ENVIRONMENTAL VARIABLES AND THE DEVELOPMENT OF PHYTOPLANKTON ASSEMBLAGES IN A HYPER-EUTROPHIC AFRICAN RESERVOIR

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ABSTRACT

The development of phytoplankton assemblages in relation to environmental variables was investigated in Lake Chivero between 2003 and 2006. The study showed that there has been a decline in dominance of cyanobacteria in Lake Chivero, instead phytoplankton development exhibited two states: (i) a clear state with lesser dominance of *Microcystis* and proliferation of cryptophytes and chlorophytes (eukaryotic algae) and (ii) a turbid state with domination by *Microcystis* and gradual exclusion of other species.

The decline of *Microcystis* domination was linked to a change in the relationship between nitrate and orthophosphate concentration. While the environment in Lake Chivero is nutrient saturated, domination of *Microcystis* was only favoured during a "gap" of high nitrate concentration with relatively higher dissolved oxygen levels, which occurred during the turbid state. As the *Microcystis* biomass built up and accumulated within the 0-5 m zone light became a limiting factor and other species were competitively excluded. This state was short-lived and after attainment of maximum biomass, the system collapsed and reverted to the clear state. For fifteen months the lake was in a clear state during which the phytoplankton community showed a typical successional pattern with cyanobacteria dominating in summer, diatoms in winter and chlorophytes and cryptomonads dominating during the hot dry period. There was generally marked dominance by cryptomonads despite high nutrient levels, high mean pH (7-9.7), high temperature (17.4 – 26.8°C) and mean N:P ratio of 10.5 which should favour cyanobacteria according to the proposed theories of cyanobacterial dominance.

Assessment of the water quality showed that although the lake is nutrient-rich, the major determinant of the development of the phytoplankton assemblage was the temporal changes in the availability of nitrogen and phosphorus. When phosphorus was abundant and nitrogen limiting, the lake existed in a clear state, however the lake shifted to a turbid state, dominated by cyanobacteria, at a critical nitrogen concentration of about 0.3 mg l^{-1} and was maintained in that state within a range of 0.3 and 1.7 mg l^{-1}. Nitrogen availability was the major determinant of the state of the lake with respect to cyanobacteria dominance although other factors like predator-grazer pressure could have affected the

phytoplankton assemblage. The clear state was a metabolic relatively low-oxygen hyper-hypereutrophic state favoured mainly by osmo-mixotrophic cryptophytes.

The algal assemblage was dominated by chlorophytes (ruderal plants) and cryptomonads, which are neither, true opportunists nor true ruderals. This assemblage was favoured by non-equilibrium dynamics. Species replacement of ruderal plants and cryptomonads by specialists in enclosures showed that turbulence is favouring dominance by opportunistic species in the lake. Observations from nitrogen addition experiments in microcosms and field observations showed that smaller species with highest ability to utilize N over *Microcystis aeruginosa* are now more competitively successful in Lake Chivero. The TN:TP ratio which for the greater part of the study period fell within the optimal resource ratio for cryptophytes rather the absolute concentration of N and P seemed to have favoured cryptophytes.

The decrease in cyanobacterial dominance, which had become a widespread public concern, is central to the management of the lake. It appears, as shown by the shift between the two states and the period spent in each state, that hyper-eutrophy at the recent level in Lake Chivero with total phosphorus > 1 mg l^{-1} and ammonium at levels of 3 mg l^{-1}, would normally not develop cyanobacterial dominance.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 INTRODUCTION

A large number of man-made lakes throughout Southern Africa were constructed for water supply for human consumption, industry, and agriculture and for various uses such as recreation and fisheries (Thornton 1987). Most of these lakes are located near urban centers and consequently serve as recipients for treated sewage and industrial municipal wastewater. Two typical cases are Hartbeespoort Dam in South Africa and Lake Chivero in Zimbabwe, both of which have been sinks for sewage and industrial wastewater since they were constructed and as a result are hyper-eutrophic.

Hyper-eutrophic systems are very nutrient-rich and consequently may suffer from frequent and severe episodes of "nuisance" cyanobacterial blooms and collapses resulting in massive fish kill, cattle mortality from algal toxins, foul beaches and impaired recreation use (UNEP 2000). Hyper-eutrophic waters have been defined as those with mean phosphorus concentration of approximately 100 $\mu g\ l^{-1}$, mean and maximum chlorophyll *a* concentrations of 25 and 75 $\mu g\ l^{-1}$ respectively and low transparency (OECD 1982).

Since they receive high and uncontrolled nutrient input (Barica 1980), hyper-eutrophic lakes and reservoirs are "over-fertilized" by inorganic nutrients (Leentvaar 1980) and represent the ultimate stage of the eutrophication process (Barica 1981, UNEP 2000). Such systems have low ecological stability, with periods of rapid phytoplankton development followed by population crashes due to unsustainably high levels of production (Barica 1981, Barica 1993, Robarts *et al.* 2005).

Leentvaar (1980) proposed three states of hyper-eutrophy with respect to plankton periodicity: acute, temporary and permanent hyper-eutrophy. In permanent hyper-

eutrophy, a permanent bloom is established in a steady state that is maintained by permanent overloading of excess nutrients either from bottom sediments or from anthropogenic activities within the catchment. Periodicity is lost because the permanent blooming species are more able to use nutrients than the other species and their biomass does not decrease throughout the year.

Temporary hyper-eutrophy is manifested as the development of a temporary algal bloom during the growing season. A bloom is initiated following a temporary increase in nutrients either after turnover or after concentration of nutrients in the water following a dry spell that lowered the lake level. When a bloom occurs during the growth season it interrupts the periodicity of plankton although in both cases the temporary bloom will be part of the normal plankton periodicity.

Acute hyper-eutrophy occurs when mechanical disturbances in a river basin leads to a sudden nutrient increase. The typical example is when mechanical stirring of bottom sediment and water releases nutrients for a short-time period thereby causing an algal bloom. Under acute hyper-eutrophy normal phytoplankton periodicity occurs, but can be interrupted by a short-term bloom.

1.2 CHARACTERISTICS OF HYPER-EUTROPHIC LAKES

The characteristics that differentiate hyper-eutrophic lakes from other eutrophic systems relate primarily to external nutrient loading, exponential algal growth and their shallowness (Barica 1980, Ulhmann 1980, UNEP 2000). The typical characteristics of hyper-eutrophy are defined by Mur (1980) as "*so great an enrichment of a (fresh) water system with minerals that a strong increase of biomass and a strong decrease in species number results*".

The high nutrient loading and the consequent high biomasses creates a shadowy light climate which exerts a heavy selective pressure on the phytoplankton assemblage thereby selecting a few, or only one dominant species of phytoplankton, most often cyanobacteria

(Mur 1980). The environmental conditions will constraint species diversity to one or two dominant species (Kruk *et al.* 2002) resulting in the mass growth of one or more dominant species throughout the year, usually suppressing the normally occurring periodicity of other species. The permanently blooming species lose periodicity and competitively exclude other species since they are more tolerant of light shading.

Light is considered to be the principal limiting factor influencing phytoplankton dynamics in hyper-eutrophic systems since nutrients are present in sufficient quantities not to be limiting. As a large cyanobacterial biomass builds up, bioturbidity increases thereby causing self-shading. Self-shading from high cyanobacterial biomass reduces light availability and imparts a selective pressure towards cyanobacterial dominance, since cyanobacteria can develop well at low light intensities (Barica 1980) while excluding species that are less tolerant of low light levels. Additional factors that favour dominance of cyanobacteria in hyper-eutrophic systems are discussed in Section 1.3.

Algal growth in hyper-eutrophic systems progresses exponentially as a result of excessive nutrient loading (UNEP 2000). Figure 1.1 illustrates the generalized pattern of algal growth in oligotrophic to mesotrophic lakes, eutrophic lakes and in hyper-eutrophic lakes (UNEP 2000). Under nutrient-poor conditions (1), since the system is stable the initial algal exponential growth eventually levels off as it attains equilibrium with nutrient supply. With increasing eutrophication (2) the system will start to exhibit signs of instability as it oscillates and may undergo a series of partial bloom crashes. Under excessive nutrient loading (3), the system becomes unstable. Primary productivity undergoes extreme fluctuations and oscillations, with periods of high primary productivity that are followed by periods of respiration only as a result of algal blooms collapsing and decomposing (Barica 1975). Generally the build-up of high biomasses results in an unstable, nutrient-deficient system liable to catastrophic die-off on a massive scale (Barica 1975). The system collapses when nutrients become inadequate for sustaining the magnitude and the physiological requirements of the bloom (UNEP 2000).

Nutrients are not exhausted during the vegetative period in hyper-eutrophic systems (Uhlmann 1980) because external nutrient loading is often several orders of magnitude greater than critical levels for shallow eutrophic lakes (UNEP 2000). Temporary nutrient

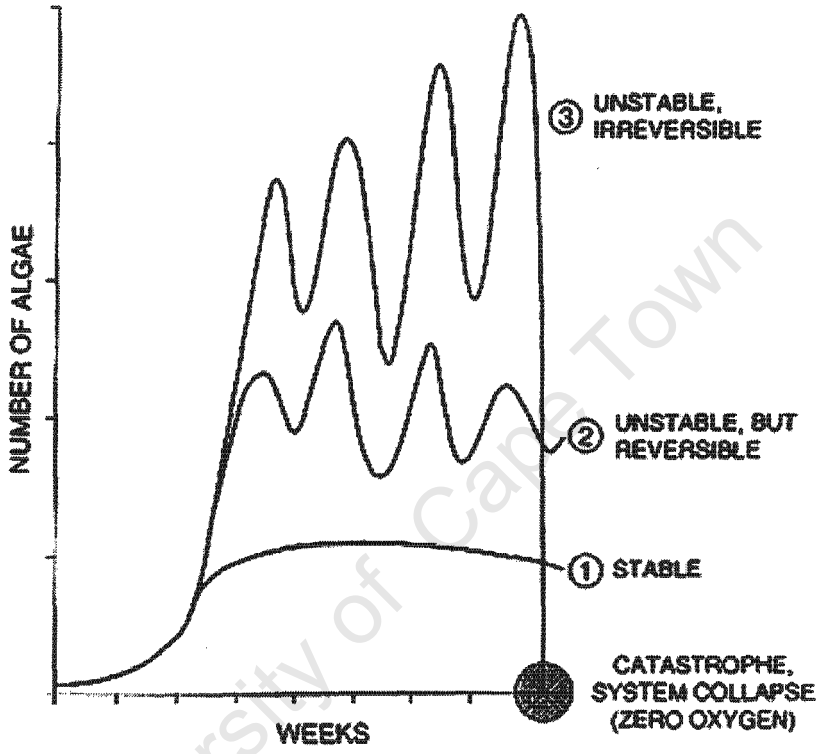


Figure 1.1 **Generalized pattern of algal growth in 1: oligotrophic to mesotrophic lakes, 2: eutrophic lakes and 3: hyper-eutrophic lakes, undergoing bloom collapses (Source: UNEP 2000-modified from Barica 1993).**

depletion occurs only at the crashing stage, after which nutrients are immediately regenerated. A steady state supported by permanent loading of excess nutrients originating from sources other than *in situ* biomass has to be maintained in order to sustain permanent hyper-eutrophy (Leentvaar 1980). Internal loading of nutrients through oxic and anoxic regeneration can be a significant source of nutrients in hyper-eutrophic systems even when external sources are reduced (UNEP 2000, Al Bakri & Chowdhury 2000).

Since oxygen production is linked to changes in algal productivity it exhibits extreme seasonal and diurnal fluctuations, with high amplitudes of maxima and minima and pronounced oscillations (Robarts *et al.* 2005). Periods of high algal productivity result in oxygen supersaturation. Leentvaar (1980) records >100% and Barica (1974), 300% with a high potential for oxygen production throughout the year (Leentvaar 1980). Due to the inherent instability in hyper-eutrophic systems (Barica 1980), though, periods of excessive growth of potentially noxious algal blooms are usually followed by crashes, sometimes creating anoxic conditions when the algal bloom collapses and decomposes, and sometimes resulting in massive fish kills (Robarts *et al.* 2005, Mhlanga *et al.* 2006). The system therefore easily dis-equilibrates, from a state of super-saturation to anoxic conditions (Uhlmann 1980). The periodic crashes of populations and cyclic anoxia, help to re-establish equilibrium and steady state conditions, however (Barica 1980).

Most hyper-eutrophic systems are shallow and un-stratified except for brief periods (UNEP 2000). Due to shallowness such systems are highly sensitive to fluctuations in the physical environment resulting in them being subject to changes in temperature, wind action and irradiance to a much greater extent than deep water bodies (Uhlmann 1980). Wind periodically re-suspends sediments and enhances the mixing of near-bottom anoxic, nutrient-rich layers with surface layers (UNEP 2000) thereby releasing nutrients in the system. A typical case is Okeechobee Lake (Florida, USA) where, despite reduction in external nutrient input, wind re-suspends sediments, thus providing adequate internal source of phosphorus (Chen & Sheng 2005).

1.3 MODELS OF CYANOBACTERIAL DOMINANCE

Cyanobacteria are the most successful taxonomic group in eutrophic water bodies. Their early evolutionary history (Lazcano & Miller 1994) is considered to have enabled them to develop qualities that favour them in competition with other organisms (Engström-Öst 2002). Their success has been explained by a number of hypotheses based on these qualities (reviewed in Sommer *et al.* 1986, Shapiro 1990, Hyenstrand *et al.* 1998). Hyenstrand *et al.* (1998), for instance, reviewed nine single-factor theories that postulate

differences between cyanobacteria and eukaryotic phytoplankton, which are used to explain cyanobacterial success. These theories are discussed below.

(i) A low TN:TP ratio is considered to be a major factor favouring cyanobacteria dominance in many lakes (Schinder 1977, Smith 1983). Aquatic ecosystems with a TN:TP ratio ≤10 are generally considered to be N-deficient, whereas those with TN:TP ≥20 are considered to be P-limited (Grayson *et al.* 1997) although Rhee (1982) suggests that a slightly higher ratio of ≤16 as indicating nitrogen limitation, whilst higher ratios indicate limitation by phosphorus. It is assumed that a molar ratio of TN:TP around 16 is favourable for cyanobacteria growth (Reynolds 1984, Harris 1986). The dominance by cyanobacteria in most water bodies impacted by urban effluent has been linked to low prevailing TN:TP ratios (Kalff & Knoechel 1978). *Microcystis* tends to dominate when the ratio falls below 10 while *Anabaena* is favoured at values below 2:1 (Walmsley & Butty 1980, Thornton 1987).

There is still no conclusive evidence of the role TN:TP ratio plays in cyanobacterial dominance however. For instance in Laguna de Bay (Philippines) and other eutrophic tropical lakes, concentrations of both nitrogen and phosphorus appear to have more pronounced impacts on algal population and assemblage structure than their ratios (Cuvin-Aralar *et al.* 2004). In some lakes phosphorus alone is still considered a better predictor of both total and relative cyanobacterial biomass than total nitrogen or the ratio of TN:TP (Trimbee & Prepas 1987, Mäkelä *et al.* 2005), thereby deviating from the predictions of Smith (1983). Downing *et al.* (2001), from a study of ninety-nine temperate lakes, showed that cyanobacteria are more strongly correlated with variation in total phosphorus and total nitrogen than in the ratio of TN:TP. TN:TP ratios also had very low predictive value for the temporal variation of cyanobacteria in twenty-six lakes studied by Mäkelä *et al.* (2005) in Southern Finland.

(ii) The "high pH/low CO_2" hypothesis suggests that cyanobacteria can out-compete other algae in water with a high pH or low CO_2 content because they can actively

utilize HCO_3^- (Shapiro 1973, 1984, 1990). Low pH (< 6.0) favours eukaryotes, which cannot utilize HCO_3^- and high pH (>8.0) favours cyanobacteria, which can.

(iii) Cyanobacteria also gain a competitive advantage by being able to regulate their position in the water column (Reynolds *et al.* 1987). This vertical migration is enabled by the regulation of intracellular aerotopes/gas vesicles (Walsby 1994) and density changes caused by intracellular carbohydrate dynamics (Gibson 1978). Vertical migration is most apparent during periods of stratification (Talling 1987, Reynolds *et al.* 1983) and during this period cyanobacteria can competitively exclude other species.

The role of buoyancy is typically illustrated in Hartbeespoort Dam (South Africa) where dense surface accumulations of *Microcystis aeruginosa* at the onset of winter control the underwater light climate, thus excluding other taxa (Hambright & Zohary 2000). Buoyancy and colony size, both of which increase with abundance, confer a double ecological advantage for *M. aeruginosa*, which makes it a formidable and successful competitor in Hartbeespoort Dam (Robarts & Zohary 1984). Buoyancy imparts ecological advantages to cyanobacteria because when at the surface they are ensured of access to CO_2 during periods of high photosynthetic activity (Paerl & Ustach 1982). This contrasts to the situation in Zeekoevlei (South Africa) where the buoyancy-driven upward motion of *M. aeruginosa* is counteracted by frequent mixing (Harding 1996). Zeekoevlei is non-stratifying and experience constant wind action throughout the year, a situation that though, has not exclusively selected for *M. aeruginosa*, which sometimes exist with chlorophytes as co-dominants (Harding 1992).

Buoyancy alteration is ecologically advantageous by allowing cyanobacteria to exploit favourable nutrient gradients throughout the water column and surface sediments (Reynolds & Walsby 1975, Mur *et al.* 1999, Mitrovic *et al.* 2001). Some cyanobacteria like *Gloeotrichia echinulata* bring to the surface phosphorus reserves taken up from the pore-water of the anoxic bottom sediments and store them intracellularly, thereby further enhancing their growth (Istvánovics *et al.*

1993, Pettersson *et al.* 1993). Presumably this relationship with phosphorus also keeps the phosphorus values higher than they would otherwise be, and reduces "permanent" losses to the sediments.

(iv) Cyanobacteria are favoured by high water temperatures because they have high temperature optima for growth (Robarts & Zohary 1987). They grow best at water temperatures exceeding 15°C with optimal growth rates at 25°C or more (Robarts & Zohary 1987). As temperatures exceed 20°C, the chances for cyanobacterial dominance increase, particularly in nutrient-enriched water bodies (Paerl 1996) but temperature alone does not determine the occurrence of a species (Robarts & Zohary 1987).

(v) The "inorganic nitrogen hypothesis" suggests that ammonium favours the development of non-nitrogen-fixing cyanobacteria, whereas nitrate-nitrogen favours the development of eukaryotic phytoplankton. Nitrogen scarcity obviously favours the development of nitrogen-fixing species (Blomqvist *et al.* 1994) like *Anabaena* that has heterocysts. Nitrogen fixing cyanobacteria dominate when TN:TP supply is low and nitrogen limiting.

(vi) The "trace-element hypothesis" suggests the importance of trace metals like iron as factors limiting cyanobacterial growth due to the element's involvement in carbon and nitrogen metabolism (Rueter & Petersen 1987). For example in Lake Erken, Hynestrand *et al.* (2000) established that iron availability could be a limiting factor for cyanobacterial development. Through enclosure experiments they observed that additions of iron increases the growth of *Gleotrichia echinulata*. Iron is biochemically involved in nitrate reduction and nitrogen fixation and is also important for photosynthesis and energy distribution in the cell (Rueter & Petersen 1987). Under conditions of high nitrogen and phosphorus loading, restricted availability of iron may control cyanobacterial growth (Paerl 1996) as observed by Nagai (2005) in Lake Kasumigaura. Added iron can also

shift populations towards cyanobacterial dominance (Parr & Smith 1976 cited by Rueter & Petersen 1987).

(vii) The grazing resistance hypothesis is based on the indigestible size, low nutritional value, grazing resistant coverings and toxicity associated with certain species of cyanobacteria, which are considered to deter feeding by zooplankton (Haney 1987). The filamentous or colonial morphology and formation of aggregates reduce feeding rate or clog the feeding appendages of suspension feeding zooplankton (Webster & Peters 1987) while the algal toxins can inhibit zooplankton feeding or cause zooplankton mortality (DeMott & Moxter 1991).

(viii) Allelopathy is considered as one of the competitive strategies of cyanobacteria effected through specific secondary metabolites that decrease number of certain phytoplankton species (Suikkanen *et al.* 2006). The microcystin produced by cyanobacterial have been observed to inhibit protein phosphatases in eukaryotic cells (Yoshizawa *et al.* 1990) and microalgal growth (Signh *et al.* 2001). For example allelopathic substances in extracts of two bloom forming cyanobacteria species *Nodularia spumigena* and *Aphanizomenon flos-aqua* from the Baltic Sea decreased the abundance of the cryptophyte *Rhodomonas* sp. and a diatom *Thalassiosira weissflogii* (Suikkanen *et al.* 2004). The extracts however also increased the abundance of other cyanobacteria, a chlorophyte *Oocystis* sp., the dinoflagellate *Amphidinium* sp. and nanoflagellates (Suikkanen *et al.* 2006). The effect of cyanobacterial toxins is assumed to be through the inhibition of electron transport in the vicinity of photosystem II during photosynthesis (Smith & Doan 1999). There is no consensus on the ecological role of cyanobacterial toxins; suggestions include cell-cell signaling for defense against grazers and competitors (Kaebernik & Neilan 2001). Lewis (1986) noted that if they can suppress or stimulate the growth of other phytoplankton then they play an important role in the competition between species.

(ix) Cyanobacteria are better adapted to grow at low light conditions because they have low requirements for light energy (Zevenboom & Mur 1980, Smith 1986,

Mur & Scheurs 1995). This has been observed in shallow lakes where the dominance of the phytoplankton assemblage shifted to species with low-light energy requirement for growth such as non-N_2-fixing cyanobacteria after eutrophication has created much lower irradiance in the water due to high ambient algal biomass (Zevenboom & Mur 1980). The phycobiliproteins present in cyanobacteria enable them to harvest light in the green, yellow and orange part of the spectrum (500-650nm) such that they can live in an environment with only green light (Cohen-Bazir & Bryant 1982 cited in Chorus & Bartram 1990). The relatively higher growth rate of cyanobacteria than other phytoplankton organisms when light intensities are low is also enhanced by their lesser requirement for energy to maintain cell function and structure (Van liere *et al.* 1979). It has been observed that *Microcystis* spp. are able to offset the effects of photo-inhibitive UV radiation encountered by near-surface populations (Paerl *et al.* 1985).

In summary, factors favouring cyanobacterial dominance are numerous and complex. Many factors interactively determine which genera and species become established and dominate in a specific ecosystem. Other factors that affect cyanobacterial dominance include mixing regimes, transparency, carbon availability and predation. The Nile tilapia (*Oreochromis niloticus*) for example, can contribute to the eutrophication of a water body by both top-down and bottom-up forces, the latter by supplying considerable amounts of nutrients to the pelagic zone, thus promoting the fast growing algae (Figueredo & Giani 2005). This discussion shows that many factors affect cyanobacteria dominance. Part of this study was to determine the role of physical-chemical factors on phytoplankton dynamics in Lake Chivero, particularly their influence on the dominance of cyanobacteria.

1.4 ALTERNATE STABLE STATES

The idea that communities can be found in one of several possible alternative stable states, as proposed by Lewontin (1969), has been a recurring theme in ecology since the late 1960s, and according to Beisner *et al.* (2003) is now experiencing a resurgence of

interest (Scheffer *et al.* 1993, Scheffer *et al.* 2001, Okey 2004). The idea is that biological communities have multiple states, or more than one local attractor, and this is envisioned as a valley in a dynamical landscape wherein a strong disturbance can push a "ball" (ecosystem) from one local "basin of attraction" to another (May 1977). The simple device used to illustrate the shifting between alternative stable states is a "ball-in-cup" analogy (Dent *et al.* 2003, Beisner *et al.* 2003). The ecosystem (the ball) can move among locally stable attractors (the cups) on a stability landscape controlled slowly by geomorphology or living organisms (DeAgnelis & Waterhouse 1987). The effects of shape, height and steepness of the cup on the ball represent the community's resistance to be changed by external forces (Boesch 1974).

Two notions, based on community and ecosystem perspectives, that have been proposed to describe how communities shift from one stable state to another are discussed by Beisner *et al.* (2003). According to the community perspective, which arises directly from traditional population and community ecology, a sufficiently large perturbation has to be applied directly to state variables (e.g. populations density) in order to shift the community from one state to another. This idea pre-supposes the simultaneous existence of different states under the same set of conditions; the environment is regarded as constant. Once shifted the community will persist in the new state unless subject to another perturbation. With the ecosystem perspective all potential stable states are not supposed to be present at all times. The change in a parameter or environmental driver will cause the community to switch from one state to another, as observed in shallow lakes subject to eutrophication (Scheffer *et al.* 2001). The resultant alternative state will be a locally stable equilibrium point, which may or may not have existed before the parameter perturbation (Beisner *et al.* 2003).

Multiple community states or major ecosystem state shifts are reported from a wide range of environments ranging from savanna woodlands (Dublin *et al.* 1990) and sand dunes (Adema *et al.* 2002) to streams (Strange *et al.* 1993) and marine environments (Okey 2004). Within freshwater ecosystems, best known examples of multiple states or

alternative stable states come from lake ecosystems (Moss 1990, Scheffer 1990, Dent *et al.* 2002), examples of which are discussed below.

Bi-stability (or more generally multistability) has been observed in lakes (Scheffer 1990) where the lake systems switched between discrete states generally termed "stable states", although according to Scheffer *et al.* (2001), the term "dynamic regime" is preferred than "stable state" which implies a constant environment. Entire lakes can shift in a catastrophic way from one state to the other (Scheffer 1999). A typical example is switches that occur following eutrophication from non-point pollution (Dent *et al.* 2002), a phenomenon that has been very noticeable over the past decades in water bodies that receive excessive nutrient loadings. Prior to nutrient loading the water will be clear. Excessive nutrient loading stimulates algal growth and under extreme circumstances, the system will 'flip' to a condition in which phytoplankton blooms persist.

According to the stability landscapes model a lake system might exist in a turbid equilibrium state under heavy nutrient loading whereas in a pristine state, it would have existed in the only other possible state, a clear-water state. This probably applies to hyper-eutrophic systems in southern Africa where immediately after impoundment the system is in a clear-water state but with continuous enrichment it switches to permanent turbidity. Zeekoevlei, although not an impoundment, previously experienced an alternated dominance of macrophytes (*Potamogeton pectinatus*) and phytoplankton (Harrison 1962 cited in Harding 1996) but is now continuously dominated by *M. aeruginosa* and chlorophytes (Harding 1996). Restorative measures, involving bio-manipulation and nutrient reduction, have been use to forcibly switch such systems to an alternative and stable clear-water state (Annadotter *et al.* 1999), although no success has been reported in southern Africa. So eutrophication in some cases has proved reversible, and sometimes irreversible (Carpenter *et al.* 1999). When a stressor is alleviated in a hysteretic system restoration to the previous state will not occur until the stressor is reduced to lower levels than those that caused the catastrophic shifts to the degraded state (Okey 2004).

This phenomenon of "flipping" (Scheffer 1999) has also been observed in shallow eutrophic lakes with respect to water clarity. Shallow lakes with intermediate to high total phosphorus concentrations can develop a stable state characterized by abundant submersed aquatic vegetation and clear water or an alternative state characterized by dense phytoplankton blooms and turbid water (Moss *et al.* 1994, Jeppesen *et al.* 1998). Such systems are said to exist in two alternative stable states. The shallow lakes and ponds where the strongly contrasting ecosystem states have been observed are of an average depth of < 3m, have surface areas ranging from 1 ha to > 100 km^2, do not stratify and have intense sediment-water interaction (Scheffer 2001). Due to their shallowness these lakes are vulnerable to stochastic episodes that may cause a shift from one state to another (Scheffer 1997).

According to Scheffer (2001) the two states in shallow lakes represent alternative attractors with distinct stabilizing feedback mechanisms. The internal feedback mechanisms will act to keep the system in a particular state, or cup, the strength of which is represented by the depth of the cup (Dent *et al.* 2002). A shift only occurs when an external driver overcomes the internal stabilizing feedback mechanisms. According to Dent *et al.* (2002) internal feedbacks in an ecosystem act to keep it in a particular state by increasing the system's resilience resulting in it being difficult to push it out of a cup.

In shallow lakes the change from a clear state to a turbid state consequently trigger a series of ecological changes including loss of submerged vegetation and the associated fauna that use weed beds for shelter and food (Moss 1998, Jeppesen *et al.* 1998). The buffering that occurs in each state enables the existence of two potential habitats, since it enables a stable community to exist through competitive exclusion and habitat modification (Moss 1998).

Nutrient loading (bottom-up control), predatory fish and invertebrates (top-down control), climatic events (floods/droughts) or some combination of external and internal factors may regulate these alternative states (Bayley & Prather 2003). According to Bayley & Prather (2003) nutrient status, depth and invertebrate predators seem to be the

most important determinants of alternative states in shallow lakes. Clear-water states exist between 50 and 150 μg l^{-1} total phosphorus and the potential to "flip" to the alternative stable state occurs if nutrients levels increase above this threshold (Scheffer *et al.* 1993, Moss *et al.* 1994). The systems where Bayley & Prather (2003) observed alternative state were shallow (< 2 m depth), surrounded by wetland complexes, rich in phosphorus (123 μg total P l^{-1}) and low in available nitrogen (18 μg l^{-1} $NH_4^+ + NO_3^-$). In North America, the alternative states have been observed in prairie lakes, in regions of intense agricultural activity (Zimmer *et al.* 2001).

Trophic cascades (Carpenter *et al.* 1995) have been shown to lead to lakes exhibiting two states: states with either small *Daphnia* populations dominated by small individuals and high algal biomass (turbid state) or large abundant *Daphnia* and relatively low algal biomass (clear-water state) (Dent *et al.* 2002). The role of trophic cascades has been observed in hyper-eutrophic systems like sewage lagoons, whereby two states are exhibited. For instance a clear-water period can occur during a warm summer period when grazing by *Daphnia*, in the absence of fish, suppresses algal growth (Uhlmann 1980). According to Uhlmann (1980) at the same nutrient load in a sewage pond, dense phytoplankton growth can occur following suppression of large zooplankton. At the same nutrient load, the water can either exhibit chlorophyll *a* concentration of more than 2000 μg l^{-1} or can be remarkably clear with chlorophyll *a* concentrations two orders of magnitude lower (Uhlmann 1980). These alternative stable states therefore occur under the same set of external conditions.

In shallow lakes, regulation of phytoplankton biomass through top-down grazing by planktivorous fish and zooplankton grazers contributes towards maintaining a system in a stable state (Carpenter & Kitchell 1993). For example grazing by large-sized zooplankton (*Daphnia*) can change the community structure of phytoplankton in shallow lakes and in the process reduce shading thereby allowing an increase in submerged macrophytes (Jeppesen *et al.* 2000). On the other hand planktivorous fish can feed extensively on large-sized zooplankton which cause the algal community to shift to small-celled species that shade out submerged aquatic vegetation (Mitchell 1989). Intervention by removal of

planktivorous fish can have "cascading effects" which results in improvement of macrophyte cover and water clarity (Hanson & Butler 1994)

It cannot be presupposed that a lake ecosystem will switch between two alternative states since these could be limited to particular situations and even in shallow lakes to an intermediate range of nutrient levels (Scheffer 1999). According to Moss (1998), in shallow lakes a forward switch to the alternative algal assemblage is easier at higher nutrient load because the gradual addition of nutrients will have eroded the resilience of the clear-water state (Scheffer *et al.* 1993) making the entire system more prone to catastrophic shifts toward an algae-dominated, turbid-water state (Beisner *et al.* 2003).

