http://waikato.researchgateway.ac.nz/

Research Commons at the University of Waikato

Copyright Statement:

The digital copy of this thesis is protected by the Copyright Act 1994 (New Zealand).

The thesis may be consulted by you, provided you comply with the provisions of the Act and the following conditions of use:

- Any use you make of these documents or images must be for research or private study purposes only, and you may not make them available to any other person.
- Authors control the copyright of their thesis. You will recognise the author's right to be identified as the author of the thesis, and due acknowledgement will be made to the author where appropriate.
- You will obtain the author's permission before publishing any material from the thesis.

PHYTOPLANKTON DYNAMICS IN LAKE TARAWERA

A thesis
submitted in partial fulfillment
of the requirements for the degree
of
Master of Philosophy
at
The University of Waikato
by
AMANDA BALDWIN

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

The University of Waikato

2009

Abstract

This study examined the dynamics of phytoplankton populations in deep (maximum depth 84 m), monomictic, oligotrophic Lake Tarawera in the Rotorua lakes district of New Zealand. Specifically, components of the annual phytoplankton assemblage; a summer deep chlorophyll maximum, a winter areal phytoplankton maximum and nitrogen fixation in ephemeral cyanobacterial surface blooms, are investigated.

Alternate hypotheses for the formation of the observed diatom deep chlorophyll maximum (DCM) in Lake Tarawera were tested. (1) That growth rates of phytoplankton are higher in the metalimnion than in the epilimnion, perhaps supported by higher concentrations of nutrients in the metalimnion. (2) That the DCM is formed by phytoplankton becoming entrained in more turbulent water at the thermocline and persists while the depth of the thermocline remains above the euphotic depth.

Lake water concentrations of total inorganic nitrogen and total phosphorus measured monthly over a year at 10 m depth intervals at a mid lake station, provided no evidence that the metalimnion had higher nutrient concentrations than those in the epilimnion. The productivity of DCM phytoplankton and epilimnion phytoplankton (5 m) was measured c. monthly over the six months that the DCM was present with *in situ* ^{13}C uptake incubations. Productivity was consistently

higher in 5 m incubations (c. 5 μg C L^{-1} h^{-1}) than in DCM incubations. The growth of DCM phytoplankton did not appear to be limited by either nitrogen or phosphorus as *in situ* incubations, over four days, with single or combined additions of these nutrients did not significantly increase chlorophyll *a* concentrations. The depth of the DCM was similar to the depth of the thermocline while the thermocline remained above the euphotic depth, but this relationship ceased as the thermocline deepened in autumn and a DCM was no longer discernable. Light availability at the thermocline therefore appears to be a key factor in regulating the position and persistence of DCM phytoplankton. Though other studies have demonstrated higher growth of DCM phytoplankton, these maybe incidental, as all DCM formed by negatively buoyant phytoplankton appear to coincide with the depth of the thermocline.

The seasonal areal maximum of chlorophyll *a* phytoplankton biomass occurred in winter in Lake Tarawera, following the onset of whole lake mixing. This increase was preceded by high (32 μg C L^{-1} h^{-1}) carbon uptakes in *in situ* ^{13}C surface incubations and increase in total phosphorus concentrations in surface waters. Lake mixing may redistribute nutrients throughout the water column, and may also alleviate light limitation. This is because the euphotic depth (25 – 30 m) is approximately half the average lake depth (55 m), so during lake mixing phytoplankton cells are in the euphotic zone half of the time. The simultaneous increase in nutrients and light is likely to support the observed higher phytoplankton productivity and biomass in winter.

Though Lake Tarawera is oligotrophic, surface blooms of cyanobacteria, occur from time to time. *In situ* incubations of phytoplankton with excess nutrients added, showed that nitrogen was the nutrient most limiting to phytoplankton growth, and ratios of TN:TP from lake water samples also indicated that nitrogen was more likely to be limiting than phosphorus. Acetylene reduction assays, carried out, *in situ*, during three shoreline bloom events over a two year period confirmed the presence of nitrogen fixation. Nitrogen fixation followed a daily pattern, with highest rates of 40 nmol $(10^6$ heterocysts$)^{-1}$ hr^{-1} at midday. Though ratios of TN:TP in surface water samples were lowest during winter mixing, surface blooms were only identified in late autumn, toward the end of the stratified period. Some of the highest concentrations of inorganic nitrogen in surface waters coincided with the occurrence of nitrogen fixation and may have been a consequence of nitrogen leaking from heterocysts. The overall contribution nitrogen fixation to the lake nutrient budget is highly uncertain, but preliminary estimates attribute between 0.04% and 8% of 'new' nitrogen inputs to nitrogen fixation in Lake Tarawera.

Acknowledgements

I am grateful to many people who helped me while I carried out this research project.

During this study, I was supported by a post-graduate scholarship from Environment Bay of Plenty and academic supervision by Professor David Hamilton and Professor Warwick Silvester of the University of Waikato. Dr Julie Hall of the National Institute of Water and Atmosphere also gave supervisory support.

Anjana Rajendram of the University of Waikato stable isotope unit carried out analyses of stable isotopes on the mass spectrometer and I was assisted, at different times, with field work by Andrew Lang, Brian Coxhead, Nina von Westernhagen, Andrea Dekrout and Mark Cullingford.

Thank you finally to my family and partner for your love, support and perspective.

Table of Contents

Figure captions

Preface

This thesis is organised into four chapters. Chapter one outlines the rationale for the research and gives a literature review on the formation of deep chlorophyll maxima and winter blooms. An overview of the study site, Lake Tarawera, is given along with its cultural and aesthetic significance. This chapter also summarises literature pertaining to surface blooms and nitrogen fixation by heterocystous cyanobacteria.

Chapter two contains the main body of the research and has been prepared to be submitted for publication. In this chapter, findings from in-lake investigations into the formation of the DCM and winter diatom bloom are presented.

In the third chapter nitrogen fixation by cyanobacteria in ephemeral shoreline blooms is demonstrated. The occurrence of nitrogen fixation is then discussed in terms of nitrogen limitation and nutrient concentrations in the lake, in order to consider the occurrence of surface blooms and nitrogen fixation in the lake.

Chapter four provides general conclusions arising from the study, discusses the associated management implications and provides recommendations for future research.

1.0 General Introduction

1.1 Rationale

This study sought an understanding of the spatial and temporal variations of phytoplankton populations in a deep temperate oligotrophic lake where blooms of cyanobacteria occur occasionally. The study comprised two main components; 1) observations of the seasonal and spatial variations in phytoplankton biomass in the lake, and 2) tests for nitrogen fixation in shoreline samples from the lake.

The first part focused on the deep chlorophyll maximum (DCM) and the variables contributing to the highest phytoplankton biomass occurring in winter. Physical and biological mechanisms have been proposed for the formation of deep chlorophyll maxima (DCMs). While some researchers have proposed that DCMs are accumulations of phytoplankton entrained in more turbulent water around the thermocline (e.g. Priscu and Goldman 1983), others (e.g., Moll et al. 1984) have shown that growth of DCM phytoplankton has greater biomass than that of surface populations and is supported by more abundant nutrient supply at depth (Comacho et al. 2003).

This research set out to understand the relative importance of biological and physical mechanisms leading to DCM formation, as opposed to surface-dominated populations. The first objective for this study was separated into three primary questions relating to distributions of phytoplankton:

- what forces drive the formation of a deep chlorophyll maximum (DCM)?
- what leads to the highest phytoplankton biomass occurring in winter in some deep lakes?
- what factors promote the formation of surface blooms of cyanobacteria in an oligotrophic lake?

In most lakes, phytoplankton biomass is greatest from spring through autumn (e.g., Bleiker and Schanz 1989; Havens et al. 2007). It tends be lower in winter due to factors such as ice cover (when this exists), cooler temperatures and shorter daylight periods. In a small number of deep lakes in temperate regions, however, phytoplankton biomass is greatest in winter (Vincent 1983; Alococer and Lugo 2003).

Another feature of interest is the formation of surface blooms of cyanobacteria in oligotrophic New Zealand lakes such as Taupo and Tarawera. As some cyanobacteria are able to overcome nitrogen limitation by fixing atmospheric nitrogen, it is useful from a water quality management perspective to understand the occurrence, magnitude and relevance of nitrogen fixation in a lake. A further objective of this study was to identify and quantify the occurrence of nitrogen fixation in Lake Tarawera, which is oligotrophic but has a high proportion of heterocystous cyanobacteria.

1.2 Phytoplankton in warm monomictic lakes

Warm monomictic lakes never freeze and are thermally stratified for substantial periods of the year (Lewis 1983). In summer, surface waters heat up, and the

density difference between the surface (epilimnion) and deeper (hypolimnion) layers prevents mixing. Cooling of the epilimnion in winter breaks down thermal stratification and the lake mixes fully. In New Zealand, a temperate maritime climate and relatively high wind speeds favour the warm monomictic regime in deeper lakes, and permanent or frequent mixing in shallow lakes (Hamilton 2005).

Changes in lake mixing are associated with distinct changes in phytoplankton biomass and productivity (Spigel et al. 2003). In deep oligotrophic lakes in the central volcanic plateau of North Island, New Zealand, phytoplankton biomass may be greatest in or near the thermocline (Vincent 1983; Ryan 2005); this sub-surface peak of phytoplankton is known as a 'deep chlorophyll maximum'. Cyanobacteria in thermally stratified lakes may also accumulate at the surface due to their buoyancy (Walsby 1969). Depending on the relationship between light and depth (Vincent 1983), winter blooms of diatoms may also be a feature of some deep temperate lakes and appear at the onset of winter mixing (Gibbs et al. 2002).

1.3 Deep Chlorophyll Maxima

Phytoplankton sometimes accumulate well below the surface of a water body in a layer commonly referred to as a 'deep chlorophyll maximum' (DCM). Some researchers have proposed physical mechanisms to explain DCM formation, (Derenbach 1979). For example, when water thermally stratifies, negatively buoyant phytoplankton may sink through surface waters until reaching the thermocline where they remain entrained (Priscu and Goldman 1983; Huisman et

al. 1999, 2002). Other microbiota and particles, including protozoa, bacteria, organic matter and mesozooplankton have also been reported to form subsurface peaks, often associated with the thermocline (Adrian and Schipolowski 2003; Chapin et al. 2004; Ediger et al. 2004; Fairbanks and Wiebe 1980; Gervais 1998; Lampert and Grey 2003).

DCMs may also form as a consequence of elevated growth rates of phytoplankton in the metalimnion compared with surface waters (Moll et al. 1984; Steele and Yentsch 1960). In many water bodies, nutrient concentrations are higher in deeper waters, when the lake is stratified, while light availability is greater at the water surface. DCMs may therefore form at depths that represent a compromise between the acquisition of deep nutrients and light (Klausmeier and Litchman 2001; Camacho et al. 2003) but can also occur in response to damaging ultraviolet radiation in the surface waters (Saros et al. 2005). Differences in zooplankton grazing pressure with depth may also lead to subsurface phytoplankton peaks (Coon et al. 1987; Sawatzky et al. 2006).

Phytoplankton cells may have physiological adaptations that lead to DCMs. For example, some marine phytoplankton are known to reduce their sedimentation rates (Waite et al. 1997; Boyd and Gradman 2002). Phytoplankton may also increase concentrations of chlorophyll within cells to compensate for low light conditions (Coon et al. 1987).

1.4 Winter phytoplankton biomass maximum

During seasonal stratification in deep lakes, macronutrients may accumulate in the hypolimnion (Gibbs et al. 2002). In winter, when the lakes begin to mix fully again, nutrients are circulated into euphotic waters, where they are available to phytoplankton. Because diatoms are negatively buoyant, they require turbulent mixing to maintain them in the euphotic zone. Winter mixing circulates diatoms through strong gradients of light as well as nutrients (Gibbs et al. 2002). Diatoms generally respond favourably to these conditions and have high growth rates. When mixing weakens or nutrients have been consumed from the surface waters (Spigel et al. 2003) the bloom declines and diatoms sink out of the euphotic zone (Viner 1984). Full lake mixing will not increase phytoplankton biomass in very deep lakes (e.g., Lake Waikaremoana, max. depth 248 m) as in this lake phytoplankton do not receive sufficient light to support increases in productivity when circulated through the entire water column (Vincent 1983).

Negatively buoyant diatoms only remain in the water column if there is sufficient turbulence to offset losses due to sedimentation. Turbulence is created by unstable excess energy arising predominantly from wind or convective cooling, which is transferred to smaller and smaller scales (Sanford 1997). A "turbulence window" (Huisman et al. 2002) or optimal range of turbulence, allows phytoplankton populations to "outgrow" sinking and mixing rates. If turbulence is greater than the critical range, phytoplankton become uniformly distributed. If the water column depth exceeds a critical depth then the light received by

phytoplankton becomes too low to support net growth. When turbulence is less than a critical range phytoplankton will sink to the bottom (Huisman et al. 1999).

The growth of a phytoplankton population is controlled by cell growth and division rates, and losses due to sedimentation and grazing. For a population to persist, growth rate must be greater than the loss rate. The relationship between growth and settling is defined by the growth number, G (Condie 1997), which describes the relative time scale for mixing and net growth:

$$G = \frac{T_s}{T_g}$$

where G is net growth and T_s is sedimentation rate and T_g is growth rate. A reaction-advection-diffusion equation can also be applied to the relationship between sinking and growth (Huisman et al. 2002) but the dimensionless relationship defined by G is more useful as it is not restricted to the sedimentation or growth rates of a particular species or specific environmental conditions (O'Brien et al. 2002).

1.5 Surface blooms

Cyanobacteria are photosynthetic protists, also commonly referred to as blue-green algae. Species of cyanobacteria are found in nearly all ecosystems including soils (e.g., Garcia-Pichel 2001; Nayak and Prassana 2007) and extreme environments including rocks (Büdel 1999). Cyanobacteria are also important members of the phytoplankton in aquatic ecosystems (Vincent 2009).

Cyanobacteria can accumulate at the surface of lakes, forming 'blooms'. Cyanobacterial blooms are of particular significance to lake management as they can be toxic (Chorus and Bartram 1999) and some species may also fix atmospheric nitrogen, contributing to nitrogen loads in the lake system (Smith 1983).

These cyanobacterial blooms tend to occur during summer under calm, nutrient-enriched conditions (Smith 1983, Schindler 1988) but may also occur in oligotrophic lakes (Pridmore and Etheridge 1987). The accumulation of cyanobacteria at the surface of lakes is generally assisted by their buoyancy. While turbulence is required to maintain negatively buoyant diatoms in the water column, cyanobacteria have gas vacuoles which keeps them buoyant in calm conditions (Walsby 1969). Vacuoles are made of protein and are filled with gas, making the cell less dense than the surrounding water (Oliver 1994). Limitation of protein or carbohydrate components through, for example, insufficient inorganic carbon supply, may limit the distribution and dominance of buoyant cyanobacteria by limiting vacuole synthesis (Klemer et al 1996). Cyanobacteria show some degree of control over their position in the water column due to physiological adaptations such as variable storage of photosynthetically fixed carbohydrate, which is used as ballast (Booker and Walsby 1981; van Rijn and Shilo 1985; Wallace and Hamilton 1999).

Cyanobacterial surface blooms tend to occur during summer when a lake water column is stratified, nutrients are abundant, the ambient ratio of N:P is low, and pH is high. Weather conditions, including temperature and wind, may be

dominant in influencing the timing of a surface bloom (Smith 1983; Paerl 1988; Schindler 1988; Dokulil and Teubner 2000). For example, low wind velocity, an absence of precipitation and higher than average solar radiation were found to be typical weather conditions during the formation of surface blooms on Lake Mendota in Wisconsin (Soranno 1997). Winds may also influence the spatial distribution of surface blooms and Kanoshina et al. (2003) found that wind-forced advection was significant in determining the spatial distribution of surface blooms in the Gulf of Finland.

Nitrogen fixation

Cyanobacteria may play a unique role in aquatic systems by increasing ambient lake water nitrogen through the fixation of atmospheric N_2. Nitrogen is an essential element for photosynthesis and growth (Loban and Harrison 1997). While inorganic nitrogen may be limited in the water (Smith 1983) around 78% of the atmosphere is made up of nitrogen. Atmospheric molecular nitrogen (N_2) is held together by a triple bond and is very stable. Only prokaryotes are able to reduce N_2 to a form that cells can use to make proteins. Among cyanobacteria, some 125 strains have been shown to be able to fix N_2 (Stewart 1980).

Some cyanobacteria use the enzyme nitrogenase to fix nitrogen. The nitrogenase enzyme complex is made up of two specialised proteins: the 'molybdenum-iron protein' (MoFe) also called dinitrogenase, and the 'iron protein' or dinitrogen reductase. Dinitrogen (N_2) binds to the metal iron (Fe) in the molybdenum-iron protein and the Fe protein reduces the MoFe protein (Howard and Rees 1996).

Nitrogenase proteins are damaged by oxygen, limiting nitrogen fixation to anoxic conditions. Among many nitrogen fixing cyanobacteria, including all planktonic freshwater cyanobacteria, there are specialised non-photosynthetic cells known as heterocysts, where nitrogenase is contained and nitrogen fixation takes place.

A large amount of energy is required to convert N_2 to a form that can be used by cells. Equation 1 (Paerl and Zehr 2000) shows the stoichiometry of the (nitrogenase-enzyme mediated) nitrogen fixation process and the ATP requirements:

$$N_2 + 8H^+ + 8e^- + 16\text{MgATP} \rightarrow 2NH_3 + H_2 + 16\text{MgADP} + 16P_i \quad (1)$$

As it is so energetically expensive to fix atmospheric dinitrogen, cyanobacteria assimilate and compete directly for inorganic forms of nitrogen whenever possible. Cyanobacteria will generally use ammonium rather than nitrate and are less able to compete for ambient nitrate than other species (Presing et al. 1998). They are, however, able to assimilate ammonium at very low ambient concentrations (Presing et al. 1998) and better able to persist in low ambient nitrogen conditions than other species, even without fixing N_2 (Horne and Commons 1987).

Ambient total phosphorus concentrations have been positively correlated with the number of heterocysts per filament (Tonno and Noges 2003) because of high nutritional requirements needed to support nitrogen fixation. Cyanobacteria are generally poor phosphorus competitors (Tilman et al. 1982); therefore they are

more likely to be present in higher ambient phosphorus levels. Ambient ratios of total nitrogen to total phosphorus (TN:TP) have been used to predict the occurrence of nitrogen fixation (Howarth et al. 1988). Fixation has been associated with TN:TP lower than the Redfield mass ratio of 7.2, equivalent to an atomic ratio of 16 (Horne and Commins 1987). In shallow polymictic lakes, however, nitrogen fixation may occur at ratios as high as c. 19 (Howarth et al. 1988). In shallow lakes the entire water column is in contact with the basal sediments so the recycled phosphorus may be rapidly incorporated into the biomass (Tonno and Noges 2003).

