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Correlations Between A Cyanobacteria Bloom's Decline and Environmental Dynamics.

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Abstract

Lake Kainui is an ombrogenous peat lake that is eutrophic and has frequent cyanobacterial blooms. This research aimed to elucidate the factors that correlated with cyanobacterial bloom decline in Lake Kainui. Abiotic and biotic variables in Lake Kainui were monitored weekly across the bloom decline phase. Physicochemical parameters measured included nutrients, micronutrients, water temperature, dissolved oxygen (DO), pH and meteorological variables. Planktonic and particle-associated bacterial community composition (BCC) was assessed through time using molecular fingerprinting (ARISA). Total algal, cyanobacterial, planktonic bacteria, planktonic viruses and planktonic crustacean abundances were determined using microscopy.

The bloom decline coincided with multiple factors including; an increasing ratio of TN:TP, changes in the ratio of mixing depth (z_{mix}) to euphotic depth (z_{eu}) and pH. The lake was stratified for several weeks before the decline and the resulting lake stability potentially created an adverse environment for cyanobacterial dominance. Changes in planktonic biota were various. Virus dynamics were positively correlated with phytoplankton dynamics. If virus abundance was negatively correlated to phytoplankton diversity, it would potentially mean that viruses were controlling the phytoplankton assemblage, however virus abundance was positively correlated to phytoplankton diversity, which suggests that viruses had a positive impact on the bloom. The positive effect was most likely due to to viruses upregulating the amount of dissolved organic matter through lysis. Bacterial abundance showed no clear correlation with other variables, but

bacterial community composition (BCC) shifted as the bloom declined. Planktonic Cladocera spiked just after the bloom decline. *Bosmina* was the dominant species of Cladocera, but was probably too small to ingest colonial *Microcystis*, which suggests that the *Bosmina* may be consuming degradation products produced by *Microcystis*. These observations indicate that if planktonic biota played a role in bloom dynamics it was most likely due to shifts in the biota causing shifts in trophic cycling that alternately favour or disfavour blooming.

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Chapter 1. Literature Review. Explaining cyanobacterial bloom decline.

1.1 The cyanobacteria and cyanbacterial decline.

In a pelagic lake ecosystem the cyanobacteria are distinguished from the majority of other phytoplankton because they are photosynthesizing prokaryotes. Cyanobacteria can exist as either colonies or as single cells suspended in the water column. Being prokaryotes, the cyanobacteria are evolutionarily distal to the other phytoplankton and so it can be anticipated that they have many morphological and behavioural adaptations that differentiate them from algae and are able to exploit different facets of what is, superficially, the same ecological niche. Like other phytoplankton they are able to form large and persistent blooms, where one or more species visibly dominates the lake water. These blooms can suddenly and dramatically crash (Havens, 2008).   Explanations of such terminations can be couched in a reversal of conditions favouring the bloom, such as the limitation of nutrients (Seip 2000; Huisman and Hulot, 2005), limiting micronutrients (Reynolds, 2006), lake mixing (Steinberg and Hartmann, 1988), shading or excessive irradiance (Sigee, 2005). Alternately, the cause of a bloom crash could be because of stress caused by excessive predation.   This predation stress could be from a sudden increase of grazing planktonic organisms such as protozoa, heterotrophic nanoflagelates, ciliates (Brabrand et al., 1983) and crustacea (Gliwicz, 2004, Sigee, 2005).   Alternately, it could be from bacteria or viruses.

This literature review addresses these various causes of bloom decline. It first surveys the three axioms that are conventionally used to explain bloom dynamics:

meteorology, lake physico-chemistry and grazing. It then separately surveys the importance of bacteria and viruses as biological forces capable of terminating and regulating blooms.

1.2 The three best axioms for explaining blooms: meteorology, lake physico-chemistry and grazing rates.

Abiotic factors in cyanobacterial dynamics.

Cyanobacteria are favored by certain environmental conditions that occur throughout the natural cycle of a lake. Many of these would favor any phytoplankton, for example abundant nutrients, however, cyanobacteria can also become abundant in conditions that would exclude other phytoplankton. Therefore, if cyanobacterial succession or crashes are caused by abiotic factors it is likely due to either the environment becoming too harsh or, possibly, because the environment returns to being benign and they lose their competitive advantage. Blooms themselves can create an environmental shift that selects for cyanobacteria, either sustaining the current population or selecting for a change in cyanobacterial composition (Scheffer, 1998).

1.2.1 Nutrients

Across a broad range of lake morphologies it appears that total phosphorus (TP) levels less than 20 mg m^{-3} will limit levels of chlorophyll-a (*chl-a*) production (Seip et al., 2000). For levels between 20 and 200 mg m^{-3} the driving factors become unclear (Seip et al., 2000). Cyanobacteria are generally out-competed

when phosphorus is limiting, though there are exceptions. Picocyanobacteria have superior phosphorus uptake kinetics due to their size (Huisman and Hulot, 2005). *Planktolyngbya*, *Anabaena* and *Aphanizomenon* can have a competitive advantage in environments where phosphorus is complexed to organic matter because they produce an alkaline phosphatase that can liberate the nutrient (Huisman and Hulot, 2005).

Generally a low ratio of total nitrogen to total phosphorus ratio (TN/TP) favors sustained dominance of cyanobacteria (Smith, 1983), however, the importance of this driver varies between studies and between species (Dokulil and Teubner 2000). Cyanobacterial species that can fix nitrogen are favored where concentrations of dissolved organic nitrogen are low enough that the physiological expense of nitrogen fixation is favoured (Huisman and Hulot, 2005). This is corroborated by an extensive literature review by Hornes and Commins (1987) that indicated that nitrogen concentration needs to fall below 50-100 $mg\,m^{-3}$ before nitrogenase activity is induced. During lake stratification there is often a much greater reservoir of dissolved nutrients below the epilimnion, so cyanobacteria that can regulate buoyancy are favoured because they can control their exposure to sunlight and take advantage of nutrients occurring down to the sediment interface (Reynolds, 2006). Cyanobacteria that do not fix nitrogen tend to be favoured when the dominant dissolved form of nitrogen is ammonium and algae tend to be favoured when nitrate dominates (Oliver and Ganf, 2000). Nitrogen is used in synthesis of gas vacuoles, therefore nitrogen depletion can mean that species that rely on buoyancy lose some competitive advantage (Oliver and Ganf, 2000)

Micronutrients have been less well studied, but trial and error development of laboratory media has indicated that some trace element levels limit cyanobacterial growth (Reynolds, 2006). Many ions that constitute cyanobacteria are available in lake water at concentrations that far exceed cellular requirements, including; sodium, potassium, calcium, magnesium, and chlorine (Reynolds, 2006). Trace metals that might be in concentrations limiting to cyanobacteria include; iron, manganese, zinc, copper, molybdenum and cobalt (Reynolds, 2006). Iron, molybdenum, cadmium, cobalt, manganese, zinc and boron limitation have occasionally been observed *in situ* or in mesocosm studies (Hyenstrand et al., 2001; Huisman and Hulot, 2005; Reynolds, 2006). Reuter and Pettersson (1993) have shown how cyanobacteria have a greater dependence on micronutrients than Eukaryotic algae therefore, changes in micronutrient concentrations may cause succession to algae (Dokulil and Teubner, 2000).

1.2.2 Physical and metrological factors impacting bloom species.

pH

The most important effect of pH on cyanobacteria is an indirect one, where pH influences CO_2 concentrations in the water. A high pH is generally an advantage to cyanobacteria because of their efficient CO_2 uptake (Dokulil and Teubner 2000). Shapiro (1997) has shown that low-CO_2/high-pH is not an important factor in initiating blooms but it can favor the dominance of cyanobacteria. That high pH favours cyanobacteria is hysteretic, because it is cyanobacterial photosynthesis that most likely raised the pH. Photosynthesis raises pH because it

removes CO_2 and because proton concentration in water is in equilibrium with CO_2 concentration pH is increased (Hamilton et al., 2004).

Dissolved oxygen (DO)

Oxygen depletion of the water column has been shown to competitively favour cyanobacteria (Trimbee and Prepas, 1988). Cyanobacterial blooms can also cause oxygen depletion in subsurface waters because blooms increase the concentration of organic matter passing through the water column and this matter consumes oxygen as it is decomposed. This is another example of hysteresis, where blooms create an environment that selects for them.

Sunlight and Irradiance

Whether cyanobacteria are favoured by low light conditions depends on the species of cyanobacteria. *Planktothorix agardhii* is low light adapted, *Microcystis* and *Aphanizomenon* can grow in higher light environments (Huisman and Hulot, 2005). The photosynthetic efficiency of cyanobacteria at low light levels might come at the price of being damaged at high light levels because the phycobilisomes that increase the amount of harvested light might be readily photo-oxidised (Mur, 1983). Photoinhibition of cyanobacteria has been shown even at reasonably low light levels (Mur, 1983). Levels of irradience are important in selecting which phytoplankton dominates the assemblage. Maximum rate of photosynthesis in cyanobacteria occurs at a very low and efficient irradiance level of 5 µmol of photons $m^{-2}s^{-1}$, and cells become photo-inhibited at over 200 µmol photons $m^{-2}s^{-1}$ (Horne and Goldman 1994 reproduced in Sigee, 2005).

Cyanobacteria also express protective enzymes against phototoxidation and their ability to do so might influence their vulnerability to decline or succession. These same photoprotective enzymes might indirectly affect community succession. For example, Shilo (1982) observed that under high irradiance *Microcystis* made a Manganese based superoxide dismutase (SOD) persisted while those that made only the ferric based SOD isoenzyme would succumb to photooxidation. It is likely that photoprotection is linked to micronutrient availability because of the requirement for enzyme co-factors.

Temperature

Cyanobacteria exist across a wide temperature range. However there is an assumption that bloom-forming cyanobacteria tend to tolerate high temperatures where they can out-compete other phytoplankton (Dokulil and Teubner, 2000). Oliver and Ganf (2000) identified that assumptions that cyanobacteria perform better than algae under higher temperatures are inferred from environmental studies. The problem is that these studies did not sufficiently establish that decline was not because of an effect of high temperatures, such as lake stabilization, rather than temperature *per se*. Oliver and Ganf (2000) conclude that assumptions about the negative affects of high temperature are unfounded.

Stratification and Mixing

Lake mixing can be forced by wind turbulence acting on the water surface (Reynolds, 1989). Diurnal changes in atmospheric temperature relative to water column temperature also cause lake mixing by convection (Reynolds, 1989).

Lake mixing might be an important factor controlling *Microcystis* distribution, which are fairly robust against changes in light and nutrients. Dokulil and Teubner (2000) hypothesise that water mixing could have a detrimental effect on the genus by robbing it of the selective advantages of buoyancy. Although light mixing gives buoyant cyanobacteria an advantage so long as the mixing force fails to entrain the cyanobacteria in the turbulent motion it creates (Huisman and Hulot, 2005). Lake turbulence can create shear forces that may break cyanobacteria cell walls or destroy colonial integrity (Moisander et. al., 2002).

Mucilage is a mass of loosely associated hydrophilic polysaccharides that have been suggested to play a role in plankton buoyancy. These polysaccharides are by themselves relatively dense, but when saturated with water they have a density near that of water, facilitating the cells control of buoyancy in the water column (Reynolds, 2006). Cells that regulate their buoyancy can do so by educing existing vacuoles through a turgor pressure-collapse mechanism (Reynolds, 1989). Alternatively a cell also can catabolise the photosynthetically derived polysaccharide that ballast the cell (Reynolds, 1989).

Mixing might also be disadvantageous to photoautotrophs because it prevents them from adapting the expression levels of photoprotective pigments. For example, laboratory and field observations suggest that a reason why *Microcystis aeruginosa* blooms are favoured by periods of lake stratification is that they have time to optimise their photosystems to the local irradience levels, any significant mixing prevents this from occurring (Oliver and Ganf, 2000).

1.2.3 Top Down Control: Grazing.

Zooplankton

The most widespread and significant phytoplankton grazers are the crustacea (Reynolds, 1997) and the remainder of this section focuses on them. Some eutrophic lakes experience a 'summer clear-water' phase despite optimum bloom conditions (Lampert and Sommer, 2007). Grazer's have often been observed to have a role in this phenomenon (Lampert and Sommer, 2007). The grazers that are likely to most significantly impact phytoplankton populations are filter feeding, pelagic cladocerans such as *Daphnia* and *Bosmina* (Gliwicz, 2004). However, the nutritional value of cyanobacteria is low. The phosphate concentration of cyanobacteria is variable, though it tends to reflect the phosphate richness of its environment (Huisman and Hulot, 2005). Therefore in eutrophic waters phosphate levels should be sufficient to sustain a grazing population and it is most likely the low intra-cellular concentration of poly-unsaturated fatty acids in cyanobacteria that is the main obstacle to a bloom supporting a grazing population (Huisman and Hulot, 2005).

Copepods are precise in their food selection and timed in their reproduction and feeding rates (Reynolds, 1997). Therefore, although copepods exert a little control on a prey species, they are unlikely to have a devastating impact on their prey if it is already at bloom proportions. In contrast, cladocerans have more indiscriminant feeding patterns, *Daphnia* for instance, will consume until their food source is exhausted and then starve (Reynolds, 1997). Laboratory studies have shown that cladocerans such as *Bosmina* may be capable of discriminating food, however they rarely, if ever, reject a food even if it is of poor nutritional

value. This is because the energetics of evacuating food from their feeding apparatus makes the rejection too costly (Gliwicz, 2004). Being prolific and (practically) indiscriminant feeders means that they have a more predictable impact on lower trophic levels, because they remove everything that is inside the upper and lower size limits of their feeding apparatus (Gliwicz, 2004).

Phytoplankton have various defensive phenotypes against grazing. Filamentous colonies are difficult for filter-feeders to manipulate for ingestion, though larger cladocerans are able to overcome this by fragmenting the colonies (Gliwicz, 2004). The excretion of extra-cellular mucilage could be a defense that makes a colony too large to be filtered. The large size of many colonial cyanobacteria may also exclude them from being grazed (Reynolds, 2006). However, Yang et al. (2006) found that the number of single cells in cultures of *Microcystis aeruginosa* remained relatively high when grazed by crustacea and that colony formation only occurred when cells were grazed by a protozoan. Another defensive feature of mucilage is that it makes ingested cells relatively impervious to digestion. It has been hypothesized that some Chroococales might even passage through the gut of zooplankton just to obtain limiting nutrients (Gliwicz, 2004).

Intracellular toxins have received considerable attention as a potential defense from grazing. Some cyanobacterial isolates have been shown to concentrate higher quantities of toxin in the presence of grazers, which suggests that this is a defense trait (Jang et al., 2003). Cladocerans might be able to detect prey toxicity but they still continue to feed indiscriminately on the cells, although at a lower rate (Gliwicz, 2004). The longevity of a cladoceran community feeding on toxic

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phytoplankton is therefore proportionate to its ability to withstand poisoning and to supplement its diet with benign cells.

Zooplankton can have a positive impact on the photosynthetic community that they do not consume through grazing. Cladocerans in particular increase the concentration of soluble nutrients in the water column making these available to nanoplankton (Gliwcz, 2004; Lambert and Sommer, 2007). It has been speculated that the mechanism for this increase in soluble nutrients could be that the cladocera excrete nutrients to 'farm' their prey, however, the hypothesis that is most favoured is the 'sloppy feeder' hypothesis – that is, cladocera allow the resuspension of partially digested prey cells including soluble nutrients and enzymes such as phosphotases (Gliwcz, 2004; Lambert and Sommer, 2007). The 'farming' of prey is therefore only an accidental offshoot of the grazers' 'sloppy feeding'.

1.3 Cyano-lytic Bacteria

1.3.1 Taxonomy of cyano-lytic bacteria

Almost all orders in the bacterial domain contain clusters of bacteria that prey on other bacteria (Martin, 2002; Jurkevitch, 2007). Examples of cyanolytic-bacteria are represented inside most of these clusters of predatory bacteria.

The first cyanolytic-bacteria isolated was identified as a *Myxobacteria* from the γ–protoeobacteria (Shilo, 1967), however 16S rRNA gene sequencing has

recently reassigned this organism and its relatives to *Lysobacter* of the

γ−protoeobacteria (Wilkinson, 2005). Numerous relatives of this organism have

since been isolated and studied (eg: Stewart and Brown 1971; Daft et al. 1973;

Stewart and Daft 1976, Stewart and Daft 1977; Walker and Higginbotham 2000).

Other cyanolytic species isolated from the γ−protoeobacteria include *Cellvibrio*

(Granhall and Berg, 1972) and *Moraxella* (Kim et al., 1997). *Aclagines*

denitrificans (Manage, 2000) of the β−protoeobacteria, and *Bdellovibrio*

(Burnham et al., 1976) of the δ−protoeobacteria are also known to be cyanolytic.

Bdellovibrio is unusual amongst cyanolytic-bacteria because it is an obligate

predator that lives inside the periplasmic space of its host – *Phormidium luridum*.

All others are facultative predators that survive by other means.

Cytophaga of the order Cytophagaceae are one of the most reported genera of

algicidal bacteria (Stewart and Brown, 1969; Rashidan and Bird, 2000). The

order Actinobacteria also contains numerous predatory bacteria (Mann, 2007).

Yamoto et al. (1998) took 83 strains of Actinomycetes from lake sediment and

found half of these positive when assayed for cyanolytic properties. Safferman

and Morris (1962) found a quarter of Actinomycetes from soil were also

cyanolytic. Individual cyanolytic *Streptomyces* (Gunnison and Alexander, 1975;

Yamoto et al., 1998) have been isolated and characterised. Cyanolytic properties

have also been identified in *Bacillus* (Reim et al, 1974; Wright and Thompson,

1985; Nakamura et al., 2003; Ahn et al., 2003) and a cyanolytic *Flexibacter*

(Sallal, 1994).

1.3.2 Host specificity of cyanolytic bacteria

Laboratory tests have found cyanolytic-bacteria to have varying host ranges. For example, there have been *Cytophaga* assayed that lyse only one species of cyanobacteria (Rashidan and Bird, 2000). In contrast, *Lysobacter sp.* SG3 has algicidal activity against numerous filamentous cyanobacteria (Walker and Higginbotham 2000;Wilkinson, 2005). Daft (1975) inoculated the *Lysobabacter* CP-1 directly into environmental water samples taken from cyanobacterial blooms. All samples, which included nine Nostocales, five Chroococcales and one Oscillatoriales, lysed within sixty hours, while controls did not. The trend for cyanolytic bacteria is a broad host range, lysing most, but not every strain in a defined phylogenetic clade (Table 1).

Table 1. Host range of some isolated cyanolytic bacteria

The first numeral in each column denotes how many species were lysed and the last number is the number of species from that clade that were assayed. If there is a number followed by an *i,* it indicates the number of species inhibited by the bacterium but not lysed.

	Uni-cellular Chroococcales	Microcystis	Other Chroococcales	Anabaena	Nostoc	Other Nostoccales	Phormidium	Plectonema	Lyngbya	Oscillaatoria	Other Oscilaoriales	Heterotrophic Bacteria	Algae
Lysobacter **FP-1**[1]	3,3	-	-	0,2	1,1	-	-	1,1	-	1,2	2,2	5,8	0,2
Lysobacter **CP-1**[2]	-	1,1	-	8,8	4,5	2,2	3,6	5,12	2,3	-	-	-	-
Lysobacter **CP-1**[3]	-	5,5	-	5,5	-	4,4	-	-	-	1,1	-	-	-
Lysobacter **CP-2**[2]	-	1,1	-	7,8	4,5	1,2	3,6	5,12	2,3	-	-	-	-
Lysobacter **CP-3**[2]	-	1,1	-	7,8	4,5	1.2	2,6	6,12	1,3	-	-	-	-
Lysobacter **CP-4**[2]	-	1,1	-	7,8	4,5	1,2	1,6	4,12	1,3	-	-	-	-
Lysobacter **SG-3**[4]	-	0,1	-	6,7	-	-	-	0,1	1,1	5,5	-	-	0,3
Alcaligenes denitrificans[5]	-	3,3	-	2,2	-	2,2	-	-	-	1,1	-	-	2i,9
Moraxella sp. **CK-1**[6]	0,3	2i,2	1,1	3,3	1,1	-	0,1	-	-	1,1i,2	-	-	-
Cytophaga **C1**[7]	0,3	0,1	0,1	1,1	0,2	-	0,3	-	0,1	-	-	0,6	-
Cytophaga **C2**[7]	3,3	1,1	1,1	0,1	0,2	-	0,3	-	0,1	-	-	0,6	-
Actinomycete **D5**[8]	2,2	-	-	-	3,3	1,1	1,1	1,1	2,2	-	-	-	0,18
Actinomycete **BB49**[8]	2,2	-	-	-	3,3	1,1	1,1	1,1	2,2	-	-	-	7,1i,18
Actinomycete **BB53**[8]	2,2	-	-	-	1,2i,3	1,1	1,1	1,1	1,2	-	-	-	2,18
Streptomyces phaeofaciens[9]	-	2,2	-	1,1	-	-	-	-	-	-	-	-	0,1
Cellvibrio[10]	-	-	1	1,1	2,2	1,1	-	-	-	-	1,1i	-	-
Flexibacter flexilis[11]	1,1	-	-	1,1	2,2	-	-	1,1	0,1	2,2	-	-	-
Flexibacter sancti[11]	1,1	-	-	1,1	2,2	-	-	1,1	0,1	2,2	-	-	-
Saprospira **sp. PdY3**[12]	2,2	0,1	1,2	3,3	-	-	1,1	1,1	-	-	-	-	-

[1]Shilo 1970, [2]Daft and Stewart 1971, [3]Daft 1975, these assays were performed on subsamples of actual lake blooms, [4] Walker and Higginbotham 2000, [5]Manage et al. 2000, [6] Kim et al. 1997, [7]Rashidan and Bird 2000, [8]Safferman and Morris 1962, [9]Yamamoto 2002, [10]Granhall and Berg, 1972, [11]Sallal 1994, [12]Shi, 2006

1.3.3 Ecology of Heterotrophic Bacteria and Cyanobacteria

Cyanobacterial bloom crashes coinciding with bacterial density "spikes"

Multiple studies have correlated bloom dynamics to bacterial dynamics, suggesting that bacteria are not only capable of lysing cyanobacteria, but that their presence throughout a bloom can influence its dynamics. Salomon et al. (2003) isolated 13 bacteria from the mucilage of *Nodularia* filaments. Five of these had

an inhibitory effect, while three had a stimulatory effect on *Nodularia* growth in laboratory trials. In a three month lake study, Daft et al. (1975) observed *Chl-a* density to peak and crash within a week and this corresponded with the termination of an *Anabaena circinalis* bloom. This peak and crash was in concert with a peak and crash in lytic bacteria density and a peak in total bacterial density. In another lake the same researchers observed two more peaks in lytic bacteria, the first corresponding to a *Microcystis* bloom and the second to a mixed bloom of *Aphanizomenon*, *Anabaena* and *Microcystis* (Daft et al., 1975). In another two-year lake study, Fallon and Brock (1979) found that *Microcystis* plated lawns inoculated with lake water yielded less than one PFU·mL^{-1}. However, predator density increased over a thousand fold when there were blooms of *Aphanizomenon*, *Anabaena* or *Microcystis*. Predator density maxima were roughly correlated with sharp declines in *Chl-a* concentration. Their experimental design excluded viruses from forming plaques on the lawns, but plaque-forming-units were still not homogenous, being composed of either protozoa, motile coccoid rods or – most commonly – of non-motile bacterial rods, two of which were isolated and determined to be *Cytophaga*. In a five month lake study using *Anabaena* and *Synechococcus* plated lawns Rashidan and Bird (2000) repeatedly isolated strains of *Cytophaga*. They found that plaque forming units (PFU's) in the lawns increased dramatically as blooms declined. This indicated that cyanolytic bacteria might have played a role in the blooms demise.