Resilience, defined by Holling (1973) as the magnitude of disturbance that can be absorbed before a system flips from one state to another, gets eroded as the system get excessively enriched. Using the ball-in-cup analogy, resilience is related to the characteristics of the basin (cup) that act to retain the ball, i.e. steepness of the slope and the area (or width) (Beisner *et al.* 2003). A small perturbation may not successfully shift the community to another state, because the system is sufficiently resilient. However when resilience is lost, the system will become fragile and can be easily tipped into a contrasting state by stochastic events (Scheffer *et al.* 2001. The community's resilience will consequently determine how much disturbance (e.g. magnitude, frequency, severity) a community can endure and still retain to its previous state (Holling & Clark 1975).

The alternative stable states theory has also been used to define different states that occur with increasing salinity in wetlands in the southwest of Western Australia (Davis *et al.* 2003). Davis *et al* (2003) identified contrasting vegetation states that were closely associated with different salinities. Increasing salinity caused a loss of freshwater species of submerged macrophytes resulting in cyanobacteria and halophilic bacteria assuming dominance at high salinities.

The predominance of either floating plants or submerged plants in temperate ponds and ditches and tropical lakes, has also been considered as two alternate stable states by

Scheffer *et al.* (2003). They illustrated this phenomenon with the case of Lake Kariba, where a floating macrophyte *Salvinia molesta* dominated during the filling phase when the lake was enriched with nutrients from decaying terrestrial vegetation (Mitchell 1969, Marshall & Junor 1981). When *Salvinia* declined as the lake stabilized, benthic vegetation and mussels assumed dominance and locked up large amounts of nutrients (Machena & Kautsky 1988) and recently a floating plant, *Eichhornia crassipes*, has encroached in localized enriched areas in the lake (Mhlanga 2001). According to Scheffer *et al.* (2003), shifts between the two alternate states - floating and submerged plants - have been driven by the amplitude of water-level fluctuations, whereby strong fluctuations during the early years favoured floating plants and suppressed submerged plants (Scheffer 1997).

The dominance of filamentous cyanobacteria of the family Oscillatoriaceae in shallow eutrophic lakes has been considered to be one of two alternative stable states of the algal assemblage by Scheffer *et al.* (1997). Through analysis of the patterns exhibited by cyanobacterial dominance in the lakes they concluded that the algal assemblage is a hysteretic system with two alternative equilibria. Hysteresis is expected from differences in physiology between cyanobacteria and algae, with the latter being superior competitors under conditions of low light and also promoting such conditions by causing higher turbidity per unit of phosphorus than other algae (Scheffer *et al.* 1997). Evidence of alternative stable states must include the testable or observable attributes of hysteresis (Okey 2004).

In Lake Kinneret, Zohary & Hambright (2005) showed that alternate stable states occurred using a 34-year record of phytoplankton, zooplankton and physico-chemical parameters. The alternate states were characterized by the presence and absence of spring blooms of the dinoflagellate, *Peridinium gatunense*.

In conclusion it appears that a specific set of external variables does not necessarily correspond to a single definite equilibrium state in aquatic ecosystems. In cases discussed above there was more than one steady state (bistability). In this thesis the shift observed

in Lake Chivero between a clear state and a turbid state was investigated within the context of the possibility of the existence of alternative stable states within the algal assemblage. The nutritional state of Lake Chivero is far above even the worst hyper-eutrophic state mentioned in Schaeffer (1999) for lakes in the temperate zone. In that context Lake Chivero, despite its size would be put in the category of sewage ponds. There is a parallel between the unstable eutrophic lakes that can exist in two different unstable states and the hyper-hypereutrophic sewage pond that oscillate between a turbid and a clear state. The explanation for the existence of the two states in Lake Chivero was also investigated within the context of equilibrium and non-equilibrium theoretical views of community organization. The concepts of equilibrium, non-equilibrium, stability and steady state are discussed in section 1.5.

1.5 EQUILIBRIUM, STABILITY AND STEADY STATES

A notion proposed in classical ecological thought is that ecological succession process tends towards "maturity", a "balanced state" or an "equilibrated state" (Odum 1969, Christensen 1995). The successional process involves the colonization by pioneer species with progressive modification by subsequent arrivals until the system attains a climactic steady state, which will be dominated by competitively stronger species (Reynolds 1993). A steady state is also considered as the successional climax, equilibrium state or pronounced dominance pattern where the algal assemblage consists of cyanobacteria or non-edible large sized phytoplankton (Naselli-Flores *et al.* 2003). According to Reynolds (1993), the outcome of succession, reached exclusively and uni-directionally through internal, mainly biotic mechanisms has often been regarded as being at equilibrium. To avoid contentions over use of the term "equilibrium", Reynolds (1993) proposed that this successional climax can be considered as the achievement of the succession's relatively most stationary and mostly nearly equilibrated state.

Within the plankton assemblage, initial invaders in the successional process are the ruderal plants (R). Ruderal plants are tolerant to turbulent (mixing) transport and light gradients. They are prolonged (centric diatoms, cyanophytes) or needle shaped (pinnate

diatoms, desmids), large (flattened discoid centric diatoms) all with a relative high surface: volume ratio. They are then followed by specialists (S: large; low metabolic activity, and low growth rate *in situ*; high nutrient storage capacity; enhanced resistance to sinking and grazing losses) with more complex adaptations to competed resources (Sommer 1981, Reynolds 1988).

According to the competitive exclusion principle, which states that if several species compete for the same resource one of them will eventually excludes all the others (Hardin 1960), species diversity is suppressed as ecological equilibrium is approached. The prediction is that the successional process should be towards the establishment of a low-diversity equilibrium (Sommer *et al.* 1993). This ecological equilibrium can only be attained under conditions of undisturbed succession when less adapted species are excluded through competitive exclusion. At equilibrium, biomass is partitioned among but a few species that are co-dominant since competitive exclusion will have operated in favour of dominant species (Rojo & Cobelas 1993). Steady state conditions or equilibrium is most likely to develop in large and deep lakes with low water renewal times and moderate trophic states (Salmaso 2003). In nature, however, stabilized ecological equilibrium is never attained because the successional process is constantly interrupted by externally imposed disturbances (Reynolds 1993).

Equilibrium is rarely attained in pelagic environments because the environment is rarely uniform due to constant disruption or disturbance (Sommer 1985). This concept is embodied within Connell's (1978) intermediate disturbance hypothesis (IDH). The IDH suggests that disturbances of intermediate frequency or intensity, relative to the time period necessary for species succession to lead to equilibrium conditions, can act to maintain high species diversity in communities that would otherwise be dominated by a few superior competitors by allowing successional "pioneer" species to invade repeatedly (Hambright & Zohary 2000). Intermediate disturbances with the appropriate intensity, frequency and duration prevent competitive exclusion and so maintain high species diversity (Reynolds 1993).

Fluctuations and oscillations around equilibrium occur as a lake system responds to perturbations caused by changes in temperature, wind action and irradiance (Uhlmann 1980). As a response the internal or external disturbances can cause a sudden departure from a previous state. Strong perturbations can cause a rapid adjustment toward the previous or a new steady state (Uhlmann 1980). The severity and frequency of disturbance will determine species composition, succession state, productivity, competitiveness and diversity of a given ecological system (Reynolds 1993).

Disturbances that could enhance species diversity in phytoplankton assemblages include water column mixing due to storms or autumnal cooling and substantial changes in zooplankton grazing pressure (Padisák 1994, Sommer 1995). According to Reynolds (1993), when disturbance is "sufficiently frequent" the community may become dominated by species capable of surviving the disturbances or which are capable of quickly reaching reproductive maturity. This is termed "plagioclimactic equilibrium". A typical example is Zeekoevlei, a system highly disturbed by wind, which consequently supports a "plagioclimatic equilibrium" perpetually dominated by *M. aeruginosa* (Harding 1996).

Practical criteria for determining whether a given "phase" in a seasonal sequence of a natural phytoplankton assemblage is at equilibrium or not have been proposed by Sommer *et al.* (1993). They recognize that it is usually difficult to determine whether a system is at equilibrium due to lack of chemical data, or to insufficient sampling frequency, or to any other cause. For practical purposes Sommer *et al.* (1993) proposed that equilibrium in a natural phytoplankton assemblage occurs if (i) one, two or a maximum of three species contribute not less than 80% of biomass with no significant changes in total biomass and their existence or coexistence persists for a period of more than two weeks.

This working definition was produced during the 8th Workshop of the International Association of Phytoplankton Taxonomy and Ecology (Sommer *et al.* 1993). Attainment of equilibrium of between one to three species is based on the competitive exclusion

theory (Hardin 1960), which suggests that phytoplankton species number in an assemblage at equilibrium will be limited to the number of simultaneously limiting resources, generally three or fewer (Hambright & Zohary 2000). The competitive exclusion theory therefore allows the phytoplankton succession to tend towards equilibrium of between one and three species at any phase of its seasonal development (Sommer *et al.* 1993) because usually fewer than three resources will be simultaneously limiting (Rojo & Cobelas 1993). This, as previously discussed, is however in contrast to the high species diversity that occurs in natural phytoplankton assemblages (Harris 1986), and is explained by the "paradox of the plankton" (Hutchinson 1961). The "paradox of the plankton" formulated by Hutchinson (1961) refers to the coexistence in turbulent surface water of a large number of phytoplankton species despite their dependence on the same resources in contradiction to theories of competitive co-existence (Hardin 1960).

A typical case where equilibrium has been persistently attained for seven years, with one species dominating, is Hartbeespoort Dam (South Africa) a hyper-eutrophic lake. According to Hambright & Zohary (2000) the subtropical climate with low wind speeds provided an environment that enabled the development each year of equilibrium or at least near-equilibrium environmental conditions with regard to nitrogen and phosphorus. Consequently phytoplankton dynamics was influenced mainly by competitive exclusion such that *M. aeruginosa* often constituted > 90% of the phytoplankton biomass. The primary limiting factor was light while nitrogen and phosphorus were in excess of phytoplankton requirements throughout the year and temperature never dropped below 12 ^{o}C. The large blooms of buoyant *M. aeruginosa* that developed in the upper waters competitively excluded non-buoyant taxa that are not adapted to light limitation. This observation supports Sommer *et al.*'s (1993) definition of equilibrium. Light, as one primary limiting resource, enabled dominance by *M. aeruginosa*, within the context of Hardin's (1960) competitive exclusion theory (Hambright & Zohary 2000).

Stability (resilience to external forcing) means that an ecological system remains relatively unchanged or that it has the ability to return to an equilibrium state if subjected to minor or temporary disturbances (Uhlmann 1980, O'Neill *et al.* 1989). According to

Bodin & Wiman (2004) an ecological system in equilibrium, should not be assumed to be "stable" because stability only relates to the systems capacity to remain in that state. Their definition of stability is the capability of the system to withstand forces tending to move it away from equilibrium.

A stable phytoplankton assemblage should therefore be able to recover its characteristic composition and relative abundances following an environmental disturbance (Pickett & White 1985). Stability, as in the case of equilibrium, can only be attained in the absence of any externally imposed disturbances and under such circumstances algal succession should in one to two months lead to a "stable equilibrium state" in which most species have been out-competed by one or a few dominants (Scheffer 1999). A "stable equilibrium state" is therefore a state at which a system will remain or, if moved away, to which the population will return (Scheffer 1999).

A stable system rapidly returns to equilibrium and fluctuates less than an unstable one (Uhlmann 1980). This has been discussed in section 1.2 with the case of algal communities in oligotrophic to hyper-eutrophic lakes. Figure 1.1 shows the decline in stability with increase in trophic status. A hyper-eutrophic water body is highly unstable since it can easily dis-equilibrate due to high biomasses and high metabolic rates (Uhlmann 1980). Uhlmann (1980) illustrates this high instability in hyper-eutrophic water bodies with respect to the diurnal oscillatory fluctuations of oxygen around the arithmetic mean. A system can readjust between two alternative stability levels; L_1 with high dissolved oxygen up to 25 mg l^{-1} during the day, and L_2 with depleted levels at daybreak. The transient phase will occur between L_1 and L_2 and in this phase the system will not be at equilibrium.

The three terms equilibrium, stability and steady state (meaning "balance") are sometimes used either inter-changeably or together in the literature. As cited in Teubner *et al.* (2003) "steady state", a thermodynamic term, is used to describe the persistence of biota and relatively stable conditions in an ecosystem. A steady-state phytoplankton assemblage would be expected to meet the criteria proposed by Sommer *et al.* (1993) as

discussed previously for equilibrium. A steady-state outcome, as previously discussed for equilibrium, occurs wherein one or relatively few species achieve overwhelming dominance though competitive exclusion (Hardin 1960) or, according to Reynolds (1993), a "climactic steady state" occurs wherein the competitively determined best-fit species achieve unchallenged dominance. Sommer (1995, 1989) through experimental investigation into the outcome of inter-specific competition in isolates of natural phytoplankton, showed that some thirty to sixty days were required to achieve a steady-state equilibrium, although according to Reynolds *et al.* (1993) in nature most recognizable ecosystems are far from being at steady state due to natural variability.

When a phytoplankton assemblage is at steady state there will be little change of individual species biovolumes and no net change of standing crop (total phytoplankton biovolume) (Teubner *et al.* 2003). By calculating Bray-Curtis similarity index to determine the stability of species composition between monthly samples, Teubner *et al.* (2003), suggested that a "perfect steady state or" or "perfect equilibrium" theoretically occurs when the calculated value is 1 (100%). This will indicate no change in individual bio-volumes of species from month to month. Net change values of zero for standing crop, indicating a balance between production and losses would also indicate "perfect equilibrium" or steady state.

This discussion shows that in nature an equilibrium or steady state phytoplankton assemblage can only be achieved under constant environmental conditions in a lake. This can only occur when environmental constancy persists over twelve to sixteen generations (Reynolds 1993) or three to six weeks (Sommer 1983). However continuous constant environmental conditions do not occur in nature because of a great variety of factors, which may have an influence on phytoplankton succession (Teubner *et al.* 2003), so the community will constantly deviate from achieving a stable, climactic equilibrium or steady state. Re-current forcing will cause a permanently non-equilibrated state and according to Reynolds (1993) the severity and frequency of the intermediate disturbances will ultimately determine the state of assemblage organization and diversity. Even under carefully controlled, constant laboratory conditions equilibrium is never attained

(Kersting 1985). The algae will therefore continuously display irregular fluctuations, now considered as "chaos" (Scheffer 1991). This notion of change, chance or chaos argues that disturbance and stochasticity dominate the shaping of communities (Sousa 1984, Picket *et al.* 1992)

To rationalize the two realms of thought, balance *versus* chaos or equilibrium *versus* non-equilibrium, Reynolds (1993) proposed a single explanatory theory, which proposes that intermediate disturbances reconcile the equilibrium and non-equilibrium arguments. Reynolds (1993) argues that the organization state of planktonic assemblages depends upon the extent of the progress towards equilibrium achieved since the last disturbance was sustained. This embodies both the competitive exclusion principle (Hardin 1960) and the intermediate disturbance hypothesis (Connell 1978) and agrees with Beisner *et al.*'s (2003) suggestion that there is no need to continue splitting non-equilibrium mechanisms from equilibrium mechanisms in an attempt to understand community regulation. Their effect cannot be considered in isolation because they both regulate and organize communities. A system's equilibrium consequently develops within the context of natural variability and disturbance regimes in addition to the ever-changing biotic forces within the community (Okey 2004).

CHAPTER 2

STUDY AREA

2.1 INTRODUCTION

Lake Chivero is hyper-eutrophic basing on the existing physical and chemical data (this chapter) and has manifested all the typical symptoms of eutrophication including existence of permanently turbid waters due to the perpetual predominance of cyanobacteria mainly *M. aeruginosa.* When this project was formulated the objective was to investigate cyanobacterial dynamics and toxin production in Lake Chivero since blooms of toxic cyanobacteria were a management concern because they posed a potentially serious health hazard to consumers. When the study commenced it was however interesting that there was a marked improvement in water clarity in the lake. The lake exhibited a "clear state" for fifteen months after which it shifted to a " turbid state" for eight months and then reverted to the clear state. The focus of the study then changed and instead investigated the development of the phytoplankton assemblages and some ecological aspects during these two states in relation to environmental variables. The work reported in this thesis focuses on the opportune coincidence of the alternate manifestation of a clear and a turbid state in a hyper-eutrophic lake where the phytoplankton assemblage has previously been dominated by cyanobacteria.

2.2 JUSTIFICATION OF THE STUDY

Most of the limnological work on Lake Chivero was carried out in the 1970s and there is limited recent limnological information that might be useful in managing problems in the lake (Nhapi 2004). The information on phytoplankton ecology in the lake is outdated, the only detailed study having been undertaken in 1968/69. The present study was therefore undertaken to determine how environmental variables influence the development of phytoplankton assemblages in the lake.

Ecological instability and periodic crashes of phytoplankton populations have not been proved within the phytoplankton assemblage in Lake Chivero, although they are

common in hyper-eutrophic systems. The lake was considered to support permanent blooms of cyanobacteria (Munro 1966, Falconer 1973, Marshall & Falconer 1973). The thrust of this thesis is to understand the development of phytoplankton assemblages in Lake Chivero in the context of our expectations of hyper-eutrophic systems.

One would expect Lake Chivero, a hyper-eutrophic lake, to be permanently turbid. It is an interesting coincidence, however, that during this study the system, which had been renowned for being permanently turbid due to high algal biomasses, switched to a clear state. The focus of this study was therefore to determine whether the reversion to a clear state was linked to changes in phytoplankton assemblages. It was hypothesized that the lake was presently in a clear metastable state but that a slight disturbance in terms of the internal nutrient balance would shift it to a turbid state that would be maintained until the nutrient balance dropped below a critical level.

The tenet of this thesis was to explain the clear state, a phenomenon that had not been reported before in the lake. I also asked whether this state would be persistent or whether it represented a single event that would not repeat, and whether a steady-state phytoplankton assemblage had developed during the clear state.

Access to qualitative phytoplankton samples collected from Lake Chivero in 1960 (1960-06-15) and in 1983 (1983-09-18) from Lund University, Sweden enabled comparison of phytoplankton taxa and species characteristics to be analysed and compared to observations made between 2003 and 2006. This enabled qualitative "snapshot" comparisons of phytoplankton compositions in Lake Chivero in 1960, 1983 and 2003 to 2006.

2.3 OBJECTIVES OF THE STUDY

The main objectives of the study were to increase knowledge of the development and dynamics of phytoplankton assemblages in a hyper-eutrophic water body and specifically to understand the reasons for the occurrence of cyanobacterial blooms and

their potential ecological implications. The development and dynamics of phytoplankton assemblages in Lake Chivero was investigated with the aim of:

(1) establishing principal environmental variables influencing dynamics of the phytoplankton assemblage
(2) characterizing Lake Chivero's phytoplankton in terms of the currently understood models of phytoplankton periodicity and succession based on phytoplankton biomass
(3) determining whether the proposed models of cyanobacterial dominance (Chapter 1 Section 1.3) can be used to explain patterns observed in species diversity, abundance and changes in this hyper-eutrophic system
(4) determining whether the clear state in Lake Chivero was linked to the development of a steady state phytoplankton assemblage

The knowledge gained, especially on the occurrence of cyanobacterial blooms and their potential ecological implications, will be useful in the development of a management plan for the lake.

2.4 ORGANIZATION OF THE THESIS

In this study I have examined the environmental variables and the development of phytoplankton assemblages in Lake Chivero. The first chapter gave a theoretical background in which the context of this thesis is developed. The current chapter gives a description and background information of the study site including the problem statement, the objectives to be addressed, and the scope.

In chapter 3 the water quality of the reservoir is described using physical and chemical parameters obtained at three stations in the lake over a 23-month period. Chapter 4 describes the development and dynamics of the phytoplankton assemblage over the same 23-month period in relation to the physical and chemical features presented in chapter 3. Chapter 5 examines the process of cyanobacterial blooming with respect to the relationship between nitrogen and phosphorus concentrations, spatial and temporal dynamics and vertical distribution of algae during the bloom. In chapter 6 the survival strategies of some species of the phytoplankton assemblage in

Lake Chivero and responses of species isolated from environmental variability during short periods of time is discussed. The chapter presents findings of studies on responses of phytoplankton assemblages isolated from some allogenic processes over short periods of time in enclosures. Chapter 7 discusses the effects of varying nitrate and TN:TP ratio on phytoplankton assemblages in microcosm experiments. Chapter 8 presents a general discussion on the study, management implications and conclusions.

2.5 SYNTHESIS OF AVAILABLE DATA

2.5.1 Geographical and ecological setting of Lake Chivero

Lake Chivero (17° 54′ S; 30° 48′ E; formerly Lake McIlwaine) was built between 1952 and 1953 on the Manyame River, 37 km southwest of Harare, at an altitude of 1363 m above sea level (Figure 2.1). It is located in the same catchment as, and is downstream of, the city it supplies, and thus has received sewage effluent since 1952 (Marshall & Falconer 1973). At impoundment its main function was to supply water to the City of Harare but it now fulfils a multi-purpose role, which includes supporting a fishery, recreational use and irrigation.

The lake has a capacity at full supply level of 250 x 10^6 m^3, and a surface area of 26 km^2. Munro (1966) recorded a mean depth of 9.5 m and a maximum depth near the lake spillway of 27.4 m. However, as a result of deposition of black, FeS- and nutrient-rich sediments over the last fifty years, the maximum depth recorded at the spillway during this study was 20 m at highest water level. A summary of the main morphometric and hydrological characteristics of the lake is given in Table 2.1.

The highest hydraulic load (80%) into the lake is from the Manyame River (Ballinger & Thornton 1982), which rises 72 km to the southeast. The catchment area of the Manyame River includes the towns of Chitungwiza, Ruwa, the eastern parts of Harare and parts of Seke Communal Lands. The waters of the Manyame are highly contaminated, Nhapi (2004) recently measuring high levels of total nitrogen (3.0 mg l^{-1}) and total phosphorus (0.7 mg l^{-1}) in the river. Contamination is from sewer overflows from Chitungwiza and waste stabilisation pond effluents from Donnybrook and Ruwa wastewater treatment works.

The Marimba and Mukuvisi streams flow directly into the headwaters of the lake while fifteen small seasonal streams enter the lake at various points (Munro 1966). About 50% and 56% of the total river flow in the Marimba and Mukuvisi rivers respectively is partially treated wastewater (Nhapi 2004). The ecology of Lake Chivero is intricately linked to the land-use activities within its catchment: about 10% urban development and 90% rural lands (Magadza 1997). Ten percent of the catchment lies within two major urban centres, Harare and Chitungwiza, and the rest is in rural and commercial farming areas. Three urban centres, Harare, Chitungwiza and Ruwa, sit directly upstream of Lake Chivero (Zanawe 1997, Figure 2.1).

The estimated urban population of Harare and Chitungwiza is around 2.4 million people (Nhapi 2004). Since the lake was impounded the population within the catchment has increased significantly (Table 2.2) and this has resulted in a corresponding increase in water uptake and waste production. The other urban settlements within the catchment, namely Chitungwiza, Ruwa, Norton and Epworth, have also expanded. As a result urban run-off and industrial and sewage effluent loading into the lake has increased and this

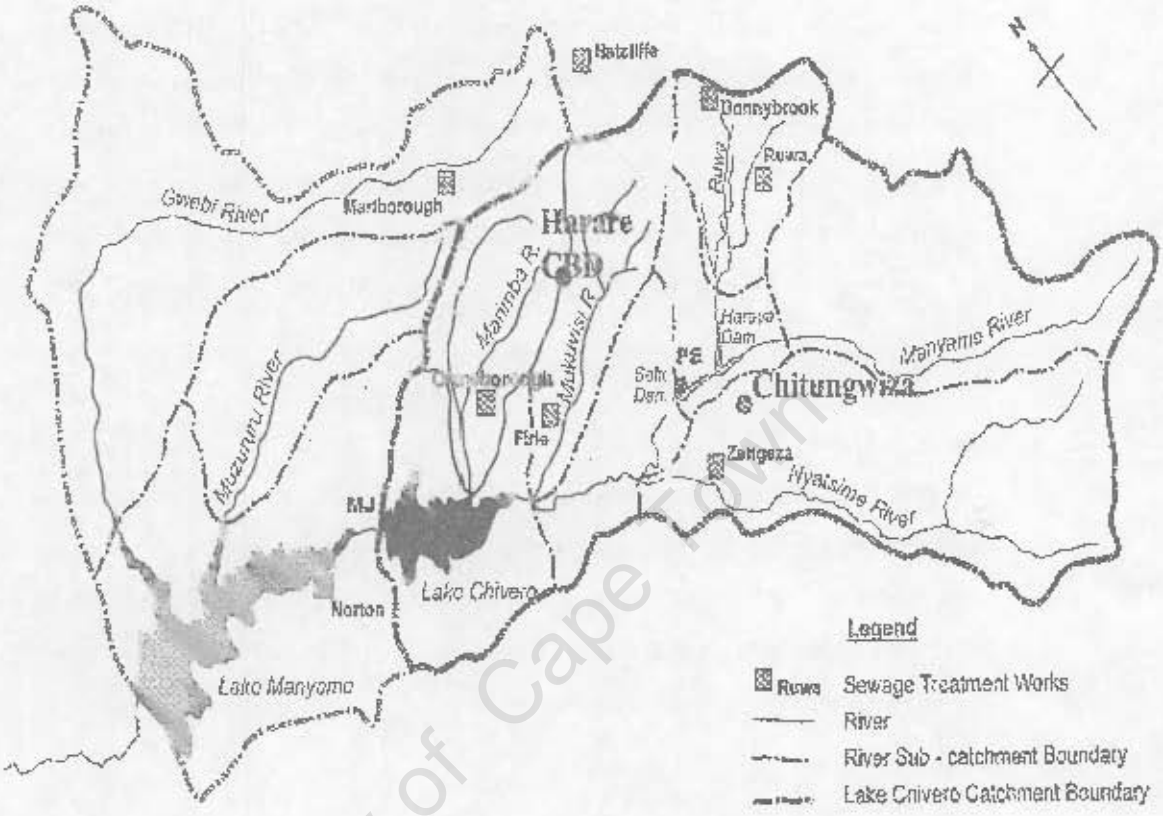


Figure 2.1 **Location of Lake Chivero in relation to the city of Harare and the principal water treatment works, wastewater treatment plants and major rivers (MJ = Morton Jaffray Waterworks, PE = Prince Edwards Waterworks) (Source: Nhapi 2004)**

continues to negatively impact on the trophic status of the lake. The other source of pollutants into the lake is leached fertilisers from small-scale but extensive urban agriculture.

Table 2.1 Morphometric characteristics of Lake Chivero (Source: Burke & Thornton 1982)

Characteristic	
Full supply volume	250 X 10^6 m^3
Full supply surface area	26.30 km^2
Catchment area	2227 km^2
Shoreline length	74 km
Maximum depth	27.43 m
Mean depth	9.4 m
Maximum breadth	8.0 km
Mean breadth	1.68 km
Length	15.7 km
Renewal time	0.82 years

Table 2.2 The population of towns in the Chivero catchment (Source: Nhapi 2004)

Urban Area	Area km^2	1969	1982	1992	2002
Harare	447.1	386,000	658,000	1, 189,103	1,862,000
Chitungwiza	42.0	15,000	172,000	274,912	388,000
Epworth	11.1	-	-	62,630	88,000
Ruwa	31.4	-	-	1,447	56,000
Total	531.6	401,00	830,000	1,548,092	2,394,000
% of national population		8	11	15	19

The geology at the site of the dam comprises an exposed fractured banded ironstone surface that overlies a solid dolerite outcrop (Burke & Thornton 1982, Munzwa 1982). The lake is underlain by granite except for a portion of the northern flank with meta-sediments and metavolcanics. A ridge of hills that form the abutment of the lake comprises of a narrow belt of schists and banded ironstones. The catchment of the main inflow, the Manyame River, is underlain by rocks of Archaean age.

2.5.2 Climate and hydrology characteristics

Precipitation over the Lake Chivero catchment is seasonal. The rainy season (summer) spans November to April and the average daily temperature during this period is 20 °C with a mean diurnal variation of 12 °C (Ballinger & Thornton 1982). Most of the rain falls from November to February. The rainfall pattern at Lake

Chivero during the study period is shown in Figure 2.2a. The hot dry season, with an average daily temperature of 22 °C, occurs from September to November. The cold dry season, with an average daily temperature of 14 °C, occurs from May to August. Mean annual rainfall is approximately 700 mm. The minimal recorded value is 410 mm and the maximal, 1236 mm (Ballinger & Thornton 1982). Rainfall is virtually restricted to the period between November and April.

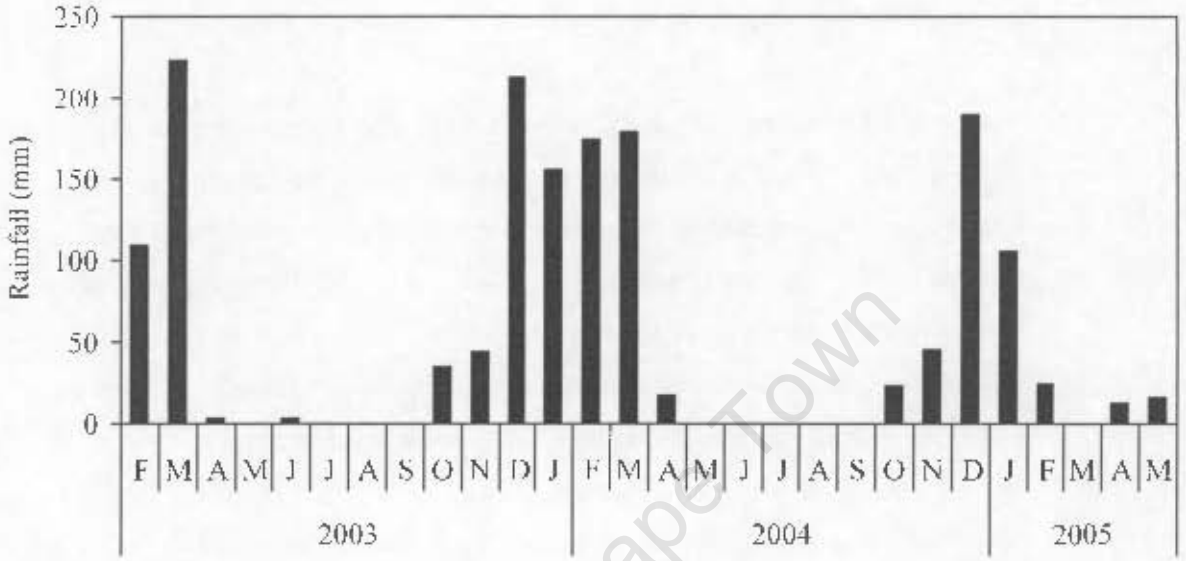
Lake-level fluctuation follows a seasonal pattern that is influenced by rainfall and riverine inflow. The lake level normally varies within about two metres of full supply level per annum with draw-down levels of up to 4 metres occurring during periods of below-average rainfall when there is reduced inflow (Ballinger & Thornton 1982).

During the study period the water level was lowest in February 2003 after which the reservoir filled to capacity by the end of the rainy season in April (Figure 2.2b) and spilled between April and May. The water level dropped from May and reached the lowest level in January 2004. The lake level was lower in February 2003 than in January 2004. The lake started re-filling in February 2004. The decline in lake level is primarily a response to abstraction by the City of Harare, which takes 60% of the outflow capacity while evaporation accounts for approximately 30% (Ballinger & Thornton 1982).