Temperature has been shown to influence nitrogen fixation. Tonno and Noges (2003) suggest that low temperature might have caused a delay between the predicted onset of fixation, based on hydro-chemical conditions, and their observed onset of fixation. Nitrogen fixation may also be positively correlated with irradiance, though inhibition at high intensities has also been observed (Lewis and Levine 1984) as it does with carbon fixation (MacIntyre et al. 2002).

Measurements of nitrogen fixation

Measuring the rate of nitrogen fixation requires samples to be incubated in a closed system to determine changes in nitrogen species with time. Acetylene gas can be used to measure the rate of nitrogen fixation. In the presence of acetylene the nitrogenase enzyme will reduce acetylene to ethylene ($C_2H_2 \rightarrow C_2H_4$) in preference to reducing N_2. The potential rate of nitrogen fixation can then be inferred from the amount of C_2H_2 that has been converted to C_2H_4. The relationship between the production of C_2H_4 and the potential N_2 fixation rate is

usually about 4:1 and is related to the loss of electrons associated with the nitrogenase enzyme (Stewart 1980). Comparisons between acetylene reduction assays and ^{15}N uptake may vary from 1.5:1 – 25:1 (Turner and Gibson 1980), which is due, in part, to of varying amounts of nitrogen leakage (Gallon et al. 2002). Acetylene reduction is therefore more appropriate for ecological studies seeking to understand nitrogen addition to the aquatic system (gross nitrogen fixed) and ^{15}N studies best suited to questions about net fixation; sequestration and acquisition by individual cells.

1.6 The importance of Lake Tarawera and the Rotorua lakes

The Rotorua region contains 12 major lakes that are of significant regional and national importance. The lakes are used for a range of activities including picnicking, walking, swimming, scenic driving, boating and fishing (Bell and Yap 2004). Tourism is also highly important to the region, making water quality and lake management concerns important issues for this sector (Hooker and Taylor 2005). Lake Tarawera in particular is renowned as a high quality rainbow trout fishery and for its clear water.

The Rotorua lakes are of significance to Te Arawa, the iwi that has mana whenua (territorial rights) of this region. Titles of 13 of the region's lakebeds (including Lake Tarawera) have been vested in Te Arawa. A deed of settlement was signed in 2004 between Te Arawa and the Crown to redress cultural, historical, spiritual and financial issues associated with the Crown removing ownership of all but one

of the lakes from Te Arawa in 1922. The Te Arawa Lakes Trust is the governing body which manages Te Arawa's interest in the lakes.

1.7 Risk of eutrophication and mechanisms to protect Lake Tarawera

While progression to eutrophication is a natural process in lakes over a long time scale (Hutchinson 1973) many lakes internationally (OECD 2008) and within New Zealand (PCE 2004) have shown accelerated rates of eutrophication due to recent human activities. These activities include the use of nitrogen based fertilisers, which have become more readily available since the discovery of the Haber Bosch process. In New Zealand there has been a major increase in agricultural and horticultural productivity associated with expansion of these fertilisers (PCE 2004).

International attention has been given to the impact of nutrients, in particular reactive nitrogen, in the Nanjing declaration (Erisman 2004). The Declaration recommends a shift towards management of land use practices to control the non-point source discharges, and to manage catchments as a whole by defining allowable nutrient limits (Erisman 2004).

In New Zealand the increase in fertilisation and conversion of land into dairy farms has been important to economic development of the country (Clark et al. 2007) but has been detrimental to the health of waterways (Hamilton 2005). Mechanisms to protect waterways from diffuse nutrient pollution in New Zealand

have been largely voluntary and councils have focused on point source discharges though consents (Drummond 2006).

Lake Tarawera is currently an oligotrophic lake (Scholes and Bloxham 2007). It could however, be vulnerable to water quality deterioration from possible changes in land use within its immediate catchment, but also through indirect inputs via the catchments of lakes Okareka, Okataina, Rotokakahi, Rotomahana and Tikitapu. These lakes indirectly discharge to Lake Tarawera through surface water streams arising from the lakes themselves and from groundwater, as Lake Tarawera is situated at the topographically lowest point of a broad hydrological landscape (Davy and Bibby 2005). All of these lakes whose water flows to Lake Tarawera have higher trophic levels (Scholes and Bloxham 2007).

To manage the declining water quality in many of the regions lakes, Environment Bay of Plenty has introduced 'Rule 11' to the Regional Water and Land Plan, to manage the use of nitrogen and phosphorus within lake catchments. By restricting excess fertilizer from farming through Rule 11, in conjunction with improved sewage treatment, riparian enhancement and changes to stock management practices, reduction in nutrient loads to the Rotorua lakes may be successful.

1.8 Study objectives

This study investigated the relative importance of physical and biological factors that drive phytoplankton dynamics in a deep oligotrophic lake. Phytoplankton

features of particular interest in this study were the DCM, the increased phytoplankton biomass in winter and the occurrence of surface blooms and nitrogen fixation of cyanobacteria.

The literature provides evidence for DCM formation by physical, biological and physiological means. To understand if Lake Tarawera's DCM is formed by increased growth the following hypothesises were tested:

1. That concentrations of available nutrients are higher in the metalimnion than the epilimnion.
2. That growth rates of DCM phytoplankton are higher than growth rates of phytoplankton at the lake surface.
3. That there is a strong relationship between the depth of the thermocline, euphotic depth and the persistence of the DCM.

Phytoplankton biomass throughout the water column has been observed to be higher in winter in some deep oligotrophic lakes in temperate regions (Vincent 1983, Alcocer and Lugo 2003). The following hypotheses were investigated to elucidate the formation of the winter phytoplankton biomass peak:

4. That increased phytoplankton biomass during winter is preceded by higher rates of phytoplankton growth.
5. That whole lake mixing coincides with an increase in nutrients in the epilimnion.

Surface blooms of cyanobacteria are most common in eutrophic lakes but are sometimes observed in oligotrophic lakes. Heterocystous species may also fix atmospheric nitrogen, particularly if nitrogen is limiting compared with phosphorus. In this study surveillance for the occurrence of ephemeral surface blooms was carried out and the following hypotheses tested:

6. That surface blooms of cyanobacteria that occur in Lake Tarawera actively fix atmospheric nitrogen.
7. That nitrogen fixation occurs at times of nitrogen limitation.

1.9 References

Alococer, J., and A. Lugo, 2003. Effects of El Niño on the dynamics of Lake Alchichica central Mexico. Geofisica Internacional **2003**:523-528.

Adrian, R., and T. Schipolowski. 2003. Bacterial and protozoan mass accumulation in the deep chlorophyll maximum of a mesotrophic lake. Archiv fur Hydrobiologie **157:** 27-46.

Bell, B., and M. Yap. 2004. The Rotorua Lakes. Evaluation of less tangible values, p. 71. Nimmo-Bell & Company Ltd.

Bleiker, W., and F. Schanz. 1989. Influence of environmental factors on the phytoplankton spring bloom in Lake Zurich. Aquatic Sciences **51** 47-58.

Booker, M. J., and A. E. Walsby. 1981. Bloom formation and stratification by a planktonic blue-green alga in an experimental water column. British Journal of Phycology **14:** 141-150.

Boyd, C., and D. Gradman. 2002. Impact of osmolytes on buoyancy of marine phytoplankton. Marine Biology **141:** 605-618.

Büdel, B., 1999. Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. European Journal of Phycology **34**: 361-370.

Camacho, A., W. Wurtsbaugh, M. Miracle, X. Armengol, and E. Vicente. 2003. Nitrogen limitation of phytoplankton in a Spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment approach. Journal of Plankton Research **25:** 397-404.

Chapin, B., F. J. Denoyelles, D. Graham, and V. Smith. 2004. A deep maximum of green sulphur bacteria ('*Chlorochromatium aggregatum*') in a strongly stratified reservoir. Freshwater Biology **49:** 1337-1354.

Chorus, I., and J. Bartram. 1999. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. World Health Organisation. Chapman & Hall, London, 416 pp.

Clark, D. A., J. R. Caradus, R. M. Monaghan, P. Sharp, and B. S. Thorrold. 2007. Issues and options for future dairy farming in New Zealand. New Zealand Journal of Agricultural Research **50:** 203-221.

Condie, S. A., and M. Bormans. 1997. The Influence of Density Stratification on Particle Settling, Dispersion and Population Growth. Journal of Theoretical Biology **187:** 65-75.

Coon, T., M. M. Lopez, P. J. Richerson, T. M. Powell, and C. R. Goldman. 1987. Summer dynamics of the deep chlorophyll maximum in Lake Tahoe. Journal of Plankton Research **9:** 327-344.

Davy, B., and H. Bibby. 2005. Seismic reflection imaging of the Haraharo Caldera boundary beneath Lake Tarawera, Okataina Volcanic Centre, New Zealand. New Zealand Journal of Geology and Geophysics **48**: 153-166.

Derenbach, J. B., H. Astheimer, H. P. Hansen, and H. Leach. 1979. Vertical microscale distribution of phytoplankton in relation to the thermocline. Marine Ecological Progress Series **1:** 187-193.

Dokulil, M. and K. Teubner. 2000. Cyanobacterial dominance in lakes. Hydrobiologia **438**: 1-12.

Drummond, L. 2006. Managing the environmental effects of agriculture under the RMA: non-point source discharges. New Zealand Journal of Environmental Law **10:** 20-23.

Ediger, D., S. Tugrul, and A. Yilman. 2004. Vertical profiles of particulate organic matter and its relationship with chlorophyll-a in the upper layer of the NE Mediterranean Sea. Journal of Marine Systems **55:** 311-326.

Erisman, J. W. 2004. The Nanjing declaration on management of reactive nitrogen. BioScience **54:** 286-287.

Fairbanks, R., and P. Wiebe. 1980. Foraminifera and chlorophyll maximum: vertical distribution, seasonal succession, and paleoceanographic significance. Science **209:** 1524-1526.

Gallon, J. R., and others 2002. Maximum rates of N_2 fixation and primary production are out of phase in a developing cyanobacterial bloom in the Baltic Sea. Limnology and Oceanography **47:** 1514-1521.

Garcia-Pichel, F., López-Cortés, F., and U. Nübel 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Applied and Environmental Microbiology **67** (4), 1902-1910.

Gervais, F. 1998. Ecology of cryptophytes coexisting near a freshwater chemocline. Freshwater Biology **39:** 61-78.

Gibbs, M. M., J. C. Rutherford, and I. Hawes. 2002. Lake Taupo Long Term Monitoring Programme 2000-2001, with a review of accumulated data since 1994, p. 73. NIWA.

Hamilton, D. 2005. Land use impacts on nutrient export in the central volcanic plateau, north island. NZ Journal of Forestry**: 49** (4): 27-31.

Hamilton, D., Hawes, I and Davies-Colley, R. 2004. Physical and chemical characteristics of lake water. Chapter 21 *In:* Freshwaters of New Zealand, pp 21.1-21.19. [Eds] .Jon Harding, Paul Mosley, Charles Pearson and

Brian Sorrell. New Zealand Hydrological Society and New Zealand Limnological Society 700 pp.

Havens, K., Beaver, J and T. East, 2007. Plankton biomass partitioning in a eutrophic subtropical lake: comparison with results from temperature lake ecosystems. Journal of Plankton Research **29** (12): 1087-1097.

Hooker, M., and S. Taylor. 2005. Lake water quality perception survey, p. 15. Key Research and Marketing LTD. Mount Manganui, New Zealand.

Horne, A. J., and M. L. Commins. 1987. Macronutrient controls on nitrogen fixation in planktonic cyanobacterial populations. New Zealand Journal of Marine and Freshwater Research **21:** 413-423.

Howard, J., and D. Rees. 1996. Structural basis of biological nitrogen fixation. Chemistry Review **96:** 2965-2982.

Howarth, R., R. Marino, J. Lane, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 1. Rates and importance. Limnology and Oceanography **33:** 669-687.

Huisman, J. 1999. Critical depth and critical turbulence: Two different mechanisms for the development of phytoplankton blooms. Limnology and Oceanography **44:** 1781-1787.

Huisman, J., M. Arrayas, U. Ebert, and B. Sommerijer. 2002. How do sinking phytoplankton species manage to persist? The American Naturalist **159:** 245-254.

Hutchinson, G. E. 1973. Eutrophication. American Scientist **61:** 269-279.

Klausmeier, C. A., and E. Litchman. 2001. Algal games: the vertical distribution of phytoplankton in poorly mixed water columns. Limnology and Oceanography **46:** 1998-2005.

Klemer, A.R., Cullen, R.J.,Mageau,M.T.,Hanson, K.M., and R. A. Sundell. 1996. Cyanobacterial buoyancy regulation: the paradoxical roles of carbon:' Journal of Phycology **31** (1) 47-53.

Kanoshina, I.,Lips, U., and J Leppänen. 2003. The influence of weather conditions (temperature and wind) on cyanobacterial bloom development in the Gulf of Finland (Baltic Sea) Harmful Algae. **2**(1): 29-41.

Lampert, W., and J. Grey. 2003. Exploitation of a deep-water algal maximum by Daphnia: a stable-isotope tracer study. Hydrobiologia **500:** 95-101.

Lewis, W. 1983. A revised classification of lakes based on mixing. Canadian Journal of Fisheries and Aquatic Sciences **40**:1779-1787.

Lewis, W., and S. Levine. 1984. The light response of nitrogen fixation in Lake Valencia, Venezuela. Limnology and Oceanography **29:** 894-900.

Loban, C., and P. J. Harrison. 1997. Seaweed Ecology and Physiology. Cambridge University Press. Cambridge, United Kingdom. 376 pp.

MacIntyre, S., J. R. Romero, and G. W. Kling. 2002. Spatial-temporal variability in mixed layer deepening and lateral advection in an embayment of Lake Victoria, East Africa. Limnology and Oceanography **47:** 656-671.

Moll, R., M. Brahce, and T. Peterson. 1984. Phytoplankton dynamics within the subsurface chlorophyll maximum of Lake Michigan. Journal of Plankton Research **6:** 751-766.

Nayak, S. and R. Prasanna 2007. Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. Applied Ecology and Environmental Research **5** (2): 103-113.

O'Brien, K. R. 2002. The effects of turbulent mixing on the vertical distribution and biomass of phytoplankton. Ph.D thesis. The University of Western Australia.

OECD 2004. Eutrophication of waters: Monitoring, management and control. Organization for economic cooperation and development. Paris, France. http ://www.oecd.org/publications.

Oliver, R. L. 1994. Floating and sinking in gas vacuolated cyanobacteria. Journal of Phycology **30:** 161-173.

Paerl, H. 1988. Nuisance phytoplankton blooms in coastal estuarine and inland waters. Limnology and Oceanography **33** (4/2): 823-847.

Paerl, H. W., and J. P. Zehr. 2000. Marine nitrogen fixation. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley Liss Inc.

PCE. 2004. Growing for good: Intensive farming, sustainability and New Zealand's environment. Parliamentary Commissioner for the Environment, Wellington, New Zealand.

Presing, M., L. Voros, S. Herodek, and G. Abrusan. 1998. The significance of various nitrogen sources for phytoplankton growth in shallow lakes and hypertrophic reservoirs. International Review of Hydrobiology **83:** 361-388.

Pridmore, R. D., and M. K. Etheredge. 1987. Planktonic cyanobacteria in New Zealand inland waters: distribution and population dynamics. New Zealand Journal of Marine and Freshwater Research **21:** 491-502.

Priscu, J. C., and C. Goldman. 1983. Seasonal dynamics of the deep chlorophyll maximum in Castle Lake, California. Canadian Journal of Fisheries and Aquatic Sciences **40:** 208-214.

Ryan, E., D. Hamilton, J. Hall, and V. Cassie-Cooper. 2005. Phytoplankton composition and biomass along horizontal and vertical gradients. Verhandlung Internationale Vereingung de Limnologie. 8 - 14 August 2004. 548 .

Sanford, L. P. 1997. Turbulent mixing in experimental ecosystem studies. Marine Ecological Progress Series **161:** 265-293.

Saros, J., S. Interlandi, S. Doyle, T. Michel, and C. Williams. 2005. Are the deep chlorophyll maxima in alpine lakes primarily induced by nutrient availability, not UV avoidance? Arctic, Antarctic and Alpine Research **37:** 557-563.

Sawatzky, L., W. Wurtsbaugh, and C. Luecke. 2006. Effects of nutrient limitation, light limitation, and invertebrate grazers on phytoplankton living in deep chlorophyll layers. Journal of Plankton Research **28:** 65-86.

Schindler, D. W. 1988. Whole ecosystem experiments: replication versus realism: the need for ecosystem scale experiments. Ecosystems **1**(4): 323-334.

Scholes, P., and M. Bloxham. 2007. Rotorua lakes water quality 2007 report, 53.p Environment Bay of Plenty. 2008/4.

Smith, V. 1983. Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton. Science **221:** 669-671.

Soranno, P.A. 1997. Factors affecting the timing of surface scums and epilimnetic blooms of blue-green algae in a eutrophic lake. Canadian Journal of Fisheries and Aquatic Sciences. **54**: 1965-1975.

Spigel, R. H., C. Howard-Williams, I. Hawes, and M. James. 2003. Predictions of water quality changes in Lake Taupo under different nitrogen loadings:

Further refinement and application of a coupled hydrodynamic- ecosystem model, p. 21. National Institute of Water and Atmosphere. CHC2002-042.

Steele, J. H., and C. S. Yentsch. 1960. The vertical distribution of chlorophyll. Journal of the Marine Biology Association **39:** 217-226.

Stewart, W. D. P. 1980. Systems involving blue-green algae (cyanobacteria). *In* F. J. Bergensen [ed.], Methods of evaluating biological nitrogen fixation. John Wiley & Sons Ltd.

Tilman, D., S. S. Kilham, and P. Kilham. 1982. Phytoplankton community ecology: the role of limiting nutrients. Annual Review of Ecology and Systematics **13:** 349-372.

Tonno, I., and T. Noges. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. Hydrobiologia **490:** 23-30.

Turner, G. L., and A. H. Gibson. 1980. Measurement of nitrogen fixation by indirect means, p. 111-138. *In* F. J. Bergensen [ed.], Methods for evaluating biological nitrogen fixation. John Wiley & Sons Ltd.

van Rijn, J., and M. Shilo. 1985. Carbohydrate fluctuation, gas vacuolation, and vertical migration of scum-forming cyanobacteria in fish ponds. Limnology and Oceanography **30:** 1219-1228.

Viner, A. B., 1984. Resistance to mixing in New Zealand lakes. New Zealand Journal of Marine and Freshwaters Research **18:** 73-82.

Vincent, W. F. 1983. Phytoplankton production and winter mixing: Contrasting two oligotrophic lakes. Journal of Ecology **71:** 1-20.

Waite, A., A. Fisher, P. A. Thompson, and P. J. Harrison. 1997. Sinking rate vs. volume relationships illuminate sinking control mechanisms in marine diatom. Marine Ecology Progress Series **157:** 97-108.