Fallon and Brock (1979) raise important doubts over whether the correlation of predator bacteria density to bloom dynamics allows an ecologist to infer a cause. The bacteria might have caused the crash, or the crash might have been otherwise

caused and the bacterial are proliferating as a result of released substrate. Likewise, demonstrating in the laboratory that a bacterium is lytic does not mean that it is necessarily lytic *in situ*. For example, *Lysobacter* are obvious candidates for causing bloom crashes. However, there are no ecological studies identifying them as having any causal role in bloom dynamics. Fraleigh and Burnham (1988) theorised that Lysobacter requires population densities of over 10^6 cells$^.$ml^{-1} to display lytic behaviour and such concentrations have never been observed *in situ*. It is clear that more studies are required that capture changes in heterotrophic bacterial dynamics throughout bloom events, not only to identify cyanobacteriolytic organisms but also to identify any environmental and physical variables that might be required for cyanolytic behaviour.

There are also bacteria that exhibit positive chemotaxis toward cyanobacterial toxins (Gallucci and Paerl, 1982; Pearl and Gallucci 1985). This could be because the toxins function as attractants facilitating the establishment of relationships with cyanobacteria that are syntrophic or mutualistic (Pearl and Gallucci, 1985; Steppe 1996, Brunberg, 1999). It could also be that the bacteria metabolise the toxins (Maruyama et al., 2003). It is plausible that toxin producing cyanobacteria should select for a community with toxin degrading bacteria. A crash, where the cyanobacterial community lyse, should result in a brief proliferation of such bacteria. Ecological studies of bacterial community composition associated with blooms should identify a compositional change favouring such (useful) organisms.

1.3 Cyanophage: viruses infecting cyanobacteria

1.4.1 Cyanophage morphology

Up until 2008, all characterised cyanophages were tailed coliphages (viruses with tails, protein coats and double stranded DNA). The coliphages are divided into three phenotypically related phage families; myovirus, siphovirus and podovirus (Mann, 2007). The myoviridae (T4 being the best known) have long contractile tails and heads with basic icosahedral symmetry. The siphoviridae have long non-contractile tails. The podoviridae have heads with basic icosahedral symmetry and short non-contractile tails. The most commonly isolated cyanophages have been the myoviridae (Suttle, 2000). The siphoviridae are the least frequently isolated. Apart from the odd exception when a siphovirus was found that infected *Lyngbya* (Mann, 2007) these have been found to infect unicellular Chroococcales (Mann, 2007). Numerous podovivrus have also been observed to infect *Prochlorococcus* (Mann, 2007). The most notable podovirus is the frequently isolated LPP phage that infects Oscillatoriales (Mann, 2007).

Until recently *all* characterised cyanophages were coliphages, however, research undertaken by Deng and Hayes (2008) has ended that generalisation. Deng and Hayes (2008) isolated viruses from cyanobacterial infested water using standard enrichment techniques, primarily algal lawns, the same technique used to discover the first cyanophage (Safferman and Morris, 1963). They found both RNA viruses and a filamentous virus, suggesting that numerous unique and distinct cyanophage have yet to be isolated.

1.4.2 Host based classification of cyanophage

Viruses are simple biological structures, therefore, their phenotype provides minimal information for taxonomists. It is likely that viral genomics will revolutionise this, however, in the meantime host range is considered an adequate approximation for cyanophage classification.

The cyanobacterial order Oscilatoriales was the first in which cyanophage lysis was discovered. Safferman and Morris (1963) isolated the strain LPP-1 on *Plectonema* lawns and named it after the genera of filamentous cyanobacteria it was active against: *Lyngbya, Plectonema* and *Phormidium*. Since then numerous strains of LPP have been isolated and characterised (Safferman and Morris, 1963; Padan et al., 1967; Safferman and Morris, 1967; Ohki and Fujita, 1996).

Cyanophage infecting unicellular Chroococcales are the most frequently studied. *Synechococcus*, the most prominent primary producer in oceans is infected by numerous phage (Khudyakov, 1977; Martin and Benson, 1988; Ortmann et al., 2002) as is its close relative *Prochlorococcus* (Sullivan et al., 2005). Unambiguous cases of a cyanophage infecting *Microcystis aeruginosa* have occurred recently (Tucker and Pollard, 2004; Yoshida et al., 2006). Cyanophage have also been identified that infect Nostocales. For instance, phage have been identified that lyse *Anabaena* (Hu et al., 1981, Phlips et al., 1990), *Nostoc* (Hu et al., 1981) and *Aphanizomenon* (Granhall, 1972).

Using host specificity for classifying cyanophages is complicated because of host-cell resistance. Consequently, viruses are distinct from many bacterial predators in that they have narrow host ranges. Some viruses infect more than one genus (Safferman and Morris, 1963), others just one strain (Yoshida et al., 2006). Yoshida et al., (2006) provide an example of how host defence can limit a virus to an infinitesimal host range. They used a strain of *M. aeruginosa* from Lake Kasumigaura (Japan) to enrich for and isolate viruses from Lake Mikata. They isolated a single virus and conducted a host range assay against many strains of *M. aeruginosa*. Of the eleven strains of *M. aeruginosa* the virus only lysed the one strain it was isolated with, it wouldn't even lyse the *M. aeruginosa* strain taken from Lake Mikata, which was the lake where the virus originated. The variability of resistance no doubt has an ecological basis, for example representatives of *Synechococcus* marine-cluster A are more susceptible to infection than marine-cluster B (Suttle, 2000). The most likely reason is that cluster A are open water organisms and have less frequent contact with viruses (i.e., less selective pressure) than the coastal cluster B organisms. Chemostat experiments have demonstrated that a susceptible host can eventually become resistant to a phage after a period of sustained contact (Wommack and Colwell, 2000).

Using hosts to classify phage is most problematic if phage infect paraphyletic clades and whether a cyanophage can infect cyanobacteria from paraphyletic clades has, until recently, been difficult to determine. Suttle (2000) concludes that because most of the viral-phylogeny problem supervened on various taxonomic confusions of the host organism and that the reappraisal of cyanobacterial phylogenies has had the upshot of unifying what were apparently paraphyletic

viruses (Suttle, 2000). However, there are still some interesting cases. *Synechococcus* is divided into Marine-cluster A and cluster B. Viruses have been isolated that infect organisms across these clades but not all members of the clade (Suttle, 2000).

We must conclude that while host-based classification may be some utility within individual studies, that host range is not sufficiently lawful to be the basis of a cladistic system. Mann (2007) gives what he considers to be the only two possible generalisations about cyanophages' host specificity: marine cyanophage do not infect freshwater hosts and vice versa and phages infecting unicellular hosts do not infect filamentous species. Identifying conserved viral genes might instead be a more fertile ground for implementing a functioning cladistic system.

Conserved cyanophage genes

Despite the vast heterogeneity of viral genomes some conserved genes have been identified that can be used to cluster viruses. Almost all known cyanophage are coliphage, containing a protein capsid. Cyanophage have been studied using PCR primers designed for capsid genes such as; the capsid assembly gene g20 (Marston et al., 2003, Muhling et al., 2005), viral sheath protein gene g91 (Yoshida et al., 2008) and major protein capsid gene (MCP; Baker et al., 2006). Many homologues of cyanobacterial genes are conserved across cyanophage genomes (Sullivan et al., 2005). Numerous phage that infect *Synechococcus* and *Prochlorococcus* carry *psbA* and *psbD,* genes coding for homologues of the D1 and D2 proteins involved in photosynthesis (Sullivan et al., 2006). These proteins are highly photo-labile and cyanobacteria upregulate their expression during

photosynthesis. When a phage hijacks the reproductive machinery of the cell it is advantagous to code for such proteins. Primers for *psbA* have been incredibly successful in studying phage of marine picocyanobacteria (e.g., Clokie et al., 2006).

1.4.3 Virus ecology

Aquatic virus ecology began when Bergh et al. (1989) demostrated that viral concentrations in water systems were immense. Using transmission electron microscopy they counted viruses in the order of 10^8 viral like particles (VLP) mL^{-1}. This fact resulted in the inference that viruses must play an important role in water ecosystems.

Pelagic viral abundance is generally ten times that of bacteria (Suttle, 2000). Studies correlating viral abundance to other variables typically find a strong positive correlation between bacterial secondary production and viral production. Correlations between environmental parameters or indicators of primary production such as *Chl-a* are generally weak (Wommack and Colwell, 2000). In their extensive literature review Wommack and Colwell (2000) summarise that only over small time-scales and distances are viral abundances predictable through bacterial abundance and that over greater time scales primary production emerges as an entity that drives viral productivity. However, in some studies, discussed below, change in primary production does immediately correlate with viral production.

Lake trophic status has been shown to have contradicting bearing on viral abundance, with only some studies showing that lakes of higher trophy have more viruses (Wommack and Colwell, 2000). Combining the variation in viral abundance that occurs seasonally with that occurring across trophic status, Betteral (2003B) conducted a seasonal study of a eutrophic and an oligotrophic lake. VLP in the eutrophic lake were on average 5.3×10^7 VLP·mL^{-1}, which was twice that of the oligotrophic lake (2.9×10^7 VLP·mL^{-1}). The virus:bacteria ratio (VBR) was greater in the eutrophic lake than the oligotrophic, apparently because of trophic status. Maranger and Bird (1995) conducted a survey of 22 lakes of different trophy and found it unlikely that VBR correlated with lake trophic status.

While virus and bacteria abundance are fairly constant, the community composition of planktonic viruses and bacteria can vary remarkably. The "killing the winner" hypothesis states that high viral activity will promote high bacterial community diversity (Thingstad and Lignell, 1997). The theory assumes that if a virus is specific to a high-density prey, then it will be more likely to encounter its prey, which will lead to a proliferation of viral particles and reduce the prey to low density. This explains bacterial community succession because it suppresses the "superior competitor for mineral nutrients" (Thingstad and Lignell, 1997). It also explains why total-virus and total-bacteria counts are constant because when a "superior competitor" is lysed another competitor succeeds (Wommack and Colwell, 2000). Hewson and Fuhrman (2007) have used the "kill the winner" model to relate changes in bacterial community structure using DNA fingerprinting methods (ARISA) to changes in total-viruses abundance (by epi-fluorescent microscopy - EFM). They tested this method across several marine

locations and found that only some locations showed the predicted correlation between bacterial diversity total-viruses. Locations that didn't show strong correlations were regions with high phytoplankton that had abundant available resources. However, Hewson and Fuhrman found a more consistent correlation between viral abundance and bacterial composition using a functional gene (*nifH*) and applying the DNA fingerprinting tool TRFLP. This suggests that viral effects can be better seen in a compartmentalised section of the bacterial population. Lymer et al. (2008) showed that dynamic shifts in bacterial community composition and viral composition are weakly related. They hypothesised that this was because the community fingerprinting techniques they were using (PFGE and TRFLP) lack sufficient resolution to indicate a true correlation, or that the effect of viruses eliminating a bacterial population is so abrupt that it is difficult to see the emergent pattern.

Wilhelm and Suttle (1999) infer that viruses can create a closed trophic loop because lysed cells create particulate matter too small for grazing organisms to ingest, but suited to bacterial uptake. An increase in viruses should lead to a spike in heterotrophic bacteria and a high proportion of phage should keep productivity at that level.

Viruses and primary production

Although viral production doesn't often correlate with primary production, there are exceptions. The following are observations of viral spikes coinciding with declines in primary producer abundance. In a year long study Manage et al., (1999) observed oscillations in *M. aeruginosa* abundance, where crashes

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coincided with increases in cyanophage PFU's. Coulombe and Robinson (1981)

conducted a long-term study of a lake prone to blooms of *Aph. flosaquae* and

identified three crashes as being most likely due to physical causes, but a fourth

bloom coincided with an increase in VLP and was unlikely to be underpinned by

environmental factors. Another study observed blooms of the filamentous mat-

forming *Lyngbya majuscula* to rapidly shrink from eight square kilometres to less

than one in less than seven days (Hewson et al., 2001). Viruses belonging to the

Siphonoviridae family were observed by electron microscopy to coincide with this

demise. In laboratory experiments, viral isolates can in some cases almost

entirely eliminate the cyanobacterial host (Tucker and Pollard, 2005). During a

laboratory-scale experiment van Hannen et al. (1999) observed a mixed

population of *O. limnetica* and *P holandica* lyse, this coincided with an increase

in VLP. The experimenters observed VLP inside lysed cyanobacteria filaments.

A later laboratory scale experiment (Gons et al., 2002) took water from the same

lake that contained a mixed assemblage of cyanobacteria. Coccoid cyanobacteria

persisted while filamentous cyanobacteria disappeared from the assemblage after

two weeks and this event coincided with a spike in virus numbers. There is also

evidence of how a virally mediated cyanobacteria decline restructures the

composition of the heterotrophic bacteria community. Jardallier et al. (2005)

conducted a 96 hr mesocosm experiment and found that in virus treated

mesocosms cyanobacteria declined, heterotrophic bacteria production and

abundance increased and the heterotrophic community composition changed. The

preferential lysis of cyanobacteria apparently favoured the dominance of other

heterotrophic bacteria.

Not only do viruses restructure heterotrophic composition but they can restructure cyanobacterial composition. Yoshida et al., (2008) investigated how cyanophages shaped the composition and abundance of cyanbacterial blooms through time. Yoshida et al., (2008) ascertained the abundance of the *M. aeruginosa* population through time by using quantitative PCR (QPCR) to measure fluctuations in the abundance of the phycocyanin intergenic spacer gene. To monitor intraspecific changes in the *Microcystis* community they used *mcy*A, a microcystin encoding gene. They observed two blooms, with significantly less *M. aeruginosa* containing the mcyA gene in the second bloom. The QPCR peaks in cyanophage gene g91 (for viral sheath protein) weakly correlated with the observed intraspecific flutuations, suggesting a relationship where the cyanophage influenced the intraspecific community structure of the cyanobacteria. Muhling et al., (2005) found that despite a prominent crash in marine *Synechococcus* being caused by phosphorus limitation, other dynamics between pico-cyanobacteria and cyanophage were covariant, including both abundance and composition changes.

1.5 Concluding remarks

The lake environment presents numerous events that increase the possibility of bloom collapse. A collapse can be due to the removal of positive factors that favour blooms, such as declining nutrients (Oliver and Ganf, 2000), changes in lake stability (Dokulil and Teubner 2000), declining pH (Dokulil and Teubner 2000) and changes in light penetration (Mur 1983, Sigee, 2005). Alternatively a negative factor might be introduced to the lake such as grazing plankton (Reynolds, 1989; Lampert and Sommer, 2007), predatory bacteria (eg. Daft et al.,

1975; Rashidan and Bird, 2000) or cyanophage (eg. Manage et al., 1999; Hewson et al., 2001; Jardallier et al., 2005). Fluctuations in bloom-associated biota need not always be negative, phytoplankton have been observed to benefit from the presence of zooplankton (Gliwcz, 2004; Lambert and Sommer, 2007), bacteria (eg. Salomon et al., 2003) and viruses (Suttle, 2000) and the removal of such agents might be to the detriment of the bloom.

Cyanobacterial blooms are an undesirable and global problem. The immediate crashing of a bloom can also have detrimental effects on lake ecosystems by causing the release of numerous intracellular cyanotoxins and by affecting water column anoxia that can result in massive fish kills (Havens, 2008). These are two reasons for wanting to understand, diagnose and possibly predict bloom collapse. Identifying predatory organisms that negatively impact cyanobacterial blooms is one avenue to achieving this.

To understand the role of predatory organisms in bloom decline Lake Kainui, a Waikato peat lake, was monitored over a five month period. Over this period *Chl-a* measurements were first taken on a daily basis and later on every alternate day. When a period of bloom decline occurred a comprehensive set of environmental variables was obtained to ascertain which of thesew variables drove the decline. Variables measured included nutrients, micronutrients, water temperature, dissolved oxygen (DO), pH and meteorological parameters were measured. Bacterial diversity was assessed using molecular fingerprinting (ARISA). Total algal, cyanobacterial, planktonic bacteria, planktonic viruses and planktonic crustacean abundances were determined using microscopy.

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Chapter 2 Explaining a cyanobacterial bloom's decline.

2.1 INTRODUCTION.

Cyanobacterial blooms are an escalating problem globally. They are symptomatic of lake eutrophication (Sigee, 2005) and often a consequence of anthropogenic inputs into water-bodies. The negative implications for aquatic ecosystems and their human users include; blooms are unsightly, they can restructure existing lake biota, they can alter lake chemical stoichiometry, bloom forming species often produce secondary metabolites that can cause odours and off flavours in water and fish flesh (Izaguirre. et al., 1982; Walker and Higginbotham, 2000; Peter, 2008) and, most notoriously, some of their secondary metabolites can be toxic to humans, livestock and other organisms (Carmichael, 1992; Codd et al., 2005).

There is a vast literature on the causes and preconditions for cyanobacterial blooms (e.g., Sverdrup, 1953; Reynolds, 2006). The present study investigates bloom termination, the corollary of bloom formation. Bloom termination is a phenomenon that is just as complex and as environmentally relevant as bloom formation. Blooms can suddenly and dramatically crash (Havens, 2008). Understanding the causes of cyanobacterial bloom crashes could provide valuable insights into plausible methodologies for lake restoration. In depth-knowledge of these processes may enable a means for switching lakes from being in bloom to a "clearwater" state.

Studies addressing the termination of blooms often investigate inversions of conditions known to cause blooms. The rubrics of 'bottom-up control' and 'top-

down control' define this approach. Bottom-up controls are traditionally the limitation of nutrients (Seip et al., 2000; Huisman and Hulot, 2005), however, other factors can reverse the trend of a bloom such as; limiting micronutrients (Reynolds, 2006), changes to lake stability (Steinberg and Hartmann, 1988) and irradiance (Sigee, 2005). Top-down control refers to planktonic grazing of phytoplankton by protozoa, heterotrophic nanoflagelates, ciliates (Brabrand et al., 1983) and crustacea (Gliwicz, 2004; Sigee, 2005).

Planktonic cyanolytic bacteria are another means by which cyanobacteria can be controlled. Over the last few decades it has become clear that bacteria are a ubiquitous but dynamic component of pelagic ecosystems. Shilo (1967) was first to report the isolation of a bacterium that lysed cyanbacteria. Since then numerous bacteria have been isolated that lyse one or more genera of cyanobacteria (e.g., Safferman and Morris, 1962; Daft et al., 1973; Yamamoto, 1998). Studies of cyanolytic bacteria have tended to be laboratory based, however there are a few ecology studies that have recorded decreases in cyanobacterial abundance coinciding with increases in the abundance of cyanolytic bacteria (Daft et al., 1975; Fallon and Brock, 1979; Rashidan and Bird, 2000). Bacteria can also inhibit phytoplankton by out-competing them for limiting nutrients (Sigee, 2005). Heterotrophs are known to help sustain cyanobacterial blooms by entering into commensal relationships with them (Salomon et al., 2003; Jiang et al., 2007). Bacteria also play a significant role in aquatic ecosystems as decomposers of phytoplankton (Sigee, 2005; Reynolds 2006). Understanding how bacterial dynamics correlate with phytoplankton dynamics is therefore important for understanding phytoplankton dynamics.

The way in which bacteria influence the flux of cyanobacterial populations can be studied by culture independent DNA techniques. PCR based genetic fingerprinting techniques have emerged as an efficient method to measure trends in bacterial community composition (BCC). Genetic fingerprinting is a convenient and efficient method for studying the relationship of the microbiota to bloom decline. Changes in phytoplankton community composition (PCC) are likely to change the composition of available nutrients, and because different bacteria are optimised for different energy sources it can be anticipated that BCC will also change (Reynolds 2006). Assessing changes in BCC that co-occur with changes in phytoplankton have proved useful in understanding the complex interaction between these micro-organisms (e.g., Eiler and Bertilsson, 2004; Tuomainen et al., 2006). Automated ribosomal intergenic spacer analysis (ARISA) is a DNA fingerprinting technique in which total community DNA is amplified using a fluorescently labeled primer. An automated capillary sequencer then detects the fluorochrome. The primer pair amplifies non-coding DNA intergenic to genes coding the 16S and 23S ribosomal subunits (Fisher and Triplett, 1999). Length heterogeneity of this intergenic region gives high phylogenetic resolution and the technique has often been used to characterise lake bacterioplankton (e.g., Yannarell and Triplett 2005, Kent et al., 2006; Newton et al., 2006).