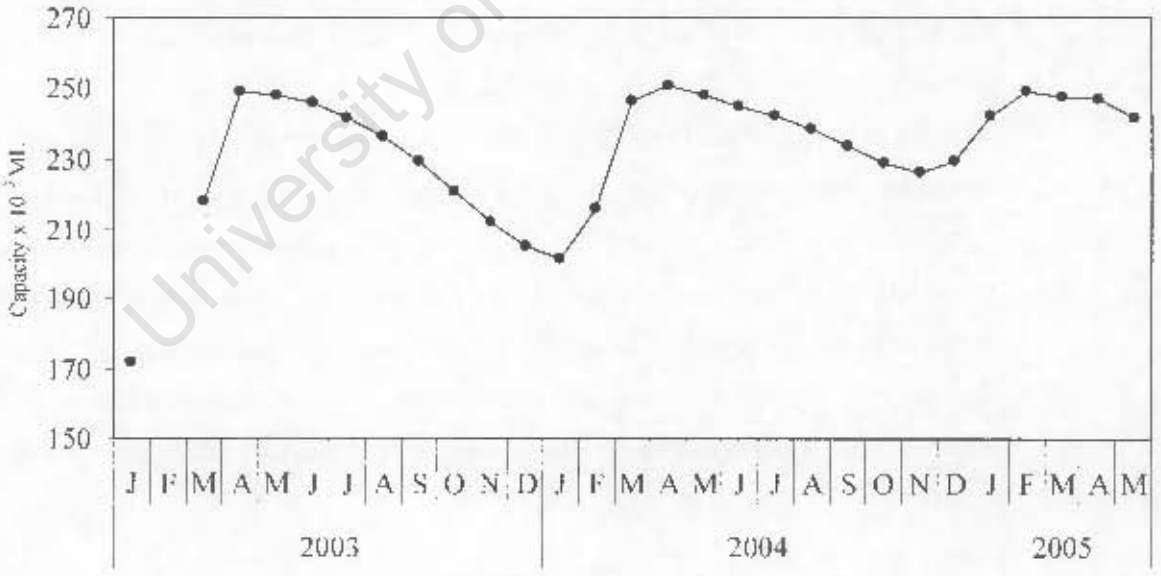
Large drawdowns affect water chemistry, mainly nutrient concentrations (Marshall & Falconer 1973, Marshall 1978) and probably phytoplankton. The effect is mainly through the variability in residence time, which depends on the rainfall pattern. Years of extremely high rainfall or drought (e.g. 1985 and 1995) can radically change water

Figure 2.2 **Rainfall patterns (Source: Records from Lake Chivero, National Parks and Wildlife Management Authority) (a) and lake level fluctuations (Source: Records from Zimbabwe National Water Authority) (b) in Lake Chivero (2003 –2005).**

(a)



(b)



residence times (Thornton 1987) which must have significant impact on the physico-chemical environment, which will then affect ecological processes in the lake, including phytoplankton dynamics. Dilution occurs during high rainfall periods and evaporative concentration during low rainfall (Scott *et al.* 1979).

The physical limnology of Lake Chivero is influenced mainly by air temperature and winds (Ward 1982). It is a warm monomictic lake with a single winter overturn. Lake Chivero is very susceptible to wind action due to its open morphometry. It has a shallow profile with large littoral areas (Thornton 1987). Overturn sometimes occurs when summer storms cool the surface waters. The consequent small maximum difference between surface and bottom water temperatures results in a high degree of instability thereby easily allowing turnover to occur (Munro 1966).

The mean wind strength in the area is 2.6 m s^{-1} and the maximum is 9.0 m s^{-1}; the prevailing winds are easterly to north-easterly (Ward 1982, Munro 1966).

2.5.3 Pollution problems in the tributaries of Lake Chivero

Scarcity of water in Southern Africa, and the consequent lack of a reliable supply of water, has led in several countries to the development of policies of reintroduction of "treated" municipal wastewaters into watercourses (Thornton 1987). These introductions of treated effluents as water conservation measures has resulted in enrichment and extremely high productivity of several impoundments in the region. At present Lake Chivero is a system under extreme pressure both as a source of water and as a means of waste removal for the city of Harare, two diametrically opposing functions.

The effluent generated from wastewater treatment plants upstream is brought into the lake through its tributaries. Machena (1997) observed that the Mukuvisi had lost its self-purification properties so that effluent entered the lake in an "unpurified" state. In 1997 Chitungwiza alone was estimated to discharge about 50 000 m^3 d^{-1} of effluent into the lake (Magadza (1997) and this quantity must have increased with the expansion of the town. Continuous increase of sewage loading into the lake is illustrated by an increase from 30 000 m^3 day^{-1} in 1960, to 40 000 m^3 day^{-1} in 1964 up

to 160 000 m^3 day^{-1} in the 1980s (Marshall 1994). The summary of the nutrient inputs in Lake Chivero from its three main tributaries is shown in Table 2.3.

Table 2.3 Summary of nutrient inputs into Lake Chivero from its three tributaries (Source: Nhapi 2004)

Source	Flow	Total nitrogen		Total phosphorus	
	m^3 y^{-1}	mg l^{-1}	kg yr^{-1}	mg l^{-1}	kg yr^{-1}
Marimba River	70,980,000	13.6	965,328	2.6	184,548
Manyame River	341,529,474	3.0	1,024,588	0.7	239,071
Mukuvisi River	89,440,667	8.9	796,022	2.4	214,658
Direct Area runoff	22,799,510	4.1	93,478	0.3	6,840

2.5.4 Limnochemistry of Lake Chivero

Extensive research has been carried out on the limnochemistry of Lake Chivero, and the lake is considered to be one of the best studied in Southern Africa (Magadza 2003). The major interest in limnological research initiated by the University of Zimbabwe from the early 1960s (summarized in Thornton & Nduku 1982) was to understand the eutrophication process. Nutrient dynamics follow a seasonal cycle associated with rainfall and runoff. The main nutrient peak in surface water is in summer while a secondary nutrient peak occurs at overturn (Robarts *et al.* 1982) or during other overturn events (Thornton & Nduku 1982). Assessments of the seasonal cycles of nutrients in the lake were done a long time ago when it was observed that nitrate and phosphorus exhibited similar patterns with the maxima occurring in spring (September – November) and summer (December – April) and minima during winter (May – August). The Harare City Council (recently responsibility was taken by Zimbabwe National Water Authority) routinely measures nutrient levels in the lake, although these data are not available.

Assessment of temporal changes in conductivity, phosphorus (Figure 2.3) and ammonia show marked changes in the trophic status of Lake Chivero. In 1960 conductivity was 100 µS cm^{-1}, orthophosphate was 0.04 mg l^{-1} and ammonia 0.1 mg l^{-1} but had increased to 170 µS cm^{-1} , 0.22 mg l^{-1} and 0.4 mg l^{-1} between 1962 and 1968 respectively (Marshall 2005) when the lake assumed hyper-eutrophic status

(Magadza 2003). A decrease of conductivity to 126 μS cm^{-1}, orthophosphate to 0.04 mg l^{-1} and ammonia to 0.04 mg l^{-1} occurred between 1970 and 1980 when sewage effluent was diverted to pass through irrigated pastureland (Thornton 1981). The diversion of sewage effluent through irrigated farmland resulted in a reduction of total phosphorus loading from 288.1 tonnes yr^{-1} to 74.6 tonnes yr^{-1} (Thornton 1980). During this period orthophosphate concentration decreased from 0.2 mg l^{-1} to 0.002 mg l^{-1} (Thornton 1981).

The lake currently receives nitrogen loading of 2879 tonnes yr^{-1} and phosphorus loading of 645 tonnes yr^{-1} (Nhapi 2004), which has reversed the recovery trend of the early eighties (Magadza 2003). Average levels of 2.0 mg l^{-1} total nitrogen (range 1.7 to 2.4 mg l^{-1}) and 0.6 mg l^{-1} total phosphorus (range 0.5 to 0.8 mg l^{-1}) recorded by Nhapi (2004) and total nitrogen concentrations ranging from 0.3 to 8.4 mg l^{-1} and total phosphorus concentrations ranging from 1 to 5 mg l^{-1} recently recorded by Ndebele & Magadza (2006) show that the lake is highly enriched. The effluent still passes through pastureland but due to excessive hydraulic loading their efficiency to strip nutrients has declined resulting in high nutrient outflows to the rivers (Nhapi & Tirivarombo 2004).

Inputs of nitrate-nitrogen, the main source of nitrogen in Lake Chivero, also significantly increased between 1962 and 1970 but, unlike phosphorus, nitrogen continued to increase following effluent diversion until 1974 (Figure 2.3b). These high levels were attributed to nitrogen-fixing crops that were grown in the pastures (Thornton 1981). Ammonia is also high, with concentrations of up to 3.5 mg l^{-1} (Moyo 1997). Highest levels occurred between 1992 and 1996, a period that was preceded by severe drought in Southern Africa when inflows in the lake consisted mostly of recycled wastewater (Magadza 2003).

Internal loading from sediments contributes significantly to maintain a state of permanent hyper-eutrophy in Lake Chivero. It was estimated in the 1970s that 56.2 metric tonnes of phosphorus was being lost from the water column to the sediments annually (Nduku 1976). Only about 59% of the phosphorus presently entering the lake flows out, the rest being locked up in the sediments and in plant biomass (Nhapi 2004). Thus the sediment sink in Lake Chivero is a significant internal reservoir of

phosphorus. Phosphorus release from the sediment was estimated by Nduku (1976) to occur at a rate of between 0.1 and 0.6 g m^{-2} y^{-1}, boosting the productivity of the lake.

The increase in conductivity from 7.2 µS cm^{-1} in 1968/69 (Falconer 1973) to values up to 778 µS cm^{-1} recorded by Magadza (1997) (Figure 2.3c) indicates a gradual built-up of salts in the lake. Saline characteristics developed between 1990 and 1996 when conductivity increased from about 295 µS cm^{-1} to 800 µS cm^{-1} (Magadza 1997). Conductivity levels are influenced by water level fluctuation. Concentration during drought periods results in high levels while dilution during periods of high river inflow results in slightly lower levels (Falconer 1973). The input of sewage effluent high in sodium and chloride also increases conductivity in the lake (Magadza 1997).

Poor flushing particularly of bottom waters results in the accumulation of nutrients and enhances a state of permanent hyper-eutrophy. An average hydraulic retention time of 1.6 years was recently estimated (Nhapi 2004) but a desirable retention time for effective phosphorus flushing is 6 months (Thornton 1980). Adequate flushing only occurs during periods of extremely high rainfall, which is rare. Nutrients accumulate in the lake, particularly during drought periods when the lake does not spill, essentially acting as a sink for the effluents flowing into it. Due to increased water demand and reduced catchment run-off the hydrobiology of Lake Chivero is considered to be a closed loop between abstracted water and returned effluent for a considerable part of the hydrobiological season (Magadza 2003). Poor flushing further enhances a state of permanent hyper-eutrophy. The high nutrient concentrations in the lake could also partly be due to evaporation from the lake during the dry season.

The water temperature in Lake Chivero ranges between 15 °C and 25 °C and maximum surface temperatures rarely exceed 25 °C (Marshall & Falconer 1973). The surface-to-bottom temperature gradient in the lake is small, not exceeding 4 °C (Marshall 1997). The thermocline is usually at a depth of 10 m (Munro 1966). Minimum temperatures are reached in August (Munro 1966).

Stable annual stratification occurs from October to March (Marshall & Falconer 1973, Mitchell & Marshall 1974) and deoxygenation of the hypolimnion occurs during the stratification period. Anoxia persists throughout summer, when oxygen stratification is most pronounced, until overturn occurs in winter (Marshall & Falconer 1973). Magadza (2003) observed that between 1988 and 1996 episodes of oxygen deficiency had become increasingly severe.

Prior to 1968 the pH was around 7.5 but rose to 9 between 1968 and 1976 (Thornton 1981) and now fluctuates between 7 and 9, the higher values resulting from photosynthesis by high algal biomasses (Thornton 1981).

During the 1970s, average Secchi disc transparencies varied from 0.6 to 1.6 m (Robarts 1979) while recently Nhapi (2004) recorded an average Secchi disc measurement of 1.4 m. The low transparencies can be attributed to high standing algal biomasses, especially the presence of permanent algal blooms. Such high algal biomasses and low transparencies suggest that light is a major factor limiting phytoplankton growth (Robarts 1981). The depth of the euphotic zone ranged between 1.3 and 3.6 m and was inversely correlated with algal standing crop. The phytoplankton in Lake Chivero has high self-shading potential and this prevents the development of large phytoplankton populations in the euphotic zone (Robarts 1981). For instance in 1975/1976 chlorophyll *a* concentrations ranged between 12 and 95 $\mu g\ l^{-1}$, which is lower than levels in Hartbeespoort Dam (NIWR 1985) and Zeekoevlei (Harding 1996), two systems of similar trophic status.

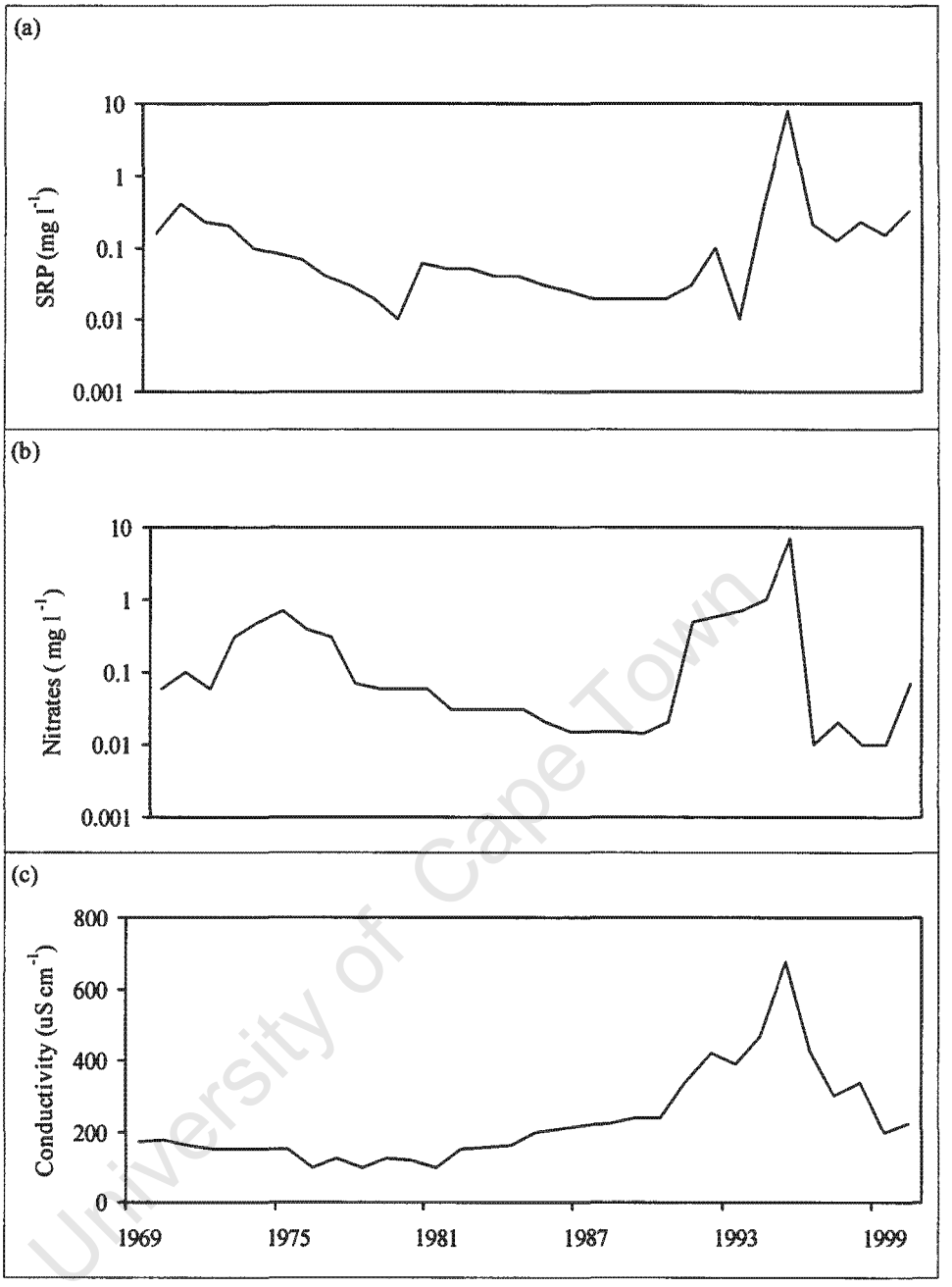


Figure 2.3 **Variations in (a) orthophosphate, (b) nitrates and (c) conductivity in Lake Chivero (Source: adapted from Marshall 2005 & Nhapi 2004)**

2.5.5 Eutrophication of Lake Chivero

Marshall (1997, 2005) reviewed the impact of eutrophication on Lake Chivero from its formation to the year 2000. The typical manifestations include excessive growth of algae, infestation by water hyacinth, and massive fish mortalities caused by oxygen deficiency and ammonia toxicity. Concurrent with eutrophication has been the establishment of dense blooms of cyanobacteria, which make water purification difficult and impart unpleasant tastes and odours to Harare drinking water. Five- to ten-fold increases in nitrogen and phosphorus concentrations resulted in increases in chlorophyll *a* concentrations, which reached 150 $\mu g\ l^{-1}$ and did not fall below ~ 50 $\mu g\ l^{-1}$ throughout 1968 - 1969 (Falconer 1973). Generally the deterioration in water quality was recognizable by the extensive algal growth resulting in unsightly surface scums or blooms by the end of the 1950s (Thornton 1981).

A decrease in chlorophyll *a* to an average of 15 $\mu g\ l^{-1}$ occurred after sewage effluent was diverted to pasture irrigation between 1970 and 1975 (Thornton 1980). An interesting observation when chlorophyll *a* concentrations decreased was the re-appearance of significant populations of other eukaryotic phytoplankton genera, with cyanobacteria being confined to a shorter part of the year.

Recurrent fish kills in Lake Chivero have also been major consequences of eutrophication. Increased productivity of the lake results in large accumulations of organic matter which decay and cause extensive deoxygenation of bottom water, leading to massive fish kills at turnover (Thornton 1981). Ammonium levels increase in the hypolimnion during the time of deoxygenation and when sudden overturn occurs, insufficient oxidation of ammonia result in fish kills (Marshall 1997). During the early years fish kills occurred at turnover when deoxygenated water from the hypolimnion also high in ammonia was brought to the surface, causing anoxic and toxic conditions. A massive fish kill in 1996 was linked to deoxygenation and ammonia toxicity, and possibly algal toxins (Moyo 1997). Recently Magadza (2003) noted that summer fish kills in the lake are now a common phenomenon. Mhlanga *et al.* (2006) established that a recent fish kills of *Oreochromis niloticus* was linked to depressed oxygen levels caused by high oxygen demand from massive algal die-off when a cyanobacterial bloom collapsed (Appendix 1).

Eichhornia crassipes (water hyacinth), a weed that had been present in small quantities in the Mukuvisi river, appeared almost immediately in Lake Chivero after dam closure as a response to nutrient enrichment (Munro 1966, Thornton 1981). The first outbreak of water hyacinth occurred soon after construction of the dam wall but was controlled by spraying with 2,4-D between 1953 and 1958 (Marshall 2005). Physical removal and chemical control using 2,4-D also successfully controlled another outbreak that occurred in 1970 when a drop in lake level, following a drought in 1967/1968, exposed dormant water hyacinth seeds. The most severe outbreak, which covered approximately 35% of the lake surface, occurred in 1985 (Chikwenhere & Phiri 1999). It was brought under control in 1992 by spraying with 2,4-D, after which biological control with two species of weevil, *Neochetina eichhorniae* and *Neochetina bruchi,* was instituted. Biological control has been successful and by 2000 weed coverage was reduced to 3-5% of the total area of the lake (Chikwenhere 2001).

Generally in terms of nutrient availability the lake is said to have gone through stages of mesotrophy (c. 1962), eutrophy (c. 1968) and hyper-eutrophy (c. 1970) in rapid succession (Thornton 1981). Following the short-lived improvement of water quality after the nutrient diversion programme, the lake bordered on mesotrophy (Thornton 1980).

2.5.6 Algal dynamics in Lake Chivero

Microcystis aeruginosa became the dominant cyanobacteria immediately after impoundment (Munro 1966) when, together with *Anabaena* sp., it occurred in large populations of approximately 1-3 million colonies l^{-1}, and formed noxious surface scums (Marshall & Falconer 1973).

Falconer (1973) carried out the only detailed long-term study on algal dynamics in Lake Chivero in 1968/69 and found that the phytoplankton assemblage consisted of six species. Three were cyanobacteria: *M. aeruginosa, Anabaena* sp. (mistakenly identified as *Anabaena flos-aquae*) and *Anabaenopsis tanganyike* and other three were diatoms: *Aulacoseira* (= *Melosira*) *granulata* var *granulata, A. granulata* var

angustissima and *A. italica*. *Microcystis aeruginosa* was the dominant species and contributed most to the biomass. It showed seasonal changes with a noticeable decline in winter, populations building up in the epilimnion from August, and large populations present by November 1969. Munro (1966) also observed seasonal changes in abundances of *M. aeruginosa,* populations developing throughout summer and declined during winter. Populations of *M. aeruginosa* showed a strong relationship to the seasonal development of thermal stratification and populations did not occur below 10 m depth.

During Falconer's (1973) study *Anabaena* sp. was common in the lake and co-occurred with *M. aeruginosa*. It exhibited three distinct maxima, one in January 1969, another in November–December 1969 when temperature and nutrients were high following stratification, and the third (and highest) at turnover in June/July, when *Anabaena* occurred as a monoculture at a concentration of 3 x 10^6 coil turns l^{-1}. Munro (1966) also reported a winter peak of *Anabaena* when oxygen tensions were lowest in the epilimnion. *Anabaena* sp. followed the thermal stratification cycle and never occurred in high numbers below a depth of 8-10 m during thermal stratification. It was distributed throughout the water column at overturn.

Anabaenopsis tanganyike occurred in the epilimnion from February to April 1969 and for the rest of the time it was uncommon or absent. Its filaments (4 x 10^5 l^{-1}) occurred exclusively in the upper layers of the epilimnion. The other dominant species in the lake were diatoms of the genera *Aulacoseira,* but only *A. granulata* var *granulata* was observed in appreciable numbers. Falconer (1973) observed two maxima of *Aulacoseira* species. The November (1968) peak coincided with the rainy season and the March to May (1969) peak fell during winter. The March to May peak comprised mainly *A. granulata* var *granulata* and *A. granulata* var *angustissima*. *Aulacoseira granulata* was observed to be uniformly distributed throughout the water column.

Falconer (1973) also regularly observed very small nannoplanktonic diatoms and a chlorophyte, *Pediastrum clathratum,* was occasionally encountered in shallow inlets and marginal vegetation. A dinoflagellate, *Ceratium hirundinella* cf. *brachyceroides,* was detected in deep water during December 1968. *Pediastrum clathratum* and

Ceratium hirundinella were remnants of a less eutrophic flora only occurring when local conditions were ideal.

In 1962/63 Munro (1966) also recorded chlorophytes of the genera *Volvox*, *Eudorina* and *Pediastrum,* and the desmid *Staurastrum,* as being common in the lake in winter and spring when algae diversity became high. Other species recorded during this period were *Actinastrum* sp., *Scenedesmus* sp., *Chlorella* sp. and *Ceratium* sp. (Thornton 1982). Altogether thirteen species were recorded in a partial species list for Lake Chivero. In a recent study Ndebele (2003) identified twenty-five phytoplankton species from three main taxonomic groups: cyanobacteria, chlorophytes and diatoms. Ndebele (2003) observed two dominant phytoplankton species, *M. aeruginosa* and *Aulacoseira* sp., which represented 64.3% and 19.3% of the total biomass respectively. This study was undertaken for a period of only two months (March and April 2003) and thus could not capture temporal patterns. A low Shannon-Weiner diversity index of 0.9 was calculated for the phytoplankton of Lake Chivero in 2003 (Ndebele 2003).

In the early days, chlorophyll *a* was shown to exhibit peaks coinciding with summer, winter and spring, the three growing seasons in the lake (Falconer 1973, Thornton 1980). In the years 1968/1969 chlorophyll *a* peaks occurred in January (150 $\mu g\ l^{-1}$), November (116 $\mu g\ l^{-1}$) and June/July (100 $\mu g\ l^{-1}$) and the range in chlorophyll *a* concentration in the lake was 50 to 150 $\mu g\ l^{-1}$ (Falconer 1973). Falconer (1973) established an epilimnetic maximum of chlorophyll *a* concentration of 150 $\mu g\ l^{-1}$. Two largest peaks occurred in the epilimnion during the summers of 1968-1969 and 1969-70 respectively when light and temperature were maximal. The third peak occurred during winter when temperature, oxygen, nitrogen and phosphorus were at their lowest levels. Thornton (1980) observed peaks in February (75 $\mu g\ l^{-1}$), June/August (44 $\mu g\ l^{-1}$) and September/October (16 $\mu g\ l^{-1}$). Chlorophyll *a* concentration ranged from 2 to 45 $\mu g\ l^{-1}$ with a mean of 15 $\mu g\ l^{-1}$ (Thornton 1980).

Robarts *et al.* (1982) described the seasonality pattern of phytoplankton in Lake Chivero from studies carried out in 1975/6. During this period *M. aeruginosa* dominated for most of the year, particularly during summer, while *Anabaena* sp. and or *A. tanganyike* were prevalent particularly during early spring. *Aulacoseira* spp.

dominated during winter. This pattern is similar to that previously described by Falconer (1973). Algal diversity was highest during winter and spring. Although *M. aeruginosa* was perpetually dominant, a "seasonal paradigm" of phytoplankton succession was exhibited.

Falconer (1973) observed that vertical distribution of *M. aeruginosa* and *Anabaena* sp. was influenced by the annual cycle of temperature stratification. The permanent summer thermal stratification was the main factor that influenced vertical phytoplankton distribution whereby the phytoplankton population remained in the euphotic zone for most of the year, and concentrations were never appreciable below 10 metres. Phytoplankton populations rarely exceeded 5×10^4 colonies l^{-1} below the thermocline and followed the pattern of thermal stratification. The phytoplankton formed surface maxima in January, July, September and December 1969. Falconer (1973) observed surface scums of phytoplankton, particularly on calm days, when the algae would float up to the surface and formed large concentrations by midday. Ndebele (2003) recently established that the highest phytoplankton concentrations were in the top 3 m with biomasses ranging from 0.8 to 7.6 mg l^{-1}.

A few studies have estimated phytoplankton biomass in Lake Chivero. Kritzberg & Hultin (2000) estimated mean monthly biomasses in summer ranging between 5 and 6.8 mg l^{-1}. In October 1998, Annadotter *et al* (2005) estimated an average biomass of 25.7 mg l^{-1} (range 10.9 to 46.9 mg l^{-1}) while Ndebele (2003) recently reported an average phytoplankton biomass of 4.9 mg l^{-1} (range 0.8 – 7.6 mg l^{-1}) between March and April 2003. Data are too limited to determine the trends in biomass changes in the lake.

From the synthesis of the few studies carried out on phytoplankton in Lake Chivero in the past, it can be said that the dominant alga in the lake was *M. aeruginosa,* which occurred together with *Anabaena* sp., *A. tanganyike* and *A. granulata.* Other genera that occurred in the lake but that were insignificant in terms of biomass were *Lyngbya, Staurastrum, Chlorella, Ceratium* and *Actinastrum.* Algal blooms were common and the main bloom species was *M. aeruginosa.*

The question that the present study will investigate is: "To what extent has the algal assemblage changed in Lake Chivero, 51 years after its impoundment?" Considering the instability of hyper-eutrophic systems, it can be hypothesized that the assemblage has changed from that previously described by Falconer (1973). In a system like Lake Chivero fluctuations and oscillations around the equilibrium state would be expected, as the system will be responding to perturbations caused by changes in temperature, wind action and irradiance. I hypothesize the existence of two distinct algal assemblages: one dominated by cyanobacteria under relatively stable environmental conditions and another dominated by eukaryotic algae under unstable environmental conditions.

2.5.7 Cyanotoxin production in Lake Chivero

The predominance of cyanobacteria in Lake Chivero could be a potential health risk to consumers since toxins can be harmful to human beings (Carmichael & Falconer 1993, Chorus & Bartram 1999) and other members of the biota (Nizan *et al.* 1986, Carmichael 1992, Mastin *et al.* 2002). The danger was recognized in Lake Chivero in the 1960s when Zilberg (1966) observed that children living in areas of the city supplied from Lake Chivero developed gastro-enteritis each year at the times when natural blooms of *M. aeruginosa* were decaying in the reservoir. Marshall (1991) established a correlation between incidences of gastro-enteritis and toxic cyanobacteria blooms in Lake Chivero.

Despite potential health concerns there is limited information on toxin production in Lake Chivero (Johansson & Olsson 1998, Hultin 1999, Kritzberg & Hultin 2000). In a recent study Annadotter *et al.* (2005) established that cyanobacteria lipopolysaccharide endotoxin caused an acute febrile reaction following drinking tap water or taking a bath in Harare. After a shower, within 2-5 hours a transient flu-like syndrome with fever, muscle pains, chest tightness and respiratory-tract symptoms developed. These symptoms were associated with massive blooms of cyanobacteria in Lake Chivero and consequently formation of high concentrations of lipopolysaccharide endotoxin yielding high concentrations in tap water. On that occasion, the algal assemblage was dominated by *M. aeruginosa* and *Microcystis botrys*. Concentrations of endotoxins ranged from 1 000 to 7 750 EU ml^{-1} while

endotoxins in Harare tap water (collected at a hotel in Harare) ranged from 60 to 205 EU ml^{-1}. Treated water in South Africa and Namibia had levels of 5 to 71 EU ml^{-1} (Burger *et al.* 1989) and in Finland 14 EU ml^{-1}.

In a separate study, Ndebele & Magadza (2006) detected microcystins in the lake. The average microcystin concentration was 19.9 µg l^{-1}, ranging from 18 to 22.5 µg l^{-1}. They however did not determine concentrations in drinking water for potential health effects to be inferred. Since the WHO (1998) guideline value for microcystin concentrations in the treated water is 1 µg l^{-1} 'tolerable daily intake for microcystins for lifetime exposure' it appears that due to dominance of cyanobacteria and high toxin concentrations detected in the lake, utilization of drinking water from Lake Chivero has potential health risks.

Within Southern Africa toxic cyanobacterial blooms have been reported from South Africa (Harding & Paxton 2001) and Kenya (Mwaura *et al.* 2004, Ballot *et al.* 2004). Toxic *Microcystis* has been reported in Hartbeespoort dam and the Vaal River (Harding & Paxton 2001). Seventeen reservoirs in South Africa demonstrated positive toxicity by mouse bioassay and livestock deaths between 1976 to 1986 (Harding & Paxton 2001). Between 1997 and 1999 cyanobacterial incidences were reported in eight reservoirs (Harding & Paxton 2001), involving deaths of cattle, sheep, giraffe and fish. Limited information on toxin production is available from water reservoirs (Rapala *et al.* 2002) in Southern Africa, including Lake Chivero. Occurrence of microcystins and lipopolysaccharide endotoxins in Lake Chivero during this study period is reported in Appendix 2.

