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THE ROLE OF VIRAL SYSTEMS IN NUTRIENT CYCLING

A thesis submitted in partial fulfilment of the requirements for the degree of

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by

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WAIKATO

Te Whare Wānanga o Waikato

"In wine there is wisdom, in beer there is Freedom, in water there is bacteria."

Benjamin Franklin

Plate 1: *Lake Okaro viewed from Rainbow Mountain (Photo by Deniz Özkundakci)*

Plate 2: *Lake Tikitapu viewed from Tarawera Road Lookout (Photo by Marie Dennis)*

ABSTRACT

The Te Arawa/Rotorua lakes located in the central North Island of New Zealand have significant cultural, historic, social and economic value. Anthropogenic changes in land use have led to a decline in water quality in some lakes. A number of lakes have accelerated eutrophication with recurring cyanobacterial blooms and periods of bottom-water anoxia. Whilst there has been extensive research undertaken on phytoplankton dynamics in freshwater lakes there is little information on the abundance and activity of viral-like particles (VLPs) and bacteria. VLPs are the most abundant biological entities in aquatic environments and play an important role in carbon and nutrient cycling, reproducing either by cell lysis or replication in the host cell. Bacterial community structure is thought to be closely linked to the viral community and bacterial taxa have a key role in biochemical cycling in freshwater systems.

Two Te Arawa/Rotorua monomictic lakes differing in their trophic status; oligotrophic Lake Tikitapu and eutrophic Lake Okaro, were sampled over a 12-month period at the surface (epilimnion) and bottom (hypolimnion) and through a discrete layer of the thermocline during stratification. The thermocline is a region where there may be steep clines in physiochemical parameters (e.g., dissolved oxygen) which may strongly affect the distribution of, and environmental factors that influence, prokaryotes, protozoa and viruses. Physiochemical variables, nutrients, microbiological and molecular analyses were undertaken on samples in order to compare and contrast changes occurring within and between the two lakes.

Both lakes were strongly stratified for c. 8 months from September 2009 to June 2010 as indicated by Schmidt stability values > 1, which contributed to well-lit but nutrient-limited surface waters for phytoplankton productivity. With increasing duration of stratification a deep chlorophyll maximum formed in both lakes with Chlorophyta and Euglenophyta the dominant phytoplankton.

Viral-like particle abundance in both lakes exceeded bacterial abundance by a factor of c. 100, with maximum VLP and bacterial abundances in both lakes c. 10^8 cells mL^{-1} and 10^6 cells mL^{-1}, respectively. Bacterial abundance in both lakes was similar in the epilimnion and hypolimnion during stratification with the exception of a peak (2 x 10^7 cells mL^{-1}) in the epilimnion of Lake Okaro in

February 2010 (and in the hypolimnion (9.74×10^6 cells mL^{-1}) in March 2010 of in Lake Tikitapu. Viral-like particle abundance was variable but the epilimnion and hypolimnion tracked in both lakes between August 2009 and January 2010 after which Lake Okaro epilimnion and hypolimnion remained steady with Lake Tikitapu hypolimnion showing considerably higher VLP abundance than the epilimnion before tracking together in June 2010.

The abundance of the following bacterial functional genes was monitored through the study; *nif*H (encoding the nitrogenase reductase protein), *dsr*A (encoding the sulphate reductase protein), *mcr*A (encoding the methyl coenzyme M reductase protein), *amo*A (encoding the ammonium oxidising protein and *nos*Z (encoding for the nitrous oxide reductase protein). The occurrence of the *nif*H gene correlated with increased abundance of cyanobacteria capable of fixing nitrogen in the epilimnion of both lakes while the *dsr*A gene was more abundant in Lake Okaro, likely due to higher organic matter concentrations and greater duration and spatial extent of reducing conditions in that lake. Abundance of *mcr*A was expected to be high in the anoxic waters of the nutrient rich sediment of Lake Okaro but there was very low abundance. The *amo*A genes were detected when concentrations of ammonium were elevated in the bottom waters of both lakes. Both lakes showed the presence *nosZ* genes with high abundance occurring in Lake Okaro in December 2009 through all levels and also in December 2009 in Lake Tikitapu in the epilimnion and hypolimnion. With denitrification reliant on the availability of nitrate (NO_3-N) and dissolved organic carbon levels and performed by obligate and facultative anaerobes, conditions need to be precise for the process to occur.

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My neglected children, who I have put off swimming in lakes for life - I promise to stop commenting on the state of the water from now on. Thank you all for believing in me and supporting me.

Stu you will probably never read this let alone understand what I've written, but thanks for all the proof-reading offers.

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ABBREVIATIONS

SI (Systeme Internationale d'Unités) abbreviations for units and standard notations for chemical elements and formulae are used throughout this thesis. Other abbreviations that are commonly used or not defined in the text are listed below.

bp	base pair(s)
conc.	concentration
CTD	conductivity, temperature and depth
ºC	degrees Celsius
DCM	deep Chlorophyll-*a* maxima
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DO	dissolved oxygen
EtOH	ethanol
EDTA	ethylenediamine-tetra-acetic acid
Fig.	figure
FC	flow cytometry
F	forward (primer)
NCBI	National Centre for Biotechnology Information
NOx	nitrogen oxide
PCR	polymerase chain reaction
Q-PCR	quantitative polymerase chain reaction
R	reverse (primer)
rpm	revolutions per minute
std	standard
TE	tris-EDTA
v/v	volume for volume
w/v	weight for volume

CHAPTER 1: INTRODUCTION

1.1 Project background

The Te Arawa/Rotorua lakes are located in the central North Island of New Zealand within the Taupo Volcanic Zone. They have significant cultural, historic, social and economic value in the region and are widely utilised for recreation and tourism. However, as a consequence of increased anthropogenic activities such as urban and agricultural development in the catchment, there has been a decline in water quality and accelerated eutrophication in a number of lakes (Özkundakci *et al.*, 2010). Increasing trophic status in some lakes has resulted in recurring cyanobacterial blooms and periods of anoxia. Although there has been considerable research on the phytoplankton of these lakes, there is little information on other planktonic organisms, particularly the abundance and activity of bacteria and viruses, their effect on cell lysis, and their potential effects on nutrient cycling.

Viral and bacterial interactions are recognised as having a major role in the functioning of aquatic ecosystems. Much of the research to date has been directed toward marine ecosystems (Short *et al.*, 2002; Weinbauer *et al.*, 2004; Wilhelm *et al.*, 2008; Hewson, 2009). The range of freshwater lakes that have been studied include ultraoligotrophic Antarctic lakes (Kepner *et al.*, 1998; Sawstrom *et al.*, 2007) through to lakes of more elevated trophic status (Lymer, *et al.*, 2008a; Middleboe *et al.*, 2008; Tijdens *et al.*, 2008). These studies contain limited data regarding the bacterial community composition in freshwater lakes and in particular the micro-organisms of functional significance which are involved in carbon and nitrogen cycling. The interactions between prokaryotes, protozoa and viruses at varying levels of oxic state within a freshwater system are of particular interest as well as the effect the trophic state has on the distribution of these organisms.

In this study the viral, bacterial and phytoplankton component of two Rotorua lakes of varying trophic status were assessed over a 12 month period. Comparative evaluation of a highly eutrophic and an oligotrophic lake was used in this study to provide insights into the relationship between physiochemical variables and the structure and function of the microbial component of these lakes.

1.2 Overview of study lakes

Rotorua's lakes have generally been monitored routinely since the 1970s. The Bay of Plenty Regional Council has statutory responsibility for lake water monitoring. It follows the New Zealand Ministry for Environment (MfE) protocols for assessing trophic state for each of the Rotorua lakes, allowing for trends in Tropic Level Variable (TLx) and Trophic Level Index (TLI) values to be monitored annually (Burns *et al.*, 2000). Increasing anthropogenic activities such as farming and urbanisation have increased the load of nutrients to many lakes.

Soils in the catchments of the Rotorua lakes are light pumice soils, low in nitrogen, originating from a variety of ash showers with high allophane content (McColl, 1972). Lakes Okaro and Tikitapu are surrounded mainly by Rotomahana mud which is hydrothermally altered rhyolytic ejecta from Lake Rotomahana. Natural thermal springs feed into some lakes, which tend to be high in dissolved phosphorus originating from minerals found in the sediments associated with thermal activity.

Lake Okaro

Lake Okaro is a small (0.32 km^2, maximum depth 18 m) explosion crater lake formed about 800 years ago, located southeast of Rotorua (38° 15' S, 176° 25' E) (Figure 1.1). Water enters the lake via two small streams on the north-west side, and drains from an outflow in the south-east side (Forsyth, 1988). The catchment area is 3.89 km^2, of which more than 95% is in pasture and utilised mostly for dairy farming, resulting in high nutrient inputs into the lake. Lake Okaro is a warm, monomictic lake and historically has stratified for c. 7 months during summer and is fully mixed for 5 months (McColl, 1972). However, climatic changes have altered this cycle, resulting in a current stratification period of c. 8 months from September to June, with c. 4 months mixing (Özkundakci, *et al.*, 2010).

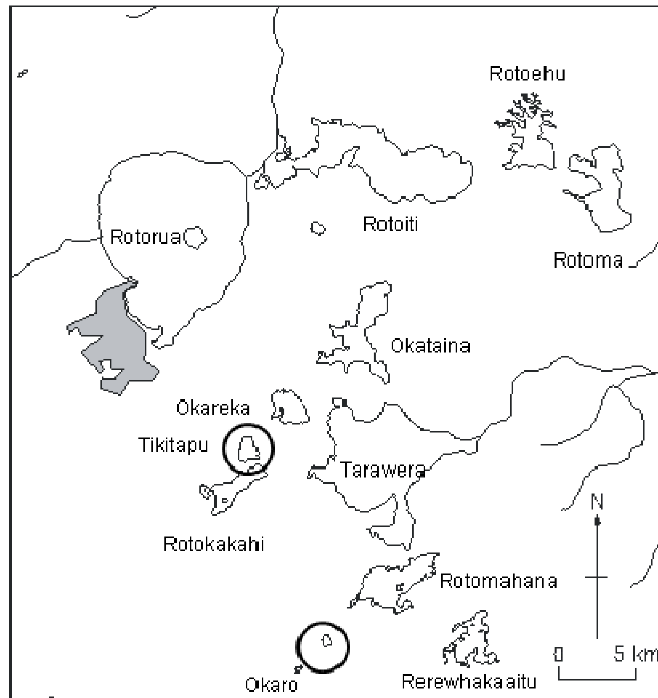

Figure 1.1 *Te Arawa/Rotorua lakes. Study lakes, Okaro and Tikitapu, are circled.*

In 1962 the waters of the hypolimnion of the lake were not anoxic (Jolly, 1968) but between 1965 and 1986 they were anoxic for 21 weeks of each stratification period (Forsyth, 1988), increasing to 28 weeks between 2004 and 2008 (Özkundakci *et al.*, 2010). Persistent cyanobacterial blooms were reported from 1963 (Forsyth, 1988), and the lake was the study site for an international investigation into cyanobacterial blooms (Vincent *et al.*, 1987). Lake water quality was at its worst in the 1990s. The lake is currently classified as super-eutrophic (Environment Bay of Plenty, 2006).

Historical high nutrient loading has produced nutrient-rich sediments and coupled with prolonged anoxia of the bottom waters has resulted in substantial remobilisation of nutrients from lake sediments (Burns *et al.*, 2009). Consequently, the lake has been the subject of a number of restoration procedures, including construction of a wetland (Tanner *et al.*, 2005), riparian protection, alum application (Paul *et al.*, 2008) and sediment capping using an aluminium-modified zeolite compound (Özkundakci *et al.*, 2010).

Lake Tikitapu

Lake Tikitapu is a small (1.4 km^2, maximum depth 27.5 m), circular lake of moderate depth, located 9 km south-east of Rotorua (38° 12' S, 176° 20' E) (Figure 1.1), formed from a lava dam around 13,000 years ago. The lake has a vivid blue colour which has been attributed to rhyolite and pumice on the lake bed.

It is fed only by rainwater and small subsurface flows. There are no visible outflows, but subsurface groundwater drains towards neighbouring Lake Tarawera. The catchment area is primarily native bush and forestry plantation, with only 3% input from pasture. Lake Tikitapu is a warm, monomictic lake and historically stratified for c. 7 months during summer and mixed fully for c. 5 months (McColl, 1972).

Low nutrient inputs have resulted in good water quality. Until recently Lake Tikitapu was classed as oligotrophic. However, Bay of Plenty Regional Council monitoring has revealed an increase in the TLI three yearly average, putting the lake at risk of being classified as mesotrophic. While there has been a reduction in annual average total nitrogen and total phosphorus levels over the past 17 years, there has been an increase in that of Chlorophyll-*a* (Scholes, 2010). A corresponding decrease in Secchi disk visibility (viz. water clarity) suggests that the lake is undergoing a rapid decline in water quality (Scholes, 2010). The waters are also low in alkalinity, calcium and silicon, resulting in very low levels of diatoms (McColl, 1972). The lake is extensively used for recreational purposes including boating, swimming, triathalons and trout fishing.

1.3 Microbial loop

1.3.1 Phytoplankton

Phytoplankton are the principal source of primary productivity in many waters, using light energy to convert carbon dioxide into organic nutrients and oxygen through photosynthesis (Paerl *et al*., 2001). True algae are classified as eukaryotic organisms while blue–green algae, or cyanobacteria, are considered to be bacteria and classed as prokaryotes. Both groups utilise characteristic light-harvesting pigments for photosynthesis.

Cyanobacteria are able to photosynthesise both aerobically and anaerobically (Wiegand *et al*., 2005). They contain Chlorophyll-*a*, phycocyanin and phycoerythrin. Some species are able to fix nitrogen, giving them a competitive advantage over other phytoplankton in nitrogen-limited environments. Some species also produce toxins which are harmful to aquatic organisms and humans (Paerl, 2008). The most common cyanobacteria in Lake Okaro are *Anabaena* sp. and *Microcystis aeruginosa* (Environment Bay of Plenty, 2000) whilst cyanobacterial blooms have not previously been recorded in Lake Tikitapu.

Chlorophyta, or green algae, contain a large concentration of Chlorophyll-*a* and are favoured by nutrient-rich conditions in freshwater systems (Paerl *et al.*, 2001). High Chlorophyta density can be indicative of eutrophication, and Chlorophyta often co-dominate with cyanobacteria (Paerl *et al.,* 2001). Green algae dominate the phytoplankton flora of Lake Tikitapu as there are few diatoms present (Environment Bay of Plenty, 2000).

Bacillariophyta, or diatoms, have an intricate cell wall (frustule) which is comprised of silica. Around 20-25% of global organic carbon fixation is thought to be carried out by diatoms (Round *et al.*, 1990), and they are important in the cycling of silica (Egge *et al.*, 1992). They may have a better ability to utilise low-nitrogen conditions (Egge *et al.*, 1992). Diatoms are one of the most dominant phytoplankton species in the Rotorua lakes, with the exception of Lake Tikitapu where low silica content prevents high abundances (Environment Bay of Plenty, 2000).

Chrysophyta, or golden algae, contain a mixture of chlorophyll as *a* and *c* and flucoxanthin (Addison *et al.*, 2007) and are photosynthetic, but in the absence of light can become hererotrophic, preying on bacteria and diatoms. They are generally not found in either Lakes Tikitapu or Okaro (Environment Bay of Plenty, 2000).

Dinoflagellates are free-swimming protists that are encased in a hard covering called a theca. They contain chlorophylls *a* and *c* and peridinin, however some species are non-pigmented heterotrophs that prey on small bacteria and other algae. They prefer well-illuminated waters and typically inhabit surface waters (Paerl *et al.*, 2001). Dinoflagellates can be strongly seasonal in abundance in Lake Okaro (Environment Bay of Plenty, 2000).

Cryptophyta are flagellated unicellar organisms containing Chlorophyll-*a,* phycoerethrin and phycocyanin. In extreme situations they are able to form cysts in order to survive. Lake Okaro had high numbers of the cryptophyte *Chroomonas minuta* between 1993 and 1995 (Environment Bay of Plenty, 2000).

The appearance of phytoplankton blooms is a prime indicator of water quality deterioration (Paerl *et al.*, 2001). Reductions in water clarity due to eutrophication can induce the dominance of surface-dwelling phytoplankton, particularly buoyancy-regulating cyanobacteria (Hamilton *et al.*, 2010). Cyanobacteria thrive in productive waters and are able to partially counter turbulence by migrating between well-lit surface waters and the nutrient-rich bottom waters due to buoyancy adjustment. These organisms are also highly tolerant to extreme conditions such as very high light levels, high temperatures, degrees of dessication and nutrient deprivation (Paerl *et al.*, 2001).

Climatic changes associated with global warming may have a more marked effect on phytoplankton assemblages than the trophic state of a lake, leading to early stratification and late destratification in lakes, together with an increase in overall temperature (Paerl, 2008). Global warming effects on patterns of precipitation and drought have a marked effect with increased precipitation introducing additional nutrient loads from groundwater and surface discharges. These inputs are offset by flushing of water bodies, however drought conditions then increase residence time, capturing nutrient loads and resulting in phytoplankton blooms. Consequently, a reduction in mixing and elevated temperature provide conditions conducive to lower diversity of cyanobacteria (Ryan, 2006) which have a competitive advantage over green algae and diatoms (Paerl *et al.*, 2008).

1.3.2 Bacteria

Aquatic bacteria drive transformations and the cycling of most biologically active elements in freshwater food webs (Newton *et al.*, 2011). They are the principal degraders and mineralisers of organic compounds and also act as biomass producers, trophic couplers and eukaryotic predators, fuelling the food web and having a profound effect on elemental fluxes and water quality in aquatic ecosystems.

Bacteria are present in freshwater systems in concentrations from 10^4 to 10^6 cells mL^{-1} and thus play a critical role in regenerating and mobilising nutrients in freshwater food webs (Berdjeb *et al.*, 2011; Newton *et al.*, 2011). One of the most important contributions of bacteria in the pelagic food web is the conversion of dissolved organic carbon (DOC) into particulate organic matter, thereby providing a dynamic link to higher consumers in the food web (Smith *et al.*, 1997).

Changes in the phytoplankton community due to grazing can alter the composition of the dissolved organic carbon pool, resulting in bacterial selection based on changing DOC pools (Sawstrom *et al.*, 2007; Lymer *et al.*, 2008b). Bacterial abundance is controlled not only by nutrient availability, sedimentation and tropic state but also by predators including protozoa, metazoa and phages (Tijdens *et al.*, 2008a). Predators tend to control bacterial growth in nutrient-poor lakes, particularly in the hypolimnion. Conversely, it is nutrients that control bacterial growth in oligotrophic lakes, with primary production constrained by concentrations of macro and trace nutrients (Wilhelm *et al.*, 2008; Berdjeb *et al.*, 2011).

Lymer *et al.* (2008b) found a higher abundance of bacteria in mesotrophic lakes compared with oligotrophic ones. Tijdens *et al.* (2008a) found that bacterial abundance was the only environmental variable that significantly correlated with temporal variations in viral community assemblages in a highly eutrophic, shallow and turbid lake where the bacterial community followed a clear seasonal cycle. Bacterial abundance fluctuated 2.3-fold over a 10-month study period. Similarly, Koskinen *et al.* (2011) studied the microbial and community structure in the Baltic Sea and found that physical and chemical factors played a major role in influencing abundance and community structure.

Stable geochemistry in an aquatic ecosystem leads to stable microbial communities (Rodriguez-Brito *et al.,* 2010). However, when bacterial strains increase in abundance, the probability of changes in abundance from viral infection increases due to increased encounter rates between viruses and bacteria (Thingstad *et al.,* 1997).

1.3.3 Bacterial Functional groups

Bacterial taxa involved in lake ecosystem processes remain largely undescribed (Newton *et al.*, 2011).

Biochemical cycling in freshwater systems is reliant on a delicate equilibrium between abiotic and biotic factors. Of particular interest are the nitrogen (essential for metabolism and causal for eutrophication) and carbon (involved in photosynthesis) cycles, with populations and communities likely to be strongly influenced by the overall availability of electron acceptors, steep redox clines and organic material from plant debris and sediment release (Newton *et al.*, 2011).

Primary productivity in aquatic systems is reliant on light levels, concentration of inorganic nutrients, temperature and trophic state. Thus productivity and community structure are driven by factors associated with vertical stratification in lakes. The epilimnion can be the zone of highest biological activity, with high oxygen saturation and adequate light leading to large fluctuations in primary productivity associated with autotrophs (Eckert *et al.*, 2001). The lower epilimnion is still oxic, but declining dissolved oxygen levels paralleled with declining temperatures tend to favour increased heterotrophic microbial activity (Eckert *et al.,* 2001). Reduced biological activity can be observed in the hypolimnion, with enhanced microbial activity by anaeriobiosis observed in bottom waters (Eckert *et al.*, 2001). Of particular interest is the anoxic-oxic zone interface where thermal stratification is strongest and the most prominent oxygen gradients are found. It is in this zone that the most heterotrophic microbial activity takes place. Micro-organisms of functional significance at this interface are 1) the anaerobic denitrifiers, 2) methanogens, which are usually present in anoxic waters, 3) aerobic ammonia oxidisers, which play an important role in nitrification, and 4) sulphate reducers, which are usually present in waters rich in decaying organic matter and major contributors to the carbon and sulphur cycles. The oxidation of ammonia to nitrate is a key component of nitrogen cycling and is performed by autotrophic nitrifiers, with the first step catalysed by ammonia monooxygenase (AMO) to oxidise ammonia to hydroxylamine (Rotthauwe *et al.*, 1997).

Denitrification is a respiratory process that reduces nitrate or nitrite to molecular nitrogen (N_2), performed by anaerobic bacteria. This process is important in aquatic systems as it can decrease the amount of available nitrogen to phytoplankton (Scala *et al.*, 1998)

Nitrogen fixation is mediated by a variety of autotrophic and heterotrophic bacteria, with the majority of aquatic ecosystem fixation undertaken by cyanobacteria (Howarth *et al*., 1988). Planktonic nitrogen fixation is high in eutrophic systems but low in oligotrophic and mesotrophic systems which have a small input of nitrogen from benthic nitrogen fixation (Howarth *et al*., 1988).

Sulphate-reducing bacteria are anaerobes that use sulphate as a terminal electron acceptor in the degradation of organic compounds. They are able to oxidise small organic molecules heterotrophically or use molecular hydrogen as an electron donor and carbon dioxide as a carbon source autorophically (Ben-Dov *et al.*, 2007). They are ubiquitous in anoxic habitats and play an important role in the sulphur and carbon cycles (Muyzer *et al.*, 2008).

Methanogens are also integral to carbon cycling, anaerobically catalysing the production of methane and carbon dioxide through the degradation of organic matter in anaerobic conditions. Methanogens are widespread in freshwater lake sediments (Steinberg *et al.*, 2008) and where methane is present in bottom waters and the thermocline (Madigan, 2003).

Berdjeb *et al.* (2011) found significant variation in bacterial community structure with depth, regardless of stratification or mixing, which suggested that community responses to depth differences in environmental conditions are more important than seasonal succession. Likewise, Lymer *et al.* (2008b) found that bacterial communities changed over a season and that different environmental variables were important for predicting bacterial community composition. The most abundant microbial species persist but display a redistribution of their relative abundances while other microbial strains ebb and flow (Rodriguez-Brito *et al.,* 2010).

1.3.4 Viruses

Until the 1990s, viral abundance in natural waters was thought to be insignificant. Knowledge of the diversity and activity of freshwater viruses lags behind that of marine environments (Wilhelm *et al.*, 2008). With the discovery that viral abundance can be up to 2.4 x 10^8 viral like particles (VLP) mL^{-1}, their contribution to the microbial loop and carbon cycling is now known to be significant (Bergh *et al.,* 1989, Tijdens *et al.*, 2008b). Viruses are a complete package that transit from one host to another, passively infecting by random encounter, therefore they do not require enzymes for metabolism or ribosomes for protein production (Lymer *et al.*, 2008b).

Viral lysis can contribute up to 70% of cyanobacterial mortality and 90-100% of bacterial mortality in freshwater systems (Tijdens *et al.,* 2008a). Tijdens *et al.* (2008a) found no correlation between viral abundance and plankton, bacteria or cyanobacteria but found a positive correlation with Chlorophyll-*a.* However, viral and bacterial abundance should correlate because of the parasitic nature of viruses. A possible explanation for this observation by Tijdens *et al.* (2008a) may be a time shift between viral infection and host cell death. In order for a virus to be abundant it has to have infected and lysed the host cell which then will have suffered declining numbers.

Host specificity can result in temporal changes in the composition of host communities that reflect seasonal patterns (Lymer *et al.*, 2008a). Changes in bacterial and viral community composition can occur on very short time scales such as days (Hewson, 2009). Phosphorus released by viral lysis is bound in organic complexes which require the activity of enzymes before the phosphorus can be assimilated by micro-organisms. Thus viral-driven phosphorus release may be coupled to a preference for organisms that can assimilate phosphorus from organic complexes (Wilhelm *et al.*, 2008). Viral lysis can disrupt the flow of energy and carbon by increasing recycling and respiratory loss lower down the food web. It transfers particulate organic matter into DOC, making it available for incorporation into new biomass. The recycled DOC is used less efficiently by uninfected bacteria (Sawstrom *et al.*, 2007).

Berdjeb *et al.* (2011) found that viral activity had a high host specificity resulting in selective mortality which was reliant on differences in host taxa, sensitivity to environmental variables, age of the system and the locally unique perturbation frequency. However, Lymer *et al.* (2008b) found that viral host dynamics are not as tightly host-specific as has generally been assumed. Viral abundance is thought to vary on seasonal scales especially in freshwater lakes with definite seasonal cycles and differing trophic status (Weinbauer *et al.,* 2002; Wilhelm *et al.*, 2008). In low-nitrogen, oligotrophic systems productivity is low and the recycling microbial loop prevails, with viruses acting as the catalysts to accelerate transformation of nutrients from particulate to dissolved form. Bacteria are also more susceptible to mortality from viral lysis in these systems (Sawstrom *et al.*, 2007). Tijdens *et al.* (2008b) found a higher viral abundance in the spring/summer period in a shallow eutrophic lake. Sawstrom *et al.* (2007) has suggested that the change from lytic to lysogenic behaviour may be a strategy for viruses to survive the unproductive winter.

1.4 Study objectives

The objective of this study was to compare relationships between physiochemical variables and phytoplankton, bacterial and viral abundance in two lakes of differing trophic status (one highly eutrophic and one oligotrophic) in the Rotorua Lakes region. Of particular interest was the bearing these factors have on each other and the vital ecological role they play in carbon and nutrient cycling.

The specific study objectives were:

- To test for relationships between, and interactions amongst, the abundance and community composition of phytoplankton, bacteria and viruses, and how these may vary between two lakes of widely differing trophic state.
- To examine how seasonal variations in the supply of substrates affects the abundance and community compositon of phytoplankton, bacteria and viruses.
- To examine how the distribution and abundance of micro-organisms varies within the water column, focusing on the role of vertical stratification and the strong gradients of oxygen that occur around the thermocline layer.

CHAPTER 2: MATERIALS AND METHODS

2.1 Sample collection

Sample collection began 13 August 2009 and was completed 10 August 2010. Samples were initially collected on a monthly basis then weekly from 11 November 2009 to 29 June 2010. Conductivity, temperature and depth (CTD) vertical profiles (SBE 19 plus SEACAT Profiler, Seabird Electronics Inc., USA), also including photosynthetically active radiation (PAR; Seabird Electronics Inc., USA), dissolved oxygen (sensor), pH (sensor) and fluorescence (sensor), were taken from stations at the deepest point in Lake Okaro (38^o18'1.7"S, 176^o23'38.5"E) and Lake Tikitapu (38°11'37.4'S, 176°19'43.6"E) prior to water sampling. Temperature, PAR, dissolved oxygen, pH and fluorescence were plotted against depth using a laptop computer immediately following the casts to ascertain the depth of the anoxic-oxic layer where it was present in the water column. Individual samples (5 L) were then taken from the epilimnion and hypolimnion using a Schindler-Patalas trap. Discrete depth samples were taken in the anoxic-oxic layer for bacterial and viral analysis using a sampling apparatus (Plate 2) to take samples at 10 cm intervals through a 50 cm layer. The sampler was lowered to the level of the thermocline and left for 5 min to provide time for the water to equilibrate following any disruption by the sampler. The bottles were then uncorked and filled with water (250 mL).

Samples for total phosphorus were taken monthly at 0-4 m (integrated sample) and 14m and 20m depths respectively for Lake Okaro and Tikitapu, from the above sampling stations as part of a Natural Environment Regional Monitoring Network conducted by Bay of Plenty Regional Council.

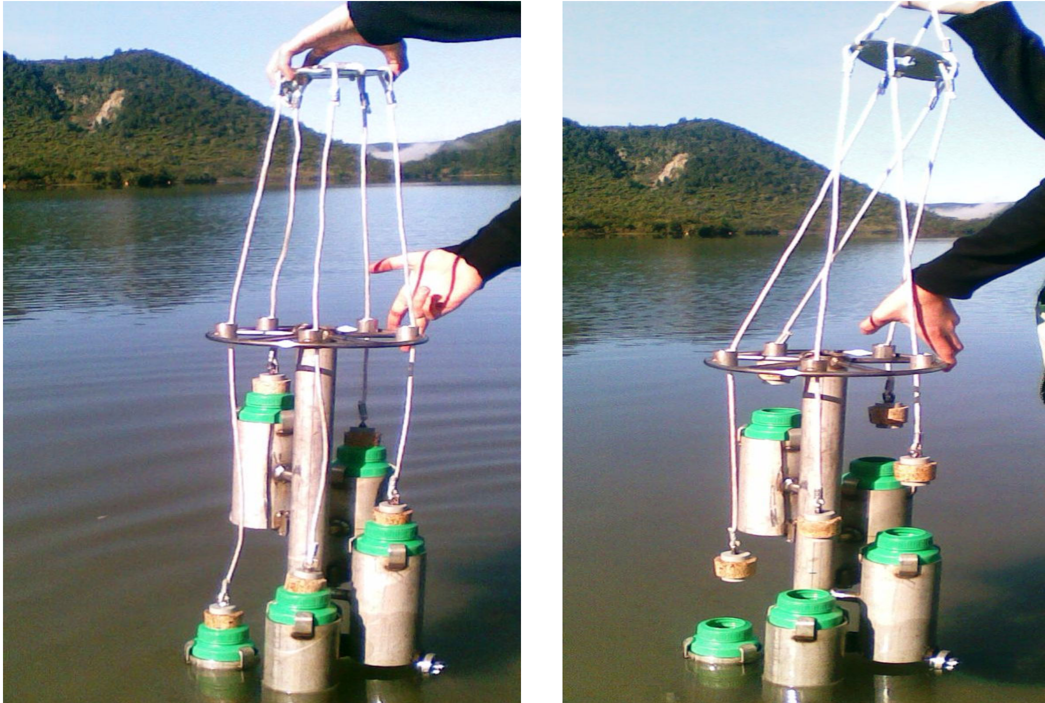

***Plate 2**: Discrete sampler a) bungs inserted prior to lowering into the water and b) after lowering and the bungs have been removed allowing the bottles to fill.*

2.1.1 Bacterial and viral counts

Sub-samples for enumeration by epifluorescence and flow cytometry were transferred immediately from the Schindler-Patalas trap into prepared 15 mL Falcon tubes (Raylab NZ Ltd) containing paraformaldehyde/phosphate-buffered saline solution (PBS) at a final concentration of 2% (m/v). Tubes were then snap-frozen in liquid nitrogen and stored at -80 ºC.

2.1.2 Definition of lysogenic and lytic bacteria

Sub-samples for enumeration of bacteria in the induction assay for lysogenic bacteria were collected then transferred to two 15 mL Falcon tubes (Raylab NZ Ltd) containing either mitomycin C (Sigma Aldrich NZ Ltd) (1 µg mL^{-1}) or no additive (control). Tubes were kept in the dark at 2-4 ºC for 24 h prior to fixation at a final concentration of 2% with a 0.02 µm-filtered glutaraldehyde solution followed by storage at 4 ºC.

2.1.3 Manual phytoplankton identification/enumeration

Sub-samples (250 mL) were collected into clean polycarbonate containers containing Lugols Iodine (1% final concentration) (Pridmore *et al.*, 1987), kept on ice then stored at 4 °C for later enumeration and identification of phytoplankton to species level.

2.1.4 Bacterial productivity

Sub-samples (250 mL) were collected into previously autoclaved glass Schott bottles (Thermo Fisher Scientific NZ Ltd) and kept in the dark at water temperature of c. 15 ºC, similar to temperatures *in situ*. Analysis of productivity was undertaken within three hours.

2.1.5 Nutrients and Chlorophyll-a

Sub-samples (60 mL) were filtered through GF/C microfiber filters (MicroAnalytix, NZ) and the filtrate collected into 50 mL Falcon tubes, kept on ice and then frozen at -20 °C for later dissolved nutrient analysis. The filters were wrapped in tin foil, kept on ice and then frozen at -20 °C for subsequent Chlorophyll-*a* analysis. Sub-samples (50 mL) were collected into 50 mL Falcon tubes, kept on ice and then frozen at -20 °C for subsequent total nitrogen analysis.

2.1.6 Organic carbon

Sub-samples (250 mL) were collected into acid-washed (10% HNO_3) polycarbonate containers, kept on ice and then stored at 4 °C for subsequent organic carbon analysis.

2.1.7 Samples for DNA extraction

Sub-samples (20 mL) were collected in sterile plastic syringes and filtered through a 0.22 µm MF-Millipore membrane filter (ThermoFisher Scientific, NZ) supported in a Swinnex 25 mm filter holder (Thermo Fisher Scientific, NZ). Each filter was transferred into a sterile 1.7 mL Eppendorf tube, snap frozen in liquid nitrogen and stored at -80 ºC.

2.2 Growth and maintenance of bacterial and viral strains

2.2.1 Bacteria

Cultures of *Pseudomonas putida* (KT2440) (Landcare Research, Canterbury, NZ), *Escherichia coli* (JC5466) and *Novosphingobium nitrogenifigens* strain Y88^T (ATCC BAA-1340; DSM 19370; *nifH* accession number DQ660368; (Addison *et al.*, 2007b) were grown on nutrient agar (Fort Richard Laboratories Ltd, New Zealand) then cultured into nutrient broth (Fort Richard Laboratories Ltd, NZ) and grown at 30 °C and 150 rpm overnight. Samples were then fixed in 2% formaldehyde (final concentration) and stored at 4 °C in the dark until analysis of bacterial abundance for method validation.

2.2.2 Pure phages

Pure phages, coliphage (infecting *E. coli*) and NZRM 2331 (infecting *P. aeruginosa*) were obtained from the Cawthron Institute (Nelson, NZ) and Environmental Science and Research (Christchurch, NZ). Phages were shipped as freeze-dried mass and reconstituted in nutrient broth prior to regeneration with host bacteria by the double layer agar method (Adams, 1959).

2.2.3 Phytoplankton

Cultures representing the groups Bacillariophyta (diatoms), Chlorophyta (green algae), Chrysophyta (chrysopytes), Cryptophyta (cryptophytes), Cyanophyta (cyanobacteria), Dinophyta (dinoflagellates) and Euglenophyta (euglenophytes) were sourced from the Cawthron Institute (Nelson, NZ) micro-algae culture collection and maintained in 50 mL plastic pottles (Biolab, NZ) containing 30 mL of MLA enrichment medium (Bolch *et al.*, 1996; Gorham *et al.*, 1964) at light intensity of 300 µmol m^{-2} s^{-1} on a 12:12 h light:dark cycle at 20 °C.

2.3 Enumeration Methods

2.3.1 Flow Cytometry

Samples were analysed using a FACSVantage™ SE flow cytometer (Becton Dickinson (BD) Biosciences, USA) equipped with the BD FACSDiVa™ digital processing electronics and software option. The sheath fluid was PBS (Lorne Laboratories Ltd., UK), adjusted to pH 7.0 and delivered through a 70 µm nozzle at 131 kPa. Daily instrument optimisation was performed using SPHERO™ fluorescent beads (BD BioSciences, USA). A target number of 1000 cells s^{-1} was used to obtain optimal cell counts by flow cytometry (FC).

2.3.2 Cell counts by flow cytometry

Cell counts were obtained using an internal standard of diluted TruCount™ beads (BD BioSciences, USA). A fluorescent bead stock was prepared by adding 1 mL of TE-Buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8) to a TruCount™ tube containing a known quantity of beads (n = 51,088). A sub-sample (100 µL) of bead stock was added to 1000 µL of prepared sample and a fixed number (n = 50) of beads counted directly by FC. Absolute cell counts were determined using the following equation:

$$\{cell\ count = \frac{number\ of\ beads\ in\ 100\ \mu L\ bead\ stock}{x/y} \times \frac{number\ of\ events\ per\ population}{number\ of\ events\ per\ bead\ population}\}$$

where x = final volume of sample and dilution buffer and y = volume sample

2.3.3 Algal enumeration by flow cytometry

Samples were vigorously shaken to disperse algal aggregates, then passed through a 40 µm strainer (Falcon, USA) to remove zooplankton and large debris and prevent clogging of the flow cell. Minimal losses of filamentous species were observed. Samples (350 mL) were then centrifuged (4000 rpm, 10 min) to concentrate phytoplankton populations. The supernatant was discarded and 2 mL of concentrated sample retained for FC. A target number of 1000 cells s^{-1} was used to obtain optimal cell counts. Measured parameters included forward scatter (FSC), side scatter (SSC), Chlorophyll-*a* fluorescence (650-700 nm), phycoerythrin fluorescence (575 nm), indicative of phytoplankton, and allophycocyanin (APC) fluorescence (660 nm), indicative of cyanobacteria.
Cell populations were resolved using a dual gating procedure based on two-dimensional cytograms of APC-PE fluorescence.

2.3.4 Viral and bacterial enumeration by flow cytometry

Samples for viral and bacterial enumeration were quickly thawed at 35 ºC (samples were still cold) and then two 1 mL aliquots taken and diluted either 1:10 in TE buffer or left undiluted.

These were then incubated with SYBR Green I (Invitrogen Molecular Probes, USA) at a final concentration of 0.5/10,000 dilution of the stock solution supplied by the manufacturer, at room temperature in the dark for 5 min, followed by incubation at 80 ºC in the dark for 10 min. Samples were cooled for 5 min, then analysed by FC. Triggering (the threshold level above which the amplitude will rise when a cell is present) was set on green fluorescence with a sample flow rate yielding below 1000 events s^{-1}.

Cell populations were resolved using a dual gating procedure based on two-dimensional cytograms of green fluorescence and SSC (Brussaard, 2009).

2.4 Induction assay for lysogenic bacteria

Bacterial and viral enumeration was carried out using flow cytometry as described above. Mitomycin C-treated samples and control samples were compared to ascertain if there was a significant difference in abundance between the two (Sawstrom *et al.*, 2007).

2.5 Bacterial enumeration by epifluorescence

Samples were thawed at 35 ºC (samples were still cold) and a 2 mL sub-sample was vacuum-filtered through 0.22 µm MF-Millipore Membrane filter (ThermoFisher Scientific, NZ), dried with a Kimwipe (ThermoFisher Scientific, NZ) and placed sample-side up on a clean Petri dish (Raylab NZ Ltd).

The filter was then stained with 300 µL of 2X SYBR Gold (final concentration of 1/5000 dilution of the stock solution supplied by the manufacturer) (Invitrogen Molecular Probes, USA) and incubated at room temperature in the dark for 15 min.

Any residual stain was vacuum-filtered through the filter and the filter wiped with a Kimwipe (ThermoFisher Scientific, NZ) to remove any remaining moisture. The filter was mounted onto a clean slide prepared with a drop of mounting solution (50% glycerol: 50% TE buffer) and topped with another drop of mounting oil. A clean cover slip was applied and the slide examined under blue-green excitation using oil immersion. Slides were examined using an Olympus BX61 microscope equipped with analysis LS Research software (Olympus, USA) at 1000× magnification. Photographs were taken using an Olympus Colourview (Olympus, USA) digital camera under bright field.

Bacterial counts were undertaken using Image J particle counting software (National Institute Health) with at least 300 cells counted from 20 random view fields (Noble *et al.*, 1998; Chen *et al.*, 2001; Wen *et al.*, 2004).