Planktonic viruses are another component that can induce cyanobacterial bloom decline. The significance of viruses in structuring aquatic ecosystems was highlighted when Bergh et al. (1989) discovered that virioplankton occurred at

densities of $10^8 \cdot mL^{-1}$. This demonstrated that viruses are a significant component of aquatic ecosystems. Their importance has been reflected in the number of ecological studies published on marine virioplankton, with >100 publications per annum over the last 4-5 years and freshwater virioplankton averaging 30 publications per annum over the last 4-5 years (Middleboe et al., 2008). This highlights the need to include virioplankton in lake ecosystem studies in New Zealand.

Safferman and Morris (1963) isolated the first cyanolytic virus. Since then hundreds have been isolated (e.g., Wommack and Colwell, 2000; Suttle, 2000, Section 1.4, Chapter 1). Multiple *in situ* studies on viral dynamics have shown declines in cyanobacteria correlate with peaks in numbers of a particular strain of virus, a correlation that might indicate population control (Coulombe and Robinson, 1981; Manage et al., 1999; Hewson et al., 2001). Laboratory experiments have also recorded correlations between cyanobacteria decline and increase in viral particles (van Hannen et al., 1999; Gons et al., 2002; Tucker and Pollard, 2005). Confirming the presence of cyanophage is usually undertaken using a technique that specifies and enumerates a particular virus, such as lawn plaque assays (Safferman and Morris 1963, Clokie et al., 2006, Deng and Hayes, 2008), or by targeting cyanophage specific genes (Marston et al., 2003; Sullivan et al., 2005; Baker et al., 2006; Yoshida et al., 2008). In their extensive literature review Wommack and Colwell (2000) surmise that over small time-scales trends in viral abundance are predictable through bacterial abundance (BA), however, over longer time scales primary production emerges as an entity that drives viral productivity. Maranger and Bird's (1995) study of 22 lakes is a significant

exception to BA being a better determinant of viral production than chlorophyll-a (*Chl-a*). Maranger and Bird (1995) include an analysis of other virioplankton studies' findings in their results, and find a strong correlation between virus like particles (VLPs) and BA (r^2=0.72) for 'corrected' VLP values. Relationship of BA to *Chl-a* are however stronger (r^2=0.81; Maranger and Bird, 1995). In their experimental work Maranger and Bird (1995) found no correlation of VLPs to BA, but weak correlations with *Chl-a* (r^2=0.52), bacterial production (r^2=0.52) and concentrations of total phosphorus (r^2=0.57). They attribute this correlation of viral to *Chl-a* and not BA to either a greater influence of algae specific viruses in lake catchments, or the inability of short term studies to pick out trends of VLPs to BA in lakes (Maranger and Bird, 1995). There are therefore grounds to expect that massive changes in *Chl-a*, such as a bloom termination, should be related to changes in virioplankton. Viruses are known to structure BCC and PCC in several ways. The first is the 'kill the winner' model (Thingstad and Lignell, 1997), premised on the fact that as a phage's prey becomes more abundant then predator-prey contact rates increase as well as the number of hosts incubating progeny phage. It is called 'kill the winner' because any dominant (winning) organism is more likely to be eliminated by viruses rather than a non-abundant organism. This model would predict that viral productivity would increase as prey diversity decreases. A second important way that viruses structure bacteria is by creating a 'closed trophic loop' also known as the 'viral shunt' (Wilhelm and Suttle, 1999). This model hypothesises that if there is high viral lysis, then a lot of nutrients will be available in a soluble form in the water column. This will encourage the growth of small organisms such as bacteria and phytoplankton because larger organisms will be unable to assimilate small food.

It is possible that the dynamics of planktonic biota do influence bloom decline, but in an indirect way, for example by competing with cyanobacteria for a limiting nutrient. To conceive of how these indirect influences operate on bloom dynamics it is important to conceptualise a model of trophic interaction. The most intuitive trophic model is that nutrients travel through a trophic cascade from phytoplankton, to herbivorous grazers, to planktivorous mesofauna (e.g., fish) to carnivorous macrofauna (e.g., piscivores). Sheldon et al. (1972) divided the water column into different size fractions, counted the number of particles (including organisms) in each fraction and also looked at how quickly organisms in each size fraction reproduced. By counting the balance of particles in each size fraction they were able to lend support to this hypothesis of a trophic cascade. They suggested that by either removing a significant percent of planktivores or increasing piscivores, biomanipulation could be used to control phytoplankton. This trophic relationship is now understood to be too simplified, but the basic principle has been successfully illustrated internationally (Hansson et al., 1998; Sondergaard et al., 2008). Azam et al., (1982) elaborated on the trophic cascade model, by using new techniques for counting microbes (epifluorescent microscopy), for estimating microbial biomass and for measuring bacterial productivity. Using a rationale very much like Sheldon et al., (1972) they tried to account for the number of bacteria in terms of the numbers of their predators, but found a deficit, which they balanced by hypothesising the microbial "loop". The microbial loop states that nutrients travel from cyanobacteria (and all other organisms), back to dissolved organic matter (DOM), to bacteria, to flagellates and then to zooplankton (Azam et al., 1983). Therefore the microbial loop has

more organisms filling the steps from phytoplankton to zooplankton. Additionally, with each step up the trophic ladder some nutrients will snake back down and increase the concentration of DOM and dissolved minerals and these will stimulate further bacterial growth. The viral mediated trophic loop ('viral shunt') is another elaboration on the microbial loop, in which the active lysis of organisms increases the pool of DOM that can be taken up by nanoplankton (<2 μm). Zooplankton can be 'messy eaters' and therefore redirect nutrients to the bottom of the trophic ladder (Gliwcz, 2004; Lambert and Sommer, 2007). The numerous kinds and modifications that can occur in a specific lake ecosystem have necessitated a variety of trophic models that take the form trophic webs (Carpenter et al., 1985; Wilhelm and Suttle, 1999; Rowe and Schallenburg, 2004). Successful remediation of a turbid eutrophic lake by biomanipulation therefore requires an assessment of all trophic paths before biomanipulation is commenced.

Lake Kainui, Waikato, New Zealand, has been monitored sporadically over the last decade by Environment Waikato (Appendix 1, Figure 16) and the results of this monitoring reveal that the lake is capable of supporting phytoplankton up to densities over 150,000 cells·mL^{-1} and that phytoplankton cell numbers fluctuate greatly. There are three contrasting records of Lake Kainui's trophic status. A 1983-4 study assigned borderline hypertrophic status to the lake when species composition and biomass were used as trophic indicators (Etheredge, 1987). The same study found nutrients concentrations consistent with eutrophic and the abundance of phytoplankton in Lake Kainui had a local peak in autumn that dropped through winter and spring. Phytoplankton grew rapidly in late spring/early summer and again peaked in December of 1983. Concentrations then

decreased to an annual low in January 1984, however, they increased again in late summer. Measurements taken in 1991 assigned oligotrophic status to Lake Kainui and also a low pH (less than 7; Waikato District Council 1991).

This study aimed to elucidate factors that were involved in cyanobacterial bloom decline with an emphasis on bacteria and viruses as possible mediating factors. This study surveyed Lake Kainui over a period of approximately five months. To identify a bloom decline the lake was visited first daily, then every alternate day to check for significant declines in *Chl a* concentration. Once a decline was identified biological parameters measured were crustacea counts, total bacterial abundance (BA), total virus like particles (VLP) and enumeration of phytoplankton genera and cyanobacteria species. These were taken to test the hypothesis that an increase in phytoplankton diversity (indicating the reversal of a monospecific bloom) and/or a decrease in *Chl-a* would correspond to a rapid climb in either VLP or BA. To find if abiotic factors played a more significant role in bloom decline than biotic ones, measurements were made of meteorology, pH, dissolved oxygen (DO), temperature, conductivity, photosynthetically available radiation (PAR), nutrients, micronutrients.

2.2 MATERIALS AND METHODS.

2.2.1 Fieldwork

Study Lake

Lake Kainui, also known as Lake D (37.67713°N,175.23269°E), is an

ombrogenous peat lake. The lake has a surface area of 25 ha and a maximum

depth of 6.7 m. It is situated 20 km north of Hamilton city, New Zealand and its

catchment is predominantly dairy pasture reclaimed from peat marshes. Recent

surveys have found no submerged macrophytes, several introduced fish species

and an introduced freshwater jellyfish also populates the lake (Boothroyd et al.,

2002; Fergie 2003, www.LERNZ.co.nz).

Field Sampling Design

Whole water column samples for assessing lake chemistry and biology were taken

from four points across a lake transect (Fig. 1). Composite, integrated water

samples were collected at 18:30 hr on a weekly basis from 6 of November 2007

through to 25 March 2008. Samples were then taken daily through a week in

January (8 January 2008 to 15 January 2008) when *Chl-a* concentrations were

minimal. An integrated column sampler of 0.5 cm diameter was lowered through

the water column to just above the sediment, the sampler tube was stoppered at

the top to retain the sample before the tube was extracted from the lake. This was

repeated four times at each site. Water from each sampling site was stored at 4 °C

until further analysis. All reusable plastic-ware was washed in either dilute

hypochlorite (Janola) or 5% hydrochloric acid. *Chl-a* was initially sampled daily

then every second day for the remainder of the five months. Samples were then

collected as described above, processed on site and stored at 4 °C in the dark until further analysis.

Meteorology

Daily and hourly meteorological data from a NIWA meteorological station situated at Ruakura (37.77879°N,175.31271°E) was obtained from the CliFlo service on the NIWA website (http://cliflo.niwa.co.nz/). Variables measured include mean air temperature, sun irradiance, gust speed, rainfall and soil moisture content.

Figure 1. Aerial photograph of Lake Kainui (Lake D). Lake chemistry and biology samples were taken from the four sample points marked in yellow.

200 m

1000 ft

Google maps (retrieved: http//maps.google.com)

In situ physical measurements

Dissolved oxygen, temperature, conductivity and pH were measured with a Sonde (6000 UPG model YSI Sonde, Yellow Springs Instrument, Ohio) at the water surface, at 2 m depth and just above the lake sediment once a week at 17:30 hr. Conductivity, temperature, depth, beam transmission, photosynthetically available radiation (PAR), dissolved oxygen (DO) (Sea-Bird Electronics Inc. Washington) and chlorophyll fluorescence (Chelsea Instruments Ltd.) were recorded vertically through the water column at six points along the transect (Fig. 1) with an SBE 19 plus SEACAT profiler (Sea-Bird Electronics Inc. Washington). Reading were taken every seven to ten days. A thermistor data logger (Tidbit Stowaway) was used to capture hourly temperature change at 70 cm depth. Data stored on the thermistor was periodically downloaded using BoxCar Pro 4.3 (Onset Computer Corporation, 2002).

Data from the Seacat profiler was visualised using Ocean Data View (Schlitzer, 2008). To obtain values pertaining to lake stratification (i.e., Schmidt stabilty, thermocline depth), the temperature and depth data from the Seacat profiler and the corresponding hourly wind speed and direction values were analysed in MATLAB with the aid of the GLEON Lake Analyser program (Kroiss, 2008). Because Lake Kainui's bathymetry is not known, for the purposes of analysis the lake's topology was approximated by treating the depths recorded along the sampling transect as representative of the entire lakes topology.

The euphotic zone was defined as the volume between the lake's surface down to the area where there was one percent of surface irradiance and it was assumed that

the light extinction coefficient (K_d) was constant though the surface layer. K_d was determined using a rearrangement of the Beer Lambert law:

$$K_d = -\ln(I_z/I_0)/z \qquad\qquad (1)$$

where I_z is PAR at vertical depth z, I_0 is the sub-surface PAR and z is the depth at which I_z was taken.

2.2.2 Preparation and Storage of Samples

Duplicate *Chl-a* samples were collected by filtering 200 ml of lake water and 5 ml saturated magnesium carbonate solution through 25 mm GF/C filters (Whatman). Samples were wrapped in aluminium foil and stored at 4°C until further analysis.

For total nutrient analyses 100 ml of unfiltered water was collected and a further 100 ml of GF/C (Whatman) filtered water was collected for measuring soluble nutrient and micronutrient concentrations. Samples were kept frozen at -20 °C.

For DNA fingerprinting analysis lake water was filtered through GF/C filter papers (Whatman) and then through 0.22 μm polycarbonate filters (Millipore) until the flow was blocked. Filtrate volume was recorded and the filters were kept frozen at -20 °C until further analysis.

Lake water samples (50 ml) were preserved with Lugols iodine (1% final concentration) for phytoplankton identification and enumeration. Lake water samples (15 ml) were stored in 2% formaldehyde (Bloem and Vos, 2004) for bacteria enumeration. Four litres of lake water was first filtered through 45 μm

nylon mesh (Madison filter, Auckland, NZ) and the retentate stored in 90%
ethanol for zooplankton identification and enumeration. The filtrate was kept for
enumeration of viruses and was subsequently strained through 5 μm polester mesh
(Madison filter, Auckland, NZ), GF/C filters (Whatman) and 0.2 μm Sartobran P
filter-capsules (Sartorius). The filtrate was then concentrated by tangential flow
diafiltration on a Proflux M12 (Millipore) using a Hellicon S10 spiral cartridge
with a 30 kDa cutoff (Millipore). Input and output pressure never exceeded 10
PSI. Samples were stored in sterile 500 ml glass Schott bottles in the dark at 4 °C
(Frederickson et al., 2003, Clokie et al., 2006B) until counted.

2.2.3 Analyses

Lake Chemistry

Chlorophyll-a was daily extracted from filters using acetone and measured for
absorbance with and without acidification across 635 nm, 645 nm, 665 nm and
750 nm wavelengths (Pridmore et al., 1983).

Nutrients were measured on a Lachat QuickChem Flow Injection Analyser (FIA+
8000 Series, Zellweger Analytics, Inc.) using standard methods to measure
phosphate (PO_4-P), nitrite (NO_2-N), nitrite (NO_3-N) and ammonium (NH_4-N).
Total nitrogen (TN) and total phosphorus (TP) samples were treated with a
persulphate digestion, then measured using the columns that measure PO_4-P, and
NO_3-N (Ebina et al., 1983).

Micronutrient samples were acid digested with dilute HNO_3 then measured with a
Perkin-Elmer Elan DRC11 Inductively Coupled Plasma - Mass Spectrometer (GE

Healthcare, Auckland, New Zealand). The following micronutrients were measured: Li[7], B[10], Na[23], Mg[24], P[31], K[39], Ca[43], V[51], Cr[52], Fe[54], Mn[55], Co[59], Ni[60], Cu[63], Zn[68], Ga[71], As[75], Se[82], Sr[88], Ag[109], Cd[111], In[115], Ba[137], Tl[205], Pb[207], Bi[209] and U[238].

Microscopy

Genera and species of phytoplankton were identified and enumerated by settling known volumes (1 – 10 mL) of Lugols iodine stained lake water in viewing chambers after a method adapted from Utemöhl (1958). An Olympus IX71 microscope was used under bright field with objectives at 200× (Olympus UPlan FLN 20x/0.050 Ph1), 400× and 650× (Olympus UPlan FLN 40x) and a phase objective (Olympus UPlan FLN 40x/0.75 Ph2) at 650×. Cyanobacteria were identified to species level using the taxonomic keys of Pridmore and Etheredge (1987), Baker (1991), Baker (1992) and Baker and Fabbro (2002).   Algae were identified to the genus level using the taxonomic key of Pridmore (1987).

Phytoplankton growth or loss rate was determined using the equation of Reynolds (1997)

$$R_n = \ln(N_t/N_0)/t \tag{2}$$

where N_t is the immediate biomass of phytoplankton, N_0 is the preceding biomass of phytoplankton and t is the time interval between the two measurements.

Bacteria and virus fractions were both separately stained with the fluorescing intercalating DNA dye, SYBR gold (Invitrogen, New Zealand: Chen 2001). Samples were diluted with 0.02 μm filtered TE buffer so that not more than 20

cells/particles were visible in a field of view. Bacteria were immobilised on 0.2 μm black polycarbonate filters (Poretics). The viral fraction was immobilized on 0.02 μm anodisc filters (Whatman).

Epifluorescent microscopy was used to enumerate bacterial abundance (BA) and total virus like particles (VLP; Noble et al., 1998; Chen 2001) using a Leica DMRD, powered by a mercury short arm lamp (HBO, 50W/AC, OSRA Germany). An N2.1 filter cube was used (excitation BP 515-560 nm/deichroic mirror 580 nm/ suppression filter LP 590 nm) in conjunction with a PL-Fluostar 100× objective.

Enumeration and identification of zooplankton was undertaken on an Olympus SZ-40 stereomicroscope at 20×-40× magnification. Zooplankton were identified using the taxonomic key of Chapman and Lewis (1976).

DNA fingerprinting of microbial community
DNA was extracted from filters using CTAB (Aldrich and Cullis, 1993) and quantified using a ND-1000 Nanodrop spectrophotometer (Nanodrop Inc., Delaware, USA). The quality of extracted DNA was confirmed by agarose-gel electrophoresis.

For automated ribosomal intergenic spacer analysis (ARISA; Fischer and Triplett 1999) each 50 μl reaction mixture contained 30 ng of diluted template DNA, 1U Platinum Taq (Invitrogen), 200 μmol of each dNTP, 750 μg of bovine serum albumin (BSA), 10x reaction buffer (Invitrogen), 300 μM of $MgCl_2$ and 0.25 μM

of each primer 1406F (5' FAM-TGYACACACCGCCCGT 3') and 23S (5'
GGGTTBCCCCATTCRG 3'; Fischer and Triplett 1999). PCR was conducted
using a PTC-200 Peltier Thermal Cycler (MJ Research Inc., Masachusetts, USA).
The thermocycler protocol commenced with 2 min at 94°C followed by thirty
cycles of 94°C for 45 sec, 55°C for 30 sec and 72°C for 2 min, with a final
extension at 72°C for 7 min. Triplicate 50 µl samples were pooled and presence
of amplicon was confirmed by agarose-gel electrophoresis. Amplicon were
purified using a PCR cleanup kit (Qiagen) and diluted 1:10 with sterile water and
2 µl of the product mixed with 0.25 µl of ROX-labelled genotyping internal size
standard 1200LIZ (Applied Biosystems, USA). Sequence lengths were
determined by running 2 µl of diluted amplicon through a ABI 3130XL Genetic
Analyser DNA sequencer (Applied Biosystems, USA).

Sequence lengths were identified on the ABI Peakscanner software (Applied
Biosystems, USA) with a threshold for the blue channel (FAM) set to a zero to
capture both peaks and noise and the orange channel (LIZ) set to fifty to capture
the internal standard's peaks. The results were then exported to Microsoft Excel
to discriminate true ARISA-fragment peaks.

Discrimination of true ARISA-fragment peaks was undertaken according to the
method of Abdo et al., (2006). This method identifies 'true' PCR peaks from
background noise by describing the pattern of noise that the sequencer generates.
This is achieved by assuming that the mean of the 'noise' peaks is zero, so that
any value falling outside three standard deviations from this mean is a true peak.
True peaks are removed and a new standard deviation recalculated. This

procedure is repeated till all peaks within three standard deviations have been identified. This method and its iterations were performed using Microsoft Excel.

Relatedness of phytoplankton composition, bacterial community composition (BCC) and abiotic variables across individual samples were plotted using non-parametric multidimensional scaling (MDS). Biotic MDS plots were based on matrices generated with Bray-Curtis similarity. Abiotic variables were also plotted on MDS using Euclidean distance as a measure of relatedness. ARISA data was plotted based on community composition (presence/absence) and semi-quantitatively treating peak area as relative abundances. Shannon Weaver Diversity indices from ARISA results were also ascertained using Primer 6 (PRIMER-E, Ltd., UK).

Anatoxin-a Screening

Sub-samples of lake water (10 ml) were thawed and formic acid added (0.1% v/v). Samples were sonicated (10 min) and then centrifuged (3,500 g, 10 min). An aliquot of the supernatant was analysed directly for anatoxins using liquid chromatography mass spectrometry (LC-MS). Anatoxins were separated by liquid chromatograph (LC; Acquity uPLC, Waters Corp., MA) using a 50 × 1 mm Acquity BEH-C18 (1.7 μm) column (Waters Corp., MA). Mobile phase A (water) and mobile phase B (95% acetontrile) both contained 0.1% ammonium formate pH 4 and were used at a flow of 0.3 ml min^{-1}. A rapid gradient from 100% A to 100% B was applied over 2.5 min. Injection volume was 5 μl. The Quattro Premier XE mass spectrometer (Waters-Micromass, Manchester) was operated in electron spray ionization (ESI+) mode with capillary voltage 0.5 kV,

desolvation gas 900 l hr^{-1}, 400°C, cone gas 200 l hr^{-1} and cone voltage 25 V.

Quantitative analysis was by multiple reaction monitoring (MRM) using mass

spectrometry- mass spectrometry (MS-MS) channels set up for anatoxin-a (166.1

> 131.0; 166.1 > 149.1; Rt 1.3 min). The instrument was calibrated with dilutions

in 0.1% formic acid of authentic standards of anatoxin-a (A.G. Scientific, USA).

Biotic Composition, Richness and Diversity and their Correlation to Abiotic Variables.