Wallace, B., and D. Hamilton. 1999. The effect of variations in irradiance on buoyancy regulation in *Microcystis aeruginosa*. Limnology and Oceanography **44:** 273-281.

Walsby, A. E. 1969. Permeability of blue-green algal gas-vacuole membranes to gas. Proceedings of the Royal Society of London B **173:** 235-255.

2.0 The formation of a deep chlorophyll maximum and winter bloom in an oligotrophic lake

2.1 Introduction

A deep chlorophyll maximum (DCM) may occur in freshwater and oceanic systems (Acinas et al. 1997), including tropical lakes (Zapata-Anzola et al. 2006), temperate lakes (Gervais 1998; Cole et al. 2002; Modenutti et al. 2004), and in polar regions (Bell and Laybourn-Parry 1999; Roberts et al. 2000). There is no single unifying theory on how DCMs form, and various physical (Derenbach 1979) and biological mechanisms (Moll 1984) have been proposed for their existence. Physical theories relate primarily to the occurrence and position of the DCM in relation to the thermal structure of a water body, with DCM depth often closely aligned to thermocline depth. At this depth the formation of a DCM may be made possible by a suitable turbulence regime – 'a turbulent window' – where negatively buoyant phytoplankton accumulate in sufficient density to offset natural losses (Priscu and Goldman 1983; Huisman et al. 1999, 2002). The occurrence of vertical gradients of bacteria, cryptophytes, protozoa, mesozooplankton, organic matter and particles supports the hypothesis that the thermal structure dictates the vertical distribution of all particles, including phytoplankton, and that they do not form subsurface peaks as a consequence of a growth advantage (Adrian and Schipolowski 2003; Chapin et al. 2004; Ediger et al. 2004; Fairbanks and Wiebe 1980; Gervais 1998; Lampert and Grey 2003).

Conversely, a DCM may also form in response to elevated growth rates relative to losses from respiration, death, sedimentation and grazing (Moll et al. 1984; Steele and Yentsch 1960). These higher growth rates may be supported by the presence of nutriclines, or simply by greater availability of nutrients at depth (Bell and Laybourn-Parry 1999; Gervais 1998; Roberts et al. 2000). Thus DCM formation may reflect the prevailing balance; a compromise between the acquisition of resources, i.e., optimum light towards the surface and greater nutrient availability at depth (Klausmeier and Litchman 2001; Camacho et al. 2003). Other biological explanations for DCM formation include a reduction in grazing by zooplankton (Coon et al. 1987; Sawatzky et al. 2006), physiological adaptation by phytoplankton cells to reduce sedimentation rates at depths of the DCM (Boyd and Gradman 2002; Waite et al. 1997), enhanced growth in the metalimnion away from damaging ultra-violet light (Saros 2005) and chromatic adaptation whereby cells living at depth with lower light have a greater proportion of chlorophyll (McManus and Dawson 1994).

Diatom blooms are most frequently reported to occur as seasonal peaks in biomass, during summer (Bailey-Watts 1976) or through autumn and spring (Miretzky and Cirelli 2004). Seasonal maxima of diatom biomass in winter are apparently less commonly reported, but have been observed in lakes in New Zealand (Vincent 1983) and Mexico (Alcocer and Lugo 2003) in association with seasonal redistribution of nutrients and adequate irradiance during winter circulation (Davies et al. 2004). When a water body begins to stratify or when nutrients have been consumed from surface waters, the bloom declines and

diatoms sink out of the surface mixed layer (Viner and White 1987; Spigel et al. 2003).

To understand the formation, persistence and breakdown of the DCM in Lake Tarawera, we tested three possible explanations for why the DCM forms. One explanation is that gross growth rates of phytoplankton, indicated by carbon uptake rates, are higher at the DCM than in the surface mixed layer. The second is that nutrient limitation is strongly alleviated in the DCM compared with surface waters. Thirdly, we examined how the vertical phytoplankton distribution may be driven by thermal stratification and light availability and consider the importance of the DCM to the winter bloom and phytoplankton dynamics in Lake Tarawera.

2.2 Methods

2.2.1 Study site

Lake Tarawera (176° 25' E, 38° 12' S) is a large (41.6 km^2), deep (max. depth 84 m), oligotrophic lake located in the central volcanic plateau of North Island, New Zealand. It lies in the Horoharo collapse caldera, which was formed by two ignimbrite eruptions; Matahina 220 thousand years ago (ka); and Rotoiti 65 ka (Nairn 2002). The catchment area of Lake Tarawera is 445 km^2, of which more than one-half is made up of five surrounding lake catchments that ultimately drain into the lake (Nairn 2002; Davy and Bibby 2005).

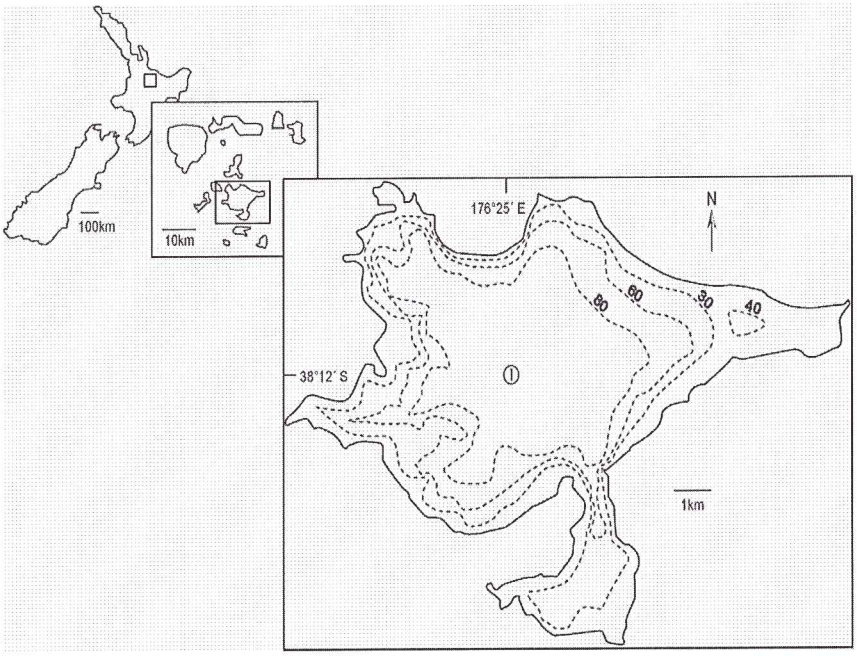

Figure 2.1. Location of Lake Tarawera amongst lakes of the Rotorua district (left- hand box) and showing central lake sampling station 1 and bathymetry (right hand box).

2.2.2 Lake profile data

All sampling for this study was carried out in Lake Tarawera at a central lake station (Figure 2.1). Sampling was undertaken approximately monthly, from July 2004 to July 2005. Water samples were taken at 10 m depth intervals from 0 to 70 m using a 12 L Schindler-Patalas trap. Sub-samples of this water were analysed for nutrients, phytoplankton composition and chlorophyll *a*.

Water for nutrient analysis was sub-sampled to 60 mL, filtered (GF/C glass-fibre filter with nominal pore size 0.45 μm), transported on ice and then stored at -20°C. A Lachat Instruments flow injection analyser (Zellweger Analytics 2000) was used to analyse samples for soluble reactive phosphorus (SRP) (Diamond 2000), ammonium (NH_4) (Prokopy 1992) and oxidised nitrogen (NO_x and NO_2) (Wendt 2000). Concentrations of NO_3 were calculated by subtracting NO_2 from NO_x.

Samples for total phosphorus (TP) were collected from the surface using a 13 m integrated sampling tube. Deep samples were collected from 80 m using the Schindler Patalas trap. Samples were transported on ice and then stored at -20° C. Nitric acid was used to digest samples before analysis, using an inductively coupled plasma mass spectrometry instrument (APHA 2005).

Two-hundred mL sub-samples for chlorophyll *a* and phaeophytin *a* analyses were filtered (GF/F) immediately after collection, transported in liquid nitrogen and stored at -20°C before extraction with 90% acetone. Extracts were measured spectrofluorometrically, with standard equations used to determine chlorophyll *a* and phaeophytin (Arar and Collins 1997). Phytoplankton degradation was assessed by comparing the percentage of phaeophytin to chlorophyll *a*. Phytoplankton were collected from 10 m sub-samples and preserved with 2% Lugol's iodine. Cells were subsequently sedimented (Utermöhl 1958), then identified using an inverted microscope.

Depth profiles of temperature, chlorophyll-fluorescence and photosynthetically active radiation were carried out with a Seabird Electronics 19plus Seacat conductivity-temperature-depth (CTD) profiler fitted with a fluorometer (Chelsea Instruments Ltd) and a photosynthetically active radiation sensor (PAR, Licor Inc.). Additional profiles were carried out in conjunction with *in situ* productivity and nutrient limitation experiments. Temperature (T) and fluorescence data from CTD casts were averaged into 1 m depth (z) bins. The thermocline was defined as the depth at which dT/dz = minimum, with visual inspection always used to confirm that this minimum corresponded to the seasonal thermocline. Chlorophyll-fluorescence was calibrated with acetone-extracted chlorophyll *a* concentrations, from samples taken at 10 m intervals at the central lake station. To account for possible solar quenching (Smyth et al. 2004), CTD fluorescence profile data points from 0-10 m were removed from profiles. These data were replaced with the solvent-extracted chlorophyll *a* data.

The spatial and temporal distribution of water temperature, SRP (as PO_4-P), NO_3-N and NH_4-N and chlorophyll *a*-corrected fluorescence were displayed in contour plots using Ocean Data View (Schiltzer 2005).

2.2.4 Subsurface photosynthetically active radiation (PAR)

An isolume value of 5 μmol m^{-2} s^{-1} PAR was set to approximate the compensation irradiance for phytoplankton. The depth at which this level occurred was estimated from the PAR attenuation coefficient (K_d) and from the surface PAR value. Subsurface PAR profiles from the CTD were used to derive K_d, assuming Beer's Law to describe the exponential decay of PAR. Surface PAR was calculated as a monthly mean value preceding each sample day, using hourly total irradiance data from Rotorua Airport climate station, 15 km from Lake Tarawera. PAR was assumed to be 45% of total solar irradiance (Kirk 1994).

2.2.5 Nutrient limitation

Nutrient limitation of phytoplankton was investigated using *in situ* nutrient spiking incubations. Water was collected with a Schindler-Patalas trap from 5 m depth and from the depth of the DCM, defined by the peak in fluorescence in the metalimnion based on CTD profiles. When there was no DCM present, samples were taken from 25 m depth. Water was then incubated in 3 L clear PET® bottles with excess NH_4Cl (to 10 mg L^{-1} NH_4-N) or KH_2PO_4 (to 1 mg L^{-1} PO_4-P), both nutrients at these concentrations, or no added nutrients (control) for four days (Stein 1973; White et al. 1985). In each trial three replicate bottles were used for each of the six treatments and two controls treatments of nutrients and depth. Chlorophyll *a* samples were taken before and after each incubation and analysed spectrofluorometrically as described above. Chlorophyll *a* concentrations from each trial were averaged for mixed and stratified lake conditions.

2.2.6 Productivity

Carbon uptake rates of phytoplankton at 5 m and at the DCM were measured monthly by incubating phytoplankton *in situ* with ^{13}C enriched $NaHCO_3$ (Hama et al. 1983). Samples from 5 m and the DCM (or 25 m) were collected with a Schindler-Patalas trap, as described above, and sub-samples were incubated in 280 mL glass bottles together with 0.1 mg of 98% $NaH^{13}CO_3$. Water collected from 5 m was incubated at 5 m and DCM water was incubated at the level of the DCM. Incubations were four hours duration (1000 – 1400 hr NZSDT). Dark bottles were also incubated *in situ* to control for non-photosynthetic carbon uptake, while bottles filled with reverse osmosis water were used to control for residual carbon from ^{13}C isotope enrichments. At least four replicate incubation bottles were used for each treatment. Following incubations, bottles were immediately placed in the dark on ice, before each bottle was filtered onto a pre-ashed GF/C filter within one hour. Four replicate samples of ambient water, of equal volume to treatment bottles, from both 5 m and DCM (or 25 m) were also filtered onto pre-ashed GF/C filters to assess natural abundances of ^{12}C and ^{13}C. Dried samples from filters were analysed for mass of particulate organic carbon and atom % ^{13}C and $\delta^{13}C$. Mass spectroscopy was performed using a Dumas Elemental Analyser (Europa Scientific ANCA-SL) interfaced with an isotope mass spectrometer (Europa Scientific 20-20 Stable Isotope Analyser). Rates of carbon uptake rate, (U_C; $\mu g\ L^{-1}\ h^{-1}$) were calculated as:

$$U_C = \frac{C(a_{is} - a_{ns})}{t(a_{ic} - a_{ns})}$$

where a_{is} is the atom % of ^{13}C in the incubated sample, a_{ns} is the atom % of ^{13}C in the natural sample, a_{ic} is the atom % of ^{13}C in inorganic carbon, C is particulate organic carbon (POC) in the incubated sample ($\mu g\ C\ L^{-1}$), and t is time (h).

2.3 Results

2.3.1 Mixing and stability

A contour plot of water temperature derived from monthly CTD data shown in Figure 2.2 demonstrates vertically orientated isotherms during winter-spring mixing, from July to the beginning of October, with temperature varying less than 1 °C (between 11 and 12 °C) over all depths. In November surface waters began to warm rapidly and by March they exceeded 21°C, while hypolimnetic waters remained between 11 and 12 °C. From November to March a strong vertical temperature gradient formed, indicated by close proximity of horizontally oriented isotherms (Figure 2.2).

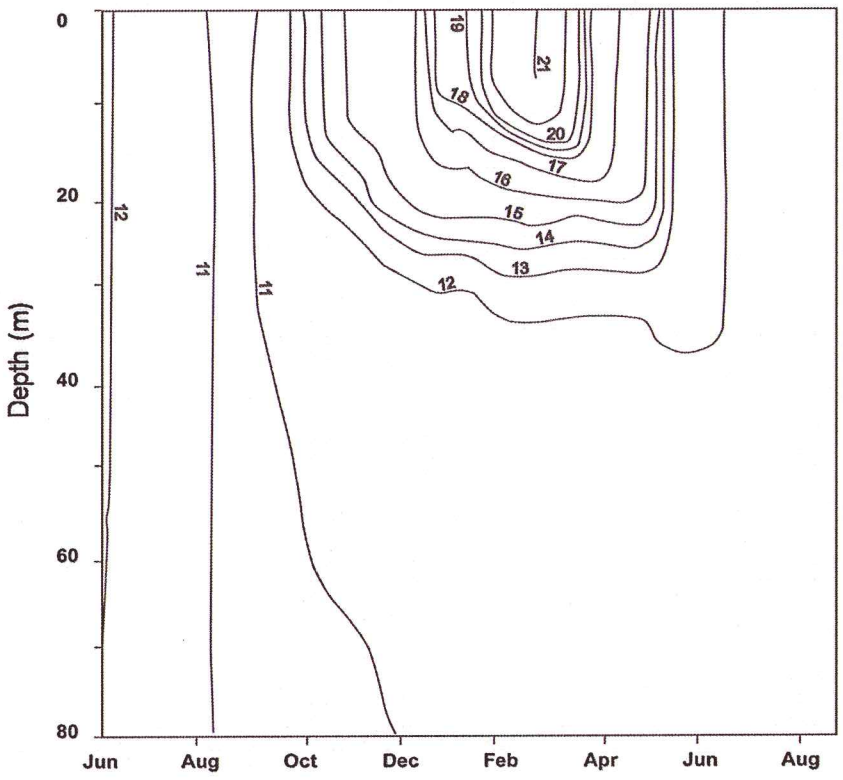

Figure 2.2. Contours of temperature with depth and time, over sampling period at Station 1.

2.3.2 The relationship between mixing depth, euphotic depth and the DCM

The depths of the DCM, thermocline and euphotic zone for each monthly profile are shown in Figure 2.3. A weak thermocline and DCM first appeared in October. From November to January the thermocline deepened along with the DCM, but the DCM remained below the thermocline. By late January the DCM was at the depth of the thermocline (26 m) and then became shallower through February (18 m) and March (15 m). By March the DCM was 2 m above the thermocline, while in April, the DCM and thermocline had deepened to 20 m. By June the thermocline had continued to deepen to 37 m and by July there was no discernable thermocline. The thermocline shown at 80 m in Figure 2.3, prior to October 2004

and in August 2005, represents complete mixing though the lake depth. In April there was a small chlorophyll *a* peak at 8 m, well above the thermocline, then no clear DCM or vertical peak in chlorophyll fluorescence in monthly samples after this time.

From November to April, the depths of the DCM and thermocline are slightly shallower than the euphotic depth (z_{eu}) (Figure 2.3), but by May the depth of the thermocline had decreased below z_{eu}. From this time the depths of the DCM and the thermocline diverged strongly and the chlorophyll fluorescence peak was close to the water surface.

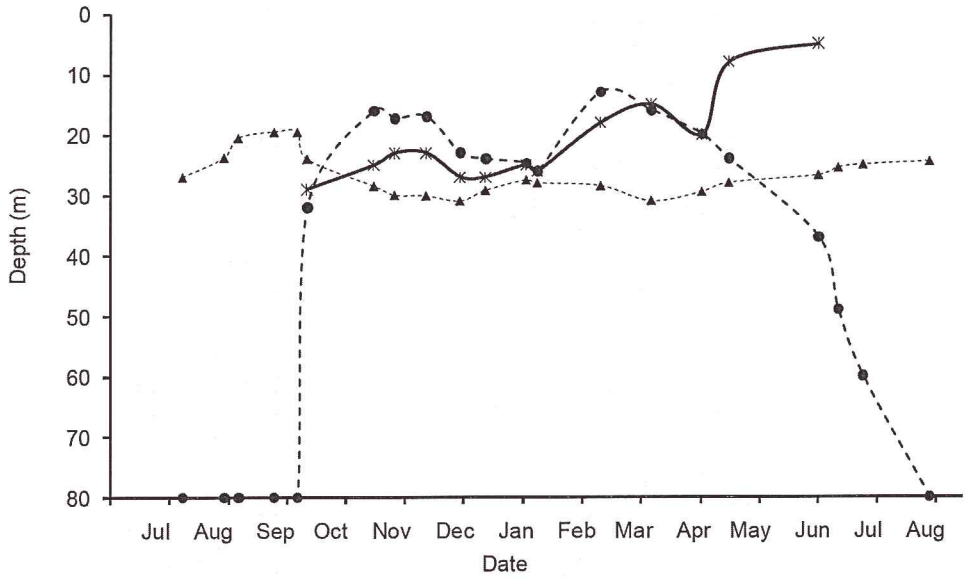

Figure 2.3. Depth of thermocline (dashed line with circles), DCM (asterisks with solid line) and the depth of the euphotic zone (dotted line with triangles).