CHAPTER 3

SPATIAL AND TEMPORAL VARIATION OF PHYSICAL AND CHEMICAL CHARACTERISTICS IN LAKE CHIVERO (2003 - 2004)

3.1 INTRODUCTION

Environmental characteristics indicative of hyper-eutrophic ecosystems are well documented (Barica & Mur 1980, Van Liere & Mur 1980, Leentvaar 1980). These conditions have been determined in hyper-eutrophic systems in Southern Africa - Hartbeespoort dam (Robarts & Zohary 1984, NIWR 1985, Zohary 1985), Zeekoevlei (Harding 1996) - and even in Lake Chivero (Munro 1966, Marshall & Falconer 1973, Magadza 2003). However, in these three systems, a clear state where the lake shifted into an alternative stable state for a long period has not been reported since the lakes became hyper-eutrophic. These systems are perpetually turbid due to persistent annual dominance of *M. aeruginosa*.

Lake Chivero could have been assumed to exist in a sustained turbid equilibrium state with a perpetual dominance of *M. aeruginosa*, a phenomenon which Harding (1996) proposed could be common in southern hemisphere lakes as he had observed in Zeekoevlei. The development of a clear state in Lake Chivero from February 2003 was atypical in a system that has principally been characterised by algal blooms since 1960. The clear state, which lasted from February 2003 to April 2004, was followed by a turbid state when a bloom developed from May 2004 to December 2004 (Chapter 4). Further monitoring in February, May, December 2005 and April 2006 showed that the lake had reverted to a clear state (Chapter 5). It was of biological and theoretical interest to determine the physical and chemical characteristics prevailing during these two contrasting states.

According to Heo and Kim (1997), increases in phosphorus loading of lakes often lead to nitrogen becoming the limiting nutrient and the phytoplankton assemblage can show

perennial changes caused by the change in the limiting nutrient through eutrophication. I have hypothesized (Chapter 4) that the decline in cyanobacterial dominance was caused by change in proportions of nitrogen to phosphorus in the lake. This chapter reports on the principal physical and chemical factors influencing the establishment of these two states and the spatial and temporal changes in abiotic conditions during the two states. Spatial and temporal variations in total phosphorus, orthophosphate, nitrate, ammonium, TN:TP ratio, total nitrogen, dissolved oxygen, conductivity, turbidity, pH and Secchi depth transparency are described using data obtained from the lake between 2003 and 2004.

Some of the theories explaining dominance by cyanobacteria in a water body (Chapter 1 Section 1.3) are linked to nutrient dynamics. The objective was also to establish which of these theories might apply to Lake Chivero. Turbidity and Secchi depth transparency are optical parameters that provide an indication of the underwater light climate in a water body, essential for understanding the seasonal trends in relation to the development of the phytoplankton assemblage. Although dissolved oxygen level and pH are influenced by phytoplankton productivity, there is no current data on their spatial and temporal dynamics in Lake Chivero despite their being of interest since they have been linked to fish-kills (Moyo 1997). Variations in phytoplankton biomass, species making up the assemblage and chlorophyll *a* are discussed in Chapter 4. The questions that this study addresses by comparing the clear and turbid states are:

(i) is limnochemistry stable and therefore predictable?
(ii) is limnochemistry spatially uniform?
(iii) are nutrients (N and P) perpetually high in the lake?
(iv) has the degree of eutrophication reduced compared to that reported in previous studies?
(v) do the two states exhibit different physical and chemical characteristics?

3.2 MATERIALS AND METHODS

Physical and chemical parameters were monitored monthly from February 2003 to December 2004 at three stations (1 to 3, Figure 3.1). Sampling on each occasion was carried out between 10.00 am and 12.00 noon because it was considered to be the period of maximum productivity in the lake. Integrated samples were collected into a bucket from 0 - 2 m, 2 - 4 m and 4 - 6 m at Station 1, 0 - 2 m and 2 - 3 m at Station 2 and 0 - 2 m and 2 - 3.5 m at Station 3. The water samples were collected into 2-litre polythene bottles that had been acid-washed and rinsed well with distilled water.

Temperature, pH, conductivity, turbidity and dissolved oxygen were immediately measured on site using field meters. Temperature was measured with a mercury thermometer. The pH was measured with a WTW pH 330i (Geotech Environmental Equipment, Inc., Denver, Colorado, USA) meter calibrated using two-stage calibration against buffers at pH 7 and 9. Conductivity was measured with a WTW Cond 330i (Geotech Environmental Equipment, Inc., Denver, Colorado, USA) meter and reported at 25^{0}C. Turbidity was measured with a Hach Field Turbidimeter after calibration with standards of 10 NTU and 100 NTU. Dissolved oxygen was determined by a WTW Oximeter 330 (Geotech Environmental Equipment, Inc., Denver, Colorado, USA) meter that was calibrated in water vapor-saturated air using the OxiCal®-SL calibration vessel. A standard 20-cm diameter disc painted into black and white quadrants was used to measure Secchi depth.

While in the field the collected samples were stored in a cooler box with ice. On arrival in the field laboratory unfiltered samples for total nitrogen and total phosphorus were separated and immediately frozen. Samples for nitrate, orthophosphate and ammonium analysis were filtered through Whatman Glass Fibre 47 mm filters to remove silt particles and then stored under refrigeration. Samples were then transported under ice to the analytical laboratory where they were immediately processed.

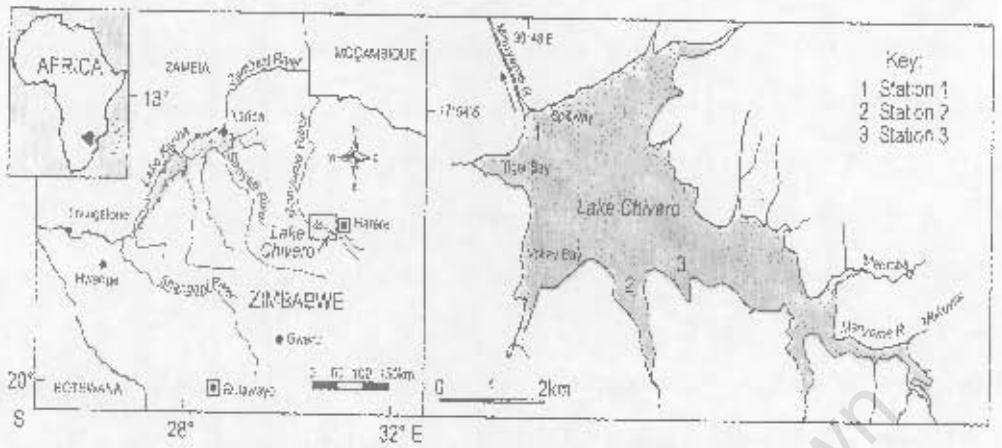


Figure 3.1 Map of Lake Chivero showing the sampling stations (Source: Mhlanga *et al.* 2006)

Concentrations of orthophosphate, nitrate, ammonium, total nitrogen and total phosphorus were determined by standard methods for chemical analysis of freshwater (Golterman *et al.* 1978). Internal calibration was done using standards of known concentration prepared in the laboratory. The concentration of the standards, which bracketed the concentration of the solutes, was used for calibration. Their respective absorbencies were used to draw a linear relation between absorbance and concentration (Beer's law). The equation is

$y = bx+a$

where y = concentration

X = absorbance

b = a "constant", the slope, with units y/x

a = a "constant", the concentration at zero absorbance, units of y.

The concentrations of the samples were read from the line using the measured absorbencies.

Orthophosphate was determined colourimetrically at a wavelength of 882 nm after addition of molybdate antimony mixed with ascorbic acid. The concentration of nitrate was determined by the cadmium-copper reduction method (Golterman *et al.* 1978) where

nitrate is reduced to nitrite in columns containing cadmium, which has been treated with copper. The absorbance of colour that developed after mixing the effluent with sulphalamide and N-I Napthylethylenediamine was measured at 545 nm. Ammonium (NH_4-N) was determined by the indophenol blue method (Golterman *et al*, 1978). This is a colorimetric method where the absorbance of a blue colour, which develops when ammonium reacts with phenol nitroprusside and an alkaline hypochlorite is measured at 635 nm. Total nitrogen was determined by the alkaline persulphate method. Total phosphorus was determined colorimetrically after digestion of unfiltered samples with 8% freshly prepared potassium persulphate. In this process polyphosphates and some organic phosphorus compounds are oxidised to orthophosphate. The intensity of the blue colour that developed in the reaction between phosphates and molybdate antimony in the presence of ascorbic acid was measured at 882 nm.

3.2.1 Data analysis

Data manipulations and statistical computations were performed using STATISTICA 7 computer software package. The Pearson correlation test was applied to ascertain significant correlations between variables. This was tested on the whole data set collected over 23 months at stations 1 to 3. Pearson correlation tests were also carried out between physical and chemical data collected from the deep station (station 1) and two shallow stations (mean of station 2 and 3). Kruskal-Wallis tests ($p < 0.05$) were used to test for differences in physical and chemical characteristics between the two years (2003 and 2004), between the clear and the turbid state, between the deep and the shallow zone and among the three sites, for each of the physical and chemical variables.

Multivariate descriptive analyses were processed employing Principal Components and Classification Analysis (PCCA) to physical and chemical data through covariance matrix with data transformation by ranging using the programme STATISTICA 7. All twelve variables collected on each date per site were used. The euphotic depth (Z_{eu}), where the light intensity is 1% of the surface intensity, was estimated from the Secchi depth (Z_{SD}) using an empirical relation that links the attenuation of light with depth according to a

Beer-Lambert law and the assumption that, at the Secchi depth, the light intensity is 16% of the incident light intensity (Lemmin 1995): $Z_{eu} = 2.5\ Z_{SD}$.

3.3 RESULTS

3.3.1 Spatial and temporal variation of physical variables

The clear and turbid states were easily distinguished with respect to physical and chemical characteristics. Turbidity, conductivity, total nitrogen, nitrates and TN:TP ratio were significantly higher ($p < 0.05$) while temperature, Secchi depth transparency, total phosphorus and orthophosphate were significantly lower ($p < 0.05$) in the turbid than in the clear state (Table 3.1). The clear state had higher phosphorus and transparency levels while the turbid state had higher nitrogen and lower transparency levels (Table 3.2).

The temporal variation in physical and chemical characteristics in the euphotic zone followed comparable patterns at the three stations (Figures 3.2 – 3.5). The mean euphotic zone ranged from 2.4 m to 5.8 m and was deepest during the clear state, up to 6 m at Station 1. Conductivity (Figure 3.2a) was significantly higher in 2004 than in 2003 ($p < 0.05$, Table 3.1). In 2003 the average conductivity in the lake ranged between 327 and 420 µS cm^{-1} with an average of 383 µS cm^{-1}. It increased as the lake level dropped, reaching a maximum of 420 µS cm^{-1} in December when the lake had reached the lowest level recorded during this study. In 2004 conductivity in the lake ranged between 404 and 502 µS cm^{-1} with an average of 446 µS cm^{-1}. In both 2003 and 2004 conductivity was lowest between February and May, when the lake was filling as it received river inflows. During this period in 2004 there was a marked decrease in conductivity, but this had not been apparent during the previous year. Generally the patterns exhibited in both years were similar, indicating that precipitation (lake level fluctuation) is the major influence on ionic concentration in the lake. Differences in conductivity levels between the deep and shallow zone ($p > 0.05$, Table 3.1) and between the three sites ($p > 0.05$, Table 3.3) were not statistically significant.

The average turbidity (Figure 3.2b) in the lake ranged between 3.2 and 28 NTU and showed a trend linked to increases in algal biomass. In 2003 the highest average turbidity of 9.7 NTU was recorded in February, after which it decreased, reached a lowest average level of 2.5 NTU in May, and then fluctuated between 3.2 and 4.9 NTU between June and December 2003 (Figure 3.2b). Turbidity was significantly higher in 2004 than in 2003 ($p < 0.05$, Table 3.1). The turbidity in 2004 ranged between 3.9 and 28 NTU. A marked increase in turbidity occurred between May and November 2004 when it increased from 6.9 NTU to reach the highest level of 28 NTU. Peaks in turbidity of 9.7, 15.8 and 28 were recorded in February 2003, March 2004 and November 2004 respectively. Differences in turbidity between the deep and shallow zone ($p > 0.05$, Table 3.1) and between the three stations ($p > 0.05$, Table 3.3) were not statistically significant.

Table 3.1 **Kruskal-Wallis test for differences in physical and chemical characteristics during the clear and turbid states, between the years 2003 and 2004 and between the deep and shallow zones. These data are for the period February 2003 to December 2004. Figures marked with * are significant at $p < 0.05$ while the rest are not.**

Variable	Clear vs turbid state	2003 vs 2004	Deep vs shallow zone
Conductivity	H = 29.972 p = 0.000*	H = 10.858 p = 0.001*	H = 0.020 p = 0.886
Turbidity	H = 19.449 p = 0.000*	H = 8.727 p = 0.003*	H = 2.825 p = 0.093
Secchi disc transparency	H = 11.039 p = 0.001*	H = 3.802 p = 0.051*	H = 6.545 p = 0.011*
pH	H = 0.630 p = 0.427	H = 0.853 p = 0.356	H = 0.267 p = 0.606
Dissolved oxygen	H = 1.869 p = 0.172	H = 1.752 p = 0.186	H = 0.792 p = 0.374
Temperature	H = 8.256 p = 0.004*	H = 0.009 p= 0.926	H = 0.302 p = 0.583
Nitrates	H = 38.269 p = 0.000*	H = 10.242 p = 0.001*	H = 0.004 p = 0.947
Ammonium	H = 2.521 p = 0.112	H = 3.412 p = 0.065	H = 0.626 p = 0.429
Total nitrogen	H = 10.816 p = 0.001*	H = 1.833 p = 0.176	H = 0.148 p = 0.701
Orthophosphate	H = 6.098 p = 0.014*	H = 2.004 p = 0.157	H = 1.044 p = 0.307
Total phosphorus	H = 6.098 p = 0.014*	H = 1.671 p = 0.196	H = 0.365 p = 0.546
TN: TP ratio	H = 21.089 p = 0.000*	H = 6.061 p = 0.014*	H = 0.392 p = 0.531

Secchi disc transparency (Figure 3.2c) ranged between an average of 0.9 and 2.3 m and varied with changes in algal biomass. It was lowest in February 2003 (1.2 m) and in November 2004 (0.9 m), when phytoplankton biomass reached peaks in the lake, and persistently dropped as the algal bloom developed from May to November 2004. In 2003 an increase in Secchi disc transparency occurred between March and April while a uniform level was maintained from April to July.

Table 3.2 **Summary of the pooled water chemistry data for the clear state (February 2003 to April 2004) (n = 45) and the turbid state (May to December 2004) (n = 24) in Lake Chivero from February 2003 to December 2004 (depth-integrated mean of stations 1 to 3 ± sd, range in parentheses).**

Variable	Unit	Clear state	Turbid state
Conductivity	$\mu S\ cm^{-1}$	394 ± 37 (323 – 448)	457 ± 32 (402 – 505)
Turbidity	NTU	5.6 ± 3.3 (2.0 – 17.5)	12.6 ± 8.4 (2.2 – 28.5)
Secchi disc transparency	m	1.9 ± 0.5 (1.0 – 2.8)	1.5 ± 0.4 (0.8 – 2.5)
pH		8.0 ± 0.6 (7.0 - 9.5)	8.3 ± 0.7 (7.12 – 9.7)
Dissolved oxygen	$mg\ l^{-1}$	5.1 ± 2.0 (1.8 – 9.5)	6.2 ± 3.1(3.2 – 16.7)
Temperature	°C	23.5 ± 2.7 (18.0 – 26.8)	21.3 ± 2.9 (17.4 – 26.5)
Nitrates	$mg\ l^{-1}$	0.13 ± 0.20 (0.004 – 0.91)	0.82 ± 0.49 (0.14 – 2.03)
Ammonium	$mg\ l^{-1}$	0.23 ± 0.56 (0.014 – 2.64)	0.35 ± 0.46 (0.01 – 1.43)
Total nitrogen	$mg\ l^{-1}$	7.25 ± 3.92 (1.5 – 17.03)	11.21 ± 4.69 (1.55 – 17.10)
Orthophosphate	$mg\ l^{-1}$	0.51 ± 0.60 (0.003 – 3.26)	0.56 ± 0.15 (0.36 – 0. 84)
Total phosphorus	$mg\ l^{-1}$	1.38 ± 1.27 (0.27 – 7.12)	0.72 ± 0.16 (0.42 – 1.12)
TN:TP ratio		7.47 ± 4.67 (1.1 – 24.5)	16.2 ± 7 (1.5 – 30.2)

A drop in Secchi depth transparency occurred in August to an average of 1.3 m at all stations and it then fluctuated between 1.3 and 2 m from August 2003 to March 2004. Uniform levels of 2.5 m (February to May 2004), 1.5 m (February to September 2004) and 2 m (April to July 2004) were maintained at Stations 1, 2 and 3 respectively (Figure 3.2c).

After this Secchi disc transparency dropped at each station and reached the lowest average level of 0.9 m in November 2004. Mean Secchi disc transparency was significantly lower in 2004 than in 2003 ($p = 0.05$, Table 3.1) and was significantly higher in the deep (i.e. at station 1 Figure 3.2) than in the shallow zone ($p < 0.05$, Table 3.1). Secchi disc transparency was significantly higher at station 1 than at station 3 ($p < 0.05$, Table 3.3) and station 2 ($p < 0.05$, Table 3.3) but was similar at stations 2 and 3 ($p > 0.05$, Table 3.3).

Table 3.3 **Kruskal-Wallis test for differences in physical and chemical characteristics between the three sites. These data are for the period February 2003 to December 2004. Figures marked with * are significant at $p < 0.05$ while the rest are not.**

Variable	Station 1 vs Station 3	Station 1 vs Station 2	Station 3 vs Station 2
Temperature	H = 0.203	H = 0.588	H = 0.131
	p = 0.652	p = 0.455	P = 0.717
pH	H = 0.131	H = 0.609	H = 0.365
	p =0.717	p = 0.435	p = 0.546
Conductivity	H = 0.116	H = 0.004	H = 0.203
	p = 0.733	p = 0.947	p = 0.652
Turbidity	H = 2.332	H = 2.863	H = 0.000
	p = 0.127	p = 0.091	p = 0.991
Dissolved	H =0.753	H = 0.914	H = 0.001
oxygen	p = 0.385	p = 0.339	p = 0.974
Secchi disc	H =5.172	H = 7.984	H = 0.771
transparency	p = 0.023*	p= 0.005*	p = 0.380
Ammonium	H= 0.326	H = 0.165	H = 0.017
	p = 0.568	p = 0.684	p = 0.895
Nitrate	H= 0.002	H = 0.095	H = 0.203
	p = 0.965	p = 0.758	p = 0.652
Orthophosphate	H= 6.495	H = 0.406	H = 4.541
	p = 0.011*	p= 0.524	p = 0.033*
Total	H = 0.008	H = 0.165	H = 0.174
phosphorus	p = 0.930	p = 0.684	p = 0.676
Total nitrogen	H= 0.464	H = 0.088	H = 0.156
	p= 0.496	p = 0.767	p = 0.069
TN: TP ratio	H= 0.479	H = 0.223	H = 0.088
	p= 0.489	p = 0.637	p= 0.762

Changes in pH between February 2003 and December 2004 are shown in Figure 3.3a. The average pH varied between 7 and 9.5. The temporal pattern exhibited during the clear state was different from that exhibited when an algal bloom developed in the lake but there was no statistically significant difference in pH between the two years ($p > 0.05$, Table 3.1). In 2003 the highest pH occurred in February. The pH then decreased from 9.5 in February 2003 to 7 in June 2003. It then increased to 8.3 in July 2003 and thereafter fluctuated between 7.5 and 8.5 until December. Prior to the development of the bloom in 2004 the average pH in the lake was 7.5 but as the bloom developed the pH increased to reach the highest average level of 9.5 in November 2004 (Figure 3.3a). Differences in pH

between the deep and shallow zone ($p > 0.05$, Table 3.1) and between the three sites ($p > 0.05$, Table 3.3) were not statistically significant.

The temporal changes in the concentration of dissolved oxygen between February 2003 and December 2004 are shown in Figure 3.3b. In 2003, when there was no bloom in the lake, the average concentrations ranged between 2.9 and 8.2 mg l^{-1} and showed pulses that are positively correlated with chlorophyll *a* concentrations ($r = 0.45$, $p < 0.05$) and pH ($r = 0.69$, $p < 0.05$). In 2004 the average concentration ranged between 2.1 and 10.9 mg l^{-1}. Following the onset of the bloom in May 2004, dissolved oxygen gradually increased in the lake and reached a maximum average concentration of 10.8 mg l^{-1} in November 2004, after which levels dropped as the algal bloom crashed in December 2004. Dissolved oxygen concentrations recorded prior to the collapse of the bloom were 8.4 mg l^{-1}, 16.7 mg l^{-1} and 12.2 mg l^{-1} at Stations 1, 2 and 3 respectively.

An assessment of temporal changes in dissolved oxygen from February 2003 to February 2004 (Figure 3.3b) showed that lowest dissolved oxygen levels in the lake occurred during the period around January to March (except for February 2003 when cyanobacterial biomass was high). Periods of high dissolved oxygen concentration occurred between July and August, and between October and November. Differences in dissolved oxygen concentration at the three sites ($p > 0.05$, Table 3.3) and between the deep and shallow zone ($p > 0.05$, Table 3.1) were not significant.

The pattern exhibited in 2003 when there was no bloom was different from that in 2004 when a bloom developed in the lake for 6 months, although there was no significant difference in concentrations ($p > 0.05$, Table 3.1).

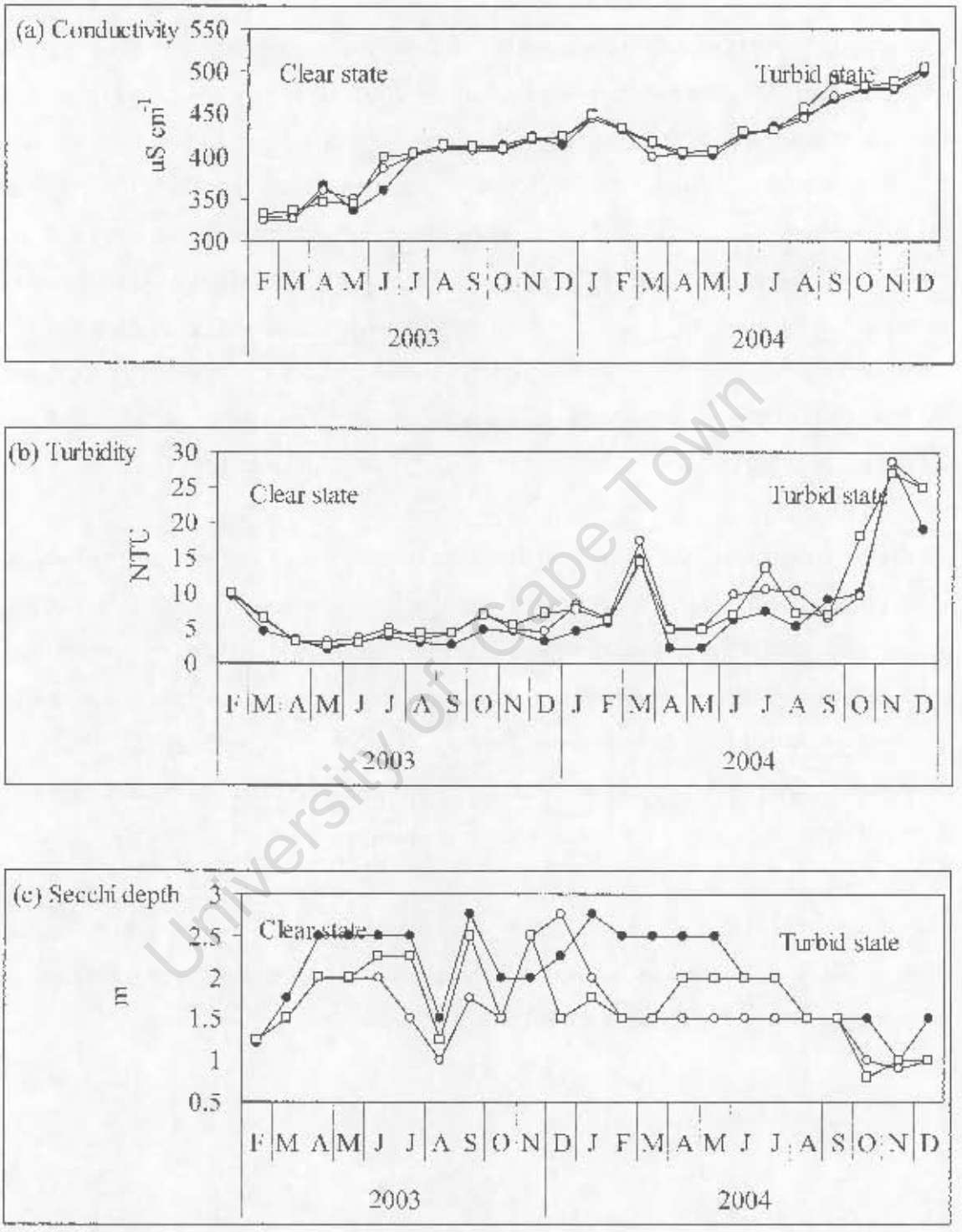


Figure 3.2 **Changes in conductivity (a) (µS cm^{-1}), (b) turbidity (NTU) (c) and Secchi depth (SD) transparency (m) at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •; Station 2 = o and Station 3 = □). Note different Y-axes.**

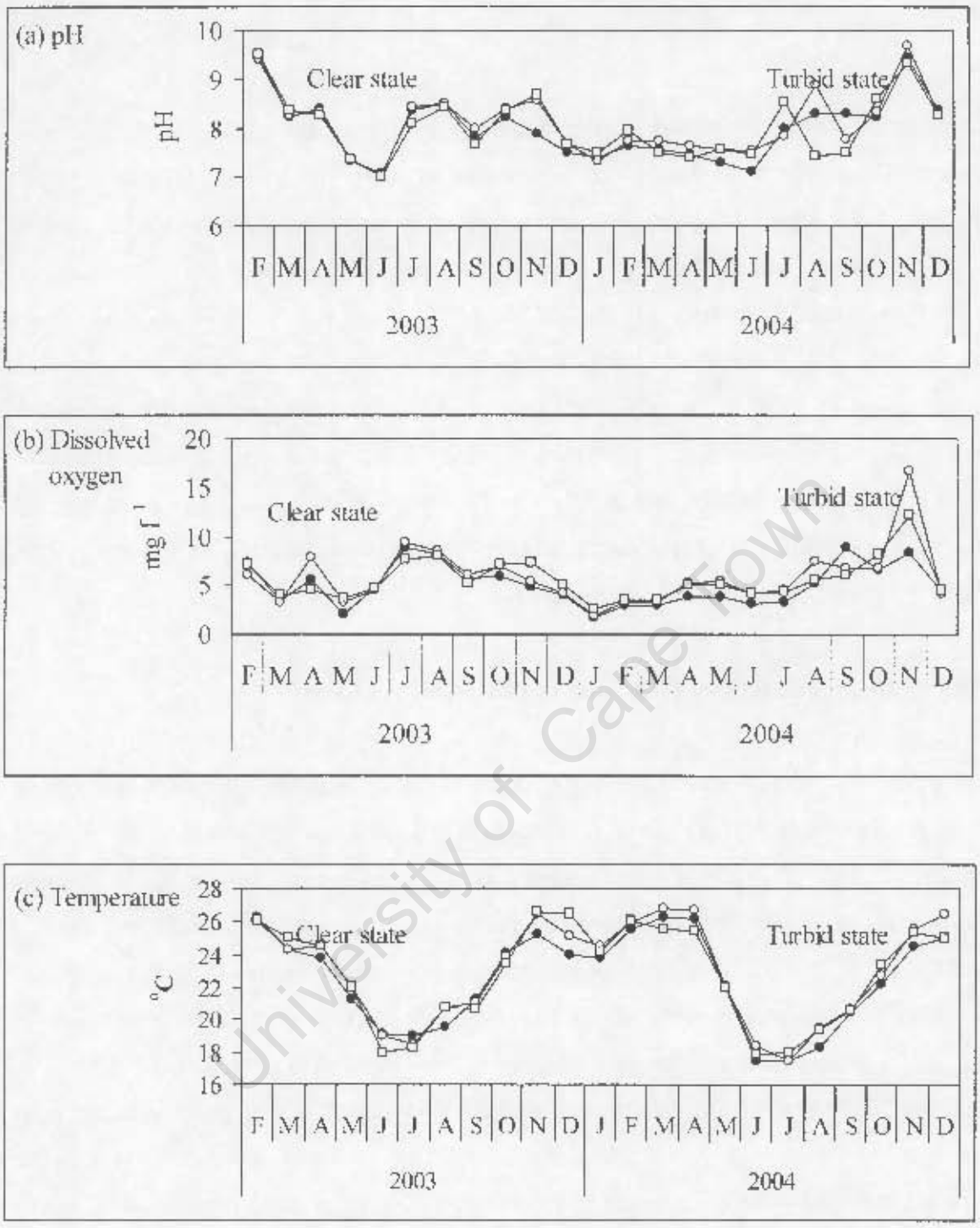


Figure 3.3 **Changes in (a) pH, (b) dissolved oxygen (mg l^{-1}) and (c) water temperature (^{o}C) at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •, Station 2 = ○ and Station 3 = □). Note different Y-axis.**

Changes in water temperature during the study period are shown in Figure 3.3c. The patterns during the two years mirrored each other and displayed a warm monomictic thermal cycle. There was no significant difference in temperature values during the two years ($p > 0.05$, Table 3.1). Water temperature was highest in summer and spring and lowest in winter (averages of 18.6 °C and 17.6 °C in July 2003 and July 2004 respectively). The temperature decreased from 26.1 °C in February 2003 and reached a lowest level of 18.6 °C in July 2003 (Figure 3.3c). The temperature then increased, reaching a maximum of 26.1 °C in November 2003. The same pattern was repeated in 2004 but the lowest temperature in July was 1°C lower in 2003. Differences between the deep and the shallow zone ($p > 0.05$, Table 3.1) or between the three stations ($p > 0.05$, Table 3.3) were not statistically significant.

3.3.2 Spatial and temporal variation of chemical variables

The concentration of nitrate was fairly uniform in the lake in 2003 (Figure 3.4a), with an average concentration of 0.1 mg l^{-1}. An increase to an average concentration of 0.3 mg l^{-1} occurred in June after which levels dropped in July to an average of 0.1 mg l^{-1}. A slight increase occurred in August and thereafter the concentration declined to a lowest average of < 0.1 mg l^{-1} in November. Nitrate concentrations remained low at an average of < 0.1 mg l^{-1} at all stations until March 2004 when the average concentration increased to 0.2 mg l^{-1}. Nitrate concentrations were higher in 2004 (up to 2 mg l^{-1}) than in 2003 (0.1 mg l^{-1}) (Figure 3.4a). There was a significant difference in mean concentration between the two years ($p < 0.05$, Table 3.1). The notable feature in 2004 was the increase in nitrate concentration at all stations from an average concentration of 0.2 mg l^{-1} in March to the highest average concentration of 1.7 mg l^{-1} reached in June (Figure 3.4a) thereafter declining until November. The period of increase in nitrate concentration coincided with a shift from a clear to a turbid state in response to the development of an algal bloom (Chapter 4). The peak of nitrate concentration occurred in June in both years, probably indicating a similar source at that time of the year. Differences between the deep and the shallow zone were not significant ($p > 0.05$, Table 3.1) and the pattern exhibited at all three stations was similar ($p > 0.05$, Table 3.1).

The temporal changes in ammonium concentrations in the lake between February 2003 and December 2004 are shown in Figure 3.4b. The concentration of ammonium was very low (average 0.1 mg l^{-1}) and fairly uniform at all stations throughout the study period except for a high average concentration of 2.1 mg l^{-1} in March 2003 (Figure 3.4b). A slight increase occurred in March 2004 (average 0.4 mg l^{-1}) at station 2 (0.5 mg l^{-1}) and station 3 (0.7 mg l^{-1}) after which levels declined then increased again from August to November 2004 but not above the March 2003 concentration.