BEST analyses and multidimension scaling were performed in the statistical

package Primer 6 (PRIMER-E, Ltd., UK). Correlation of biotic to abiotic plots

and the principle variables underpinning those correlations were assessed by

BEST analysis indicated by Spearman rank correlation (ρ). BEST analysis selects

environmental variables that may explain biotic patterns.

2.3 RESULTS

2.3.1 Chlorophyll-a trends over five months

The concentration of *Chl-a* through November and December 2007 was variable

but high, reaching levels exceeding 90 μg l^{-1} (Fig. 2) and generally remaining

above 60 μg l^{-1} (Fig. 2). However, in late December and early January *Chl-a*

declined at a rate of -2.5 μg l^{-1} day^{-1} reaching a minimum of 3.5 μg l^{-1} on 10

January (Fig. 2) indicating a pronounced decline in the bloom. By the 22 of

January *Chl-a* had increased above 20 μg l^{-1} and from this period onward *Chl-a*

remained between 20 and 40 μg l^{-1} (Fig. 2).

The ten week period from 11 November 2007 until the 29 of January 2008

contained the greatest fluctuation in *Chl-a.* This period was chosen as the focal

point of the study and each week from this period will subsequently be referred to

as weeks 1 through 10 respectively. Where measurements were made outside of

this timeframe, results are supplied in the appendices.
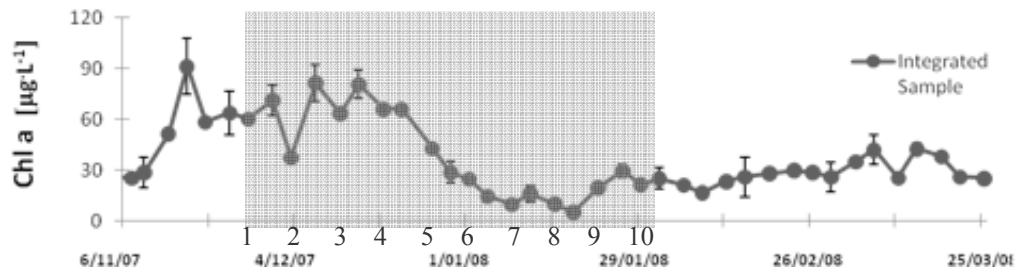
Figure 2. Change in *Chlorophyll-a* concentration in Lake Kainui from 6 November 2007 through to 27 March 2008. The shaded area indicates the 10 week period that is focussed on in subsequent analyses (27 November 2007 to 29 January 2008) and is subsequently referred to as weeks 1 through 10.

2.3.2 Meteorology

The mean daily air temperature averaged 18.4 ^{o}C with a maximum of 23.1 ^{o}C and

a minimum of 13.6 ^{o}C (Fig. 3A). The maximum daily air temperature ranged

from 30.8 ^{o}C down to 18.9 ^{o}C. Mean daily wind speed was 9.37 m's^{-1} with a peak

of 16.3 m's^{-1} (Fig. 3B). Rain was sporadic and sparse with a total rainfall of 76.1

mm over the ten weeks. The greatest rainfall occurred in week 4 (40.5; Fig. 3E).

Daily solar radiation averaged 23.0 MJ'm^{-2}, the maximum occurring in week 10

(34.21 MJ'm^{-2}; Fig. 3D). Soil moisture content consistently declined through

summer, from 35.1% in week 1 to 13.7% in week 10 (Fig. 3C).
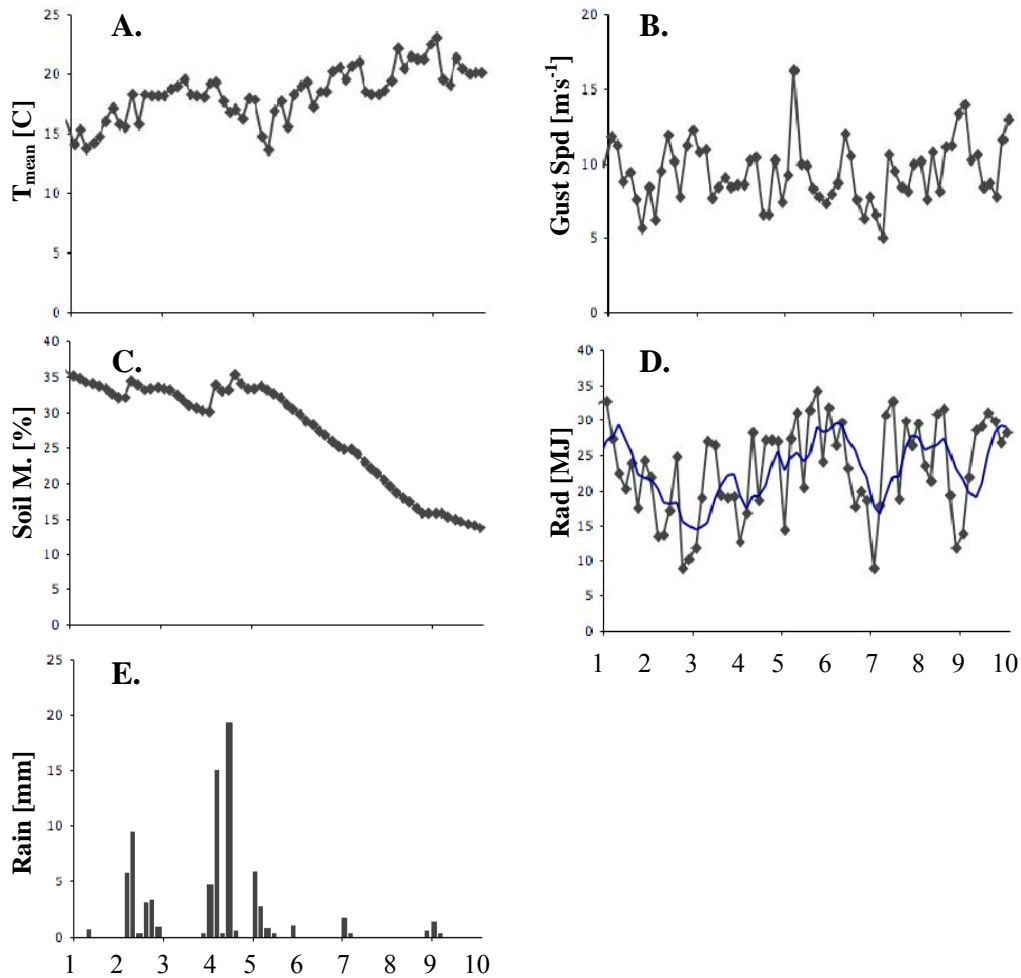
Figure 3. Meteorological data for 10 week interval. A. Temperature, B. Near surface gust speed, C. Percent of ground soil moisture saturation, D. Solar radiation and E. Rainfall. Data downloaded from the NIWA meteorological station situated at Ruakura.

2.3.3 Physico-chemical variables

The manually measured water temperature taken at 17:30 pm over the 10 weeks had a mean of 23.9 °C ranging from 20.7 °C (±0.1, week 3) up to 27.3 °C (±0.7, week 6) (Fig. 4A). The mean temp at 2 m depth was 23.1°C ranging from 20.7 °C (±0.1, week 3) to 25.2 °C (±0.7, week 8) (Fig. 4B). Results from the data logger were inconsistent. This was most likely due to changes in water level resulting in the logger gradually becoming closer to the water surface (Appendix 10). Lake Kainui was thermally stratified on weeks one and two and from weeks four

through eight (Fig. 5F). Schmidt stability varied across the ten-week period, ranging from 8.1 to 11.8 through the stratified weeks and decreasing to be in the range of 0.05 to 1.6 during the mixed periods (Table 1).

Surface dissolved oxygen (DO) levels averaged 8.1 mg·l^{-1} (Fig. 4C). Periods of stratification coincided with periods of anoxia in the hypolimnion. Local maxima for dissolved oxygen occurred on week 2 (10.5 mg·l^{-1}) and week 4 (9.0 mg·l^{-1}) of the ten week period, either side of a period of isothermy. Surface pH was correlated with the integrated *Chl-a* concentration. Mean pH was 8.2 for the two weeks and ranged from 10.1 down to 7.1. During periods of low *Chl-a* pH was near neutral. Conductivity varied little through the measured period (Fig. 4D).

The euphotic zone on week 8 was three times deeper than that in week 1 through to week 4 (Fig. 5A). The ratio of mixing depth to euphotic depth (z_{mix}:z_{eu}) reached a maximum in week 3 and then trended down. There was a correlation between the ratio of z_{mix}:z_{eu} to the rate of change of *Chl-a* (r^2=0.63; Fig. 13). Beam transmission also increased as *Chl-a* declined (Fig. 4C).

BEST analysis found that the environmental variables that best explained observed patterns in phytoplankton dynamics were pH, conductivity and temperature. The Speareman's correlation coefficient (ρ) for these was 0.803.
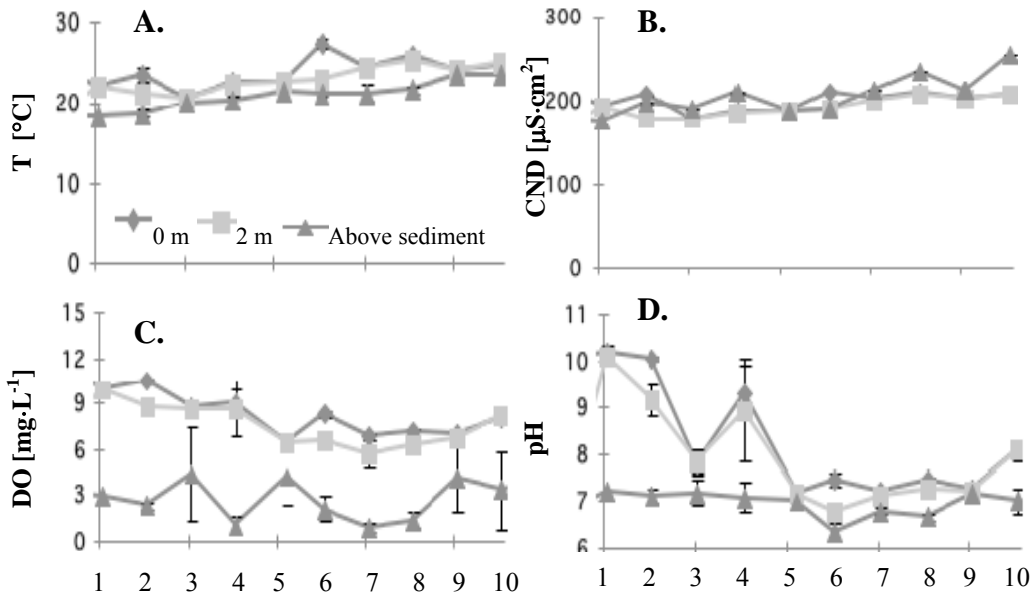
Figure 4. Physical parameters measured at Lake Kainui over 10 week interval. A. Temperature, B. conductivity, C. dissolved oxygen and D. pH.
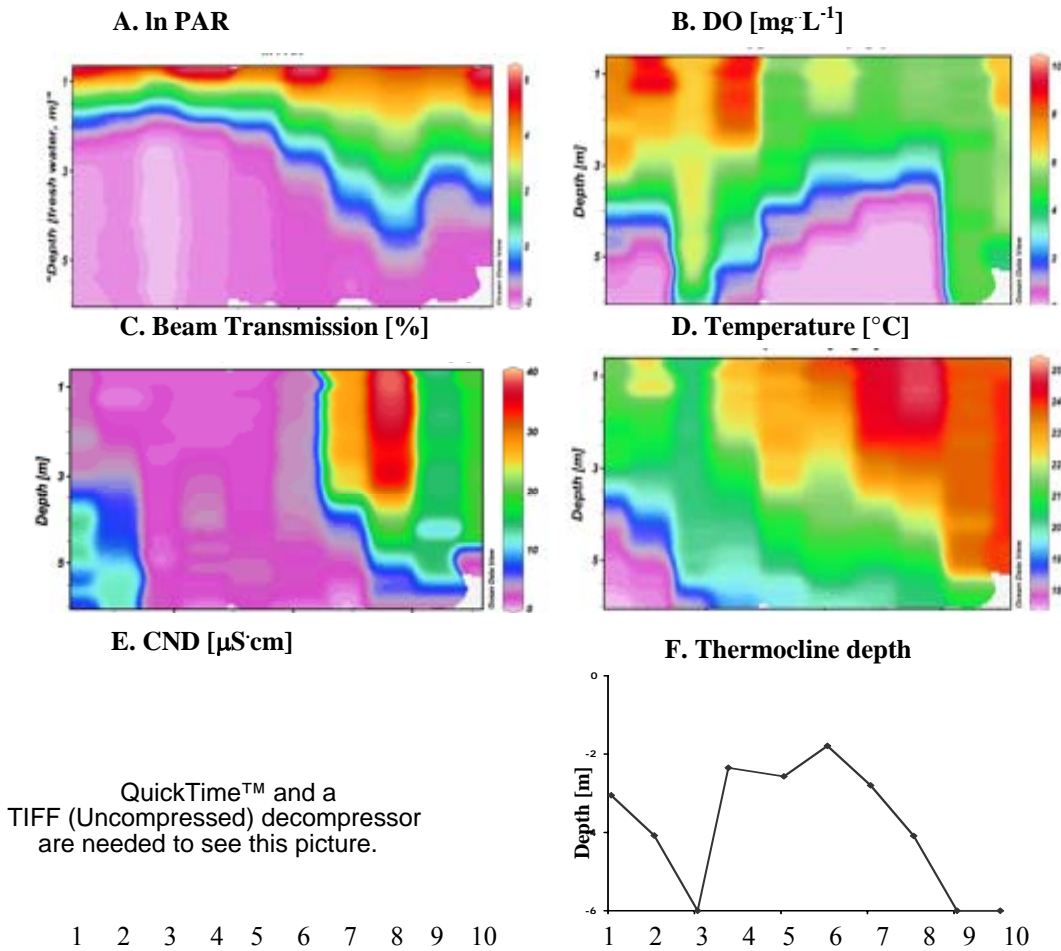

Figure 5. Physical characteristics of Lake Kainui measured over 10 week interval. Figures A-E show weekly and interpolated data. Figure F shows the depth of the mixed layer.

Table 2. Values and co-efficients of lake mixing for Lake Kainui over 10 week interval. Where depth reads #N/A refers to periods of complete lake mixing.

	Thermocline Depth	Epilimnion Depth	Hypolimnion Depth	Schmidt Stability
1	3.05	2.85	3.55	9.87
2	4.08	2.08	5.28	8.51
3	#N/A	#N/A	#N/A	0.17
4	2.35	2.15	3.55	8.36
5	2.57	2.27	2.87	8.44
6	1.79	0.89	2.09	8.05
7	2.8	2.6	3.1	11.27
8	4.09	2.09	5.29	11.78
9	#N/A	#N/A	#N/A	0.12
10	#N/A	#N/A	#N/A	0.05

2.3.4 Lake Chemistry

Nutrients

The concentration of nitrite (NO_2-N) in the water column showed little variation about its mean of 0.005 mg·L^{-1} (Fig. 6E). The mean concentration of nitrate (NO_3-N) in the water column was 0.07 mg·L^{-1} and ranged from 0.1 mg·L^{-1} to 0.05 mg·L^{-1} (Fig. 6G). Ammonium (NH_4-N) had a mean value of 0.06 mg·L^{-1} and ranged from 0.393 mg·L^{-1} to 0.007 mg·L^{-1} (Fig. 6C) Peaks in NH_4-N were not correlated with periods of hypoxia.

Total phosphorus (TP; Fig. 6B) and total nitrogen (TN; Fig 6D) concentrations followed the same trend as *Chl-a*, peaking in week 4 then declining to their minima in January. However, while TN and TP concentrations covaried ($r^2=0.95$) the ratio of TN:TP *did* vary with the ratio being approximately 15 until week 6 then doubled to 30 on week 8 (Fig. 6F). Using the trophic state indices of Carlson (1977) Lake Kainui was consistently eutrophic from November 2007 through to March 2008 (Appendix 5 Fig. 19).

The BEST analysis identified TN, TP and nitrite as the nutrients that best

explained phytoplankton dynamics ($\rho = 0.857$).
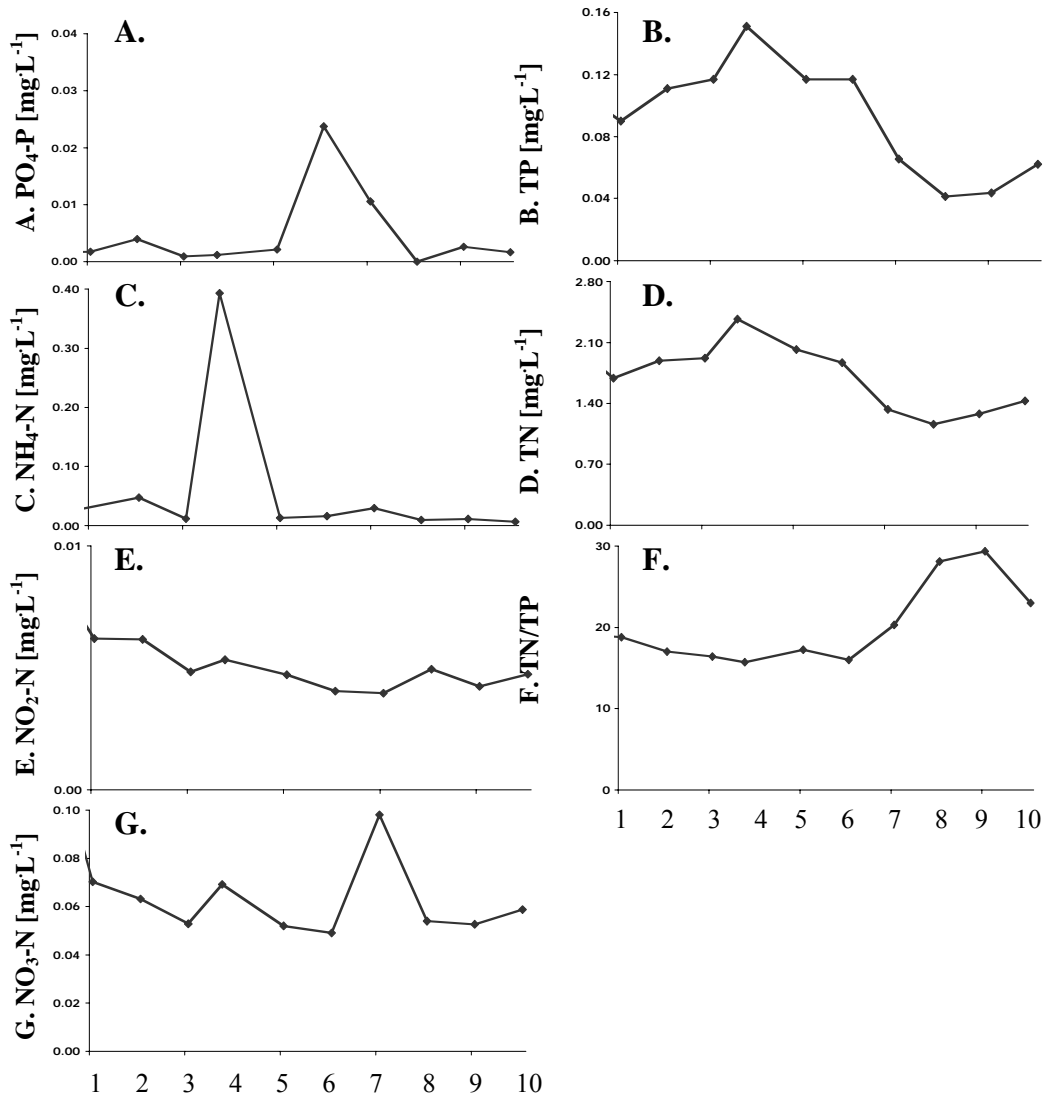
Figure 6 Variation in the concentration of nutrients in integrated water samples taken from Lake Kainui for ten week interval.