2.6 Manual phytoplankton counts

Phytoplankton samples were analysed using an inverted Olympus microscope (Olympus IX71, Olympus NZ) and Utermöhl settling chambers (Utermöhl, 1958) according to a protocol adapted from USEPA (Keith, 2003) and Hotzel *et al.*, (1999). Identification to species level was made with standard taxonomic text (Baker *et al.*, 1999; McGregor, 2001; John *et al.*, 2002).

2.7 Bacterial production

Bacterial production was evaluated using a protocol derived from Nagata, (1987) and Fuhrman *et al.*, (1982) for measurement of uptake of [^{3}H] thymidine-labelled with the radioisotope-labelled tritium – [methyl-^{3}H] thymidine. Duplicate 5 mL samples of water were transferred into bijou bottles and [methyl-3H] thymidine (70 to 90 Ci mmol^{-1}, Perkin-Elmer NEN, New Zealand Scientific Ltd) was added to make a final concentration of 5 to 6 nM.

Control samples were inactivated with 1% formalin prior to thymidine addition. Samples were incubated around *in situ* water temperature (± 2 ºC) in the dark for 1.5 h in order to incorporate the labelled radioisotope into the bacterial biomass. Uptake was halted by the addition of unlabelled thymidine (Sigma Aldrich, NZ) at a final concentration of 10 mM. The samples were then chilled quickly in a water/ice slurry water bath (1-2 ºC) and extracted for 15 min.

Chilled trichloroacetic acid (TCA) was added to a final concentration of 5% w/v and the samples filtered through 0.2 µm MF-Millipore membrane filters and washed with 5% TCA. The papers were dehydrated over silica gel under a bell jar and transferred to scintillation vials. An aliquot (10 mL) of dioxane base scintillation fluid was added and the samples counted by liquid scintillation in a Beckman Scintillation counter. The number of moles of thymidine incorporated was calculated from the formula:

$$U = dpm\ (SA)^{-1} 4.5\ x\ 10^{-13}$$

where U is moles of thymidine, dpm is disintegrations per minute on the filter, SA is specific activity of the thymidine in Ci mmol^{-1} and 4.5 x 10^{-13} is the number of curies per dpm.

2.8 Nutrients

Nutrient analyses were performed using an Aquakem 200cd discrete photometric analyser (Thermo Scientific, USA) with Aquakem Konelab software version 7.2. Analyses for ammonium (NH_4^+), nitrite (NO_2^-) total oxidized nitrogen (NOx) and dissolved reactive phosphorus (PO_4^{3-}) were carried out using the standard Aquakem methods (Thermo Scientific, USA). Nitrate (NO_3^-) was calculated by subtracting NO_2-N values from NOx values post analysis. Total nitrogen (TN) was determined using a modified simultaneous persulfate digestion method (Ebina *et al.*, 1983) based on EPA Methods 353.1 and 365.3 respectively (Keith, 1996). Calibration standards for TN were digested identically to samples. Ammonium and phosphate were analysed according to Methods for the Examination of Waters and Associated Materials, Ammonia in Waters 1981 and Phosphorus in Waters, Sewage and Effluents 1981 (Eaton *et al.,* 2005).

Samples were analysed for NOx on the discrete analyser using modified EPA Methods 353.1. Milli-Q water was used in preparing all standards and reagents. Stock standards were prepared from analytical reagent-grade chemicals, and stored in clean bottles at 4 °C. Working standards were prepared by diluting stock standards with Milli-Q water. Quality control standards were run after every 40 samples.

Total phosphorus was determined using a modified simultaneous persulfate digestion method (Ebina *et al.*, 1983) based on EPA Methods 353.1 and 365.3 respectively (Keith, 1996) and subsequent analysis using a Flow Injection Analyser.

2.9 Chlorophyll-*a*

Chlorophyll-*a* was determined according to a protocol adapted from Arar and Collins (Arar *et al.*, 1997). Filters were ground manually in a mortar and pestle and Chlorophyll-*a* extracted by steeping the filter in 90% $MgCO_3$-buffered acetone for 24 h. The slurry was centrifuged for 10 min at 3300 rpm and fluorescence of an aliquot of supernatant measured before and after acidification to 0.003 N with 0.1 N HCl to correct for phaeophytin degradation products, on a 10 AU Fluorometer (Turner designs).

2.10 Organic Carbon

Organic carbon was determined using an Elementar HiToc analyser and auto-injector (Elementar Analysensysteme GmbH, Hanau, Germany) to comply with APHA standard method 5310B (Eaton *et al.*, 2005). To determine total carbon, a sample was injected into a catalyst furnace to oxidise carbon to carbon dioxide. The inorganic component was determined by injecting the sample into a reactor containing dilute acid, where the inorganic component, assumed to be carbonate, was converted to carbon dioxide.

The carbon dioxide from each reaction was removed in a carrier gas stream to separate infrared detectors and the output integrated and expressed as elemental carbon. Organic carbon was calculated as the difference between the total and inorganic values.

2.11 Changes in abundances of bacterial functional genes

2.11.1 Water

Water free of RNA/DNA was sourced from Invitrogen™ (New Zealand) for use in PCR reactions and for re-suspension of DNA.

2.11.2 Buffers and solutions

Table 2.1 *Common buffers and solutions used in all DNA and PCR reactions.*

Solution reference name	Components
TE buffer	10 mM Tris-HCl, 1 mM EDTA, pH 8.0
Phosphate buffer	0.1 M NaH_2PO_4, pH 8.0
SDS Lysis buffer	10% w/v SDS, 0.5 M Tris, 0.1 M NaCl
CTAB/NaCl solution	0.7 M NaCl, 10% w/v CTAB
Chloroform:Isoamyl	22:1 Chloroform:Isoamyl alcohol
Phenol:Chloroform:Isoamyl	2.5:2.4:1 Phenol:Chloroform:Isoamyl alcohol

2.11.3 DNA extraction and purification

Total genomic DNA was extracted from stored membrane filters using the PowerWater® DNA Isolation Kit (GeneWorks Pty Ltd, Australia).

The resultant DNA was then purified using the Promega Wizard Purification system (In Vitro Technologies, NZ) and eluted with 50 µL nuclease-free water and stored at -20 ºC. DNA concentrations of all samples were determined by ND-1000 UV-Vis Spectrometer (NanoDrop Technologies, USA).
DNA was viewed by electrophoresis using 1% agarose gel, stained with ethidium bromide and the bands visualised on a UV transilluminator.

2.11.4 Quantification of functional genes

The abundance of selected functional genes: nitrogenase reductase (*nifH*), ammonia monooxygenase (*amoA*), methyl coenzyme M reductase (*mcrA*), dissimilatory sulfate reductase (*dsrA)* and nitrous oxide reductase (*nosZ*) were estimated in selected DNA extracts using quantitative PCR (qPCR) and normalised with respect to a universal bacterial 16S rDNA qPCR assay.

All samples from the monthly sampling frequency phase were included for DNA extraction and samples were included from a random selection of dates from both the hypolimnion, epilimnion and through the thermocline during the weekly sampling phase.

2.11.5 QPCR standards

Oligonucleotide primers developed from previously published work were sourced from Sigma-Aldrich (Australia). Table 2 lists the primers used and their sequences and reference sources.

Table 2.2 *Oligonucleotide primers and their sequences used for quantitative PCR.*

Primer pair	Sequence (5'3')	Target gene	Product size (bp)	Reference
338F	ACTCCTACGGGAGGCAGCAG	16S	181	Park *et al*., (2005)
518R	ATTACCGCGGCTGCTGCTGG			
PolF	TGC GAY CCS AAR GCB GAC TC	*nifH*	360	Poly *et al*., (2001)
PolR	ATSGCCATCATYTCRCCG GA			
nosZF	CGYTGTTCMTCGACAGCCAG	*nosZ*	259	Kloos *et al.,* (2001)
nosZR	CATGTGCAGNGCRTGGCAGAA			
amoA-1F	GGGGTTTCTACTGGTGGT	*amoA*	491	Rotthauwe *et al.,* (1997)
amoA-2R	CCCCTCKGSAAAGCCTTCTTC			
mlasF	GGTGGTGTMGGDTTCACMCARTA	*mcrA*	464	Steinberg *et al*., (2009)
mcrAR	CGTTCATBGCGTAGTTVGGRTAGT			
DSR1F	ACSCACTGGAAGCACG	*dsrA*	222	Ben-Dov *et al.,* (2007)
RH3-dsr-R	GGTGGAGCCGTGCATGTT			

The nitrogen-fixing isolate *Novosphingobium nitrogenifigens* strain $Y88^T$ (ATCC BAA-1340; DSM 1930; *nifH* accession number DQ660368) (Addison *et al*., 2007) was used as a standard for *nifH* qPCR (Bowers *et al*., 2008).

Acquisition of standards for qPCR of ammonia oxidisers, methanogens, sulphate reducers and nitrate reducers was performed by a modified phenol-chloroform extraction of genomic DNA from the settled biomass of a sequencing batch reactor thought to contain these functional genes, which was fed with municipal waste at SCION (Rotorua, NZ). A small amount of biomass was vortexed with 1.5 mL 5M NaCl, the supernatant decanted and the pellet resuspended in 300 µL phosphate buffer. The pellet was then reacted with 300 µL chloroform-isoamyl alcohol and 300 µL SDS lysis buffer in a FastPrep machine at 4.5 m s^{-1} for 40 s. Cell debris was pelletised by centrifuging at 14000 rpm for 5 min and the supernatant transferred to a clean microcentrifuge tube and reacted with 567 µL TE buffer, 30 µL 10% SDS and 3 µL 14 mg L^{-1} proteinase K (Gibco BRL). The mixture was vortexed and incubated at 37 ºC for 1 h and 100 µL 5M NaCl added, together with 80 µL CTAB/NaCl solution, mixed and incubated at 65 ºC for 10 min. An equal volume of chloroform-isoamyl alcohol was added and mixed prior to centrifugation at 3000 rpm for 20 min. The supernatant was transferred to a clean tube and the chloroform-isoamyl alcohol addition repeated. The resulting supernatant was then transferred to a clean tube and an equal volume of phenol-chloroform-isoamyl alcohol added, the mixture shaken, vortexed and then centrifuged at 3000 rpm for 20 min. The supernatant was transferred to a clean tube and 500-600 µL 100% ethanol added, mixed and centrifuged at 14000 rpm for 10 min. The supernatant was drawn off and the pellet left to dry in a laminar flow cabinet before the DNA was resuspended in 50 µL of sterile water.

The resultant DNA was then cleaned using a GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Australia) and DNA determined by ND-1000 UV-Vis Spectrometer (NanoDrop Technologies, USA). DNA was viewed by electrophoresis using 1.5 % agarose gel, stained with ethidium bromide and the bands visualised on a UV transilluminator.

2.11.6 PCR identification of functional genes for use as standards

All PCR reactions were carried out in 9700 or 2700 PCR machines (Applied Biosystems). All PCR reactions were undertaken in a total volume of 25 µL using Taq polymerase (Roche, USA). Table 3 lists the PCR setup and cycling conditions for each gene.

Table 2.3 *PCR conditions for functional gene analysis*

	mcrA	***amoA***	***nosZ***	***dsrA***
DNA vol (~10-20μg mL^{-1})	0.5	1.0	0.5	1.0
Final conc (μM)	0.25	0.2	0.2	0.15
dNTP conc (μM)	0.2	0.2	0.2	0.2
Taq U μ^{-1}	0.03	0.1	0.1	0.1
MgCl (mM)	2	2	3	2
PCR buffer	1x	1x	1x	1x
Initial denaturation (min)	1:00 at 95ºC	2:00 at 95 ºC	3:00 at 94 ºC	3:30 at 95 ºC
No. cycles	40	35	35	35
Denaturation (min)	0:30 at 95 ºC	0:15 at 95 ºC	0:15 at 95 ºC	0:15 at 95 ºC
Annealing (min)	0:45 at 55 ºC	1:00 at 57 ºC	1:00 at 57 ºC	1:00 at 62 ºC
Elongation (min)	0:30 at 72 ºC	0:50 at 72 ºC	0:40 at 72 ºC	0:30 at 72 ºC
Final extension (min)	7:00 at 72 ºC	5:00 at 72 ºC	5:00 at 72 ºC	7:00 at 72 ºC

2.11.7 DNA viewing by electrophoresis

Separation by electrophoresis was undertaken using 1.5 % agarose gel. Aliquots of DNA (4 µL for PCR products and 2 µL for extracted DNA) were loaded into each well with 4 µL orange gel loading dye (Sigma- Aldrich, Australia). Gels were run at 120V, for 40 min then stained with ethidium bromide and the bands visualised on a UV transilluminator (GelDoc™ 2000, Bio-Rad). DNA bands were compared to PicoGreen quantification dyes (Molecular probes, Invitrogen) run on the same agarose gel. PCR products of the correct size were used for cloning.

2.11.8 Cloning

All cloning reactions were performed using the TOPO TA Cloning® Kit (with pCR® 2.1 TOPO) (Invitrogen, USA) under permit GMD05083. The resulting colonies were individually cultured overnight in LB media (Per L^{-1}; 10 g Tryptone, 5 g yeast extract, 10 g NaCl, 15 g purified agar, pH 7 and 50 µg mL^{-1} ampicillin). Plasmids were then prepared using a Pure Link Quick Plasmid Miniprep Kit (Invitrogen, USA) and sequenced with the M13 forward and M13 reverse primers and the PCR products cleaned using the following: 20 µL PCR product, 0.75 µL ExoI and 1.5 µL FastAP (Fermentas enzymes, Global Science and Technology, NZ). The reaction was run at 37 ºC for 30 min, 85 ºC for 15 min with a 4 ºC hold.

2.11.9 Sequencing

All sequencing reactions were performed with the ABI PRISM® Big Dye™ Terminator Cycle Sequencing Ready Reaction kit (Version 1.1) (Applied Biosystems). An aliquot of 2 µL of each template DNA was sequenced using 3.2 pmol primer, 1 µL Ready Mix (Applied Biosystems) and 3.5 µL Big Dye Sequencing buffer (Applied Biosystems) in a final volume of 20 µL. The sequencing reactions were carried out with the following PCR profile: 96 ºC for 1 min, 25 cycles of 96 ºC for 10 s, 55 ºC for 5 s, 60 ºC for 4 min, with a final hold at 4 ºC.

2.11.10 Purification of sequencing reactions

Sequencing reactions were purified using the Sephadex G-50 method (GE Healthcare, Australia) using Whatman 96 well terminator removal unifilter plates (Global Science and Technology, NZ) to remove excess primer and dye.
Purified products were stored at -20 ºC in the dark prior to sequence analysis on an ABI PRISM® 9700 16 Capillary Genetic Analyser (Applied Biosystems).

2.11.12 Electrophoresis of sequencing reactions

The purified sequencing reactions were made up to a total volume of ~20 μL with distilled water and a 96-well septae mat was placed onto the plate before the reactions were denatured at 96 ºC for 5 min. The plate was immediately transferred onto ice for 2 min. The sample names and plate locations were loaded into a sample sheet for the ABI PRISM® 9700 16 Capillary Genetic Analyser (Applied Biosystems) and, once placed in the machine, the plate was linked to the sample sheet. The samples were electrophoresed using either the POP4® polymer and a 36-cm capillary array (using the rapid sequence protocol), or the POP6® polymer and standard sequencing protocol. The electrophoresis and analysis protocols were standard and supplied with the machine.

2.11.13 Raw sequence analysis of 9700 files

Each sequence file was analysed post-electrophoresis to obtain an optimal sequence electropherogram using Sequence Analysis Software (version 3.7, Applied Biosystems). Each sequence was trimmed to remove the TT adaptor.

Ambiguous or messy sequences were also edited or deleted as appropriate. All sequences were then aligned using the Sequencher™ Sequence Analysis package (version 4.2) (GeneCodes Corporation) into contigs of homologous sequences with the minimum requirement for homology set to 90% over 20 bases.

2.11.14 Sequence alignment to publicly available sequences

To verify that the primers were amplifying the correct genes, sequences were compared to publicly available sequences using the NCBI Blastn and Blastx programs (http://blast.ncbi.nlm.nih.gov/Blast.cgi/) (Altschul *et al.*, 1990). Matches of the selected sequences were of >97% similarity to the correct genes.

2.11.15 Quantitative PCR

All qPCR reactions were performed using a LightCycler instrument (Roche) using Roche LightCycler consumables and FastStart DNA Master SYBR Green kits.

The presence of the correct sized amplicon was confirmed by loading 4 µL of the resulting PCR product onto agarose gel (see Section 2.14.1) and by performing melting curve analysis on the LightCycler. A water control was included in each experiment and if DNA contamination was observed then the results discarded and the experiment repeated. For all qPCR experiments a 4–point, 5-fold dilution series standard curve was prepared using the appropriate plasmid. All the qPCR's used the same mix as follows: FastStart DNA MasterPLUS SYBR Green 1 reagent mix (Roche) was mixed with $MgCl_2$ at a fixed concentration of 1mM for all reactions. A reaction mix for 32 reactions was prepared by adding 57 µL FastStart Reaction mix with 7 µL LightCycler FastStart Enzyme, 64 µL of the appropriate gene specific forward (F) and reverse (R) primer (final concentration 1 µM) and 132 µL water. Amplification reactions were then carried out in a total volume of 10 µL containing 2 µL of appropriate DNA and 8 µL of the master mix (Table 2.4).

Table 2.4 *qPCR conditions for functional gene quantification*

	16S	***nosZ***	***AmoA***	***mcrA***	***nifH***	***dsrA***
DNA vol (µL) (~10-20µg mL^{-1})	2	2	2	2	2	2
Water vol (µL)	4	4	4	4	4	4
Faststart SYBRplus Mix 5X vol (µL)	2	2	2	2	2	2
($MgCl_2$ Mmol)	1	1	1	1	1	1
Primers used	338F & 518R	nosZF & nosZR	amoA-1F & amoA2R	mlasF & mcrAR	nifHpolF & nifHpolR	DSR1F & RH3dsR
Conc (µmol)	0.4	1.0	1.0	1.0	1.0	1.0
Standard used	*Escherichia coli* K-12 (JC5466)	Plasmid DNA containing *nos-Z* (2.15.10)	Plasmid DNA containing *amo-A* (2.15.10)	Plasmid DNA containing *mcrA* (2.15.10)	*Novosphingobium nitrogenifigens* Y88	Plasmid DNA containing *dsrA* (2.15.10)
Cycle data						
Initial denaturation (min)	95°C for 10 min	95°C for 10 min	95°C for 10 min	95°C for 10 min	95°C for 10 min	95°C for 10 min
No. cycles	40	45	45	45	45	45
	95° C for 5 s	95°C for 15 s	95°C for 10 s	95°C for 10 s	95° C for 5 s	95° C for 15 s
	55° C for 20 s	60° C for 13 s	55° C for 4 s	55° C for 4 s	54° C for 4 s	60° C for 13 s
	72° C for 20 s	72° C for 30 s	72° C for 20 s	72° C for 20 s	72° C for 15 s	72° C for 30 s
Melt curve (temp)	65° C to 95° C	60° C to 95° C	65° C to 95° C	65° C to 95° C	65° C to 95° C	60° C to 95° C

2.14 Data analysis

Isopleth plots were made using Ocean Data View software (version ODV 4.3.10). Lake physical states were calculated using Lake Analyser (version 3.2) for Schmidt Stability, Wedderburn number and thermocline depth. (Read *et al.,* 2011).

A paired t-test was used to determine if differences between samples treated with Mitomycin C and untreated (control) were significant. A normality test was performed on data using Minitab 15.1.0.0 (Minitab Inc., USA) to determine if the data followed a normal distribution. Log 10 transfer of data was then performed to normalise data and a paired t-test on the differences between treated and untreated samples performed using Minitab 15.1.0.0 (Minitab Inc, USA). If the difference was significant then the sample was deemed to contain Lysogenic bacteria.

Principal component analysis was undertaken using Statistica 9.1 (Statsoft, USA). Variables analysed were log abundances of algae, viruses, bacteria, nitrogen species, phosphorus species, temperature, conductivity, fluorescence and oxygen percentage saturation.

CHAPTER 3: RESULTS

3.1 Physical variables

3.1.1 Temperature and thermal structure

Lake Okaro

Surface water temperature ranged from 8.5 to 22.5 °C and bottom temperatures from 7.9 to 10.0 °C between 17 August 2009 and 10 August 2010 (Figure 3.1). The metalimnion top (metaT) ranged in depth from 4.5 to 12.5 m during stratification, based on a metalimnion density definition of 0.2 kg m^{-3} m^{-1}. Schmidt stability (S) (Read *et al.*, 2011) was calculated between 17 August 2009 and 10 August 2010 (Figure 3.2) indicating that the lake was stratified (S>1) from 7 September. The duration of stratification was approximately 8 months until turnover which occurred in June 2010.

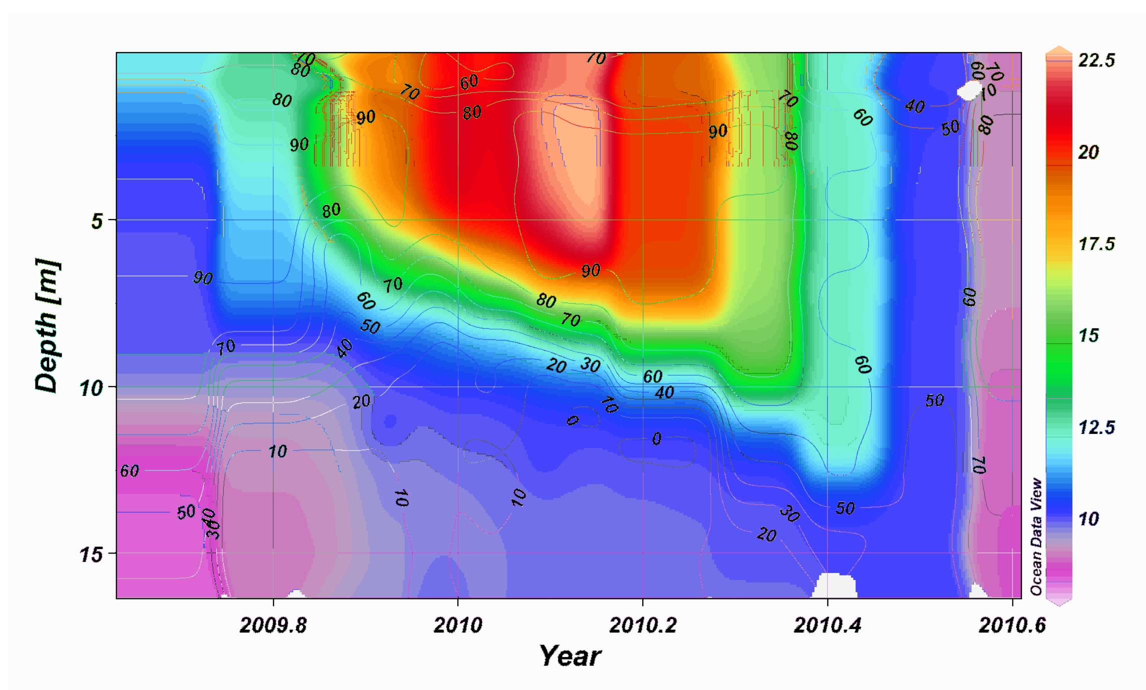

Figure 3.1 *Isopleths of temperature (°C) (see colours), depth (m) (see left-hand axis) and oxygen saturation (%) (contour lines) in Lake Okaro, 17 August 2009 to 10 August 2010.*

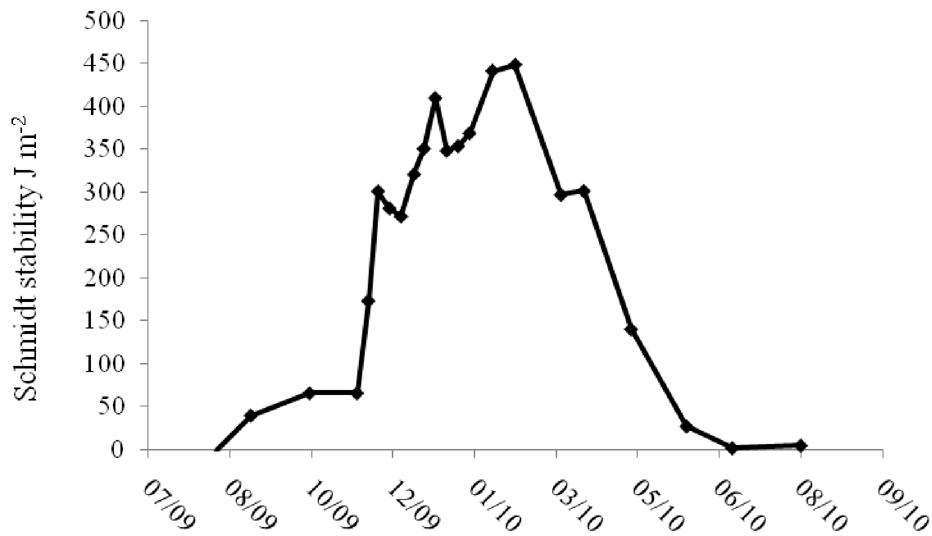

Figure 3.2 *Schmidt stability in Lake Okaro, 17 August 2009 to 10 August 2010.*

Lake Tikitapu

Surface water temperature ranged from 8.9 to 21.6 °C and bottom temperature from 9.1 to 12.11 °C between 17 August 2009 and 10 August 2010 (Fig 3.3). The top of the metalimnion (metaT) ranged in depth from 6.85 to 18.87 m using metalimnion threshold density definition of 0.05 kg $m^{-3}m^{-1}$. Values of Schmidt stability (S) indicated that the lake was stratified (S>1) prior to the beginning of sampling on 17 August 2009 until 10 August 2010 (Figure 3.4), when turnover had occurred.

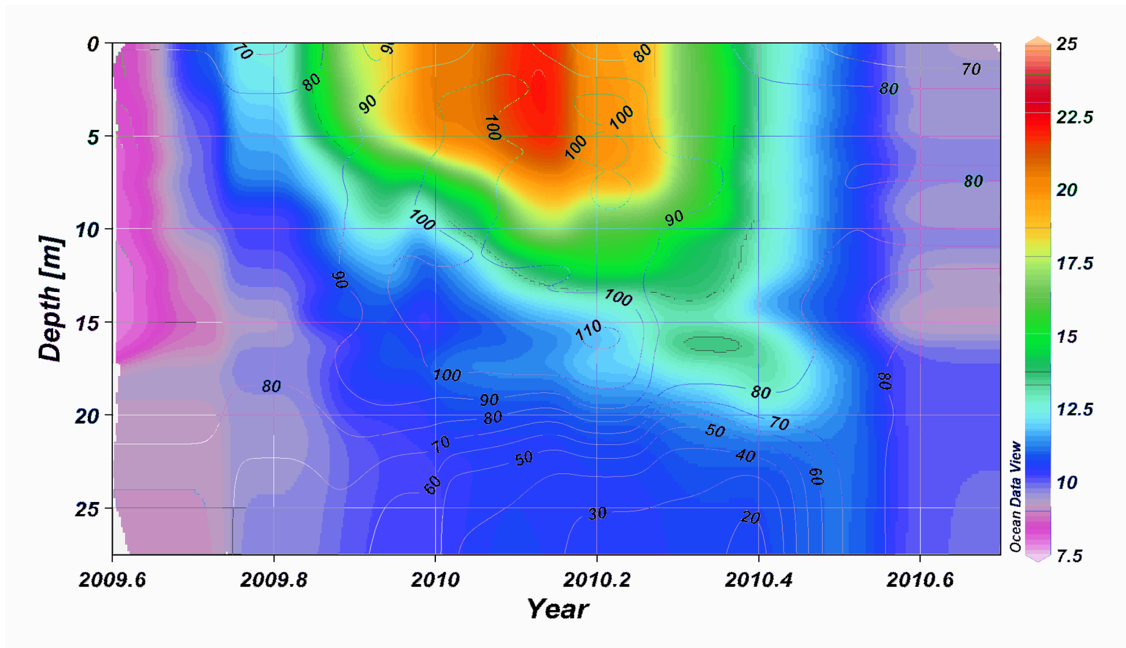

Figure 3.3 *Isopleths of temperature (^oC) (see colours), depth (m) (see left-hand axis) and oxygen saturation (%) (contour lines) in Lake Tikitapu, 17 August 2009 to 10 August 2010. Time scale is given as decimal year.*

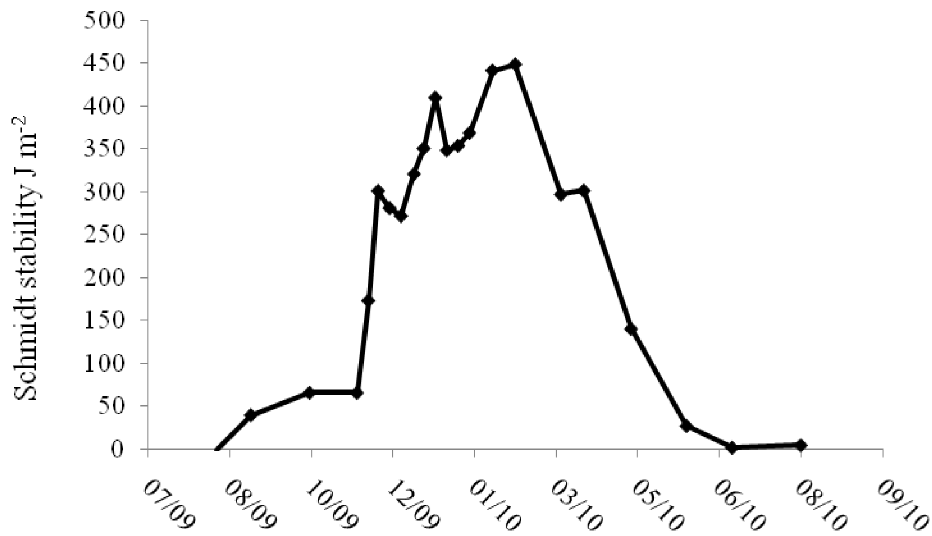

Figure 3.4 *Schmidt stability in Lake Tikitapu, 17 August 2009 to 10 August 2010.*

3.1.2 Dissolved oxygen

Lake Okaro

Oxygen saturation in bottom waters of Lake Okaro continuously exceeded 40% when the lake was mixed on sampling dates between 17 August 2009 to 17 September 2009 (Figure 3.1). Oxygen saturation was then low (<10%) throughout the hypoliminion and generally between 70-80% in the mixed epilimnion. Two mixing events occurred on 1 December 2009 and 5 January 2010 resulting in increased oxygen saturation at lower depths (Figure 3.1).

Lake Tikitapu

Oxygen saturation levels exceeded 70% throughout the water column from 17 August 2009 to 13 September 2009 (Figure 3.3). Following stratification the mixed epilimnion waters remained above 70% saturation, while waters below based on the metalimnion dropped to below 60% saturation. Turnover had occurred when sampling was concluded on 10 August 2010 and at this time the water column dissolved oxygen exceeded 80% saturation (Figure 3.3).

3.2 Chemical variables

3.2.1 Nutrients

Lake Okaro

The highest nutrient values for Lake Okaro were all recorded in the waters below the metalimnion (hypolimnion) (Figures 3.5 to 3.6). Total phosphorus (TP) concentrations were highest in the hypolimnion peaking at 0.39 mg L^{-1} on 27 April 2010 (Figure 3.4a). Concentrations of TP in the mixed top waters (epilimnion) were stable remaining under 0.1 mg L^{-1} throughout the sampling period. Epilimnion and hypolimnion TP concentrations were converged (c. 0.07 mg L^{-1}) at the beginning of sampling prior to stratification and again after turnover at a similar concentration. Phosphate (PO_4–P) concentrations were also higher in the hypolimnion than the epilimnion, increasing after stratification and then converging following lake turnover. The highest concentration recorded in the hypolimnion was on 9 December 2010 (0.103 mg L^{-1}) (Figure 3.5b).

There were large fluctuations in concentrations of PO_4-P in summer samplings at all levels. The highest concentration recorded in the epilimnion was on 29 June 2010 (0.039 mg L^{-1}).

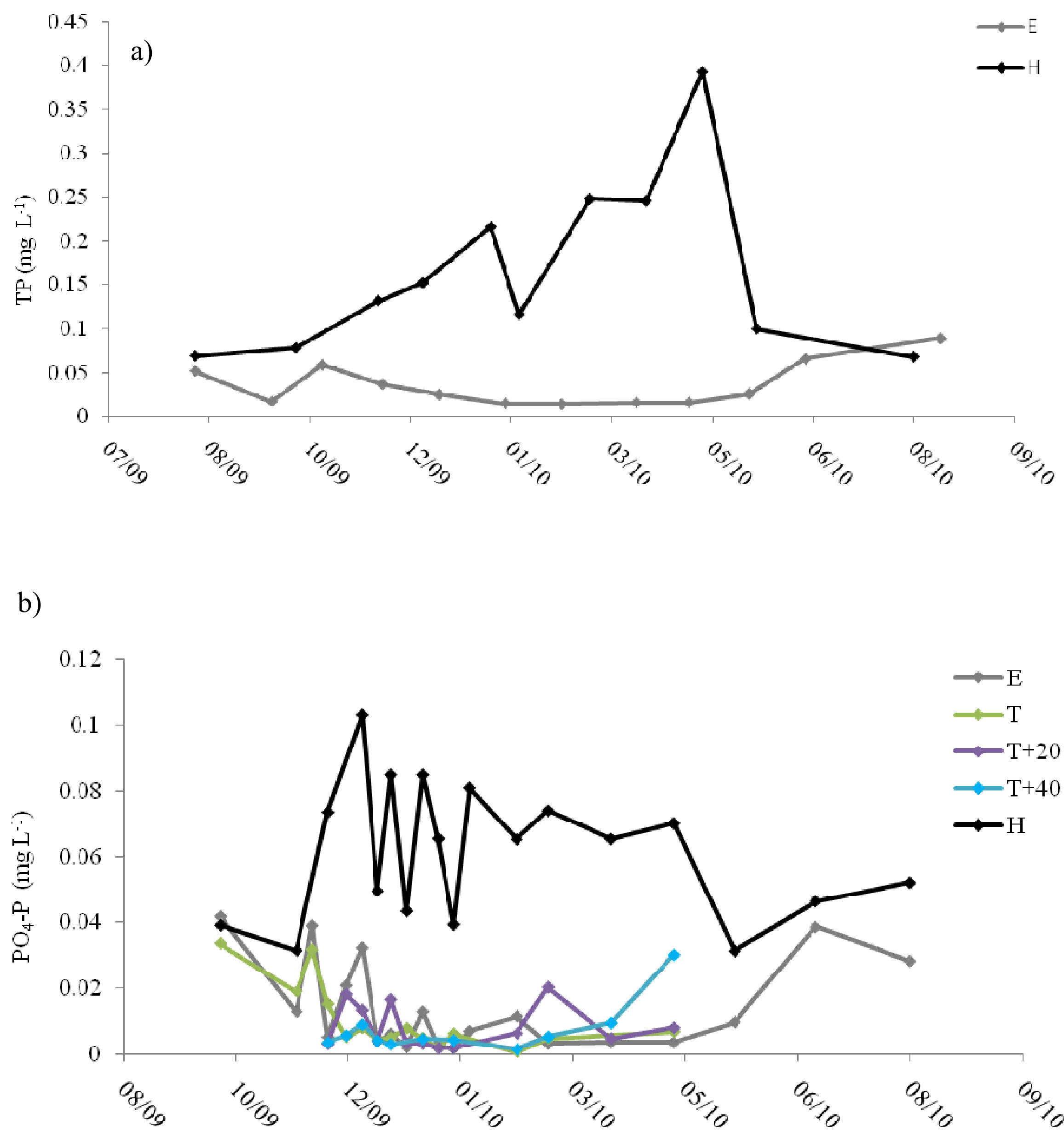

Figure 3.5 *Lake Okaro (a) Total phosphorus (TP) in the epilimnion (E) and hypolimnion (H), from 18 August 2009 to 23 August 2010, and b) phosphate (PO_4–P) concentrations in the epilimnion, thermocline region (the top of the thermocline (T), 20 cm below (T+20), and 40 cm below (T + 40)) and the hypolimnion, from 17 August 2009 to 10 August 2010.*

Total nitrogen (TN) concentrations fluctuated in the summer months with higher concentrations in the hypolimnion at the end of summer before convergence in concentrations amongst different depths at the time of turnover (Figure 3.6a). The highest TN concentration in the hypolimnion was recorded on 10 November 2009 (1.510 mg L^{-1}) and the highest in the epilimnion on 16 December 2009 (1.264 mg L^{-1}).

Ammonium (NH_4-N) concentrations (Figure 3.6b) were generally higher in the hypolimnion, apart from a peak 40 cm below the thermocline in May 2010. The highest NH_4-N concentration in the hypolimnion was recorded on 2 March 2010 (1.070 mg L^{-1}) and the highest concentration in the epilimnion on 7 October 2009 (0.393 mg L^{-1}). Nitrate (NO_3-N) concentrations were highly variable with several peaks on any given sampling day occurring at any one of the levels sampled during the study (Figure 3.6c). The highest concentration of NO_3-N occurred in the hypolimnion on 26 January 2010 (0.097 mg L^{-1}) and in the epilimnion on 2 December 2009 (0.09 mg L^{-1}).

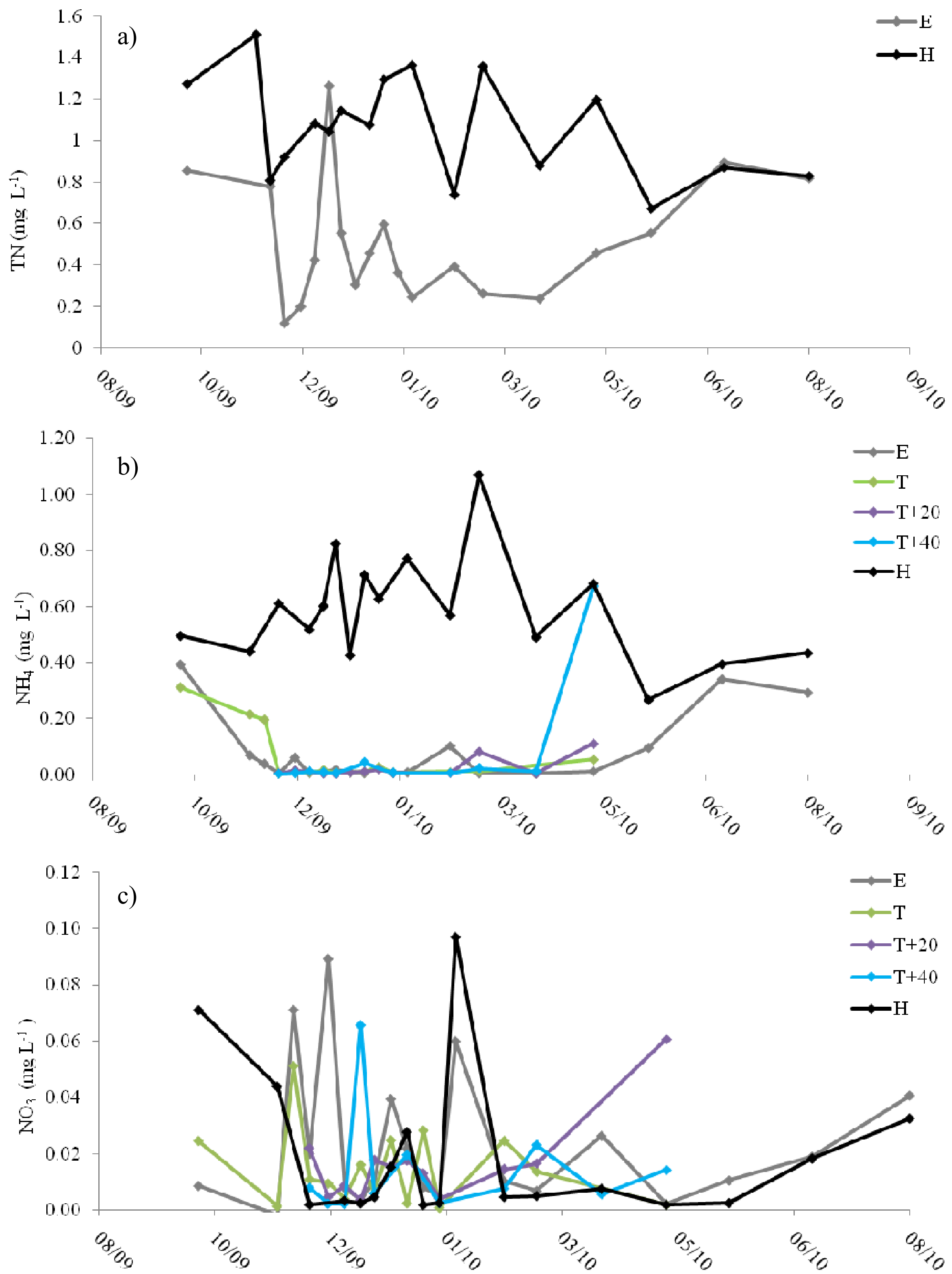

Figure 3.6 *Lake Okaro a) total nitrogen (TN) in epilimnion (E) and hypolimnion (H) b) ammonium (NH_4-N) and c) nitrate (NO_3-N) in epilimnion, thermocline region (the top of the thermocline (T), 20 cm below (T+20) and 40 cm below (T+40)) and the hypolimnion, from 17 August 2009 to 10 August 2010.*

Lake Tikitapu

Total phosphorus (TP) concentrations in the mixed epilimnion and hypolimnion followed similar temporal trends (Figure 3.7a) apart from a peak in the epilimnion on 29 October 2009 (0.021 mg L^{-1}) and in the hypolimnion on 19 May 2010 (0.02 mg L^{-1}). Apart from these two peaks concentrations remained below 0.01 mg L^{-1}. Phosphate (PO_4-P) levels in the epilimnion and hypolimnion (Figure 3.7b) followed similar temporal trends to those of TP. There was a peak in the epilimnion and hypolimnion on 29 December 2009 (0.021 and 0.022 mg L^{-1} respectively). Epilimnion PO_4-P concentrations increased slightly following turnover. Thermocline phosphate concentrations showed very little variation and no major peaks.