2.3.3 Nutrient profiles

Concentrations of SRP ranged from 0.8 to 15 μg L^{-1} across all depths and throughout the sampling year (Figure 2.4a). Highest concentrations occurred near the bottom of the lake during late-summer stratification. Lowest concentrations occurred throughout the water column in July and August when the lake was fully mixed. The mean concentration of NO_3-N over all depths and months was 2.7 μg L^{-1}. A peak of NO_3-N occurred in May, when some values were as high 15 μg L^{-1} (Figure 2.4b). Concentrations of NH_4-N were also correspondingly low, typically <5 μg L^{-1} over summer. During the stratified period surface concentrations were particularly low (<2.5 μg L).

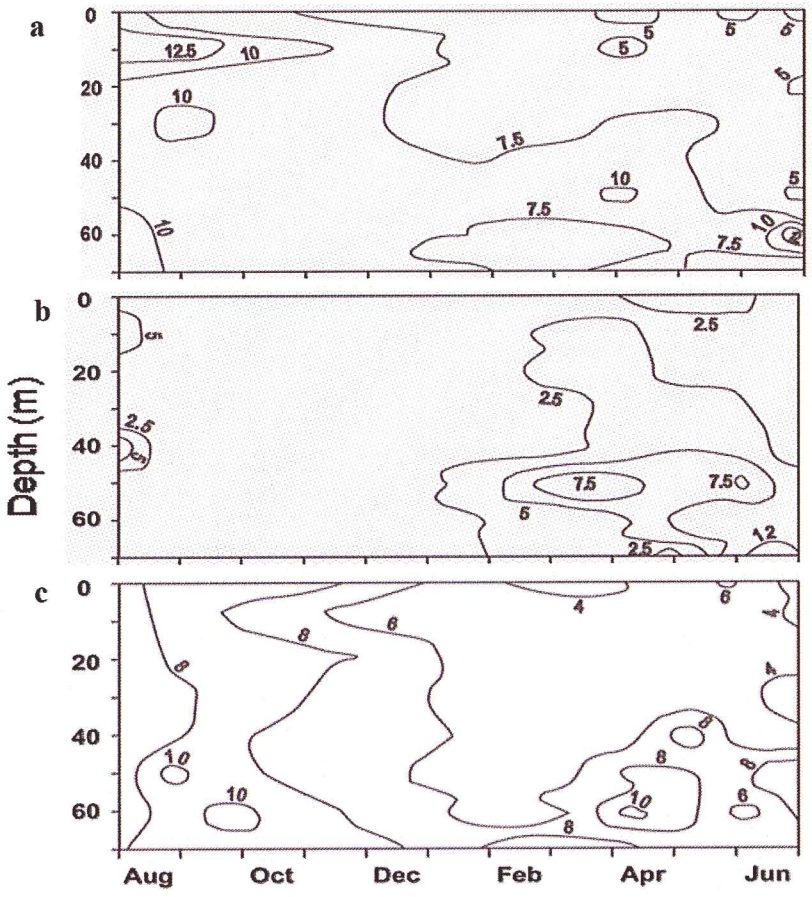

Figure 2.4. Concentrations of SRP ($\mu g\ L^{-1}$)(a), NH_4-N ($\mu g\ L^{-1}$)(b) and NO_3-N ($\mu g\ L^{-1}$)(c) over depth, from July 2004 to July 2005.

2.3.4 Total phosphorus

During winter mixing in 2004, concentrations of total phosphorus (TP) from integrated surface samples (0-16 m) and at 82 m were similar, between 9 and 11 $\mu g\ L^{-1}$ (Figure 2.5). Following stratification TP in the integrated surface samples increased to a peak of 14 $\mu g\ L^{-1}$ in December, after which time there was a nearly

linear decline until May, when concentrations reached an annual minimum of 4 μg L^{-1}. At the onset of mixing in June 2005 total phosphorus from the integrated surface sample increased to 24 μg L^{-1}. Concentrations of total phosphorus at 82 m were consistently higher during the stratified period, averaging 16.3 μg L^{-1}, with the greatest depth differential in May, at the end of the summer stratification period.

.

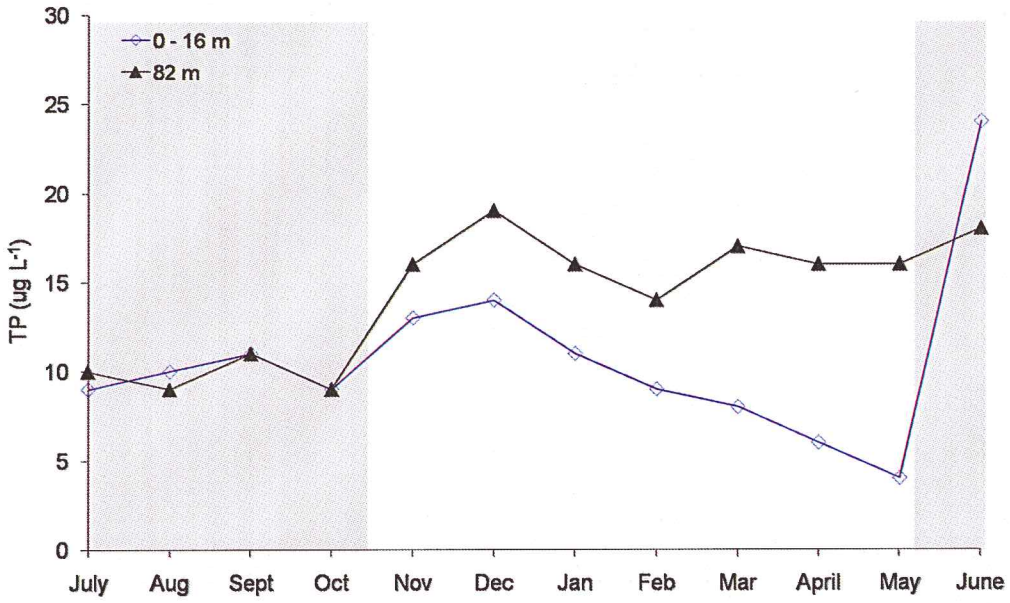

Figure 2.5. Concentration of total phosphorus from July 2004 to July 2005 at 0-16 m (open diamonds) and 82 m (triangles). Shaded regions of the graph represent times when the lake is mixed.

2.3.5 Chlorophyll a

Figure 2.6 shows monthly chlorophyll fluorescence (F) profiles at the central lake station over the one-year sampling period, with values normalised to chlorophyll *a* concentrations using linear regression from samples taken at 10-m depth intervals

and analysed for pigment concentration using solvent extraction. Isopleths of F are aligned vertically during the period of water column mixing. The highest F values occurred during winter mixing (July to September 2004, some exceeding 1.75 μg L^{-1}. During stratification F was generally lower and more heterogeneous throughout the water column, as indicated by horizontally aligned isopleths. Values were around 1 μg L^{-1} near the surface, increasing to around 1.3 μg L^{-1} between 20 and 30 m with a decline to around 0.8 μg L^{-1} below 30 m.

The percentage of phaeophytin, as a proportion of chlorophyll *a*, is shown in Figure 2.7. Data were separated into either mixed (October 2004 to April 2005) or stratified samples (June to September 2004) with the month of May 2005 not included in the analysis as it was considered to represent a transitional phase between lake mixing and stratification. When the lake was mixed there was no significant relationship ($p>0.05$) in the proportion of phaeophytin to chlorophyll *a* and lake depth (Figure 2.7 a) and the proportion ranged from 23 – 50% over all depths. During the stratified period however, there was a strong relationship between the proportion of phaeophytin to chlorophyll *a* and lake depth ($r^2 = 0.693$, $p<0.01$; Figure 2.7 b). Overall, the proportion ranged from 13 to 67%.

Samples taken from the DCM during stratification were mostly diatoms, with the dominant species comprising *Cyclotella* spp, *Stephanodiscus* spp., *Aulacoseira granulata*, *Asterionella formosa* and *Fragilaria crotonensis*. In April and May, surface phytoplankton comprised mostly of the cyanobacteria *Anabaena lemmermanni and Anabaena circinalis.*

During winter the phytoplankton population was vertically homogenous and comprised the same dominant species as the DCM; *Cyclotella* spp, *Stephanodiscus* spp., *Aulacoseira granulata*, *Asterionella formosa* and *Fragilaria crotonensis*.

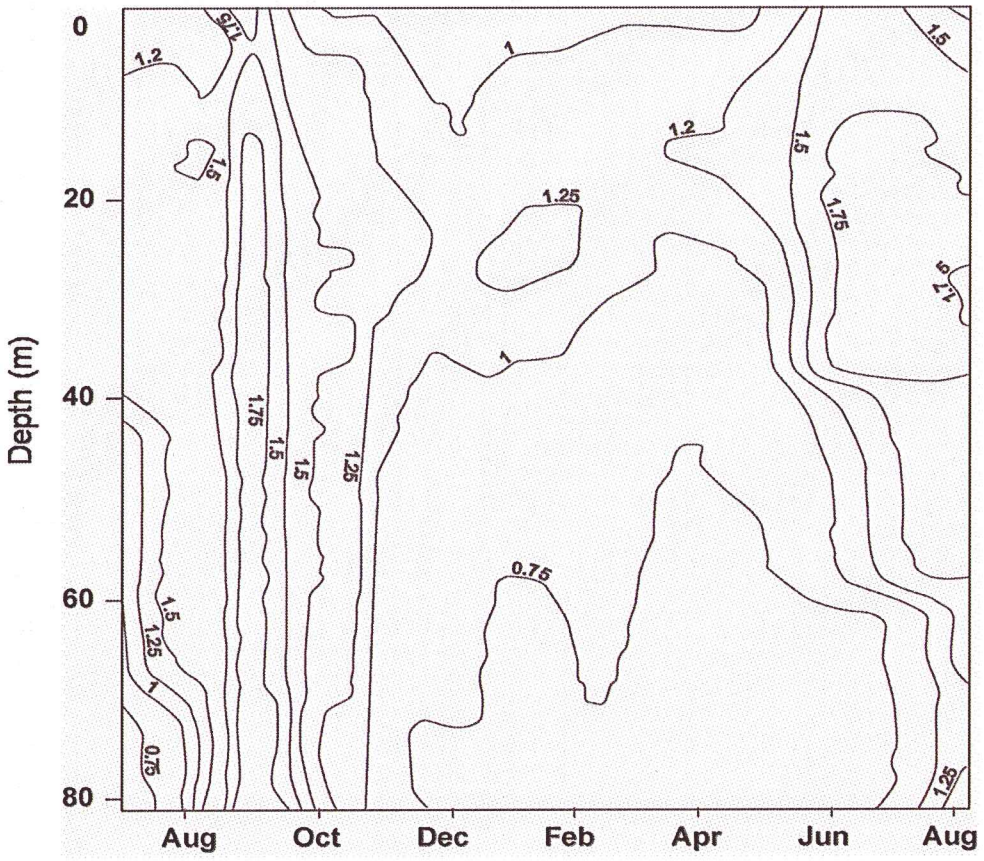

Figure 2.6. Chlorophyll a*-corrected fluorescence (µg L $^{-1}$) from 81 m CTD profiles over the sampling period at station 1.*

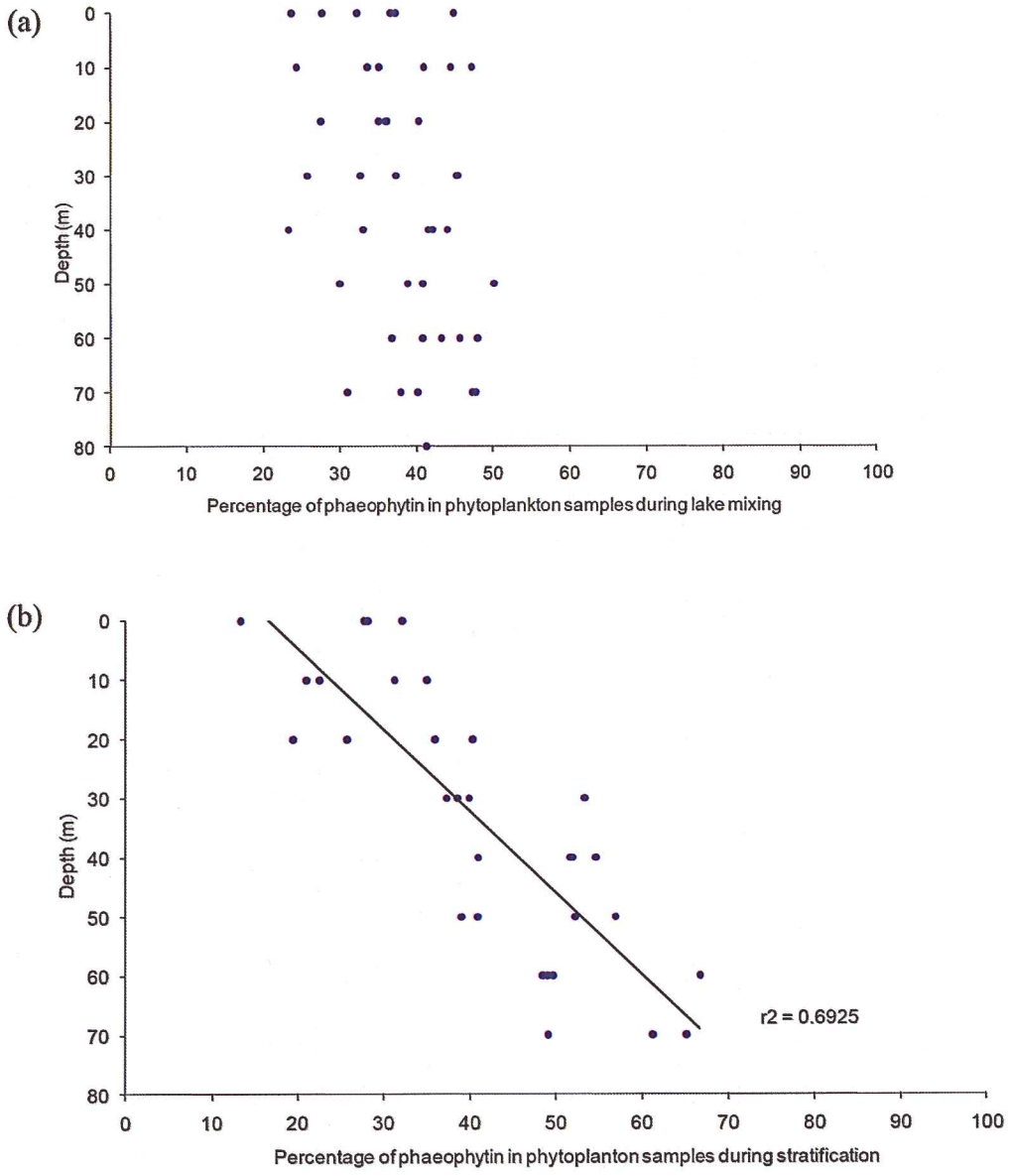

Figure 2.7.Proportion of phaeophytin in monthly chlorophyll samples over the lake depth during (a) whole lake mixing (July – October 2004 and June 2005) and (b) stratified months (Nov 2004-January 2005 and April 2005) Data from February and March 2005 was lost so could not been included. Data from May 2005 was not included as this month was a transition between mixing and stratification.

2.3.6 Carbon uptake

Rates of carbon uptake were lower at the DCM than at 5 m (thermally stratified conditions), and lower at 25 m than 5 m for each of the monthly samples (mixed conditions) (Figure 2.8). Carbon uptake rates at 5 m in June 2005 were particularly high compared to other incubations (32 μg L^{-1} h^{-1}). The second highest carbon uptake rates were in May 2005 and August 2004 when uptakes rates of 6 μg L^{-1} h^{-1} were recorded (Figure 2.8). Carbon uptake rates in incubations from the DCM were negligible but 25m incubations in May and June show a small amount of activity (0.7 μg L^{-1} h^{-1}).

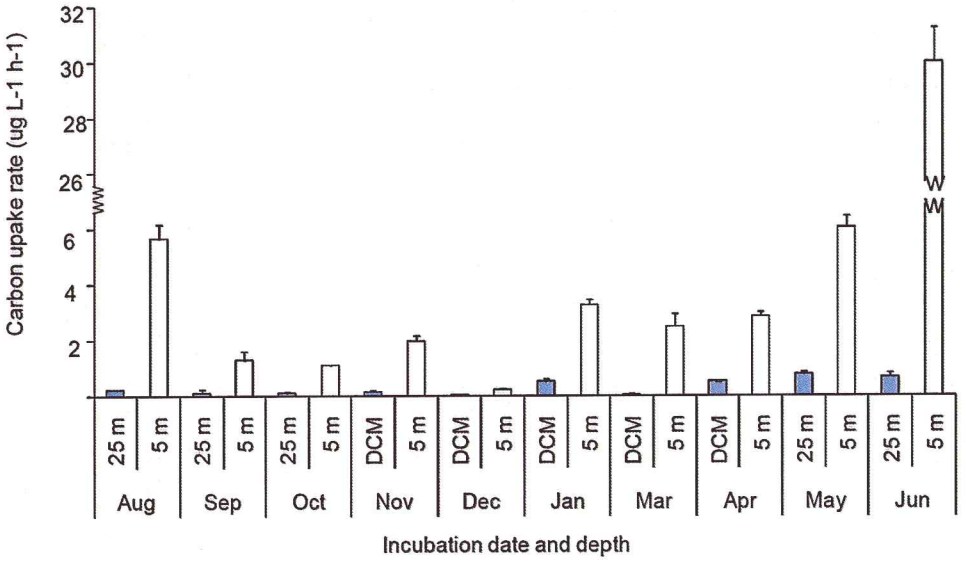

Figure 2.8. Comparison of photosynthetic carbon uptake rates at 5 m and at the DCM. Where no DCM was present, deep incubations were conducted at 25 m. Error bars show one standard deviation of mean.

2.3.7 Light and nutrient limitation incubations

Figure 2.9 shows the percentage increase of chlorophyll *a* at 5 m depth and for DCM samples in four-day nutrient incubations, above chlorophyll *a* concentrations of the incubated controls. While some patterns are indicated, variance between samples was high and in some instances standard deviations (SD) were greater than the mean. Insufficient replication, due in part to a high rate of incubation bottle loss, contributed to the high variability in the results. Percentage increases in chlorophyll *a* were higher at 5 m than at the DCM or 25 m (Figure 2.9). The smallest apparent increase in chlorophyll *a* resulted from phosphorus addition, with no apparent change (0% increase SD 31%) when the lake was mixed and a mean increase of 21 % (SD 54%) during stratified months. Increases in chlorophyll *a* at 5 m appear greater with nitrogen addition compared with phosphorus addition. During mixing (July–October 2004 and June–July 2005), addition of nitrogen increased chlorophyll *a* by 102.6 % (SD 25%) compared with 126 % (SD 97%) during stratified months (November 2004 – May 2005). The greatest increases in chlorophyll *a* were from incubations with both nitrogen and phosphorus addition, by 148 % (SD 103%) during mixing and by 225.6 % (SD 187%) during stratification.