Ammonium was generally higher in 2004 than 2003, although the differences were not significant ($p > 0.05$, Table 3.1). In March 2004 peaks were recorded only at stations 2 and 3 while in March 2003 peaks occurred at all the stations (Figure 3.4b). Differences between the deep and shallow zone were not significant ($p > 0.05$, Table 3.1), nor were the patterns exhibited at the three sites ($p > 0.05$, Table 3.3).

Two periods could be distinguished with respect to total nitrogen, total phosphorus and orthophosphate in the lake in 2003: February to July, when the concentrations were high, and August to December, with lower concentrations (Figures 3.4a & 3.5a & b). Total nitrogen concentration fluctuated widely at all stations between 2 and 14.7 mg l^{-1} (Figure 3.4c). There was no significant difference between the mean for all stations for the two years ($p > 0.05$, Table 3.1). In 2003, two periods could be distinguished with respect to total nitrogen concentrations. A higher average concentration of 9.2 mg l^{-1} occurred between February and July with a lower average concentration of 3.9 mg l^{-1} from August to December. In 2004 the pattern was reversed: lower values occurred only between January and February (average concentration of 4.8 mg l^{-1} and 4.2 mg l^{-1} respectively) while for the rest of the period concentrations fluctuated between 8.2 and 14.8 mg l^{-1}. Differences between the deep and shallow zone were not significant ($p > 0.05$, Table 3.1) and the pattern exhibited at the three stations was similar ($p > 0.05$, Table 3.3).

A different pattern was exhibited by orthophosphate concentration during the two years (Figure 3.5a). In 2003 two periods can be distinguished with respect to orthophosphate concentration. A high average concentration of 0.9 mg l^{-1} occurred between February and

June 2003, followed by a decrease in July to a lower average concentration of 0.3 mg l^{-1}, which was maintained until December. This pattern was seen at station 1 and station 2 while at station 3 a relatively lower average concentration of 0.1 mg l^{-1} was maintained throughout, except for a slight increase to 0.3 mg l^{-1} in June.

The highest orthophosphate concentrations occurred in February and May 2003 at station 1 (3.3 mg l^{-1} and 1.4 mg l^{-1} respectively) and station 2 (1.3 mg l^{-1} and 1.9 mg l^{-1} respectively). In 2004 the average concentration in the lake ranged between 0.3 and 0.7 mg l^{-1}. Levels were slightly higher between January (average concentration 0.3 mg l^{-1}) and May (average concentration 0.7 mg l^{-1}) prior to the development of the bloom. The concentration dropped following the onset of the bloom to an average concentration of 0.4 mg l^{-1} between June and September 2004. This was followed by an increase to an average concentration of 0.7 mg l^{-1} in December. The patterns exhibited between station 1 and station 3 and between station 3 and station 2 were significantly different ($p < 0.05$, Table 3.3) but no significant differences were recorded between the deep and the shallow zones ($p > 0.05$, Table 3.1).

The patterns exhibited by total phosphorus concentration were different in 2003 and 2004 (Figure 3.5b). As with orthophosphate, two periods could be distinguished with respect to total phosphorus concentrations in 2003: February to July with a high average concentration of 2.4 mg l^{-1} and large fluctuations (range 0.7- 3.9 mg l^{-1}), followed by a decrease in August after which total phosphorus concentrations remained fairly uniform at 0.7 mg l^{-1} at all stations. In 2004 the total phosphorus concentration was fairly uniform in the lake (Figure 3.5b), with no significant differences between the deep and shallow zone ($p > 0.05$, Table 3.1) or between the three stations ($p > 0.05$, Table 3.3). The average concentration during the year ranged between 0.6 and 0.8 mg l^{-1}.

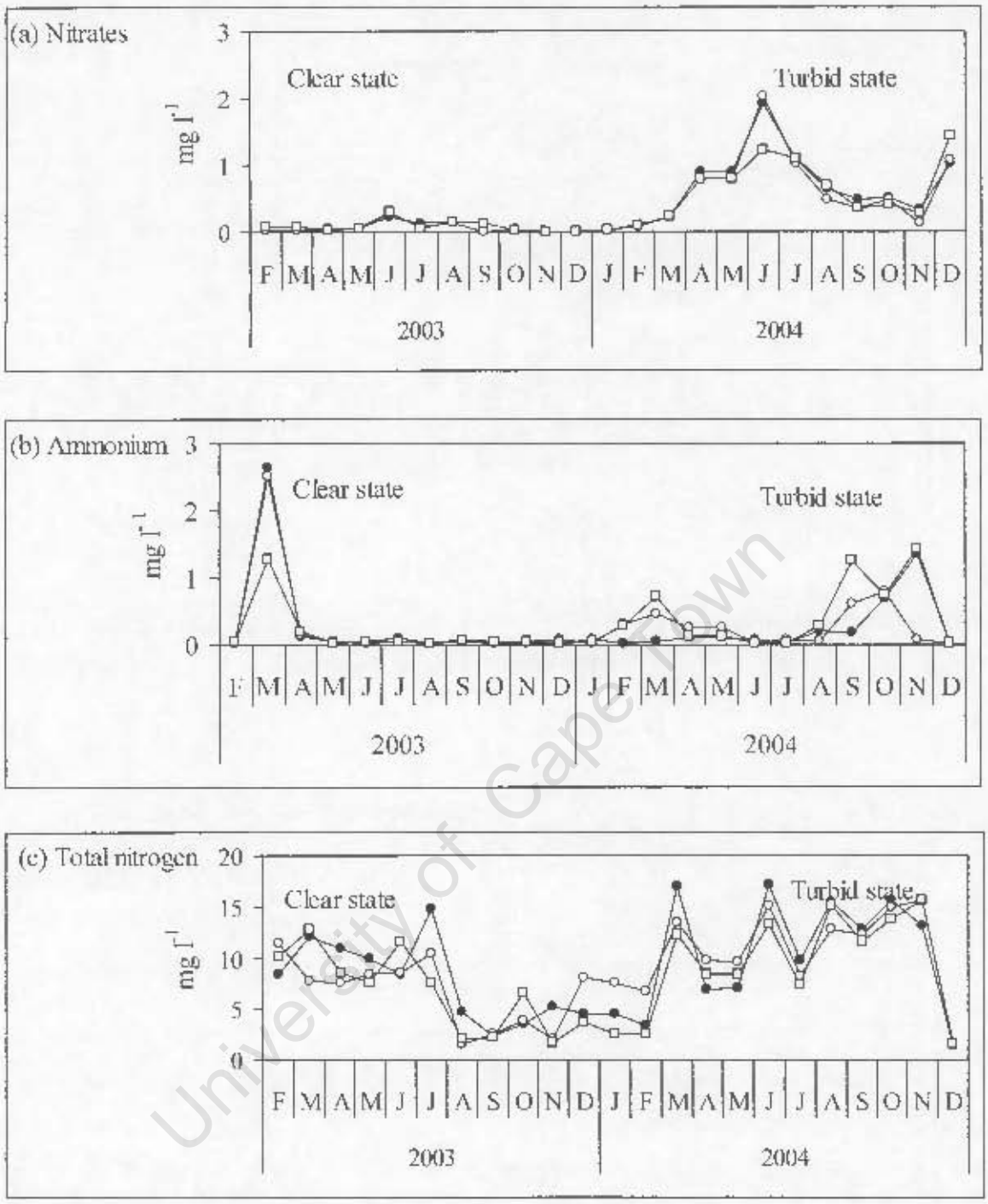


Figure 3.4 **Spatial and temporal variations in the concentrations of (a) nitrate (mg l^{-1}), (b) ammonium (mg l^{-1}) and (c) total nitrogen (TN) (mg l^{-1}) at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •; Station 2 = o; Station 3 = □). Note different Y-axis.**

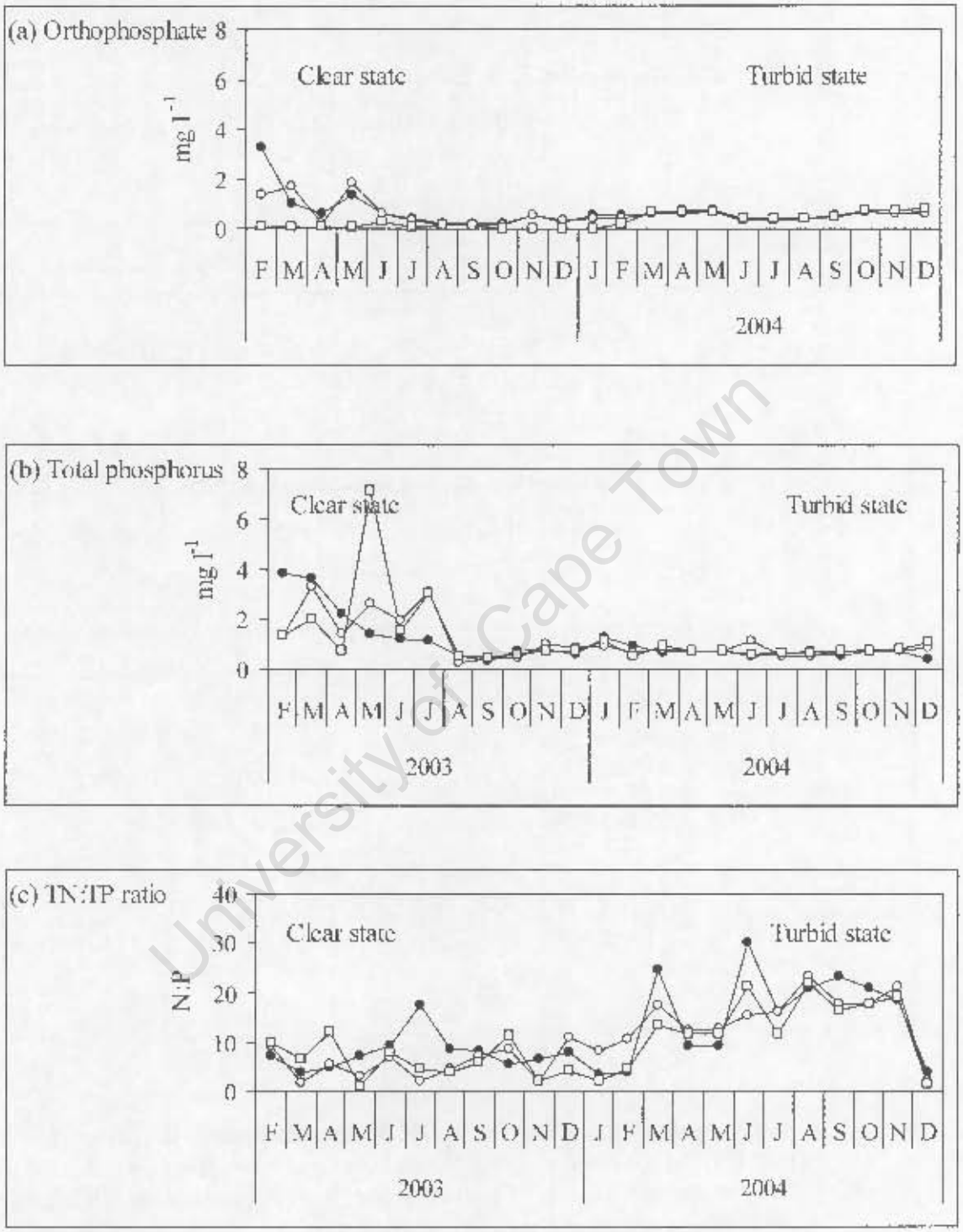


Figure 3.5 **Spatial and temporal variation in the concentration of (a) orthophosphate (mg l^{-1}), (b) total phosphorus (TP) (mg l^{-1}) and (c) TN:TP (N:P) ratio at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •; Station 2 = o; Station 3 = □). Note different Y-axis.**

The TN:TP ratio was below 30 during the whole study period (Figure 3.5c). Peaks of 17.4, 24.5 and 30.3 were recorded at station 1 in June 2003, March 2004 and June 2004 respectively (Figure 3.5c). Otherwise in 2003 the ratios fluctuated between 1.9 and 24 but were below 10 for most of the time. The TN:TP ratio was significantly higher in 2004 than in 2003 ($p < 0.05$, Table 3.1). In 2004 the ratio fell below 10 only in January and February while for the rest of the year it fluctuated between 11 and 22.2. Differences between the deep and the shallow zone ($p > 0.05$, Table 3.1) and between the three sites ($p > 0.05$, Table 3.3) were not significant.

Summaries of the physical and chemical characteristics at the three sampling stations and in the lake are shown in Tables 3.4 and 3.5 respectively. The lake water was rich in nutrients.

Table 3.4 Summary of physical and chemical characteristics (23 months average ± sd) at three sampling sites in Lake Chivero between February 2003 and December 2004. n = 23 for each variable.

Variable	Unit	Station 1	Station 2	Station 3
Conductivity	$\mu S\ cm^{-1}$	391.3 ± 39.6	392.1 ± 38.4	395.4 ± 39.0
Turbidity	NTU	4.9 ± 3.3	6.2 ± 3.8	6.1 ± 3.1
Secchi disc transparency	m	2.2 ± 0.5	1.8 ± 0.5	1.8 ± 0.4
pH		8 ± 0.6	8.1 ± 0.6	8.1±0.6
Dissolved oxygen	$mg\ l^{-1}$	4.9 ± 2.2	5.3 ± 2.3	5.3 ± 1.8
Temperature	^{o}C	23.1 ± 2.6	23.4 ± 2.7	23.4 ± 3.0
Nitrates	$mg\ l^{-1}$	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Ammonium	$mg\ l^{-1}$	0.2 ± 0.7	0.3 ± 0.7	0.2 ± 0.4
Total nitrogen	$mg\ l^{-1}$	7.9 ± 4.6	7.1 ± 3.5	6.5 ± 4.3
Orthophosphate	$mg\ l^{-1}$	0.7 ± 0.8	0.6 ± 0.6	0.1 ± 0.2
Total phosphorus	$mg\ l^{-1}$	1.4 ± 1.1	1.4 ± 1.0	1.5 ± 1.8
TN: TP ratio		8.4 ± 5.8	6.9 ± 4.3	6.3 ± 4.0

Table 3.5 **Summary of the pooled water chemistry data for the euphotic zone of Lake Chivero from February 2003 to December 2004. n = 69 for each variable.**

Variable	Unit	Minimum	Maximum	Mean ± sd
Conductivity	$\mu S\ cm^{-1}$	323	505	416 ± 46
Turbidity	NTU	2	28.5	8.1 ± 6.48
Secchi disc transparency	m	0.8	2.8	1.8 ± 0.52
pH		7.0	9.7	8.0 ± 0.64
Dissolved oxygen	$mg\ l^{-1}$	1.8	16.7	5.5 ± 2.46
Temperature	^{o}C	17.4	26.8	22.8 ± 2.95
Nitrates	$mg\ l^{-1}$	Trace	2.03	0.4 ± 0.47
Ammonium	$mg\ l^{-1}$	0.01	2.6	0.3 ± 0.52
Total nitrogen	$mg\ l^{-1}$	1.5	17.1	8.6 ± 4.58
Orthophosphate	$mg\ l^{-1}$	Trace	3.3	0.53 ± 0.49
Total phosphorus	$mg\ l^{-1}$	0.3	7.1	1.2 ± 1.1
TN:TP ratio		1.1	30.2	10.5 ± 6.93

3.3.3 Relationships among variables

Pearson correlation coefficients (r) for the physical and chemical variables measured in Lake Chivero between February 2003 and December 2004 are shown in Table 3.6. Conductivity significantly correlated negatively with Secchi disc transparency, orthophosphate and total phosphorus and positively with turbidity, dissolved oxygen, nitrates and TN:TP ratio. The pH values were significantly correlated positively with turbidity and dissolved oxygen and negatively with Secchi disc transparency. Turbidity correlated positively with dissolved oxygen, nitrates and TN:TP ratio and negatively with Secchi disc transparency. Nitrates correlated positively with total nitrogen and TN:TP ratio and negatively with total phosphorus. Ammonium correlated positively with total nitrogen. Total nitrogen correlated positively with TN:TP ratio. Orthophosphate correlated positively with total phosphorus. Secchi disc transparency correlated positively with TN:TP ratio. These correlations show that the clear state is a metabolic low oxygen hyper-hyereutrophic state.

3.3.4 Principal Component Analysis

The results from a Principal Components and Classification Analysis of physical and chemical variables explained 70.6% of the variance in environmental data in four axes. The four PCCA axes accounted for 27.5%, 18.5%, 14.6% and 10% of the variance respectively. Figure 3.6 shows the results of the PCCA as an ordination biplot of the variables. Seven variables contributed more than the rest of the variables ($r > 0.5$) to ordination as shown on Factor 1: pH, conductivity, turbidity, dissolved oxygen, Secchi depth, total nitrogen and TN:TP ratio (Table 3.7). For Factor 2 temperature, pH, nitrates and total phosphorus were the most important variables ($r > 0.5$, Table 3.7). The biplot of the sampling dates is shown in Figure 3.7 with most of the sampling dates situated close within the upper two quadrants. Closeness of sites indicates limited spatial differences among the three sites. Factor 1 (27.5%) clearly separated the turbid state (numbers 17-23) from the clear state (numbers 1–16). So PCCA Factor 1 represented the two states, whereas Factor 2 (19.9%) separated the samples according to their temporal variation. At the positive side of Factor 2 are samples collected during the turbid state associated with high nitrate, total nitrogen and TN:TP ratio (Figure 3.6) confirming the link of cyanobacterial dominance to the high nitrate period (Chapter 4). At the negative side of Factor 2 are located samples collected during the early part of the clear state (numbers 1-4) associated with orthophosphate and total phosphorus (Figure 3.6), which represented their highest values at the beginning of the clear state.

Table 3.6 **Pearson correlation coefficients (r) for the physical and chemical variables measured in Lake Chivero between February 2003 and December 2004. Abbreviations and units: EC = electrical conductivity (µS cm^{-1}), TURB = turbidity (NTU), DO = dissolved oxygen (mg l^{-1}), SECC = Secchi disk transparency (m), $NH4^+$ = ammonium (mg l^{-1}), NO_3 = nitrate (mg l^{-1}), SRP = orthophosphate (mg l^{-1}), TP = total phosphorous (mg l^{-1}), TN = total nitrogen (mg l^{-1}) and N:P = TN:TP ratio. n = 69 for each variable. (* $p < 0.05$, ** $p < 0.01$)**

	pH	EC	TURB	DO	SECC	$NH4^+$	NO_3	SRP	TP	TN
EC	0.046									
TURB	0.502**	0.565**								
DO	0.696**	0.237*	0.364**							
SECC	-0.538**	-0.319**	-0.602**	-0.449**						
NH_4^+	0.178	-0.062	0.204	-0.299*	0.028					
NO_3	-0.217	0.384**	0.257*	-0.154	-0.169	-0.126				
SRP	0.154	-0.253*	0.151	-0.145	-0.063	0.237	0.015			
TP	0.000	-0.534**	-0.181	0.059	-0.098	0.233	-0.254*	0.328**		
TN	0.072	0.033	0.233	-0.206	0.212	0.326**	0.254*	0.190	0.072	
N:P	0.056	0.368**	0.298*	-0.215	0.254*	0.075	0.420**	-0.018	-0.389**	0.795**

(a)

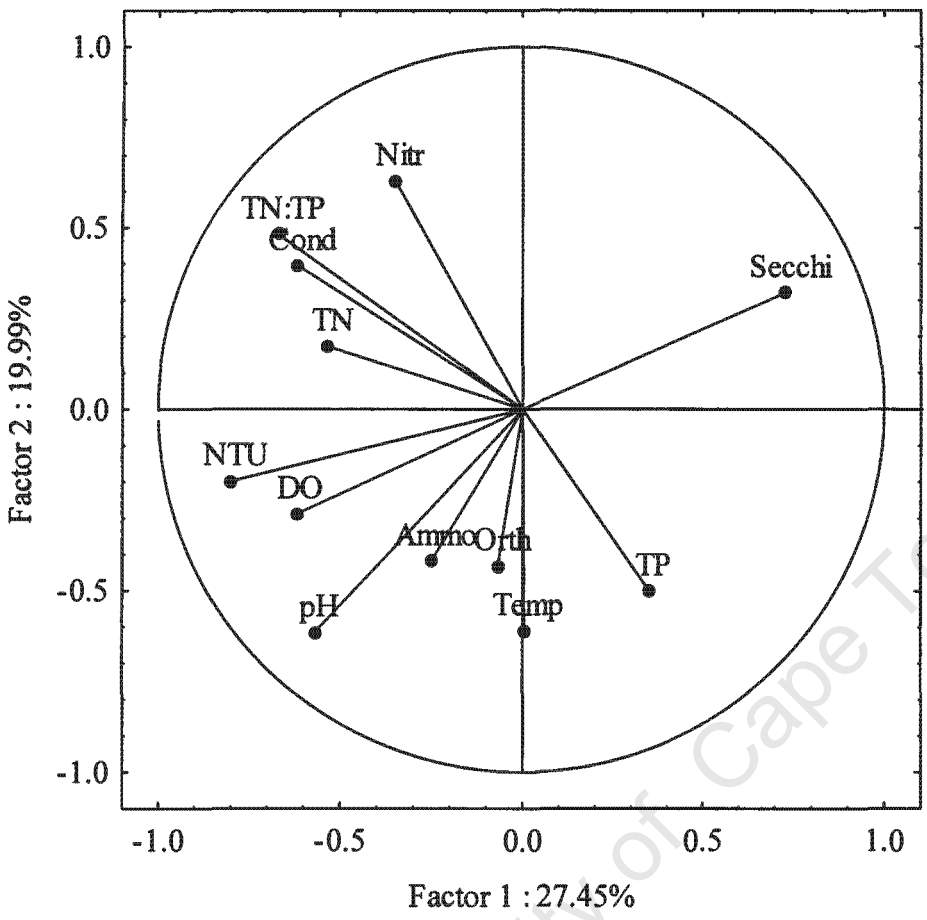


Figure 3.6 **PCA as an ordination biplot of the relationship of the physical and chemical variables. (Nitr = nitrates, Cond = conductivity, TN = Total nitrogen, TP:TP = TN:TP ratio, NTU = turbidity, DO = dissolved oxygen, TP = Total phosphorus, Temp = Temperature, orth = orthophosphate, Ammo = Ammonia, Secchi = Secchi depth)**

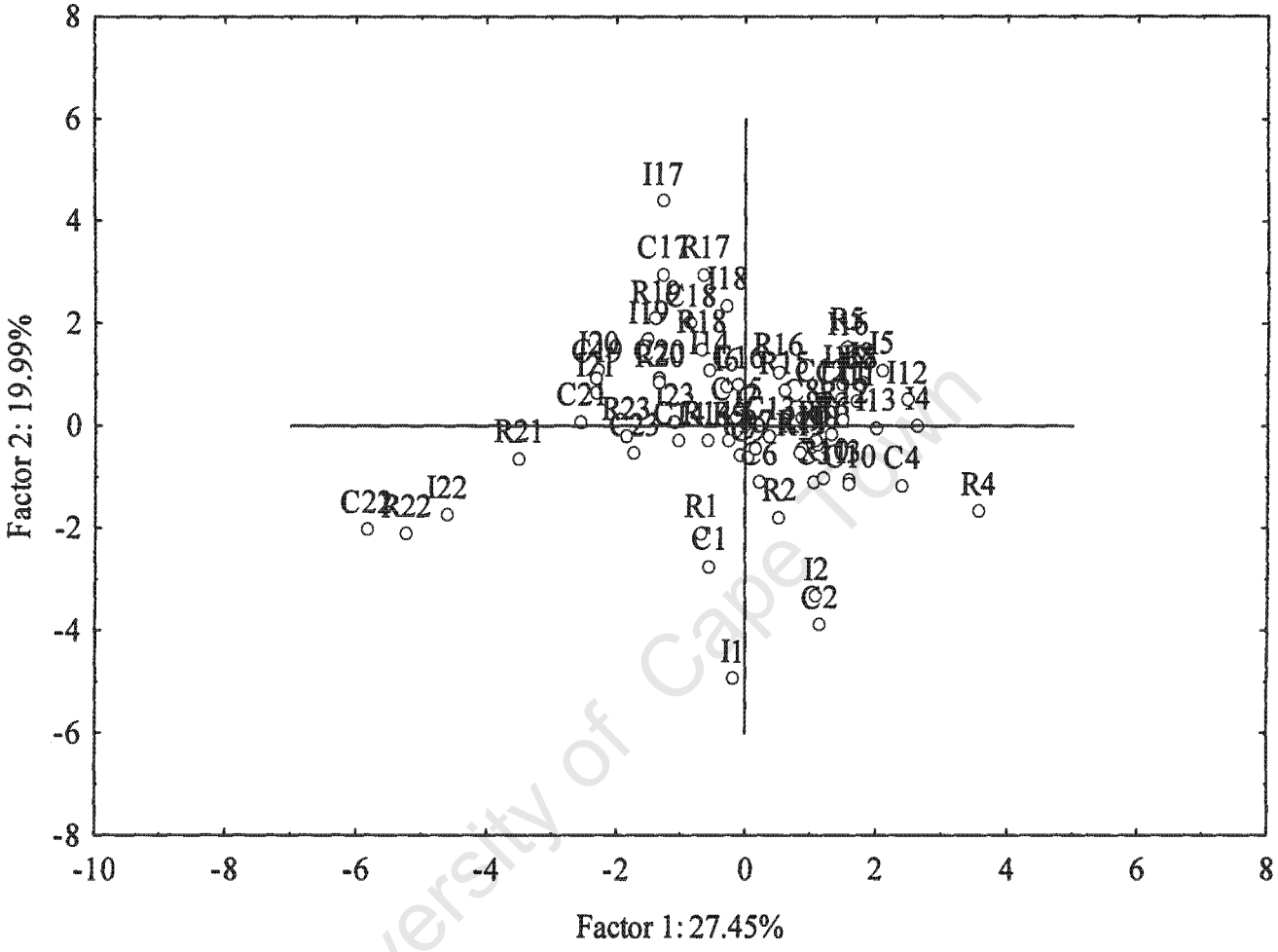


Figure 3.7 **PCA as an ordination biplot of the spatial and temporal variation at the three sites (I = Site 1, C = Site 2 and R = 3; number 1 to 23 denotes sampling months starting from 1 = February 2003….. 23 = December 2004).**

Table 3.7 Physical and chemical variables correlations (n = 69) with principal components

Variable	Factor 1	Factor 2
Temperature	0.004	-0.611
pH	-0.569	-0.614
Conductivity	-0.614	0.394
Turbidity	-0.799	-0.197
Dissolved oxygen	-0.619	-0.288
Secchi depth	0.728	0.320
Ammonium	-0.250	-0.417
Nitrate	-0.349	0.629
Orthophosphate	-0.068	-0.430
Total phosphorus	0.351	-0.50
Total nitrogen	-0.531	0.171
TN:TP ratio	-0.668	0.481
Explained variance	27.5%	19.9%

3.4 DISCUSSION

The study was interesting because it was undertaken during a period when the lake switched between clear and turbid states, although algal blooms had previously been considered to be a permanent feature (Munro 1966, Robarts 1979, Magadza 2003). A striking contrast was observed in the pattern of nitrate dynamics, which could have been a trigger resulting in a shift between a clear and a turbid state. During the clear state the lake was richer in phosphorus while nitrate concentration was lower and relatively uniform except for a slight increase in June 2003. Nitrate concentration was higher during the turbid state, and notably seven times higher in June 2004.

The most striking observation is that when nitrate levels reached 0.8 mg l^{-1} in May 2004, the lake switched from a clear to a turbid state following the development of an algal bloom that lasted for eight months (Chapter 4 & 5). When nitrates dropped to 0.3 mg l^{-1}, the bloom collapsed, and the lake switched back to the clear state. Tezuka (1985) also observed that, in general, dissolved inorganic nitrogen is depleted during cyanobacterial blooms and suggested this as an important factor regulating the appearance of algal blooms in Lake Biwa in Japan. The dynamics of nitrate appeared to be one of the major

factors that triggered the development of an algal bloom in Lake Chivero, and could have a significant role in shifting the lake between the two states.

The clear state persisted between February 2003 and April 2004 although bio-available phosphorus concentration and nitrogen were above levels of 10 μg l^{-1} and 40 μg l^{-1} respectively, which are considered sufficiently high to trigger nuisance cyanobacterial growth in lakes and reservoirs provided that other physical and chemical parameters are conducive to their development (Oliver *et al.* 1998). The role of nitrate as a bloom trigger at a "critical concentration" is supported by the observation that although orthophosphate and total phosphorus concentrations were high between February 2003 and April 2004, the lake remained in a clear state because nitrate levels were probably below the concentration essential for triggering a bloom. The turbid state persisted for eight months when nitrate concentrations were > 0.3 mg l^{-1} and a shift to a clear state occurred when nitrate concentrations fell below this level in December 2004. From these observations it can be concluded that the critical range of nitrate concentration where the lake is likely to shift to a turbid state ranges between 0.3 and 1.7 mg l^{-1} assuming that other relevant conditions are favourable. Heo & Kim (1997) also observed that cyanobacterial blooms occurred in a nitrogen-rich environment in Lake Soyang, Korea. This, as observed in Lake Chivero, is also contrary to the typical observation that cyanobacterial blooms are rare in waters of high nitrogen level.

The dynamics of the TN:TP ratio also provide indications of the possible role of nitrogen availability as a trigger for switching the system from a clear to a turbid state. The TN:TP ratio was >10 (range 11- 22) during the turbid state and <10 during the clear state. According to Grayson *et al.* (1997) aquatic ecosystems with TN:TP ratios < ~ 10 are considered to be N-deficient while those with TN:TP ratios > ~ 20 are considered to be P-limited. Based on this criterion, Lake Chivero can be considered to have been N-limited during the clear state, with regard to cyanobacterial blooms. As such the system remained in a clear state until May 2004 when the TN:TP ratio was > 11. When the TN:TP dropped following a decline in total nitrogen, the system shifted back to a clear state. This supports the observation that when nitrogen and phosphate are not limiting,

the best TN:TP ratio for cyanobacterial growth ranges between 10:1 and 16:1 (Pearson 1990).

According to Smith (1983) TN:TP ratios below 29 promote the dominance of cyanobacteria while lower ratios provide conditions suitable for the development of nitrogen-fixing cyanobacteria (Ashton 1979, 1981). The TN:TP ratio was below 25 during the present study. Based on Ashton's work the phytoplankton assemblage in Lake Chivero should be dominated by nitrogen-fixing cyanobacteria. This aspect is discussed in Chapter 4 with observations made on the development of algal assemblages.

High air temperatures and high incidence of solar radiation provide an environment conducive to the development of cyanobacterial blooms and thereby a turbid state (Rahman *et al.* 2005, Ballot *et al.* 2005). Growth rates of cyanobacteria are optimal within a range of 25 to 35°C (Ganf 1974, Robarts & Zohary 1987). Temperatures measured during the present study, except between May and August, were near the optimal range for cyanobacteria and fell within the range 14 to 25 °C, as was previously recorded by Thornton & Nduku (1982). Temperature however, seemed not have been a major factor since it was lower during the turbid than the clear state and the switch to the turbid state occurred when both water and air temperatures were lowest.