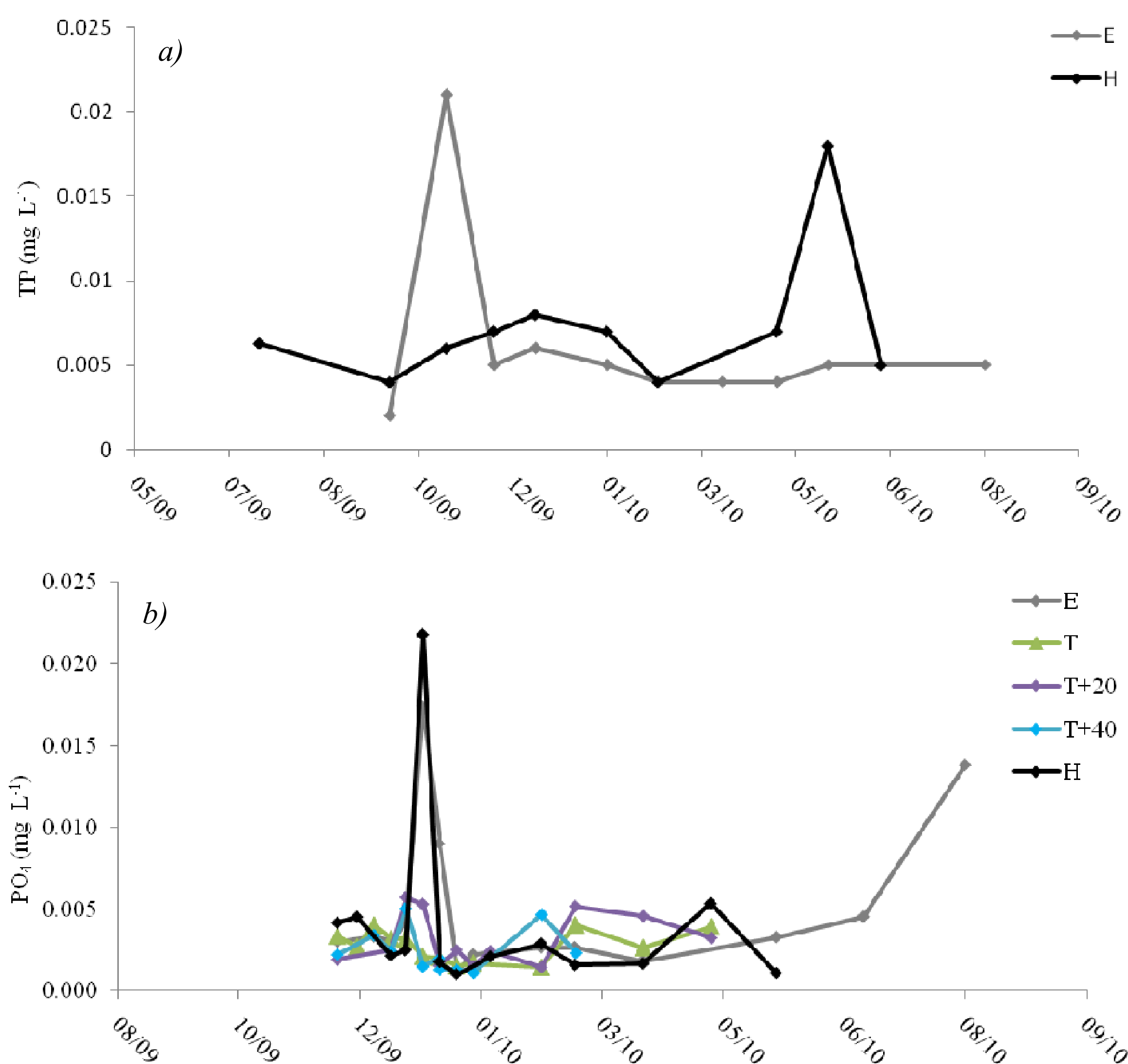

Figure 3.7 *Lake Tikitapu a) total phosphate (TP) in the epilimnion (E) and hypolimnion (H), from 29 September 2009 to 10 August 2010, and b) phosphate (PO4-P) in the epilimnion, thermocline region (the top of the thermocline (T), 20 cm below (T+20) and 40 cm below (T+40)) and the hypolimnion, from 17 August 2009 to 10 August 2010.*

Concentrations of TN in the epilimnion and hypolimnion of Lake Tikitapu were relatively constant (Figure 3.8a). The highest concentration was recorded in the hypolimnion on 7 October 2009 (0.450 mg L^{-1}). Concentrations of NH_4-N oscillated through time in the epilimnion of Lake Tikitapu, with high levels on 24 November 2009 (0.046 mg L^{-1}), 5 January (0.045 mg L^{-1}), 2 March 2010 (0.023 mg L^{-1}) and 24 May 2010 (0.022 mg L^{-1}). Ammonium-N concentrations in the thermocline followed similar trends to hypolimnion concentrations (Figure 3.8b). Nitrate-N concentrations (Figure 3.8c) peaked at the top of the thermocline and 40 cm below the thermocline on 16 December 2009 (0.226 mg L^{-1} and 0.3052 mg L^{-1}, respectively).

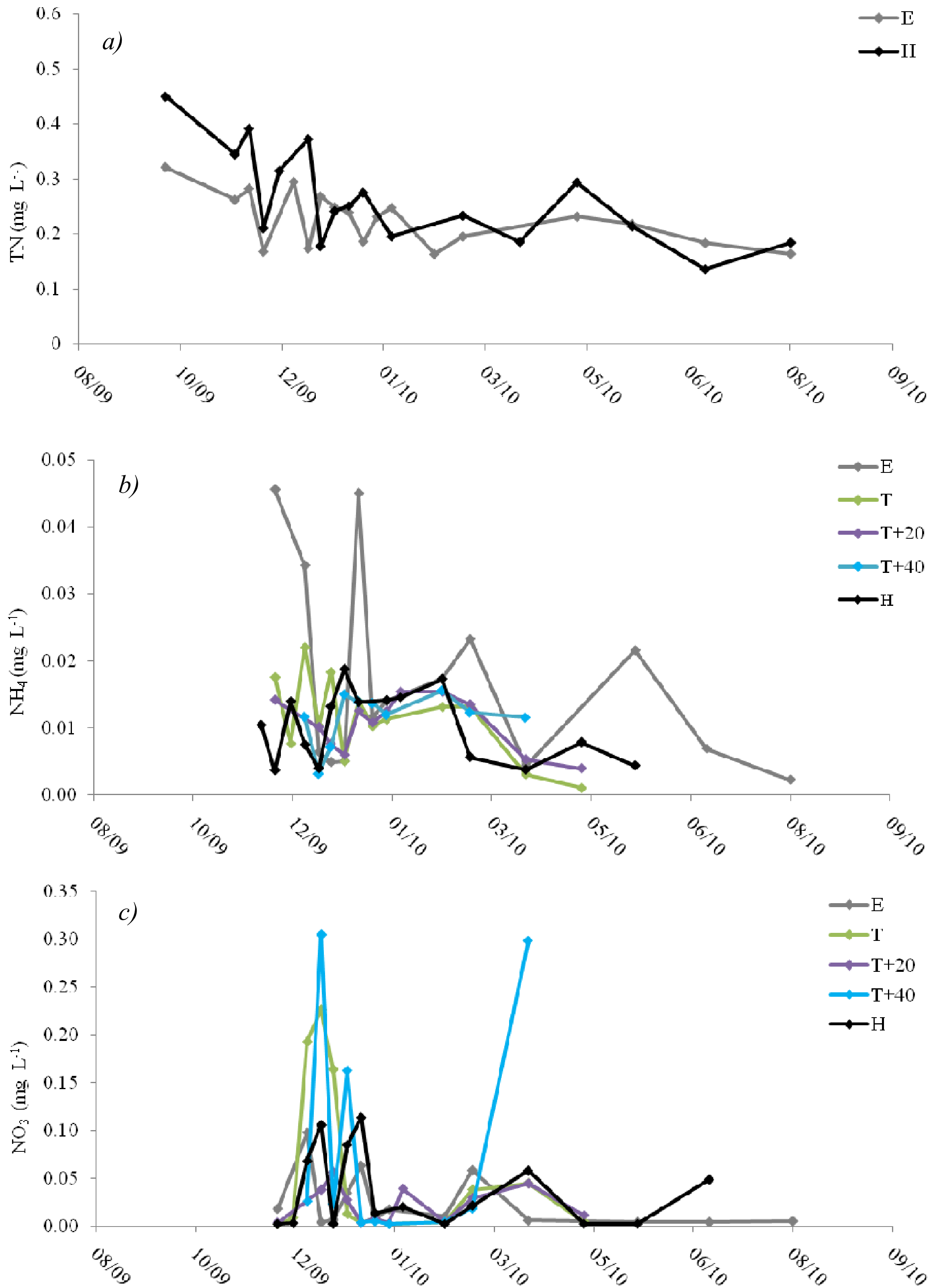

***Figure 3.8** Lake Tikitapu a) total nitrogen (TN) in epilimnion (E) and hypolimnion (H) b) ammonium (NH_4-N) and c) nitrate (NO_3-N) in epilimnion, thermocline region (the top of the thermocline (T), 20 cm below (T+20) and 40 cm below (T+40)) and the hypolimnion, from 17 August 2009 to 10 August 2010.*

3.2.2 Dissolved Organic Carbon

Lake Okaro

Dissolved organic carbon (DOC) concentrations in the epilimnion and hypolimnion of Lake Okaro ranged from 1 to 9 mg L^{-1} during the study (Figure 3.9). High concentrations occurred in the epilimnion and hypolimnion on 8 September 2009 (5 and 8 mg L^{-1}), in the hypolimnion on 22 December 2009 (5 mg L^{-1}), in the epilimnion on 5 January (5 mg L^{-1}), 2 February 2010 in the hypolimnion and epilimnion (both 5 mg L^{-1}) and 1 June 2010 in the hypolimnion and epilimnion (both 4 mg L^{-1}).

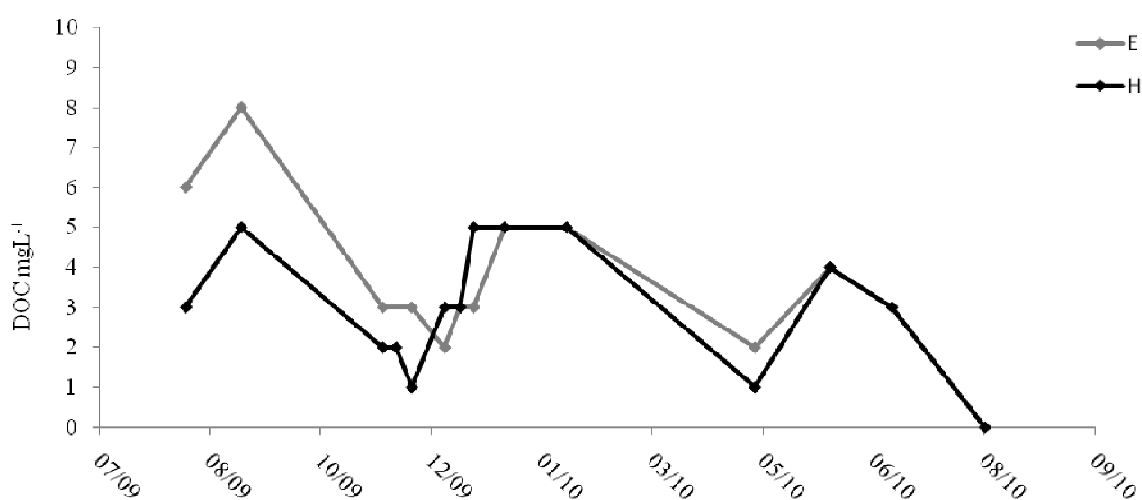

Figure 3.9 *Concentrations of dissolved organic carbon (DOC) in Lake Okaro epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

Lake Tikitapu

Concentrations of DOC in the epilimnion and hypolimnion of Lake Tikitapu were below 10 mg L^{-1} (Figure 3.10) but showed some variability, with peaks in the hypolimnion on 9 September 2009 (6 mg L^{-1}), 2 February 2010 (5 mg L^{-1}), 1 and 29 June 2010 (both 3 mg L^{-1}), and in the epilimnion on 9 September 2009 (4 mg L^{-1}) and 1 June 2010 (5 mg L^{-1}).

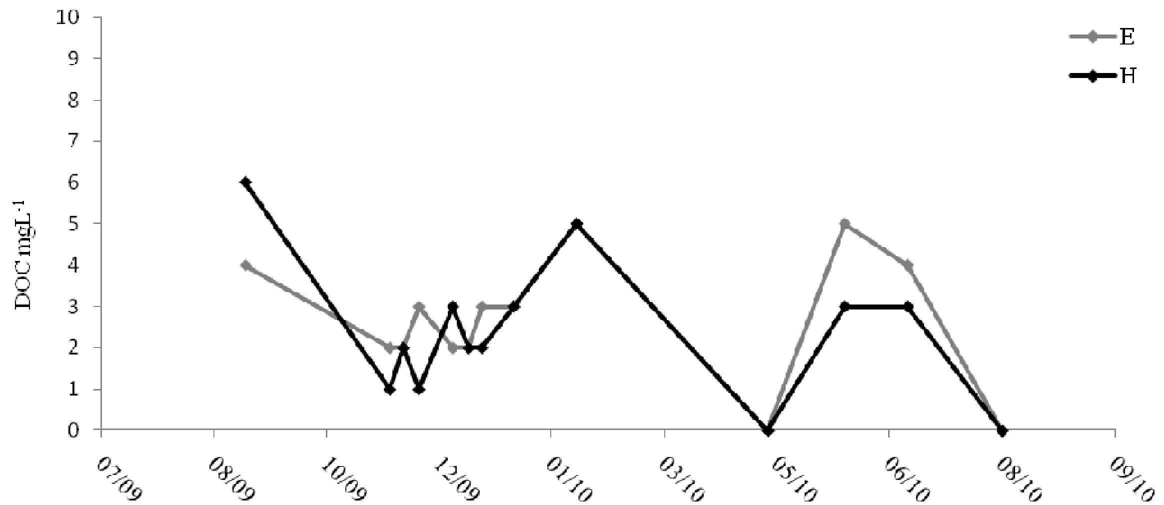

Figure 3.10 *Concentrations of dissolved organic carbon (DOC) in Lake Tikitapu epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

3.3 Biotic parameters

3.3.1 Fluorescence

Lake Okaro

Fluorescence maxima in the water column was concentrated in the mixed surface waters prior to stratification after which the maxima was at a depth similar to the thermocline, i.e. a chlorophyll fluorescence depth maximum (DCM). The fluorescence maxima followed the thermocline moving down as it deepened. Once the lake mixed again, after turnover, the fluorescence maxima decreased and was spread through the mixed layers of the lake. Maximum chlorophyll fluorescence occurred on 19 January 2010, 27.3 µg L^{-1} at the surface (Figure 3.11). The DCM spanned a depth less than 5 m below the thermocline during stratification, and there was a peak of 90.8 µg L^{-1} at a depth of 7.3 m on 2 February 2010.

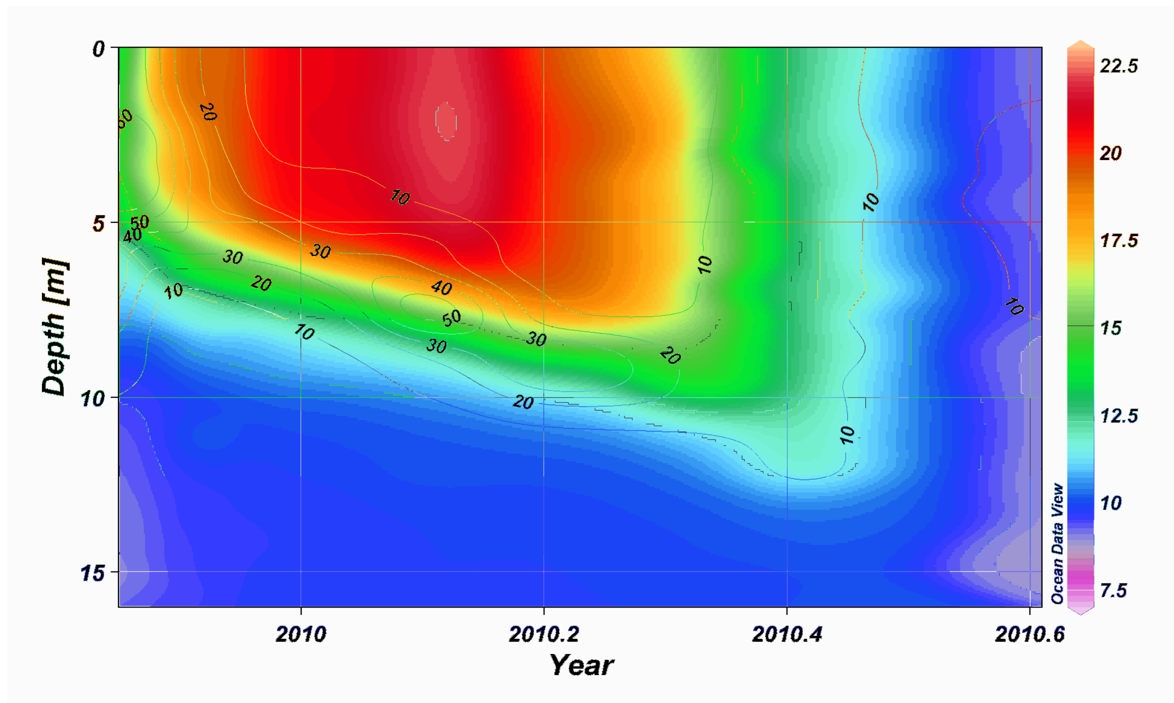

Figure 3.11 *Isopleths of temperature (°C) (see colours), depth (m) (see left-hand axis) and fluoresence (µg L^{-1}) (contour lines) in Lake Okaro, 11 November 2009 to 10 August 2010.*

Lake Tikitapu

Fluorescence maxima remained well below the mixed surface waters in Lake Tikitapu throughout the sampling period. Prior to stratification, fluorescence was low and concentrated below 12 m (Figure 3.12). With stratification the maxima was observed around the 5 m level after which it followed the thermocline as a distinct DCM. The DCM was located well below the thermocline at around 22 m, and was broad extending 5-10 m during stratification. The maximum fluorescence in the DCM at any time was 19.3 µg L^{-1} at a depth of 16.4 m on 5 January 2010. Following turnover in June the DCM disintegrated and the fluorescence was relatively evenly distributed through all depths.

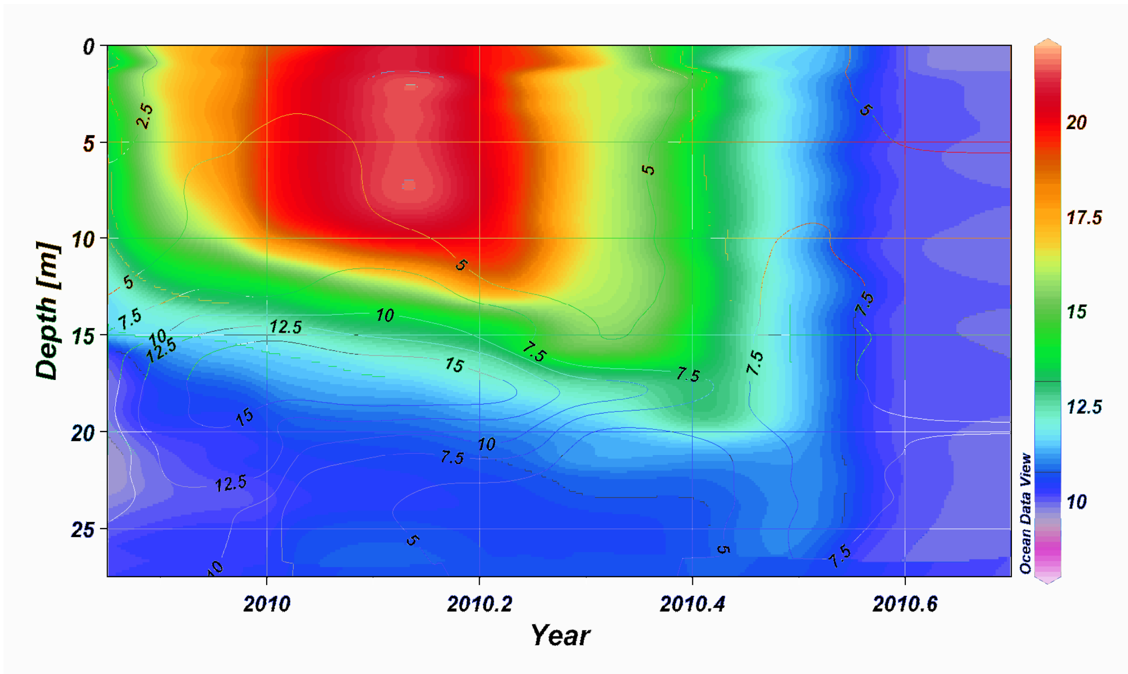

Figure 3.12 *Isopleths of temperature (^oC) (see colours), depth (m) (see left-hand axis) and fluoresence ($\mu g\ L^{-1}$) (contour lines) in Lake Okaro, 11 November 2009 to 10 August 2010.*

3.3.2 Phytoplankton abundance

Lake Okaro

A range of species were present in the epilimnion and hypolimnion in Lake Okaro, at total concentrations below 5×10^4 cells mL^{-1} for the majority of the sampling period. Total phytoplankton counts increased in the epilimnion just prior to lake turnover, with a maximum of 2.23×10^5 cells mL^{-1}. Total phytoplankton counts in the hypolimnion mirrored this increase to a lesser extent with a maximum of 1.29×10^5 cells mL^{-1} on 1 June 2010. At this time microscopic examination determined that the counts were dominated by Euglenophyta in the epilimnion and Chlorophyta (green algae) in the hypolimnion (Figure 3.13). Euglenophyta and Chlorophyta co-dominated until 9 December 2009 when Bacillariophyta dominated. Bacillariophyta remained in the epilimnion for the remainder of the sampling period giving way to dominance by Euglenophyta and increased counts of dinoflagellates on 29 June 2010. Cyanobacteria species were found in the epilimnion on 7 September 2009 and again on 17 and 24 November 2009, with a maximum of 2.38×10^5 cells mL^{-1}.

Of the latter concentrations 1.98×10^4 cells mL^{-1} were filamentous *Anabaena* sp. Cyanobacteria persisted in the epilimnion at moderate densities from 9 December 2009 through to 1 June 2010 at densities between 2.9×10^1 and 2.4×10^4 cells mL^{-1}. Microscopic examination confirmed that the cyanobacterial species were mostly *Anabaena* sp. and *Merismopedia* sp.. Cyanobacteria were also present in the hypolimnion from 7 September 2009 through to 30 March 2010, at a maximum density of 5.12×10^3 cells mL^{-1} on 16 December 2009. The only cyanobacteria present in the hypolimnion between 16 and 29 December 2009 and between 30 March and 25 May 2010 were *Anabaena* sp. *Merismopedia* sp. were present in the hypolimnion from 16 December 2009 through to 30 March 2010.

In both the epilimnion and hypolimnion through the sample period the dominant green alga was *Dictospaerum* sp., the dominating diatom was *Fragilaria* sp. and the dominating euglenophyte was *Clamydomonas* sp.

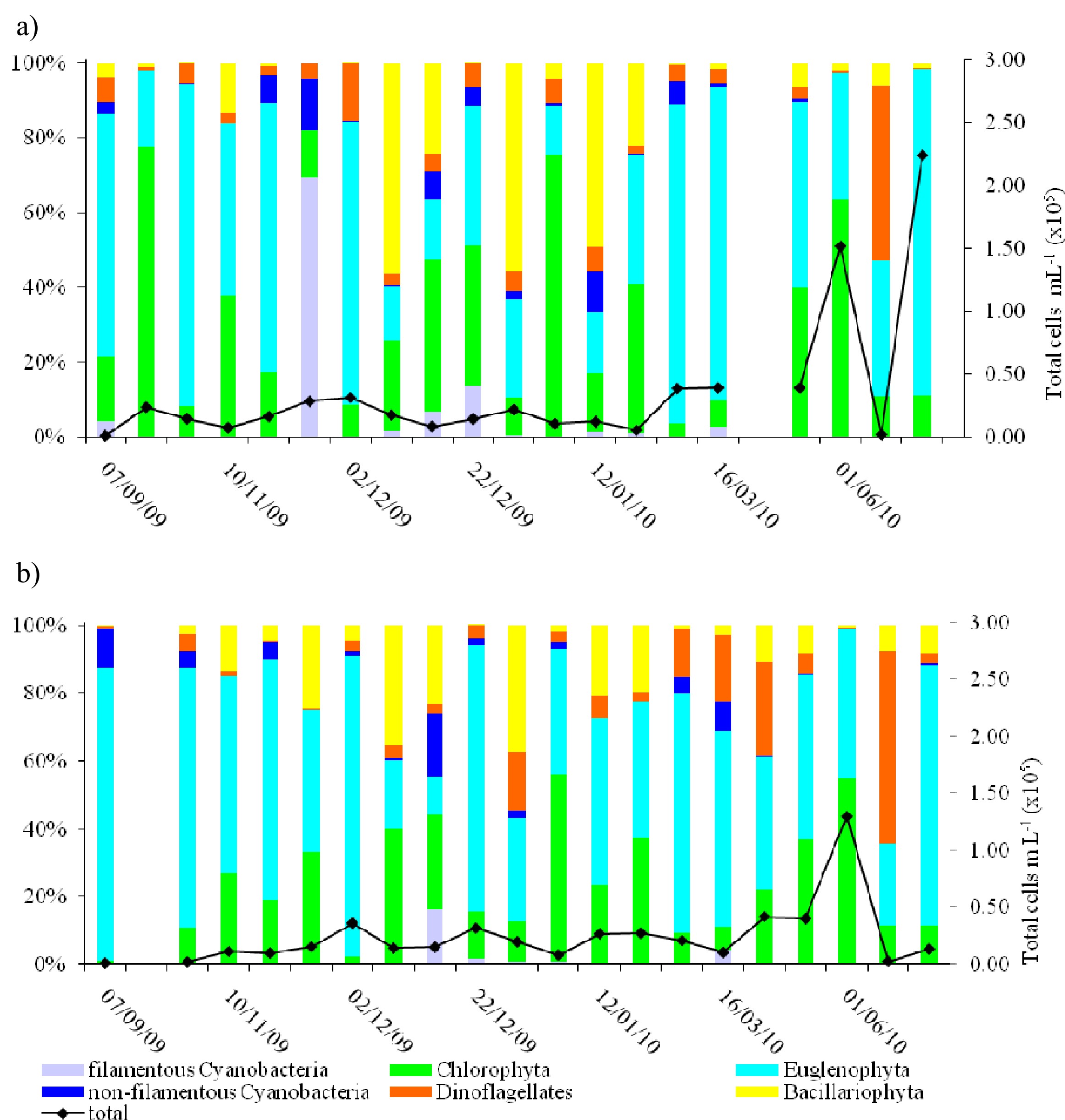

Figure 3.13 *Percentage abundance (primary axis) and total cell concentrations (secondary axis) of phytoplankton in Lake Okaro in a) the epilimnion and b) the hypolimnion, 17 August 2009 to 10 August 2010.*

Lake Tikitapu

There were two main peaks in total phytoplankton concentrations in the epilimnion of Lake Tikitapu. On 16 December 2009 concentrations were 2.64 x 10^5 cells mL^{-1} and were dominated by Euglenophyta, and on 5 January 2010 concentrations were 2.54 x 10^5 cells mL^{-1} dominated by Chlorophyta (Figure 3.14).

Total phytoplankton counts in the hypolimnion fluctuated reaching a maximum on 5 January 2010 with 1.90 x 10^5 cells mL^{-1}. Euglenophyta dominated in the epilimnion until 17 November 2009 after which domination fluctuated between Chlorophyta and Euglenophyta. A similar pattern of dominance was repeated in the hypolimnion. There was one occurrence of a cyanobacterium genus, identified as *Pseudanabaena,* in both the epilimnion and hypolimnion on 7 September 2009. Dinoflagelates were dominant in the epilimnion on 12 December 2009 and in the hypolimnion on 22 December 2009 and 27 April 2010. Bacillariophyta were present in the epilimnion and hypoliminon from 7 September 2009 to 29 June 2010 with higher counts in both the epilimnion and hypolimnion on 29 June 2010. In both the epilimnion and hypolimnion the dominant green alga was *Cosmarium* sp., the dominant euglenophyte was *Chlamydomonas* sp.. The only Bacillariophyta species present was *Synedra acus.*

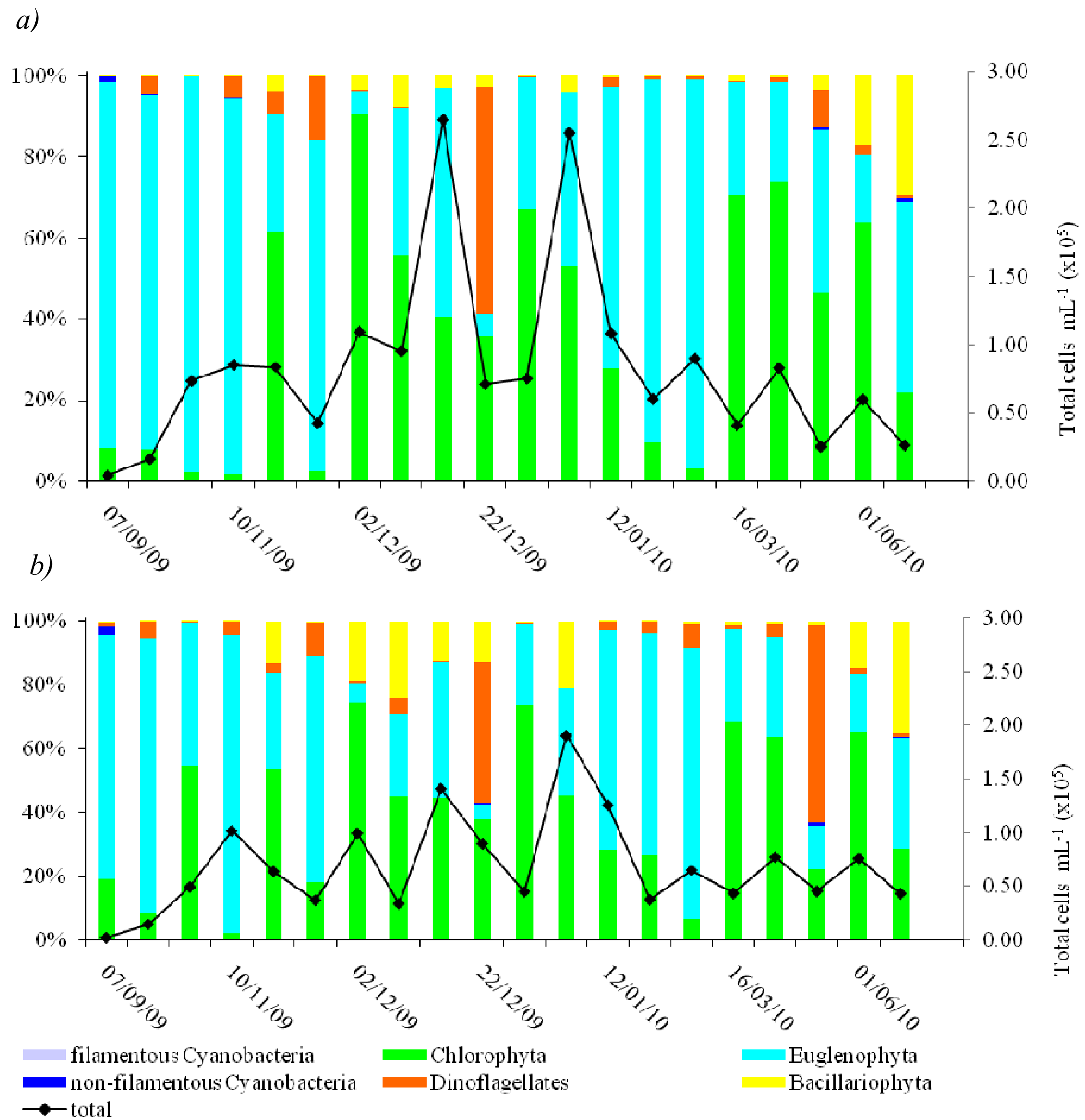

Figure 3.14 *Percentage abundance (primary axis) and total cell concentrations (secondary axis) of phytoplankton in Lake Tikitapu a) epilimnion and b) hypolimnion, 17 August 2009 to 10 August 2010.*

3.3.3 Bacterial abundance

Lake Okaro

Bacterial abundance was reasonably stable at all depths sampled (epilimnion, thermocline depths and hypolimnion) in Lake Okaro (Figure 3.15 and Fig 3.16).

There was a major peak in abundance (2 x 10^7 cells mL^{-1}) in the epilimnion on 16 February 2010 (Figure 3.15), however there was no corresponding peak in abundance at any of the thermocline depths. There was no corresponding hypolimnion sample taken on this date.

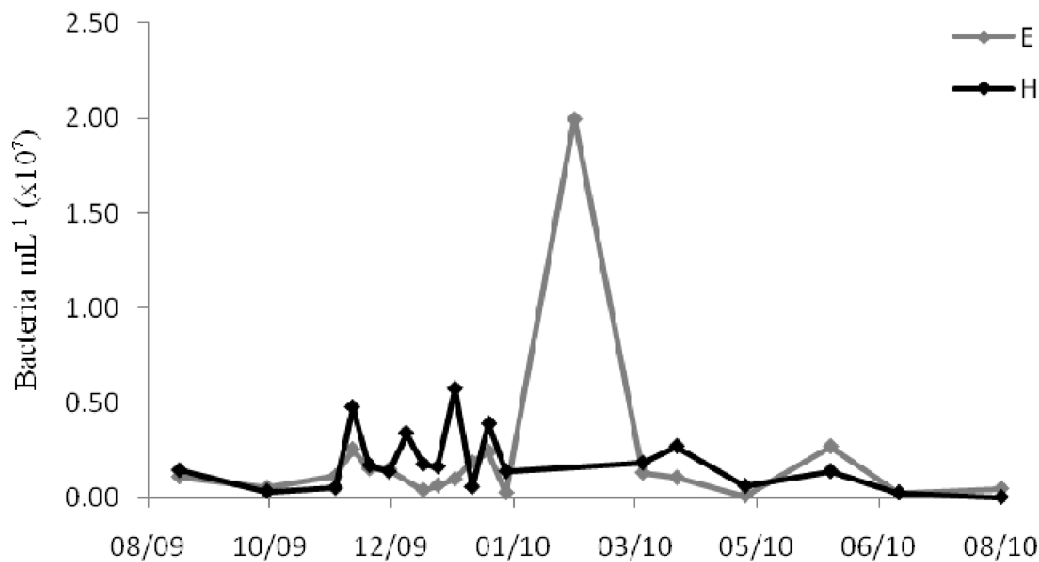

Figure 3.15 *Bacterial abundance in Lake Okaro at epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

Within the thermocline there were two major peaks in abundance, at the 40 cm below the top of the thermocline on 24 November 2009 (1.99 x 10^7 cells mL^{-1}) and at the 20 cm below the top of the thermocline on 16 March 2010 (1.63 x 10^7 cells mL^{-1}) (Figure 3.16). Abundance at all other depths sampled followed a similar trend, with counts between 9.45 x 10^2 and 5.75 x 10^6 cells mL^{-1}.

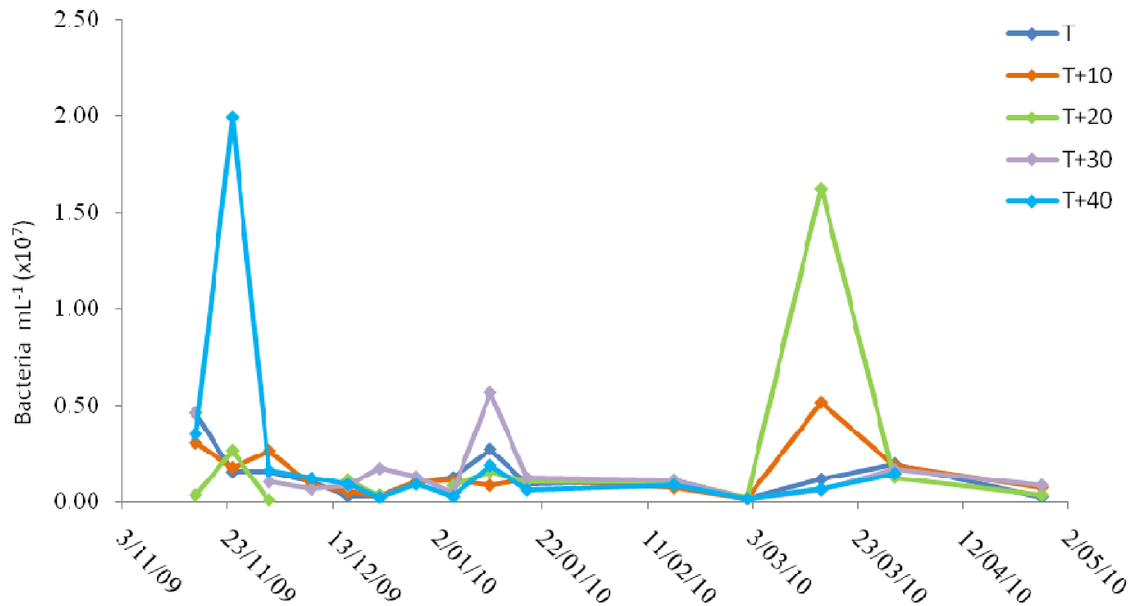

***Figure 3.16** Bacterial abundance in Lake Okaro at thermocline region (top of thermocline (T), 10 cm below (T+10), 20 cm below (T+20), 30 cm below (T+30), and 40 cm below (T+40)), 17 November 2009 to 27 April 2010.*

Lake Tikitapu

Bacterial abundances in the epilimnion and hypolimnion of Lake Tikitapu were very similar with the exception of a peak in the hypolimnion on 16 March 2010 (9.74 x 10^6 cells mL^{-1}) which was not observed in the epilimnion (Figure 3.17) but at all levels in the thermocline (Figure 3.18).