Samples incubated for four days at 25 m during the period of mixing showed an average increase in chlorophyll *a* of 34 % (SD 43%) above the control in response to nitrogen addition; 9 % (SD 36%) with phosphorus and 3.1 % (SD 3%) with nitrogen and phosphorus. Samples incubated at the DCM showed a mean

increase of 4.3 % (SD 11%) with nitrogen, 7.7% (SD 15%) with phosphorus and 15.6% (SD 1.2%) with nitrogen and phosphorus. Standard deviations for different treatments (Figure 2.9) are again large and overlap. Due to the high variability between treatment replicates and between samples, as well as replicate treatments that were lost during the incubation, results are not statistically significant though they may nevertheless provide some indication of general trends.

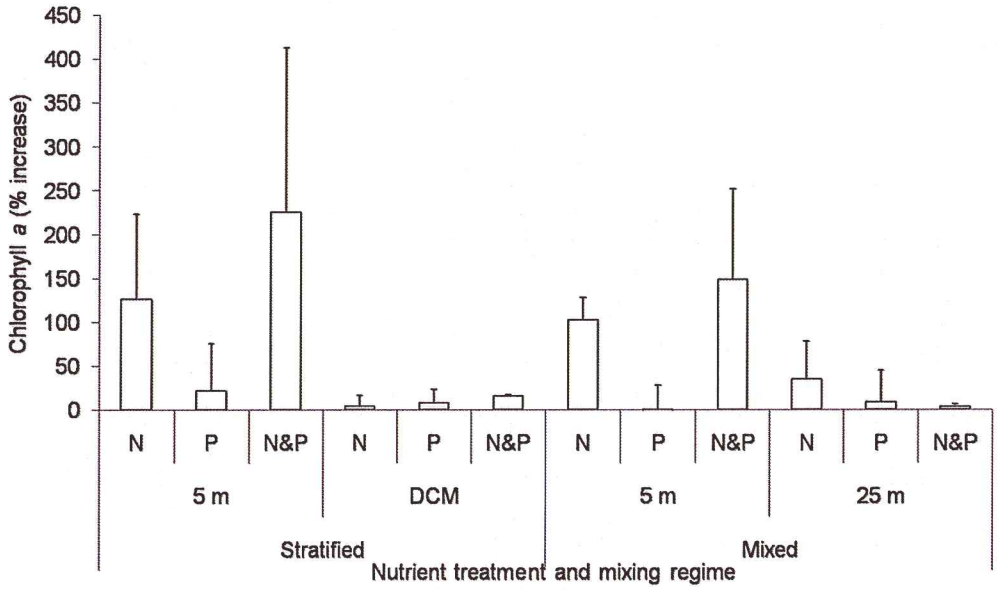

Figure 2.9. Mean percentage increase in chlorophyll a concentrations after in situ incubation for 4 days, with additions of N, P or N+P, compared with control incubations (no added nutrients). During mixing, when no DCM was present, incubations were at 25 m. Error bars show one standard deviation of the mean.

2.4 Discussion

Both volumetric carbon uptake and carbon uptake per unit chlorophyll were consistently lower at the DCM than at 5 m depth. The deep incubations showed very little carbon uptake activity, indicating growth limitation of the DCM population. The inorganic nutrients SRP, NO_3-N and NH_4-N were low throughout the water column profile and there was no indication that nutrients were greater in the metalimnion to support a higher biomass at the DCM. Experimental nutrient addition to DCM phytoplankton has been shown to increase chlorophyll *a* and primary productivity in other lakes (Gross et al. 1997; Wurtsbaugh et al. 2001), but this response was not observed in Lake Tarawera. Nutrient addition to the DCM and 25 m depth samples resulted in little stimulation of phytoplankton biomass, suggesting that while ambient levels are low, nitrogen and phosphorus are not the primary factors limiting phytoplankton growth in the DCM. The limited response of DCM phytoplankton to nitrogen and phosphorus could potentially be due to micronutrient limitation (Vrede and Tranvick 2006) and further investigation is required to eliminate micronutrients as a limiting factor.

A more likely explanation for limited carbon uptake and stimulation of DCM phytoplankton by nutrient addition is that the phytoplankton are light limited. The correlation between the depth of the DCM and thermocline persisted while the DCM remained within the euphotic zone (above the 5 $\mu mol\ m^{-2}\ s^{-1}$ isolume) but

once the thermocline deepened below the euphotic zone there was no longer a chlorophyll peak associated with this region.

As a consequence of light limitation, chlorophyll pigments in phytoplankton may degrade to phaeophytin (Lorenzen 1965) a molecule similar to chlorophyll but without the central Mg^{2+} ion required for electron transfer. Previous studies (Descy 2005) have found increased phaeophytin proportions with depth in lakes during stratification, similar to the pigment ratio profiles found in Lake Tarawera. DCM phytoplankton had significantly more phaeophytin, indicating they were physiologically compromised compared with phytoplankton from surface samples. It is consistent that these physiologically compromised phytoplankton are less able to take up carbon and to utilise additional nutrients compared with phytoplankton from surface samples.

Though other authors have shown evidence that the metalimnion may provide some DCM populations with more conducive growth conditions (Saros et al. 2005; Klausmeier and Litchman 2001) this investigation did not, as productivity of DCM phytoplankton was lower than productivity of phytoplankton at 5 m. Passive accumulation of phytoplankton therefore appears more likely. Negatively buoyant diatoms can only remain in the surface waters if there is sufficient turbulence (Huisman et al. 2002). The existence of thermal stratification provides resistance to turbulent mixing (Sanford 1997), resulting in diatoms sedimenting

out under gravity until the thermocline, where their descent slows, leading to an accumulation of cells at this depth (Woods 1972).

DCM phytoplankton may remain viable under low light conditions (Tilzer et al. 1977) until winter mixing commences, which circulates them through strong light gradients. The DCM phytoplankton may then act as an inoculum, contributing to the high productivity and biomass recorded in June at the onset of winter mixing. The size and status of the DCM population may therefore have implications for the size of the winter phytoplankton population as it may, in effect, provide the initial source of cells for the winter population.

Carbon uptake rates of phytoplankton incubated at 5 m during the stratified period were slightly lower, but consistent with uptake rates of around 6 $\mu g\ L^{-1}\ h^{-1}$ reported in deep oligotrophic Lake Taupo (Vincent et al. 1983), also located in the central volcanic plateau region of North Island, and in two deep oligotrophic lakes of South Island (Schallenberg and Burns 1997).

Unlike DCM incubations, phytoplankton at 5 m showed a positive growth response to nutrient additions. The greatest increase in chlorophyll *a* was in response to the addition of both nitrogen and phosphorus together, followed by nitrogen alone. These results indicate that nitrogen is the nutrient most likely to limit phytoplankton growth during incubations. Nitrogen limitation of

phytoplankton growth has also been identified in other lakes within the central volcanic plateau (White et al. 1986; Burger et al. 2007).

At the onset of winter mixing (June 2005) a 'bloom' occurred, with increased chlorophyll *a* through the entire water column (Figure 2.6) and the highest carbon uptake rates recorded over the investigation period (Figure 2.8). Prior to June 2005 the greatest phytoplankton biomass in the water column was associated with the DCM in the metalimnion where, as discussed above, cells appeared to be light limited. Water column mixing alleviates light limitation and gives phytoplankton from the entire water column a period of time in the euphotic zone. Improvement in phytoplankton health is demonstrated by lower and homogeneous ratios of phaeophytin to chlorophyll *a* throughout the depth of the lake.

Turbulent mixing of the water column may also support the winter bloom in Lake Taupo (Vincent 1983), but full lake mixing does not increase phytoplankton biomass in all deep lakes. If the mixing depth exceeds a critical depth, the light received by phytoplankton becomes too low to support net growth (Huisman et al. 1999). Very deep lakes such as Lake Tahoe (501 m max. depth) have lower phytoplankton biomass during mixing (Tilzer and Goldman 1978) as does Lake Waikaremoana (248 m max. depth), which is also apparently too deep for a winter bloom (Vincent 1983, Howard Williams et al. 1986).

Winter mixing also plays a role in distributing nutrients through the water column. During stratification TP showed a clear seasonal separation between surface waters and bottom waters from November to May, coinciding with thermal stratification. Similar accumulation of phosphorus has been reported in the hypolimnion of other lakes within the central volcanic plateau (Gibbs et al. 2007; Burger et al. 2006) and may be a result of releases from the bottom sediments (Burger et al. 2007). At the onset of lake mixing, TP in the surface waters increased from 4 $\mu g\ L^{-1}$ in May to 24 $\mu g\ L^{-1}$ in June which apparently supported the observed increase in phytoplankton productivity and biomass.

DCMs have been proposed as environmental indicators, as they may be sensitive to changes associated with eutrophication (Gross et al. 1997; Moll and Stroemer 1982; Shortreed and Stockner 1990). A direct response of DCM phytoplankton to the addition of nutrients was not observed in this study, but cannot be ruled out due to the inconclusive nature of the results. Increased nutrient additions to the lake might also impact the DCM indirectly, if for example surface phytoplankton increase in biomass in response to additional nutrients, their proliferation may result in considerable shading to DCM populations (Christensen et al. 1995). Any reduction in light from proliferations of the surface population may impact on the ability of DCM phytoplankton to subsist through the summer and may have implications for the initiation of the subsequent winter bloom.

DCMs have also been reported to contribute significantly to the overall primary productivity of lakes and as an energy source for other trophic levels (Barbiero and Tuchman 2004; Hanson et al. 2007). DCM primary productivity in this study was extremely low, however, and would likely provide a limited food supply to zooplankton (Barbiero and Tuchman 2004) compared with the productivity of surface waters.

While some DCM populations receive additional nutrients and a more suitable light environment compared with conditions in the surface mixed layer (Abbott et al. 1984; Coon et al. 1987), these factors may be incidental. DCMs of negatively buoyant phytoplankton only occur in stratified water bodies, indicating that the metalimnion has a primary role in their formation. Adequate conditions to support phytoplankton growth or at least to levels of subsistence must, however, also be present for the DCM to persist. In this study a relationship between the DCM and thermocline depths in the presence of adequate light was observed, but this association was lost as the thermocline deepened to below the estimated compensation depth.

2.5 References

Abbott, M., K. Denman, T. M. Powell, P. J. Richerson, R. Richards, and C. R. Goldman. 1984. Mixing and the dynamics of the deep chlorophyll maximum in Lake Tahoe. Limnology and Oceanography **29**: 862-878.

Acinas, S., F. Rodriguez-Valera, and C. Pedros-Alio. 1997. Spatial and temporal variation in marine bacterioplankton diversity as shown by RFLP fingerprinting of PCR amplified 16S rDNA. FEMS Microbiology Ecology **24**: 27-40.

Adrian, R., and T. Schipolowski. 2003. Bacterial and protozoan mass accumulation in the deep chlorophyll maximum of a mesotrophic lake. Archiv. fur Hydrobiologie **157**: 27-46.

Alcocer, J., and A. Lugo. 2003. Effects of El Niño on the dynamics of Lake Alchichica, central Mexico. Geofisica Internacional 2003: 523-528.

Arar, E. J., and G. B. Collins. 1997. In vitro determination of chlorophyll a and phaeophytin a in marine and freshwater algae by fluorescence, p. 22. National Exposure Research Laboratory. Office of Research and Development . U.S Environmental Protection Agency.

Bailey-Watts, A. 1976. Planktonic diatoms and some diatom silica relations in a shallow eutrophic Scottish loch. Freshwater Biology **6**: 69-80.

Barbiero, R., and M. Tuchman. 2004. The deep chlorophyll maximum in Lake Superior. Journal of Great Lakes Research **30**: 256-268.

Bell, E., and J. Laybourn-Parry. 1999. Annual plankton dynamics in an Antarctic saline lake. Freshwater Biology **41**: 501-519.

Boyd, C., and D. Gradman. 2002. Impact of osmolytes on buoyancy of marine phytoplankton. Marine Biology **141**: 605-618.

Burger, D., D. Hamilton, J. Hall, and E. Ryan. 2007. Phytoplankton nutrient limitation in a polymictic eutrophic lake: community versus species-specific responses. Archiv. fur Hydrobiologie **169:** 57-68.

Burger, D. F., D. P. Hamilton, C. P. Pilditch, and M. M. Gibbs. 2006. Sediment phosphorus release during stratification in polymicitic Lake Rotorua. Verhandlungen Internationale Vereinigung de Limnologie **29:** 811-814.

Camacho, A., W. Wurtsbaugh, M. Miracle, X. Armengol, and E. Vicente. 2003. Nitrogen limitation of phytoplankton in a Spanish karst lake with a deep chlorophyll maximum: a nutrient enrichment approach. Journal of Plankton Research **25:** 397-404.

Chapin, B., F. J. Denoyelles, D. Graham, and V. Smith. 2004. A deep maximum of green sulphur bacteria ('Chlorochromatium aggregatum') in a strongly stratified reservoir. Freshwater Biology **49**: 1337-1354.

Christensen, D., S. R. Carpenter, and K. Cottingham. 1995. Predicting chlorophyll vertical distribution in response to epilimnetic nutrient enrichment in small stratified lakes. Journal of Plankton Research **17:** 1461-1477.

Cole, P., C. Luecke, W. Wurtsbaugh, and G. Burkart. 2002. Growth and survival of Daphnia in epilimnetic and metalimnetic water from oligotrophic lakes: the effects of food and temperature. Freshwater Biology **47:** 2113-2122.

Coon, T., M. M. Lopez, P. J. Richerson, T. M. Powell, and C. R. Goldman. 1987. Summer dynamics of the deep chlorophyll maximum in Lake Tahoe. Journal of Plankton Research **9**: 327-344.

Davies, J.-M., W. Nowlin, and A. Mazumder. 2004. Variation in temporal [^{14}C] plankton photosynthesis among warm monomictic lakes of coastal British Columbia. Journal of Plankton Research **26(7):** 763-778.

Davy, B., and H. Bibby. 2005. Seismic reflection imaging of the Haraharo Caldera boundary beneath Lake Tarawera, Okataina Volcanic Centre, New Zealand. New Zealand Journal of Geology and Geophysics **48**: 153-166.

Derenbach, J. B., H. Astheimer, H. P. Hansen, and H. Leach. 1979. Vertical microscale distribution of phytoplankton in relation to the thermocline. Marine Ecological Progress Series **1:** 187-193.

Descy, J. P.and others 2005. Phytoplankton pigments and community composition in Lake Tanganyika. Freshwater Biology **50**: 668-684.

Diamond, D. 2000. Determination of ortho phosphate in waters by flow injection analysis colorimetry, p. 22. Lachet Instruments.

Ediger, D., S. Tugrul, and A. Yilman. 2004. Vertical profiles of particulate organic matter and its relationship with chlorophyll-a in the upper layer of the NE Mediterranean Sea. Journal of Marine Systems **55**: 311-326.

Fairbanks, R., and P. Wiebe. 1980. Foraminifera and chlorophyll maximum: vertical distribution, seasonal succession, and paleoceanographic significance. Science **209**: 1524-1526.

Gervais, F. 1998. Ecology of cryptophytes coexisting near a freshwater chemocline. Freshwater Biology **39**: 61-78.

Gibbs, M. M. 2007. Lake Taupo long-term monitoring programme 2005-2006, p. 83. Environment Waikato. NIWA client report Ham2008-028.

Gross, H., W. Wurtsbaugh, C. Luecke, and P. Budy. 1997. Fertilization of an oligotrophic lake with a deep chlorophyll maximum: predicting the effect on primary productivity. Canadian Journal of Fisheries and Aquatic Sciences **54**: 1177-1189.

Hama, T., Miyazaki, Y., Ogawa, T., Iwakuma, M.Takahashi, A.,Otsuki and S. Ichimura. 1983. Measurement of photosynthetic production of a marine phytoplankton population using a stable 13C isotope. Marine Biology **73**: 31-36.

Hanson, C., S. Peasant, A. Waite, and C. Pattiaratchi. 2007. Assessing the magnitude and significance of deep chlorophyll maxima of the coastal eastern Indian Ocean. Deep Sea Research Part II: Topical Studies in Oceanography **54**: 884-901.

Howard-Williams, C., K. Law, C. Vincent, J. Da Vies, and W. F. Vincent. 1986. Limnology of Lake Waikaremoana with special reference to littoral and pelagic primary producers. New Zealand Journal of Marine and Freshwater Research **20:** 583-597.

Huisman, J. 1999. Critical depth and critical turbulence:Two different mechanisms for the development of phytoplankton blooms. Limnology and Oceanography **44**: 1781-1787.

Huisman, J., M. Arrayas, U. Ebert, and B. Sommerijer. 2002. How do sinking phytoplankton species manage to persist? The American Naturalist **159:** 245-254.

Kirk, J. T. O. 1994. Light and photosynthesis in aquatic ecosystems, 2 ed. Cambridge University Press.

Klausmeier, C. A., and E. Litchman. 2001. Algal games: the vertical distribution of phytoplankton in poorly mixed water columns. Limnology and Oceanography **46**: 1998.

Lampert, W., and J. Grey. 2003. Exploitation of a deep-water algal maximum by Daphnia: a stable-isotope tracer study. Hydrobiologia **500**: 95-101.

Lorenzen, C.J. 1965 a note on the chlorophyll and phaeophytin content of the chlorophyll maximum. Limnology and Oceanography. **10**:482-483.

McManus, G. B., and R. Dawson. 1994. Phytoplankton pigments in the deep chlorophyll maximum of the Caribbean Sea and wester tropical Atlantic Ocean. Marine Ecological Progress Series **113**: 199-206.

Miretzky, P., and A. F. Cirelli. 2004. Silica dynamics in a Pampean lake (Lake Chascomus, Argentina). Chemical Geology **203**: 109-122.

Modenutti, B., E. Balserio, C. Callieri, C. Queimalinos, and R. Bertoni. 2004. Increase in phytoplankton efficiency as a strategy of planktonic organisms exploiting deep lake layers. Freshwater Biology **49**: 160-169.

Moll, R., M. Brahce, and T. Peterson. 1984. Phytoplankton dynamics within the subsurface chlorophyll maximum of Lake Michigan. Journal of Plankton Research **6**: 751-766.

Moll, R., and E. Stroemer. 1982. A hypothesis relating trophic status and subsurface chlorophyll maxima in lakes. Archive fur hydrobiologie **94**: 425-440.

Nairn, I. A. 2002. Geology of the Lake Okataina volcanic centre. Institute of Geological and Nuclear Sciences. Lower Hutt, New Zealand. 156p.

Priscu, J. C., and C. Goldman. 1983. Seasonal dynamic of deep chlorophyll a maximum in Castle Lake, California. Canadian Journal of Fisheries and Aquatic Sciences **40**: 208-214.

Prokopy. 1992. Ammonia in surface and waste waters, p. 8. Lachat Instruments.