As a hyper-eutrophic lake, Lake Chivero was characterized by high conductivity, nitrogen and phosphorus levels and pH >8, which should favour the dominance of cyanobacteria (Shapiro 1990, Paerl 1996). The shift to a clear state was not caused by a decline in nutrient levels in the lake. Orthophosphate concentration has previously been reported to range from 0.3 to 0.5 mg l^{-1} (Magadza 1997) and more recently from 0.9 to 1.2 mg l^{-1} (Rommens *et al.* 2003). During the present study a higher maximum concentration of 1.9 mg l^{-1} was recorded, indicating further enrichment.

Hyper-eutrophic systems are characterised by unbalanced nutrient and oxygen regimes (Barica 1980). Seasonal nutrient and dissolved oxygen cycles exhibit extreme fluctuations with high amplitudes of their maxima and minima and pronounced

oscillations (Barica 1974) when a non-steady state is destroyed (Barica 1980). Extreme fluctuations were apparent in the temporal development of nutrients and oxygen.

Nutrients were not exhausted during the vegetative period as expected in hyper-eutrophic systems (Leentvar 1980) but periods of high and low nutrients linked to algal uptake or external and internal loading were exhibited. The temporal change in dissolved oxygen showed that presently there are both periods of low and high dissolved oxygen levels in the lake. Dissolved oxygen levels reached super-saturation levels during the turbid state, while periods of low dissolved oxygen levels occurred when algal biomasses were low during the clear state. The epilimnion was not permanently super-saturated with oxygen as previously observed (Marshall & Falconer (1973), however.

The period of low dissolved oxygen levels occurred around January – March, except for February 2003 when cyanobacterial biomass was high (Chapter 4). Magadza (1997) noted a trend of increasingly severe episodes of oxygen deficiency in Lake Chivero from 1988 to 1996. From the information now available it is apparent that high levels of dissolved oxygen occur as a result of high photosynthetic rates when algal biomasses are large. As the algae die off around February to March when summer is coming to an end, dissolved oxygen declines in the lake.

Die-offs of massive algal populations in hyper-eutrophic lakes can result in oxygen depletion (Barica 1981). It was observed during the present study that oxygen regimes were unbalanced and the implications for a fish-kill of *Oreochromis niloticus* with other parameters previously been implicated in fish deaths in the lake (Burke & Thornton 1982, Moyo 1997) ammonia, pH and temperature is discussed in Appendix 1.

Most hyper-eutrophic systems have high bioturbidity due to dense algal growth. During the present study bioturbidity was low for most of the time except when an algal bloom developed (Chapter 4). Dense algal growth during the algal bloom period significantly reduced transparency. Robarts (1979) previously recorded Secchi disc transparency ranging from 0.6 - 1.6 m in Lake Chivero. Except during the turbid state, the

transparency recorded during the clear state was higher than levels previously recorded by Robarts. Lower levels of transparency recorded by Robarts coincided with the period when algal blooms were reported to be permanent in the lake. Ndebele (2003) recorded an average transparency of 1.1 m between March and April 2003, which falls in the range recorded during the same season in the present study. The implications of high bioturbidity on competitive exclusion of non-buoyant algal taxa are discussed in Chapter 5.

Nitrogen and phosphorus dynamics during the present study did not exactly follow the pattern described by Thornton & Nduku (1982). They observed that nitrogen and phosphorus exhibited a similar pattern with their maxima in spring and summer and their minima during winter. As observed by Thornton & Nduku (1982) orthophosphate concentrations were highest in summer and spring, the period of river inflows, which could be the source. Minimal nitrate concentrations did not occur in winter; indeed concentrations were highest during this period. The sediments may have been the source of nutrients during that period since that is when turnover occurred (Marshall & Falconer 1973) and nutrients could have been released into the hypolimnion. Maxima of ammonium occurred in March, and between September to December in 2004, a deviation from late winter months (June-August) maxima previously observed by Thornton & Nduku (1982). Deviations from the observations of Thornton & Nduku (1982) might be an indication that natural fluctuations may be lost or masked in human-impacted ecosystems.

From the temperature profile data for 2004 (Chapter 5) turnover occurred in June, since the lake was isothermal. The high nitrate levels in June 2004, might therefore be attributed to nitrification of ammonia brought into circulation from deeper waters. It is not clear why nitrates were low in June 2003, nor why the spring nitrate maxima did not occur during this study. Since the likely source of nutrients in spring is runoff, however, it is likely that early rains did not bring in much in the way of nutrients. Low levels for all nutrients for the rest of the year may be attributable to consumption by phytoplankton (Chapter 4 & 5).

Thornton & Nduku (1982) suggested that orthophosphate distribution in Lake Chivero influenced algal growth patterns in 1969 and 1979. This suggestion was based on the observation that chlorophyll *a* maxima coincided with orthophosphate peaks. This aspect is discussed in detail in Chapter 4. However it was noted that during the present study chlorophyll *a* maxima were not linked to peaks of orthophosphate, possibly indicating changes in the pattern of nutrient dynamics, which may have implications for phytoplankton community dynamics.

Most tropical lakes have pronounced seasonal fluctuations that usually correspond to variations in rainfall, runoff or vertical mixing within the lake (Melack 1996). A clear seasonal pattern in 2004 could have been masked by continuous entry of nutrient-rich sewage effluents through the rivers. Conductivity showed variations linked to changes in lake level as observed by Falconer (1973) although the two years were not comparable especially with respect to conductivity, which indicates instability and unpredictability.

Due to the shallow profile and large littoral areas, the lake is susceptible to continuous mixing by wind (Thornton 1987), as is Zeekoevlei (Harding 1996). Lack of spatial differences among the sites and between the shallow and deep zone is an indication of through mixing by wind of the waters of Lake Chivero.

3.4.1 Comparisons with Zeekoevlei and Hartbeespoort dam

Two hyper-eutrophic systems to which the physical and chemical limnology of Lake Chivero can be compared with are Hartbeespoort dam and Zeekoevlei in South Africa. All three systems have high nutrient levels due to the impact of progressive urbanization and development in the catchment. Although the three systems are all alkaline, pH is significantly higher all year round in Zeekoevlei and Harbeespoort dam (Figure 3.8) and this can be attributed to the higher algal biomasses and photosynthetic rates in these systems. The average chlorophyll *a* concentration in Zeekoevlei is 233 $\mu g\ l^{-1}$ while in Hartbeesport dam levels are even higher (5 $\mu g\ l^{-1}$ to 1 000 $\mu g\ l^{-1}$) (NIWR 1985). In Lake Chivero the maximum chlorophyll *a* concentration of 92.8 $\mu g\ l^{-1}$ attained during the

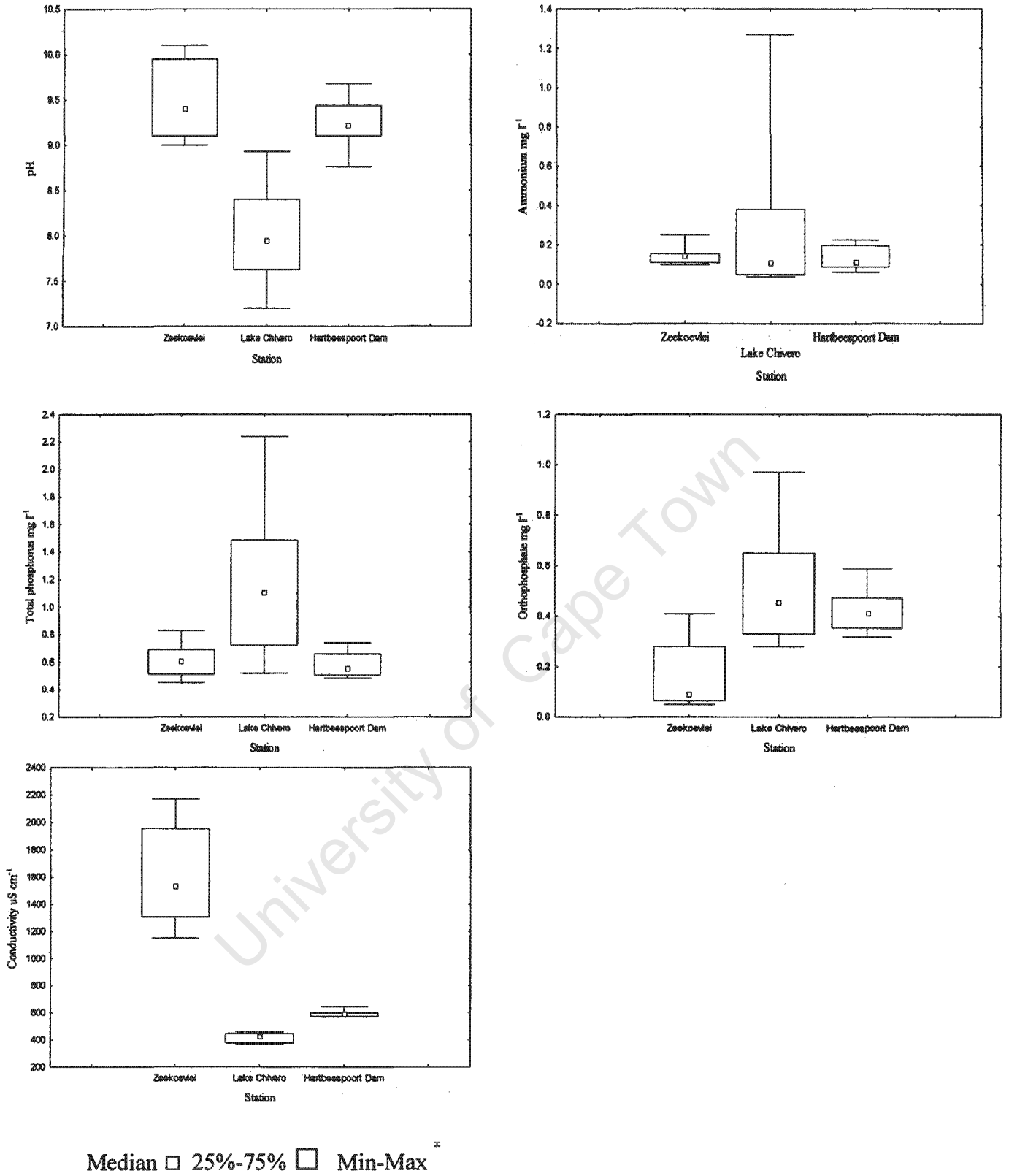


Figure 3.8 Comparison of selected physical and chemical conditions in Lake Chivero, Zeekoevlei[1] and Hartbeespoort dam[1]. ([1]Source: Kira 1994).

bloom period at one of the stations (Chapter 5), is far lower than levels recorded in the other two systems.

Due to the higher algal biomasses and the resultant higher photosynthetic rates, dissolved oxygen is comparatively higher in Zeekoevlei and Hartbeespoort dam than in Lake Chivero. In case of Zeekoevlei, dissolved oxygen was above 9 mg l^{-1} all year round between 1981-1990, whereas in Lake Chivero such a level was only attained in November 2004 when high algal biomasses had built-up in the lake.

Zeekoevlei is reported to have year-round non-limiting concentrations of nitrogen and phosphorus with mean annual values of 3.6 and 0.55 mg l^{-1} respectively (Kira 1994). Comparison of nitrogen and phosphorus concentrations in the three systems showed that total phosphorus is significantly higher in Lake Chivero than in the other two (Figure 3.8). Ammonium and orthophosphate concentrations are similar in Lake Chivero and Hartbeespoort dam while total nitrogen and orthophosphate are significantly higher in Lake Chivero than in Zeekoevlei (Figure 3.8). Generally with respect to nutrients Lake Chivero seems to have higher levels although the three systems are all hyper-eutrophic. Since nitrogen and phosphorus are the key regulatory nutrients for algae it can be hypothesized that the phytoplankton communities in these systems should be similar. Light is also a major factor influencing algal dynamics. In Zeekoevlei Secchi depth transparency is less that 0.5 m which is explained partly by high algal biomass and the shallowness of the system. In Lake Chivero higher levels of up to 2 m were recorded during this study while in Hartbeespoort dam it ranges between 2.9 – 3 m. Being a coastal relict estuary, Zeekoevlei has higher conductivity than Lake Chivero (Figure 3.8) and is also much shallower with a mean depth of 1.9 m and a maximum depth of 5.2 m (Kira 1994).

CHAPTER 4

PHYTOPLANKTON DYNAMICS

4.1 INTRODUCTION

Phytoplankton dynamics in Lake Chivero have been influenced by the trophic status of the lake, mainly continuous enrichment caused by high nutrient loading. The lake has consequently been permanently turbid due to the predominance of blooms of *M. aeruginosa*, which have been reported to be a permanent feature in the lake (Magadza 2003).

At the commencement of the study Lake Chivero was expected to be permanently turbid. It is an interesting coincidence, however, that during this study the system, which is renowned for being permanently turbid due to high phytoplankton biomasses, switched to a clear state. The thrust of this study was therefore to determine whether the shift to clear water was linked to changes in phytoplankton assemblages responding to changes in the physical and chemical environment. It was hypothesized that the lake was presently in a "clear metastable state" but a slight disturbance in internal nutrient balance would shift it to a turbid state that would be maintained until the nutrient balance dropped below a critical level. Metastability means that an ecological system can maintain itself over a limited range of conditions but may eventually undergo significant alterations if constraints continue to change (O'Neill *et al.* 1989).

The focus was to describe the clear state and to compare it to the turbid state. I also intended to establish whether the clear state would persist or whether it represented a single event that would not repeat, and whether a steady-state phytoplankton assemblage, as defined in Chapter 1 Section 1.5, would develop during the two states. The development and stability of the phytoplankton assemblage in Lake Chivero was therefore investigated within this context and under the assumption that the two states support distinct phytoplankton assemblages.

Phytoplankton closely tracks both short and long-term environmental changes in lake ecosystems (Salmaso 2002). Comparison of investigations carried out in recent years with those made many years ago may provide indications of change. In order to determine possible indications of change of the phytoplankton assemblage current findings were compared to a previous study by Falconer (1973) and to qualitative samples collected in 1960 (15-06-1960) and in 1983 (18-09-1983) obtained from Lund University, Sweden. Due to paucity of long-term historical information on phytoplankton dynamics in Lake Chivero, this only provides a "snapshot" comparison.

Functional classification and prediction of succession of freshwater phytoplankton (Reynolds *et al.* 2002) hypothesizes that succession leads to a climax in the pelagic environment similar to that seen in terrestrial vegetation (Grime 1979). Consequently phytoplankton assemblages or associations *sensu* Reynolds *et al.* (2002) are expected to achieve a steady state or reach equilibrium (Chapter 1 Section 1.5) at some stage of their seasonal succession. During this study I wanted to establish whether phytoplankton assemblages or associations *sensu* Reynolds *et al.* (2002) achieved steady state or reached equilibrium during the clear and turbid states and whether functional groups could be a valid and useful concept to describe species succession in a hyper-eutrophic lake. Reynolds (1997) recognises three classes of planktonic autotrophs, namely colonists (C), stress (S) and disturbance tolerators and ruderals (R). The occurrence of these groups reflects the energy availability, resource availability and degree of disturbance within a system. I hypothesized that the physical and chemical environment would be different during the clear and the turbid state (Chapter 3) and tested whether this would be reflected in the phytoplankton associations.

A wide range of single-parameter models has been proposed to explain the dominance of cyanobacteria in aquatic ecosystems (Chapter 1, Section 1.3). During this study I wanted to determine the applicability of the proposed theories on cyanobacteria dominance in explaining the patterns observed on species diversity, abundance and changes and whether the patterns observed conform to expectations in a hyper-eutrophic lake.

I expected to answer the following questions:

(i) has the phytoplankton assemblage in Lake Chivero changed since 1960, 1982, 1983 in terms of species composition, species diversity and periodicity?

(ii) what factors influence phytoplankton dynamics in the lake?

(iii) did distinct steady-state phytoplankton assemblages occur during the clear and turbid states?

(iv) what ecological conditions occurring during these two periods may lead to steady-state conditions?

(v) did coherent phytoplankton associations that can be ascribed to trait-separated functional groups according to Reynolds *et al.* (2002) occur during the study period?

(vi) is the phytoplankton assemblage similar to two other hyper-eutrophic systems in southern Africa, Zeekoevlei and Hartbeespoort dam?

4.2 MATERIALS AND METHODS

4.2.1 Sampling

From February 2003 to December 2004 phytoplankton and chlorophyll *a* concentration were monitored monthly at three stations (Figure 3.1). Station 1, located near the water intake tower, is approximately 20 m deep, while Stations 2 and 3 were in the shallow zone (maximum depth approximately 5 m). Water samples for phytoplankton and chlorophyll *a* analysis were collected using a Ruttner sampler from the following integrated depth intervals: 0 - 2 m, 2 - 4 m and 4 - 6 m at Station 1; 0 - 2 m and 2 - 3 m at Station 2; and 0 - 2 m and 2 – 3.5 m at Station 3. Phytoplankton biomass and chlorophyll *a* concentration were measured in the samples from each depth and a mean was calculated for the euphotic zone for each site.

Phytoplankton samples for quantitative analysis were immediately preserved with Lugol's iodine at the time of sampling. Phytoplankton samples for qualitative analysis were collected using a 10-µm plankton net and preserved with formalin acidified with Acetic acid.

4.2.2 Quantitative phytoplankton analysis

Utermöhl's sedimentation method was used to identify and enumerate phytoplankton (Utermöhl 1958, Cronberg 1982) using a Nikon inverted phase contrast microscope. Lugol-fixed samples were settled in 2, 5, 10 and 25 ml plankton chambers for 4 to 12 h depending on the size of the chamber. Single cells were counted and filamentous algae measured with an eyepiece graticule, where a grid divided in equal-sized squares covered the whole field of view. The filamentous algae were measured with the square grid in the eyepiece and the total length of filaments per diagonal of the chamber or field of view were calculated. Colony-forming cyanobacteria were mostly counted as colonies and multiplied by the estimated number of cells per colony. Approximately 60 - 100 cells of the dominant species were counted under 400X magnification in order to obtain acceptable reliability in estimations. Cell volumes were estimated from the mean cell dimensions and cellular shape of each species (Rott 1981, Sun & Liu 2003). On each sampling date mean cell dimensions of phytoplankton were calculated for all species on the basis of measurements of 10-20 individuals. For the calculation of fresh weight the specific density of phytoplankton cells was assumed to be 1.0 (Cronberg 1997).

4.2.3 Qualitative investigation of phytoplankton

Qualitative investigations of net samples were done using normal light microscopy at different magnifications. The species were identified and their abundances noted. Cleaned diatoms were mounted in styrax and identified under an Olympus 1X70 microscope using phase contrast. Phytoplankton species and species characteristics in qualitative samples collected in Lake Chivero in 1960 (15-05-1960) and 1983 (18-09-1983) that were obtained from the Department of Limnology, Lund University in Sweden

were analysed and compared to observations made between 2003 and 2006. Although probably not adequate, this enabled a qualitative snapshot comparison of phytoplankton composition in Lake Chivero in 1960, 1983 and between 2003 and 2006 to be made.

The literature referred to for taxonomic identification included Komárek & Cronberg (2001), Cronberg & Komárek (2004) and a series of books by Huber-Pestalozzi (1938-1983). Diatoms preparations showed that two dominant species were *Aulacoseira granulata* and *Cyclotella* sp. The cryptophyte, which was represented mainly by *Cryptomonas* sp., could not be identified to species because this requires Transmission Electron Microscopy and Scanning Electron Microscopy.

4.2.4 Chlorophyll *a* analysis

Chlorophyll *a* concentrations were determined by the acetone extraction method (Golterman *et al.* 1978). Known volume (approximately 500 ml) of water samples were filtered using a vacuum pump through Whatman glass fibre filters of 0.7 μm (Whatman International, Maidstone, UK) within less than six hours of collection. The filters were kept at 0°C before chlorophyll *a* extraction (Wetzel & Liken 2000). Filters were cut into small pieces and put in a 10 ml centrifuge tube where 7 ml of 90% acetone was added. The chlorophyll *a* was extracted in an ultrasonicator for 10 minutes after which extraction was continued for 1 to 3 hours in a refrigerator. After extraction samples were centrifuged at 3000 r.p.m. for 10 minutes in order to eliminate suspended material. Chlorophyll *a* concentration in extracts was measured with a spectrophotometer (Hitachi Spectro-photometer, Kebo Lab, Stockholm, Model 100-40) at 630, 645, 665 and 750 nm. Chlorophyll *a* concentration was calculated according to the following equation:

$$[\text{Chl-a}]\ (\mu g\ l^{-1}) = \{11.6(D665-D750)-1.31(D645-D750)-0.14(D630-D750)\} \times F$$

where F = volume of acetone (ml)/volume of sample (ml)

4.2.5 Data processing and statistical analysis

Shannon diversity and Pielou's evenness were computed using the programme Primer 6 version 6.1.5. Shannon's diversity index, H′, was computed for each sampling date using natural logarithms of species biomass (Magurran 1988) as:

$$H' = -\Sigma (ni/n) \ln (ni/n)$$

where ni is the biomass of the i^{th} species and n is the total biomass of the sample (Shannon & Weaver 1949). Only Shannon diversity was calculated because in Zeekoevlei Harding (1996) established that Shannon and Brillounin's diversity indices produced the same trend and were closely correlated.

Evenness was calculated by the formula:

$E(J') = H'/\ln S'$

where S is the number of species found in the sample (LLoyd & Ghelardi 1964) and the other variables are as in Shannon's diversity index.

The community similarity (stability of species composition) was measured by Bray-Curtis similarity between monthly samples to indicate the change in biomass of individual species from month to month. Bray-Curtis similarity is amenable to ecological data because it is independent of scale of measurement and from joint absences (Clarke 1993, Clarke & Warwick 1994). The biomass of individual species was used to calculate phytoplankton similarity between each pair of successive monthly samples (Teubner *et al.* 2003). The biomass of seven dominant species within a sample (>3% of total biomass) was included in the calculation. Analyses were done using the programme Primer 6 version 6.1.5. Raw data was fourth-root transformed to standardize abundance and to decrease level of variability among species.

I compared two distinct periods for temporal niche separation: the clear state (February 2003 to April 2004) and the turbid state (May 2004 to December 2004) focusing on determining whether steady-state phytoplankton assemblages developed.

Principal Components and Classification Analysis (PCCA) using the programme STATISTICA 7 was carried out on phytoplankton and environmental data from stations 1 to 3 in order to identify any underlying relationships among the dominant phytoplankton species and environmental variables. PCCA, which provides options to compute the principal components (factor axis), was based on correlations among variables. Phytoplankton species biomass data were transformed to natural logarithms prior to analysis in order to obtain roughly multinormal algal data and to reduce skewness in the data (Zar 1984). Seven species that occurred in most of the samples were included in the analysis. Rare species were excluded since their inclusion would weaken correlations and compounds the total analysis (Hill 1979). Twelve environmental variables were used in the analysis: conductivity (cond), turbidity (NTU), Secchi disc transparency (Secc), pH, dissolved oxygen (DO), water temperature (temp), nitrates (nitr), ammonium (ammo), total nitrogen (TN), orthophosphate (orth), total phosphorus (TP) and TN:TP ratio (TN:TP). The sixty-nine sampling dates (twenty-three dates for each of the three stations) were used as cases in PCCA.

Mann-Whitney U test was used to test whether mean values for phytoplankton biomass, chlorophyll *a* concentration, diversity, evenness, and Bray-Curtis similarity were significantly different during the clear and turbid states.

4.3 RESULTS

4.3.1 Phytoplankton species composition

Five dominant taxonomic groups represented the phytoplankton community: cyanophytes, bacillariophytes, chlorophytes, cryptophytes and euglenophytes. Two rare species in the lake were Dinophyceae and Xanthophyceae represented by *Peridinium* sp. and *Pseudostaurastrum* sp. respectively. A total of 64 phytoplankton species were identified (Table 4.1). Chlorophytes comprised 61% (39 species) of the species, cyanophytes 16% (10 species), bacillariophytes 9%, cryptophytes 3% and euglenophytes 7%. The dominant cyanophytes were *M. aeruginosa*, *M. wesenbergii* and *M. novacekii*.

Other cyanophytes occurring at very low densities were *Microcystis botrys*, *Aphanocapsa* cf. *incerta*, *Planktothrix agardhii* and *Woronchnia* sp. *Anabaena* sp. occurred at a very low frequency and was only observed in samples collected in December 2003, January 2004 and after the bloom had collapsed.

4.3.2 Comparison of species composition in 1960, 1983 and 2003-2006

Qualitative comparison of species composition in 1960, 1983 with samples collected between 2003 and 2006 is shown in Table 4.2 as presence (+) and absence (-). *Microcystis botrys, M. wesenbergii* and *Anabaena* sp. were the three dominant cyanophytes in the lake in June 1960. Dense colonies of *M. botrys* with their typical radial morphology were dominant in the sample and *Pseudoanabaena mucicola* colonised the mucilage surrounding the *Microcystis* colonies. *Anabaena* sp. had no heterocysts to enable identification to species level. *M. wesenbergii* also with *P. mucicola* extensively colonising the mucilage surrounding the colonies was dominant in 1983. *Pediastrum simplex* and *P. duplex* occurred during all the years. Other species common in 1960 were *Aulacoseira* (=*Melosira*) *italica*, *Ceratium hirundinella* and *Eudorina elegans*. *Ceratium hirundinella* was not present in 1983 and 2003 to 2006. *Aulacoseira italica* was dominant in 1960 samples. It was not present in 1983 and was not observed in samples collected from 2003 to 2006. Instead *A. granulata* had become abundant in 1983 and 2003-2006, replacing the other species. Species present in all the years included *Cosmarium* sp., *Scenedesmus* sp., *Eudorina elegans*, *Closterium setacium* and *Cyclotella* sp. By comparing the preliminary species list of Robarts *et al.* (1982) and the net samples collected from the three years, the common species in the lake have been *Anabaena* sp., *M. aeruginosa*, *M. wesenbergii*, *M. botrys*, *P. simplex*, *P. duplex* and *A. granulata*. Chlorophytes have generally been the most diverse group. Euglenophytes mainly genera *Trachelomonas* sp. was not recorded in 1960, 1982 and

Table 4.1 Phytoplankton species list for Lake Chivero

Cyanophyceae: blue-green algae
Chroococcales
Aphanocapsa cf. *incerta* (Lemm.) Cronb. and Kom.
Microcystis aeruginosa Kütz.
M. botrys Teil.
M. flos-aquae (Wittr.) Kirchn.
M. novacekii (Kom.) Comp.
M. wesenbergii Kom. in Kondr.
Woronichnia sp.
Oscillatoriales
Planktothrix agardhii (Gom.) Anagn. et. Kom.
Pseudanabaena mucicola (Naum. Et. Hub.-Pest.) Bourr.
Nostocales
Anabaena sp.
Anabaenopsis tanganyike (G.S. West) Wolosz. Et Mill.
Chlorophyceae: green algae
Volvocales
Eudorina sp.
Tetrasporales
Chlamydocapsa sp.
Chlorococcales
Actinastrum sp.
Botryococcus sp.
Coelastrum microporum Näg. in A. Br.
C. reticulatum var. *cubanum* Kom.
C. sphaericum Näg.
Chlorella sp.
Chroococcus limneticus Lemm.
Crucigenia quadrata Morren
C. tetrapedia (Kirch.) W. & G.S. West
Crucigeniella apiculata (Lemm.) Kom.
Dactylococcopsis sp.
Dictyosphaerium sp.
Gleocystis sp.
Kirchneriella sp.
Korschpalmella sp.
Merismopedia sp.
Micractinium sp.
Monoraphidium sp.
Nephrocytium sp.
Oocystis sp.
Pediastrum boryanum (Turp.) Menegh.
P. duplex Meyen
P. simplex Meyen
P. simplex var. *simplex* Meyen

P. tetras (Ehrenb.) Ralfs
Pandorina sp.
Scenedesmus acuminatus (Lagerh.) Chod.
Scenedesmus arcuatus (Lemm.) Lemm.
Scenedesmus spp.
Schroederia sp.
Tetraedron setigera

Zygnematales

Closteriopsis sp.
Closterium sp.
Cosmarium sp.
Staurastrum spp.
Staurastrum tetracerum
Euastrum sp.

Cryptophyceae: cryptomonads

Cryptomonas sp.
Rhodomonas sp.

Diatomophyceae: diatoms

Asterionella sp.
Aulacoseira granulata (E.) Simons.
Cyclotella sp.
Gyrosinga sp.
Nitzschia sp.
Synedra spp.

Dinophyceae: dinoflagellates

Ceratium brachyceros Daday
Peridinium sp.

Euglenophyceae: euglenophytes

Euglena sp.
Lepocincilis sp.
Phacus sp.
Trachelemonas sp.

Xanthophyceae: xanthophytes

Pseudostaurastrum sp.

Table 4.2 Comparison of phytoplankton species composition in net samples recorded in Lake Chivero in 1960, 1982, 1983 and 2003-2006.

	1960	1982[1]	1983	2003/2006
CYANOPHYCEAE- BLUE-GREEN ALGAE				
Anabaena sp.	+	+	+	+
Anabaenopsis tanganyike (G.S. West) Wolosz. Et Mill.	-	+	-	-
Aphanocapsa cf. *incerta* (Lemm.) Cronb. And Kom.	-	-	-	+
Chroococcus limneticus Lemm.	-	-	-	+
Dactylococcopsis sp.	-	-	-	+
Lyngbya contorta	-	+	-	-
Microcystis aeruginosa Kütz.	+	+	+	+
M. botrys Teil.	+	-	+	+
M. flos-aquae (Wittr.) Kirchn.	-	-	-	+
M. novăcerkii (Kom.) Comp.	-	-	-	+
M. wesenbergii Kom. in Kondr.	+	-	+	+
Merismopedia sp.	-	-	-	+
Planktothrix agardhii (Gom.) Anagn. et. Kom.	-	-	-	+
Pseudanabaena mucicola (Naum. et. Hub.-Pest.) Bourr.	+	-	+	+
Woronichnia sp.	-	-	+	+
CHLOROPHYCEAE- GREEN ALGAE				
Actinastrum sp.	-	+	-	+
Botrycoccus sp.	-	-	-	+
Coelastrum sp.	+	-	+	-
Coelastrum microporum Näg. in A. Br.	-	-	-	+
C. reticulatum var. *cubanum* Kom.	-	-	-	+
C. sphaericum Näg.	-	-	-	+
Chlamydocapsa sp.	-	-	-	+
Chlorella sp.	-	+	-	-
Chroococcus limneticus Lemm.	-	-	-	+
Closteriopsis sp.	-	-	-	+
Closterium sp.	+	-	+	+
Cosmarium sp.	+	-	+	+
Crucigenia quadrata Morren	-	-	-	+
C. tetrapedia (Kirch.) W. & G.S. West	-	-	-	+
Crucigeniella apiculata (Lemm.) Kom.	-	-	-	+
Dactylococcopsis sp.	-	-	-	+
Dictyosphaericum sp.	-	-	-	+
Euastrum sp.	-	-	-	+
Eudorina sp.	+	+	+	+

Fragillaria sp.	-	-	+	+
Gleocystis sp.	-	-	-	+
Kirchneriella sp.	-	-	-	+
Korschpalmella sp.	-	-	-	+
Merismopedia sp.	-	-	-	+
Micractinium sp.	-	-	-	+
Monoraphidium sp.	-	-	-	+
Nephrocytium sp.	-	-	-	+
Oocystis sp.	-	-	-	+
Pandorina sp.	-	-	-	+
Pediastrum boryanum (Turp.) Menegh.	+	+	+	+
P. simplex var *simplex* Meyen	-	+	-	-
P. duplex Meyen	+	-	-	+
P. tetras (Ehrenb.) Ralfs	-	-	-	+
P. simplex Meyen	+	-	+	+
Scenedesmus acuminatus (Lagerh.) Chod.	-	-	-	+
S. arcuatus (Lemm.) Lemm.	-	-	-	+
Scenedesmus spp.	-	+	+	-
Schroederia sp.	-	-	-	+
Staurastrum paradoxum	-	-	+	-
Staurastrum spp.	+	+	+	-
Staurastrum tetracerum Ralfs	+	-	+	+
Tetraedron setigera	-	-	-	+
Tetraedron trigonum (Näg) Hansg.	-	-	+	-
Volvox sp.	-	+	-	-
CRYPTOPHYCEAE –CRYPTOMONADS				
Cryptomonas sp.	-	-	-	+
Rhodomonas sp.	-	-	-	+
DIATOMOPHYCEAE –DIATOMS				
Asterionella sp.	-	-	-	+
Aulacoseira italica	+	-	+	-
A. granulata (E.) Simons.	+	+	+	+
Cyclotella sp.	+	-	+	+
Diatoma sp.	-	-	+	-
Gyrosinga (cf. *attenuatum*)	-	-	+	+
Navicula sp.	-	-	+	-
Nitzschia sp.	-	-	-	+
Syndera spp.	-	-	-	+
DINOPHYCEAE-DINOFLAGELLATES				
Ceratium sp.	-	+	-	

Ceratium brachyceros Daday	-	-	-	+
C. hirundinella	+	-	-	-
Peridinium sp.	-	-	-	+
EUGLENOPHYCEAE				
Euglena sp.	-	-	-	+
Lepocinclis sp.	-	-	-	+
Phacus sp.	-	-	-	+
Trachelemonas sp.	-	-	-	+
XANTHOPHYCEAE				
Pseudostaurastrum sp.	+	-	-	+
TOTAL SPECIES RECORDED	**19**	**14**	**24**	**64**

[1]Source: Robarts *et al.* 1982
+ present
- absent

1983. Generally there has been an increase in species in the lake from 19 in 1960 to a present record of 64.

4.3.3 Seasonal dynamics of phytoplankton

The development of the phytoplankton assemblage switched between a clear (February 2003 to April 2004) and turbid state (May to December 2004). The two periods exhibited different patterns as discussed below.