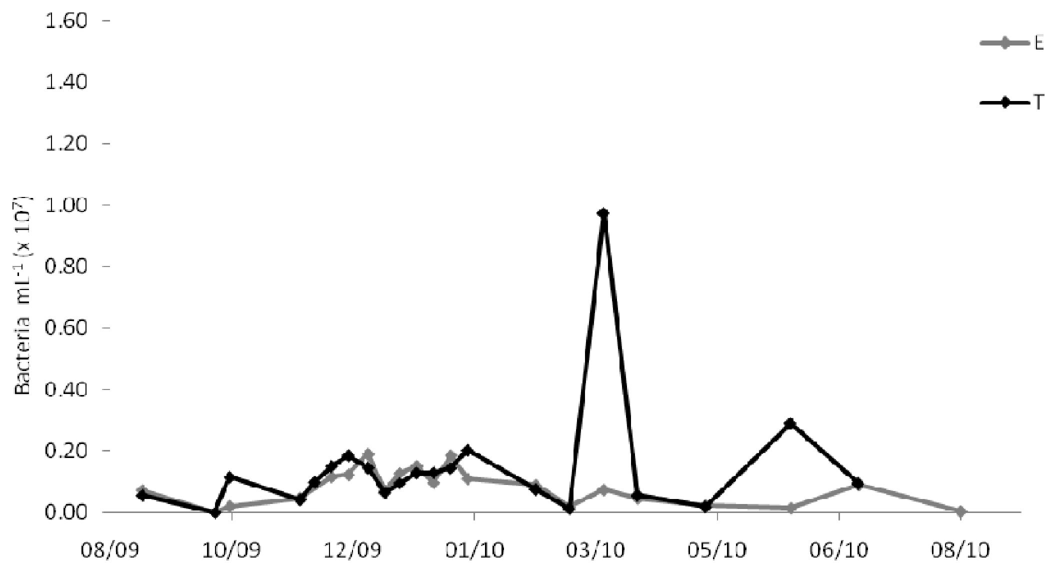

Figure 3.17 *Bacterial abundance in Lake Tikitapu in the epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

A large peak was also observed in the 20 cm below the top of the thermocline (T+20) 5 January 2010 (1.46×10^7 cells mL^{-1}) (Figure 3.18).

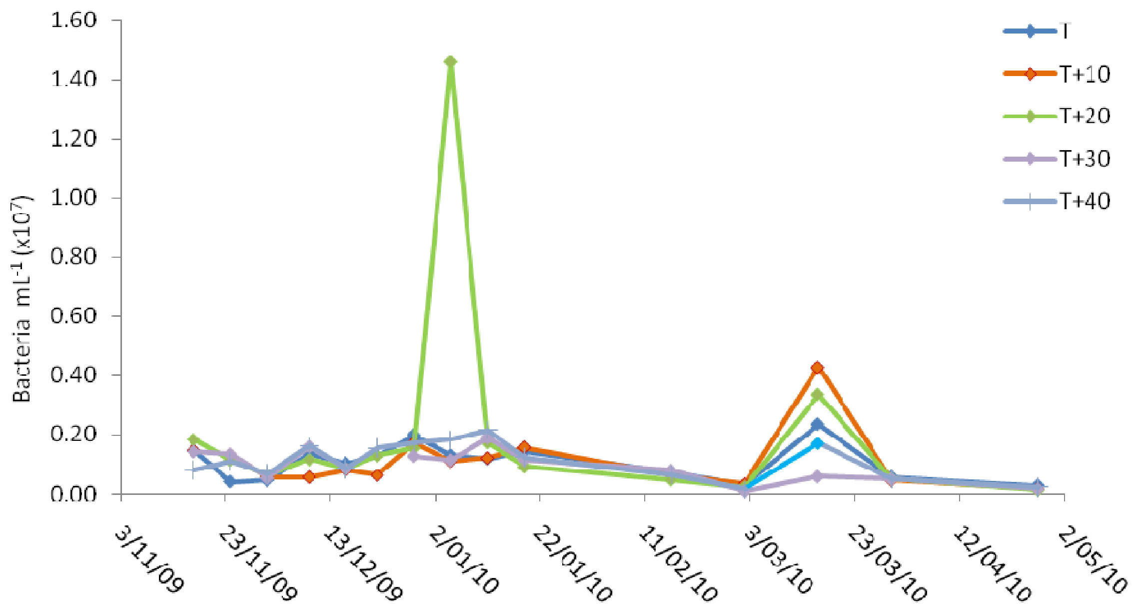

Figure 3.18 *Bacterial abundance in Lake Tikitapu in the thermocline region (the top of the thermocline (T), 10 cm below (T+10), 20 cm below (T+20), 30 cm below (T+30) and 40 cm below (T+40)), 17 November 2009 to 27 April 2010.*

3.3.4 Lysogenic bacteria

The dataset for both lakes was analysed together using Minitab 15 (Minitab Inc. USA) and returned a standard deviation for the difference between treatments of 0.095. The paired t-test gave a P value of 0.133 with 86% confidence.

Lake Okaro

There were no significant induction events (samples with increased VLP abundance) at any depth in Lake Okaro between November 2009 and March 2010 indicating that the samples analysed from this lake did not contain Lysogenic bacteria (Table 3.1).

Table 3.1 *Lysogenic fractions of bacterial populations in Lake Okaro, 24 November 2009 to 2 March 2010 (Data is converted to log 10 values). The statistical significance between paired samples untreated (control) and treated with Mitomycin C was determined by paired t-test. Significant results were P<0.133 with 86 % confidence for those differences marked by *.*

Date	Environment	Control (log 10 conversion)	Treated (log 10 conversion)	Difference (control-treated)
24/11/09	Epilimnion	7.967	7.964	0.005
	20 cm below thermocline	8.036	8.029	0.007
	Hypolimnion	7.997	7.985	0.013
16/12/09	Epilimnion	7.602	7.576	0.027
	20 cm below thermocline	7.202	7.133	0.069
	Hypolimnion	7.639	7.603	0.036
12/01/10	Epilimnion	7.711	7.715	-0.004
	20 cm below thermocline	7.850	7.855	-0.005
	Hypolimnion	8.021	8.056	-0.034
26/01/10	Epilimnion	7.354	7.332	0.021
	20 cm below thermocline	7.680	7.654	0.027
	Hypolimnion	7.488	7.474	0.014
02/03/10	Epilimnion	7.596	7.680	-0.084
	20 cm below thermocline	7.613	7.747	-0.133
	Hypolimnion	7.495	7.580	-0.085

Lake Tikitapu

There were four significant induction events in Lake Tikitapu (Table 3.1), indicating that Lysogenic bacteria were present at these times.

Table 3.2 *Lysogenic fractions of bacterial populations in Lake Tikitapu, 24 November 2009 to 2 March 2010 (Data is converted to log 10 values). The statistical significance between paired samples untreated (control) and treated with Mitomycin C was determined by paired t-test. Significant results were P<0.133 with 86 % confidence for those differences marked with *.*

Date	Environment	Control (log 10 conversion)	Treated (log 10 conversion)	Difference (control-treated)
24/11/09	Epilimnion	7.276	7.223	0.053
	20 cm below thermocline	7.837	7.833	0.003
	Hypolimnion	7.706	7.684	0.022
16/12/09	Epilimnion	7.311	7.278	0.032
	20 cm below thermocline	7.133	7.041	0.092*
	Hypolimnion	7.334	7.295	0.039
12/01/10	Epilimnion	7.315	7.304	0.011
	20 cm below thermocline	7.467	7.221	0.245*
	Hypolimnion	7.503	7.513	-0.010
26/01/10	Epilimnion	7.101	7.056	0.046
	20 cm below thermocline	7.141	7.101	0.040
	Hypolimnion	7.079	7.012	0.067*
02/03/10	Epilimnion	7.390	7.561	-0.170
	20 cm below thermocline	7.844	7.509	0.335*
	Hypolimnion	7.332	7.208	0.124*

3.3.5 Changes in functional gene abundance

The abundance of five key functional genes was assessed by quantitative PCR (QPCR). All genes were detected in all samples analysed and were classed as abundant if concentrations were above 10^{-3} genes mL^{-1}.

Lake Okaro

Quantitative PCR of functional genes in Lake Okaro showed considerable variation in abundance between different functional genes and also considerable temporal and spatial variation (Figures 3.19 and 3.20). The highest concentration of the gene *dsr*A (encoding the sulphate reductase protein; Figure 3.19a) was detected at all depths in samples taken between 24 November 2009 and 16 December 2009. The highest concentration of the gene *nos*Z (encoding the nitrous oxide reductase protein) (Figure 3.19b) (7.3 x 10^4 genes mL^{-1}) occurred in the hypolimnion on 29 December 2009, and in the epilimnion on 12 January 2010 (7.4 x 10^4 genes mL^{-1}).

The highest concentration of the gene *amoA* (encoding the ammonium oxidase protein) was less than 1.0 gene mL^{-1} throughout the study period at all depths (Figure 3.19c). The highest concentration of the gene *mcr*A (encoding the methyl coenzyme-M reductase protein (Figure 3.20a) occurred on 12 January 2010 in the 40 cm below the top of the thermocline layer (0.5 genes mL^{-1}). The highest concentration of the gene *nif*H (encoding the nitrogenase reductase protein) (Figure 3.20b) occurred in the epilimnion and hypolimnion peaking on 29 December 2009 in the hypolimnion at 3.3 x 10^5 genes mL^{-1}.

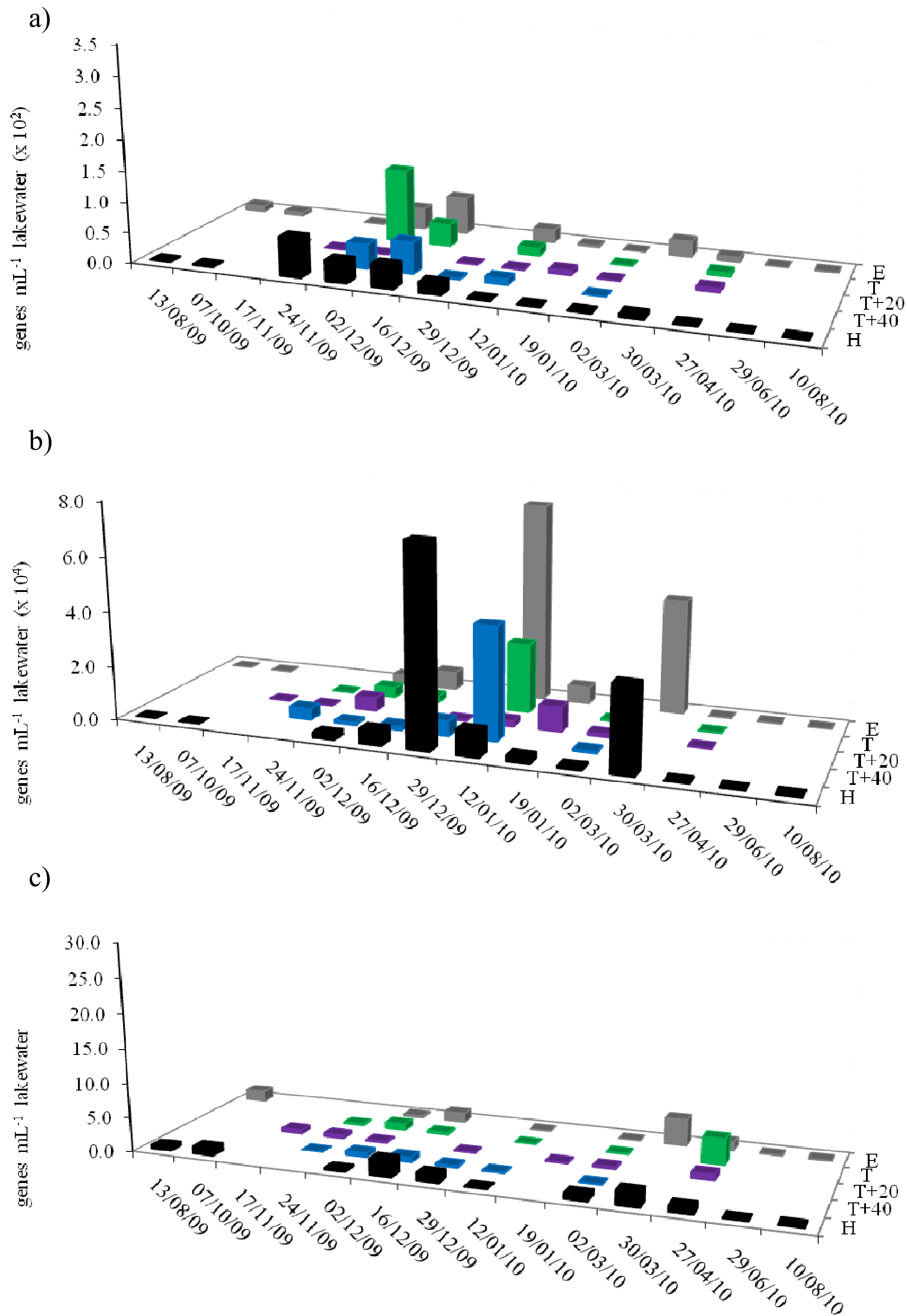

Figure 3.19 *Gene abundance in Lake Okaro epilimnion (E), thermocline region (top of thermocline (T), 20 cm below (T+20), 40 cm below (T+40)), and hypolimnion (H) for a) dsrA gene (encoding the sulphate reducing protein), b) nosZ gene (encoding nitrous oxide reductase protein), c) amoA gene (encoding ammonium oxidase protein), 17 August 2009 to 10 August 2010. Note different y-axis scales in all figures.*

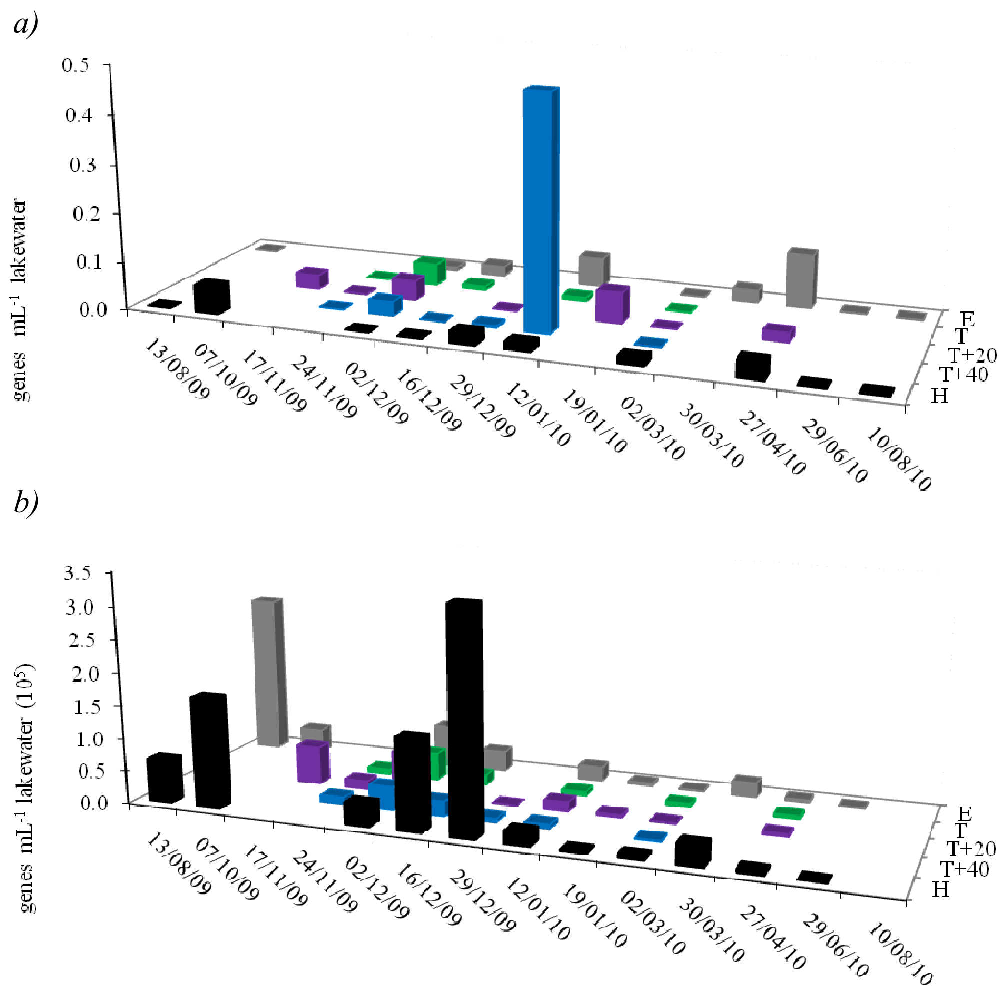

Figure 3.20 *Gene abundance in Lake Okaro epilimnion (E), thermocline region (top of thermocline (T), 20 cm below (T+20), 40 cm below (T+40)), and hypolimnion (H) a) mcrA gene (encoding methyl coenzyme-M reductase protein) and b) nifH gene (encoding nitrogenase reductase protein), 17 August 2009 to 10 August 2010. Note different y axis scales used in the two figures.*

Lake Tikitapu

Quantitative PCR of functional genes in Lake Tikitapu showed some temporal and spatial variation (Figures 3.21 and 3.22). The highest concentration of the *dsr*A gene occurred on 2 December 2009 in the top of thermocline (3.3×10^{2} genes mL^{-1}) (Figure 3.21a). The highest concentration of the *nos*Z gene (7.7×10^{4} genes mL^{-1}) (Figure 3.21b) also occurred in the top of the thermocline on 2 December 2009. The highest concentration of the *amo*A gene was in the top of the thermocline on 2 March 2010 at 2.8×10^{1} genes mL^{-1} (Figure 3.21c).

The highest concentration of the *mcr*A gene was in the epilimnion on 24 November 2009 (2.8×10^{-1} genes mL^{-1}) and on 27 April 2010 (1.79×10^{-1} genes mL^{-1}) (Figure 3.22a). There were high concentrations of the *nif*H gene in the hypolimnion, epilimnion and the top of the thermocline which peaked in the epilimnion on 2 December 2009 with 3.4×10^{5} genes mL^{-1} (Figure 3.22b).

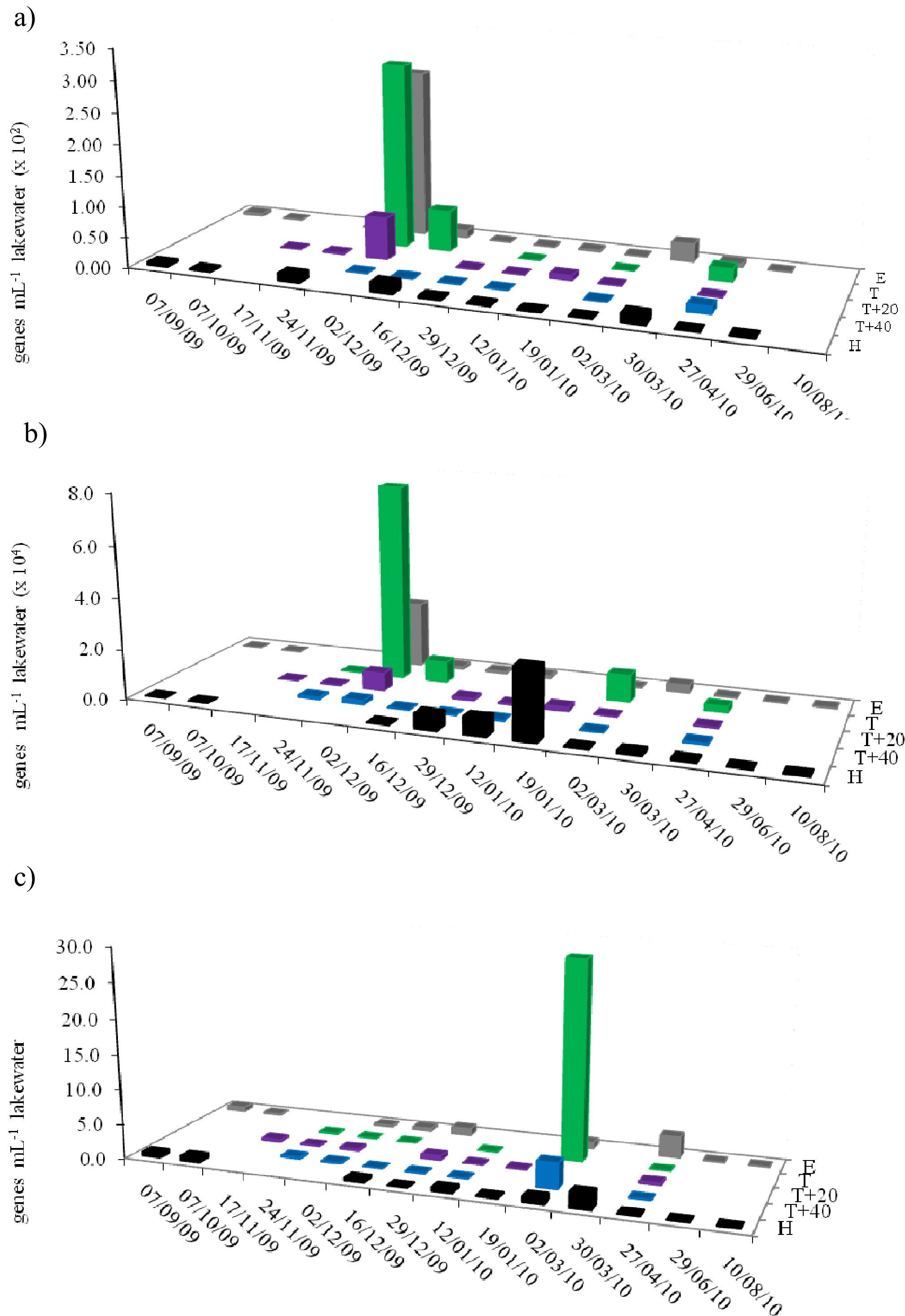

Figure 3.21 *Gene abundance in Lake Tikitapu epilimnion (E), thermocline region (top of thermocline (T), 20 cm below (T+20), 40 cm below (T+40) and hypolimnion (H) for a) dsrA gene (encoding the sulphate reducing protein), b) nosZ gene (encoding nitrous oxide reductase protein), c) amoA gene (encoding ammonium oxidase protein), 17 August 2009 to 10 August 2010. Note different y axis scales used in the all figures.*

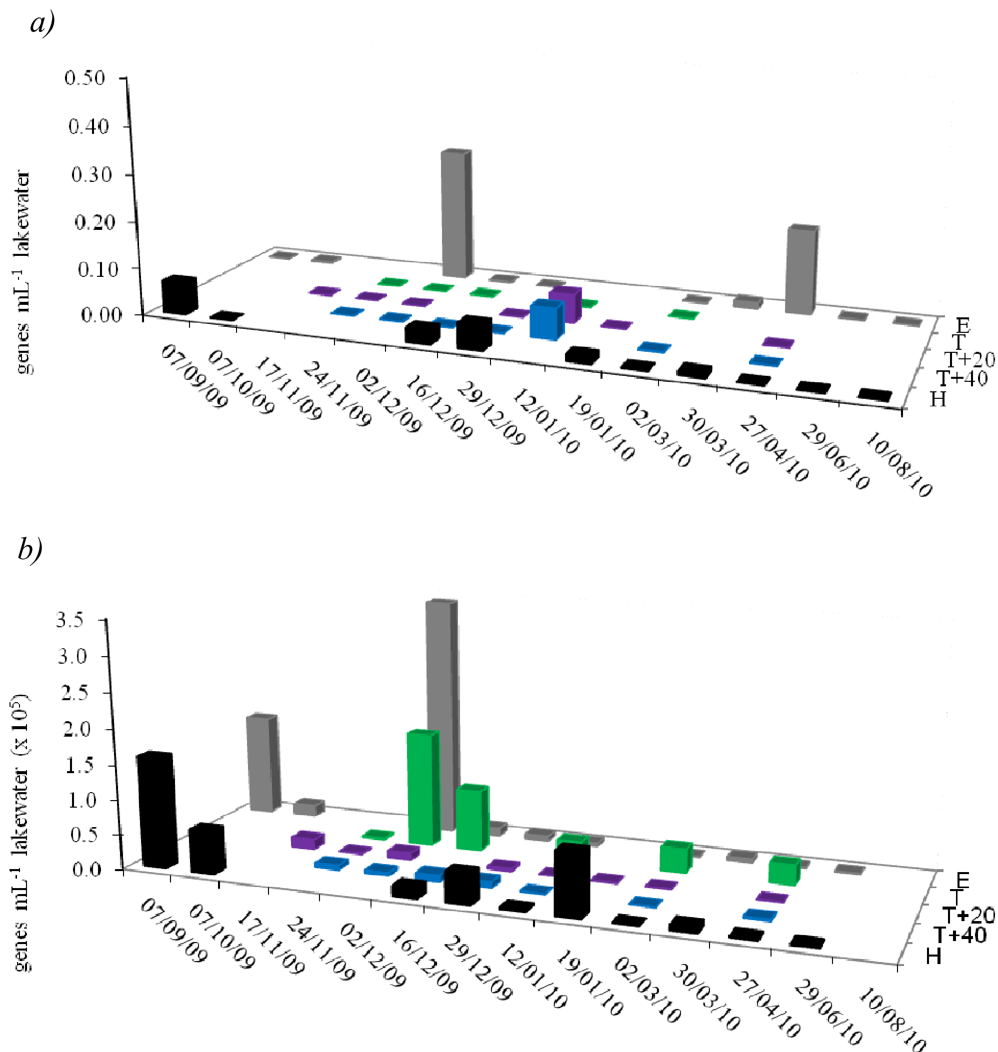

Figure 3.22 *Gene abundance in Lake Tikitapu epilimnion (E), thermocline region (top of thermocline (T), 20 cm below (T+20), 40 cm below (T+40) and hypolimnion (H) for a) mcrA gene (encoding methyl coenzyme-M reductase protein) and b) nifH gene (encoding nitrogenase reductase protein), 17 August 2009 to 10 August 2010. Note different y axis scales used in the two figures.*

3.3.6 Viral-like particle abundance

Lake Okaro

Viral-like particle (VLP) abundance was highest in the hypolimnion, with the exception of one peak in the epilimnion in November 2009 (Figure 3.23) and considerable variability at all levels sampled between 7 September 2009 and 19 January 2010. There were peaks in abundance in the hypolimnion on 10 November 2009, 9 December and 29 December 2010 (3.01×10^8, 3.63×10^8 and 2.86×10^8 VLP ml^{-1}, respectively). A simultaneous epilimnion and hypolimnion peak in VLP abundance occurred on 30 March 2010 (9.73×10^7, 1.52×10^8, respectively).

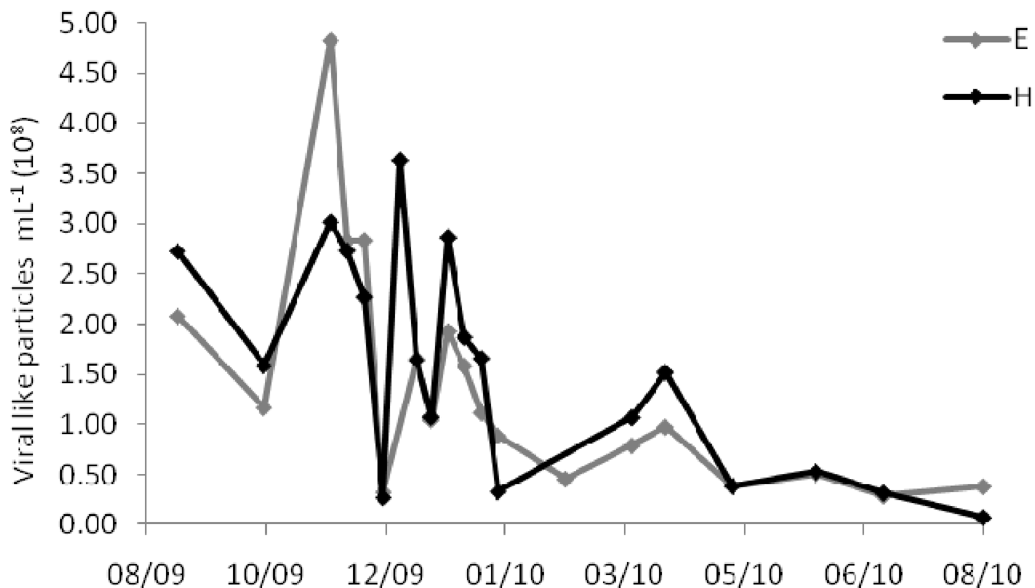

Figure 3.23 *Viral like particle (VLP) abundance in Lake Okaro at the epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

Abundance through the thermocline (Figure 3.24) was lower than in the epilimnion and hypolimnion. There was greater variability in the thermocline, particularly in early summer through all depths. The peaks at all depths in the thermocline in March 2010 reflected the peaks observed in the epilimnion and hypolimion.

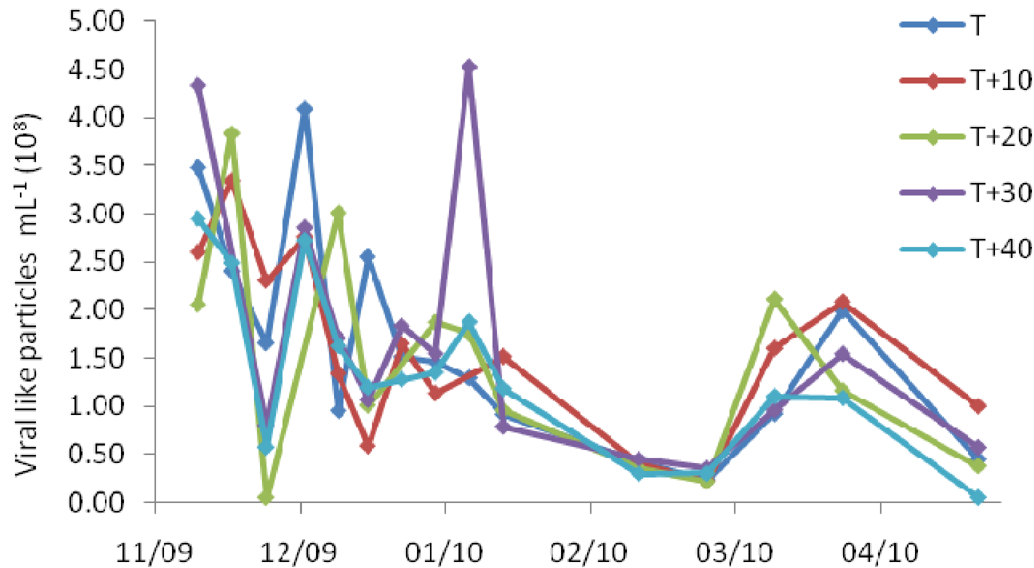

Figure 3.24 *Viral like particle (VLP) abundance in Lake Okaro at the thermocline region (top of the thermocline (T) 10 cm below (T+10), 20 cm below (T+20), 30 cm below (T+30) and 40cm below (T+40)), 17 November 2009 to 27 April 2010.*

Lake Tikitapu

Viral-like particle (VLP) abundance was greatest in the hypolimnion with peaks occurring on 2 December 2009, 19 January and 16 March 2010 (1.79×10^8, 1.57×10^8, 2.71×10^8 VLP ml^{-1}, respectively) (Figure 3.25). There was only one substantial peak in abundance in the epilimnion on 2 December 2009 (1.39×10^8 VLP ml^{-1}).

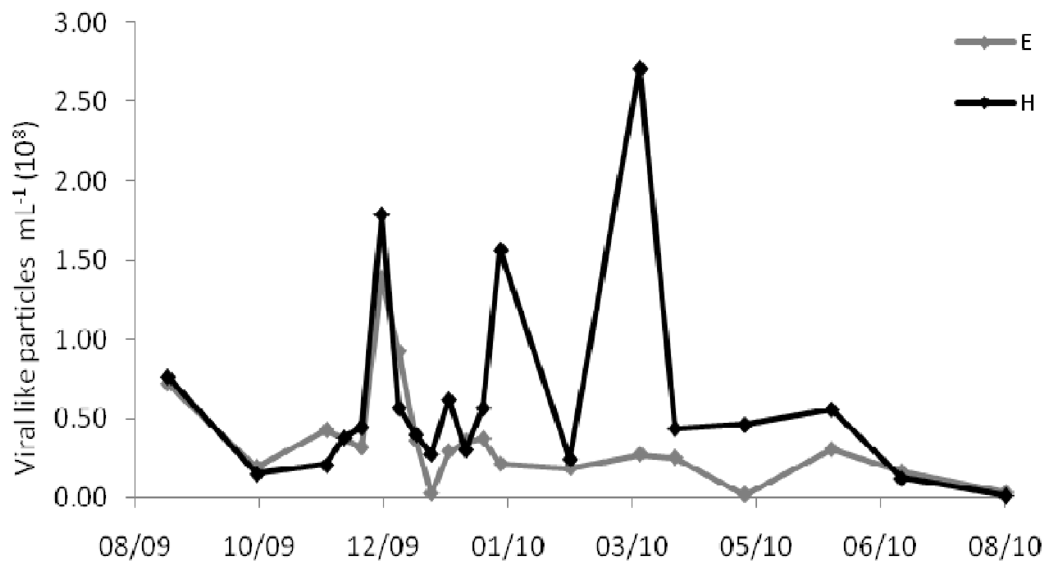

Figure 3.25 *Viral like particle (VLP) abundance in Lake Tikitapu at epilimnion (E) and hypolimnion (H), 17 August 2009 to 10 August 2010.*

Viral-like particle abundance was also lower in the thermocline (Figure 3.26) but also tended to fluctuate at all thermocline levels in the early summer months. A peak in all depths within the thermocline occurred on 16 March 2010 and coincided with a similar peak in the epilimnion and hypolimnion, with samples from 10 cm (T+10) and 20 cm below the thermocline (T+20) showing the highest abundances (1.38×10^8, 1.01×10^8 VLP ml^{-1}).

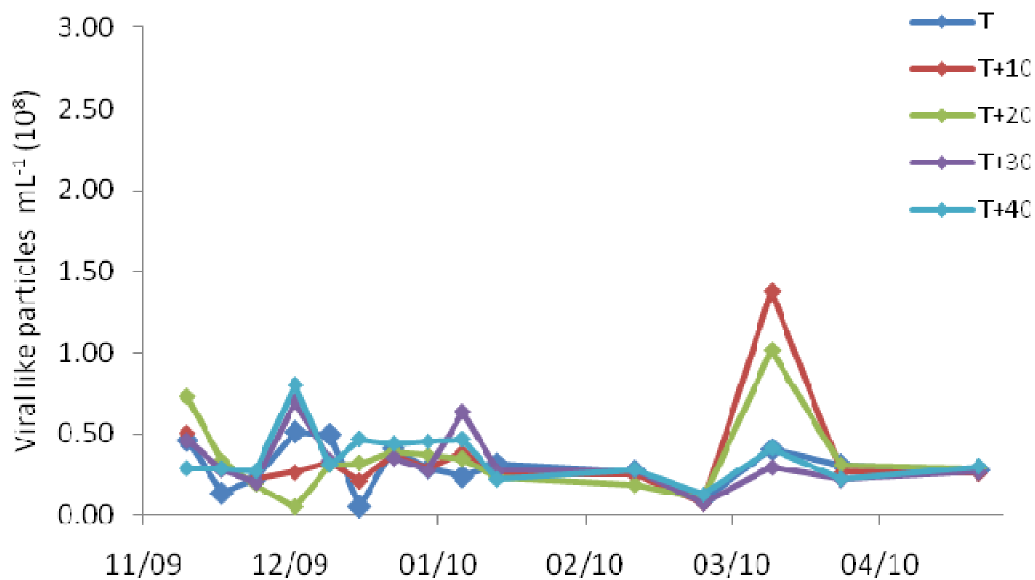

Figure 3.26 *Viral like particle (VLP) abundance in Lake Tikitapu at the thermocline region (top of the thermocline (T) 10 cm below (T+20), 20 cm below (T+20), 30 cm below (T+30) and 40cm below T+40)), 17 November 2009 to 27 April 2009.*

3.4 Statistical analysis of environmental influences on biotic variables

Principal component and classification analysis (PCA) was used to explore the relationships between the biotic and physio-chemical variables with respect to lake depth. Figures 3.27 and 3.28 shows the projection of the variables on the factor plane of the first two components for all observations in each lake in the epilimnion and hypolimnion. For all analyses there were 14 active variables and 20 active cases (Appendix III).

Lake Okaro

Eigenvalues for Lake Okaro epilimnion were 4.880 and 2.696 respectively (Figure 3.27a). Individually, component 1 explained 34.9 % of the variability in the data and component 2 explained 19.3 % of the variability in the data. Eigenvalues for Lake Okaro hypolimnion were 2.945 and 2.529 respectively (Figure 3.27b).

Individually component 1 explained 21.0 % of the variability of the data and component 2 18.07 % of the variability of the data.

In the mixed surface waters of Lake Okaro, positive correlations were observed between temperature, conductivity and oxygen saturation while VLP abundance, fluorescence and DOC concentrations showed negative correlations (Figure 3.27a). The bottom waters indicated positive correlations between algal abundance and chlorophyll-*a* levels, with negative correlations observed between oxygen concentration, NH_4-N and PO_4-P concentrations, and between oxygen saturation and conductivity (Figure 3.27b).

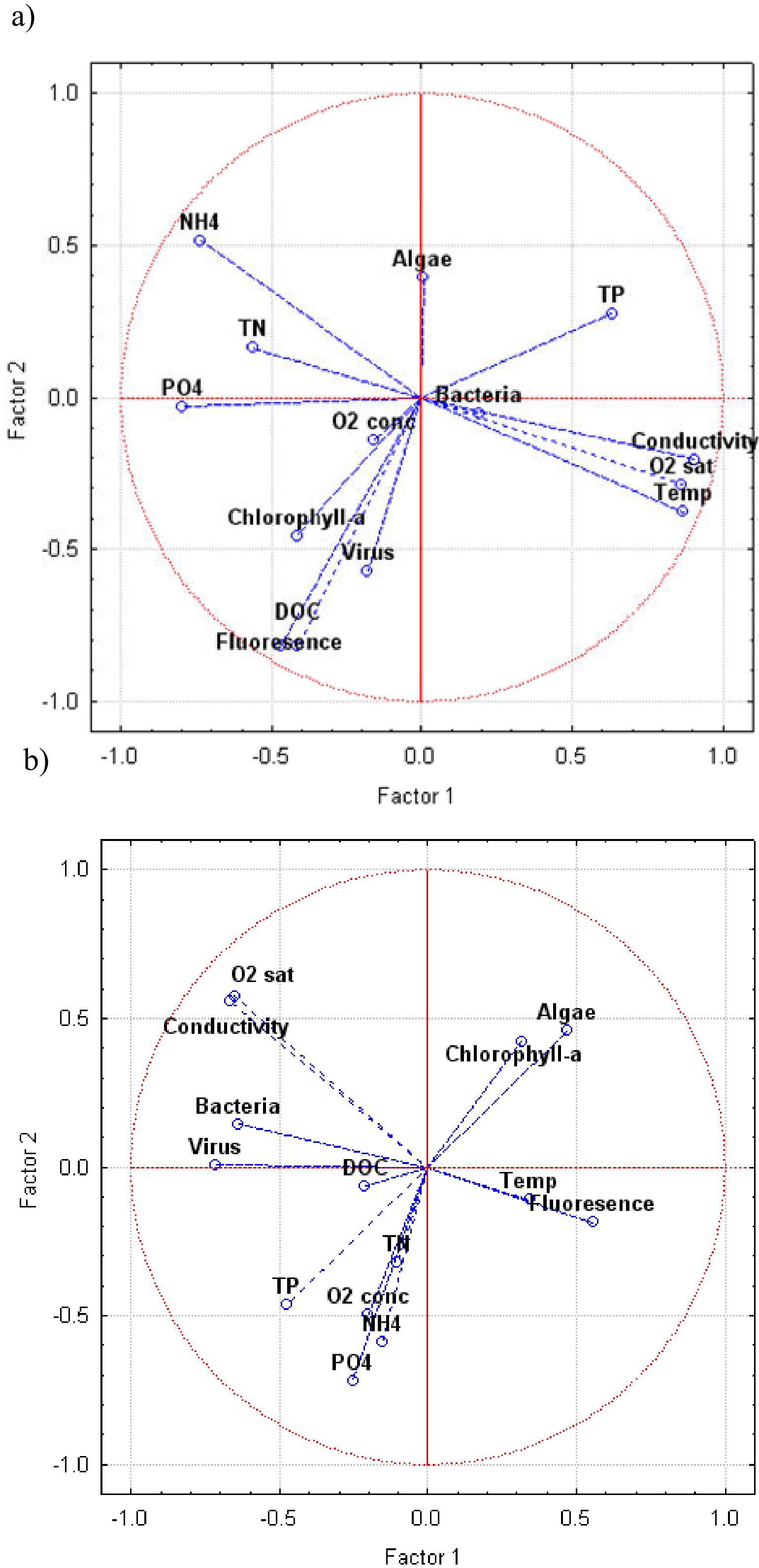

Figure 3.27 *Projection of the variables on the factor-plane (1 × 2) for biotic and physio-chemical variables in Lake Okaro for a) the epilimnion and b) the hypolimnion, for samples taken between 17 August 2009 and 10 August 2010.*

Lake Tikitapu

Eigenvalues for Lake Tikitapu epilimnion were 3.026 and 2.728 respectively (Figure 3.28a). Individually component 1 explained 21.61 % of the variability of the data and component 2 explained 19.49 % of the variability of the data. Lake Okaro hypolimnion produced eigenvalues of 3.457 and 2.608 respectively (Figure 3.28b). Individually component 1 explained 24.69 % of the variability of the data and component 2 18.63 % of the variability of the data.