Roberts, E., J. Laybourn-Parry, D. McKnight, and G. Novarinos. 2000. Stratification and dynamics of microbial loop communities in Lake Fryxell, Antarctica. Freshwater Biology **44**: 649-661.

Sanford, L. P. 1997. Turbulent mixing in experimental ecosystem studies. Marine Ecological Progress Series **161**: 265-293.

Saros, J., S. Interlandi, S. Doyle, T. Michel, and C. Williams. 2005. Are the deep chlorophyll maxima in alpine lakes primarily induced by nutrient availability, not UV avoidance. Arctic, Antarctic and Alpine Research **37**: 557-563.

Sawatzky, L., W. Wurtsbaugh, and C. Luecke. 2006. Effects of nutrient limitation, light limitation, and invertebrate grazers on phytoplankton living in deep chlorophyll layers. Journal of Plankton Research **28**: 65-86.

Schallenberg, M., and C. Burns. 1997. Phytoplankton biomass and productivity in two oligotrophic lakes of short hydraulic residence time. New Zealand Journal of Marine and Freshwater Research **31**: 119-134.

Schiltzer, R. 2005. Ocean Data View. http://www.awi-bremerhaven.de/GEO/ODV,2005.

Shortreed, K., and J. Stockner. 1990. Effect of nutrient additions on lower trophic levels of an oligotrophic lake with a seasonal deep chlorophyll maximum. Canadian Journal of Fisheries and Aquatic Sciences **47**: 262-273.

Smyth, T. J., K. L. Pemberton, J. Aiken, and R. J. Geider. 2004. A methodology to determine primary production and phytoplankton photosynthetic parameters from Fast Repetition Rate Fluorometry. Journal of Plankton Research **26**: 1337-1350.

Spigel, R. H., C. Howard-Williams, I. Hawes, and M. James. 2003. Predictions of water quality changes in Lake Taupo under different nitrogen loadings: Further refinement and application of a coupled hydrodynamic- ecosystem

model, p. 21. National Institute of Water and Atmosphere.21p. CHC2002-042.

Steele, J. H., and C. S. Yentsch. 1960. The vertical distribution of chlorophyll. Journal of the Marine Biology Association **39**: 217-226.

Stein, J. 1973. Handbook of phycological methods. Cambridge University Press. Cambridge. 448 pp.

Tilzer, M., and C. Goldman. 1978. Importance of mixing, thermal stratification and light adaptation for phytoplankton productivity in Lake Tahoe (California-Nevada). Ecology **59**: 810-821.

Tilzer, M., H. Paerl, and C. R. Goldman. 1977. Sustained viability of aphotic phytoplankton in Lake Tahoe (California - Nevada). Limnology and Oceanography **22**: 84 - 91.

Utermöhl, H. 1958. Zur Vervolkommung der quantitativen phytoplankton. Mittenilungen Internationale Vereinigung für Theoretische und Agewandte Limnologie **9:** 1-13.

Vincent, W. F. 1983. Phytoplanton production and winter mixing: Contrasting two oligotrophic lakes. Journal of Ecology **71**: 1-20.

Viner, A. B., and E. White. 1987. Phytoplankton growth, p. 494. In A. B. Viner [ed.], Inland Waters of New Zealand. DSIR Science Information Publishing Centre.

Vrede, T., and L. Tranvick. 2006. Iron constraints on planktonic primary production in oligotrophic lakes. Ecosytems **9**: 1094-1105.

Waite, A., A. Fisher, P. A. Thompson, and P. J. Harrison. 1997. Sinking rate vs. volume relationships illuminate sinking control mechanisms in marine diatom. Marine Ecology Progress Series **157**: 97-108.

Wendt, K. 2000. Determination of nitrate/nitrite in surface and waste waters by flow injection analysis, p. 24. Lachat Instruments.

White, E., K. Law, G. Payne, and S. Pickmere. 1985. Nutrient demand and availability among planktonic communities - an attempt to assess nutrient limitation to plant growth in 12 central volcanic plateau lakes. New Zealand Journal of Marine and Freshwater Research **19**: 49-62.

White, E., G. Payne, S. Pickmere, and P. Woods. 1986. Nutrient demand and availability related to growth among natural assembages of phytoplankton. New Zealand Journal of Marine and Freshwater Research **20**: 199-208.

Woods, J. D., and R. L. Wiley. 1972. Billow turbulence and ocean microstructure. Deep Sea Research **19**: 87-121.

Wurtsbaugh, W., H. Gross, P. Budy, and C. Luecke. 2001. Effects of epilimnetic versus metalimnetic fertilization on the phytoplankton and periphyton of a mountain lake with a deep chlorophyll maxima. Canadian Journal of Fisheries and Aquatic Sciences **58**: 2156-2166.

Zapata-Anzola, A., C. Rivera-Rondon, and J. Donato-Rondon. 2006. Dynamics of photosynthetic pigments in an Andean lake in Colombia. Lakes and Reservoirs: Research and Management **11**: 29-38.

Zellweger-Analytics-Inc. 2000. QuikChem 8000 Automated Ion Analyzer Ominon FIA. Zellweger Analytics Inc.

3.0 Nitrogen fixation by ephemeral surface blooms in oligotrophic Lake Tarawera

3.1 Introduction

The term 'bloom' is used subjectively throughout the literature, to describe concentrations of phytoplankton that are significantly greater than the average for a particular water body (Paerl 1988; Oliver and Ganf 2000). Blooms tend to be composed of only one or two species (Oliver and Ganf 2000) and because surface blooms can occur suddenly and are a visually obvious change in water appearance, they are of interest to the public. The occurrence of visually obvious cyanobacterial blooms can be indicative of declining water quality (Paerl and Ustach 1982) and may lead to public concern due to the sudden localised reduction in aesthetics and potential health risk to recreational bathing (Chorus and Batram 1999; Wood et al. 2006).

Surface blooms of nitrogen-fixing cyanobacteria are normally associated with lakes that have elevated nutrient concentrations (e.g., Steinberg and Hartmann 1988; McQueen and Lean 1987; Reynolds and Petersen 2000).
Schindler et al. (1977, 2008) demonstrated that low ratios of total nitrogen to total phosphorus concentrations (TN:TP) in pelagic waters are associated with the occurrence of cyanobacteria. Cyanobacteria, using specialised cells referred to as heterocysts, can circumvent nitrogen limitation by converting atmospheric nitrogen (N_2) into biologically available forms (Stewart et al. 1969). Even in

oligotrophic lakes, cyanobacterial blooms may occur when ratios of TN:TP are low (Horne and Galet 1985).

The process of breaking the N_2 triple bond during nitrogen fixation is energetically expensive, consuming 16 ATP molecules (Paerl and Zehr 2000) and requiring additional phosphorus for ATP synthesis. Due to this requirement, ambient total phosphorus concentrations have been positively correlated with the number of heterocysts per filament (Tono and Noges 2003) and nitrogen fixation tends to occur only when other available sources of nitrogen are depleted.

Nitrogen fixation is more likely to occur in lakes when ambient water temperatures exceed 20 °C (McQueen and Lean 1987, Noges et al. 2008; Gondwe et al. 2008) and when the water column is stratified (Levine and Lewis 1985; Mugidde et al. 2003; Gondwe et al. 2008) as stratification leads to a reduction of turbulence. Relatively quiescent water may enhance nitrogen fixation (Paerl 1985) as when water is more turbulent, the fragile junction between heterocysts and vegetative cells may break apart (Lang and Fay 1971). Therefore, small scale shear can limit nitrogen fixation in environments where it would otherwise be predicted to occur (Moisander et al. 2002).

Detecting the presence of nitrogen fixation *in situ* within oligotrophic lakes can be challenging due to methodological limitations (Mague and Burris 1973) and because heterocystous cyanobacteria in open water samples can be at low concentrations. The formation of surface blooms naturally concentrates heterocystous cyanobacteria, however, presenting an opportunity to carry out *in*

situ acetylene reduction assays (ARA) to measure nitrogen fixation (Stewart et al. 1967) where otherwise the sensitivity of ARA might be compromised by low concentrations of heterocysts. Acetylene reduction assay (ARA) is a simple and sensitive method for field investigations of nitrogenase activity, which is indicative of nitrogen fixation. The assay can be used to estimate gross nitrogenase activity (Gallon et al. 2002) and includes nitrogenous compounds that 'leak' from the heterocyst and are not incorporated into cells but make a contribution to biologically available nitrogen concentrations in lake water (Ward and Wetzel 1980; Gallon et al. 2002).

Lake Tarawera is an oligotrophic lake that, at times, has surface blooms of cyanobacteria (Dryden and Vincent 1986; Pridmore and Etheredge 1987). Growth of phytoplankton in Lake Tarawera has previously been shown to be limited by biologically available nitrogen in the water column (Payne et al. 1988; White et al. 1986), providing an environment that may favour nitrogen fixation.

This study considers to what extent mid-lake conditions correlate with, or promote the formation of nitrogen fixing shoreline surface blooms. Surveillance for surface blooms was carried out over a two year period along the western shoreline of Lake Tarawera for the purpose of examining their potential for nitrogen fixation. Nutrient concentrations and physical conditions were also investigated at a mid-lake station over the same two year period to test if the occurrence of cyanobacterial blooms coincided with periods when there was likely to be strong nitrogen limitation.

3.2 Methods

3.2.1 Study site

This study was undertaken at Lake Tarawera (176° 25' E, 38° 12' S), a large (41.6 km^2), deep (max. depth 84 m), oligotrophic lake in the Central Volcanic Plateau of North Island, New Zealand. Measurements were carried out at two shoreline locations and water samples for nutrient analysis were taken from a mid lake location (Station 1, Figure 3.1). In 2005, two surface blooms were examined; at Station 2 ('The Landing') and in May 2006 a surface bloom was examined at Station 3 ('Rangiuru Bay')

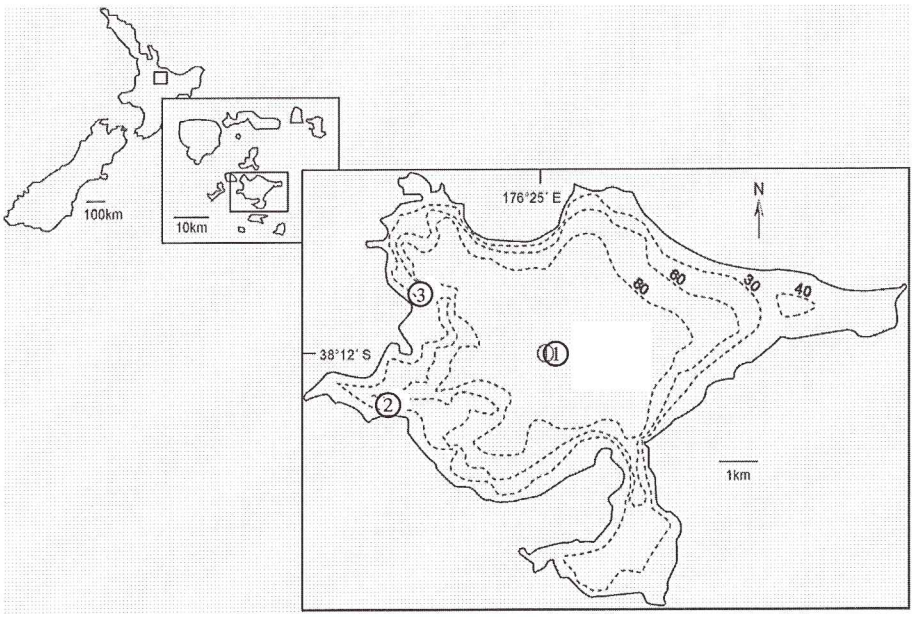

Figure 3.1. Location of Lake Tarawera and sampling and sites; Station 1 (mid-lake station), 2 ('The Landing') and 3 ('Rangiuru Bay').

3.2.2 Sampling and bloom identification

Visual inspections were made of western bays of Lake Tarawera for obvious surface phytoplankton 'blooms' on at least two occasions of every month between July 2004 and July 2006. In this research 'bloom' is used to describe a surface aggregation of phytoplankton that was observed from the shoreline. Regular contact was also maintained with lake users who provided additional observations on surface blooms. Blooms occurred at Stations 2 and 3 (see Figure 3.1 for location) over the two year period. When blooms were located, surface grab water samples were taken and inspected using light microscopy (x 200 magnification) to identify the species present and to inspect for the presence of heterocysts. Following the identification of heterocysts, tests for nitrogenase activity were carried out using acetylene reduction assays (ARAs). No ARAs were carried out if heterocystous cyanobacteria were not present.

3.2.3 Qualitative assessment of nitrogenase activity - autumn 2005

In 2005, heterocystous blooms were identified on 3 March and 5 April at Station 2. ARAs were conducted on these days to test for nitrogenase activity (Flett et al. 1976).

Studies were conducted to optimise the ARA for quantitative assessment. The protocol used and a summary of the resulting data are included as an appendix to this thesis. Quantitative assessment of nitrogenase activity by ARA was conducted during a cyanobacterial bloom which occurred from 18-22 May 2006.

Dark bottles were used to control for nitrogenase activity in the absence of light and bottles of reverse osmosis water were also assayed to control for non-specific ethene formation or contamination not relating to nitrogenase activity, though none was found. At least four replicates were made of each treatment type in each trial period. Separate incubations were conducted on 20 and 21 May 2006 over the following time periods: 08:30-10:30, 12:00-14:00; 13:00-15:00 and 16:30-18:30 hr. At the end of the incubation bottles were immediately placed on ice in the dark, and the gas phase sampled using a 28 gauge insulin needle and syringe. Syringes of the gas were stored by insertion into rubber stoppers to prevent gas leakage and transported to the laboratory for further analysis on the same day as sampling. Gas samples were analysed for ethene using a Schimadzu flame ionisation gas chromatograph.

Following ARAs, phytoplankton were preserved with 2% Lugol's iodine. Twenty mL sub-samples were subsequently sedimented, and heterocysts counted using an inverted microscope at 200X magnification. Heterocyst counts were used to calculate the number of heterocysts in each incubation bottle and then combined with ethene production data to calculate ethene production per heterocyst (Lewis and Levine 1984).

Ethene production was used to estimate nitrogen fixation using a conversion factor of 0.25 (Jensen and Cox 1983). Converting data from ethene production to nitrogen fixation enables comparison with similar acetylene reduction assay studies (Gondwe et al. 2008; Lewis and Levine 1984).

3.2.4 Nutrients

Water nutrient data for the period July 2004 to July 2006 were obtained from Environment Bay of Plenty, the regional environmental management authority, in order to examine lake nutrient status. Samples for nutrient analysis were collected once a month at Station 1 using an integrated sampling tube between 0 and 13 m depth, then transported on ice to the laboratory for analysis. Samples for total phosphorus (TP) were digested using nitric acid, before analysis using an inductively coupled plasma mass spectrometry instrument (APHA 2005). A Lachat Instruments flow injection analyser (Zellweger Analytics 2000) was used to analyse samples for soluble reactive phosphorus (SRP) (Diamond 2000), ammonium (NH_4) (Prokopy 1992) and oxidised nitrogen (NO_x and NO_2) (Wendt 2000). Concentrations of NO_3 were calculated by subtracting NO_2 from NO_x data. For the purpose of inferring liming nutrients, mass ratios of TN to TP (Howarth et al. 1988; Redfield 1958) and inorganic nitrogen (NH_4-N + NO_3-N) to SRP were calculated.

3.2.5 Surface temperature

Surface mid-lake temperatures were recorded, monthly at Station 1 from July 2004 to July 2006, using a conductivity temperature depth (CTD) profiler.

3.3 Results

3.3.1. Surface temperature

Monthly measurements of surface water temperatures at Station 1 (Figure 3.2) follow an expected seasonal pattern. The minimum recorded temperature was 11.0 °C in both August 2004 and August 2005 and the maximum surface temperatures recorded were 20.3 °C in March 2005 and 21.6 °C in February 2006. Shoreline surface blooms occurred in the months following the peak annual surface water temperature. Mid-lake surface water temperatures at the time that blooms were observed were 18 °C (March 2005), 15 °C (April 2005) and 14.5 °C (May 2006).

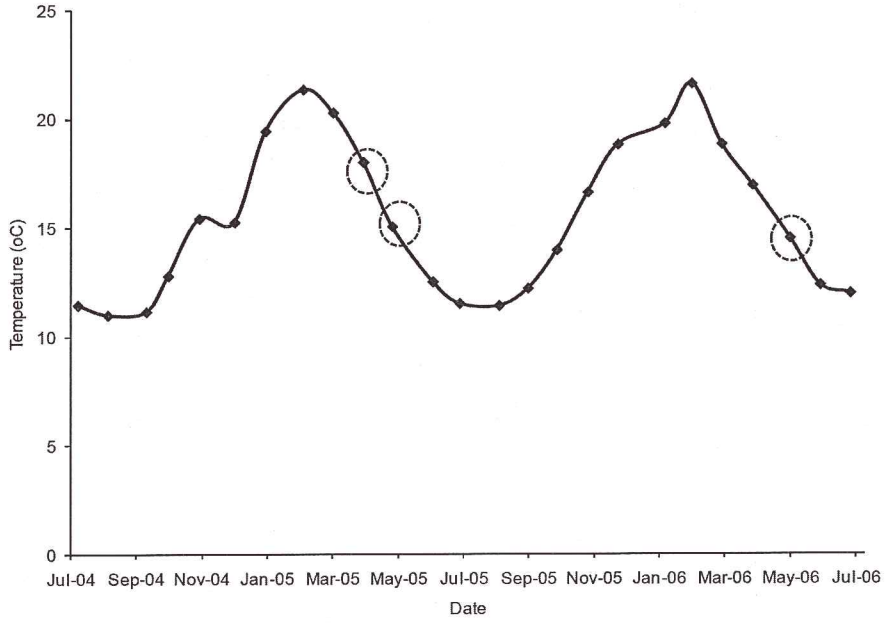

Figure 3.2. Mean Surface temperature at Station 1, recorded monthly from July 2004 to July 2006. Dashed circles indicate times when phytoplankton surface blooms were observed and nitrogenase activity was confirmed to be present in shoreline samples.

3.3.2 Total nitrogen and total phosphorus

Over the two year period, July 2004 to July 2006, TP in surface integrated samples ranged from 4 μg L^{-1} in May 2005 to 24 μg L^{-1} in June 2005 (Figure 3.3). The average concentration of TP over the entire period was 10.4 μg L^{-1}. From November 2004 to May 2005 TP integrated surface water samples showed a near linear decrease from 13 μg L^{-1} to 4 μg L^{-1}, which coincided with the period of thermal stratification. This pattern was, however, not repeated from November 2005 to May 2006, when TP in surface integrated samples did not show any particular trend, fluctuating between 7 and 13 μg L^{-1}.