Micronutrients

The concentration of most micronutrients varied, however, it was difficult to

discern any that co-varied with changes in phytoplankton. Multi-dimensional

(MDS) ordinations of sample dates based on micronutrients showed that samples

were very similar across the bloom period. (Fig. 7) However MDS showed that

the samples taken on weeks 7 and 8 differed from the other samples and the

sample taken on week 9 was a definite outlier (Fig. 7).   Copper, aluminium,

phosphorus, iron, boron, manganese and barium all showed unusual changes in

concentration compared with other micronutrients. Manganese varied inversely

with *Chl-a* concentration, showing a small spike on week 2 and a large spike on

weeks 7 and 8 (Fig. 8). Iron also shows an increase in concentration around

weeks 7 and 8 (Fig. 8).   Barium follows the bloom approximately (Fig. 8).
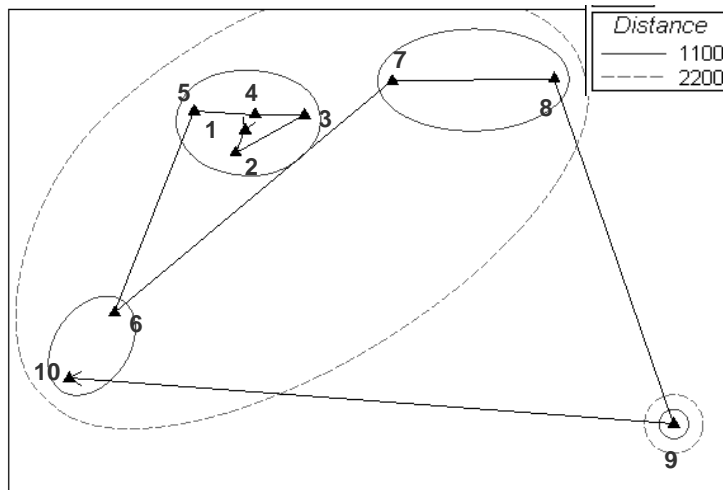
Figure 7. MDS plot of weekly samples based on micronutrients for Lake Kainui over a 10 weeks interval. Ordination based on Euclidean distance matrix.
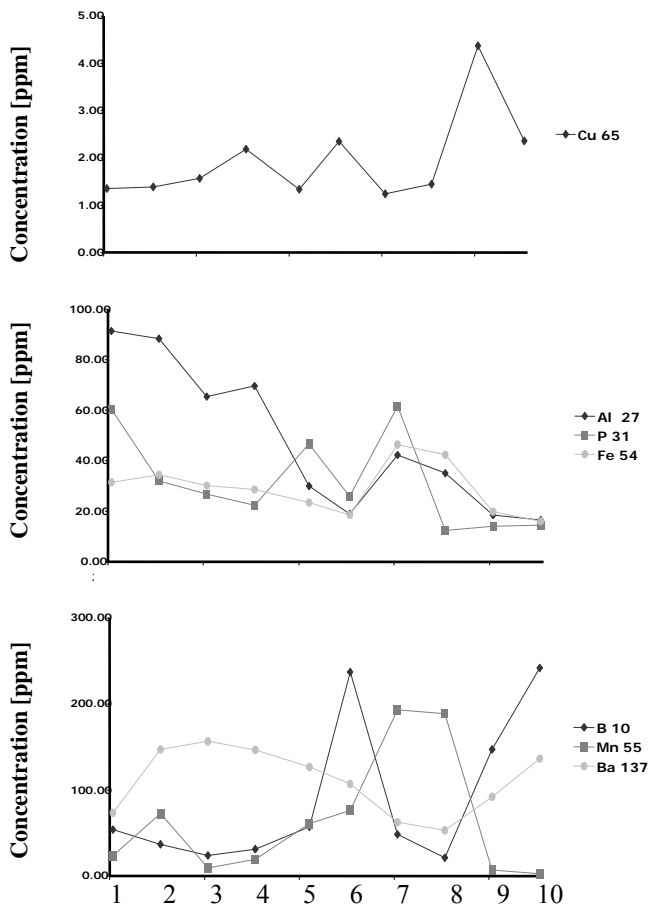
Figure 8. Micronutrient concentration changes through time from integrated water samples from Lake Kainui over ten week interval.

2.3.5 Dynamics of planktonic biota

Phytoplankton

Total phytoplankton counts ranged from 2×10^5 cells'mL^{-1} down to 2×10^4 cells'mL^{-1} over weeks 1 through 10 (Fig. 10). Lake Kainui's phytoplankton assemblage was dominated by cyanobacteria throughout; the first seven of the surveyed weeks cyanobacteria comprised 98 to 99% of the phytoplankton assemblage, then decreased to 93 to 94% over the last three weeks (Fig 10).

The cyanobacterial assemblage in Lake Kainui was dominated by *M. wesenbergii* and *Pseudoanabaena* sp (Fig. 10A). The *Pseudoanabaena* sp. was distributed roughly equally between being planktonic and being epibiotic in the mucilage of *M. wesenbergii* (Fig. 9). Both species were present through the duration of the study. Nitrogen fixing species of Nostocales were intermittently present, these included *Aph.issatchenkoi*, *An. planktonica, An.spiroides* and *An.a circinales.*
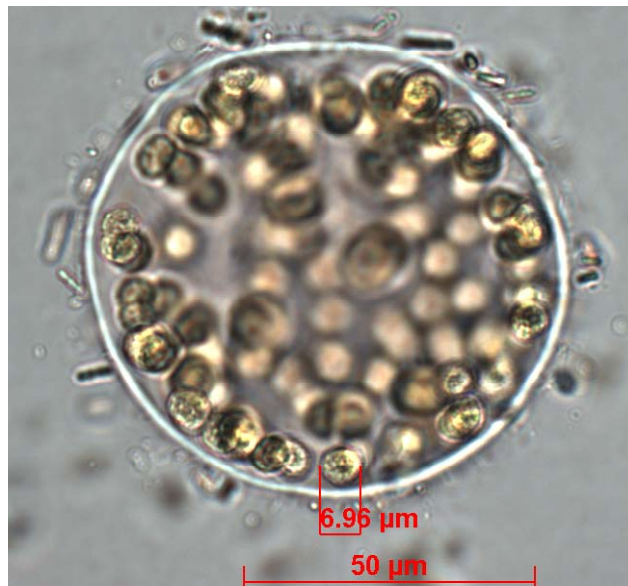
Figure 9. Colony of *Microcystis wessenbergii* cells (round) with its distinctive refractive mucilage. Rod-shaped colonies of the epibiotic *Pseudoanabaena* sp are evident about the colony periphery.

A.

Phytoplankton [cells·Ml⁻¹]

Thousands

140
120
100
80
60
40
20
0

1 2 3 4 5 6 7 8 9 10

Pseudoanabaena
M. wesenbergii
Oscilatoria sp.1
Oscilatoria sp.2
Microcystis spp
A. planktonica
Anabaena. sp
Aph. Issatchenkoi

B.

Cyanobacteris [cells·Ml⁻¹]

Thousands

2.5
2
1.5
1
0.5
0

1 2 3 4 5 6 7 8 9 10

Diatom
Charophyta
Euglenoidea
Dinoflagellates
Cryptophyta
Other
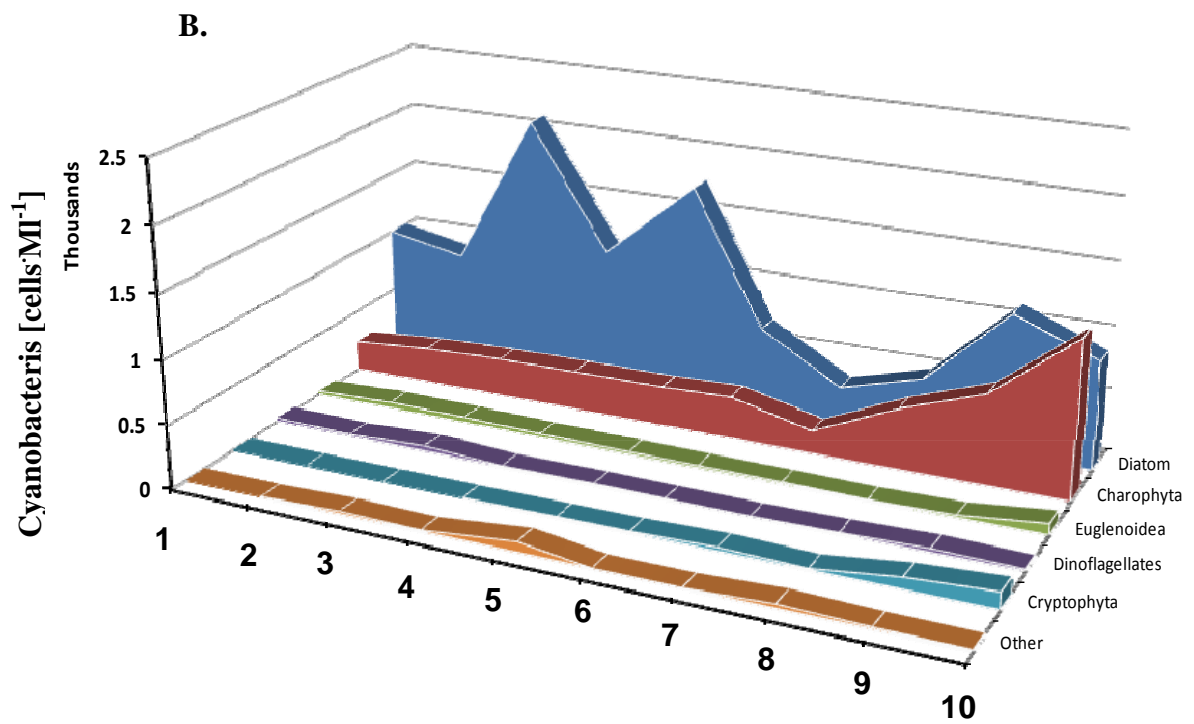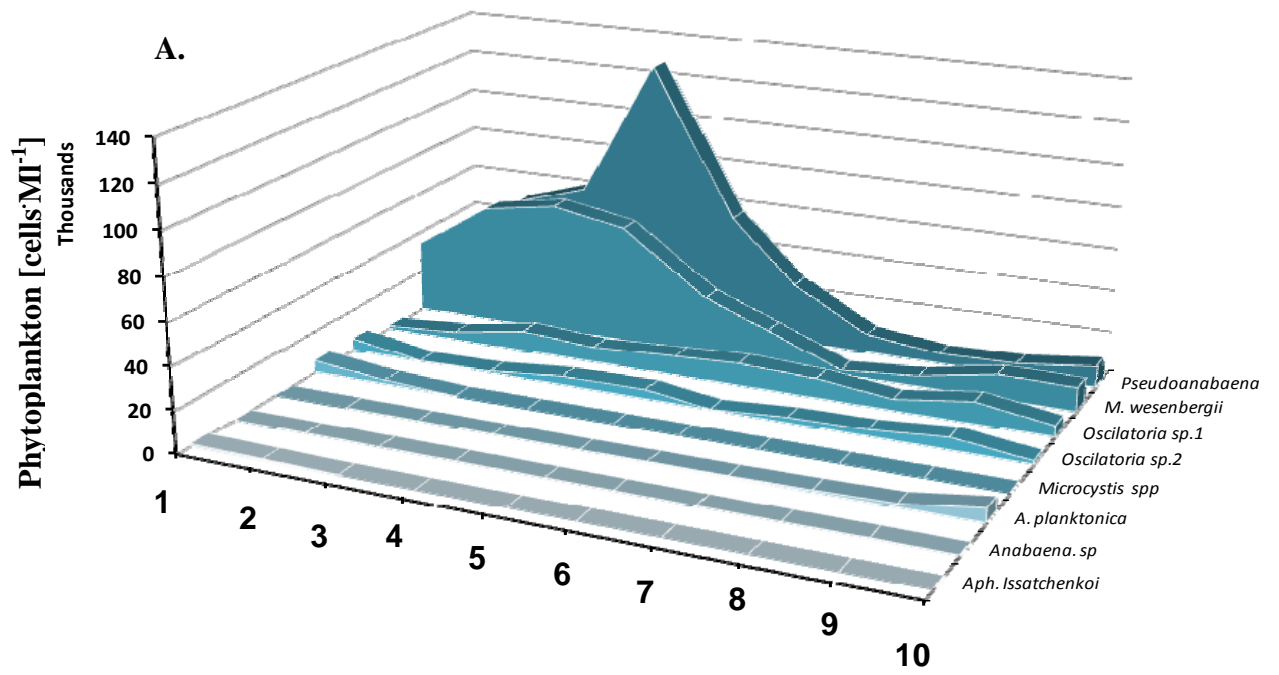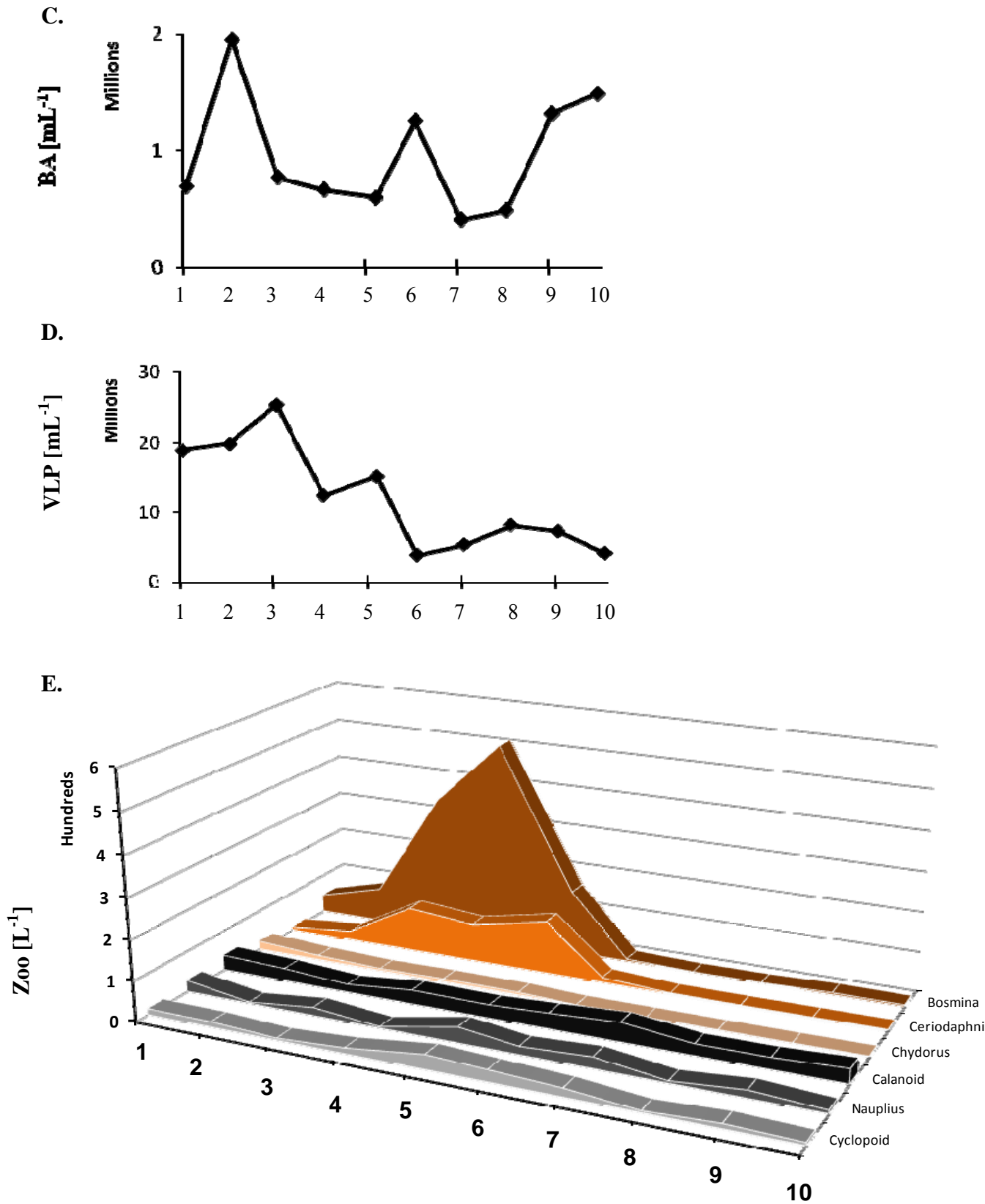
Figure 10 Abundance of planktonic biota in Lake Kainui over ten week interval. A. Relative change in algal orders abundance. B. Cyanobacteria abundance. C. Change in planktonic bacterial abundance. D. Change in planktonic viral like particle (VLP) abundance. E. Change in zooplankton abundance.

Table 3. Growth rates for orders of phytoplankton in Lake Kainui. The most frequently occurring phytoplankton orders are shown. Changes are rates of change of biomass.

Week	Cyanobacteria	Charophyta	Diatom	Euglenoidea	Dinoflagellates
1	-0.07	-0.07	-0.18	0.20	-0.19
2	0.07	0.03	-0.02	0.08	-0.10
3	0.02	0.01	0.12	-0.02	0.16
4	0.05	0.00	-0.09	-0.06	-0.26
5	-0.08	0.00	0.06	-0.04	0.05
6	-0.09	0.01	-0.16	-0.02	-0.14
7	-0.12	-0.09	-0.11	-0.04	-
8	-0.03	0.14	0.07	-0.16	-
9	0.09	0.06	0.12	0.20	0.16
10	0.00	0.08	-0.04	0.23	-0.10
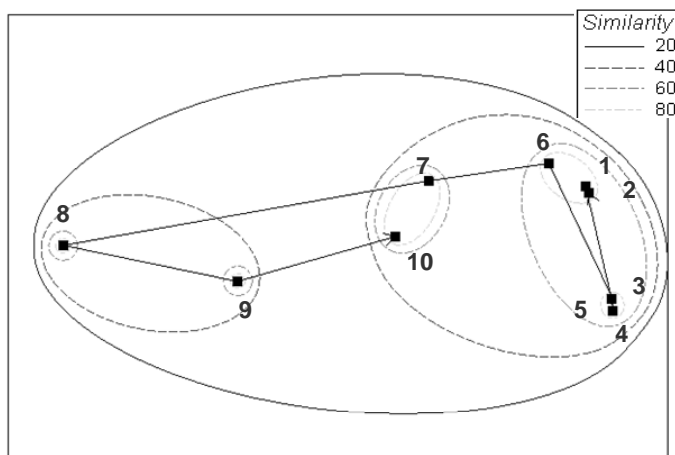
Figure 11 MDS plot showing the difference in phytoplankton community composition in Lake Kainui over ten week interval. Figure is based on the Bray-Curtis similarity matrix relating variation in biomass of species of phytoplankton composition.

Bacteria

Unicellular Planktonic Bacterial Abundance

Planktonic bacteria abundance averaged 7.7 x 10^6 cells·mL^{-1} ranging between 1.56 x 10^7 cells·mL^{-1} and 3 x 10^5 cells·mL^{-1}. Bacterial abundance did not follow phytoplankton and was not correlated with any other variables (Fig. 10C).

Bacterial Community Composition

ARISA on weekly planktonic samples gave an average number of 107 ± 10 ARISA fragment lengths (AFLs). ARISA on particle associated samples showed an average of 122 ±14 AFLs. ARISA profiles for both planktonic (Fig. 12A) and

particle associated BCC (Fig. 12B) assorted samples into two clusters with the

members of these clusters sharing >40% similarity.

A.   **B.**
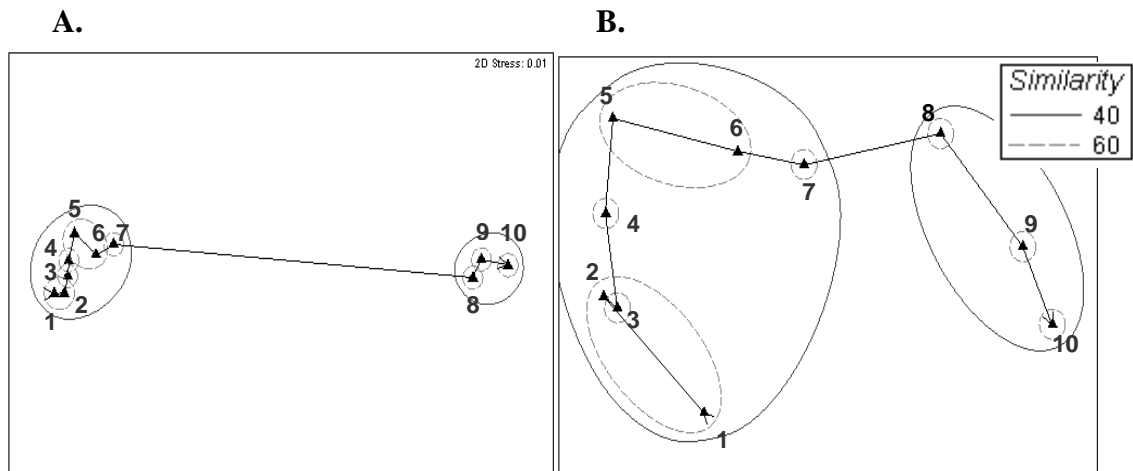
Figure 12 MDS ordinations of Bacterial Community Composition (BCC) derrived from Bray-Curtis similarity matrices of ARISA data for Lake Kainui over ten week interval. A. Planktonic Bacteria Community Composition (BCC). B. Particle associated BCC.

Virus Like Particles

Mean count of VLPs was 1.2×10^7 VLP mL^{-1} for the 10 weeks, with a maximum of

2.5×10^7 VLP.mL^{-1} and a minimum of 3.8×10^6 VLP mL^{-1} (Fig 10D). The virus to

bacteria ratio (VBR) of planktonic viruses against planktonic bacteria varied

erratically, ranging from 41 to 3 (Fig. 13).
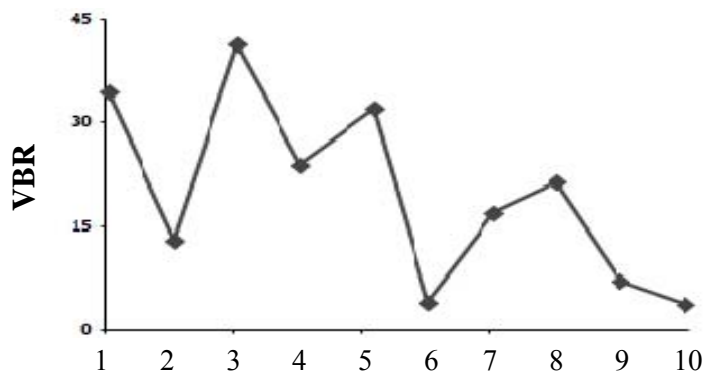
Figure 13 Virus bacterial ratio (VBR) for 10 weeks at Lake Kainui over ten week interval.

Fluctuations in the abundance of virus like particles were weakly correlated with

changes in *Chl-a* (Spearman's ρ=0.62) and the abundance of cyanobacteria

(Spearman's ρ=0.66), diatoms (ρ=0.62), and Euglenoidea (Spearman's ρ=0.65;

Table 3). Of the cyanobacteria *M. wesenbergii* was the species best correlated

with viral abundance (Spearman's ρ=0.78; Table 3). Almost all significant

correlations of biotic factors with viral abundance were positive coefficient

values, with the only exception being *An. planktonica* which increased from being

beneath detectable levels as viral numbers declined (Table 3). The correlation of

biotic community composition to viral abundance was generally not strong. The

only exception was that when viral abundance was expressed as a ratio with

cyanobacteria abundance then there was a reasonable correlation with changes in

bacterial community composition as represented by semi-quantitative ARISA

(Spearman's ρ=0.82). The abundance of VLPs also correlated negatively to

phytoplankton diversity (Spearman's ρ=-0.60). Planktonic bacterial composition

and abundance was not correlated to with planktonic viral abundance (Table 3).