Positive correlations were observed in the mixed surface layer between oxygen saturation and conductivity, and between algal abundance and temperature (Figure 3.28a). A strong negative correlation was observed between VLP and TN and NH_4-N concentrations. In the bottom waters bacterial abundance and VLP abundance was positively correlated while NH_4-N, TN and DOC were negatively correlated (Figure 3.28b).

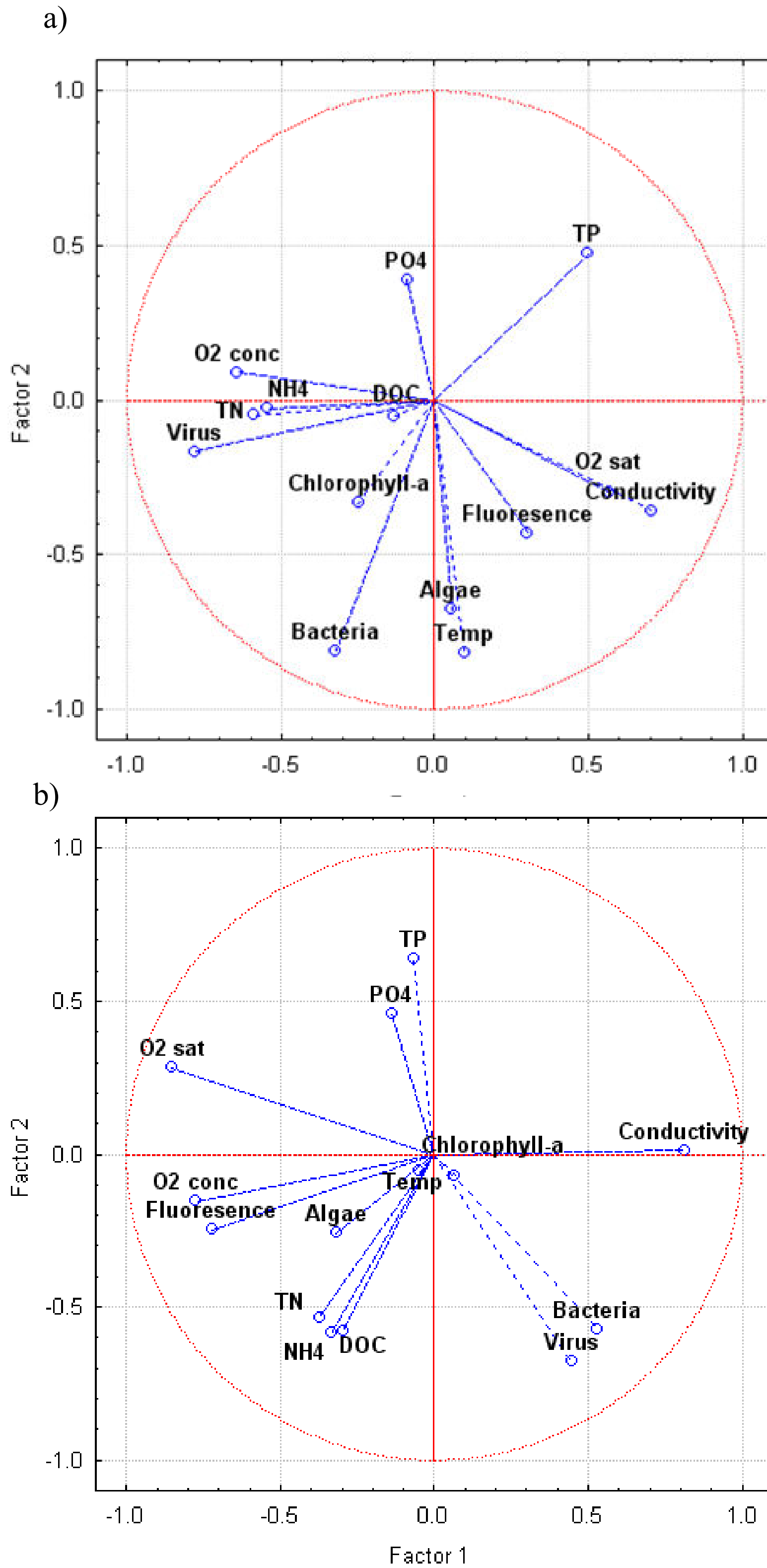

Figure 3.28 *Projection of the variables on the factor-plane (1 × 2) for biotic and physio-chemical variables in Lake Tikitapu a) epilimnion and b) hypolimnion, 17 August 2009 to 10 August 2010*

Figures 3.29 and 3.30 show the projection of the cases on the factor plane for all observations in each lake at the epilimnion and hypolimnion defined by the two most significant factors.

Lake Okaro

Eigenvalues for Lake Okaro epilimnion were 4.839 and 2.572 respectively (Figure 3.29a). Individually component 1 explained 40.33 % of the data variance and component 2 explained 21.43 % of the data variance. Eigenvalues for Lake Okaro hypolimnion were of 2.948 and 2.412 respectively (Figure 3.29b). Individually, component 1 explained 20.1 % of the data variance and component 2, 24.57 % of the data variance.

Biotic and abiotic factors showed definite groupings for seasonality with discreet clusters of factors forming for each season in both the top mixed waters (epilimnion) and the bottom waters (hypolimnion). There were some data outliers in both the hypolimnion and epilimnion for spring. The position of the winter grouping was similar for the epilimnion and hypolimnion however summer, spring and autumn all showed a shift between the hypolimnion and epilimnion.

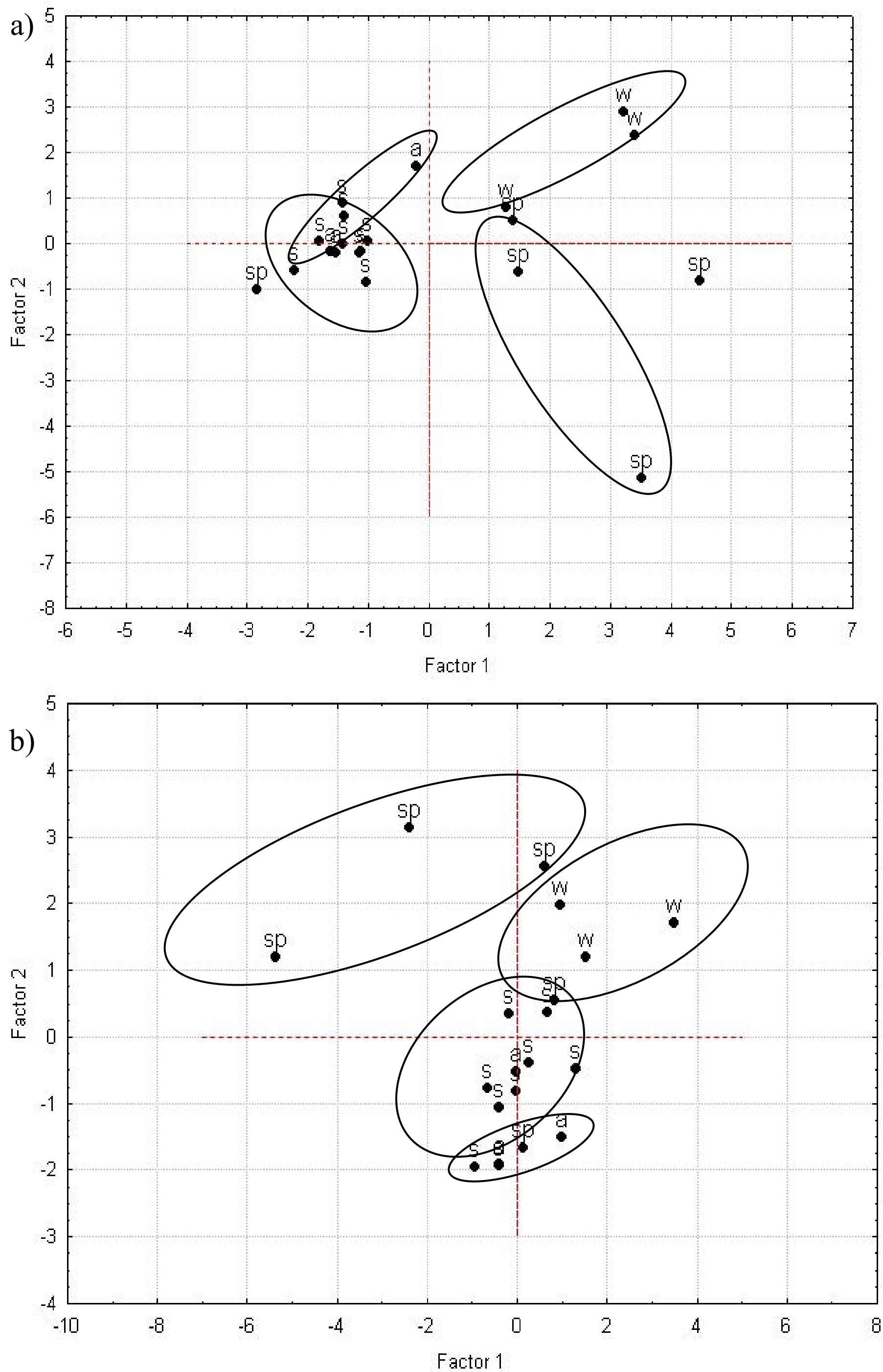

Figure 3.29 *Projection of variables for biotic and physio-chemical variables in Lake Okaro a) epilimnion and b) hypolimnion, 17 August 2009 to 10 August 2010, showing the factor coordinates for all observations defined by the two most significant factors, using the grouping variable of seasonality. Active variables: 12, active cases: 20, and factors: 2. Ellipses are drawn to better infer groupings. s=summer; sp=spring; w=winter; a=autumn.*

Lake Tikitapu

Eigenvalues for Lake Tikitapu epilimnion were 4.839 and 2.572 respectively (Figure 3.30a). Individually component 1 explained 40.33 % of the data variance and component 2 explained 21.32 % of the data variance. Eigenvalues for Lake Tikitapu hypolimnion were 2.924 and 2.785 respectively (Figure 3.30b). Individually component 1 explained 24.37 % of the data variance and component 2 explained 23.21 % of the data variance.

Biotic and abiotic factors also showed grouping around seasonality in Lake Tikitapu in both the epilimnion (Figure 3.30a) and hypolimnion (Figure 3.30b). Clustering for spring and summer was strongest in the epilimnion and hypolimnion and the grouping was similar for both with a slight shift on the axis in the hypolimnion and a larger shift for autumn. There were two outliers in the epilimnion for spring and outliers for autumn, winter and summer in the hypolimnion.

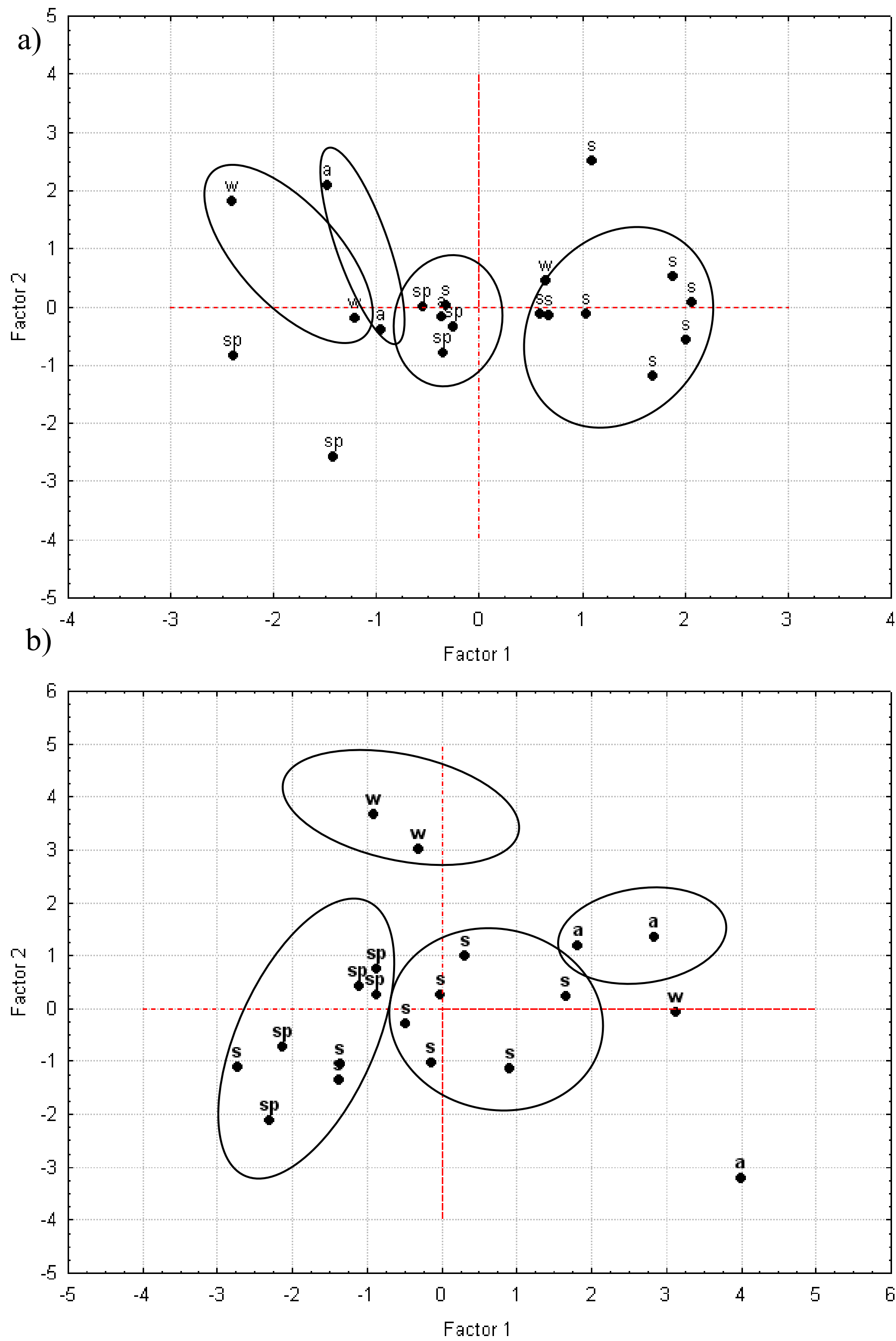

Figure 3.30 *Projection of variables for biotic and physiochemical variables in Lake Okaro a) epilimnion and b) hypolimnion, 17 August 2009 to 10 August 2010, showing the factor coordinates for all observations defined by the two most significant factors, using the grouping variable of seasonality. Active variables 12, active cases 20, and factors 2. Ellipses are drawn to better illustrate groupings. s=summer; sp=spring; w=winter; a=autumn.*

CHAPTER 4: DISCUSSION

4.1 Comparison of physical variables between lakes Okaro and Tikitapu

Lakes Okaro (classified as eutrophic) and Lake Tikitapu (classified as oligotrophic tending towards mesotrophic), are small, shallow lakes. While between lakes there was little differentiation between their respective surface and hypolimnion temperatures, there were marked differences in nutrient concentrations and physiochemical properties. Schmidt stability, or the resistance to mixing due to the potential energy inherent in stratification, was approximately two-fold higher in Lake Tikitapu compared to Lake Okaro, indicating that Lake Tikitapu had greater stratification strength and required a larger amount of energy to break down stratification. The net result of stratification is a suppression of the vertical transfer of nutrients between the top and bottom layers of a lake (Read *et al*., 2011). This results in bottom waters which are nutrient-rich and light-limited, and surface waters which are nutrient-limited and light-rich. This was the case in eutrophic Lake Okaro with total nitrogen (TN), ammonium (NH_4-N), total phosphorus (TP) and phosphate (PO_4-P) concentrations all higher in the hypolimnion than the epilimnion. The division was not as apparent in oligotrophic Lake Tikitapu which interestingly had a higher Schmidt stability. The trophic state difference between the lakes has an important bearing on nutrient differences, with strong internal loading of enriched sediments in Lake Okaro in response to prolonged anoxia. The existence of a deep chlorophyll maximum in both lakes around the thermocline depth provides a potential barrier for deeper light penetration into the bottom waters.

Stratification also provides a barrier to the transfer of carbon and oxygen between layers and one of the most apparent differences between lakes Okaro and Tikitapu is the level of oxygen saturation, with Lake Okaro becoming anoxic in the hypolimnion soon after thermal stratification had taken place but this process being more gradual in Lake Tikitapu. Prior to stratification levels of oxygen tended to be higher and relatively uniform through the water column.

Oxygen saturation in Lake Tikitapu remained between 20-100% in the hypolimnion resulting in oxic conditions which allowed for aerobic biological activity to continue.

4.2 Comparison of chemical variables between lakes Okaro and Tikitapu

Total phosphorus (TP) levels are an indicator of nutrient status in lakes. Levels of TP in Lake Okaro reinforce its classification as eutrophic with consistently high levels of TP particularly in the hypolimnion. During stratification phosphorus is rapidly exhausted in the epilimnion and accumulates in the hypolimnion, due to both sedimentation from surface waters and releases from the bottom sediments. Hypolimnion TP concentrations increased during stratification in Lake Okaro coinciding with anoxic conditions which may have triggered the release of phosphorus bound in the sediment. Subsequent utilisation and assimilation of TP by microorganisms reduced TP levels prior to lake mixing when concentrations at the epilimnion and hypolimnion converged to pre-stratification concentrations. Lake Tikitapu also had higher TP levels in the hypolimnion than the epilimnion, however a peak in epilimnion concentration in October 2009 is unexplained as there was not a sustained heavy rainfall incident (Appendix III) or any mixing events. There was considerable rainfall recorded in Lake Tikitapu on 13-16 May 2010 and again on 25 May 2010 which may have contributed to increased runoff into the lake and the subsequent increase in hypolimnion TP in May 2010.

Bacteria can assimilate large amounts of PO_4-P under oxic conditions and in effect can compete with phytoplankton for PO_4-P. Phosphate levels in Lake Okaro were higher than in Lake Tikitapu and extremely variable over the entire sample period. Under anoxic conditions PO_4-P can be released from the sediment and the variability of PO_4-P in Lake Okaro may have been due to releases from the hypolimnion Variability of PO_4-P through all depths indicates that there may have been a number of different processes acting differentially through the water column to affect concentrations.

By comparison levels of PO_4-P were relatively stable in Lake Tikitapu apart from a slight increase at lake turnover which may be due to redistribution of PO_4-P through the lake following mixing.

Total nitrogen (TN) was higher in Lake Okaro than in Lake Tikitapu. Levels in Lake Okaro were also variable, especially in summer. A peak in the epilimnion in December may have been the result of nitrogen fixation associated with a cyanobacterial bloom at the same time. Stable TN levels with little difference between the bottom and top layers in Lake Tikitapu reflect a stable system more typical of oligotrophic status.

Biological nitrogen fixation by bacteria and some cyanobacteria produces ammonium (NH_4-N) which is assimilated by cells in the presence of iron and molybdenum. Subsequent cell lysis can boost nitrogen inputs to the water column than can then ultimately become available for subsequent production. High levels of NH_4-N in the hypolimnion of Lake Okaro may be explained by the lack of conversion of NH_4-N to nitrous oxide (NOx), as under anoxic conditions this conversion is halted and NH_4-N levels then tend to build up in the hypolimnion. Under these anoxic conditions bacteria are also able to utilise NO_3-N or nitrite (NO_2-N) in place of oxygen for oxidation of organic compounds, i.e., denitrification. All nitrogen species in Lake Okaro show convergence with depth, as expected with lake mixing.

Increases in NH_4-N in the surface waters of Lake Tikitapu in December 2009 and January 2010 and again in May and June 2010 appear to coincide with major recreational events (triathlons in December, a rowing regatta in January and jet boating, waterskiing and Waka Ama in March). The increased boating and human activity could introduce an external nutrient load and also increased turbulence resulting in sediment disturbance (Garrad *et al.,* 1987; Liddle *et al.,* 1980).

4.3 Comparison of phytoplankton biomass and assemblages between Lakes Okaro and Tikitapu

The existence of a deep chlorophyll maximum (DCM) is reliant on the depth of light penetration but the pre-requisites are seasonal thermal stratification, the presence of a metalimnion and a euphotic depth below the base of the epilimnion (Hamilton *et al.*, 2010).

A DCM was observed in both Lake Tikitapu and Lake Okaro. The finding of a DCM in Lake Okaro is significant as the first observation of a DCM in this lake was also in 2009 (Simmonds, B. pers. comm.). While the peaks of fluorescence in the DCM were much lower in Lake Tikitapu than Lake Okaro they occurred around the same period in summer, between January and February 2010. Hamilton *et al.* (2010) found that in lakes with high transparency and low nutrient concentrations the fluorescence maximum is pushed into the lower part of the metalimnion almost extending into the hypolimnion. This has been observed for other deep lakes of the Taupo Volcanic Zone (e.g., Lakes Taupo and Rotoma; Hamilton *et al.,* 2009). Changes in water clarity can have a marked effect on the DCM, with potential to disrupt the DCM. In Lake Tikitapu there has seen a decrease in Secchi disk levels (Scholes, 2010) and this potentially may put the existence of the DCM in this lake at risk. If the DCM ceases to form it is likely that there will be higher surface phytoplankton populations.

Cyanobacterial blooms have dominated in Lake Okaro in the past (Environment Bay of Plenty, 2000; Ozkundakci *et al.,* 2010), however, apart from a brief period in which there was a surface bloom in December 2009, the dominant phytoplankton recorded during this study were chlorophytes and euglenophytes. Increased water clarity resulting from remedial actions in Lake Okaro (Tanner *et al.,* 2005; Paul *et al.,* 2008; Ozkundakci *et al.,* 2010) may have led to the formation of a DCM and subsequent decrease in dominance by buoyant cyanobacteria responsible for blooms.

Phytoplankton biomass based on chlorophyll fluorescence in Lake Okaro, was stable until mixing in June 2010 when total phytoplankton numbers increased due to increases in Chlorophyte and Euglenophyte abundance.

Paerl *et al.* (2001) found that phytoplankton blooms are often most likely when there is high nutrient loading accompanied by a water residence time long enough to support adequate growth and high productivity. The Lake Okaro catchment exceeded 50 mm total daily rainfall on one occasion in January and again in May 2010, providing little surface runoff into the lake during much of the remainder of the stratified period.

Euglenophytes and chlorophytes also dominated in Lake Tikitapu. The commonality with Lake Okaro of the species dominating the phytoplankton assemblage, may reflect a decline in water quality in Lake Tikitapu. Historical nutrient data from Bay of Plenty Regional Council monitoring (Appendix III) indicates that there is a slight upward trend in TP concentrations in the surface waters of Lake Tikitapu and a slight downward trend in TN concentrations with several spikes in nitrogen observed in 2001 and 2003. Total phosphorus has shown a marked decrease in both the surface and bottom waters of Lake Okaro (Appendix III) while nitrogen has remained stable.

Phytoplankton dominance can also be affected by mineral availability, with diatomaceous phytoplankton dominating only if there is sufficient silica present. Lake Tikitapu has been distinctive due to the complete lake of diatoms in the past (Ryan, 2006; Environment Bay of Plenty, 2000). In this study, the diatom *Synedra acus* was observed throughout the year in both the epilimnion and hypolimnion with a maximum of c. 6900 cells mL^{-1}. Silica availability can also be related to phosphorus availability with high phosphorus levels creating an increased demand on silica resources (Schelske *et al.*, 1971) and low silica:nitrogen levels inhibiting diatom growth (Environment Bay of Plenty, 2000). It is possible that in the past the ratio of silica:nitrogen or silica:phosphorus has had a regulating effect on silica availability and the appearance of diatoms may be an indicator of increased nutrient input from select high-loading events. Silica concentrations were not measured in this study so there was no determination of changes in silica levels.

In Lake Tikitapu there has been increased total phytoplankton abundance in December 2009 and January 2010 which may also have been stimulated with the introduction of additional nutrient input from the large number of people using the lake for direct contact recreation.

Statistical analysis showed that there was a positive correlation between temperature, phytoplankton (either measured via cell counts, chlorophyll-a or fluorescence) in both lakes. Ryan *et al.* (2006) found that in summer, lake mixing gave a more accurate prediction of phytoplankton composition than trophic state or light regime, but that the prediction could not be applied in winter months. Havens (2008) found cyanobacterial blooms increased with temperature and Tijdens *et al.* (2008) also observed the largest shifts in phytoplankton community composition in summer.

4.4 Comparison of viral-like particle and bacterial abundance between lakes Okaro and Tikitapu

Microbial and predator-prey models predict a repetitive cycle in which an increase in prey populations leads to an increase in predator populations that in turn decreases the prey population leading to its own decline (Rodriguez-Brito *et al.,* 2010). A popular theory is known as 'Kill the Winner', which predicts that viruses will rapidly reduce the population of the most abundant microbial species, preventing the best microbial competitors from building up a high biomass. Increases in VLP abundance in Lake Okaro in December 2009 and January 2010 then again in April 2010 and in Lake Tikitapu in December 2009, January 2010 and March 2010 were, however, coupled with only small variations in bacterial abundance. The low correlation coefficient values between bacterial and VLP abundance was further confirmed by the statistical analysis that showed that the hypolimnion of Lake Tikitapu was the only depth where a positive correlation was found between bacteria and viruses. It may be plausible that this was the only environment where 'normal' bacterial viral interactions were taking place without any external stressors from alterations to trophic state.

It is possible that viral lysis was regulating the proliferation of bacterial biomass. With a decrease in the number of bacteria available for VLP infection there could be expected to be a subsequent decline in VLP numbers.

However the lysis of bacteria results in more available DOM for further growth and followed by an increase in bacteria and, depending on VLP numbers and coincidental contacts, viral infection and a continuation of the cycle. There will be occasions when both VLPs and bacteria are in low numbers. This occurred in the epilimnion in both lakes in the cooler months following summer. The epilimnion in both lakes also showed periods of high VLP concentrations and low bacterial concentrations followed by low VLP concentrations and high bacterial concentrations. In the hypolimnion of Lake Okaro both VLP and bacterial numbers tracked each other but in Lake Tikitapu VLP numbers were higher than bacterial numbers until after summer when they began to track each other.

The viral loop allows for the flow and assimilation of organic carbon from lysed bacterial cells into the biomass of an uninfected community (Sawstrom *et al.,* 2006). However, Lymer *et al*. (2008b) found that the relationship between the bacterial and viral community was not strong and that viral host dynamics were not as tightly host-specific or that correlations between the two were time-shifted. Increases and decreases in bacteria, VLP and carbon in both study lakes did not coincide with each other supporting the theory that there is a potential time lag associated with the changes in abundance of the flora and carbon levels and biotic factors.

Viral-like particle abundance in both study lakes exceeded that of bacterial abundance throughout the sampling period, an observation Sawstrom *et al.* (2006) also found in ultra-oligotrophic lakes. Tijdens *et al.* (2008b) and Sawstrom *et al.* (2006) found that viral lysis was the main cause of bacterial mortality in winter and the viral loop was less important in summer in oligotrophic lakes. However in my study found there was more variation, including larger numbers of bacteria and viruses in summer than winter.

The absence of lysogenic bacterial populations in Lake Okaro and only four significant recorded instances of lysogenic bacteria in Lake Tikitapu was unexpected as Tijdens *et al.,* (2008) had identified that viral lysis removed up to 100% of the potential heterotrophic bacterial production and Bettarel *et al.* (2004) identified viral lysis as dominating over protistan grazing. Sawstrom *et al.* (2006) found that lysogenic bacteria were uncommon in ultra-oligotrophic lakes.

Sawstrom concluded that this could in partly be due to the largely unknown effectiveness of mitomycin C as an induction agent in freshwater systems (Sawstrom *et al.,* 2006) and thus induction may not have been complete for the bacterial population. Sawstrom *et al.* (2006) also found that the lytic cycle predominated in cold ultra-oligotrophic lakes and that the greatest viral impact occurs in lakes of low productivity. Given that both the study lakes had high VLP and bacterial numbers with periodic fluctuations in abundance it is possible that lytic infection was the main method of viral infection.

4.4 Functional gene dynamics

Conflicting data on whether bacterial community composition and diversity are related to a community's functional traits has resulted in cross-study comparisons of freshwater lake bacterial phylotypes (Newton *et al*., 2011). Such studies have shown that bacterial functional group dynamics are complex but seem to follow patterns in relation to nutrient levels. Temporal and spatial trends changes in functional gene abundance in this study were also complex.

The presence of high concentrations of the *nif*H gene (encoding the nitrogenise reductase protein) correlated with a period of high cyanobacteria abundance in samples from the surface waters in Lake Okaro. The dominant species at the time of high *nif*H was A*nabaena* sp. which is a nitrogen-fixing species (Paerl *et al.,* 2001). Likewise the presence of *Pseudanabaena* in Lake Tikitapu occurred when there were high concentrations of *nif*H. This species does not have heterocytes, however recent sequencing of some species from this genera has shown the presence of *nif*H genes (Singh *et al*., 1987) and N-fixation by means other than heterocytes appears likely.

The *dsr*A gene (encoding the sulphate reductase protein) and *mcr*A gene (encoding the methyl coenzyme M reductase protein) take part in the decomposition of organic matter. The *dsr*A gene was particularly abundant in the eutrophic Lake Okaro. The high amount of dissolved organic matter appeared to provide a source for bacterial activity through all depths in the lake. In contrast this gene was only detected in low abundance in Lake Tikitapu possibly due to low DOC concentrations.

The organically-rich sediment of Lake Okaro and its highly anoxic conditions in the hypolimnion likely provide an ideal environment for methanogenic activity. In the anoxic zone methane is produced from the anaerobic decomposition of organic acids through bacterial decomposition. However there was only one occasion when high concentrations of *mrcA* were detected in the hypolimnion; in January 2010. Between September 2009 and February 2010 oxygen saturation in the hypolimnion of Lake Okaro was $\leq 10\%$. As part of the remedial action taken on Lake Okaro a sediment cap using an aluminium-modified zeolite compound (Özkundakci *et al.*, 2010) was used on two occasions in 2007 and 2009 and it is also possible that this capping has inhibited the release and availability of DOC. Some methanogens do not die instantaneously by oxygen but are able sustain periods of oxygen stress which may explain the positive detection of *mrc*A genes in the surface of Lake Okaro (Liu *et al.,* 2008). It is plausible that there was movement of *mrc*A positive bacteria into the surface layer. *Mrc*A positive bacteria were also detected in the surface waters of Lake Tikitapu and in the thermocline, an area where activity may be enhanced by rapid depth transitions at oxic-anoxic interfaces where DOM may be plentiful.

Nitrification activity is detected by the presence of the *amo*A gene (encoding the ammonium oxidising protein). The activity of *amo*A was high in the hypolimnion of Lake Okaro and may be related to the high levels of NH_4-N in the hypolimnion of this lake. A similar pattern was observed in Lake Tikitapu in March and April 2010 when there were elevated NH_4-N concentrations at all sampled depths in the lake.

Denitrification as detected by the presence of the *nos*Z gene (encoding for the nitrous oxide reductase protein) occurred at all depths sampled but only in December 2009 in Lake Okaro. Denitrification is tightly coupled with loss of oxygen as it is the first electron acceptor that is reduced following loss of oxygen.

At the same time Lake Tikitapu had only low levels of *nos*Z gene detected, however, concentrations increased in December 2009 in the hypolimnion coinciding with a decrease in NO_3-N, indicating possible conversion to elemental nitrogen. Lake Tikitapu was oxygen-depleted in the hypolimnion, providing favourable conditions for denitrification to take place.

One caveat with these data is that DNA was used in the PCR analysis which can only be used to show the presence of bacteria with particular functional groups. It does not indicate that the bacteria identified were utilising this capability. The use of RNA would give a more accurate indication of the capability. Indeed inconsistency between results has also been linked to differences between DNA and RNA-based analysis (Koskinen, et al., 2010; Newton, et al., 2011) with PCR based analysis using DNA distorting community structure by underestimating or overestimating certain groups.

CONCLUSIONS

The current trophic state of lakes Okaro (eutrophic) and Tikitapu (oligotrophic) were confirmed by the physiochemical and biological data collated in this study. In Lake Okaro there was rapid oxygen depletion and loss in the hypolimnion in contrast with a more sustained period when the hypolimnion was oxic in the hypolimnion of Lake Tikitapu, despite its greater stratification strength indicated by the Schmidt stability value. The formation of a DCM is a recent phenomenon in Lake Okaro and an indication that water clarity is improving and there is now light penetration into the metalimnion. Coupled with this was the lack of sustained cyanobacterial blooms on the surface of the lake which have been previously regular occurrence since the late 1970's.

Relatively high TN and TP levels in Lake Tikitapu coupled with an upward trend in TP observed from Bay of Plenty Regional Council monitoring data over the past c. 10 years, is cause for concern. The trophic state of the lake may be changing which is likely to cause a flow-on effect with increased phytoplankton abundance and changes in species composition. In this study moderate densities of diatoms were identified for the first time in Lake Tikitapu and this may be an indicator for phytoplankton changes associated with changing levels of nitrogen and phosphorus.

Differences were observed in abundances of microbial assemblages between the two lakes with higher abundances observed in the more eutrophic Lake Okaro. This is most likely due to a greater organic load in this lake. Seasonal differences in abundance, diversity and function of the microbial assemblage within were also observed. The highest abundances and variation tended to occur in summer in both lakes with changes linked to physical events i.e. stratification and mixing. Seasonal differences in the supply of substrates, and the related differences in oxic state may ultimately affect phytoplankton and microbial abundance, distribution and community structure.

The lack of correlation between VLP and bacterial abundance was unexpected, but similar results have been obtained for other studies in freshwater systems. A possible lack of host specificity for infection and disparity between host abundance, carbon availability and biotic factors confounds the VLP and bacterial relationship. The data from this study did not provide any information on viral infection and this could be an area for future study. Very few viral genomes have been identified and the expansion of databases for these organisms could assist with identifying those viruses which are infecting freshwater bacteria, their method of infection, and also those viruses which may be alien to these environments.

The low abundance of *mcr*A genes in eutrophic Lake Okaro, particularly in the hypolimnion, was unexpected as the anoxic nature of the water should have provided the ideal environment for release of nutrients from the sediment and subsequent decomposition of organic acids. The low level of abundance of *nos*Z genes in both lakes was also interesting as denitrification should have been one of the processes supported in both lakes.

The thermocline gradient was an area of great variability in chemical composition and consequently supported changes in microorganism abundance and community structure. This gradient is still poorly understood and would benefit from further investigation using highly resolved depth monitoring.

While there has been some work undertaken to identify the bacterial component present in freshwater systems, the majority of the work has concentrated on marine systems. Thus further investigation using powerful molecular technology such as 454 Pyrosequencing to characterise the full microbial diversity would provide valuable data for comparisons between trophic state and bacteria, and also assist in the understanding of the dynamics bacteria in relation to changes in substrate availability and oxygen status at the thermocline.

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APPENDIX I: RECENT SCIENTIFIC PAPER ACCEPTED FOR PUBLICATION CONTAINING METHODS USED IN THIS THESIS

The following manuscript has been accepted for publication in Water Science and Technology Volume 64(4) August 2011, pages 999-1008. It contains the development of methods used in this study.

Application of flow cytometry for examining phytoplankton succession in two eutrophic lakes

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ABSTRACT

Flow cytometry has potential as a rapid assessment technique to evaluate phytoplankton biomass and properties. It facilitates for multi-parameter analysis of individual cells on the basis of light scattering effects induced from cellular constituents, as well as auto-fluorescence. Flow cytometry has been widely used in oceanography to quantify and sort heterogeneous algal populations and more recently in the development of bioassays for marine and freshwater algae using toxicity tests based on growth inhibition. Fluorescence emission characteristics may be especially useful in classifying cyanobacteria as they contain phycoerythrin which emits light predominantly in the 550-600 nm waveband, chlorophyll (650-700 nm emission) and allophycocyanin (660 nm emission). The objective of our study was to assess the utility of flow cytometric analysis for the rapid identification and sorting of freshwater algae and cyanobacteria species, with particular emphasis on two lakes in the Rotorua region of New Zealand. Using a selection of laboratory-cultured freshwater algae and cyanobacteria species, this study demonstrated unique light scatter and fluorescent characteristics for each species examined. These properties allowed for rapid species identification and sorting of mixed populations of laboratory cultures and water samples from the two Rotorua lakes.

Analysis of lake water samples collected over seven months demonstrated changes in abundance and community composition of phytoplankton and demonstrates that flow cytometry may be a useful technique for examining seasonal changes in phytoplankton dynamics.

KEYWORDS

Cyanophyta; flow cytometry; fluorescence; microscopy; rapid assessment

INTRODUCTION

Chlorophyll-*a* (chl-*a*) is commonly used as a proxy for total phytoplankton biomass. After a simple extraction step chl-*a* can be measured fluorometrically or spectrophotometrically. However, monitoring of chl-*a* provides little information on phytoplankton population dynamics and the resulting shifts in community structure often associated with changes in lake processes (e.g. cyanobacterial dominance during stratification) (Oliver & Ganf, 2000).

Light microscopy has traditionally been used to identify and enumerate phytoplankton. Distinguishing genera or species using morphological characteristics remains difficult, laborious, and requires a great deal of expertise (Findlay & Kling, 2001; Vuorio et al., 2007). In the past decade alternative detection methods based on molecular assays have been developed including; denaturing gradient gel electrophoresis (DGGE) (Janse et al., 2003), microarrays (Castiglioni, et al., 2004) and real-time quantitative PCR (qPCR) (e.g. Bowers, et al., 2000; Kurmayer & Kutzenberger, 2003). Molecular techniques have limitations in that they may only detect organisms from specific phyla (Castiglioni, et al., 2004) or that the number of targets that can be detected in a single assay is minimal (i.e., <3 in qPCR). To date the ability to monitor multiple phyla and therefore track phytoplankton community dynamics has not been feasible using molecular assays.

Flow cytometry (FC) is emerging as a rapid and quantitative method for environmental monitoring of phytoplankton populations based on their light scattering properties and fluorescence. Algae contain differing quantities of a variety of light absorbing pigments and even the spectra of apparently identical phycobiliproteins can differ among species (Shapiro, 2003).

The ability to use an array of parameters based on FC may be especially useful for the discrimination of algal species. Flow cytometry has recently been used in the development of bioassays for marine and freshwater algae toxicity testing (Franklin et al, 2002; 2004; 2005) and has also had extensive use in marine and freshwater ecology for the characterisation and quantification and sorting of phytoplankton populations. Portable and shipboard flow cytometers such as the Optical Planktonic Analyser (OPA) have been used for identification and enumeration of algal cells in the field (Borsheim et al., 1989; Balfoort et al., 1992; Hofstraat, et al., 1994), and more recent innovations have been the development of online *in situ* flow cytometers such as the Cytobuoy which can be located inside small buoys or on other platforms for environmental phytoplankton identification and counting (Dubelaar et al., 1999).