4.3.3.1 Clear state (February 2003 to April 2004)

A seasonal successional pattern was exhibited during the clear state. The general pattern of phytoplankton succession was similar at the three stations, although slight variations occurred (Figure 4.1). The population of cyanophytes, mainly *Microcystis* spp., dominated during the hot rainy season (between February and March 2003). Cyanophytes highest contribution to biomass was in February 2003 when it contributed 80% and 74% of the total biomass at Stations 1 and 2 respectively (Figure 4.1a & Figure 4.1b).

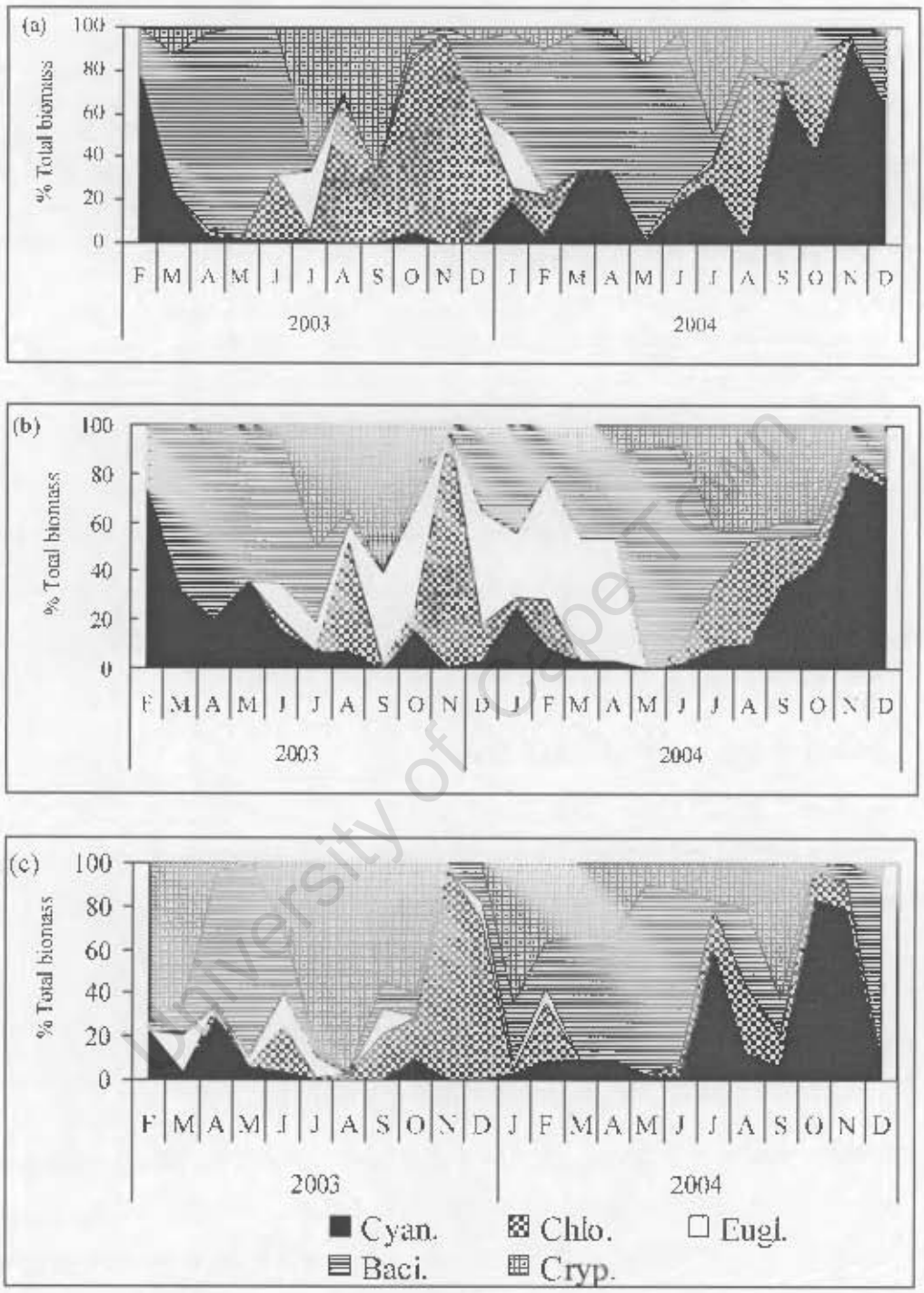


Figure 4.1 **Percentage composition of phytoplankton groups at three stations in Lake Chivero from February 2003 to December 2004 (Station 1 = a; Station 2 = b; Station 3 = c) (Cyan. = Cyanobacteria, Chlo. = Chlorophyta, Eugl. = Euglenophyta, Baci. = Bacillariophyta, Cryp. = Cryptophyta). Data for the three stations are shown to illustrate the close similarity in the periodic character of the three sites.**

Contribution of cyanophytes at Station 3 was less (22%, Figure 4.1c) with cryptophytes contributing 73% of the total biomass. *Microcystis* spp. (*M. aeruginosa*, *M. wesenbergii*, *M. novacekii*) exhibited distinct seasonal variation (Figure 4.2, Figure 4.3). During the clear state the highest average cyanophytes biomass (1.7 mg l^{-1}, mean of 3 stations) occurred in February 2003. After April 2003 cyanophyte biomass was insignificant at all the three sites (Figure 4.2, Figure 4.3). Although insignificant to biomass contribution, cyanophytes persistently occurred at Station 2 except in September, November and December 2003 (Figure 4.1b). At Station 1 cyanophytes were not detected in samples between April and December 2003 (Figure 4.1a) while at Station 3 cyanophytes were not detected in samples between July and September 2003 and between November and December 2003 (Figure 4.1c). A slight increase in percentage biomass of cyanophytes occurred at all stations in October 2003 (Figure 4.1a, Figure 4.1b, Figure 4.1c).

Bacillariophytes assumed dominance at the onset of the cool winter period (April to July 2003) and the phytoplankton assemblage changed to an assemblage of the two diatoms *A. granulata* and *Cyclotella sp.* The percentage contribution of bacillariophytes to total biomass during this period ranged from 3 to 90% (Figure 4.1). *Aulacoseira granulata* was the most dominant diatom while numbers of *Cyclotella* were sometimes very low (Figure 4.3). The biomass of *A. granulata* was highest between February and July 2003 and lowest between August 2003 and January 2004 (Figure 4.3). The biomass of *A. granulata* was below 0.5 mg l^{-1} between February 2003 and February 2004 (Figure 4.3). It increased at all stations in March 2004 and reached a maximum of 2.9 mg l^{-1} at Station 3. *Aulacoseira granulata* occurred throughout the clear state at Stations 2 and 3 but was negligible in the samples at Station 1 between August 2003 and December 2004 (Figure 4.3).

The assemblage changed to a dominance of cryptophytes and chlorophytes at the onset of the hot period from July until the beginning of the rainy season in November/December 2003 (Figure 4.1). The cryptophyte *Cryptomonas* sp. occurred throughout the clear state although its favourable growing period was between July and October when its biomass reached a maximum (Figure 4.2, Figure 4.3). The highest biomass was recorded between

July and September, the favourable period for cryptophytes in the lake. During this period cryptophytes percentage contribution ranged from 31 - 96% of the total biomass. In August a bloom of *Cryptomonas* sp. occurred at Station 3 and a biomass of 30.7 mg l^{-1} was recorded while the other stations had low biomasses. *Cryptomonas* sp. comprised 97% of the total biomass. *Cryptomonas* sp. had generally been most abundant at Station 3, such that when conditions became optimal it attained high biomasses. The dominance of cryptophytes overlapped with chlorophytes that became dominant between September and November (Figure 4.1). Initially chlorophytes only increased in diversity with no marked increase in biomass until around November when a maximum biomass of *Coelastrum microporum, C. reticulatum* var. *cubanum* and *C. sphaericum* was attained at all stations (Figure 4.2, Figure 4.3). The biomasses recorded were 6.8 mg l^{-1}, 9.8 mg l^{-1} and 9.5 mg l^{-1} at Stations 1, 2 and 3 respectively (Figure 4.2, Figure 4.3). Chlorophytes contributed over 95% of the total biomass in November. The dominant chlorophytes species contributing to most of the biomass were *Coelastrum* spp. and *Gleocystis* sp. (Figure 4.3).

Euglenophytes were represented mainly by *Trachelomonas* sp. with rare encounters of *Phacus* sp. and *Euglena* sp. They did not have a distinct growing period or clear seasonal pattern (Figure 4.3). Their contribution to biomass was small and the pattern was variable at the three sites. At Station 1 *Trachelomonas* sp. was recorded between July and August and between December and January 2004 (Figure 4.1). At Station 2 it occurred in samples between May and March 2004 (Figure 4.1) while at Station 3 it occurred for most of the period except in November and December (Figure 4.1). Desmids, relics of a previous riverine community, were observed during the clear state but were insignificant in terms of biomass contribution. The species observed were *Staurastrum* spp., *Closterim* sp. *and Cosmarium* sp.

4.3.3.2 Turbid state (May to December 2004)

The switch to the turbid state was marked by a gradual built-up in biomass of *M. aeruginosa* until it attained 93%, 81% and 79% of the total biomass at Stations 1, 2, and 3

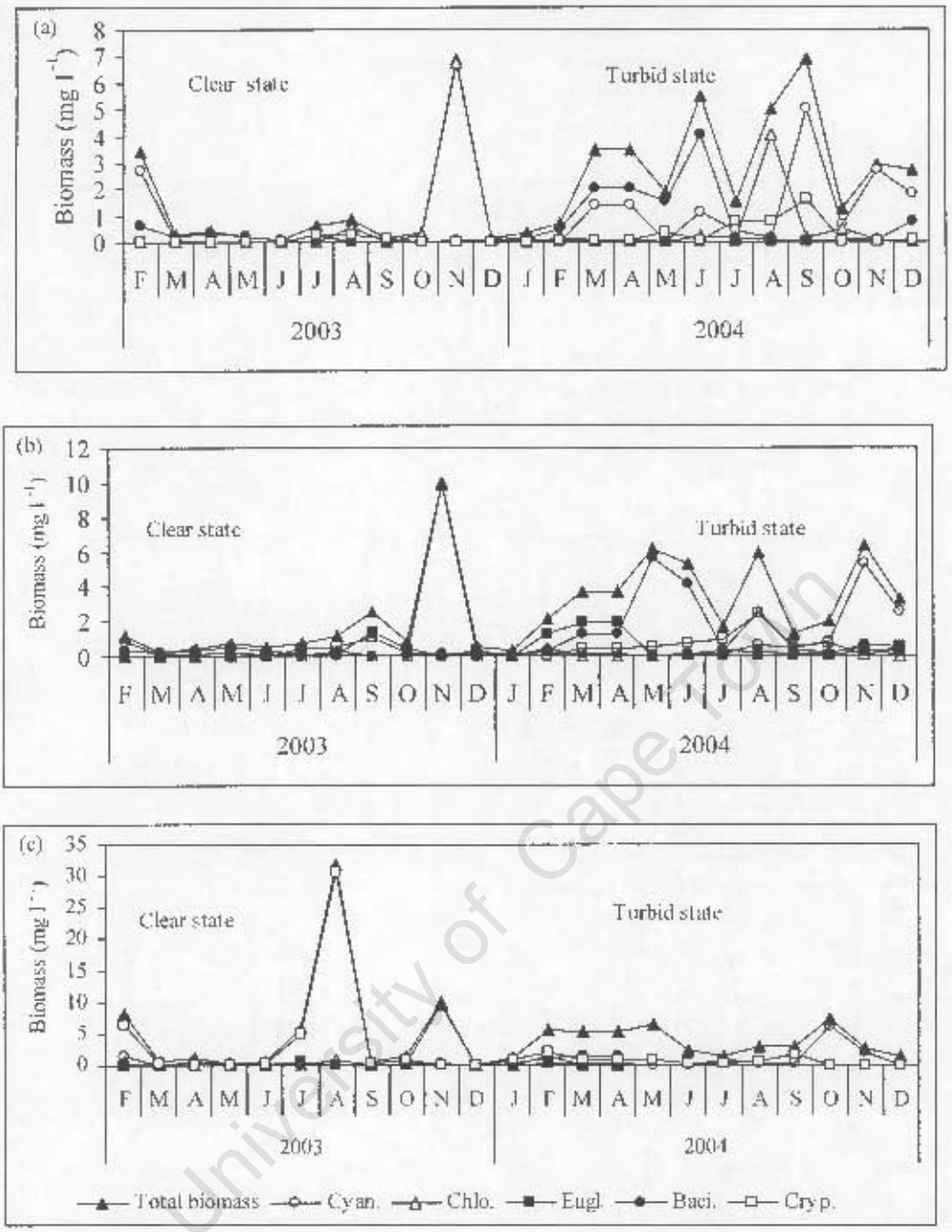


Figure 4.2 **Spatial and temporal change of algal biomass at three stations in Lake Chivero from February 2003 to December 2004 (Station 1 = a; Station 2 = b; Station 3 = c) (Cyan. = Cyanobacteria, Chlo. = Chlorophyta, Eugl. = Euglenophyta, Baci. = Bacillariophyta, Cryp. = Cryptophyta)**

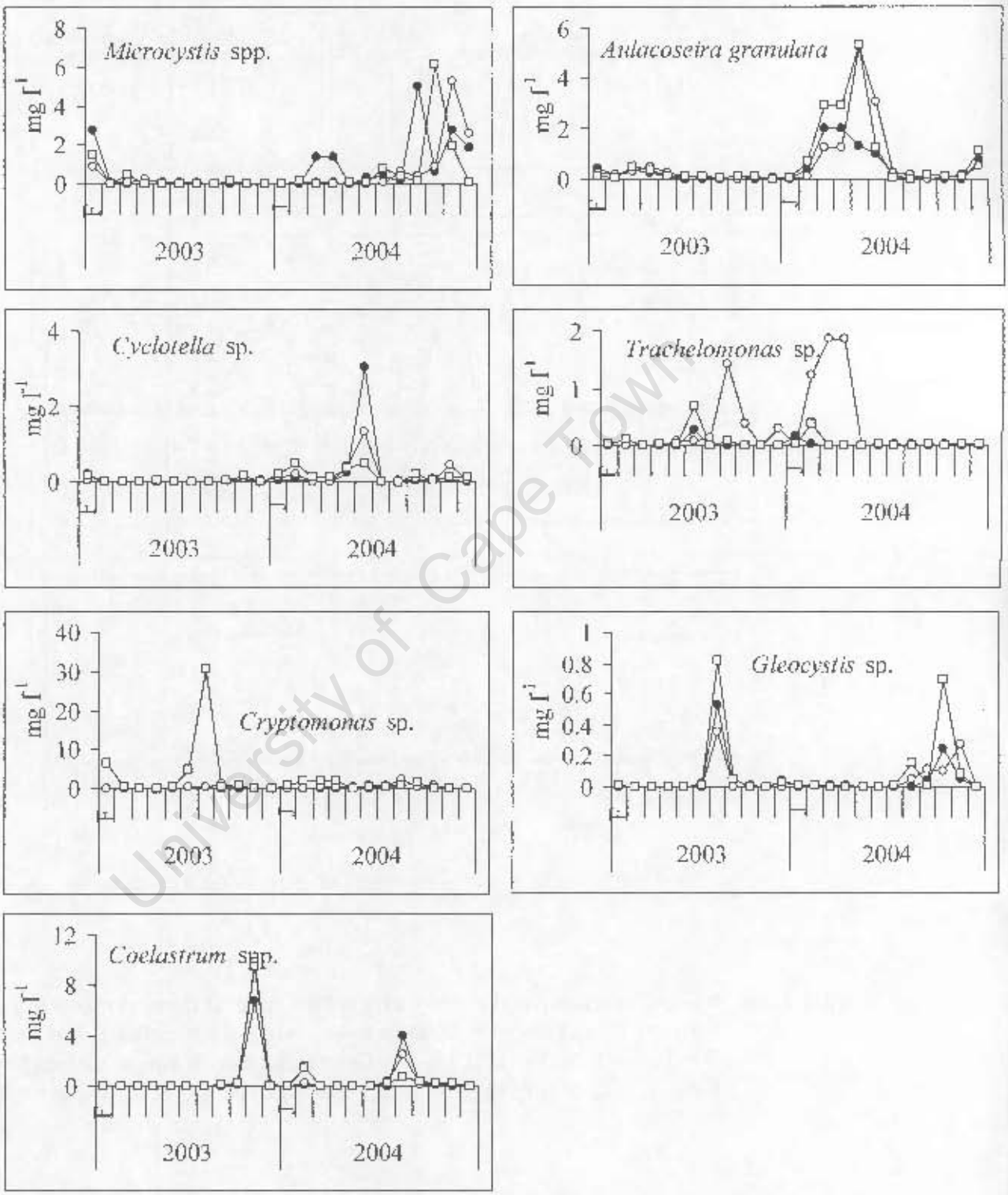


Figure 4.3 **Temporal variations of biomass (mg l^{-1}) for the seven most abundant species at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •; Station 2 = o; Station 3 = □). The axis on the graphs are different.**

respectively in November 2004 (Figure 4.1a, Figure 4.1b, Figure 4.1c). The period of maximum *Microcystis* biomass (October - December 2004) at all stations was characterized by a marked decline in *Cryptomonas* sp. biomass. Initially as the lake switched to a turbid state, the biomass of cryptophytes increased, followed by a sharp drop at maximal *M. aeruginosa* biomass in November. The dominance of bacillariophytes occurred as expected in winter (May and July), with biomasses higher than during the clear state (Figure 4.3). Except for euglenophytes, which were of lesser importance, all other species initially increased in biomass until October 2004 when *M. aeruginosa* assumed dominance (Figure 4.2). The phytoplankton bloom was mixed but *Microcystis* gradually competitively excluded other species although the bloom did not become monospecific.

4.3.4 Variation in total biomass

The clear state at Stations 1 and 2 (Figure 4.2) was characterized by lower biomasses except for the period when *Microcystis* dominated in February 2003 (during this period one could see aggregates of phytoplankton cells) and when a bloom of *Coelastrum* occurred in November 2003. The average total biomasses (during the clear and the turbid states, respectively) were 1.4 mg l^{-1} and 3.4 mg l^{-1} at Station 1 and 1.9 mg l^{-1} and 4.1 mg l^{-1} at Station 2. The high biomass of *Cryptomonas* and *Coelastrum* in August and November respectively masked the distinctness of the two states (Figure 4.2c) at Station 3 as such the average biomass during the clear state (5.2 mg l^{-1}) was higher than during the turbid state (3.4 mg l^{-1}). The total biomass was generally higher at Station 3 than at the other two stations. Overall total biomass was significantly higher (Mann- Whitney U-Test $Z = -3.32$, $p < 0.05$) during the turbid than the clear state, however.

Marked fluctuations in total biomass during the turbid state in response to changes in species dominance occurred at Stations 1 and 2 while at Station 3 the pattern was relatively uniform. Although the period from March to April 2004 was designated as a clear state due to the visual absence of blooms, it had high biomass mainly dominated by bacillariophytes.

4.3.5 Phytoplankton diversity, evenness and stability

The spatial and temporal variations of the Shannon-Wiener diversity, species evenness phytoplankton similarity (Bray-Curtis index), at three stations are shown in Figure 4.4. This was based on fresh weight biomass data. The average Shannon-Wiener diversities were 0.83 and 0.95 during the clear and the turbid state respectively. Species diversity was not significantly different (Mann-Whitney U-Test, $Z = -0.37$ $p > 0.05$) during the two states because species representation to biomass was similar since the bloom was mixed rather than monospecific. Moderate variability of diversity shows that the algal assemblage was determined by a few dominants, which contributed most of the biomass. The switch between clear and turbid state was not reflected as differences in diversity because the shift was due to the gradual change in species relative contribution to total biomass. Diversity only dropped to a low level (0.63) in November 2004 when *Microcystis* assumed a considerably higher biomass.

A marked drop in Shannon-Wiener diversity to 0.1 occurred at all stations in November 2003 (Figure 4.4.a) due to a high biomass of *Coelastrum*, which assumed a dominance of over 95% of the total biomass, and in winter (April – May 2003) when *A. granulata* dominated. Seasonal fluctuations in diversity occurred whereby during summer (December to February) diversity was high and dropped in winter (March-May) when the phytoplankton flora shifted to a dominance of 2 diatoms. It increased from June up to October (spring), a period of highest species diversity in the lake, when chlorophytes and cryptophytes dominated. The species evenness showed a similar pattern to diversity (Figure 4.4 c). It was not significantly different during the clear and the turbid state (Mann-Whitney U-Test, $Z = 0.47$, $p > 0.05$). Diversity closely correlated with evenness (equitability) (r 0.8 =, $n = 69$, $p < 0.05$). Evenness (Kruskal-Wallis Anova H ($n = 39$) = 5.1 $p > 0.05$) and diversity (Kruskal-Wallis Anova H ($n = 69$) = 4.3, $p < 0.05$) were not significantly different among the stations. Diversity was only significantly positively correlated with conductivity ($r = 0.3$) and negatively with total phosphorus ($r = 0.3$) and not with any other physical and chemical parameters.

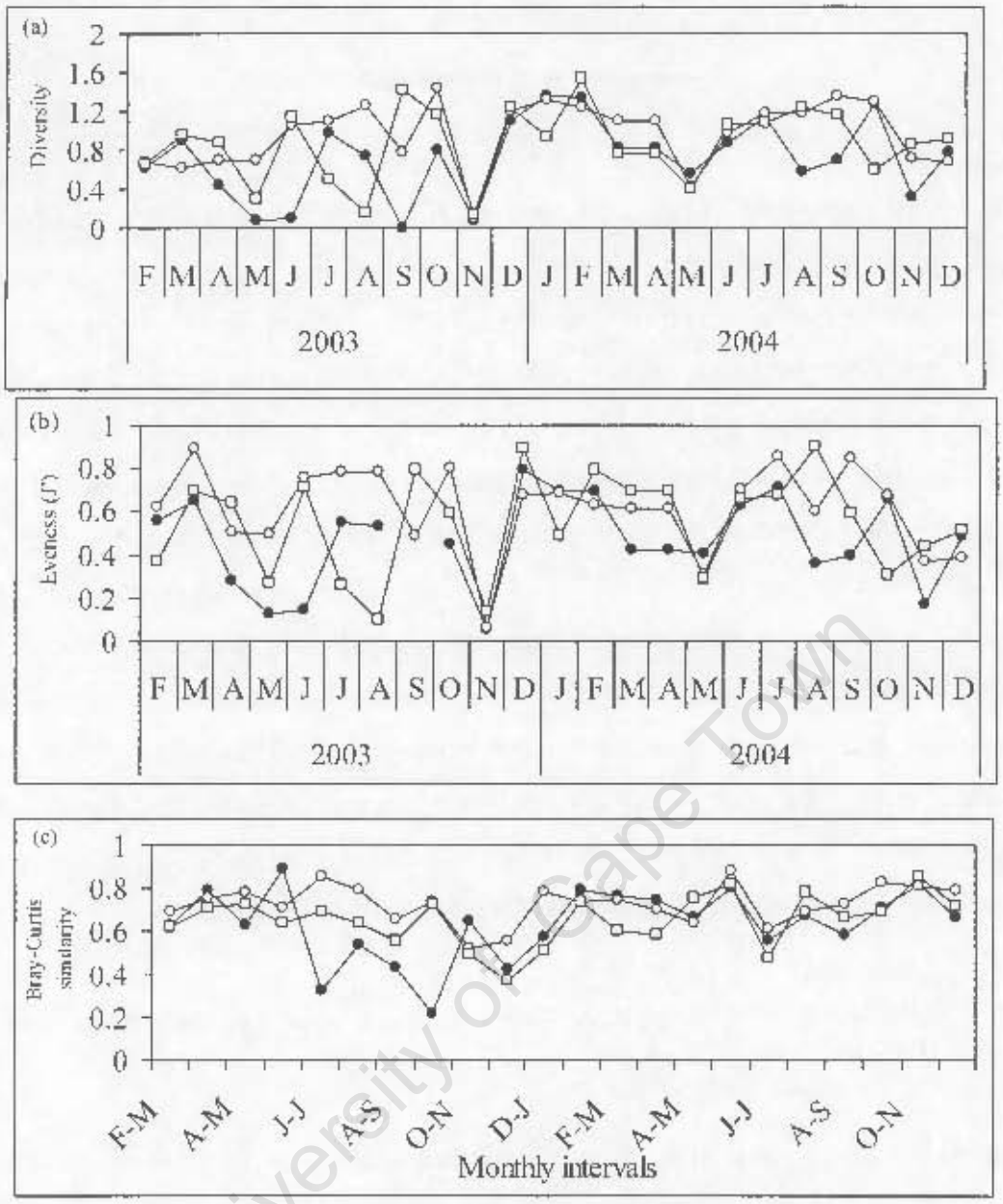


Figure 4.4 **Spatial and temporal variations of (a) Shannon-Wiener diversity, (b) Pielou's evenness and (c) Bray-Curtis similarity between successive monthly samples at three stations in Lake Chivero from February 2003 to December 2004. (Station 1 = •; Station 2 = o; Station 3 = □).**

Except for a slight drop in similarity between November/December 2003 and June/July 2004, high Bray-Curtis similarities were measured during the study period indicating a stable phytoplankton composition (Figure 4.4c) during both the clear and the turbid state. High Bray-Curtis similarity was observed over the whole sampling period because right through the period the bulk of phytoplankton biomass was consistently due to two or three dominant species. There was no significant difference (Mann-Whitney U-Test, $Z = 0.64$, $p > 0.05$) between the clear and the turbid state. Notable was the increase in similarity from July to December 2004 as the lake shifted to a turbid state. Bray-Curtis similarity was significantly higher at station 2 (Kruskal-Wallis Anova H ($n = 69$) = 6.6, $p > 0.05$) than at stations 3 and 1. However the fluctuation pattern of Bray-Curtis similarity was similar at the 3 stations except for low values calculated at Station 1 between the samples of July and August 2003, August and September 2003, and September and October 2003. This indicates that the share of biomass of individuals species during those periods were in each case quite different from that of the previous month.

4.3.6 Variation in chlorophyll *a* concentration with respect to shift between the two states

The development pattern of chlorophyll *a* concentration during the study period was distinguishable into two different periods; a clear state and a turbid state (Figure 4.5) and the concentration was significantly higher ($p < 0.05$) during the turbid state. The clear state was typified by a clearly definable seasonality pattern that was exhibited at all stations (Figure 4.5). Chlorophyll *a* concentration during the clear state showed pulses linked to seasonal changes. The concentration during the clear state ranged from 2 to 48.9 $\mu g\ l^{-1}$, with a mean of 16.1 $\mu g\ l^{-1}$. The concentration of chlorophyll *a* increased to reach a peak in April 2003, and declined thereafter to minimum levels in June when levels of 2.7 $\mu g\ l^{-1}$, 2.7 $\mu g\ l^{-1}$ and 4.1 $\mu g\ l^{-1}$ were recorded at stations 1, 2 and 3 respectively. The concentration increased again to reach a peak in July that was followed by a sharp decline in August especially at station 1 and 2 while at station 3 the August decline was not apparent, with a lowest level of 4.1 $\mu g\ l^{-1}$ being reached in September. Another increase occurred and a maximum was reached in October, which was also immediately followed

by a sharp persistent decline to an average concentration of 7.4 µg l^{-1} in January 2004, which was maintained until May 2004. The mean chlorophyll *a* concentrations during the peaks in April, July and October 2003 were 29.2 µg l^{-1}, 20.3 µg l^{-1} and 37.9 µg l^{-1} respectively (Figure 4.5).

Changes in chlorophyll *a* peaks and troughs during the clear state were related to the seasonal changes in species composition. The February to April 2003 peak, which fell within the summer period, occurred when cyanophytes dominated and the October 2003 peak occurred in spring when chlorophytes dominated. A decrease in chlorophyll *a* concentrations occurred between April and June 2003 when bacillariophytes were dominant and in August 2004 when cryptophytes dominated. A similar pattern was exhibited at the 3 stations. Chlorophyll *a* concentration was generally higher at station 2 during the peak periods. The highest chlorophyll *a* concentration occurred in October 2003 at station 2 and 3.

The shift to the turbid state from May 2004 was marked by a gradual increase in chlorophyll *a* from an average concentration of 7.1 µg l^{-1} until a maximum average concentration of 42.4 µg l^{-1} was reached in November 2004 (Figure 4.5). The pattern of increase was only uniform between May and August 2004. The highest concentration of 56.9 µg l^{-1} was reached in August 2004 at station 2 while at station 1 and 3 the concentrations were 31.5 µg l^{-1} and 16.1 µg l^{-1} respectively. The increase was persistent and marked at station 3 during the turbid state. Station 2 exhibited two collapses after attainment of maximum concentration in August 2004 and in November 2004. At station 1 after reaching a maximum concentration of 31.5 µg l^{-1} in August 2004 the concentration gradually dropped until a concentration of 6.1 µg l^{-1} was reached in December 2004.