A.
ABUNDANCE

	BA	Chl-a	Chl-a growth rate	Cyanobacteria	Charophyta	Cryptophyta	Diatom	Euglenoidea	dinoflagellates
VLP	0.03	0.62	0.22	0.66	-0.09	-0.23	0.62	0.65	0.49

	M.wesenbergii	Microcystis . spp	Planktonic Pseudoanabaena	Epibiotic Pseudoanabaena	Total Pseudoanabaena	Anabeana planktonica	Aphanizomenon issatchenkoi	Oscilatoriales sp.1	Oscilatoriales sp.2
VLP	0.78	0.51	0.58	0.60	0.52	-0.68	0.51	-0.36	0.02

B. DIVERSITY

	Planktonic BCC	Planktonic BCC (Pres.Abs)		Particle associated BCC (semi-quantitative)	Partticle associated BCC GF/C (Pres.Abs)		Phytoplankton Diversity
VLP	0.04	0.01	**VLP**	0.24	0.05	**VLP**	-0.60
VBR	0.24	0.18	**VCR**	0.82	0.33	**Vphy R**	0.47

Table 4 Spearmans correlation coefficients relating the dynamics of viral like particle abundance (VLP) to (A.) phytoplankton abundance and (B.) phytoplankton diversity indices. A. Correlation of VLP to various indicators of phytoplankton abundance. B. Correlation of VLP abundances to Shannon diversity indices for planktonic bacterial community composition (BCC), particle associated BCC and phytoplankton community composition.

Zooplankton Abundance

The Copepod population over the ten week period was fairly consistent in composition and abundance (Fig. 10E). Calanoids averaged 34 L^{-1}, ranging from 20 L^{-1} to 48 L^{-1} and Cyclopoids averaged 17.6 L^{-1} ranging from 4.5 L^{-1} to 36 L^{-1} (Fig. 10E). In stark contrast the abundance of Cladocera fluctuated dramatically. *Bosmina* sp. were numerically the most significant of the Cladocera peaking at 511 L^{-1} in week 5, then declining to 5 L^{-1} in week 7 and finally falling beneath detectable limits by week 8 (Fig 10E). *Ceriodaphnia* ranged from 132 L^{-1} down to 1.5 L^{-1} and *Chydoras* ranged from 24 L^{-1} down to undetectable concentrations (Fig. 10E).

BEST analysis of biotic variables

BEST analysis of potential biotic drivers for bloom dyanamics picked out *Bosmina*, *Chydorus* and VLP dynamics as the most likely variables to be structuring the phytoplankton community ($\rho = 0.76$). Adding particle associated BCC to these potential drivers resulted in a slight reduction in the correlation ($\rho = 0.75$).

Toxin Screening

With the exception of 6th of November 2007, anatoxin-a was found in all samples up until the week 2 (Appendix 9, Table 8). A sample that was taken from the littoral zone of the lake on the 12th of September 2007 had an anatoxin-a concentration of 8.23 $\mu g^. L^{-1}$. Toxin concentrations taken through the whole water column across the lakes centre were not so high and did not exceed 0.17 $\mu g^. L^{-1}$.

2.4 DISCUSSION

2.4.1 Phytoplankton Dynamics in Lake Kainui

Between 1983-84 Etheredge (1987) observed that the contribution of cyanobacteria to Lake Kainui's phytoplankton assemblage was 0.5% of the mean annual phytoplankton biomass and 0.2% of mean phytoplankton cell density. Boswell (1985) records a phytoplankton assemblage at Lake Kainui that was dominated by unidentified green colonial coccoids for the "first few weeks" of 1980. The assemblage soon shifted to being dominated by "similar numbers" of the algae *Ankistrodesmus, Closterium, Staurastrum, Melosira, Cryptomonas, Perinidium* and *Trachelomonas*. The more comprehensive temporal study of Etheredge (1987), which spanned July 1983 to July 1984, identified the Chlorophyta as dominant constituents, with *Zygophaceae* making up 86.5% of mean annual biomass. It is therefore surprising that Lake Kainui now regularly experiences cyanobacterial blooms. The dramatic dominance of cyanobacteria in the phytoplankton assemblage over 2007-08 invites the question about what has happened at Lake Kainui over the last twenty years to now favour cyanobacteria over other algae?

This change to a cyanobacteria-dominated assemblage may be partly attributable to the recent colonisation of Lake Kainui by cyanobacterial species not previously observed in New Zealand that are more competative than the previously established algal species. Etheredge (1987) identified 17 species of cyanobacteria across a two year study (Appendix 2, Table 7), but the only species that both her study and ours identified were *M.aeruginosa, An. circinalis* and *An.spiroides.*

None of these species was in abundances that would be considered ecologically significant in either study. Over the course of the present study *M. wesenbergii* was the dominant cyanobacteria in terms of biomass. *Microcystis wesenbergii* has only been observed in New Zealand within the last decade (Wood et al., 2005). The same is true of some of the other species that were present at lower densities in Lake Kainui such as *An. planktonica* (Wood et al., 2005) and *Aph. issatchenkoi.* However, it is possible that *Aph. issatchenkoi* was misidentified as another genus in lake surveys last century (Wood et al., 2007). There is also no record for Lake Kainui of the *Pseudoanabaena* species that numerically dominated the cyanobacteria. The occurrence of this *Pseudoanabaena* in the mucilage of *M. wesenbergii* suggests that *M. wesenbergii* may shepherd its growth.

Another explanation for the shift to cyanobacteria is that it is driven by a change in nutrient abundance. The farmland in the lake's catchment probably followed a national trend toward more intensive farming practices (Schallenberg, 2004) and it is probable that the cyanobacterial dominance is indicative of higher nutrient loads. This study measured nutrient concentrations integrated through the entire water column and the 1980s studies measured nutrient concentrations in surface waters, therefore caution must be taken in comparing the studies. It should also be noted that the 1980s data sets are limited to only a few sample points. The values recorded in this study for TP and TN through the 2008 post bloom-decline period do not differ markedly from the figures that Etheredge (1987) recorded (Table 4). The bloom peaks of December 2007 are however substantially higher. Total phosphorus (TP) reached 151 mg·m^{-3}, which is more than three times that

recorded by Etheredge and TN was more than double that recorded by Etheredge, reaching 2,370 mg·m^{-3}.

Table 5. Nutrient concentrations in Lake Kainui, 1980, 1983 and present study. 1980 and 1983 measurements are taken from surface waters, but present study is of the integrated water column.

	Jan to Mar 1980[1]	Winter 1980[1]		1983[2]		Nov 2007 to March 2008
	n=10	Site 1 n=1	Site 2 n=1	5 Sept	10 Nov	n=21
Total Phosphorus (mg·m^{-3})	-	-	-	37	45	72
Total Kjeldahl Nitrogen (mg·m^{-3})	-	-	-	815	1240	1565
Dissolved Reactive Phosphorus (mg·m^{-3})	0 - 22 (mean:10)	12	12	9	12	6
Nitrate (mg·m^{-3})	0 - 18.7 (mean:9)	84.5	87.1	17	36	97
Ammonia (mg·m^{-3})	12 – 105 (mean:49)	57.8	54.8	13	99	37

[1] Boswell (1985)
[2] Etheredge (1987)

Nutrient concentrations appear to be relevant to Lake Kainui's phytoplankton assemblage. Before the bloom declined in January 2008 Lake Kainui exhibited low ratios of total nitrogen to total phosphorus ratio (TN:TP), which are environmental conditions that favour cyanobacteria (Smith, 1983). The higher concentration of NH$_4$ relative to NO$_3$ favours species that do not fix nitrogen such as *M. wesenbergii* (Oliver and Ganf, 2000). Lake Kainui had high pre-crash concentrations of TP, but also had persistently low levels of phosphate. The latter explains the high abundance of *Microcystis* spp. because low phosphate concentration selects for gas-vacuolated cyanobacteria (Ganf and Oliver, 1982) that can migrate down the water column to deeper waters or the sediment-water interface where phosphate concentrations should be higher. The uptake rate for phosphorus is high in *Microcystis.* This genus can tolerate low intracellular P and has the ability to store high concentrations of P (Ganf and Oliver, 1982) and this would also have contributed to its success in Lake Kainui.

The lake micronutrients that varied significantly through the bloom decline in Lake Kainui (copper, aluminium, phosphorus, iron, boron, manganese and barium) are mostly implicated in phytoplankton health (Hyenstrand et al., 2001; Huisman and Hulot, 2005; Reynolds, 2006). However, without *in situ* enrichment experiments it is difficult to know if these micronutrients were limiting and therefore restricting phytoplankton development or if fluctuations in these micronuitrients are a result of variations in phytoplankton biomass.

Both *A. planctonica* and *A. issaachenckoi* are known to produce a suite of intracellular toxins (Paerl and Millie, 1996; Wood et al., 2007) and for the duration that *A. issatchenkoi* was in the water column anatoxin-a was detected at concentrations of 0.8 to 1.7 $\mu g \cdot l^{-1}$ (Appendix 9, Table 8). At these concentrations, which are well below those specified for drinking water, anatoxin-a is unlikely to be problematic for recreational purposes. However, the measured concentrations were surprisingly high relative to the low density of *A. issatchenkoi* throughout the water column (1×10^3 cells $\cdot mL^{-1}$). If this organism was to accumulate at the water's surface or littoral areas it could result in a localised concentration of anatoxin-a to dangerous levels. Illustrating this supposition, a water sample that was taken from the littoral zone in October 2007 (as a pilot sample) was found to have much higher concentrations of anatoxin-a ($8.23 \mu g \cdot L^{-1}$).

Microcystis is a genus that is well known for toxicity (Paerl and Millie, 1996). Toxins have been identified in Japanese strains of *M.wesenbergii* (Bijkerk, 2006) but not detected in *M. wesenbergii* strains isolated from 13 European lakes (Bijkerk, 2006). A more recent survey of *M. wesenbergii* from seven Chinese

lakes found none that was positive for microcystins (Xu et al., 2008). It is not known whether the *M. wesenbergii* in Lake Kainui produces microcystins.

pH

Through the course of the bloom the pH was elevated (fig 4D). In water, protons are in equilibrium with CO_2 (Hamilton et al., 2004). The removal of CO_2 by photosynthesis was therefore likely to be the cause of this high pH. Cyanobacteria are favoured over other phytoplankton once conditions of low-CO_2/high-pH are established (Shapiro, 1997). The low CO_2 and high pH are driven and maintained by the cyanobacteria's own metabolism, and so create an environment that selects for them. The dramatic cyanobacterial decline therefore caused the end of this selectively advantageous environment.

The pH can also directly influence the availability of lake nutrients. In a peat lake the dissolved humic matter can complex to phosphate and iron, making these nutrients biologically unavailable (Steinberg, 2004). This sequestration is pH dependent and happens under low pH (Steinberg, 2004). The drop in pH that coincided with the bloom decline could therefore have had implications for nutrient availability and explain why the bloom did not recover.

Table 6. Physical parameters of Lake Kainui, 1980, 1983-4 and the present study. Unless otherwise stated, measurements are taken from surface waters.

		Jan to Mar 1980[1]	Winter 1980[1]		Jul 1983 to Jul 1984[2]	Nov 2007 to Jan 2008
		range across two sites, n=10	Site 1 n=1	Site 2 n=1	n=22	n=10
Temperature (^{o}C)						
	surface	20.1 – 27.5	15	14.5	11 – 24 (mean: 18.1)	20.7 – 27.3 (mean: 23.8)
	bottom	20.0 – 20.1	13.6	14.0	11 – 22 (mean: 17.4)	18.3 – 23.7 (mean: 21.0)
Dissolved oxygen (g·m^{-3})						
	surface	5.4 – 10.6	12.6	12.6	7 – 11 (mean: 8.9)	6.5 – 10.6 (8.2)
	bottom	0.2 – 7.9	10.8	12,0	0.2 – 10.8 (mean: 7.7)	0.9 – 4.4 (mean: 2.7)
Secchi depth (m)		0.5 – 1.0	0.6	0.5	0.5 – 1.0	-
pH		6.0 – 7.2 (mean: 6.7)	6.1	6.3	7.5 – 7.8	7.1 – 10.1 8.2
Conductivity (S·cm^{-1})		111 – 132 (mean: 117)	80	78	80, 183*	6.6 – 7.6 (mean: 7.1)
Suspended solids (g·m^{-3})		1.1 - 11.2 (mean:6.5)	12	10.6	-	-

[1] Boswell (1985)
[2] Etheredge (1987)
* Only two readings for conductivity, the first is in spring 1983 and the second is in summer 1984 were taken.

2.4.2 Solar affects: temperature change, stratification, euphotic depth and drought.

Changes in water temperature were inversely correlated with algal abundance and biomass. However the water temperature never exceeded 28 ^{o}C, which is the optimum water temperature for the growth of *M. wesenbergii* isolates and the temperature that must be exceeded for succession to occur *in situ* (Imai et al, 2009). This study therefore concurs with Oliver and Ganf's (2000) generalisation that the correlation of temperature change to bloom decline is not a direct causal relationship, but a case of temperature change modulating other environmental variables and that it is these secondary variables that affect bloom health.

Euphotioc depth (z_{eu}) varied little for weeks 1 through 4, but then increased over weeks 5 throgh 8, which was the period when phytoplankton productivity

declined (Fig. 5A). The epilimnion depth (z_{mix}) varied little over weeks 4 through

7 (Fig. 5F). The ratio of z_{mix}:z_{eu} followed the same trend as the rate of *Chl-a*

concentaion change (fig. 14) is unusual because z_{mix}:z_{eu} is considered to have an

inverse relationship to phytoplankton growth rates because if mixing depth is

greater than euphotic depth then phytoplankton entrained in the mixed layer will

be exposed to less light (Sverdrup, 1953). When z_{mix}:z_{eu} is high then buoyancy

regulating phytoplankton, such as *Microcystis,* might be favoured and this could

explain why changes in z_{mix}:z_{eu} corresponded to changes in *Chl-a* and

cyanobacterial growth rates (Tilzer, 1987). Wallaced and Hamilton (2000)

predicted that *Microcystis* would out-compete phytoplankton that do not regulate

their buoyancy so long as lake mixing was not so vigorous as to inhibit buoyancy

regulation; this would not occur, until z_{mix}:z_{eu}>>5. In Lake Kanui z_{mix}:z_{eu} only

exceeded 5 in week 4, which was directly before bloom decline.
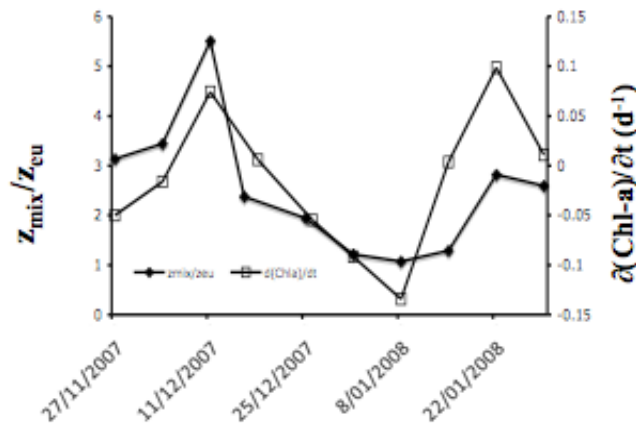

Figure 14 Left axis shows the ratio of mixing depth (z_{mix}) to euphotic depth (z_{eu}) and the right
shows the daily rate of change in *Chl-a.*

The Waikato region experienced unusually low rainfall in the summer of 2007-08,

which could have been related to phytoplankton decline. Lake Kainui is an

ombrogenous (exclusively rain-fed) lake and low rainfall would therefore have

constrained the nutrient inflow into the lake and potentially reduced the likelihood of bloom development.

It has also been found that droughts cause a reduction in dissolved organic carbon (DOC). DOC concentration was not measured in this study but it might have been relevant to bloom decline because as DOC declines the penetration of UV light into lakes increases exponentially (r^2=0.98 for a regression relationship derived from 22 lakes, Schindler et al., 1996). UV dimerises DNA and liberates toxic by-products such as free radicals and reactive oxygen species through the products of photo-mineralisation reacting with oxygen (Steinberg, 2004). Photo-mineralisation is also a self-reinforcing phenomenon whereby increased UV light penetration increases the extent of photo-oxidation of macromolecules and the resulting destruction of these also allows increased UV light penetration (Schallenberg, 2004). The period of stratification that coincided with the blooms decline meant that if UV had degraded macromolecules then these would not be replenished by lake mixing from the sediments.

2.4.3 Bacteria

Planktonic bacterial abundance (BA) in Lake Kainui

The fluctuations in planktonic bacterial abundance (BA) did not correlate with changes in phytoplankton abundance or composition. This result is not unexpected in a peat lake due to the rich supply of allochthonous carbon that decouples heterotrophic bacteria from being dependent on autochthonous carbon (Arvola et al., 1999). The smaller size of heterotrophic bacteria typically means they have superior nutrient uptake kinetics to phytoplankton and therefore would

dominate productivity during nutrient limiting conditions in a peat lake (Jones 1992; Arvola et al., 1999). Bacterioplankton abundance in Lake Kainui climbed after the bloom declined, which was a period of nutrient limitation. It is possible that Lake Kainui was in a state of trophic flux, moving from a dystrophic and predominantly allochthonous system to a eutrophic, predominantly autochthonous and 'blooming' system and then moving back again.

Planktonic Bacterial Community Composition (BCC)

There was a dramatic shift in planktonic BCC on 15 January 2008 (Fig. 12A). This was one week too early to be caused by lake mixing, so it is unlikely to be attributable to a shift from hypoxia or the entrainment of bacterial cells from deeper waters or the sediment into the water column. This shift in planktonic BCC did however co-occur with troughs in cyanobacterial and crustacean abundance. Dissolved organic carbon can be available in the water column in various forms and BCC is responsive to what that form is (Kritzberg et al., 2006). The shift in BCC could be the result of fluctuations in phytoplankton which cause a change in DOM composition. This has been observed to affect BCC in humic lakes (Kent et al., 2006; Kritzberg et al., 2006). The discrepancies between actual cell counts and predicted cell counts of bacteria in the mucilage of *Microcystis* led Worm and Søndergaard (1998) to speculate that *Microcystis* colonies 'seed' the water column with planktonic bacteria. For this reason it could be expected that a decline in *Microcytsis* would cause a decrease in the phylogenetic signal of the 'seeded' bacteria in the water column and therefore cause a significant shift in BCC. Changes in grazing pressure have also been observed to affect BCC (Kent et al., 2006), and could cause a shift towards grazing resistant phenotypes.

Studies of BCC in humic lakes have found that BCC can quickly change, especially in summer (Yannarell et al., 2003; Newton et al., 2006). However, research has also identified that several persistent phylotypes of bacterioplankton exist through these dynamic changes in BCC (Yannarell et al., 2003; Eiler et al., 2004, Newton et al. 2006). The planktonic BCC at Lake Kainui had 20 persistent phylotypes across the ten weeks and the particle associated BCC had 30 (Appendix 8 Fig. 23). This is significant because it indicates that there was either and component of the BCC that is able to adapt to conditions through a bloom decline or, more likely, that there was a sufficient continuity through the bloom to sustain some of the bacteria population. This continuity was likely to have been allocthonous input.

Particle Associated Bacterial Community Composition

Particle associated bacteria were not counted in this study, however fluorescent staining revealed a rich community of bacterial cells associated with large humic particles and cyanobacterial colonies taken from Lake Kainui. MDS analysis of particle associated BCC (Fig 12B) showed a compositional drift that was more gradual than that of planktonic bacteria, however, still clearly discernible. Many processes probably underpin this drift, but the decline in *Microcystis* most likely resulted in a decline in an environmental niche that structured particle associated BCC. Kapustina (2006) demonstrated that the BCC associated with *Microcystis* colonies is different in structure to that of planktonic bacteria. Worm and Søndergaard (1998) found that bacteria growing epibiotically on *Microcystis* colonies can constitute 37% of lake bacterial abundance and Brunberg (1999)

found a similar contribution of as much as 40% of bacterial abundance. The productivity of these epibiotic bacteria is variable. Worm and Søndergaard (1998) characterised *Microcystis* colonies as 'hotspots' of bacterial activity that can dominate bacterial production, but Brunberg (1999) found that epibiotic bacteria only dominated pelagic bacterial production on decaying sedimentary colonies, not healthy ones. This difference in productivity (which were assayed in a similar way) is probably because bacteria respond to either the environment and/or the growth phase of their *Microcystis* host. Maruyama et al. (2003) showed that the epibiotic BCC changes depending on such factors as microcystin expression (for microcystin degrading hetrotrophic bacteria) and *Microcystis* health. Jiang et al., (2007) demonstrated how *M. aeruginosa* and an epibiotic bacterium circulate phosphorus between themselves inside the *Microcystis* mucilage, which results in *Microcystis* colonies being sites of concentrated phosphorus. The direction of phosphorus flow depends on the stage in the life-cycle of each organism. The disruption of a similar phosphorus cycle in Lake Kainui could explain both why the bloom had limited recovery and the loss of phosphorus from the system.

2.4.4 Viruses

Virus Like Particles, Bacterial Abundance and chlorophyll-a

The majority of previous research into viral abundance shows a stronger correlation with BA than *Chl-a* (Wommack and Colwell, 2000), although there are exceptions (eg. Maranger and Bird, 1995; Lymer et al., 2008). A hypothesis of the current study was that a massive decline in *Chl-a* (bloom collapse) would correlate with a change in viral abundance. This was observed in this study with

VLP abundance being moderately correlated with *Chl-a* concentration (Spearman's ρ=0.62). The strongest correlation was to *M. wesenbergii* dynamics (ρ=0.78). However, without quantatifying particle-associated BA it is difficult to definitely determine whether virus abundance positively correlated with *Microcystis* abundance or the abundance of its epibiota.