There are twelve lakes within the Rotorua district of New Zealand, which have a wide range in morphologies, water clarity, trophic status and nutrient concentrations. As a consequence of increasing anthropogenic activities such as urban and agricultural development in the catchments of these lakes, there has been an increase in the frequency of cyanobacterial blooms, as well as a general deterioration in water quality (Özkundakci et al., 2010). In the past decade the severity and extent of these blooms has increased, raising environmental and human health concerns (Burns et al., 2009). Regular monitoring of these lakes entails analysis of large numbers of samples using time consuming methods, particularly in relation to the identification and enumeration of the dominant species of phytoplankton. There is also substantial temporal variability in phytoplankton biomass within lakes that is not necessarily captured at the monthly frequency adopted within the current monitoring program, which is focused mostly on samples from only one station from each lake (Burns, et al., 2009). Given the time lags that occur between sample collection and analysis of phytoplankton using traditional enumeration techniques, there is also limited time for regional authorities to be reactive in assessing immediate human health risks posed by blooms of cyanobacterial.

The factors that regulate successions of phytoplankton species and initiate blooms of cyanobacteria are still strongly debated in the literature (Huisman et al., 2005) and appear to be highly specific to the preceding physicochemical conditions and meterology as well as the waterbody of interest (Figueredo & Giani, 2001). Phytoplankton contain distinctive chlorophyll and fluorescent photosynthetic pigments. Excitation of these pigments at a defined wavelength results in distinct fluorescence emission spectra which can be used to differentiate between various species. These characteristic allow for direct measurement by FC without the need to apply dyes or stains. Thus the use of FC may help to contribute fundamental information on phytoplankton successions and bloom initiation by providing an opportunity to process large numbers of phytoplankton samples to a species level, at rates hitherto not realised with traditional light microscopy methods.

The objective of this study was to determine the feasibility of using conventional FC methods to distinguish and sort phytoplankton species based on their light scattering and fluorescent properties. Pure phytoplankton cultures were used as references for lake waters containing mixed communities. Intensive monitoring of two eutrophic lakes at a number of depths over several months was used as the basis for a detailed investigation of how FC can be used to quantify the co-existence and dominance of different phytoplankton species.

METHODS

Pure phytoplankton cultures

Cultures representing the groups Bacillariophyta (diatoms), Chlorophyta (green algae), Chrysophyta (chrysopytes), Cryptophyta (cryptophytes), Cyanophyta (cyanobacteria), Dinophyta (dinoflagellates) and Euglenophyta (euglenophytes) were sourced from the Cawthron Institute (Nelson, New Zealand) culture collection and maintained in 50 mL plastic pottles (Biolab, New Zealand) containing 30 mL of MLA enrichment medium (Gorham et al., 1964; Bolch & Blackburn, 1996) at 300 µE $m^{-2}s^{-1}$ on a 12:12 h light:dark cycle at 20°C.

Study sites

Two of the Rotorua lakes were sampled intensively for the purposes of this study. Lake Okaro is located southeast of Rotorua (Figure 1.). It is a small explosion crater lake, 0.32 km^2 in area with a maximum depth of 18 m. It is monomictic, and is continuously stratified during summer. Water enters via a small stream on the north-west of the lake and drains from an outflow in the south-east. High nutrient loading into Lake Okaro is due primarily to pastoral land use, as 95% of the lake catchment is used for dairy farming (McColl, 1972), and there is substantial remobilisation of nutrients from the bottom sediments due mostly to prolonged anoxia of bottom waters associated with seasonal stratification (Burns *et al.,* 2009). Lake Okaro has poor water quality, with summer cyanobacterial blooms, and is the subject of ongoing applications of a modified zeolite compound designed to create a phosphorus absorbing cap over the bottom sediments, and to limit phosphorus release from this layer to the overlying water column (Environment Bay of Plenty, 2009).

Lake Rotoehu is located 35 km north-east of Rotorua and is the smallest of a chain of three lakes lying on the edge of the Okataina caldera. The lake has an area of 8.1 km^2 and maximum depth of 13.4 m. Lake Rotoehu is polymictic, with periods of stratification of up to a few days (Ryan, 2006). The water source is from small streams and sub-terrainean flow. The lake has a series of arms at its northern end and geothermal springs located on the south east shore. Lake Rotoehu experiences major cyanobacterial blooms in summer months and has had a recent severe hornwort (*Ceratophyllum demersum)* infestation in the southern corner of the lake (Burns et al., 2005).

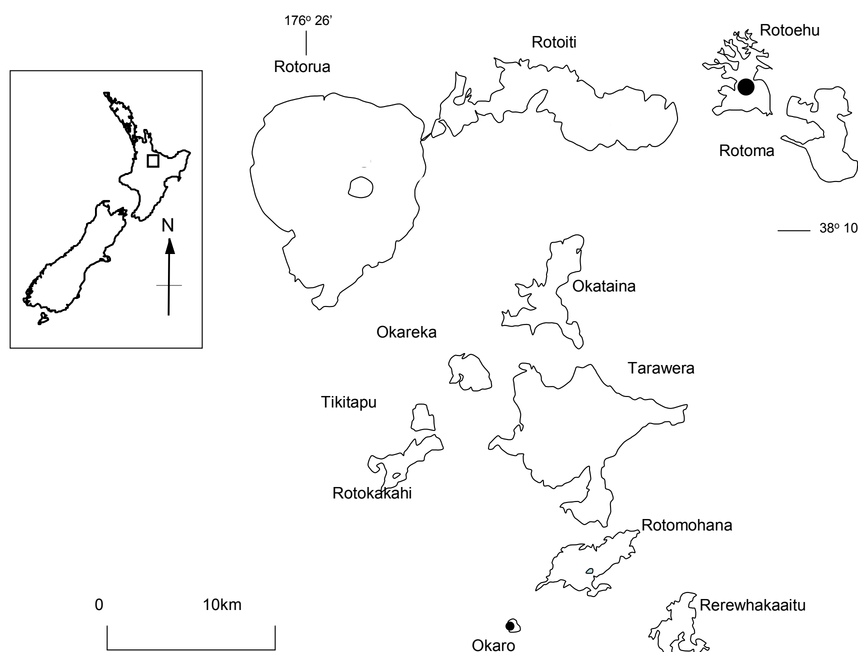


Figure 1. Lakes in the Rotorua district. Dots denote the location of sampling sites at Lake Rotoehu and Lake Okaro.

Field sample collection

Water samples were collected weekly from a station at the deepest point in lakes Okaro and Rotoehu (38°18014S, 176°23635E and 38°022943S, 176°530294E respectively) between October 2008 and February 2009. One-litre water samples were collected using a Schindler-Patalas trap at depths of 1, 3, 5, 7 and 9m, transferred to glass Schott bottles and kept on ice until analysis. Surface water temperatures were recorded using a portable depth sounder (Hummingbird PiranhaMAX230, USA).

Samples were prepared for microscopy and FC within four hours of collection. Each sample was vigorously shaken to disperse algal aggregates, then passed through a 40 µm sieve (Falcon, USA) to remove zooplankton and large debris and prevent clogging of the flow cell. Microscopic examination of debris retained on the sieve showed negligible retention of phytoplankton as intended. Samples (350 mL) were then centrifuged (5000 rpm for 10 min) to concentrate phytoplankton populations. The supernatant was discarded and 4 mL of concentrated sample retained for microscopic analysis and FC.

Morphological analysis was performed on unpreserved samples using an Olympus BX61 microscope equipped with analysis LS Research software (Olympus, USA) at 400x magnification. Photographs were taken using an Olympus Colourview (Olympus, USA) digital camera under bright field.

FLOW CYTOMETRY

Concentrated samples were analysed using a FACSVantage™ SE flow cytometer (Becton Dickinson (BD) Biosciences, USA) equipped with the BD FACSDiVa™ digital data processing electronics and software option. The sheath fluid was phosphate buffered saline solution (PBS) (Lorne Laboratories Ltd., UK), adjusted to pH 7.0 and delivered through a 70μm nozzle at 131 kPa. Daily instrument optimization was performed using SPHERO™ fluorescent beads (BD BioSciences, USA). A target number of 1000 cells per second was used to obtain optimal cell counts by FC. Measured parameters included forward scatter (FSC), side scatter (SSC), chlorophyll fluorescence (650-700nm) phycoerythrin (575 nm), indicative of phytoplankton, and allophycocyanin (APC) fluorescence (660nm), indicative of cyanobacteria. Cell populations were resolved using a dual gating procedure based on two-dimensional cytograms of APC-PE fluorescence.

CELL COUNTS

Cell counts were obtained using an internal standard of diluted TruCOUNT™ beads (BD BioSciences, USA). A fluorescent bead stock was prepared by adding 1 mL of MLA growth medium to a TruCOUNT tube containing a known quantity of beads (n=51 088). A sub-sample (100 μL) of bead stock was added to 1000 μL of algal sample and a fixed number (n=50) of beads counted directly by FC. Absolute cell counts were determined by using the following equation:

$$\text{cell count} = \frac{\text{number of beads in 100uL bead stock}}{x/y} \times \frac{\text{number of events counted per population}}{\text{number of events counted in bead population}}$$

where x = volume of sample centrifuged and y = final volume concentrated sample

Cell sorting

For sorting the drop drive was set to a frequency of 39 kHz, with an amplitude of 3V and drop delay between 14 and 16. The sheath fluid was PBS (see above). Sort criteria were defined by the populations which had been distinguished in the original pure culture cell counts. Sorting was performed using the Yield option in the DiVa software (BD Biosciences, USA), which maximized cell number recovery in sorted isolates. The sorted cells were collected into 2 mL of MLA medium (Bolch & Blackburn, 1996). The collected sort was then aseptically transferred into sterile specimen containers with 50 mL prepared MLA medium for growth studies and subsequent identification.

results

Unialgal cultures from each of the seven phyla, and also a mixture of the phyla were processed by FC and displayed as a bivariate scatterplot of SSC versus PE (Figure 2a), chlorophyll (Figure 2b), and APC (Figure 2c) fluorescence. Each dot on the scatter plots represents and individual particle.

Two-dimensional cytograms of APC-PE fluorescence of a mixed species sample indicated well defined discrete clusters of each species. The resulting cytogram (Figure 2d) was used to determine the phyla composition of field samples.

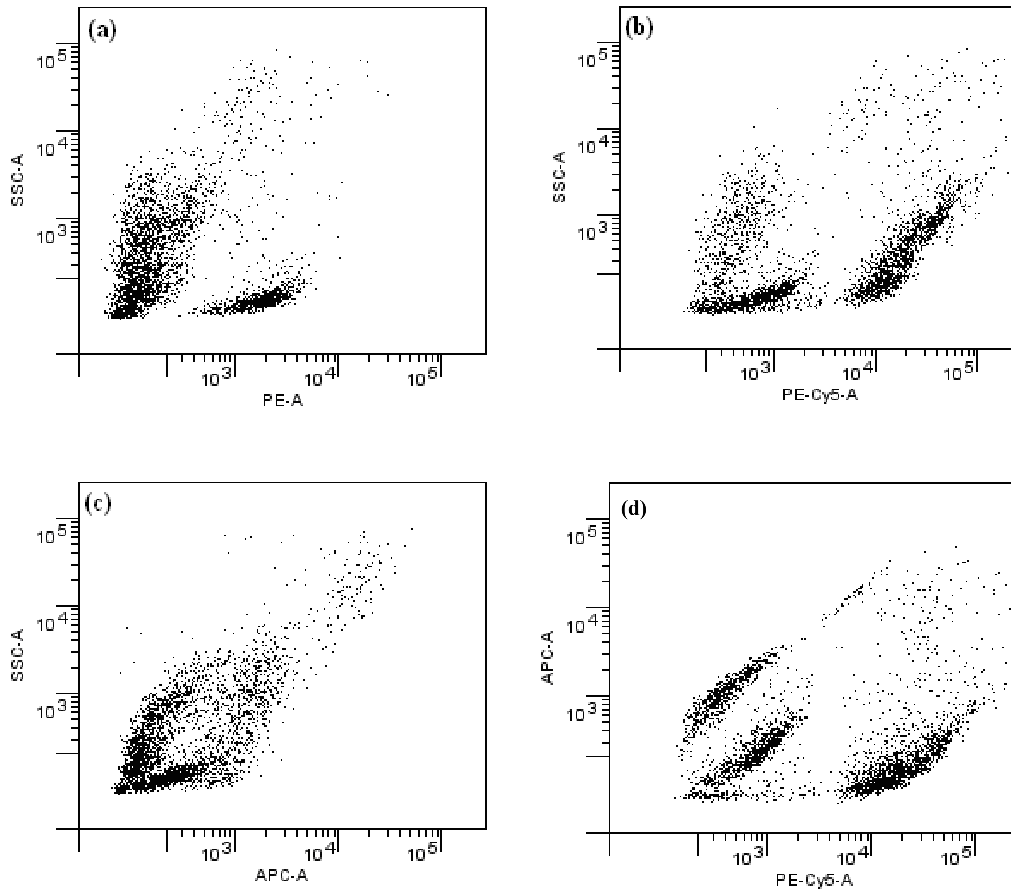
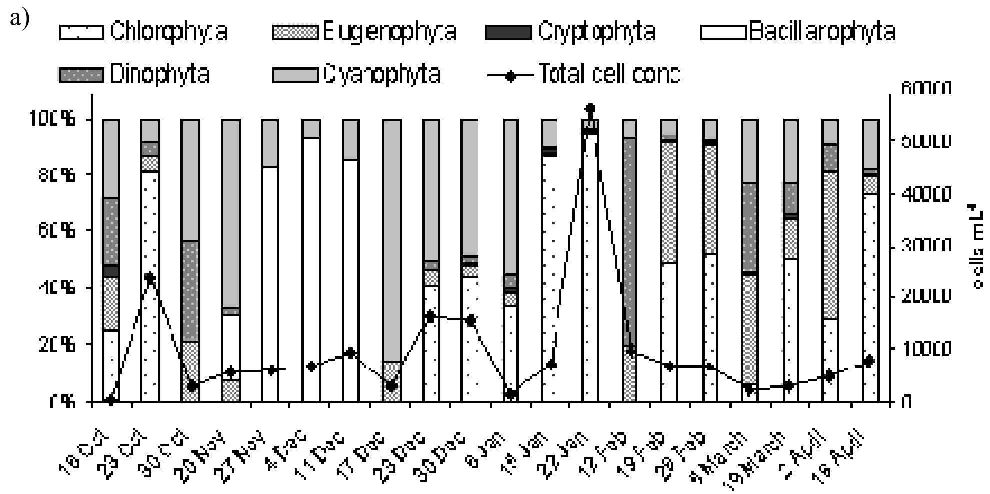


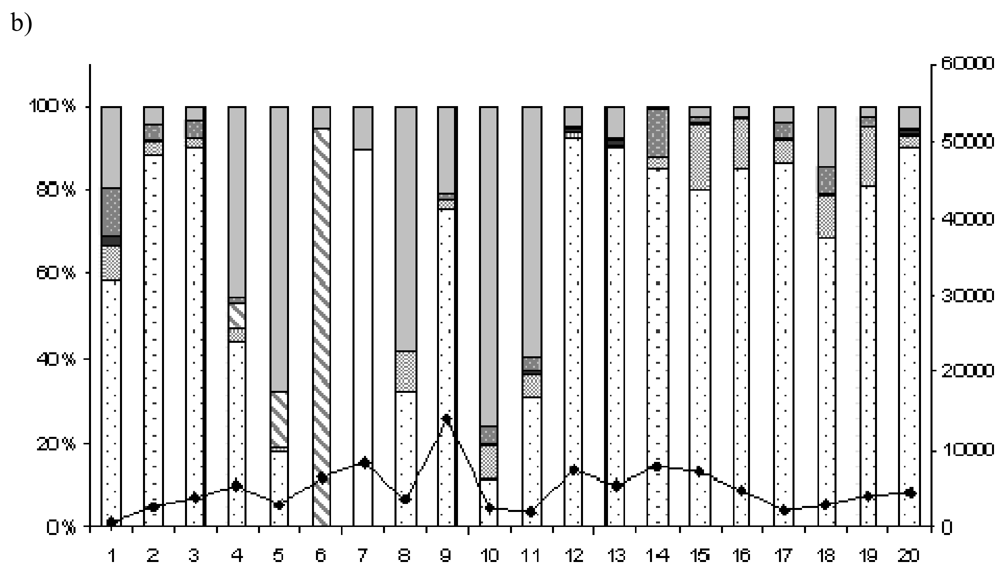
Figure 2. *Example of bivariate log scatterplot of SSC versus a) phycoerthrin (PE) fluorescence, b) chlorophyll (PE-Cy5) and c) allophycocyanin (APC) fluorescence, and d) allophycocyanin (APC) versus chlorophyll (PE-Cy5) fluorescence.*

Phytoplankton counts (cells per mL) were calculated for water samples collected from the two lakes the period 16 October 2008 to 16 April 2009 using the cluster template from the pure cultures. Figures 3 and 4 show cell concentrations determined by FC as relative abundance and total counts of the most common phytoplankton species occurring in the two lakes separated by the respective sampling depths. Surface temperatures ranged from 14 to 22°C on Lake Okaro and 14 to 27°C on Lake Rotoehu over the period of sampling.

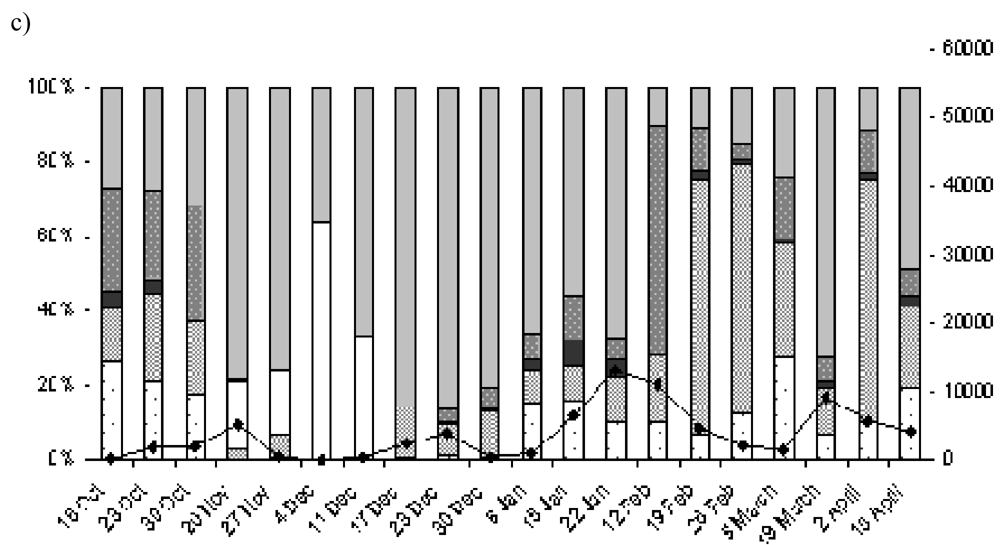
a)



b)



c)



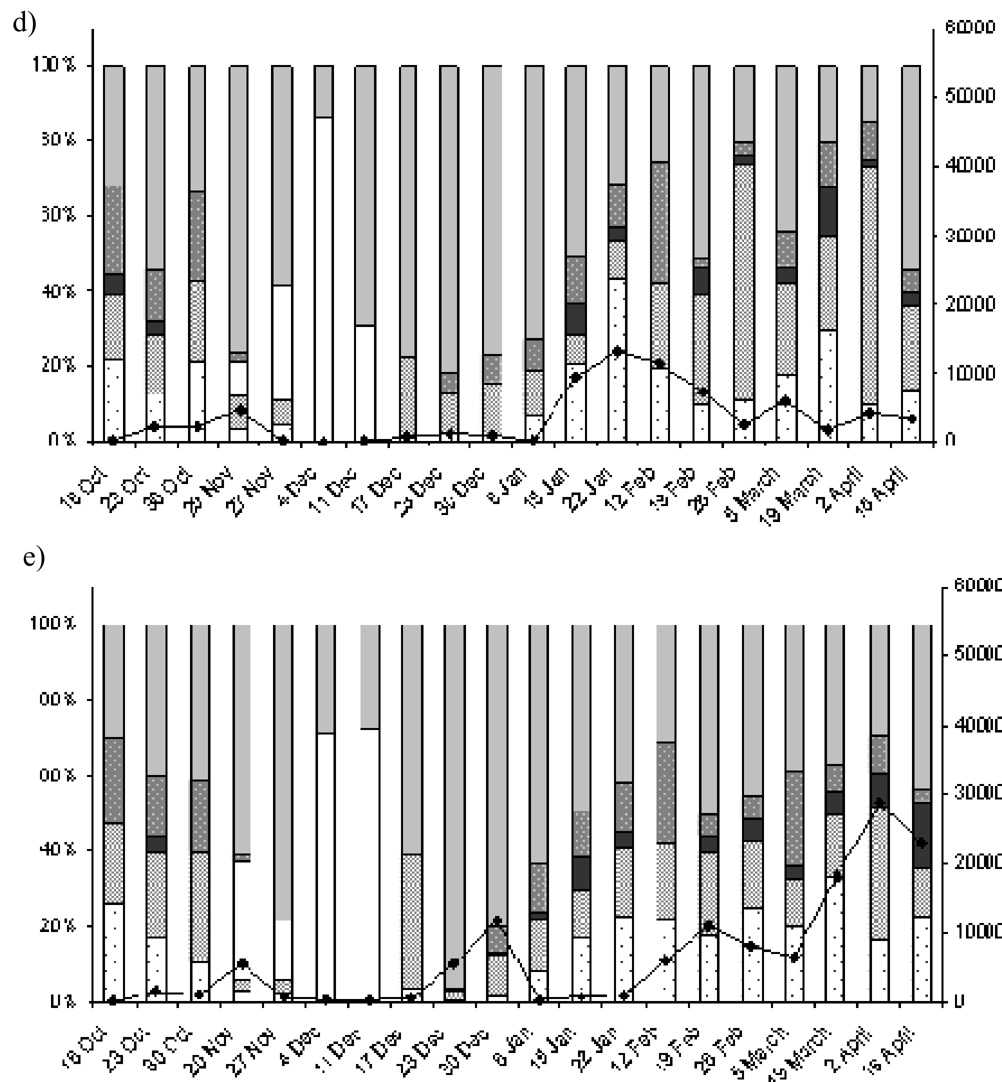
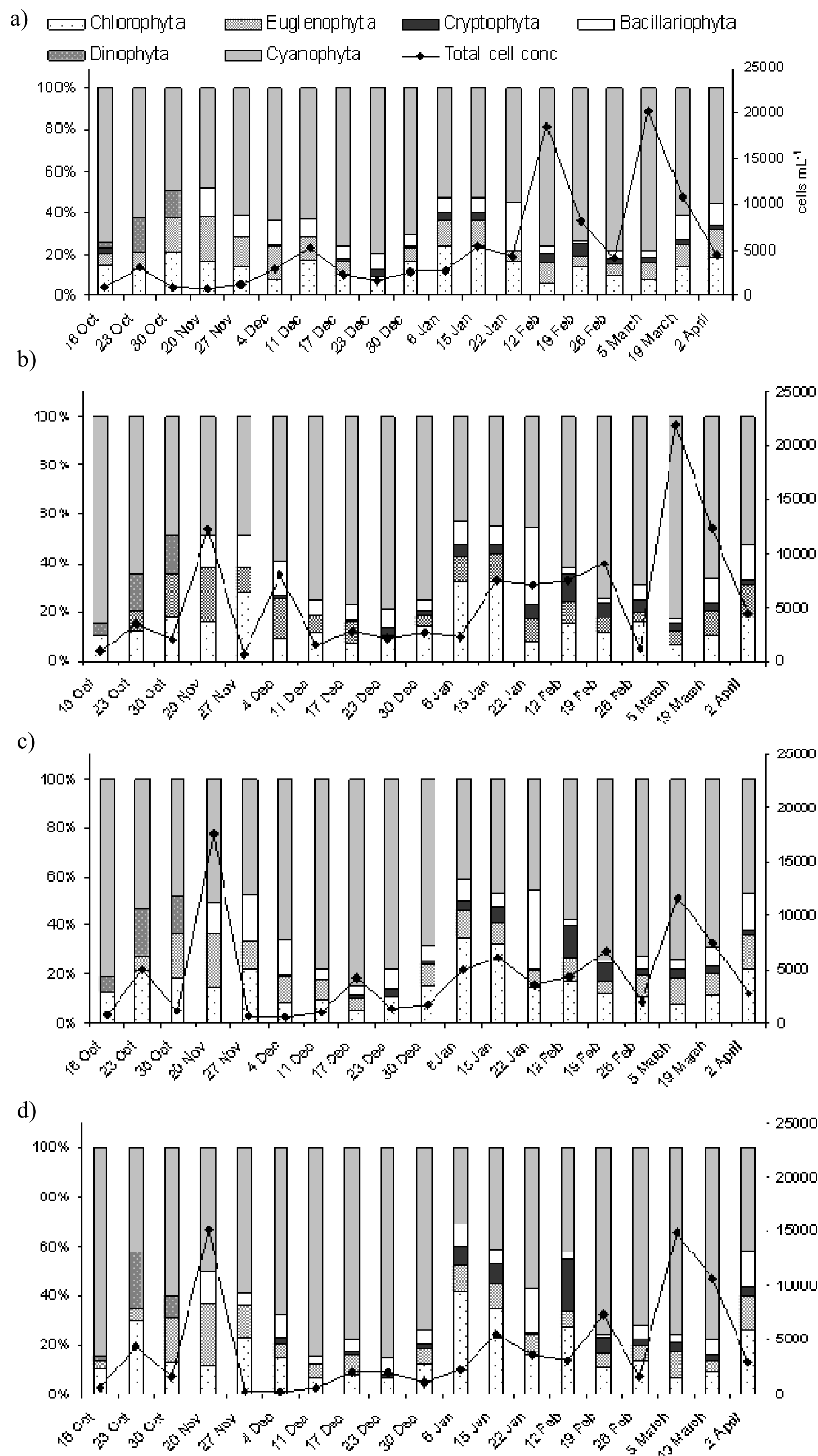


Figure 3. *Phytoplankton percentage abundance and total cell counts by flow cytometry for the period 16 October 2008 to 16 April 2009 for Lake Okaro at depths of a) 1 m, b) 3 m, c) 5 m, d) 7 m and e) 9 m.*


a)
b)
c)
d)
Chlorophyta
Euglenophyta
Cryptophyta
Bacillariophyta
Dinophyta
Cyanophyta
Total cell conc
cells mL-1
0%
20%
40%
60%
80%
100%
0
5000
10000
15000
20000
25000
16 Oct
23 Oct
30 Oct
20 Nov
27 Nov
4 Dec
11 Dec
17 Dec
23 Dec
30 Dec
6 Jan
15 Jan
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12 Feb
19 Feb
26 Feb
5 March
19 March
2 April

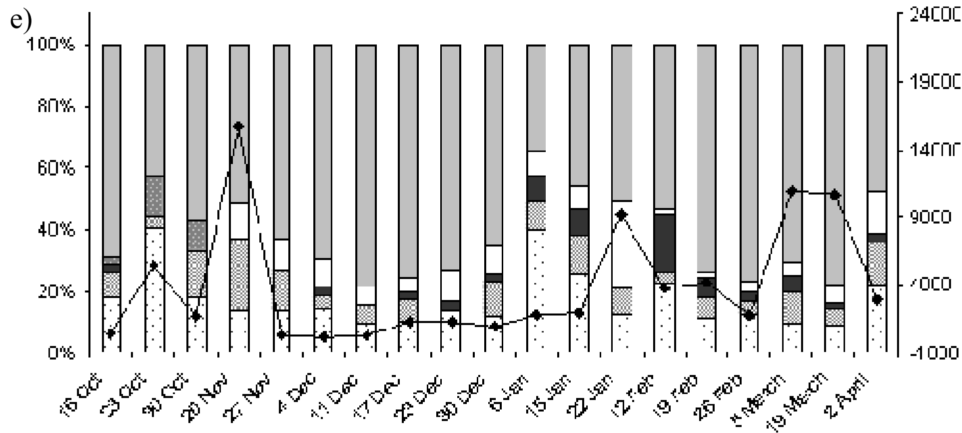


Figure 4. *Phytoplankton percentage abundance and total cell counts by flow cytometry for the period 16 October 2008 to 16 April 2009 for Lake Rotoehu at depths of a) 1 m, b) 3 m, c) 5 m, d) 7 m and e) 9 m.*

Both lakes showed a similar pattern of increased cell concentrations corresponding to a general increase in surface water temperatures which were maximal on 15 January 2009 in Lake Okaro and 26 February 2009 in Lake Rotoehu.

In Lake Okaro there were marked increases in cell concentrations from early January to mid-March at 5 m and deeper (Figure 3c-e), at a time when surface water temperatures also increased rapidly. Total phytoplankton concentrations for Lake Okaro peaked at approximately 56,340 cells mL^{-1} at 1 m, 13,830 cells mL^{-1} at 3 m, 13,100 cells mL^{-1} at 5 m, 13,010 cells mL^{-1} at 7 m and 28,580 cells mL^{-1} at 9 m depth on 22 January 2009, 23 December 2008, 22 January 2009, 22 January 2009 and 2 April 2009, respectively (Figures 3a, e). Cell concentrations varied with depth in Lake Okaro where there was a general decrease in concentrations at the surface after January and until the end of sampling while at the same time there was a reverse trend in cell concentrations at 9 m depth, with total phytoplankton concentrations decreasing at the surface to < 10,000 cells mL^{-1} and increasing at 9 m to > 20,000 cells mL^{-1} (Figures 3a, e). Between 3 and 7 m in Lake Okaro (Figures 3b-d) cell concentrations were reasonably stable without the large peaks that characterize the deeper and shallower samples from this lake.

Shifts in phytoplankton phyla were observed through the time series, with dominance of Chlorophyta in Lake Okaro in January (Figure 3a-e) reaching concentrations of 34,000 cells mL^{-1} and Bacillariophyta increasing in relative abundance throughout the water column in mid-December prior to a rapid decrease in abundance shortly thereafter. Dinophyta were present in low concentrations throughout the sampling period in Lake Okaro and throughout the water column. A fish kill was observed on Lake Okaro (data not presented) at the end of January which also corresponded with the lake taking on a brown hue, and a foul stench being emitted from water samples collected below 7 m. *Peridinium* (Dinophyta) were observed by microscopy of samples at this time, making up 67% of the total cell count (Figure 3e).

Lake Rotoehu total cell concentrations peaked at approximately 20,290 cells mL^{-1} at 1 m, 21,900 cells mL^{-1} at 3 m, 17,590 cells mL^{-1} at 5 m, 15,190 cells mL^{-1} at 7 m and 15,740 cells mL^{-1} at 9 m depth on 22 January 2009, 5 March 2009, 20 November 2008, 20 November 2008 and 20 November 2008 respectively (Figures 4a, e). Total cell concentrations were similar throughout the water column in Lake Rotoehu, with <5,000 cells mL^{-1} (Figure 4a-e), and an overall dominance by Cyanophyta. High concentrations of Cyanophyta (~8,000 cells mL^{-1}) were recorded in Lake Rotoehu in late November with a surface bloom (taken here to be concentrations of >15,000 cells mL^{-1}) but concentrations also >15,000 cells mL^{-1} at several depths (Figure 41-3) and again in February in the surface waters only (~ 14,000 cells mL^{-1}) and on 5 March at the surface as well as at several depths (>15,000 cells mL^{-1}). Dinophyta increased in abundance briefly in Lake Rotoehu in late October and early November but were relatively homogeneous throughout the water column. Both Euglenophyta and Bacillariophyta were present in low concentrations throughout the water column over the entire sampling period. Lake Rotoehu also had a fish kill (data not presented) mid-February, which coincided with observations of rotting hornwort (*Ceratophyllum demersum)* on the lake surface as well as large colonies and blooms of phytoplankton. *Microcystis sp*. was prominent in samples taken both by microscopy and FC from the surface at this time.

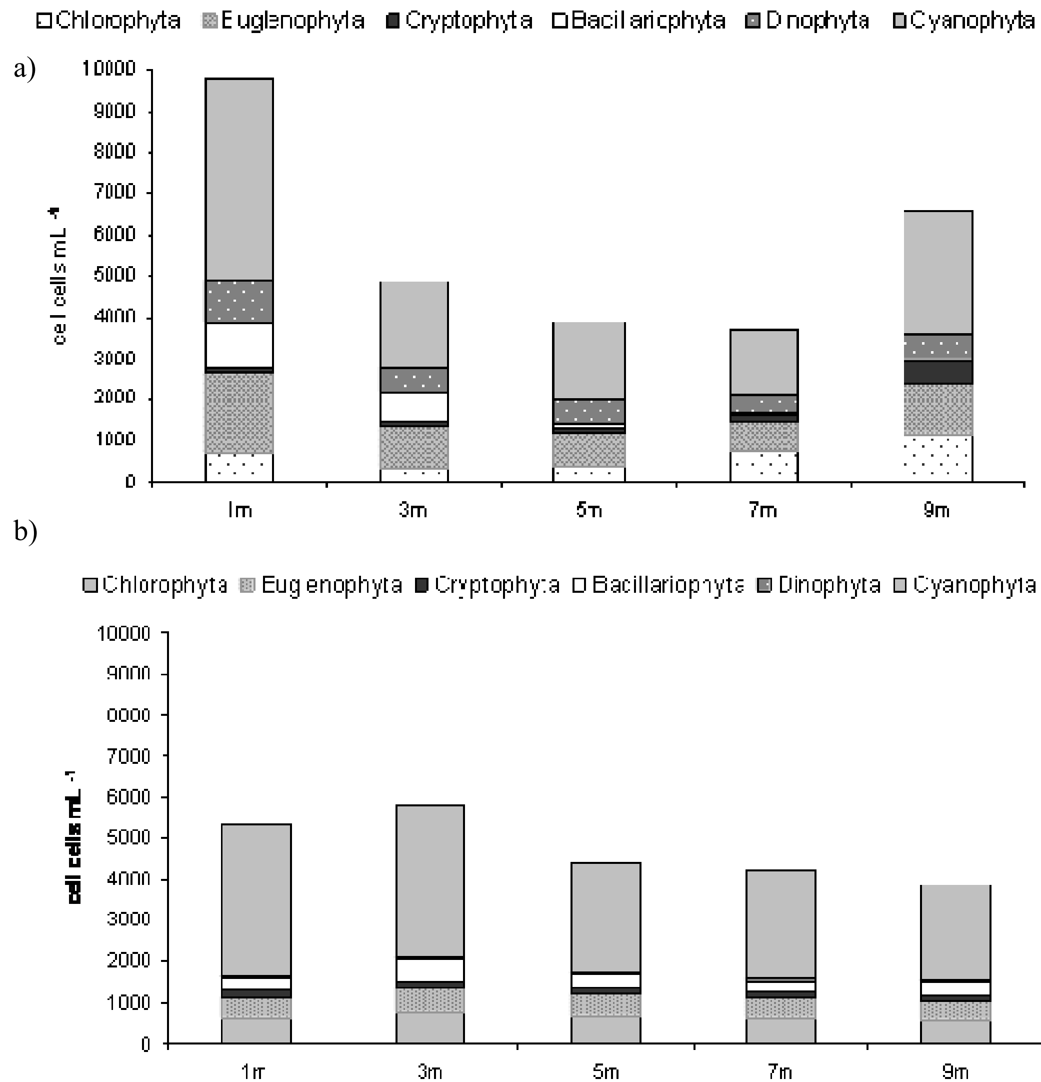
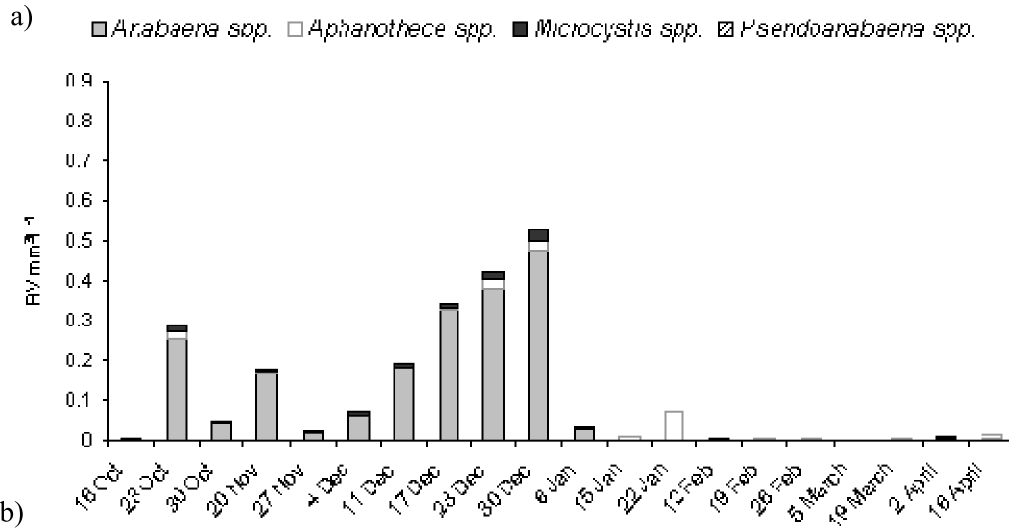


Figure 5. Average cell concentrations at varying depths for the period 16 October 2008 to 16 April 2009 for a) Lake Okaro and b) Lake Rotoehu.

Figure 5a-b shows the differences in phytoplankton between the two lakes. Lake Rotoehu was much more stable with cell concentrations very similar at all depths across all species. However Lake Okaro shows a wider depth variation in cell concentrations with a greater diversity of species and higher average cell concentrations at the surface and 9m depth.

