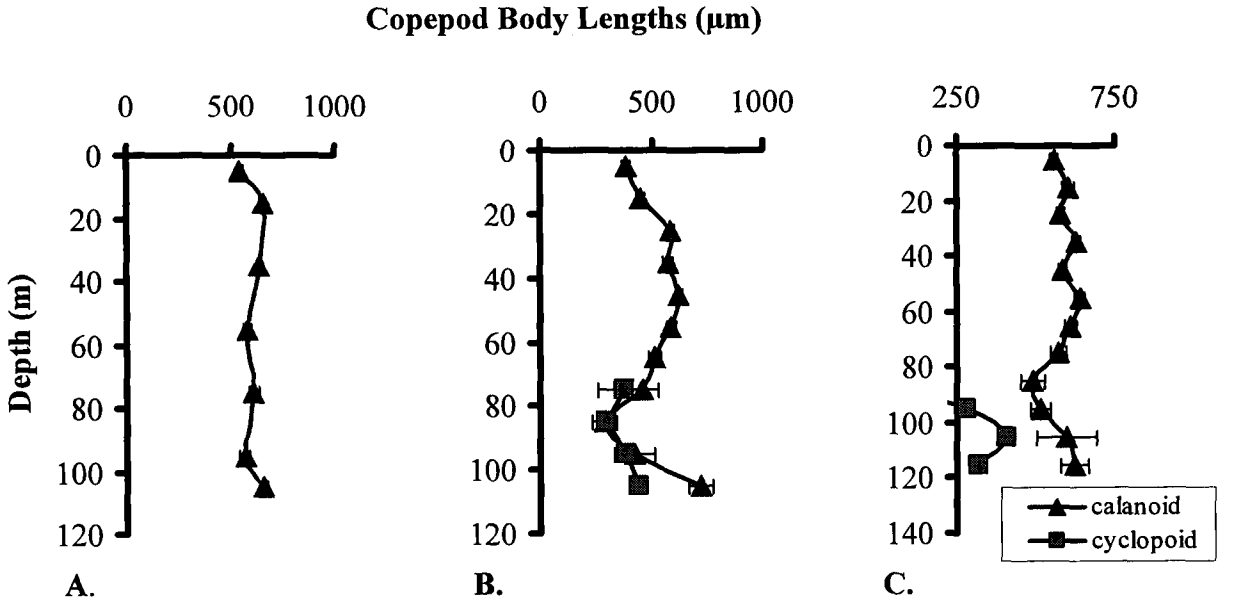

Figure 5.5 Size distributions (± se) of calanoid and cyclopoid copepods with depth. From left to right: A. 2000, B. 2002, C. 2004.

CHAPTER 6: CONCLUSIONS

This major objective of this study was to fulfill the need to better understand the processes governing the primary and secondary production of Lake Matano. Specifically, the study characterized the phytoplankton and zooplankton communities of Lake Matano and determined that Lake Matano exhibited very low production in comparison with other lakes of similar physical characteristics. In addition, chemical stresses, including nutrient limitation and metals stress were considered.

As anthropogenic demands on the lake increase, the need to gage the impact of these demands and to provide the first detailed record of the pelagic plankton community becomes more significant. As an ancient lake that has remained relatively isolated through much of its history, studies of Lake Matano's community also offer insight into factors governing community dynamics and assembly.

Reynolds *et al.* (2000) provided a qualitative model that correlates significant physicochemical characteristics with the assemblages of pelagic phytoplankton dominating a lake. The model has been applied to understanding the regulation of phytoplankton dynamics in the large lakes of the world, as delimited by lake depth and/or surface area. Lake Matano qualifies as one of the large lakes of the world. Reynolds *et al.* (2000) suggests that although the paucity of data available for Lake Matano prevented its inclusion during model development, Lake Matano may present a rewarding study. The limnology of Lake Matano and important geochemical characteristics of the lake are

reviewed in Chapter 1 and applied to Reynolds' framework for classifying the other large lakes of the world. In terms of depth, morphology and latitude, Lake Matano is comparable to Lake Tanganyika. Lake Matano is classified as a warm near-meromictic lake, based on the scheme applied to the other large lakes (Hutchinson and Loffler, 1956; Reynolds *et al.*, 2000). Deep mixing occurs throughout the year, to a depth of at least 100 m, where a slight thermal structure has been detected. As a regional frame of reference, the downstream Malili Lakes, Mahalona and Towuti, are continuous warm polymictic lakes. As a shallow, tropical lake, Lake Mahalona correlates with other lakes under this description. In the case of Towuti, the classification is atypical, as it is usually reserved for very shallow lakes.