There are three likely explainations for the correlation between *M. wesenbergii* and virus dynamics. The first is that viruses might be supporting the cyanobacteria by indiscriminantly solubulising cells and therefore making nutrients available - the viral shunt theory (Thingstad and Lignell, 1997; Wilhelm and Suttle, 1999). The second mechanism is that viruses might be specifically lysing and controlling *M. wesenbergii* - kill the winner theory (Thingstad and Lignell, 1997). A third possiblilty is that virus dynamics correlate with *M. wesenbergii* dynamics because viruses are acting on bacteria that are associated with *M. wesenbergii.*

The 'viral shunt' is a case of viruses positively influencing a component of bacterial or phytoplankton growth. By contrast the second ('kill the winner') and the third possibility are cases of viruses negatively influencing a component of the microbial population's growth. Theoretically these negative influences would restructure the bacterial community by reducing the density of abundant species so that as prey diversity increases, then relative viral abundance will decrease. To test for this, each week's viral abundance was converted into a ratio with prey abundance and the resulting value then correlated with the weekly prey diversity (Shannon-Weaver). The strongest correlation with diversity was a positive one;

the ratio of virus abundance to cyanobacteria abundance was high when particle-associated bacteria diversity was highest (Table 4). These results could be interpreted in several ways; it could support the "kill the winner hypothesis", but reflect the fact that cyanobacteria create an abundant, but transitory niche that can be colonised by novel bacteria. Alternatively it could just be an artefact of dividing VLP abundance by cyanobacterial abundance, despite the fact that Brunberg (1999) found a very consistent correlation between epibiotic bacterial abundance and *Microcystis* abundance. Hewson and Fuhrmann (2007) looked at correlations between viral abundances and bacterial community composition (ascertained by ARISA) and between viral abundances and diazotroph community composition (ascertained by terminal restriction fragment length polymorphisms; TRFLP) and were only able to draw limited conclusions about the role of viruses. Greater success has been achieved by studies that have fractioned the viral population into phylotype, either by fingerprinting the viral community (e.g. using pulsed field electrophoresis) or by following a viral 'gene of interest'. These techniques allow for correlations to be visualised between viral phylotypes and potential prey. Genetic techniques are currently restricted by the limited comprehensiveness of sequence databases for viral genes. As such, quantitation of viral strains by isolation (such as on algal lawns) is a long-standing means of speciating viruses and has been successfully used to assess the role of viral species in terminating and controlling blooms (Manage et al., 2001; Clokie et al., 2006B). To extend the present study so that it could state with confidence that viruses were negatively controlling cyanobacteria applying these techniques would be useful.

The positive correlation between VLPs and *M. wesenbergii* observed in this study indicates that *M. wesenbergii* might have benefited from the increase in solubilised organic matter that phage lysis provides. Total abundance of VLP's was negatively correlated with phytoplankton diversity (ρ=-.60) but when VLP's are converted to a ratio of phytoplankton the relationship to phytoplankton diversity was positive and weaker (ρ=0.47). It is likely that this is evidence of the viral shunt. There is high viral abundance coinciding with low diversity, but as viral abundance declines diversity changes little. The negative correlation between abundance of VLP's and *An. planktonica* is also consistent with viral shunt. It would be expected that as lysis declines there would be less biologically available nitrogen in solution and so the ability to fix inorganic nutrients would be favoured. A more detailed explanation of whether viral abundance positively impacted cyanobacterial success would have been provided by an analytic method geared to assaying the impact of viral productivity on host productivity (Winget et al., 2005). Viral dilution assays have demonstrated that viral activity can have either a positive or a negative effect on primary production (Suttle, 2000; Winget et al., 2005) and the use of such a technique would extend the range of inferences available to this study.

2.4.5 Zooplankton

Crustacean grazing pressure on cyanobacteria

A further possible factor that may be regulating cyanobacteria in Lake Kainui were the periodic rapid increases in grazing zooplankton numbers. Copepods are relatively large crustaceans that are capable of consuming large algae (Reynolds, 2006). There was a small but persistent community of copepods in Lake Kainui.

However it has been established that copepods are consistent but slow in their reproduction and consumption rates and so are unlikely to control a bloom (Reynolds, 2006). Cladocerans are the most likely candidates to cause bloom decline and there was a very pronounced peak in the Cladoceran population – of *Bosmina* in particular. The fluctuation in Cladoceran abundance in Lake Kainui tracks the fluctuation in cyanobacterial abundance and this is reminiscent of the predator prey relationship modelled by the Lotka-Volterra equation (Sigee, 2005). Further information would be required to confirm the causative effect of zooplankton, for example, specific growth rate, reproduction rate and senescence rate of both predator and prey as well as grazing rates of zooplankton. More fundamental will be to confirm that Cladocera such as *Bosmina* do in fact consume cyanobacteria *in situ* and if they do, then what species?

Of the Cladocera, *Daphnia* have been observed to consume large species of cyanobacteria (Reynolds, 2006). However it was *Bosmina*, not *Daphnia* that was observed to proliferate in Lake Kainui. The size of a *Microcystis* colony relative to a *Bosmina* makes it seem unlikely that it is mechanically possible for *Bosmina* to ingest them, however *Bosmina* might be able to break into the colony and consume individual cells. Additionally, *Bosmina* might gain nourishment from the *Pseudoanabaena* sp. that occurred both planktonically and epibiotically on *M. wesenbergii* colonies. These *Pseudoanabaena* have short trichomes (of two to four cells), are little more than a micron wide and would be a more likely to be eaten by *Bosmina*. Another possibility is that *M. wesenbergii* colonies seed the water with unicellular *Microcystis* and that the Cladocera feed off these (single spherical cells were occasionally observed in the water column, but not

frequently). Previous studies describe great variability in the response of Cladocera populations to grazing on *Microcystis* (Hanazato, 1991; Gliwicz, 2004), though the general trend is that most species/strains of Cladocera are adversely affected by large colony size or toxin production (eg. Sommer and Sommer, 2006). Ultimately, to determine if Cladocera are directly grazing cyanobacteria in Lake Kainui then gut content analysis must be performed on specimens of Cladocera collected from the lake. This is difficult to do microscopically, because Cladocerans manipulate their food to the extent that it is a non-descript bolus in their guts (Reynolds, 2006). One solution is washing the exterior of crustacea of free DNA, macerating them, and then using molecular techniques to identify the cyanobacterial DNA liberated from their gut (Oberholster et al., 2006). This is a possibility for future study to confirm that the gut content of *Bosmina* does contain *Microcystis*.

***The indirect impact of Cladocera and viruses on Microcystis wesenbergii***

Bosmina and *Ceriodaphnia* have historically dominated the planktonic crustacea in Waikato lakes (Greenwood et al., 1999) and until recently there has been little record of larger Cladocera such as *Daphnia*. *Bosmina* populations in Lake Kainui may therefore be a legacy of biogeography and if *Daphnia* were introduced to the lake they might instead dominate. International studies have found that *Microcystis* blooms can select for *Bosmina* over *Daphnia* (Hanazato, 1991). The co-occurrence of *Microcystis* and *Bosmina* has been attributed to the unlikelihood that *Bosmina* could ingest anything as large as *Microcystis* as it randomly ingests food whereas other larger Cladocera are likely to ingest the *Microcystis,* which are a of poor nutritive value and can contain intracellular toxins such as microcystin.

In a series of experiments Hanazato (1991) found that Cladocera, including *Bosmina*, grew with variable success when fed *Microcystis*, but all grew very well when fed with dead and decaying *Microcystis*. The implications of this for Lake Kainui is that Cladocera probably control *Microcystis* in a significant but *indirect* way. By grazing on the products of degrading *Microcystis* they suppress autotrophic production by outcompeting the autotrophs for recycled nutrients; in effect they redirect nutrients up into higher trophic levels. It is plausible that viral lysis acted to continually cycle nutrients back into the autotrophic and bacterial community through December and so sustained the bloom through the 'viral shunt'. The rise of the Cladoceran population in late December opened up that closed trophic loop and redirected nutrients up the food chain and out of the shunt (Fig.15) this scenario could be confirmed by measuring the relative concentrations of $\partial^{13}C$ in the Cladocera to infer if the origin of their dietary carbon is either allochthonous or autochthonous (Karlsson et al., 2003). The fatty acid profile of zooplankton is typically a direct function of their diet (because zooplankton do little in the way of fatty acid synthesis). Fatty acid biomarkers can also be used in conjunction with stable isotopes to give an indication of nutrient origin (Perga et al., 2006).

The long period of the anoxia in Lake Kainui's hypolimnion would not be suitable for predators of Cladocera (Sarlava et al., 1999). A rapid increase in Cladocera in Lake Kainui occurred several weeks into this period of anoxia, which suggests that the alleviation of predator stress might have affected their success. If the assertions above are true then biomanipulation by removing pest fish or

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restocking of Cladocera might be a relevant and effective way to approach lake

restoration in Lake Kainui.

A. Bloom-time trophic dynamics
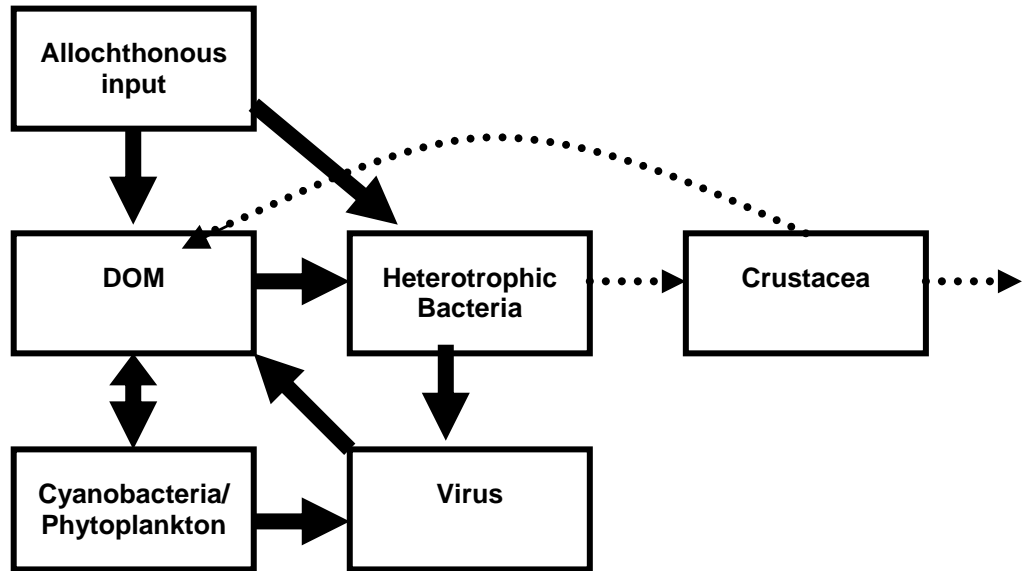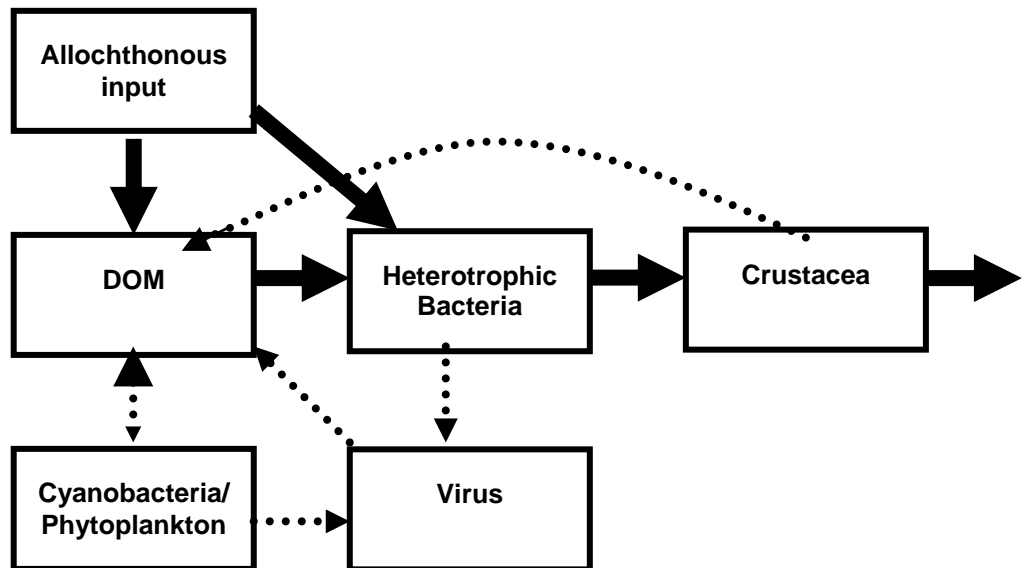

B. Post-bloom trophic dynamics

Figure 15 Conceptual diagram of change in nutrient flow in Lake Kainui. Size of arrows indicates the strength of nutrient flow through biota. Note that these diagrams do not include loss or regeneration of DOM by abiotic processes such as photoxidation. A. Shows the focusing of nutrients amongst smaller sized lake biota, which is thought to favour a sustained cyanobacterial bloom. It is hypothesized that indiscriminate viral lyses underpins the process via 'viral shunt'. B. Shows how the ascendancy of planktonic Crustacea could break this cycling and redirect nutrients up the trophic ladder.

2.5 CONCLUSION

Over a ten week period in the summer of 2007-08 Lake Kainui experienced the decline of a mixed assemblage, cyanobacteria bloom. The assemblage was dominated by *M. wesenbergii* and a *Pseudoanabaena* sp. that occurred both planktonically and epibiotically in the mucilage of *M. wesenbergii*. The bloom persisted until the end of December, when *Chl-a* gradually declined to five percent of the blooms mean value.

The object of this study was to identify if planktonic viruses or bacteria were implicated in a cyanobacteria bloom's decline. It was hypothesised that if they were not, then there would be no significant correlation between bloom decline and viral or bacterial abundance. Planktonic bacterial abundance showed no correlation to bloom decline suggesting that bacteria were not involved in bloom decline. However changes in planktonic and particle associated bacterial community composition did correspond to bloom decline. This suggests that a portion of planktonic bacteria either affected bloom decline or responded to it, but that the bacteria doing this constituted a small proportion of planktonic bacteria abundance because they did not influence total bacterial counts. Viral abundance was positively correlated with bloom decline. This could either indicate that bloom organisms were being controlled by viruses (i.e. virus abundance followed cyanobacteria abundance) or that the bloom benefited from viral activity (i.e. cyanobacteria abundance followed virus abundance). The prior would have been indicated if the ratio of virus:prey abundance was negatively correlated with prey community diversity – the "kill the winner" hypothesis. This was not the case, so the "kill the winner" hypothesis was not supported. By contrast, no results gave a

reason to reject the hypothesis that viruses were positively influencing the bloom (most likely by the "viral shunt") and therefore the decline in viral abundance might have had a causal role in bloom decline.

The next objective of this study was to ascertain if non-microbial parameters had a role in the bloom decline. The sudden increase in Cladocera abundance in weeks 3 and 4, especially of the genus *Bosmina,* indicates that these organisms may have restricted the bloom by grazing. Because *Bosmina* are too small to consume *Microcystis* colonies it is assumed that *Bosmina* were feeding on the products of degraded *Microcystis* colonies and therefore *Bosmina* negatively impacted the bloom by preventing nutrients from being recycled within the nanoplankton. Also, the decline of the cyanobacterial population and its failure to re-establish may have been related to the low rainfall and a sustained period of lake stratification, most likely by decreasing the nutrient supply to the epilimnion. Increasing irradiance may have resulted in photo-oxidation and its toxic by-products may have negatively affected cyanobacteria growth.

The results of this study suggest several recommendations. Using microscopy, this study observed no correlation between bacterial abundance and bloom decline, however genetic fingerprinting (with ARISA) showed that bacterial composition was correlated to bloom dynamics. Therefore, the genetic fingerprinting of bacterial communities is a valuable and recommended technique because it offers information that would be difficult to obtain using classical microbiology techniques. Greater confidence in how viruses and crustacea impact

bloom events could have been achieved by conducting dilution assays (Winget et al., 2005) in addition to the techniques used in the present study.

That zooplankton might indirectly regulate blooms in *Lake Kainui* by redirecting nutrients up the trophic ladder suggests that biomanipulation might be a successful way to manage cyanobacterial blooms. Removing introduced fish and freshwater jellyfish might stabilise a trophic cascade that reduces nutrients cycling back through primary producers, therefore alleviating the severity of the blooms.

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APPENDICES

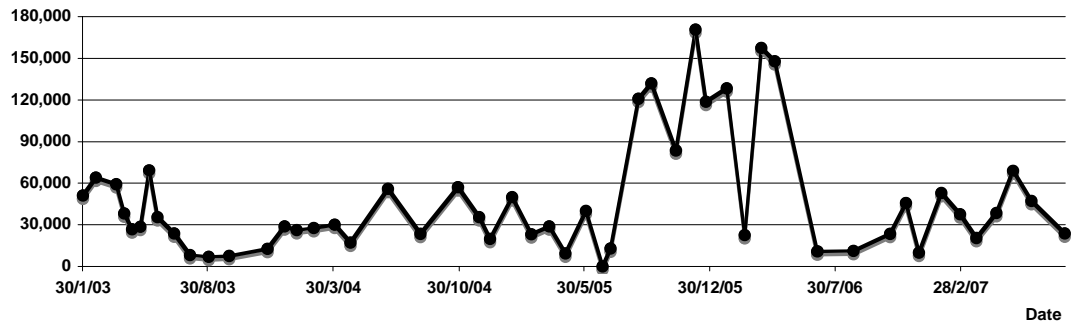## Appendix 1. Cyanobacterial concentrations in Lake Kainui.

Figure 16 Lake Kainui: total cyanobacteria abundance January 2003 to August 2007. Source; Environment Waikato.

Appendix 2. Cyanobacterial species in Lake Kainui 1983-84.

Table 7. Cyanobacteria in Lake Kainui, 1983-1984[1]

Species	Cell Density (mL^{-1})
Chroococcales	
Chroococcaceae	
Aphanocapsa elachista	0.2
Aphanothece nidulans	0.2
Chroococcus disperses	2.8
C. d. var. *minor*	1.8
C. limneticus	0.8
Merismopedia elegans	0.3
M. minima	14.6
Microcystis aeruginosa	4.0
Nostocales	
Nostocaceae	
Anabaena circinales	0.6
A. flos-aquae	0.3
A. minutissima ?	0.4
Oscillatoriaceae	
Lyngbya limnetica	0.2
Oscillatoria curviceps	0.1
O. geminata	0.1
O. limnetica	0.6
O. planctonica	0.1
O. splendida	0.2
O. subbrevis	0.2

[1]Etheredge (1987)

Appendix 3. Lake Kainui's physical parameters over five months.
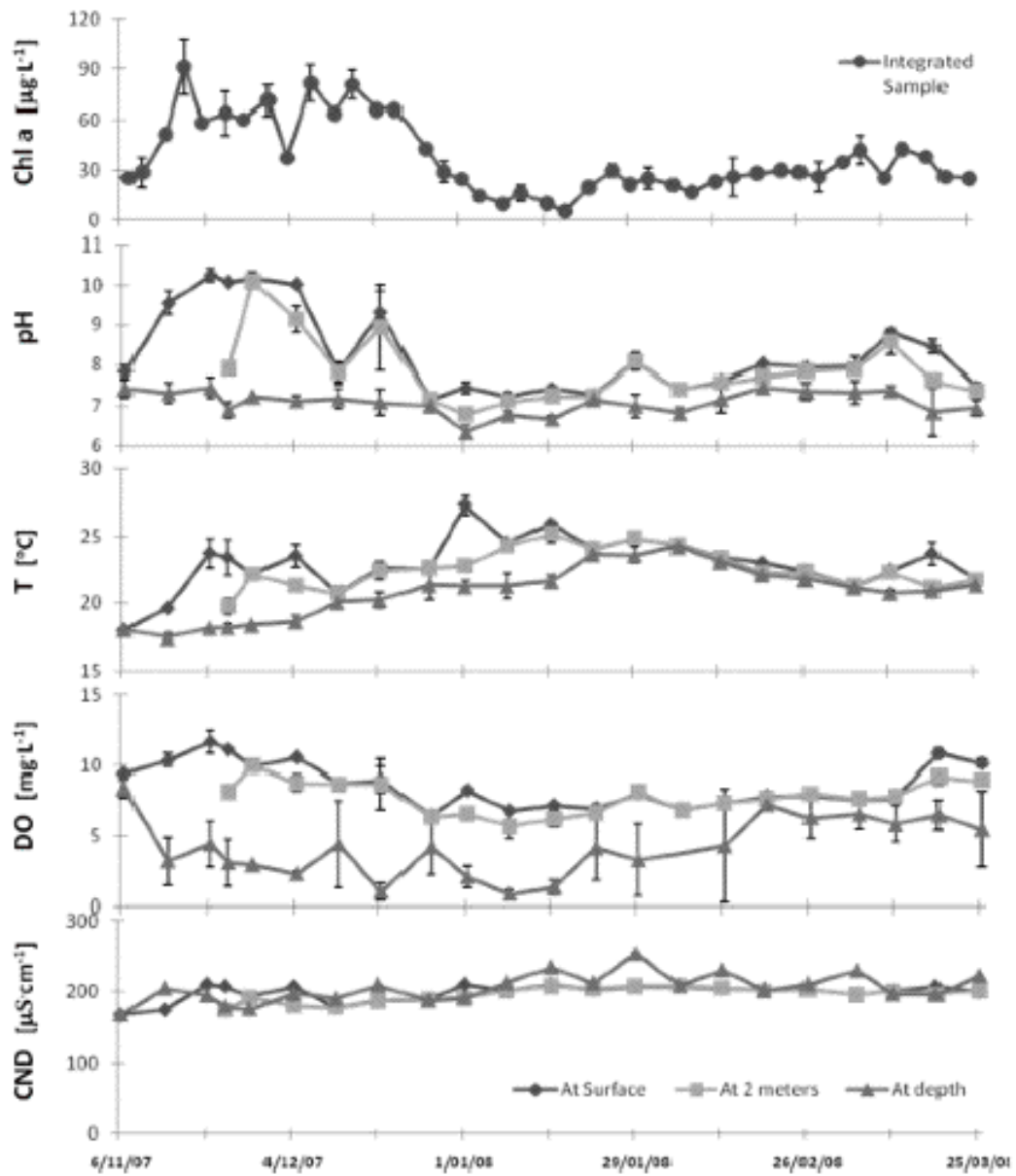
Figure 17 Physical parameters for Lake Kainui between 6 November 2007 and 27 of March 2008.