Four dominant Cyanophyta genera were identified in the lakes as *Anabaena* spp., *Aphanothece* spp., *Microcystis* spp. and *Psendoanabaena* spp. Biovolumes were calculated to account for variability in sizes of the genera (Wood et al., 2008) and are shown for the five sampling depths at Lakes Okaro and Rotoehu in Figures 6 and 7 respectively.



a)
Anabaena spp. Aphanothece spp. Microcystis spp. Pseudoanabaena spp.
BV mm³ L⁻¹
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
16 Oct
23 Oct
30 Oct
20 Nov
27 Nov
4 Dec
11 Dec
17 Dec
23 Dec
30 Dec
6 Jan
15 Jan
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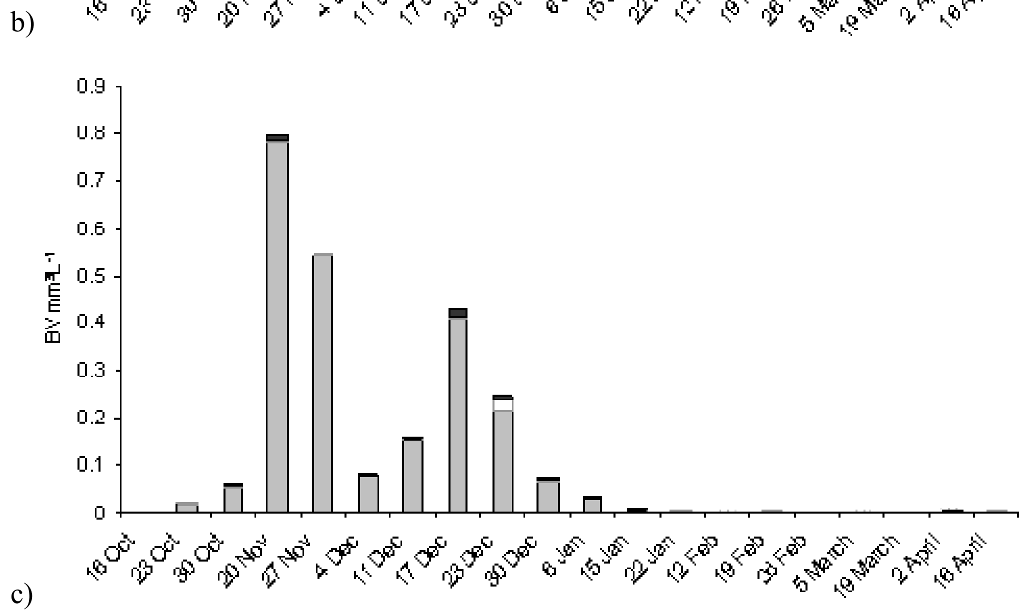
26 Feb
5 March
19 March
2 April
16 April



b)
BV mm³L⁻¹
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
16 Oct
23 Oct
30 Oct
20 Nov
27 Nov
4 Dec
11 Dec
17 Dec
23 Dec
30 Dec
6 Jan
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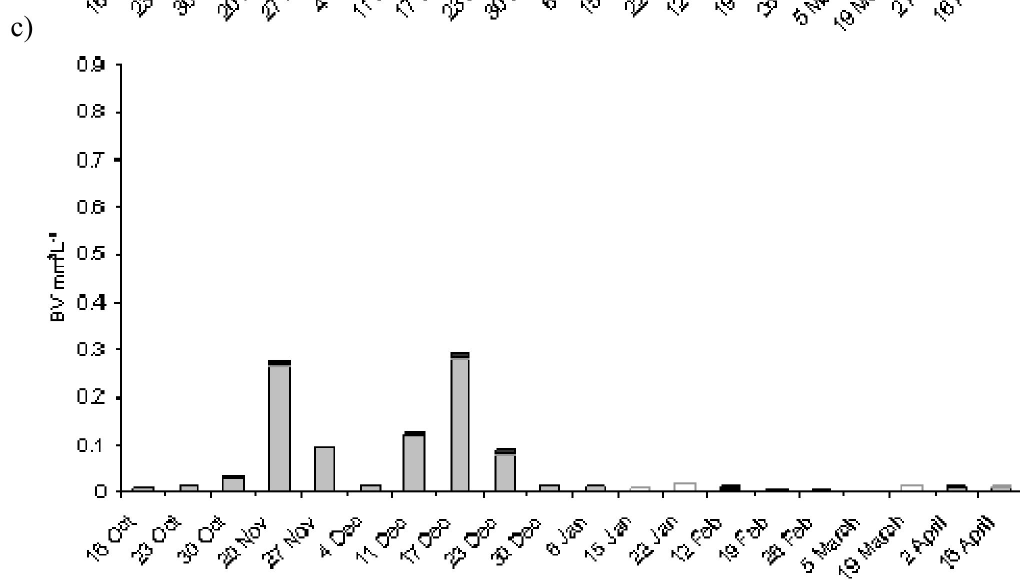
5 March
19 March
2 April
16 April



c)
BV mm³L⁻¹
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
16 Oct
23 Oct
30 Oct
20 Nov
27 Nov
4 Dec
11 Dec
17 Dec
23 Dec
30 Dec
6 Jan
15 Jan
22 Jan
12 Feb
19 Feb
26 Feb
5 March
19 March
2 April
16 April

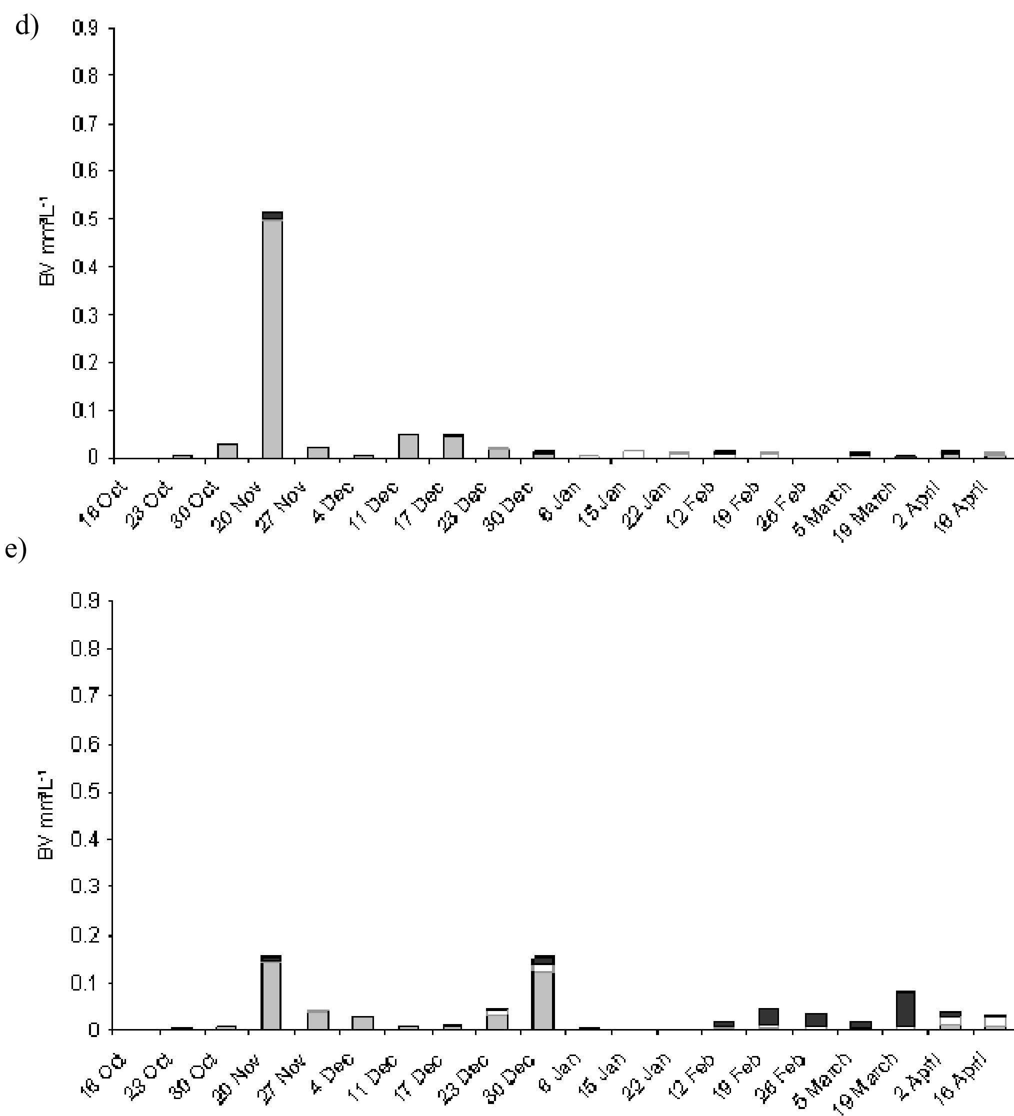
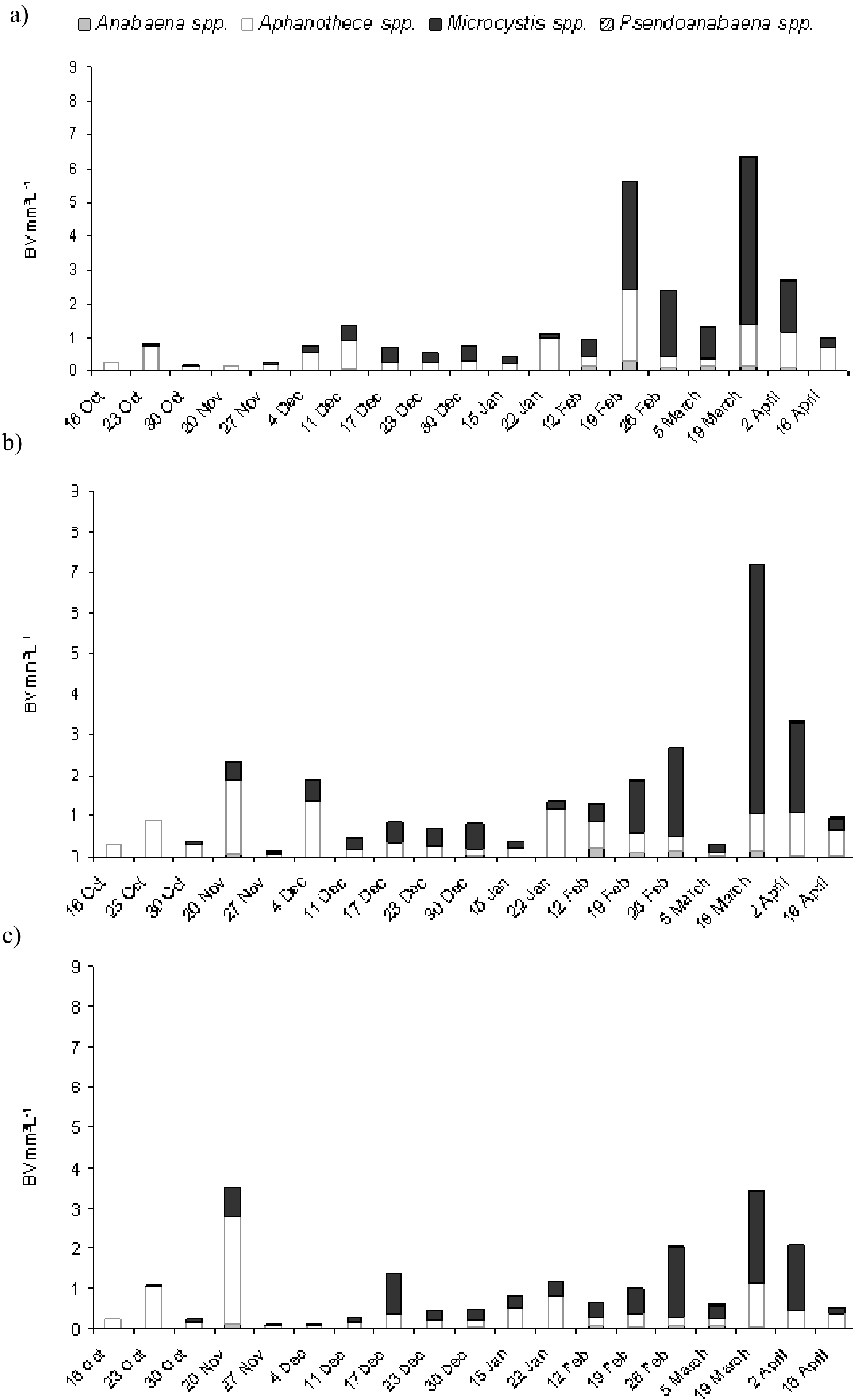


***Figure 6**. Cyanophyta species biovolumes (BV) as mm^3L^{-1} at varying depths during the period 16 October 2008 to 16 April 2009 in Lake Okaro at depths of a) 1 m, b) 3 m, c) 5 m, d) 7 m and e) 9 m.*


a)
Anabaena spp.
Aphanothece spp.
Microcystis spp.
Psendoanabaena spp.
BV $mm^3 L^{-1}$
16 Oct
23 Oct
30 Oct
20 Nov
27 Nov
4 Dec
11 Dec
17 Dec
23 Dec
30 Dec
15 Jan
22 Jan
12 Feb
19 Feb
26 Feb
5 March
19 March
2 April
16 April
b)
c)

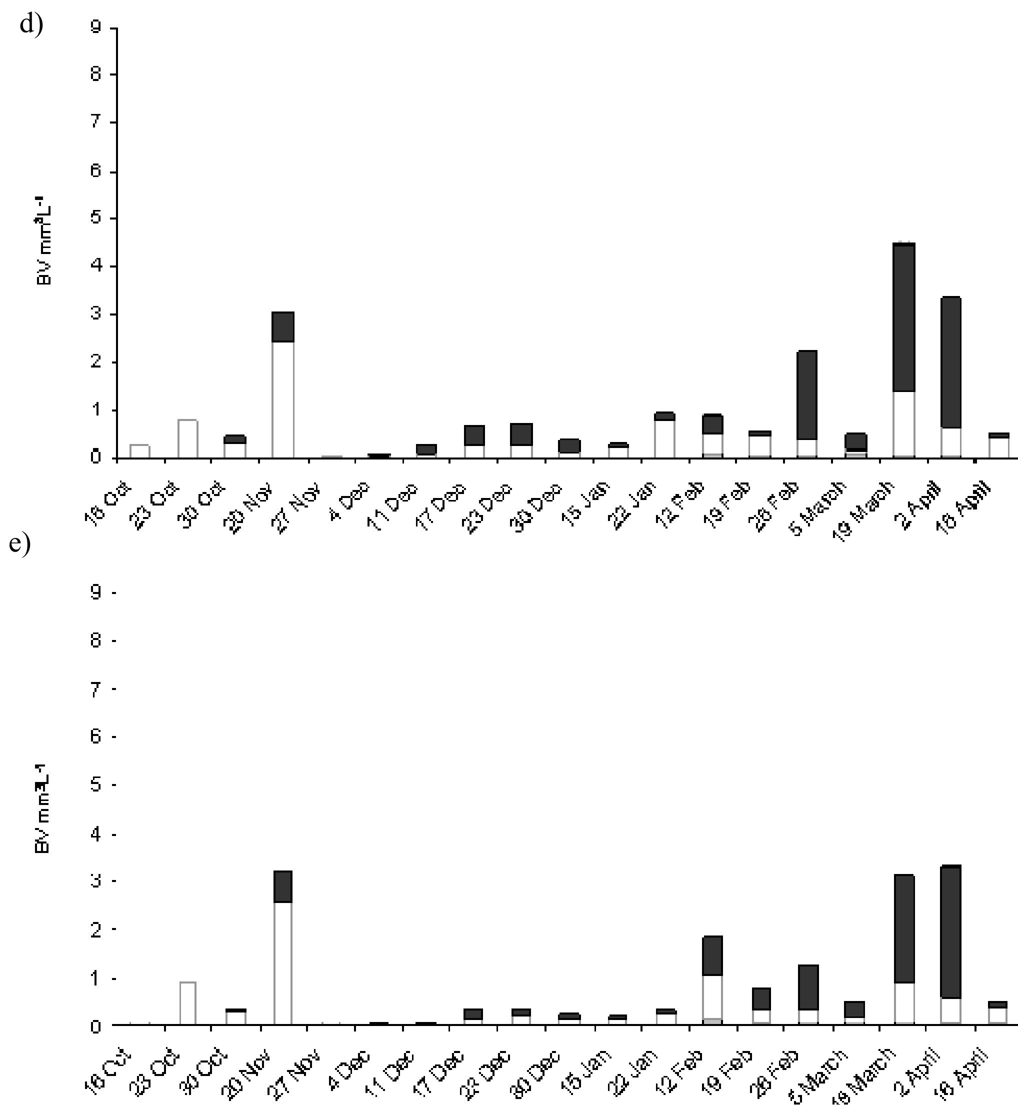


***Figure** 7. Cyanophyta species biovolumes (BV) as mm^{3}L^{-1} at varying depths during the period 16 October 2008 to 16 April 2009 in Lake Rotoehu at depths of a) 1 m, b) 3 m, c) 5 m, d) 7 m and e) 9 m.*

Anabaena spp. biovolumes were highest in Lake Okaro (Figure 6a-e), where this genus dominated throughout the water column apart from a short period during February and March when *Microcystis* spp. dominated in the deeper waters (Figure 4e). *Microcystis* spp. were dominant in Lake Rotoehu throughout the sampling period (Figure 7a-e), however there appeared to be some sort of co-existence with *Aphanothece* spp. across all depths. At no time did *Microcystis* spp. appear to strongly exclude this species.

On the other hand concentrations of *Anabaena* spp. remained relatively low in Lake Rotoehu throughout the sampling period. Approaching the end of the sampling period, notably in mid-March, there appeared to be a distinct shift towards higher biomass, together with an increase in species dominance, especially at 9 m depth, with total biovolumes generally increasing together with increasing contributions by *Microcystis* spp. and *Aphanothece.*

discussion

Aquatic environments undergo seasonal transformations in physical (e.g. stratification) and chemical (e.g. nutrient concentrations) variables that are often associated with marked changes in phytoplankton succession and biomass (Reynolds, 1997). Different phytoplankton phyla exhibit an array of physiological adaptations to environmental variables which result in these natural seasonal successions. By using FC the changes in major phyla were able to be tracked in the two eutrophic study lakes at high frequency and over several different depths that would not otherwise be possible with standard microscopy techniques. The data for the two lakes revealed marked major changes in biomass and species composition within our relatively frequent weekly sampling period and within depth intervals of as little as 2 m. While seasonal perturbations drive major transitions in species assemblages at spatial scales, often linked to prevailing thermal divisions (e.g. surface mixed layer, hypolimnion), even our highly resolved sampling revealed major changes in populations within successive monitoring events and between depth-resolved samples. It appeared that an even more highly resolved sampling routine may be required to contribute the level of process information to be able to understand how different species, especially within phyla and genera, are responding to environmental variables.

Because of its ease of use, Chlorophyll-*a* has been the method of choice to determine phytoplankton biomass at highly resolved space and time intervals, but it does not allow for the enumeration of phytoplankton species and phyla like FC. The target phytoplankton phyla investigated in this study were Bacillariophyta, Chlorophyta, Chrysophyta, Cryptophyta, Dinophyta and Cyanophyta. Each phylum has its own distinctive pigmentation and fluorescence properties that facilitated classification by FC.

Further validation would be required to fully determine the robustness of the technique, by performing a thorough comparison of FC-determined cell concentrations with those acquired using microscopic techniques.

Many phytoplankton phyla, especially Dinophyta and Bacillariophyta, are large organisms (up to 200 μm) with varying morphologies. The use of larger nozzle sizes (up to 130 μm) to prevent FC nozzle blockage could facilitate detection of more of these larger particles and extend the range of species classification and enumeration. Work has also been done designing a nozzle which is able to orient and sort asymmetrical cells (Rens et al, 1999). Newer designs will reduce the need for pre-screening and breaking up of colonies and cells prior to FC analysis, and ultimately lead to improved analytical capabilities.

Characterisation of mixed communities with FC was also possible by sorting clusters identified on bivariate plots of APC vs PECy5 fluorescence. A potential disadvantage of the current method was that the sorting and counting process appeared to be too vigorous for some of the phytoplankton cells and subsequent concentration onto slides indicated that some cells had been damaged. Sorting into a growth medium enabled subsequent microscopic examination of sorted cells with a number of species exhibiting excellent growth in the post sorting phase. As with any culture based technique, however, every attempt should be made to compare pre- and post-cultured populations, and to standardize incubation times to ensure that culturing in growth media does not itself result in differential selection of species.

Since c.2005 there has been a marked increase in numbers of small Cyanophyta (< 2 μm) such as *Aphanothece* sp. and *Aphanocapsa* sp. in the Rotorua lakes (Wood, et al., 2008). Quantification of these small Cyanophyta by microscopy is extremely difficult, time consuming and prone to error. Flow cytometric enumeration of mixed community samples containing these organisms is rapid and comparatively straight forward. Initial results can be obtained within minutes at a throughput by FC of 12 or more samples per hour by FC, for classification and quantifying cell concentrations. This high throughput has obvious time saving benefits in the analysis of large numbers of routine monitoring samples and could be extremely useful for the rapid identification and pre-warning of potential bloom situations in water bodies, especially given that it would allow more complete depth coverage.

For extended periods during this study the lakes were dominated by Cyanophyta, along with high concentrations of Chlorophyta, typical of eutrophic systems (Paerl et al., 2001). Different Cyanophyta have specific features that can give them a competitive advantage over other phytoplankton including: buoyancy regulation, the ability to utilise atmospheric nitrogen and luxury uptake of phosphorus in excess (luxury consumption) and highly resistant akinetes that can allow populations to rapidly re-establish when conditions are favourable (Paerl, et al., 2001). Lake Okaro is often stratified whereas Lake Rotoehu only intermittently stratifies (Burns, et al., 2009). Phytoplankton assemblages in stably stratified lakes are significantly different to those from intermittently stratified and mixed lakes (Ryan, 2006). Dinophyta concentrations in Lake Rotoehu were low, with dominance by Cyanophyta. Lake Okaro was also dominated by Cyanophyta but with a greater Dinophyta presence. Dinophyta have the ability to migrate between nutrient-rich deeper waters and well lit surface waters to satisfy both their nutritional and light requirements. Lakes supporting a poor light climate for phytoplankton, such as Okaro, can be dominated by filamentous Cyanophyta such as *Anabaena* sp. which have a competitive advantage by being able to tolerate low light along with low energy requirements. *Anabaena* spp. are generally surface dwelling, preferring conditions where there is water column stability, high levels of surface irradiance and mostly a lack of nutrient limitation (Paerl et al., 2001; Havens, 2008). Conditions in Lake Okaro in November and December may have supported the growth of this genus, with the lake stratifying but still nutrient-rich and with plentiful light. However, waters of Lake Okaro rapidly become anoxic with the onset of seasonal stratification and nutrients that are released from the bed sediments may start to become mixed and available for phytoplankton populations near the end of the stratification period when the thermocline deepens. The rapid increase of total cell concentrations in April may therefore be due to a number of environmental factors occurring at this time which have their own specific effect on phytoplankton succession. Other Cyanophyta such as *Microcystis* spp. can use their intermittent buoyancy to optimise light capture on surface waters and thus shade phytoplankton at lower depths, giving them a competitive advantage. The mixing in Lake Rotoehu, however, may have provided sufficient supply of growth-limiting nutrients for growth of other phytoplankton.

Mixing in this lake would be more likely to interfere with buoyancy regulation and inhibit aggregation of cells.

Microscopy has been the method of choice for phytoplankton identification and enumeration, while Chlorophyll-*a* is the standard for phytoplankton biomass. The FC protocol based on fluorescence provided a rapid throughput for enumeration and classification of a number of phytoplankton species. Further work is required to expand the range of phyla able to be classified and the application of immunochemical labelling techniques will allow for further determination of selected phyla to a species level (Hofstraat, et al., 1994; Vives-Rego et al, 2000; Brussaard et al, 2001). If the relevant environmental variables such as light, nutrients, temperature, etc., can be measured at the same high-resolution space and time scales as we have achieved with FC then we will attain an even greater understanding of the dynamics and factors that regulate phytoplankton succession and community structure.

CONCLUSION

The objective of this study was to assess the application of flow cytometric analysis in the differentiation of freshwater algae and cyanobacteria. By using multi-parameter analysis incorporating the size, density and the natural fluorescence properties of freshwater phytoplankton populations it was possible to characterise and enumerate mixed phytoplankton communities. The use of pure cell cultures provided the basis for this analysis through discreet reference plots for known phytoplankton species as well as mixed culture sorting and enumeration. The methodology was then used to demonstrate changes in abundance and composition of phytoplankton and specific Cyanophyta populations in two eutrophic Rotorua lakes. These data illustrate the potential of FC as a useful, time efficient technique for quantifying and sorting phytoplankton populations. The application to these two eutrophic lakes suggests that traditional microscopy techniques will continue to struggle to produce the resolution of sampling in time and space to be able to better elucidate rapid successions amongst populations in natural aquatic systems.

Further validation and method development is underway aimed at determining a wider range of phyla and possible differentiation between species to ensure that the FC method is robust and correlates well with traditional light microscopy methods.

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APPENDIX II: DETAILED METHODS

Culture Media Preparation

Nutrient agar

Prepared as per manufacturer's instructions.

Phage rehydration broth

Diffco nutrient broth 20g

Sodium chloride 5g

Make up to 1L in milli-Q water and autoclave.

Cloning

Kit used: TOPO TA Cloning Kit (with pCR 2.1-TOPO). Catalog number K4500-01, Invitrogen.

LB Medium and Plates

10 g tryptone

5 g yeast extract

10 g NaCl

950 mL MilliQ water

For agar: add 15 g L^{-1} purified agar before autoclaving.

Adjust to pH 7.0 with NaOH and bring volume up to 1 L. Autoclave and cool to 50 °C before adding antibiotics.

Antibiotics

Make up solution of 50 mg mL^{-1} Ampicillin in sterile water. Filter sterilise before storing at -20 °C.

Add 1 mL L^{-1} to make final 50 µg ml^{-1} concentration in media.

Make up 40 mg mL^{-1} X-gal solution in dimethylformamide (DMF). Store at -20 °C.

Step 1: PCR product set up

Use as fresh as possible PCR products, A-overhang can drop off over time, reducing ligation and transformation. Best results are obtained from adding A tails the morning of cloning.

Step 2: Setting up Ligation

Set up the TOPO cloning reaction on ice around 1pm in afternoon:

Fresh PCR product	0.5 to 4 µL
Salt Solution	1 µL
Water	Add to a total volume of 5 µL
TOPO vector	1 µL
Final Volume	**6 µL**

1. Mix reaction gently and incubate for 5 minutes at room temperature (22-23 °C). Increasing the incubation time to 20 to 30 minutes, allows more molecules to ligate, increasing the transformation efficiency.
2. Place the reaction on ice (TOPO cloning reaction can be stored at -20° C overnight).

Step 3: Preparation for Transformation

3. Equilibrate water bath to 42 °C.
4. Warm vial of S.O.C media to room temperature.
5. Warm plates at 37 °C for 30 minutes.
6. Spread 40 µL of 40 mg mL^{-1} X-gal on each LB plate and incubate at 37 °C until ready for use.
7. Thaw **on ice** 1 vial of One Shot cells for each transformation.
8. Add 2 µL of the TOPO cloning reaction into a vial of One Shot Chemically competent *E. coli* and mix gently. **Do not mix by pipetting up and down.**
9. Incubate on ice for 5 to 30 minutes.
10. Heat-shock the cells for 30 seconds at 42 °C without shaking.

11. Immediately transfer the tubes to ice for 2 minutes.
12. Aseptically add 250 µL of room temperature SOC medium.
13. Cap the tube tightly and shake the tube horizontally at 200 rpm at 37 °C for 1 hour.
14. Spread 10 µL (+ 40 µL of SOC) and 50 µL from each transformation onto a pre-warmed plates and incubate overnight at 37 °C. Plate two different volumes to ensure that at least one plate will have well-spaced colonies.
15. Store the remaining transformation mix in the fridge. Additional cells may be plated out the next day, if desired.

Step 4: Analysing Transformants

16. An efficient reaction should produce several hundred colonies.
17. Take 2-6 white or light blue colonies and culture them overnight in LB medium containing 50 µg mL^{-1} ampicillin.
18. Isolate plasmid DNA
19. Sequence the construct with the M13 forward and M13 reverse primers. Both are supplied at 0.1 µg μL^{-1} in TE Buffer.

Primer	Sequence
M13 Forward	5'-GTAAAACGACGGCCAG-3'
M13 Reverse	5'-CAGGAAACAGCTATGAC-3'

APPENDIX III: DATA

Table A.1 *Lake Okaro Water Chemistry from the epilimnion, thermocline region (top of thermocline, 20 and 40 cm below), and the hypolimnion, August 2009 to August 2010.*

Level	Date	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	(NO_3^- + $NO_2^{-)}$-N (mg L^{-1})	PO^4-P (mg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	DOC (mg L^{-1})	Chl *a* (mg L^{-1})	Temperature (°C)
Epilimnion	14 August 2009							6		
	8 September 2009							8		11.7307
	13 September 2009									
	7 October 2009	0.3930	0.0063	0.0149	0.0420	0.8545	0.0856		25.77141	12.83
	13 October 2009									12.83
	10 November 2009	0.0697	0.0039	0.0021	0.0130			3		16.02
	17 November 2009	0.0393	0.0074	0.0785	0.0392	0.7803	0.0637	48		20.16
	24 November 2009	0.0049	0.0000	0.0200	0.0052	0.1176	0.5531	3	13.311	19.06
	2 December 2009	0.0613	0.0008	0.0900	0.0209	0.198	0.461		1.93213	18.45
	9 December 2009	0.0055	0.0001	0.0080	0.0325	0.4244	0.4777	2	11.74596	19.16
	16 December 2009	0.0106	0.0001	0.0037	0.0040	1.264	0.6744	3	3.869647	20.81
	22 December 2009	0.0176	0.0001	0.0104	0.0062	0.5528	0.3288	3	2.81	21.78
	29 December 2009	0.0103	0.0011	0.0405	0.0021	0.3038	0.3981		3.78	20.09
	5 January 2010	0.0061	0.0001	0.0219	0.0129	0.4558	0.3791	5	4.38	20.38
	12 January 2010	0.0235	0.0001	0.0082	0.0027	0.596	0.4355		0.792513	20.75
	19 January 2010	0.0065	0.0001	0.0050	0.0017	0.3618	0.3724		0.848063	

	26 January 2010	0.0087	0.0001	0.0600	0.0070	0.244	0.38		2.354647	
	2 February 2010							5		22.54
	16 February 2010	0.1031	0.0001	0.0103	0.0116	0.3924	0.3834		5.889647	22.47
	2 March 2010	0.0055	0.0001	0.0069	0.0032	0.262	0.2887		1.439	
	16 March 2010									19.63
	30 March 2010	0.0041	0.0010	0.0273	0.0036	0.237	0.3491		4.652565	19.60
	27 April 2010	0.0124	0.0001	0.0023	0.0036	0.4578	0.2981	2	2.582	15.95
	24 May 2010	0.0962	0.0007	0.0112	0.0098	0.556	0.3427		20.449	
	1 June 2009							4		12.23
	29 June 2010	0.3406	0.0010	0.0199	0.0387	0.8952	0.3366	3		10.08
	10 August 2010	0.2923	0.0020	0.0427	0.0284	0.8168	0.4159		9.517	8.53
Thermocline	7 October 2009	0.3100	0.0002	0.0248	0.0336					
	13 October 2009									11.32
	10 November 2009	0.2140	0.0007	0.0022	0.0190					11.32
	17 November 2009	0.1950	0.0003	0.0514	0.0317	0.7156	0.026	3		15.91
	24 November 2009	0.0037	0.0001	0.0110	0.0152	0.398	0.5849	2		18.53
	2 December 2009	0.0124	0.0001	0.0096	0.0052					19.14
	9 December 2009	0.0074	0.0001	0.0042	0.0080			3		18.31
	16 December 2009	0.0157	0.0001	0.0162	0.0045			3		19.85
	22 December 2009	0.0071	0.0001	0.0062	0.0049			4		20.37
	29 December 2009	0.0076	0.0001	0.0249	0.0078					21.96
	5 January 2010	0.0093	0.0001	0.0026	0.0049			4		20.33
	12 January 2010	0.0271	0.0001	0.0284	0.0021					20.37
	19 January 2010	0.0080	0.0001	0.0010	0.0062					20.55
	2 February 2010							4		22.33
	16 February 2010	0.0094	0.0001	0.0247	0.0006					22.58

	2 March 2010	0.0120	0.0010	0.0147	0.0048				
	16 March 2010								19.60
	30 March 2010								19.63
	27 April 2010	0.0532	0.0001	0.0020	0.0068				16.01
	1 June 2010								12.31
Thermocline +									
20cm	17 November 2009							2	
	24 November 2009	0.0056	0.0001	0.0220	0.0032	0.4594	0.4026	2	
	2 December 2009	0.0139	0.0001	0.0046	0.0181				
	9 December 2009	0.0112	0.0001	0.0087	0.0133			3	
	16 December 2009	0.0045	0.0001	0.0037	0.0042			3	
	22 December 2009	0.0032	0.0001	0.0178	0.0165			4	
	29 December 2009	0.0075	0.0001	0.0153	0.0035				
	5 January 2010	0.0101	0.0190	0.0365	0.0033			4	
	12 January 2010	0.0189	0.0001	0.0130	0.0019				
	19 January 2010	0.0062	0.0001	0.0040	0.0021				
	2 February 2010							5	
	16 February 2010	0.0056	0.0001	0.0145	0.0064				
	2 March 2010	0.0822	0.0010	0.0175	0.0202				
	16 March 2010								
	30 March 2010	0.0046	0.0001	0.0608	0.0049				
	27 April 2010	0.1108	0.0001	0.0826	0.0081				
	1 June 2010								
Thermocline +									
40cm	17 November 2009							3	
	24 November 2009	0.0015	0.0001	0.0080	0.0037	0.4966	0.7629	2	

	2 December 2009	0.0039	0.0001	0.0024	0.0058					
	9 December 2009	0.0107	0.0001	0.0023	0.0092			3		
	16 December 2009	0.0071	0.0031	0.0687	0.0042			3		
	22 December 2009	0.0044	0.0001	0.0061	0.0031			4		
	29 December 2009									
	5 January 2010	0.0426	0.0001	0.0197	0.0048			5		
	12 January 2010									
	19 January 2010	0.0053	0.0001	0.0027	0.0044					
	2 February 2010							4		
	16 February 2010	0.0049	0.0001	0.0078	0.0016					
	2 March 2010	0.0220	0.0010	0.0242	0.0054					
	30 March 2010	0.0099	0.0001	0.0058	0.0097					
	27 April 2010	0.6725	0.0001	0.0143	0.0303					
	1 June 2010									
Hypolimnion	14 August 2009							3		
	8 September 2009							5		
	7 October 2009	0.4960	0.0074	0.0785	0.0392	1.2729	0.377			
	13 October 2009								2.159506	8.95
	10 November 2009	0.4400	0.0037	0.0476	0.0316	1.5096	0.3073	2		8.95
	17 November 2009					0.8087	0.687	2		9.24
	24 November 2009	0.6112	0.0001	0.0020	0.0736	0.9214	0.6759	1	9.25463	9.49
	2 December 2009								4.423463	9.71
	9 December 2009	0.5180	0.0001	0.0035	0.1031	1.0826	0.766	3	6.27513	9.48
	16 December 2009	0.6016	0.0001	0.0025	0.0497	1.0426	0.8242	3	3.566647	9.57
	22 December 2009	0.8240	0.0001	0.0047	0.0849	1.1438	0.6195	5	4.89	9.69
	29 December 2009	0.4258	0.0001	0.0153	0.0435	0.4528	0.4553		8.09	9.75

5 January 2010	0.7133	0.0001	0.0278	0.0849	1.0752	0.4616	5	8.61	9.61
12 January 2010	0.6275	0.0001	0.0019	0.0654	1.2944	0.5248		8.145313	9.65
19 January 2010		0.0001	0.0026	0.0394	0.156	0.6435		4.29048	9.71
26 January 2010	0.7715	0.0001	0.0972	0.0810	1.3622	0.4734		12.05065	
2 February 2010							5		9.78
16 February 2010	0.5682	0.0001	0.0047	0.0652	0.7406	0.4403		6.51248	9.74
2 March 2010	1.0695	0.0001	0.0052	0.0740	1.3576	0.4956		15.567	
16 March 2010									9.74
30 March 2010	0.4909	0.0001	0.0077	0.0654	0.8798	0.4337		34.23159	9.79
27 April 2010	0.6822	0.0001	0.0019	0.0701	1.1966	0.5083	1	25.903	9.83
24 May 2010	0.2671	0.0001	0.0026	0.0314	0.6732	0.3711		24.219	
1 June 2010							4		10.03
29 June 2010	0.3946	0.0010	0.0193	0.0465	0.8706	0.4798	3.0000		
10 August 2010	0.4340	0.0010	0.0336	0.0521	0.8302	0.4572		5.023	

Table A.2 *Lake Tikitapu Water Chemistry from the epilimnion, thermocline region (top of thermocline and 20 and 40 cm below the thermocline) and the hypolimnion, August 2009 to August 2010.*

Level	Date	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	$(NO_3^- + NO_2^-)$-N (mg L^{-1})	PO^4-P (mg L^{-1})	TN (mg L^{-1})	TP (mg L^{-1})	DOC (mg L^{-1})	Chl *a* (mg L^{-1})	Temperature (°C)
Epilimnion	8 September 2009							4		10.8533
	7 October 2009					0.3212	0.0106			
	13 October 2009								0.412206	12.0302
	10 November 2009					0.262	0.0049	2		12.0302
	17 November 2009					0.2823	0.0001	2		15.3603
	24 November 2009	0.04555	0.0019	0.01962	0.0030	0.168	0.0144	3	1.04333	16.6021
	2 December 2009								7.571297	17.5739
	9 December 2009	0.03421	0.0001	0.09777	0.0033	0.2946	0.044	2	4.945297	17.4656
	16 December 2009	0.00633	0.0001	0.00386	0.0031	0.1738	0.0371	2	2.186313	18.609
	22 December 2009	0.00486	0.0001	0.00649	0.0041	0.2682	0.0259	3	3.67	17.8966
	29 December 2009	0.00500	0.0001	0.03444	0.0174	0.2484	0.0445		3.54	20.1039
	5 January 2010	0.04500	0.0020	0.06500	0.0090	0.2394	0.0197	3	2.91	18.9911
	12 January 2010	0.01153	0.0002	0.00684	0.0012	0.1864	0.0214		3.38148	19.6757
	19 January 2010	0.01399	0.0001	0.01735	0.0022	0.2314	0.019		2.489313	20.0189
	26 January 2010					0.2476	0.0136		0.233647	
	2 February 2010							5		21.0359
	16 February 2010	0.01732	0.0001	0.00985	0.0026	0.1632	0.0104		1.47763	21.6726
	2 March 2010	0.02320	0.0008	0.05888	0.0026	0.1954	0.0424		0.833	

	16 March 2010									19.6316
	30 March 2010	0.00433	0.0006	0.00617	0.0018				1.001	19.6316
	27 April 2010					0.2316	0.1144		1.4	16.462
	24 May 2010	0.02152	0.0001	0.00439	0.0033	0.218	0.0693		2.279	
	1 June 2010							5		13.3112
	29 June 2010	0.00683	0.0010	0.00488	0.0045	0.1834	0.5159		4.148	11.8404
	10 August 2010	0.00222	0.0001	0.00505	0.0138	0.164	0.4478		3.154	8.955
Thermocline	7 September 2009									9.75
	13 October 2009									11.27
	11 November 2009									11.43
	17 November 2009							65		14.98
	24 November 2009	0.01759	0.0001	0.00159	0.0033	0.1446	0.0398	1		15.19
	2 December 2009	0.00766	0.0001	0.00842	0.0027					17.38
	9 December 2009	0.02206	0.0003	0.19277	0.0040			7		17.01
	16 December 2009	0.01032	0.0001	0.22609	0.0032			2		18.87
	22 December 2009	0.01835	0.0001	0.16363	0.0032			2		
	29 December 2009	0.00502	0.0002	0.01251	0.0021					
	5 January 2010	0.01397	0.0006	0.00420	0.0020			2		19.84
	12 January 2010	0.01026	0.0001	0.00858	0.0015					19.96
	19 January 2010	0.01130	0.0001	0.00196	0.0017					19.97
	2 February 2010							5		20.28
	16 February 2010	0.01314	0.0001	0.00452	0.0014					21.76
	2 March 2010	0.01314	0.0010	0.03868	0.0040					
	16 March 2010									19.76
	30 March 2010	0.00309	0.0001	0.04450	0.0026					19.76
	27 April 2010	0.00104	0.0001	0.00413	0.0039					16.20

	1 June 2010								10.48
Thermocline +									
20cm	17 November 2009							1	
	24 November 2009	0.01412	0.0007	0.00401	0.0019	0.2296	0.0387	6	
	1 December 2009								
	9 December 2009							19	
	16 December 2009	0.01008	0.0001	0.03772	0.0025			2	
	22 December 2009	0.00758	0.0001	0.05628	0.0057			2	
	29 December 2009	0.00593	0.0001	0.02728	0.0053				
	5 January 2010	0.01249	0.0001	0.00331	0.0016			3	
	12 January 2010	0.01074	0.0001	0.00815	0.0025				
	19 January 2010	0.01245	0.0001	0.00303	0.0015				
	26 January 2010	0.01536	0.0006	0.03951	0.0025				
	2 February 2010							5	
	16 February 2010	0.01534	0.0001	0.00442	0.0015				
	2 March 2010	0.01344	0.0009	0.03000	0.0052				
	16 March 2010								
	30 March 2010	0.00527	0.0001	0.04443	0.0046				
	27 April 2010	0.00392	0.0001	0.01133	0.0033				
Thermocline +									
40cm	17 November 2009							1	
	24 November 2009					0.2752	0.0528	6	
	1 December 2009								
	9 December 2009	0.01166	0.0001	0.02620	0.0022			19	
	16 December 2009	0.00316	0.0001	0.30531	0.0034			2	
	22 December 2009	0.00718	0.0001	0.02329	0.0024			2	