Further understanding of the pelagic community of Lake Matano requires knowledge of the organization of the primary production base. The dominant phytoplankton assemblage found in Lake Matano, as well as the downstream lakes, is one of non-N-fixing cyanobacteria (see Chapter 2). This assemblage is common to the large lakes of low latitude and atypical at higher latitude. This finding corroborates that, in considering important lake physicochemical characteristics, latitude provides the clearest indication of phytoplankton type (Reynolds *et al.*, 2000). However, the cyanobacterial dominance at low latitudes is also associated with the trend in mixing types (Chapter 2). An important aspect of the cyanobacterial assemblages is the small biovolume, which facilitates staying afloat in the water column (Reynolds *et al.*, 2000). This adaptation would be important in the meromictic and continuous warm polymictic lakes in which this assemblage proliferates, particularly in the deeply mixed Malili Lakes.

Lake Matano and the downstream lakes exhibit poor standing biomass most similar to an ultra-oligotrophic, unproductive Arctic-type lake, suggesting growth limitation of the primary production. Indeed, Lake Matano exhibits poor production at each trophic level, poor species richness and the lack of a top piscivore trophic level.

It has been proposed that lake production is constrained as a result of either nutrients (Haffner *et al.*, 2001) or metal toxicity (Fernando, 1987; Haffner *et al.,* 2001). The ultramafic catchment of the lakes is rich in heavy metals such as chromium, and poor in nutrients such as phosphorus and nitrogen. A nutrient enrichment experiment was conducted using phytoplankton from the lake, cultured in growth media prior to initiation of the experiment (Chapter 3). Higher numbers of cells were observed in the nitrogen-enriched cultures of water from the lake, relative to the control, but the effect was not significant. The trend may have resulted from transient nitrogen limitation of selected phytoplankton populations. In the P-enriched lake water, significantly higher ($p<0.01$) growth was observed than in the control cultures and nitrogen enriched cultures. In addition, community dominance shifted towards *Chlorella*. The results suggest the existence of phosphorus limitation of net primary productivity in Lake Matano. This finding deviates from the accepted norm for tropical lakes, in which nitrogen limitation is the norm, while temperate lakes are predominantly phosphorus limited (Lewis, 2000). Nitrogen limitation in tropical lakes results from the insolubility of nitrogen and heightened microbial metabolism in warmer temperatures (Lewis, 2000). In Lake Matano, the high levels of iron in the lake may scavenge phosphorus, depleting the available phosphorus. If this is the case, the ratio of N:P must be relatively high, with

phosphorus in very low supply. An outgroup was also introduced into the experiment, consisting of bottled water as a culture medium. A ready growth response of significant magnitude indicated that a growth restriction had been readily alleviated in the outgroup. The water contained nutrient levels several orders of magnitude above the level found in the lake. The experiment suggests that nutrient bioavailability plays a role in limiting primary production.

Alternatively, metal toxicity restricts primary production (Chapter 4). A process of metal removal applied to lake water for culture media enhanced phytoplankton growth in this water relative to growth in water in which the metals had not been removed. A significant change in phytoplankton composition did not occur, suggesting toxic release of the existing species.

Lastly, the diversity, richness and life history characteristics of the secondary production base of Lake Matano were examined. The factors influencing community assemblage have been classically addressed through determination of the spatial and temporal plankton distribution (Talling, 1957; Hutchinson, 1967). This approach was used to determine what factors regulate the dynamics of the pelagic zooplankton community. Fecundity, size distribution, $\delta^{13}C$ and $\delta^{15}N$ isotopic enrichment, composition and abundance were analyzed with depth in three sampling years. Results suggested that vertical structure is maintained in the well-mixed epilimnion of the lake. In tropical systems, top-down effects have traditionally been invoked as the dominant force governing plankton dynamics of freshwater systems (McQueen *et al.,* 1988). However, in

Lake Matano, the paucity of the primary production base and the lack of a significant piscivore population suggest that bottom-up effects have a greater influence on zooplankton community structure than do typical top-down effects.

This study reviews physical, chemical and biological characteristics of Lake Matano. Physicochemical characteristics of the lake were compared with those of other large lakes and with regional lakes; experiments were conducted to test chemical stress, and the potential influence of higher trophic levels on the production base was examined. Potential nutrient limitation was demonstrated in Chapter 3 while results in Chapter 4 suggested toxicity release from metals stress. Field expeditions revealed low primary and secondary production over three field sampling expeditions. This atypically low primary and secondary production in Lake Matano is consistent with the chemical stresses tested.

6.2 -- References

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