Total nitrogen (TN) concentrations in surface integrated samples from Station 1 ranged from a minimum of 18 μg L^{-1}in December 2005 to a maximum of 205 μg L^{-1} in May 2006 (Figure 3.3). The highest TN for the July 2004 to July 2005 period was 168.5 μg L^{-1} in April 2005. The average concentration of TN in surface integrated samples from July 2004 to July 2006 was 113.7 μg L^{-1}.

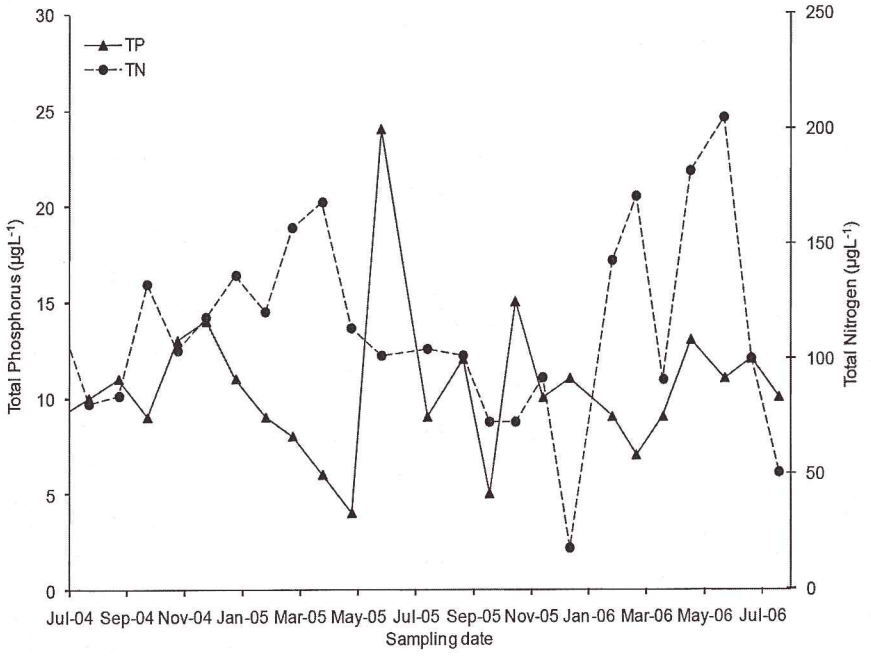

Figure 3.3 Concentrations of total phosphorus (triangles) and total nitrogen (circles) in surface integrated samples (0-13 m) at Station 1.

Figure 3.4 shows mid-lake mass ratios of TN to TP in monthly integrated surface samples at Station 1 over the period July 2004 to July 2006. Timing of surface blooms that showed nitrogenase activity is shown. Shoreline surface bloom samples were taken on 3 March and 5 April 2005, and 20 May 2006. Mid-lake TN:TP ratios at times corresponding to the occurrence of these blooms were 19.6, 28.0 and 18.6 respectively. These were among the highest TN:TP levels recorded over the investigation period, although ratios were also relatively high in May 2005 (28.3) and March 2006 (24.4) when no surface blooms were identified. The average TN:TP over the period was 12.5, higher than the 'optimal' or physiologically balanced ratio of 7.2 (Redfield 1958), which is shown as a horizontal line in Figure 3.4.

In 2004-2005 variation in TN:TP was driven largely by fluctuations in TP. This nutrient declined in surface waters from November to May and increased in surface waters when full lake mixing occurred in June. Shaded regions in Figure 3.4 indicate the approximate timing of full lake mixing. In all instances, in this study, nitrogenase activity was found to occur in autumn towards the end of the two stratified periods.

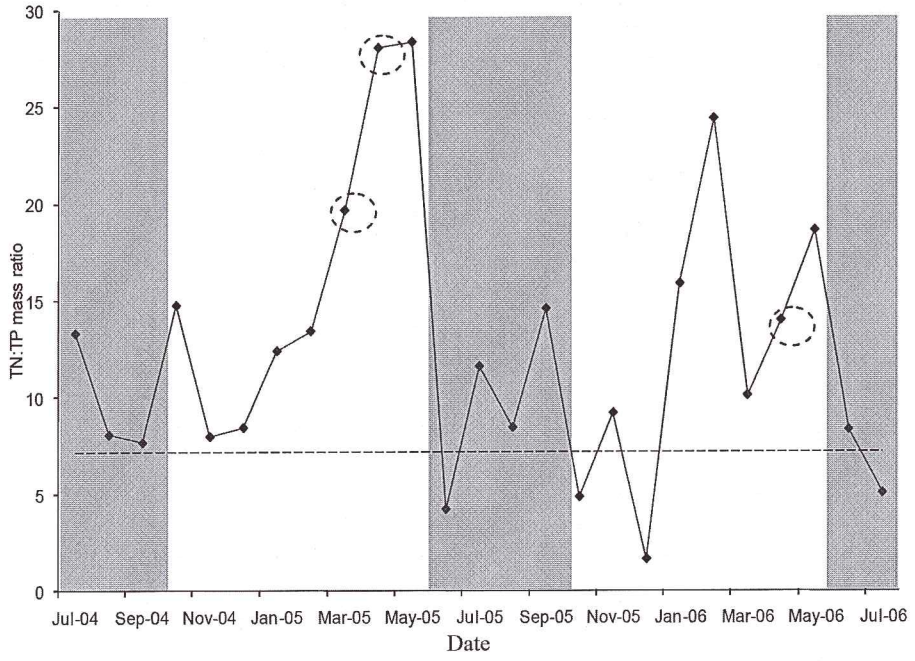

Figure 3.4. The mass ratio of TN:TP in integrated surface water samples (0 – 13 m) at station 1 in Lake Tarawera from July 2004 to July 2006. Shaded regions approximate times when the lake was fully mixing. Dashed circles indicate times when nitrogenase activity was confirmed to be present in shoreline studies. The dotted horizontal line indicates the 'optimal' ratio of TN:TP equal to 7.2

3.3.3 Ratios of inorganic nutrients

The mass ratios of inorganic forms of nitrogen to phosphorus, i.e. inorganic nitrogen (NO_3-N + NO_2-N + NH_4-N) and dissolved reactive phosphorus (PO_4-P), from surface integrated samples in the middle of the lake are shown in Figure 3.5. Nitrogenase activity was detected when ratios were high; in the mid-lake in March 2005 (2.8), April 2005 (9) and May 2006 (3.2). The average ratio over the period July 2004 to July 2006 was 1.96. Ratios were high when surface blooms were present, particularly so in April 2005 when a ratio of 9 was recorded though ratios were lower (between 1.5 and 2.5) in the months preceding observations of surface blooms in 2005. In 2006, however, the months preceding the observation of nitrogen fixation in the lake shore surface bloom did not have consistently lower ratios of inorganic nitrogen to DRP.

Average surface integrated inorganic nitrogen over the investigation period was 7.7 $\mu g\ L^{-1}$ and average DRP was 4.6 $\mu g\ L^{-1}$. The highest mid lake surface water concentrations of both inorganic nitrogen (51 $\mu g\ L^{-1}$) and DRP (16 $\mu g\ L^{-1}$) occurred in May 2006.

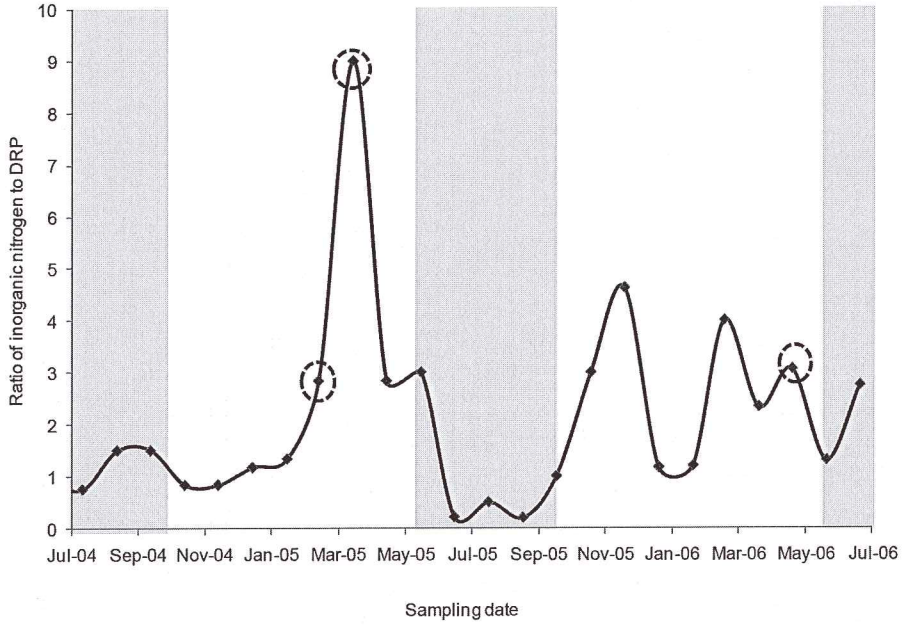

Figure 3.5 Ratios of inorganic nitrogen (NH4 -N+ NO3-N) to dissolved reactive phosphorus in Lake Tarawera at station 1 from July 2004 to July 2006 from surface integrated samples (0 to 13m). Shaded regions approximate times when the lake was fully mixed. Dashed circles indicate times when nitrogenase activity was confirmed to be present in shoreline studies.

3.3.4 Acetylene reduction assays

The dominant heterocyctous species present in Lake Tarawera was *Anabaena circinalis*. *Anabaena lemmermani*,and *Anabaena flos-aquae* were occasionally present in low numbers.

Qualitative ARAs carried out in March and April 2005 confirmed nitrogenase activity during cyanobacterial surface blooms. On 20 and 21 May 2006 quantitative measurements were made of ARA. Figure 3.6 shows the rate of nitrogen uptake (calculated from nitrogenase activity) per heterocyst in a series of 2 hour *in situ* incubations on 20 and 21 May 2006. The mean concentration of

heterocysts in the incubation bottles over the four trials was 5,591,000 L^{-1}(SD 2,237,000 L^{-1}). Nitrogenase activity was highest in 12:00 to 14:00 hr and 13:00 to 15:00 hr periods, with average nitrogen fixation calculated to be 37 and 40 nmol (10^6 heterocysts) $^{-1}$ hr^{-1} in light incubations. Nitrogen uptake was lower in the 08:30-10:30 hr incubations, with 11 nmol (10^6 heterocysts) $^{-1}$ hr^{-1} while in the 16:30-18:30 hr incubations 7 nmol (10^6 heterocysts)$^{-1}$ hr^{-1} of nitrogen was fixed.

Dark bottle nitrogen uptake ranged from 4 to 12 nmol (10^6 heterocysts) $^{-1}$ hr^{-1} and also appeared to follow a daily pattern, with higher uptake recorded in 12:00 to 14:00 hr and 13:00 to 15:00 hr incubation periods. Differences between light and dark uptake were smallest in morning and evening incubations and greatest during the middle of the day.

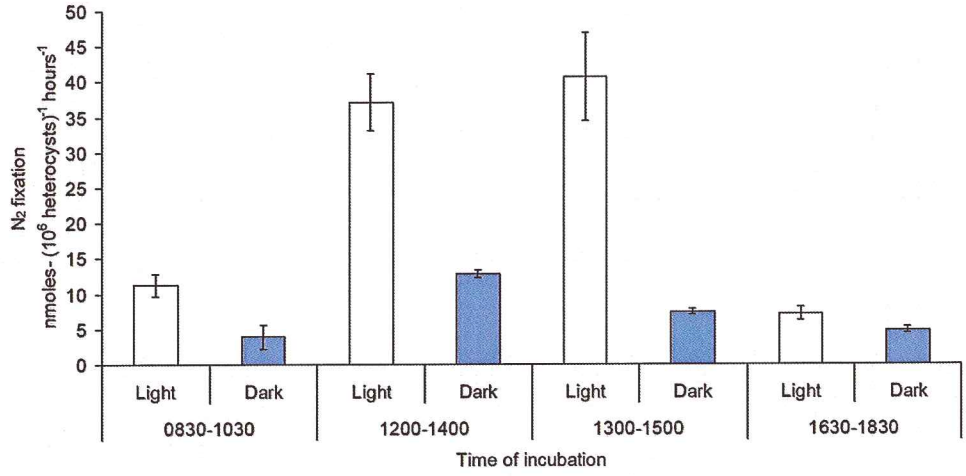

Figure 3.6. Mean nitrogen fixation as estimated by ethene production in acetylene reduction assays during a surface bloom 20 and 21 March 2006. Light treatments are shown by bars with no shading and dark treatments are shown shaded. Error bars show one standard deviation of the mean.

3.4 Discussion

The occurrence of nitrogen fixation was confirmed in two shoreline blooms in autumn (March and April) 2005 and one shoreline bloom in autumn (May) 2006. In this study, ARAs during the middle of the day (12:00 - 14:00 and 13:00 - 15:00 h) yielded 37 -40 nmol N (10^6 heterocysts)$^{-1}$ hr^{-1}, similar to the results of Lewis and Levine (1984) who found 35-40 nmol N (10^6 heterocysts)$^{-1}$ hr^{-1} during incubations in the middle of the day in Lake Valencia, Venezuela. Higher nitrogen fixation in the middle of the day is likely a consequence of a response to the high energy demand of nitrogen fixation which is supported by increased photosynthesis in higher light conditions (Lewis and Levine 1984) and warmer temperatures (Tonno and Noges 2003).

Concentrations of TN and TP show Lake Tarawera to be oligotrophic, though TP is slightly elevated. Average TP from integrated surface samples from the middle of the lake was 10.4 μg L^{-1}, slightly higher than the oligotrophic range of 4.1 to 9.0 (Burns et al. 1999), indicating slight enrichment. Average TN from surface integrated samples, however, was 113.7 μg L^{-1}, within the oligotrophic range of 73 to 157 μg L^{-1} (Burns et al. 1999). Within the Rotorua Lake group, Rotoma, Okataina and Tikitapu are also oligotrophic (Environment Bay of Plenty, 2008) and low TN:TP is typical of lakes in New Zealand's central volcanic plateau.

The mean TN:TP mass ratio of 12.5 in integrated surface samples from mid lake Station 1 over the two year period indicates that Lake Tarawera is more likely to be nitrogen limited than phosphorus. The mass ratio of TN to TP required for

balanced growth of a phytoplankton assemblage approximates at 7.2 (Redfield 1958). Lower N:P ratios may favour nitrogen fixing cyanobacteria (Smith 1983) and when N:P ratios are high cyanobacteria tend to be less common (Pick and Lean 1987).

The highest recorded TN in each of the years monitored coincided with the occurrence of nitrogen fixing surface blooms (Figure 3.3). While lower concentrations of TN might be expected, there may be a time lag between the occurrence of a low nitrogen environment and the observation of cyanobacterial blooms at the shore. A trend of increasing TN from the beginning of summer (December) through to autumn (April/May) occurred in both monitoring years (Figure 3.3). The lower TN concentrations in early summer may be associated with an increase in the proportion of nitrogen fixing cyanobacteria. Peaks of TN in Station 1 surface waters coinciding with the occurrence of shoreline surface blooms may be the result of nitrogen leakage from heterocystous cyanobacteria (Gallon et al. 2002). The existence of a time lag between low nitrogen to phosphorus ratios at Station 1 and the observation of surface blooms along the shoreline might explain why TN:TP and inorganic nitrogen: DRP were at their highest levels when blooms were observed (Figures 3.4 and 3.5). While low ratios occurred in early December and January 2005, this pattern did not recur in 2006. Ratios of TN:TP were consistently below 7.2:1 during periods of lake mixing (June to October) but no surface blooms were observed over these times (Figure 3.3).

Surface blooms observed in Lake Tarawera generally persisted for less than one week and were localised in bays or discrete stretches of shoreline. This study only investigated surface blooms that occurred along the western shore. It is likely that patchy surface blooms are patchy both in the mid-lake, in bays and along the shore-line. Whole lake surveillance is likely to have identified more bloom events. As only three blooms were identified during the study it is hard to correlate mid lake nutrient ratios to bloom formation events with certainty and future studies might usefully analyse nutrient concentrations together with relevant physiological variables for the cyanobacteria.

Water column mixing may limit the formation of nitrogen fixing cyanobacterial blooms. Because of higher light requirements of cyanobacteria, they are likely to be out-competed when the depth of the mixing zone is greater than the depth of the euphotic zone ($z_{mix} > z_{eu}$) (Steinberg and Hartman 1988). As the euphotic depth is between 25-30 m and the average depth of Lake Tarawera is 55 m, in winter the mixing depth will be far greater than the euphotic depth ($z_{mix} >> z_{eu}$) and therefore nitrogen fixation in Lake Tarawera in unlikely to occur during this period.

Lower water temperature during lake mixing may also limit nitrogen fixing cyanobacteria, as water temperatures were c. 11 °C during lake mixing. Observations of shoreline surface blooms occurred when lake surface temperatures were highest (March 2005: 20.2°C) or in the months (April 2005, May 2006) closely following the peak surface water temperatures. It has been shown that nitrogen fixation increases when ambient water temperature exceeds

20 °C (McQueen and Lean 1987; Noges et al. 2008; Gondwe et al. 2008). This may be because competition for nitrogen increases as ambient water temperature increases (Tilman et al. 1996).

The contribution of nitrogen fixation to the overall nitrogen budget of Lake Tarawera is likely to be small compared to other nitrogen inputs. To calculate the contribution of fixed nitrogen, it could be assumed that nitrogen fixation might occur in the top 1 m of water, over a period of c. 150 days, from January when surface water temperatures exceed 20 °C, until the commencement of lake mixing in June. From experiments in this study it could be assumed that higher rates of nitrogen fixation occur during the middle of the day and lower rates in the morning and evening. While the number of heterocysts in the top 1 m of lake water over the January to June period was not estimated directly in this study, some approximations may be possible. If, for example, heterocysts were at densities of c. 40000 L^{-1}, around 800 kg of 'new' nitrogen would be brought into Lake Tarawera each year through nitrogen fixation. A more conservative estimate of heterocyst density, for example 2,000 L^{-1}, would only yield around 37 kg. Around 90 tones of nitrogen has been estimated to enter Lake Tarawera each year via streams and groundwater (Hamilton et al. 2006). Based on the estimates above, fixed nitrogen may contribute between 0.9% and 0.04% of all new nitrogen additions. There is additional uncertainty in these estimates. If, for example, nitrogen fixation occurred throughout the top 10 m (rather than just the top 1 m) the nitrogen contribution from fixation would increase ten fold.

Any increase in phosphorus to the lake may increase the prevalence of heterocystous cyanobacteria, resulting in increases to nitrogen fixation and subsequent nitrogen contributions to the lake. Future efforts in lake management to protect Lake Tarawera from eutrophication and nuisance blooms, should seek to control both nitrogen and phosphorus inputs concurrently.

3.6 References

Burns, N. M., J. C. Rutherford, and J. S. Clayton. 1999. A monitoring and classification system for New Zealand lakes and reservoirs. Journal of Lake and Reservoir Management **15:** 255-271.