	29 December 2009	0.01501	0.0005	0.16316	0.0050					
	5 January 2010	0.01385	0.0003	0.00390	0.0015			3		
	5 January 2010	0.01400	0.0001	0.00400	0.0020					
	12 January 2010	0.01367	0.0001	0.00512	0.0013					
	19 January 2010	0.01196	0.0001	0.00214	0.0013					
	2 February 2010							5		
	16 February 2010	0.01556	0.0001	0.00445	0.0011					
	2 March 2010	0.01231	0.0003	0.01871	0.0047					
	16 March 2010									
	30 March 2010	0.01154	0.0001	0.29885	0.0023					
	27 April 2010									
Hypolimnion	8 September 2009							6		9.12
	7 October 2009					0.4502	0.0001			
	13 October 2009								0.500581	9.90
	10 November 2009					0.3455	0.0098	1		9.90
	17 November 2009					0.3919	0.0266	2		12.13
	24 November 2009	0.01037	0.0001	0.00183	0.0041	0.2112	0.0364	1	2.016	10.14
	2 December 2009	0.00363	0.0001	0.00305	0.0045	0.3154	0.0503		13.71546	9.97
	9 December 2009	0.01393	0.0001	0.06818				3	2.268797	10.15
	16 December 2009	0.00746	0.0001	0.10590	0.0021	0.3732	0.018	2	5.418313	10.45
	22 December 2009	0.00393	0.0001	0.00217	0.0025	0.1784	0.0215	2	1.13	
	29 December 2009	0.01314	0.0003	0.08523	0.0218	0.242	0.0421		1.02	
	5 January 2010	0.01874	0.0006	0.11427	0.0018	0.2516	0.0148	3	5.28	12.10
	12 January 2010	0.01379	0.0001	0.01314	0.0010	0.2758	0.0506		6.091647	11.21
	19 January 2010								9.205813	10.57
	26 January 2010	0.01405	0.0001	0.02004	0.0021	0.196	0.0231		4.122147	

2 February 2010							5		10.78
16 February 2010	0.01449	0.0001	0.00191	0.0029				3.93698	11.16
2 March 2010	0.01725	0.0003	0.02165	0.0016	0.2338	0.0583		2.33	
16 March 2010									10.64
30 March 2010	0.00561	0.0001	0.05792	0.0016	0.1866	0.0478		2.431	
27 April 2010	0.00373	0.0001	0.00225	0.0053	0.2942	0.0459		5.427	10.64
24 May 2010	0.00780	0.0001	0.00219	0.0011	0.2146	0.0556		2.616	
1 June 2010							3		10.48
29 June 2010	0.00434	0.0001	0.04866	0.0698	0.1364	0.2936	3	4.501	
10 August 2010					0.185	0.8794		3.727	

Table A.3 *Summary statistics for regression analysis (R-square) of biotic and physiochemical factors in Lake Okaro epilimnion. Significance is if P≤0.05.*

	Log Chlorophyll-a	Log Bacteria	Log Virus	Log Algae	Log Temp	Log Conductivity	Long TN	Log TP	Log PO4-P	Log NH4-N	Log DOC	Log O2 sat	Log 02 conc	Log Fluorescence
Log Chlorophyll-a	1	.4911	.4761	.7304	.0621	-.2117	-.0976	-.5126	.2016	-.1773	.5601	-.0424	.0961	.7527
	p= ---	p=.216	p=.233	p=.040	p=.884	p=.615	p=.818	p=.194	p=.632	p=.674	p=.149	p=.921	p=.821	p=.031
Log Bacteria	.4911	1.0000	.5713	.0529	.2100	-.0181	-.1479	-.2908	.1860	-.0017	.6186	.1848	.4798	.3352
	p=.216	p= ---	p=.139	p=.901	p=.618	p=.966	p=.727	p=.485	p=.659	p=.997	p=.102	p=.661	p=.229	p=.417
Log Virus	.4761	.5713	1.0000	.1172	.6834	.4075	.2008	-.3303	.0288	-.5501	.6767	.5906	.7856	.6508
	p=.233	p=.139	p= ---	p=.782	p=.062	p=.316	p=.634	p=.424	p=.946	p=.158	p=.065	p=.123	p=.021	p=.081
Log Algae	.7304	.0529	.1172	1.0000	.2485	.1349	-.4024	-.1016	-.3239	-.4199	-.0454	.1334	-.2977	.3512
	p=.040	p=.901	p=.782	p= ---	p=.553	p=.750	p=.323	p=.811	p=.434	p=.300	p=.915	p=.753	p=.474	p=.394
Log Temp	.0621	.2100	.6834	.2485	1.0000	.8916	-.1972	.1716	-.5953	-.8903	.0460	.8444	.5463	.1401
	p=.884	p=.618	p=.062	p=.553	p= ---	p=.003	p=.640	p=.684	p=.119	p=.003	p=.914	p=.008	p=.161	p=.741
Log Conductivity	-.2117	-.0181	.4075	.1349	.8916	1.0000	-.1026	.5821	-.7194	-.8229	-.3617	.9067	.4212	-.2725
	p=.615	p=.966	p=.316	p=.750	p=.003	p= ---	p=.809	p=.130	p=.044	p=.012	p=.379	p=.002	p=.299	p=.514
Log TN	-.0976	-.1479	.2008	-.4024	-.1972	-.1026	1.0000	.0834	.2095	.2877	.1438	.1159	.3043	.0647
	p=.818	p=.727	p=.634	p=.323	p=.640	p=.809	p= ---	p=.844	p=.618	p=.490	p=.734	p=.785	p=.464	p=.879
Log TP	-.5126	-.2908	-.3303	-.1016	.1716	.5821	.0834	1.0000	-.6339	-.2101	-.8564	.4356	.0074	-.8660
	p=.194	p=.485	p=.424	p=.811	p=.684	p=.130	p=.844	p= ---	p=.091	p=.617	p=.007	p=.281	p=.986	p=.005

Log PO4-P	.2016	.1860	.0288	-.3239	-.5953	-.7194	.2095	-.6339	1.0000	.5912	.5642	-.5014	-.0827	.4138
	p=.632	p=.659	p=.946	p=.434	p=.119	p=.044	p=.618	p=.091	p= ---	p=.123	p=.145	p=.206	p=.846	p=.308
Log NH4-N	-.1773	-.0017	-.5501	-.4199	-.8903	-.8229	.2877	-.2101	.5912	1.0000	.0780	-.6654	-.4724	-.1604
	p=.674	p=.997	p=.158	p=.300	p=.003	p=.012	p=.490	p=.617	p=.123	p= ---	p=.854	p=.072	p=.237	p=.704
Log DOC	.5601	.6186	.6767	-.0454	.0460	-.3617	.1438	-.8564	.5642	.0780	1.0000	-.1417	.4542	.8657
	p=.149	p=.102	p=.065	p=.915	p=.914	p=.379	p=.734	p=.007	p=.145	p=.854	p= ---	p=.738	p=.258	p=.005
Log O2 sat	-.0424	.1848	.5906	.1334	.8444	.9067	.1159	.4356	-.5014	-.6654	-.1417	1.0000	.4514	-.1029
	p=.921	p=.661	p=.123	p=.753	p=.008	p=.002	p=.785	p=.281	p=.206	p=.072	p=.738	p= ---	p=.262	p=.808
Log O2 conc	.0961	.4798	.7856	-.2977	.5463	.4212	.3043	.0074	-.0827	-.4724	.4542	.4514	1.0000	.2609
	p=.821	p=.229	p=.021	p=.474	p=.161	p=.299	p=.464	p=.986	p=.846	p=.237	p=.258	p=.262	p= ---	p=.532
Log Fluorescence	.7527	.3352	.6508	.3512	.1401	-.2725	.0647	-.8660	.4138	-.1604	.8657	-.1029	.2609	1.0000
	p=.031	p=.417	p=.081	p=.394	p=.741	p=.514	p=.879	p=.005	p=.308	p=.704	p=.005	p=.808	p=.532	p= ---

Table A.4 *Summary statistics for regression analysis (R-square) of biotic and physiochemical factors in Lake Okaro hypolimnion. Significance is if P≤0.05.*

	Log Chlorophyll-a	Log Bacteria	Log Virus	Log Algae	Log Temp	Log Conductivity	Long TN	Log TP	Log PO4-P	Log NH4-N	Log DOC	Log O2 sat	Log 02 conc	Log Fluorescence
Log Chlorophyll-a	1.0000	.1448	.2620	.6548	-.4212	.5504	.3012	-.4900	-.2299	-.0731	-.3358	.0916	-.3557	.2473
	p= ---	p=.710	p=.496	p=.056	p=.259	p=.125	p=.431	p=.181	p=.552	p=.852	p=.377	p=.815	p=.347	p=.521
Log Bacteria	.1448	1.0000	.4919	.4413	-.0675	.2664	.0778	.5391	.3633	.1878	.3916	.2851	.5006	.2514
	p=.710	p= ---	p=.179	p=.234	p=.863	p=.488	p=.842	p=.134	p=.337	p=.628	p=.297	p=.457	p=.170	p=.514
Log Virus	.2620	.4919	1.0000	-.1700	-.8134	-.3440	.5555	.1912	.2562	.2637	.2634	-.4793	.2268	-.4383
	p=.496	p=.179	p= ---	p=.662	p=.008	p=.365	p=.120	p=.622	p=.506	p=.493	p=.494	p=.192	p=.557	p=.238
Log Algae	.6548	.4413	-.1700	1.0000	.2473	.8352	-.2476	-.2093	-.1361	-.2055	.0028	.6919	-.1879	.5967
	p=.056	p=.234	p=.662	p= ---	p=.521	p=.005	p=.521	p=.589	p=.727	p=.596	p=.994	p=.039	p=.628	p=.090
Log Temp	-.4212	-.0675	-.8134	.2473	1.0000	.4705	-.7795	.1589	.0710	-.1964	.1580	.7193	.2798	.6170
	p=.259	p=.863	p=.008	p=.521	p= ---	p=.201	p=.013	p=.683	p=.856	p=.612	p=.685	p=.029	p=.466	p=.077
Log Conductivity	.5504	.2664	-.3440	.8352	.4705	1.0000	-.3751	-.0518	.0374	-.0661	-.1438	.7996	.1152	.8881
	p=.125	p=.488	p=.365	p=.005	p=.201	p= ---	p=.320	p=.895	p=.924	p=.866	p=.712	p=.010	p=.768	p=.001
Log TN	.3012	.0778	.5555	-.2476	-.7795	-.3751	1.0000	-.0086	.2536	.6184	-.2691	-.6578	-.2073	-.2761
	p=.431	p=.842	p=.120	p=.521	p=.013	p=.320	p= ---	p=.983	p=.510	p=.076	p=.484	p=.054	p=.592	p=.472
Log TP	-.4900	.5391	.1912	-.2093	.1589	-.0518	-.0086	1.0000	.6524	.4958	.1318	.1466	.6428	.2172
	p=.181	p=.134	p=.622	p=.589	p=.683	p=.895	p=.983	p= ---	p=.057	p=.175	p=.735	p=.707	p=.062	p=.575

Log PO4-P	-.2299	.3633	.2562	-.1361	.0710	.0374	.2536	.6524	1.0000	.7563	.1703	-.0011	.5167	.3628
	p=.552	p=.337	p=.506	p=.727	p=.856	p=.924	p=.510	p=.057	p= ---	p=.018	p=.661	p=.998	p=.154	p=.337
Log NH4-N	-.0731	.1878	.2637	-.2055	-.1964	-.0661	.6184	.4958	.7563	1.0000	.0413	-.0820	.2283	.2469
	p=.852	p=.628	p=.493	p=.596	p=.612	p=.866	p=.076	p=.175	p=.018	p= ---	p=.916	p=.834	p=.555	p=.522
Log DOC	-.3358	.3916	.2634	.0028	.1580	-.1438	-.2691	.1318	.1703	.0413	1.0000	.2705	.4041	-.1375
	p=.377	p=.297	p=.494	p=.994	p=.685	p=.712	p=.484	p=.735	p=.661	p=.916	p= ---	p=.481	p=.281	p=.724
Log O2 sat	.0916	.2851	-.4793	.6919	.7193	.7996	-.6578	.1466	-.0011	-.0820	.2705	1.0000	.1957	.7407
	p=.815	p=.457	p=.192	p=.039	p=.029	p=.010	p=.054	p=.707	p=.998	p=.834	p=.481	p= ---	p=.614	p=.022
Log O2 conc	-.3557	.5006	.2268	-.1879	.2798	.1152	-.2073	.6428	.5167	.2283	.4041	.1957	1.0000	.3292
	p=.347	p=.170	p=.557	p=.628	p=.466	p=.768	p=.592	p=.062	p=.154	p=.555	p=.281	p=.614	p= ---	p=.387
Log Fluorescence	.2514	-.4383	.5967	.6170	.8881	-.2761	.2172	.3628	.2469	-.1375	.7407	.3292	1.0000	1.0000
	p=.514	p=.238	p=.090	p=.077	p=.001	p=.472	p=.575	p=.337	p=.522	p=.724	p=.022	p=.387	p= ---	p= ---

Table A.5 *Summary statistics for regression analysis (R-square) of biotic and physiochemical factors in Lake Tikitapu epilimnion. Significance is if P≤0.05.*

	Log Bacteria	Log Virus	Log Algae	Log Temp	Log Conductivity	Log TN	Log TP	Log PO4-P	Log NH4-N	Log DOC	Log O2 sat	Log O2 conc	Log Fluorescence
Log Chlorophyll-a	.5569	.9064	.4222	.0288	.1419	.9549	.0796	-.0319	.6630	-.6119	.5556	.2299	-.1035
	p=.443	p=.094	p=.578	p=.971	p=.858	p=.045	p=.920	p=.968	p=.337	p=.388	p=.444	p=.770	p=.896
Log Bacteria	1.0000	.7473	.9762	.8385	.8894	.4658	-.5966	.2373	.2368	-.5132	.0937	.9364	.5643
	p= ---	p=.253	p=.024	p=.162	p=.111	p=.534	p=.403	p=.763	p=.763	p=.487	p=.906	p=.064	p=.436
Log Virus	.7473	1.0000	.6922	.2735	.4645	.7550	.0394	-.2296	.3311	-.8482	.1866	.4918	.3247
	p=.253	p= ---	p=.308	p=.726	p=.536	p=.245	p=.961	p=.770	p=.669	p=.152	p=.813	p=.508	p=.675
Log Algae	.9762	.6922	1.0000	.8737	.9572	.2864	-.5539	.1082	.0205	-.5658	-.1237	.9649	.7292
	p=.024	p=.308	p= ---	p=.126	p=.043	p=.714	p=.446	p=.892	p=.979	p=.434	p=.876	p=.035	p=.271
Log Temp	.8385	.2735	.8737	1.0000	.9474	-.0120	-.8482	.4334	-.0386	-.1187	-.1330	.9707	.6487
	p=.162	p=.726	p=.126	p= ---	p=.053	p=.988	p=.152	p=.567	p=.961	p=.881	p=.867	p=.029	p=.351
Log Conductivity	.8894	.4645	.9572	.9474	1.0000	.0102	-.6365	.1365	-.1835	-.4166	-.3063	.9817	.8251
	p=.111	p=.536	p=.043	p=.053	p= ---	p=.990	p=.364	p=.863	p=.817	p=.583	p=.694	p=.018	p=.175
Log TN	.4658	.7550	.2864	-.0120	.0102	1.0000	-.0431	.2171	.8550	-.3495	.7771	.1431	-.3456
	p=.534	p=.245	p=.714	p=.988	p=.990	p= ---	p=.957	p=.783	p=.145	p=.650	p=.223	p=.857	p=.654
Log TP	-.5966	.0394	-.5539	-.8482	-.6365	-.0431	1.0000	-.8397	-.2857	-.3701	-.2537	-.7346	-.1730
	p=.403	p=.961	p=.446	p=.152	p=.364	p=.957	p= ---	p=.160	p=.714	p=.630	p=.746	p=.265	p=.827
Log PO4-P	.2373	-.2296	.1082	.4334	.1365	.2171	-.8397	1.0000	.6256	.6817	.6537	.2943	-.3895

	p=.763	p=.770	p=.892	p=.567	p=.863	p=.783	p=.160	p= ---	p=.374	p=.318	p=.346	p=.706	p=.611
Log NH4-N	.2368	.3311	.0205	-.0386	-.1835	.8550	-.2857	.6256	1.0000	.1857	.9875	-.0005	-.6669
	p=.763	p=.669	p=.979	p=.961	p=.817	p=.145	p=.714	p=.374	p= ---	p=.814	p=.013	p=.999	p=.333
Log DOC	-.5132	-.8482	-.5658	-.1187	-.4166	-.3495	-.3701	.6817	.1857	1.0000	.3168	-.3405	-.5918
	p=.487	p=.152	p=.434	p=.881	p=.583	p=.650	p=.630	p=.318	p=.814	p= ---	p=.683	p=.660	p=.408
Log O2 sat	.0937	.1866	-.1237	-.1330	-.3063	.7771	-.2537	.6537	.9875	.3168	1.0000	-.1227	-.7691
	p=.906	p=.813	p=.876	p=.867	p=.694	p=.223	p=.746	p=.346	p=.013	p=.683	p= ---	p=.877	p=.231
Log O2 conc	.9364	.4918	.9649	.9707	.9817	.1431	-.7346	.2943	-.0005	-.3405	-.1227	1.0000	.7030
	p=.064	p=.508	p=.035	p=.029	p=.018	p=.857	p=.265	p=.706	p=.999	p=.660	p=.877	p= ---	p=.297
Log Fluorescence	.5643	.3247	.7292	.6487	.8251	-.3456	-.1730	-.3895	-.6669	-.5918	-.7691	.7030	1.0000
	p=.436	p=.675	p=.271	p=.351	p=.175	p=.654	p=.827	p=.611	p=.333	p=.408	p=.231	p=.297	p= ---

Table A.6 *Summary statistics for regression analysis (R-square) of biotic and physiochemical factors in Lake Tikitapu hypolimnion. Significance is if* $P \leq 0.05$.

	Log Chlorophyll-a	Log Bacteria	Log Virus	Log Algae	Log Temp	Log Conductivity	Log TN	Log TP	Log PO4-P	Log NH4-N	Log DOC	Log O2 sat	Log 02 conc	Log Fluorescence
Log Chlorophyll-a	1.0000	-.6065	-.3736	0	.6659	-.4023	.2136	.0469	.1314	.0410	.6952	.4720	.0894	.4625
	p= ---	p=.202	p=.466	p=.910	p=.149	p=.429	p=.684	p=.930	p=.804	p=.938	p=.125	p=.345	p=.866	p=.356
Log Bacteria	-.6065	1.0000	.4872	-.1838	-.0863	.7317	-.2633	.0742	-.4155	.2402	.1335	-.6740	-.3033	-.5032
	p=.202	p= ---	p=.327	p=.727	p=.871	p=.098	p=.614	p=.889	p=.413	p=.647	p=.801	p=.142	p=.559	p=.309
Log Virus	-.3736	.4872	1.0000	-.0604	-.3595	.3964	.6586	-.5952	-.9238	.5448	-.0333	-.4822	.4979	-.2623
	p=.466	p=.327	p= ---	p=.910	p=.484	p=.437	p=.155	p=.213	p=.008	p=.264	p=.950	p=.333	p=.315	p=.616
Log Algae	.0602	-.1838	-.0604	1.0000	.3624	-.7812	.0059	-.2692	.0282	.6231	-.2129	.8111	.6488	.8960
	p=.910	p=.727	p=.910	p= ---	p=.480	p=.067	p=.991	p=.606	p=.958	p=.186	p=.685	p=.050	p=.163	p=.016
Log Temp	.6659	-.0863	-.3595	.3624	1.0000	-.2446	-.0920	-.0645	.0283	.4477	.6460	.4285	.0318	.5849
	p=.149	p=.871	p=.484	p=.480	p= ---	p=.640	p=.862	p=.903	p=.957	p=.373	p=.166	p=.397	p=.952	p=.223
Log Conductivity	-.4023	.7317	.3964	-.7812	-.2446	1.0000	-.0520	.0714	-.3793	-.1697	.2139	-.9778	-.5433	-.8853
	p=.429	p=.098	p=.437	p=.067	p=.640	p= ---	p=.922	p=.893	p=.458	p=.748	p=.684	p=.001	p=.265	p=.019
Log TN	.2136	-.2633	.6586	.0059	-.0920	-.0520	1.0000	-.8392	-.7590	.4988	.0035	-.0391	.7511	.1310
	p=.684	p=.614	p=.155	p=.991	p=.862	p=.922	p= ---	p=.037	p=.080	p=.314	p=.995	p=.941	p=.085	p=.805
Log TP	.0469	.0742	-.5952	-.2692	-.0645	.0714	-.8392	1.0000	.7524	-.7373	.2277	-.0361	-.7985	-.2891
	p=.930	p=.889	p=.213	p=.606	p=.903	p=.893	p=.037	p= ---	p=.084	p=.094	p=.664	p=.946	p=.057	p=.578

Log PO4-P	.1314	-.4155	-.9238	.0282	.0283	-.3793	-.7590	.7524	1.0000	-.7135	-.1638	.4076	-.5438	.1117
	p=.804	p=.413	p=.008	p=.958	p=.957	p=.458	p=.080	p=.084	p= ---	p=.111	p=.757	p=.422	p=.265	p=.833
Log NH4-N	.0410	.2402	.5448	.6231	.4477	-.1697	.4988	-.7373	-.7135	1.0000	.1285	.2234	.7362	.5419
	p=.938	p=.647	p=.264	p=.186	p=.373	p=.748	p=.314	p=.094	p=.111	p= ---	p=.808	p=.671	p=.095	p=.267
Log DOC	.6952	.1335	-.0333	-.2129	.6460	.2139	.0035	.2277	-.1638	.1285	1.0000	-.0938	-.2510	.0059
	p=.125	p=.801	p=.950	p=.685	p=.166	p=.684	p=.995	p=.664	p=.757	p=.808	p= ---	p=.860	p=.631	p=.991
Log O2 sat	.4720	-.6740	-.4822	.8111	.4285	-.9778	-.0391	-.0361	.4076	.2234	-.0938	1.0000	.4695	.9365
	p=.345	p=.142	p=.333	p=.050	p=.397	p=.001	p=.941	p=.946	p=.422	p=.671	p=.860	p= ---	p=.347	p=.006
Log O2 conc	.0894	-.3033	.4979	.6488	.0318	-.5433	.7511	-.7985	-.5438	.7362	-.2510	.4695	1.0000	.6315
	p=.866	p=.559	p=.315	p=.163	p=.952	p=.265	p=.085	p=.057	p=.265	p=.095	p=.631	p=.347	p= ---	p=.179
Log Fluoresence	.4625	-.5032	-.2623	.8960	.5849	-.8853	.1310	-.2891	.1117	.5419	.0059	.9365	.6315	1.0000
	p=.356	p=.309	p=.616	p=.016	p=.223	p=.019	p=.805	p=.578	p=.833	p=.267	p=.991	p=.006	p=.179	p= ---

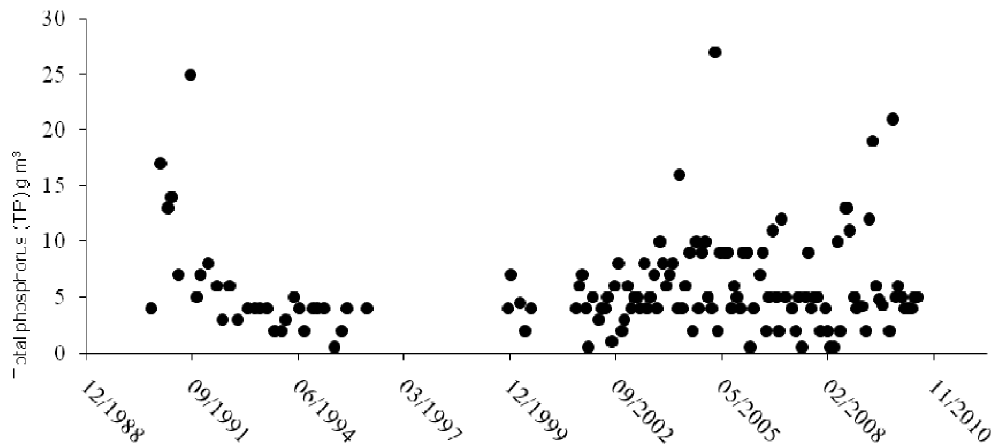


Figure A.1 *Total phosphorus (TP) concentrations at the surface of Lake Tikitapu, 29 August 1990 to 16 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

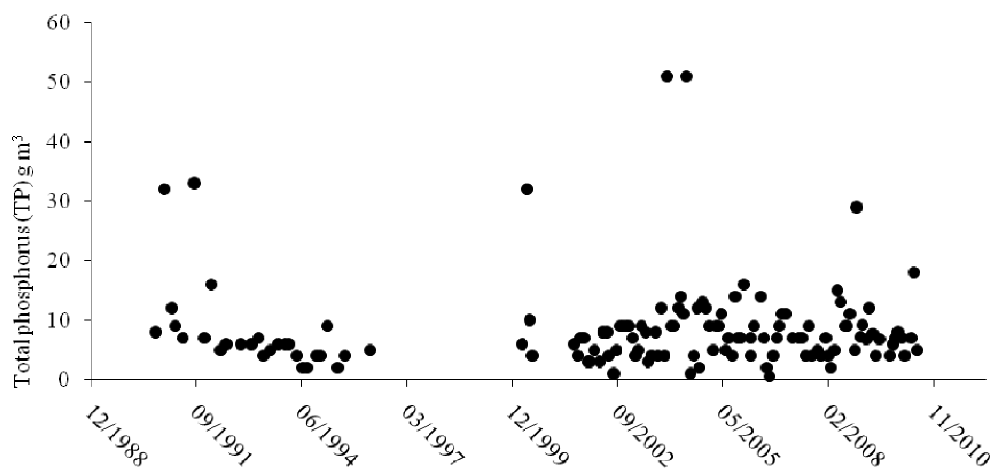


Figure A.2 *Total phosphorus (TP) concentrations in the bottom waters of Lake Tikitapu, 29 August 1990 to 16 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

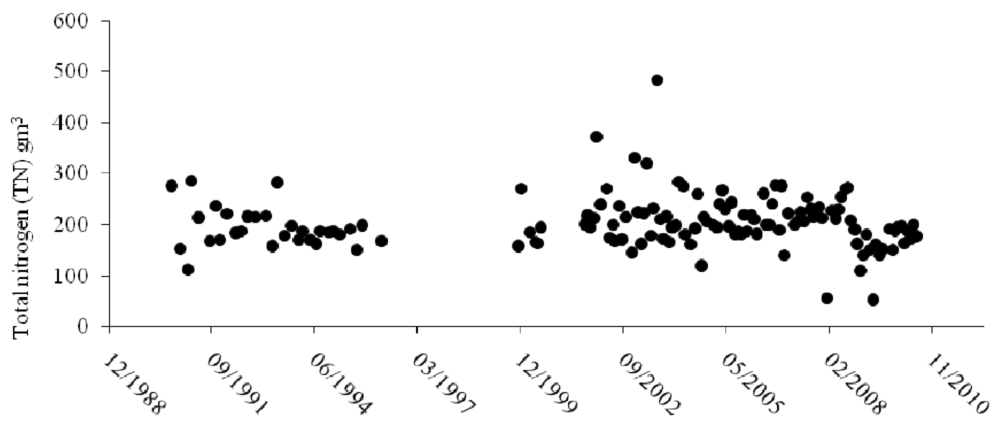


Figure A.3 *Total nitrogen (TN) concentrations at the surface of Lake Tikitapu, 29 August 1990 to 16 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

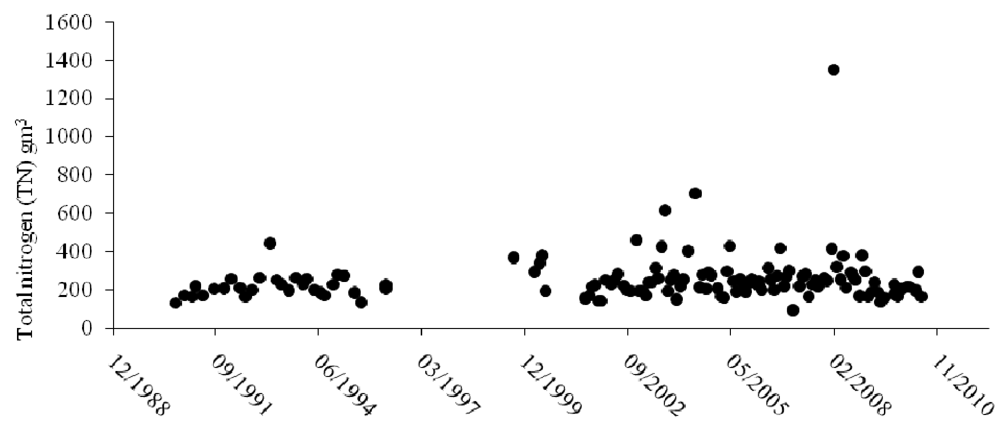


Figure A.4 *Total nitrogen (TN) concentrations at the surface of Lake Tikitapu, 29 August 1990 to 16 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

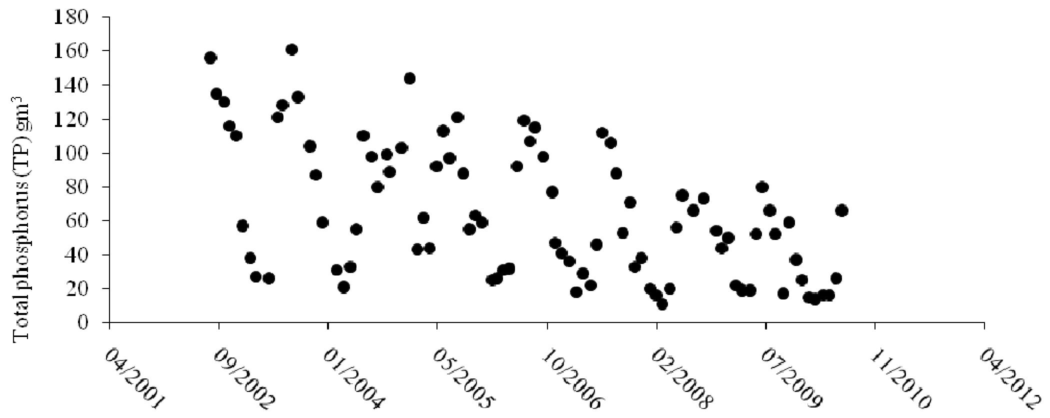


Figure A.6 *Total phosphorus (TP) concentrations at the surface of Lake Okaro, 13 July 2000 to 17 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

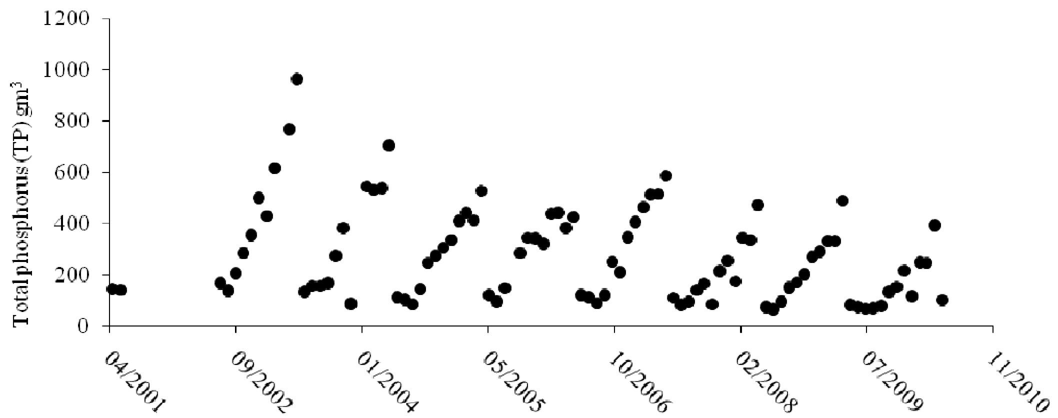


Figure A.7 *Total phosphorus (TP) concentrations in the bottom waters of Lake Okaro, 13 July 2000 to 17 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

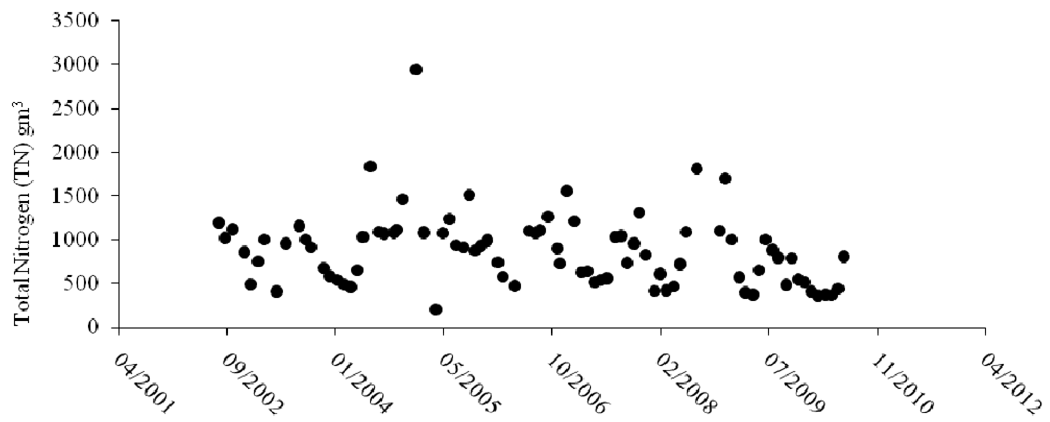


Figure A.6 *Total nitrogen (TN) concentrations at the surface of Lake Okaro, 13 July 2000 to 17 June 2010. (Data courtesy of Bay of Plenty Regional Council).*

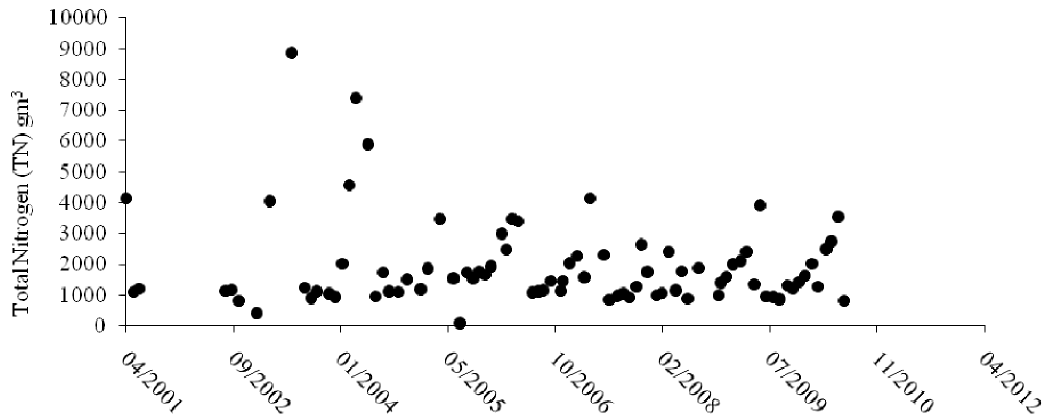
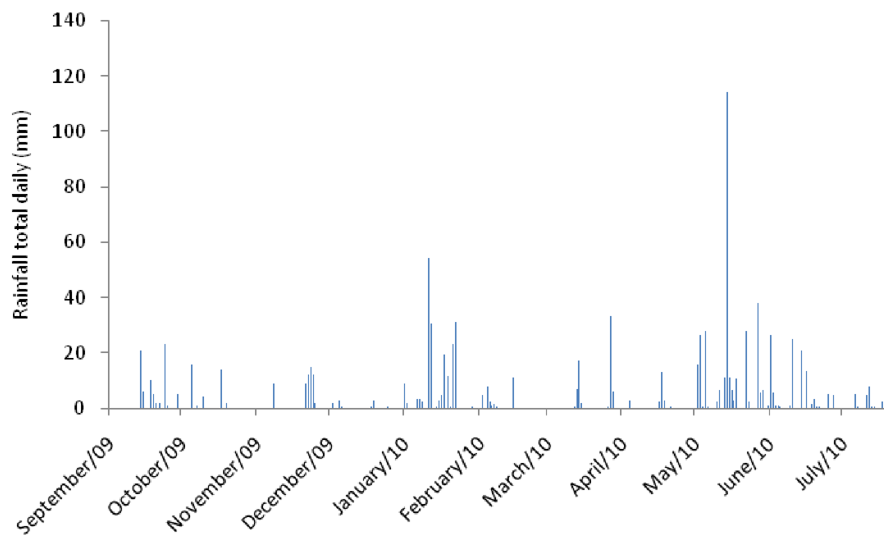


Figure A.6 *Total nitrogen (TN) concentrations in the bottom waters of Lake Okaro, 13 July 2000 to 17 June 2010. (Data courtesy of Bay of Plenty Regional Council).*



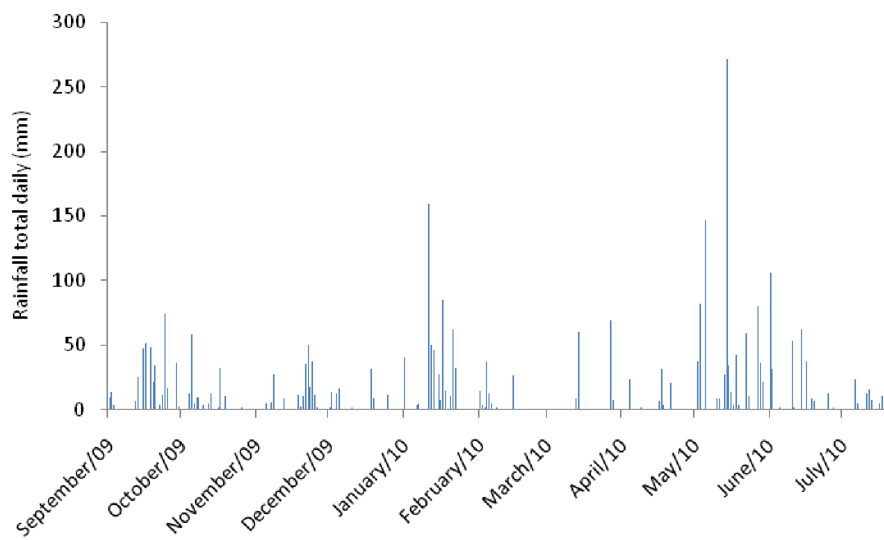


Figure A.7 *Rainfall data as total daily volume (mm) for a) Lake Okaro and b) Lake Tikitapu, September 2009 to July 2010. (Data courtesy of Bay of Plenty Regional Council).*

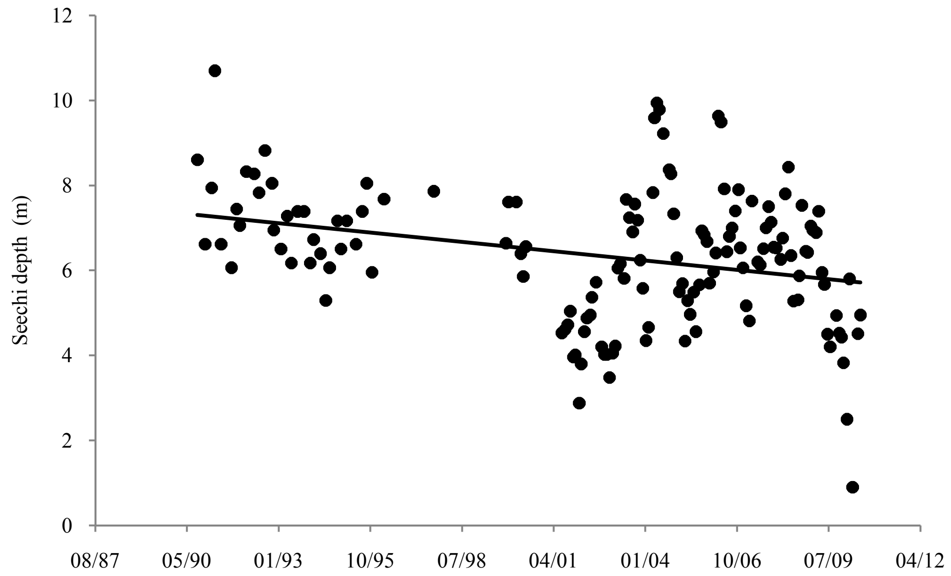


Figure A.8 *Secchi depth visibility (viz water clarity) measurements in Lake Tikitapu from August 1987 to August 2010. Data courtesy of Bay of Plenty Regional Council.*