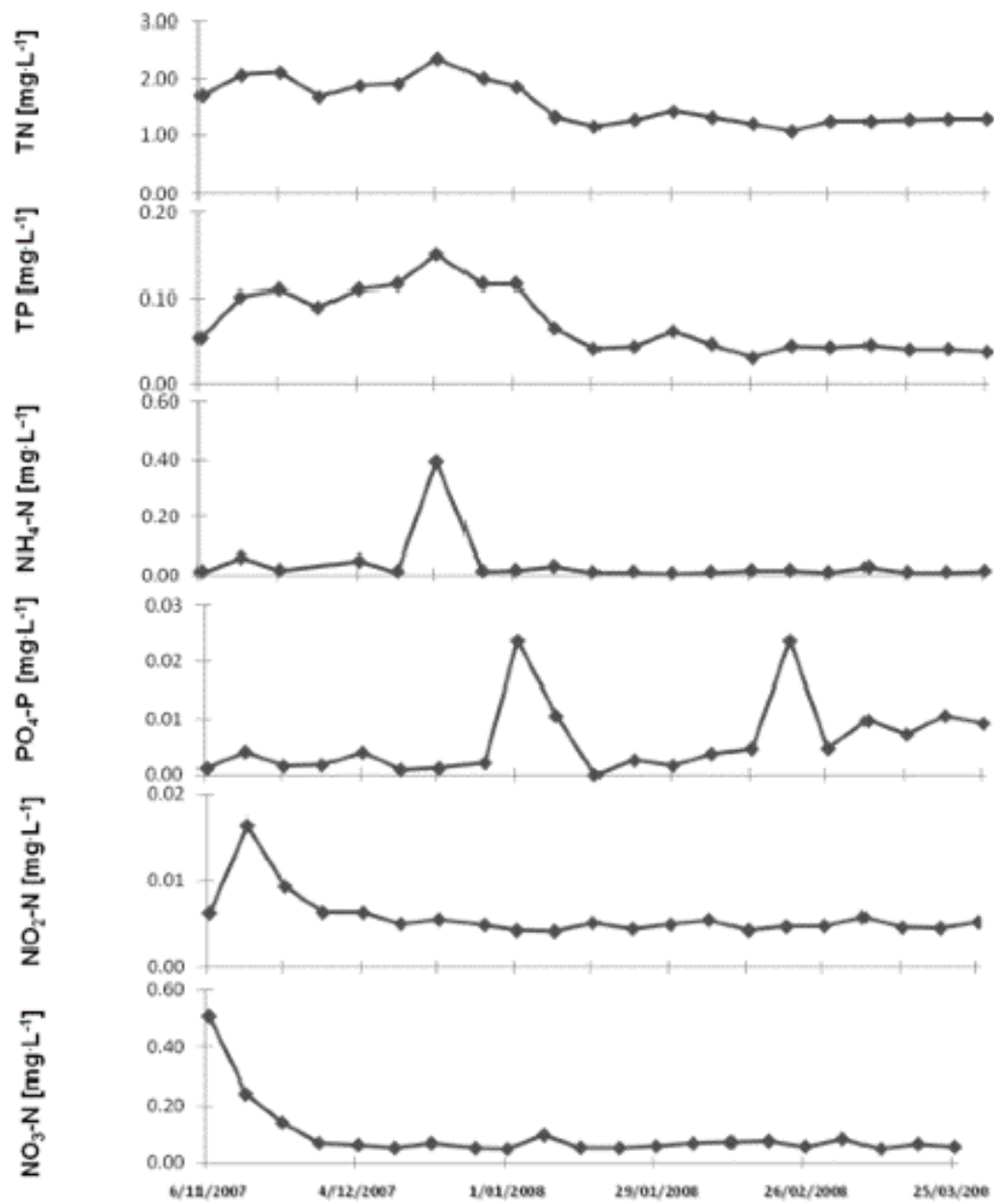**Appendix 4. Nutrient Concentrations in Lake Kainui over five months.**

Figure 18 Change in lake nutrient concentrations for Lake Kainui between the 6th of November 2007 and the 27th of March 2008.
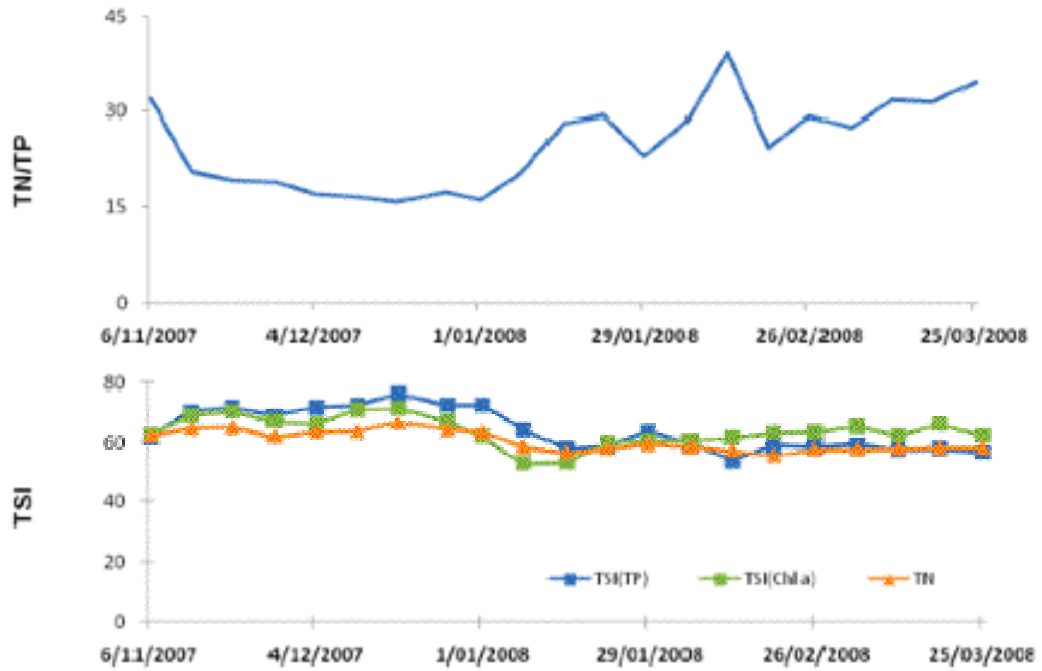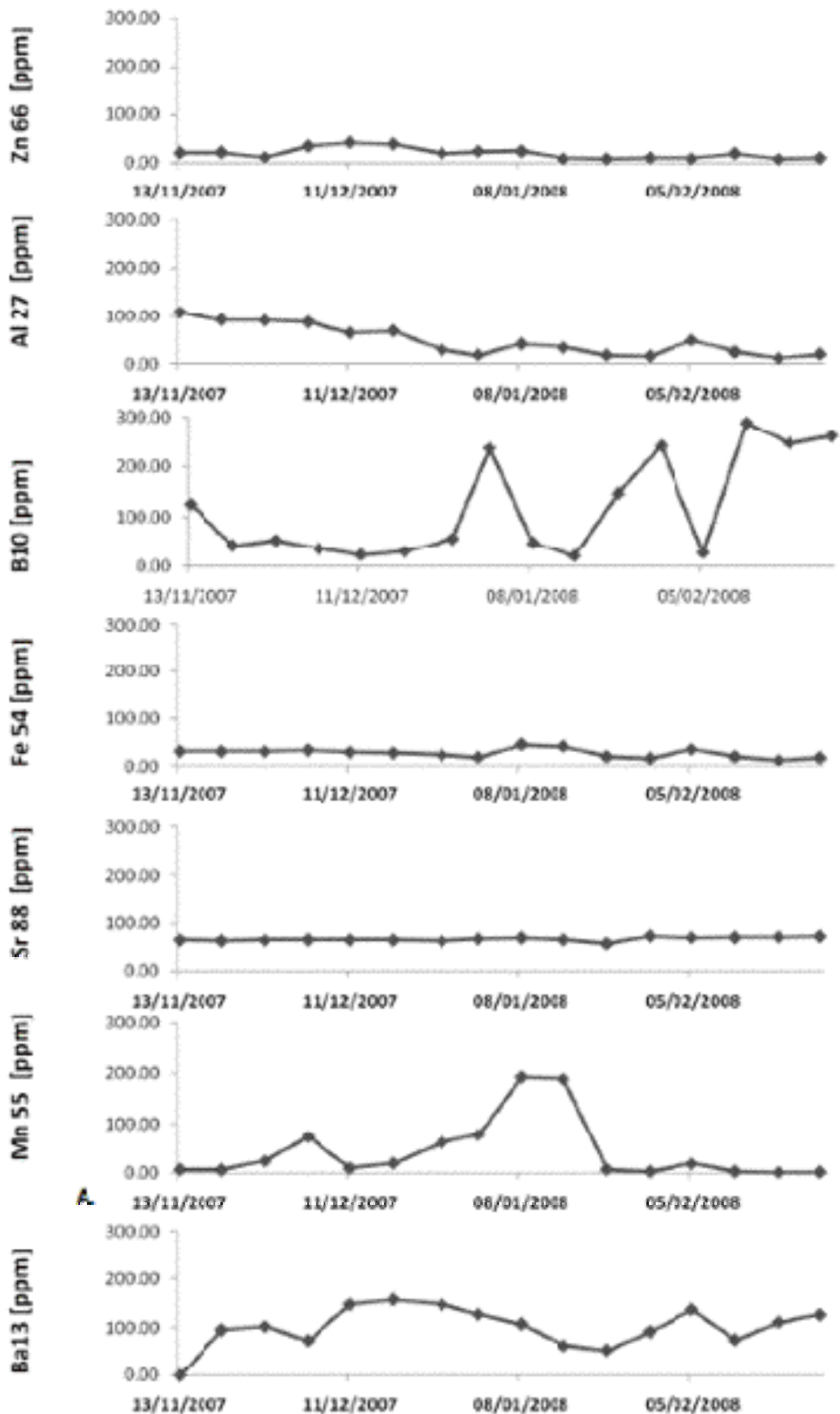
Figure 19 TN/TP ratio and Carlson trophic state index for Lake Kainui between 6 November 2007 and 27 March 2008.
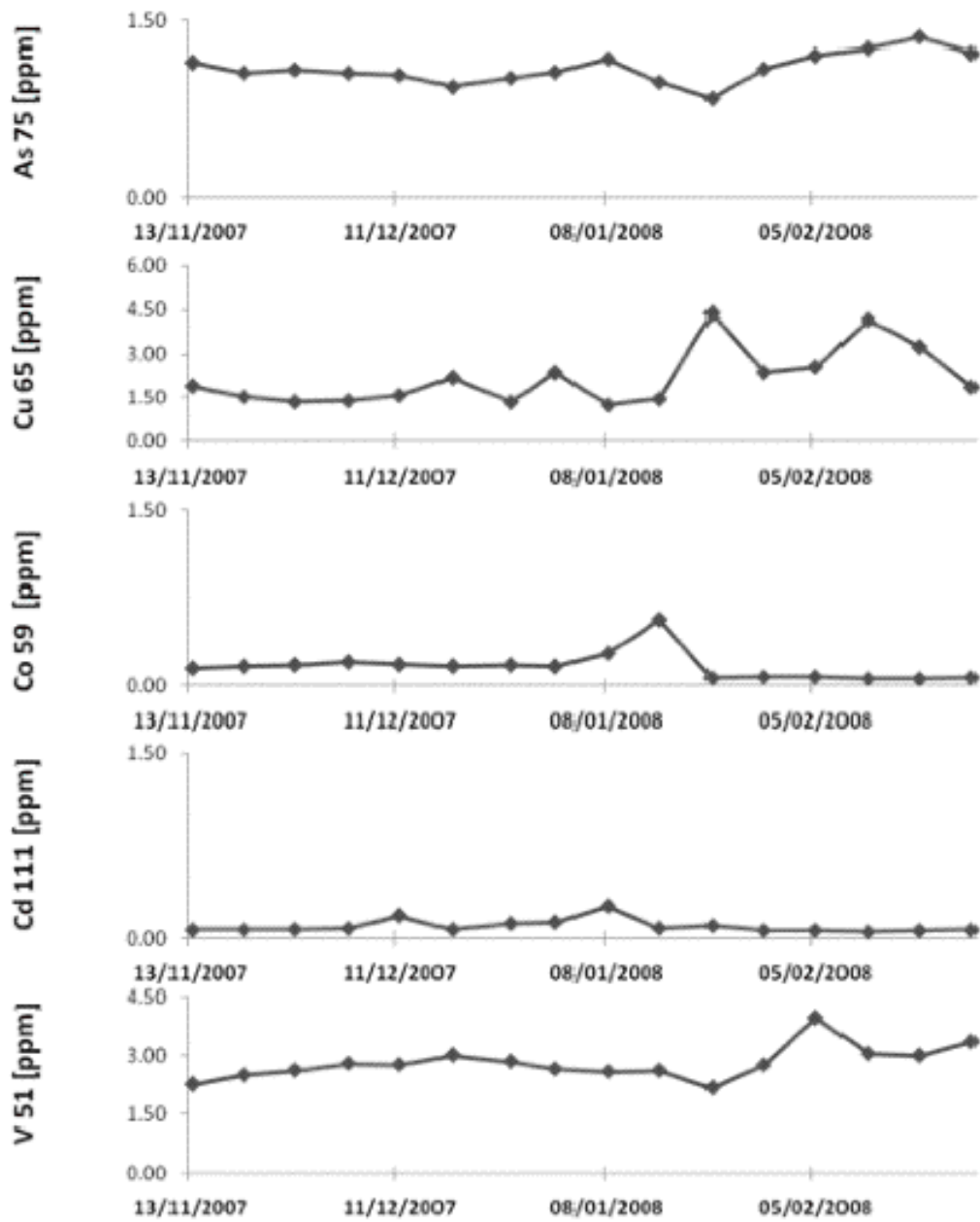
Appendix 6. Micronutrient concentrations.
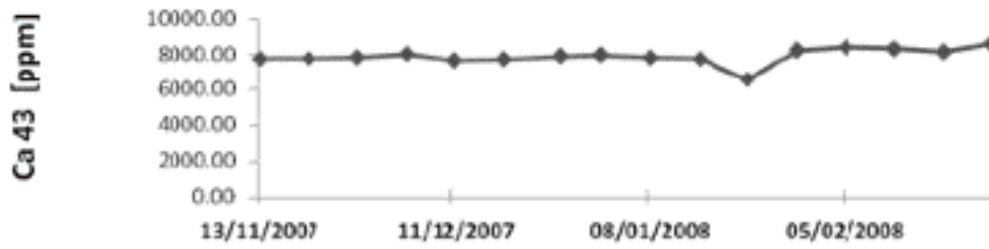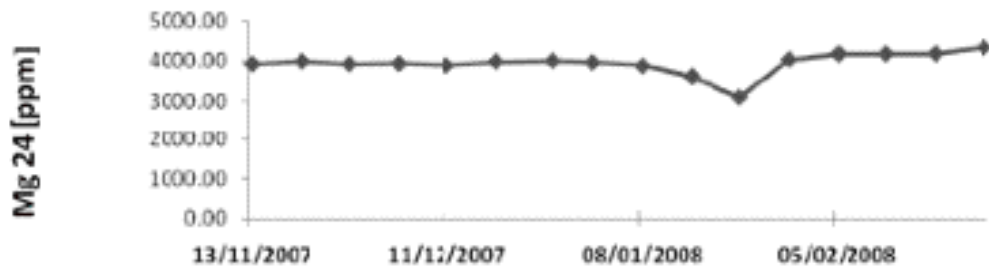
Figure 20 Change in lake micronutrient concentrations for Lake Kainui between 6 November 2007 and 27 March 2008 using integrated composite samples.

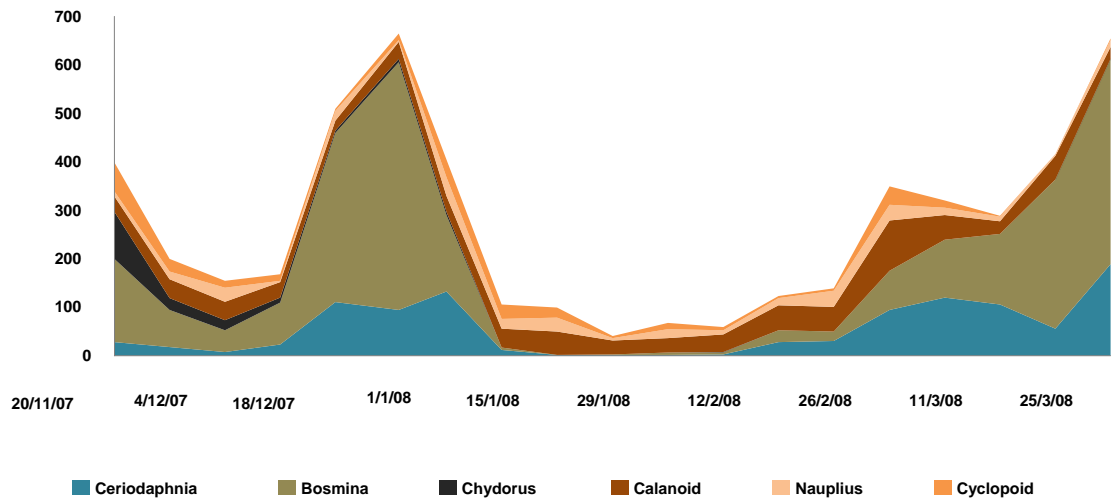**Appendix 7. Change in Zooplankton abundance over five months.**

Figure 21 Abundance of planktonic crustacea over 5 months for integrated composite samples from Lake Kainui for 20 November 2007 through to 27 March 2009.

Appendix 8. ARISA data for Lake Kainui.

Table 8 Number of AFLs detected per ARISA for Lake Kainui between 27 November 2007 and 29 January 2008.

	27/11/07	4/12/07	11/12/07	18/12/07	26/12/07	1/1/08	8/1/08	15/1/08	22/1/08	29/1/08
Planktonic	121	116	121	111	104	123	119	135	120	101
Particle Associated	135	121	138	107	99	115	122	118	126	142

Figure 22 ARISA phylotype distribution over for Lake Kainui between 27 November 2007 and 29 January 2008. Each line represents an ARISA fragment length (AFL) present in at least two samples. A coloured bar indicates that the AFL occurred at the corresponding sample point. The graph is semi quantitative as bar colours increase in tone for every order of magnitude of the AFL signal's strength. A darker bar means a stronger signal for that AFL.

Appendix 9. Toxin screen for Lake Kainui.

Table 9 Anatoxin-a concentration in lake water of Lake Kainui between 6 November 2007 and 5 February 2008.

	Anatoxin-a $\mu g \cdot L^{-1}$
06/11/2007	0.00
13/11/2007	0.16
20/11/2007	0.08
24/11/2007	0.10
27/11/2007	0.16
02/12/2007	0.17
04/12/2007	0.10
06/12/2007	0.00
11/12/2007	0.00
18/12/2007	0.00
26/12/2007	0.00
01/01/2008	0.00
08/01/2008	0.00
15/01/2008	0.00
22/01/2008	0.00
29/01/2008	0.00
05/02/2008	0.00

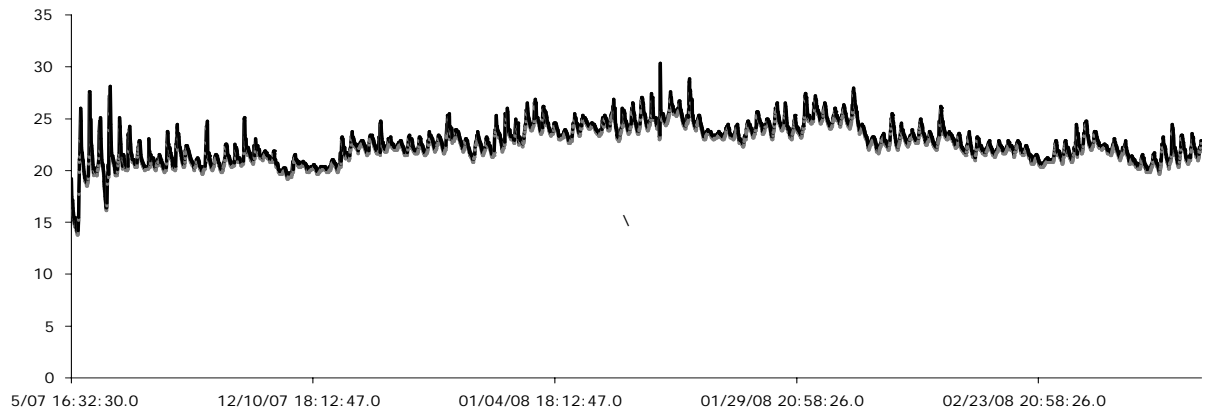## Appendix 10. Lake Kainui, change in water temperature.

Figure 23 Hourly change in water temperature in Lake Kainui from 3 November 2003 till 11 March 2008. Data was obtained from a water logger. However the logger's depth decreased as precipitation increased. The graph shows 'stepping' down, which is most likely an artefact of manually adjusting the logger's position in the water column.