Chorus, I., and J. Bartram. 1999. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. World Health Organisation.

Diamond, D. 2000. Determination of ortho phosphate in waters by flow injection analysis colorimetry, p. 22. Lachet instruments.

Dryden, S. J., and W. F. Vincent. 1986. Phytoplankton species of Lake Okaro, Central North Island. New Zealand Journal of Marine and Freshwater Research **20**: 191-198.

Environment-Bay-of-Plenty. 2008. Bay of Plenty regional water and land plan, 445 p. Environment Bay of Plenty. Whakatane.

Flett, R. J., R. D. Hamilton, and N. E. R. Campbell. 1976. Aquatic acetylene reduction techniques: solutions to several problems. Canadian Journal of Microbiology **22**: 43-51.

Gallon, J. R.and others 2002. Maximum rates of N_2 fixation and primary production are out of phase in a developing cyanobacterial bloom in the Baltic Sea. Limnology and Oceanography **47:** 1514-1521.

Gondwe, M. J., S. J. Guilford, and R. E. Hecky. 2008. Planktonic nitrogen fixation in Lake Malawi/Nyasa. Hydrobiologia **596:** 251-267.

Hamilton, D., M. Hamilton, and C. McBride. 2006. Nutrient water budget for Lake Tarawera, p. 32. Centre for Biodiversity and Ecology Research. 32p. CBER contract report 46.

Horne, A. J., and M. L. Commins. 1987. Macronutrient controls on nitrogen fixation in planktonic cyanobacterial populations. New Zealand Journal of Marine and Freshwater Research **21:** 413-423.

Horne, A. J., and D. L. Galat. 1985. Nitrogen fiation in an oligotrophic saline desert lake: Pyramid Lake Nevada. Limnology and Oceanography **30:** 1229-1239.

Howarth, R., R. Marino, and J. J. Cole. 1988. Nitrogen fixation in freshwater, estuarine, and marine ecosystems. 2. Biogeochemical controls. Limnology and Oceanography **33**: 688-701.

Jensen, B. B., and R. Cox. 1983. Direct measurements of steady-state kinetics of cyanobacterial N^2 uptake by membrane-leak mass spectrometry and comparsions between nitrogen fixation and acetylene reduction. Applied and Environmental Microbiology **45**: 1331-1337.

Lang, N., and P. Fay. 1971. The heterocysts of blue-green algae. 2. Details of ultrastructure. Proceedings of the Royal Society of London: Series B **178**: 193-203.

Levine, S. and W. M. Lewis. 1984. Diel variation of nitrogen fixation in Lake Valencia, Venezuela. Limnology and Oceanography **29**: 887-893.

Levine, S., and W. M. Lewis. 1985. The horozontal heterogeneity of nitrogen fiaxation in Lake Valencia, Venezuela. Limnology and Oceanography **30**: 1240-1245.

Lewis, W. M. 1983. A revised classification of lakes based on mixing. Canadian Journal of Fisheries and Aquatic Sciences **40:** 1779-1787.

Lewis, W., and S. Levine. 1984. The light response of nitrogen fixation in Lake Valencia, Venezuela. Limnology and Oceanography **29**: 894-900.

Mague, T. H., and R. H. Burris. 1973. Nitrogen fixation in the Great Lakes. BioScience **23:** 236-239.

McQueen, D. J., and D. R. S. Lean. 1987. Influence of water temperature and nitrogen to phosphorus ratios on the dominance of blue-green algae in Lake St. George, Ontario. Canadian Journal of Fisheries and Aquatic Sciences **44**: 598-604.

Moisander, P. H., J. L. Hench, K. Kononen, and H. Paerl. 2002. Small-scale shear effects on heterocystous cyanobacteria. Limnology and Oceanography **47:** 108-119.

Mugidde, R., R. E. Hecky, L. L. Hendzel, and W. D. Taylor. 2003. Pelagic nitrogen fixation in Lake Victoria (East Africa). Journal of Great Lakes Research **29**: 76-88.

Noges, T., R. Laugaste, P. Noges, and I. Tonno. 2008. Critical N:P ratio for cyanobacteria and N_2-fixing species in the large shallow temperate lakes Peipsi and Vortsjarv, North-East Europe. Hydrobiologia **599**: 77-78.

Oliver, R.L. and G.G. Ganf (2000). Water blooms. *In* Whitton, B.A and M. Potts [eds]. The ecology of cyanobacteria. Their diversity in space and time. Kluwer Accademic Publishers. Dordretcht. The Netherlands. 670pp.

Paerl, H. 1985. Microzone formation: Its role in the enhancement of aquatic N_2 fixation. Limnology and Oceanography **30**: 1246-1252.

Paerl, H. W., and J. F. Ustach. 1982. Blue-green algal scums: An explanation for their occurence during freshwater blooms. Limnology and Oceanography **27**: 212-217.

Paerl, H. 1988. Nuisance phytoplankton blooms in coastal estuarine and inland waters. Limnology and Oceanography **33** (4/2): 823-847.

Paerl, H. W., and J. P. Zehr. 2000. Marine nitrogen fixation. *In* D. L. Kirchman [ed.], Microbial Ecology of the Oceans. Wiley Liss Inc.

Payne, G., E. White, S. Pickmere, and P. Woods. 1988. Algal resonses to nitrogen and phosphorus additions in four central north island, New Zealand lake waters. Internationale Vereinigung für Theoretische und Angewandte Limnologie Verhandlungen **23**: 602-606.

Pick, F. R., and D. R. S. Lean. 1987. The roles of macronutrients (C, N, P) in controlling cyanobacterial dominance in temperate lakes. New Zealand Journal of Marine and Freshwater Research **21:** 425-434.

Pridmore, R. D., and M. K. Etheredge. 1987. Planktonic cyanobacteria in New Zealand inland waters: distribution and population dynamics. New Zealand Journal of Marine and Freshwater Research **21:** 491-502.

Prokopy. 1992. Ammonia in surface and waste waters, p. 8. Lachat Instruments.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. American Scientist **46**: 205-221.

Reynolds, C. S., and A. C. Petersen. 2000. The distribution of planktonic cyanobacteria in Irish lakes in relation to their trophic status. Hydrobiologia **424:** 91-99.

Schindler. 1977. Evolution of phosphorus limitation in lakes. Science **195**: 260-262.

Schindler, D. W.and others 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37 year whole ecosystem experiment. Proceedings of the National Academy of Science **105:** 32-37.

Smith, V. 1983. Low nitrogen to phosphorus ratios favour dominance by blue-green algae in lake phytoplankton. Science **221**: 669-671.

Steinberg, C. E. W., and H. M. Hartmann. 1988. Planktonic bloom-forming Cyanobacteria and the eutrophication of lakes and rivers. Freshwater Biology **20**: 279-287.

Stewart, W. D. P., G. P. Fitzgerald, and R. H. Burris. 1967. In situ studies on N2 fixation using the acetylene reduction technique. Proceedings of the National Academy of Science **58:** 2071-2078.

Stewart, W. D. P., A. Haystead, and W. Pearson. 1969. Nitrogenase activity in heterocysts of blue-green algae. Nature **224**: 226-228.

Tonno, I., and T. Noges. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. Hydrobiologia **490**: 23-30.

Vincent, W.F. 2009. Cyanobacteria. *In:* Likens, G.E. [ed]. Encyclopaedia of inland waters. Volume 3. 226-232. Oxford: Elsevier.

Ward, A. K., and R. G. Wetzel. 1980. Interaction of light and nitrogen source among planktonic blue-green algae. Archiv fur Hydrobiologie **90**: 1-25.

Wendt, K. 2000. Determination of nitrate/nitrite in surface and waste waters by flow injection analysis, p. 24. Lachat Instruments.

White, E., G. Payne, S. Pickmere, and P. Woods. 1986. Nutrient demand and availability related to growth among natural assembages of phytoplankton. New Zealand Journal of Marine and Freshwater Research **20**: 199-208.

Wood, S. A., and P. T. Holland. 2006. Survey of cyanotoxins in New Zealand water bodies between 2001 and 2004. New Zealand Journal of Marine and Freshwater Research **40**: 585-597.

Zellweger-Analytics-Inc. 2000. QuikChem 8000 Automated Ion Analyzer Ominon FIA. Zellweger Analytics Inc.

4.0 Conclusions

4.1 Research findings

4.1.1 DCM formation and persistence

In Lake Tarawera the DCM is not formed by higher phytoplankton growth rates in the metalimnion as productivity of DCM phytoplankton, as measured by *in situ* carbon uptake rates, was low compared with rates in surface waters. Nutrient additions of nitrogen, phosphorus, and nitrogen and phosphorus combined, to *in situ* incubations over four days, did not show significant increases in chlorophyll *a*, suggesting that DCM phytoplankton growth is not primarily limited by macronutrients, but by an alternate environmental influence.

Light appears to be a critical factor for phytoplankton growth during stratification in Lake Tarawera. The ratio of phaeophytin to chlorophyll increased linearly with depth indicating an increase in the physiological degradation of phytoplankton that corresponds with decreasing light availability. Further evidence for the importance of light to sustaining the DCM population is shown by the correspondence in depths of the thermocline and DCM while the thermocline remained above the euphotic depth. Once the thermocline deepened to below the euphotic depth it was no longer possible to detect a chlorophyll peak in the metalimnion.

Light available to DCM phytoplankton may be reduced at times when cyanobacterial surface blooms, like those observed in Chapter three, occur in the mid-lake. High concentrations of cyanobacteria may effectively reduce the euphotic depth. As the relationship between thermocline depth and euphotic depth is critical to the persistence of DCM phytoplankton, the duration of DCM persistence may be reduced with increasing surface phytoplankton biomass and especially with surface blooms.

DCM formation is most likely the result of the accumulation of negatively buoyant phytoplankton as they sink under gravity through epilimnetic water and become entrapped at the density transition in the metalimion. Those phytoplankton that remain entrapped around the thermocline as it deepens below the euphotic depth are unlikely to attain compensation irradiances to support their respiratory needs, and this is likely to be reflected in increased phaeophytin: chlorophyll ratios and, ultimately, in the loss of phytoplankton cells.

4.1.2 Formation of the winter areal maximum

Areal chlorophyll *a* was highest in Lake Tarawera during winter months. This increase in winter phytoplankton biomass was supported by an increase in phytoplankton productivity. In situ ^{13}C uptake at 5 m was more than five times greater in June than in any other month. This increased is likely to be supported by lake mixing redistributing nutrients from the bottom of the lake, as TP in surface integrated samples increased from 4 $\mu g\ L^{-1}$ in May, prior to mixing, to 24 $\mu g\ L^{-1}$ in June. Winter mixing may also increase phytoplankton productivity by

increasing light availability to negatively buoyant phytoplankton and maintaining them in the water column. As Lake Tarawera's euphotic depth is half the average lake depth, phytoplankton mixed through the water column will be in the euphotic zone half of the time. In deeper lakes such as Lake Waikaremona (also an oligotrophic lake of North Island New Zealand), that has an average depth of 93 m, areal phytoplankton is not highest during winter mixing. Because phytoplankton mixed through the water column of very deep lakes become light limited during long periods out of the euphotic zone the peak in annual phytoplankton biomass is not observed in winter. While higher nutrient availability following mixing helps support increased phytoplankton growth, there must be adequate light within the water column to support a winter biomass peak.

4.1.3 Surface blooms and nitrogen fixation

Though Lake Tarawera is oligotrophic, surface blooms of cyanobacteria were observed in shoreline locations, particularly in late autumn. These blooms were found to be actively fixing nitrogen and midday fixation rates per heterocyst were similar those recorded by other authors (e.g., Levine and Lewis 1984; Gondwe et al. 2008).

Low ratios of TN:TP, stimulation of surface phytoplankton by nitrogen addition to *in situ* microcosm experiments in Chapter two, and confirmation of nitrogen fixation by cyanobacteria in Chapter three, all suggest that nitrogen is a key limiting nutrient in well lit surface waters of Lake Tarawera. While low TN:TP ratios show the potential for nitrogen fixation (Smith 1983), the lowest ratios of

TN:TP occurred in winter when no blooms were observed. Winter mixing is, however, likely to limit cyanobacteria through physical damage to cyanobacterial filaments or colonies during turbulent mixing (Lang and Fay 1971) and reduced growth due to cooler water temperatures (Tonno and Noges 2003), confining nitrogen fixing cyanobacterial blooms to summer and autumn months.

Elevated concentrations of TN recorded in the mid-lake coincided with the occurrence of nitrogen fixing surface blooms along the shoreline. This increase may be from excess nitrogen leaking from actively fixing cells (Gallon et al. 2002).

The contribution of nitrogen by nitrogen fixing cyanobacteria to the Lake Tarawera nutrient budget is small compared with nitrogen inputs from other sources. As nitrogen fixation can help to compensate for limited nitrogen availability in water (Schindler 1977) it is important that phosphorus inputs are managed concurrently with nitrogen inputs to guard against future eutrophication risk. Recent research into limiting nutrients from Canada (Schindler et al. 2008) and Northern Europe (Noges et al. 2008) has shown that management of nitrogen inputs without concurrent management of phosphorus leads to increases in biomass and proportion of nitrogen fixing cyanobacteria that can cause nuisance blooms which may be toxic. Long term protection of Lake Tarawera requires the management of not only the immediate catchment, but also the catchments of lakes Okareka, Okatania, Rotokakahi, Rotomahana and Tikitapu as these ultimately produce inputs into Lake Tarawera.

Nitrogen limitation

In situ incubations with excess nitrogen and phosphorus indicated nitrogen was most limiting to phytoplankton at 5 m but no nutrient limitation was evident at the DCM where light was most limiting. Nitrogen limitation in surface waters provides opportunity for heterocystous cyanobacteria that are able to circumvent periods of nitrogen limitation through fixation of nitrogen from the atmosphere. While uncertainty remains regarding the quantity of nitrogen that nitrogen fixation contributes to Lake Tarawera, substantial contributions may be possible.

4.2 Recommendations for future research

In this study the role of light was identified as an important factor in the persistence of the DCM but incubations were limited to just two depths. *In situ* carbon uptake experiments carried out at multiple depths in the epilimnion, with corresponding measurements of photosynthetically active radiation, could be used to explore the relationship between phytoplankton growth and light with water depth. Alternately, laboratory productivity investigations could be carried out on phytoplankton under a range of controlled light conditions in order to construct photosynthesis vs. irradiance curves for phytoplankton from selected depths.

Carbon uptake studies based on a static incubation at a single depth may not accurately represent the light climate experienced by phytoplankton that are circulated through the water column. Incubations with ^{13}C *in situ* at multiple depths and for different time intervals could assist with developing a coherent picture of how phytoplankton productivity might vary over relevant time and

space scales typical of the lake environment. Alternately, mixing could be simulated during *in situ* carbon uptake experiments by tethering incubation bottles to lines mechanically lifted and lowered through the water column at a rate designed to approximate that of the natural vertical advection rate. These data could then be used to describe the transit of phytoplankton productivity through Lake Tarawera during winter mixing.

The importance of Lake Tarawera's DCM as an energy source to higher trophic levels and the extent to which these higher trophic levels may be dependent on the DCM's persistence have not been evaluated. If surface blooms of cyanobacteria increase in prevalence, the reduction in light to the DCM populations may reduce their biomass and have implications for zooplankton and fish populations. Further work could be carried out to test for relationships between distributions of lake biota and the DCM location. Use of stable isotopes (e.g., for nitrogen and carbon) may also assist with resolving the relative influence of DCM and surface phytoplankton on higher food chain levels, provided there was sufficient discrimination of the different phytoplankton assemblages (i.e., DCM and surface-layer phytoplankton).

To more accurately quantify the amount of nitrogen that nitrogen fixation adds to Lake Tarawera it is necessary to better understand the heterogeneous distribution of heterocystous cyanobacteria. Understanding the temporal and spatial distribution of heterocysts throughout the lake would then enable nitrogen fixation to be modelled using results from acetylene reduction assays at different times and locations within the lake.

Quantitative assessment of nitrogen fixation by acetylene reduction assay (ARA) was only carried out over two days. Several more days of data should be collected to confirm that the results of nitrogen uptake per heterocyst are in fact generalisable. ARA experiments should be extended through the night also so that nitrogen uptake can be more accurately estimated through the daily cycle. In addition, conducting ARAs in multiple locations and depths throughout the lake may give an indication of the spatial variability of nitrogen fixation.

4.3 References

Gallon, J. R., and others 2002. Maximum rates of N_2 fixation and primary production are out of phase in a developing cyanobacterial bloom in the Baltic Sea. Limnology and Oceanography **47:** 1514-1521.

Gondwe, M. J., S. J. Guilford, and R. E. Hecky. 2008. Planktonic nitrogen fixation in Lake Malawi/Nyasa. Hydrobiologia **596:** 251-267.

Lang, N., and P. Fay. 1971. The heterocysts of blue-green algae. 2. Details of ultrastructure. Proceedings of the Royal Society of London: Series B; **178**: 193-203.

Lewis, W., and S. Levine. 1984. The light response of nitrogen fixation in Lake Valencia, Venezuela. Limnology and Oceanography **29**: 894-900.

Noges, T., R. Laugaste, P. Noges, and I. Tonno. 2008. Critical N:P ratio for cyanobacteria and N_2-fixing species in the large shallow temperate lakes Peipsi and Vortsjarv, North-East Europe. Hydrobiologia **599**: 77-78.

Schindler. 1977. Evolution of phosphorus limitation in lakes. Science **195**: 260-262.

Schindler, D. W and others 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37 year whole ecosystem experiment. Proceedings of the National Academy of Science **105**:32-37.

Smith, V. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science **221:** 669-671.

Tonno, I., and T. Noges. 2003. Nitrogen fixation in a large shallow lake: rates and initiation conditions. Hydrobiologia **490:** 23-30.

5.0 Appendix

5.1 Quantitative assessment of nitrogenase activity

Trials were carried out to establish optimal acetylene concentrations and incubation times for the ARAs. Lake water containing heterocystous cyanobacteria from a bloom was transferred to 285 ml clear glass bottles with a 10% gas phase. Bottles were stopped with rubber bungs and varying concentrations (2%, 4%, 8%, 10%, 15%, 20% and 25%) of acetylene gas (C_2H_2) were injected into the bottles through the rubber bung using fine gauge insulin needles. The bottles were incubated for four hours and the gas phase was sampled and analysed for ethene (C_2H_4) at 0, 1, 2, 3 and 4 hrs using a Schimadzu flame ionisation gas chromatograph. From these trials, 20% acetylene was chosen as being sufficient to saturate nitrogenase uptake. Lower acetylene concentrations (4-10%) produced yields of ethene proportional to the acetylene added. There was no significant increase in ethene production when initial concentrations of acetylene were 15, 20 and 25%. Between zero and two hours ethene produced increased with time, but after two hours ethene production plateaued. A two hour incubation was chosen for subsequent incubations.