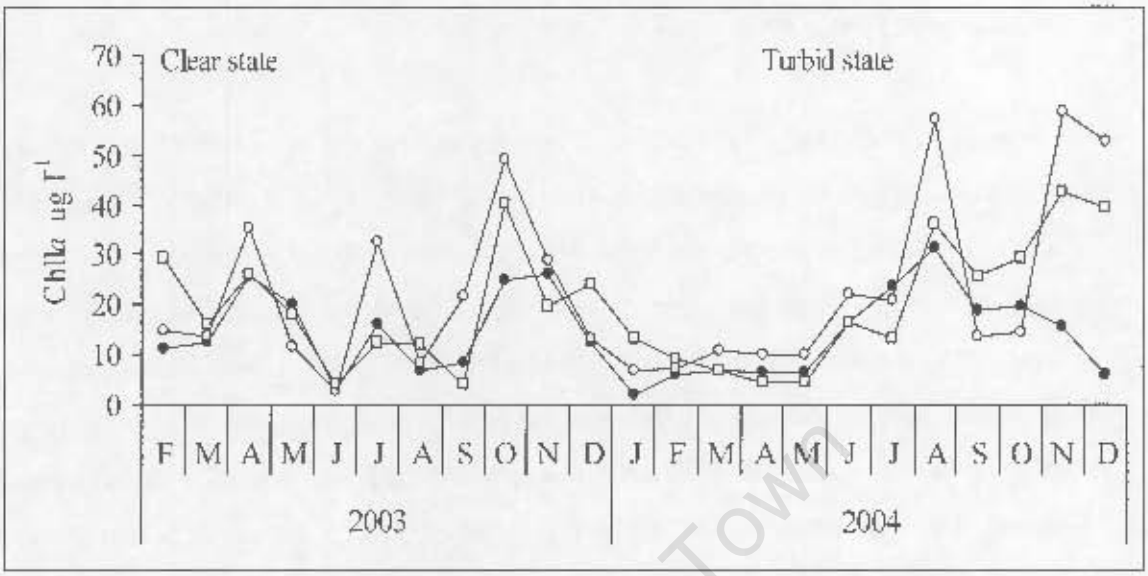


Figure 4.5 **Spatial and temporal variation of chlorophyll *a* (µg l^{-1}) at three stations in Lake Chivero between February 2003 and December 2004 (Station 1 = • - mean of integrated samples 0-2m, 2-4 m and 4-6 m; Station 2 = o - mean of integrated samples 0-2 m and 2-3m; Station 3 = □ - mean of integrated samples 0-2 m and 2-3.5 m)**

The concentration of chlorophyll *a* correlated positively and significantly with pH ($r = 0.50$, $n = 42$, $p < 0.05$) and dissolved oxygen ($r = 0.40$, $n = 42$, $p < 0.05$) but inversely and significantly to Secchi depth ($r = -0.40$, $n = 42$, $p < 0.05$) and was not correlated with any components of nitrogen and phosphorus. There was surprisingly no correlation between total phosphorus and chlorophyll *a*.

4.3.7 Relationship between major taxa divisions and physical and chemical variables

The correlations of biomass for each of the major divisions represented in Lake Chivero with the measured physical and chemical variables are presented in Table 4.3. Dominance by cyanophytes was significantly positively correlated to pH, conductivity, turbidity, nitrate, total nitrogen, TN:TP ratio, orthophosphate, total phytoplankton biomass and inversely correlated with Secchi disc transparency. Bacillariophytes were

significantly correlated to temperature, nitrate, orthophosphate, total phytoplankton biomass and negatively correlated to pH and dissolved oxygen. All taxa were negatively correlated with Secchi depth, although this was only significant for cyanophytes and cryptophytes. Chlorophytes were significantly correlated with conductivity, dissolved oxygen, TN:TP ratio, chlorophyll *a* concentration and total phytoplankton biomass and inversely correlated to orthophosphate and total phosphorus. Cryptophytes negatively correlated with temperature, orthophosphate and total phosphorus and positively correlated with conductivity, nitrate and TN:TP ratio. Only cyanophytes significantly positively correlated with pH while bacillariophytes had a negative correlation. None of the taxonomic groups were correlated to ammonium. A non-significant correlation was obtained between chlorophyll *a* concentration and cyanophytes. Bacillariophytes had a non-significant negative correlation with chlorophyll *a* concentration. Only chlorophytes had a significant positive correlation with chlorophyll *a* concentration. All taxa except euglenophytes positively correlated with total phytoplankton biomass.

4.3.8 Temporal dynamics with respect to functional (C-S-R) patterns

Three classes of planktonic autotrophs, namely growth strategists (C), specialists (S) and ruderal plants (R) have been distinguished by Reynolds (1997). Invasive fast growing species (C-species sensu Reynolds/Grime) are small, with a high surface: volume ratio (small centric diatoms, pinnate diatoms and small chlorophytes. Specialists are of large size, have low area to volume ratio, low metabolic activity and low growth rate *in situ*, have high nutrient storage capacity and have enhanced resistance to sinking and grazing. Ruderals are specialized in tolerating turbulent transport and light gradients.

The functional-group model assists in understanding why certain species of phytoplankton should be most favoured than others in the assembly of communities (Reynolds *et al.* 2002). Functional associations or functional groups consist of species with similar morphology and similar environmental requirements although they do not necessarily belong to the same phylogenetic group (Reynolds *et al.* 2002). Functional groups are useful in evaluating the responses to environmental conditions and changes in

a lake (Kruk *et al.* 2002, Naselli-Flores *et al.* 2003). I tried to use this model to explain the increasing dominance of cryptomonads and the decline of *Microcystis aeruginosa*, an S-strategist during the study period. The functional class assignments of seven dominant phytoplankton genera recorded in Lake Chivero are shown in Table 4.3. The clear state was dominated by *Cryptomonas* sp. and *Coelastrum* spp., an association ascribed to the functional grouping "B" of Reynolds *et al.* (2002). The main characteristics include small size, high SA/V, fast growth and invasive in nature. *Cryptomonas* sp. assumed fast growth and invasiveness by immediately colonizing following collapse of the cyanobacterial bloom (Chapter 5). The favourable conditions during this period could have been comparatively good light conditions resulting in a deeper euphotic zone. The collapse of the bloom created a disturbance that reset autogenic succession and thereby effectively created a "gap" that colonists can occupy. Increased light penetration allowed proliferation of colonists. It seems whenever conditions were optimal *Cryptomonas* sp. assumed high biomass, even during the clear state. This is an osmo-mixotrophic organism that partly lives on dissolved organic matter and can tolerate low dissolved oxygen levels

At the switch to the turbid state cryptophytes, chlorophytes (growth strategists) and ruderals (bacillariophytes) were initially dominant and present in high proportions. However as the environment became more turbid and light limited (low Secchi disk visibility), the large, slow growing specialist (*M. aeruginosa*) gradually assumed dominance.

Correlations (Table 4.4) showed that only cyanobacterial abundance was positively related to turbidity. This implies that as long as cyanobacteria are dominant they can competitively exclude other taxa; thereby explaining the shift between clear (low turbidity, eukaryotic algae) and turbid states (high turbidity, cyanobacteria). Also cyanobacteria significantly negatively correlated with Secchi depth, indicating that their dominance reduced light penetration. The successional pattern during the clear state from February 2003 to April 2004 can be represented as M → B → Y → J according to codes by Reynolds et *al.* (2002). The codes and the species involved are defined in Table 4.5 This reflects a seasonal shifts of: cyanophytes → bacillariopytes → cryptophytes →

chlorophytes. During the clear state the Y functional group predominated. The progression during the turbid state was too short to reflect seasonal pattern. The composite pattern for the whole period would be M → B → Y → J → B → J/Y→ M. The successional progression during the clear state seems to have represented a "dynamic metastable equilibrium" (Schumm 1977). Although the same groups remained an abrupt change occurred when the system shifted to the turbid state, resulting in minor shifts in the succession process. Generally the "strategies" (C,S,R) and the phytoplankton association (Reynolds *et al.* 2002) depicted well the clear and the turbid state.

Table 4.3 Functional class assignments of six dominant phytoplankton genera recorded in Lake Chivero during this study

Ruderals (R)	Colonists (C)	Specialists (S)
Cyclotella	(R↔C) *Coelastrum*	*Microcystis*
Aulacoseira	(R↔C) *Scenedesmus*	
	(R↔C) *Cryptomonas* (C↔S)	

4.3.9 Assessment of the development of the phytoplankton assemblage using multivariate exploratory analysis

Principal Components and Classification Analysis (PCCA) extracted fifteen eigenvalues, with the first 3 accounting for 66% of the variance (Table 4.6) explained by environmental factors. The projection of the variables on the factor-plane (1x2) shows the association of the species and environmental variables (Figure 4.6). The association of *Microcystis* with nitrate, total nitrogen, TN:TP ratio and orthophosphate supports the observation that its dominance during the study period was regulated by relative abundance of nitrate and orthophosphate. The two chlorophytes *Gleocystis* and *Coelastrum* were associated with turbidity, dissolved oxygen, pH, ammonia and

Table 4.4 **Pearson correlation coefficients (r) between phytoplankton biomass, per division with the measured physical and chemical parameters in Lake Chivero, February 2003 to December 2004. The number of cases is 69; figures denoted by an asterix* are significant at $p < 0.05$. (n = 69)**

Variable	Unit	Cyanophyta	Chlorophyta	Euglenophyta	Bacillariophyta	Cryptophyta
Temperature	°C	0.18	-0.18	0.11	***0.26****	***-0.32****
pH		***0.28****	0.22	0.03	***-0.29****	0.03
Conductivity	µS cm^{-1}	***0.37****	***0.63****	0.12	-0.03	***0.24****
Turbidity	NTU	***0.68****	0.18	0.14	0.22	0.19
Dissolved oxygen	mg l^{-1}	0.09	***0.29****	0.01	***-0.35****	0.16
Secchi disc transparency	m	***-0.55****	-0.16	-0.11	-0.08	***-0.27****
Ammonium	mg l^{-1}	0.23	-0.06	0.01	0.11	0.15
Nitrate	mg l^{-1}	***0.43****	0.13	-0.09	***0.42****	***0.44****
Orthophosphate	mg l^{-1}	***0.42****	***-0.27****	***-0.28****	***0.43****	***-0.28****
Total phosphorus	mg l^{-1}	-0.10	***-0.54****	-0.02	0.17	***-0.40****
Total nitrogen	mg l^{-1}	***0.44****	-0.04	-0.06	0.21	0.07
TN:TP	mg l^{-1}	***0.48****	***0.29****	-0.05	0.13	***0.31****
Chlorophyll *a*	µg l^{-1}	0.22	***0.29****	0.03	-0.09	0.03
Biomass	mg l^{-1}	***0.43****	***0.39****	-0.02	***0.41****	***0.46****

Table 4.5 Trait-separated functional groups of phytoplankton (Reynolds *et al.* 2002)

Code	Typical representatives	Tolerances	Sensitivities	Species during this study
B	*Aulacoseira subartica* *Aulacoseira islandica*	Light deficiency	pH rise, Si depletion, stratification	*Aulacoseira granulata*
Y	*Cryptomonas*	Low light	phagotrophs	*Cryptomonas*
J	*Pediastrum, Coelastrum, Scenedesmus, Golenkinia*	-	settling into low light	*Coelastrum* spp.
M	*Microcystis, Sphaerocavum*	High insolation	stratification, light, flushing	*Microcystis* spp.

Table 4.6 Summary of the PCCA analysis for the relationship between phytoplankton and environmental factors in Lake Chivero between February 2003 and December 2004.

Value no.	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	7.15	37.65	7.15	37.65
2	3.09	16.26	10.24	53.92
3	2.28	12.04	12.53	65.97
4	1.87	9.85	14.40	75.83
5	1.75	9.22	16.16	85.05
6	0.88	4.63	17.04	89.69
7	0.58	3.07	17.62	92.76

conductivity. *Aulacoseira granulata* tended to be closer to temperature probably because it was influenced by temperature, being restricted to the winter period. *Trachelomonas* sp., *Cryptomonas* sp. and *Cyclotella* sp. occurred together and were associated with total phosphorus.

4.3.10 A model explaining the switch from clear to turbid state

I propose a generalized model of the equilibrium states inferred from the observed development of the phytoplankton assemblage in Lake Chivero during the study period. The model assumes a critical nitrate concentration (NO_{3crit}) above which cyanobacteria dominate and below which cryptophytes and chlorophytes dominate. Once cyanobacteria dominate they can perpetuate themselves because their presence increases turbidity, resulting in the reduction in light availability and thereby competitively exclude other species. When algal dominance reaches unsustainably high levels of productivity (chlorophyll *a* concentration just below 100 $\mu g\ l^{-1}$), the systems become unstable, resulting in a collapse that re-sets the succession process back to dominance by cryptophytes and chlorophytes (clear state). The clear state was characterized by high light intensities (high Secchi depth, low turbidity), an environment that was inhibitory to cyanobacteria because they were less dominant (competitively inferior). The nutrient balances (and temporal variation of the environment) prevent competitive exclusion of other species during the turbid state. Further monitoring will enable further development and validation of this model.

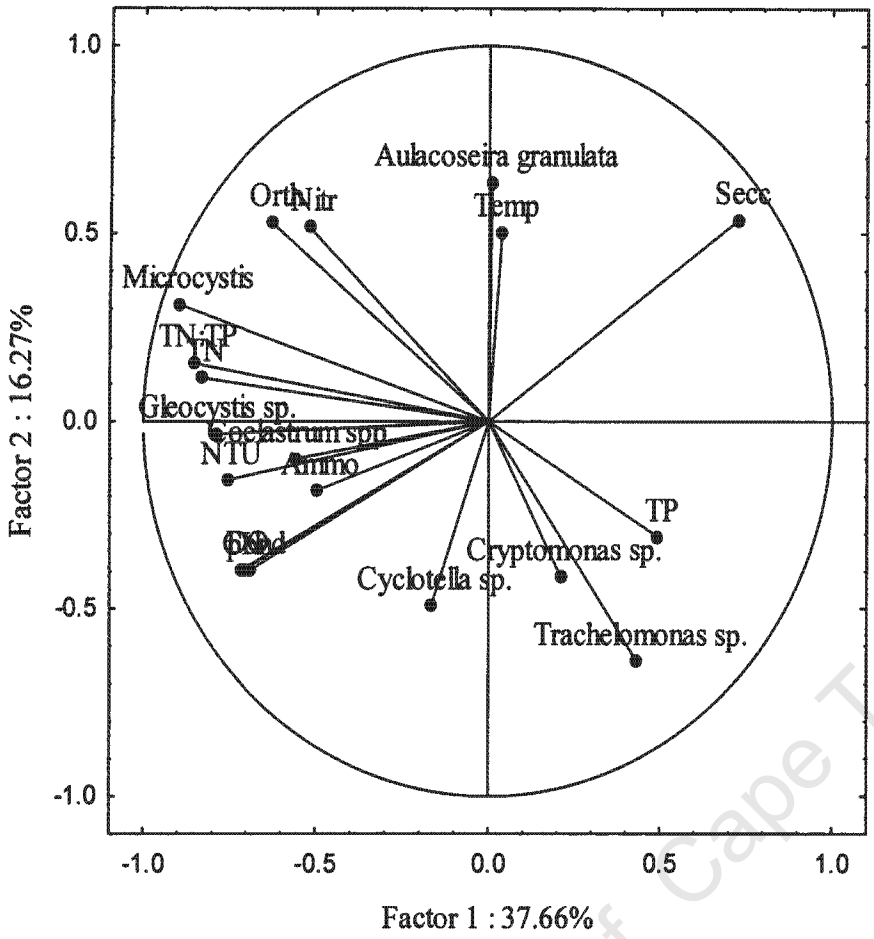


Figure 4.6 **A biplot of the relationship between physical and chemical parameters and abundant phytoplankton taxa. The abbreviations used for physical and chemical parameters area: Cond = Conductivity, Ammo = Ammonium, NTU = Turbidity, DO = Dissolved oxygen, TN:TP = TN:TP ratio, TN = Total nitrogen, Orth = Orthophosphate, Nitr = Nitrate, Secc = Secchi depth, TP = Total phosphorus.**

4.4 DISCUSSION

4.4.1 Shift between a clear and a turbid state

An important feature of complex systems is their capacity to surprise us by changing in a sudden and unpredictable way (Hansell *et al.* 1997), as happened in Lake Chivero during this study period. The patterns observed in the phytoplankton assemblage indicated that the lake exhibited two states: a longer-lasting clear state associated with low cyanobacteria abundance and a shorter turbid state with a mixture of cyanobacteria and eukaryotes. Cryptophytes, bacillariophytes and chlorophytes dominated during the clear state when Secchi depth visibility was high and turbidity low while during the turbid state Secchi disc visibility was low and turbidity high. According to the definition by Leentvaar (1980) (Chapter 1 Section 1.1) Lake Chivero exhibited temporary hyper-eutrophy with respect to phytoplankton periodicity. Prior to the study it was envisaged that there would be a permanent bloom (permanent hyper-eutrophy) maintained in a steady state, which would mask seasonal phytoplankton periodicity but instead the phytoplankton assemblage exhibited normal periodicity (particularly during the clear state).

The main question is whether the situation observed in the phytoplankton assemblage represents "bi-stability" or alternative stable states (Chapter 1 Section 1.4). The alternate existence of (i) a clear state dominated by eukaryotic algae and (ii) a turbid state dominated by cyanobacteria or a mixture of cyanobacteria and other taxa indicates existence of "bi-stability". Scheffer *et al.* (2001), in their study of shallow lakes in The Nertherlands subject to eutrophication, reckon that change in a single parameter will cause the community to switch from one state to another. Since Scheffer *et al.* (2001) were basing their observation on long time periods, the challenge is to consider whether the contrasting dynamics of nitrate observed in Lake Chivero during the two periods can be considered as a sufficiently "strong environmental driver" to have adequately caused a shift. While there could have been other underlying factors it appears that a "critical

nitrate concentration" resulting from high dissolved oxygen was a major driver that caused the shift in the phytoplankton assemblage. Furthermore, since the shift between these states was not linked to reduction in nutrient loading, a decrease in in-lake nutrient concentration or bio-manipulative processes, the change in nutrient (N and P) balance (Chapter 3) is the most plausible explanation.

The duration spent in each state, 15 months for the clear state and 8 months for the turbid state, shows that these were really distinguishable periods in the phytoplankton assemblage, each being probably a "locally stable equilibrium point" (Beisner *et al.* 2003). Although this may not have been a "major ecosystem state shift" it is of local management interest, particularly since the clear state was longer and that when the cyanobacterial bloom collapsed the lake reverted to the clear state (Chapter 5). It could be an indication that the equilibrium position in the phytoplankton assemblage is tilting towards this state, an indicator that it may be possible to "hold" the lake in this preferred state.

A typical example in lakes is a switch that occurs following eutrophication where due to excessive nutrient loading the lake "flips" into a situation where phytoplankton blooms persist (Dent *et al.* 2002). As shown in this study "bi-stability" can occur under hyper-eutrophic conditions. This however has to be investigated further particularly to establish whether the clear state can be maintained for a longer period. As in shallow eutrophic lakes this should involve determination of stabilizing feedback mechanisms (Chapter 1 Section 1.4) (Scheffer 2001). While it may not be possible to reverse the hyper-eutrophic state of Lake Chivero, it may be possible to institute management options that will maintain a phytoplankton assemblage less dominated by cyanobacteria through manipulation of N and P and enhancing the appropriate feedback mechanisms.

The major stabilizing mechanism of the clear state could be the increasing grazing pressure on cyanobacteria by *Oreochromis niloticus*, an exotic invasive tilapia fish species that now constitute approximately 80% of the catch in Lake Chivero (Crispen Phiri, personal communication, University Lake Kariba Research Station, Kariba,

Zimbabwe). Tilapias can feed on large algae (mainly cyanobacteria and diatoms) resulting in the proliferation of small-sized taxa e.g. chlorophyceans (Figueredo & Giani 2005). Biomanipulation studies in temperate lakes have shown that an increase in grazing pressure from cladocerans following a decrease in density of zooplanktivorous fishes lead to reduction of the algal biomass and an increase in water quality (Søndergaard *et al.* 1990). Control of algal biomass in tropical regions has been attributed to omnivorous filter-feeding fishes (Figueredo & Giani 2005, Kormarkova 1998), an aspect that is still (i.e. not in this thesis) to be investigated in Lake Chivero, especially the possible role of *O. niloticus* in relation to nutrient shifts as forcing factors to maintain the lake in a clear state.

Hysteresis in general terms is defined as "the retardation or lagging of an effect behind the cause of the effect: the influence of earlier treatment of a body on its subsequent reaction" (Chambers dictionary). The phytoplankton assemblages of shallow eutrophic lakes have been considered to be "hysteretic" systems (Chapter 1 Section 1.4) with two alternative equilibria consisting of (i) cyanobacteria (Family Oscillatoriacea) and (ii) other eukaryotic algae. In shallow eutrophic lakes this hysteresis is based on the fact that that cyanobacteria are adapted to low light conditions and also promote low light conditions by causing high turbidity (Scheffer *et al.* (1997). It has been recommended that support for alternative stable states should have testable or observable attributes of hysteresis (Okey 2004). The observable attributes during this study (although the data set is small) are discussed in Chapter 5 – where at the onset of the turbid state a gradual exclusion of other species occurred as turbidity and total dissolved solids increased and Secchi depth visibility and the euphotic depth decreased. A model to explain the switch from the clear to the turbid state has been proposed (Chapter 4, Section 4.3.10).

Extending the theory of alternative states to this hyper-eutrophic tropical lake, I contend that from the patterns observed during this study Lake Chivero exhibited (i) a clear state with lesser dominance of cyanobacteria and lower algal biomass of eukaryotic algae with higher biomass being attained when conditions were suitable and (ii) a turbid state with increasing cyanobacterial abundance and decreasing dominance of other species. Lake

Chivero, where the environment during the study period was favourable for the eukaryotic algal assemblage with improved clarity, presents an opportunity to undertake long-term monitoring and intensive studies in order to further validate this observation. The findings supported the hypothesis that the lake was in a "clear metastable state" during the study period but a slight disturbance in internal nutrient balance would shift it to a turbid state that would be maintained until the nutrient balance dropped below a critical level.

4.4.2 Equilibrium states in relation to species and biomass contribution

The predominance of cryptophytes and chlorophytes in the phytoplankton assemblage for 15 months (clear state) is an indication that non-equilibrium dynamics them. *Microcystis*, a specialist, was confined to a shorter period when an almost equilibrium condition was attained. Equilibrium favoured specialists and reduced the predominance of other taxa. A similar observation made in Hartbeespoort Dam by Hambright & Zohary (2000) showed that abundance of *Microcystis* was a dominating factor on the occurrences of chlorophytes, bacillariophytes and cryptophytes which tended to proliferate at low *Microcystis* abundances.

Almost steady-state conditions were approached between March and August 2003 during the clear state, while for the rest of the period the algal assemblage experienced non-equilibrium dynamics. Even during the clear state irruptions of high biomasses of chlorophytes and cryptophytes occurred but generally during the clear state biomass changes were relatively uniform (< 1 mg l^{-1}) with about 80% contributed by 3 species. Bray-Curtis similarity index was uniform and the algal assemblage almost approached equilibrium. As observed by Kruk *et al.* (2002) it is possible at higher trophic levels to attain equilibrium that is sometimes maintained for considerable periods mainly because environmental conditions constraint species diversity to one or two dominating species. Nitrate concentration, which I consider the main factor to have influenced the switch between the two states, was relatively uniform during the period when near-equilibrium was attained and this could have curtailed wide fluctuations in biomass.

It has however been noted that long-lasting equilibrium phases where three or fewer species comprise more than 80% of the phytoplankton biomass (Chapter 1 Section 1.5) are rare (J. Padisak, personal communication cited by Harding 1996). Harding (1996) however reported a unique situation in Zeekoevlei between 1992 and1993 where a long-lasting equilibrium phase was maintained in which *M. aeruginosa* dominated for six to eight months of the year while for the remaining four to six months *M. aeruginosa* co-occurred with one or two chlorophyte species. This is in contrast to the situation observed in Lake Chivero during the present study.

4.4.3 Applicability of models of cyanobacterial dominance in Lake Chivero

The phytoplankton assemblage in Lake Chivero did not exhibit a perpetual dominance of cyanobacteria during this study period as would be expected in a hyper-eutrophic lake. This implies that factors such as low TN:TP ratio, high pH and high temperatures proposed by models of cyanobacterial dominance (Chapter 1 Section 1.3) which were also characteristic of the condition in the lake during the study period (Chapter 3) were not the major determinants of cyanobacteria dominance. As a hyper-eutrophic lake, Lake Chivero was characterized by high conductivity, high nitrogen and phosphorus levels and pH >8 (Chapter 3), which should have favoured and imparted a selective advantage to cyanobacteria (Shapiro 1990).

Low TN:TP ratios (<29) have been suggested as a major factor favouring the dominance of cyanobacteria (Smith 1983, Shapiro 1990). Although the TN:TP ratios were below 20 during the entire study period, cyanobacteria were not persistently dominant. TN:TP ratios are said to become insignificant if the nutrient concentrations exceed those limiting cyanobacteria growth (Reynolds 1992), and during this study nitrogen and phosphorus levels in the lake were not limiting for cyanobacteria, which could explain the deviation as also observed by Jensen *et al.* (1994) for shallow Danish lakes.

Temperature is considered an important environmental factor influencing cyanobacterial dominance (Paerl 1996) with growth rates of cyanobacteria being optimal within a range

of 25 – 35 °C (Ganf 1974). Temperatures measured during this study, except between May and August, were near the optimal range for cyanobacteria and fell within the range 14 - 25 °C (Chapter 3), as was previously recorded by Thornton & Nduku (1982). They were also near temperatures of between 25.9 and 28 °C, optimal for bloom growth, recorded in Lake Victoria (Ochumba & Kibaara 1989). It was interesting however that the bloom commenced in June 2004 when temperatures were lowest (17-20°C) and persisted throughout the winter period until December (summer). Temperatures started to increase in November and had reached 25°C by December when the bloom collapsed. This shows that temperature could not have been a major factor limiting cyanobacterial dominance in Lake Chivero.

During the previous year (2003) temperatures were higher (winter June to August) than in winter of 2004 (Chapter 3), although blooms did not occur. Also the preceding months (January to May 2004) had higher surface temperatures of up to 27°C, although blooms were initiated only from May 2004 when temperatures had started to decline in the lake. Thus occurrence of blooms could not have been a function of temperature alone. Temperature is not a limiting factor in Chivero because blooms have also been previously reported to occur even when temperatures are lowest in winter (Junor 1964).

The pH in Lake Chivero was usually alkaline and always >6 throughout the study period (Chapter 3). Similarly to temperature, high pH/low CO_2 could play a role in influencing cyanobacterial dominance (Paerl 1996), but appears not to have been critical in the actual initiation of the cyanobacterial bloom during this study. Allelopathic effects could not have been of much significance either, because due to the 'small numbers of cyanobacteria microcystin levels were low (Appendix 1); furthermore, it has not been established whether the strain of *M. aeruginosa* in Lake Chivero is toxic.

4.4.4 Has the phytoplankton assemblage changed?

The phytoplankton assemblage in Lake Chivero, as in other eutrophic systems in southern Africa, has previously been dominated by cyanobacteria, mainly *M. aeruginosa*,

A. flos-aquae and *A. tanganyike* (Munro 1966, Falconer 1973, Marshall & Falconer 1973, Mitchell & Marshall 1974, Robarts & Southall 1977, Robarts 1979). The sampling period reported on here seems to have coincided with a period of reduced cyanobacterial abundance, since cyanobacterial blooms were not permanent or persistently abundant. There was an unusual decline in *M. aeruginosa*, while *Anabaena* sp. was rare.

Dense cyanobacterial blooms appeared in the lake immediately after its construction, when the lake became eutrophic, and became a permanent feature as the water quality continued to deteriorate (Munro 1966). Although data are not available for the period since then, visual observations over the years have indicated that blooms are the norm. Anomalously low concentrations of cyanobacteria were encountered during the present study. Nutrient loading into the lake has not decreased (see Chapter 3) and therefore the decline in cyanobacterial dominance was not due to an improvement in water quality.

Algal species dominance and composition have changed. *Microcystis novacekii*, *M. botrys* and *M. wesenbergii*, which co-occurred with *M. aeruginosa*, were not reported in former studies on Lake Chivero, but are common in eutrophic systems and have been described in Lake Kariba by Cronberg (1997). Although *M. botrys* and *M. wesenbergii* have not been previously reported from Lake Chivero, they were however present in the 1960 and 1983 samples that I identified indicating that they have always been present in the lake. It appears that all *Microcystis* species were previously considered to be *M. aeruginosa*. *Microcystis botrys* was dominant in 1960 and 1983 samples, however it is usually confused with *M. aeruginosa*. The main distinguishing characteristics are its typical radial morphology and larger cells of up to 5-6 μm in diameter. Considering its predominance in 1960 and 1983 samples, it is possible that previous reports of the single dominance of *M. aeruginosa* in Lake Chivero may have included *M. botrys*. *Microcystis wesenbergii* is easily distinguishable from the two other species due to its compactness and distinct transparent mucilage that surrounds its colonies so should not have been confused with *M. aeruginosa*. The only new record within the genus is *M. novacekii*.

The most remarkable difference between the cyanobacterial assemblage in the 1980s and that recorded in the present study was the decline in the dominance of *Anabaena* sp. and *A. tanganyike,* two heterocystous species previously reported by Mitchell and Marshall (1974) as being amongst the dominant algae in 1970-71. At that time *A. flos-aquae* was reported as co-dominating with *M. aeruginosa*, particularly during winter (around July) when oxygen tensions in the epilimnetic waters were lowest (Munro 1966). Falconer (1973) observed *A. flos-aquae* almost to form a monoculture in July 1969, whereas only a few filaments of *Anabaena* sp. were occasionally encountered in the present study. *Anabaenopsis tanganyike* was rare during this study, although it was previously reported from the epilimnitic waters, mainly between February and April (1969), while for the rest of the year it occurred only in low numbers, if at all (Falconer 1973).

According to Blomqvist *et al.* (1994), nitrogen scarcity favours the development of nitrogen-fixing species. *Anabaena* sp. and *A. tanganyike* are expected to dominate when nitrogen is limiting (i.e. at low N:P ratios) because they are capable of nitrogen fixation (Gallon 2001). Nitrogen was the primary phytoplankton growth-limiting nutrient in Lake Chivero during the early years (Robarts & Southall 1977); it seems that, at that time, low nitrogen levels favoured the occurrence of nitrogen-fixing cyanobacteria to the extent that they were dominant. According to Reynolds (1998), algal dominance is the result of a stochastic combination of environmental variables. *Anabaena* favours nitrate depletion (Sakamoto & Okino 2000), stable environmental conditions such as the absence of water turbulence, long water retention times (Reynolds *et al.* 2002) and high irradiance (Ahn *et al.* 2002). The average nitrogen load received by the lake is now four times higher than the levels estimated in 1996 (Nhapi 2004), so nitrate levels are now higher than the levels recorded when the dominance of *Anabaena* was reported. The lake has always been subject to turbulence and has a short water-retention time of 0.82 years (Marshall & Falconer 1973), so high nitrate levels are the most likely factor limiting the dominance of *Anabaena*. However, since *Anabaena* is present in low numbers, it might be expected to increase if the levels of nitrate were to decrease, although generally *Anabaena* spp. are also not common in southern African lakes and reservoirs.

Two dominant bacillariophytes in Lake Chivero during the study were *A. granulata* and *Cyclotella* sp. *Aulacoseira italica* was present in 1960 samples and absent in 1983 and 2003-2006 samples. It appears that this species has been replaced by *Aulacoseira granulata*. Falconer (1973) previously recorded 3 species of *Aulacoseira* in Lake Chivero; *A. granulata* var *granulata* as dominant while *M. granulata* var *angustissima* and *M. italica* occurred in small numbers. *M. italica* was not encountered. The dominant species was *A. granulata*. According to Talling & Talling (1965) *Aulacoseira* species are most numerous in lakes of relatively low alkalinity and salinity (Class 1). In current terms it is found in lakes with conductivities of less that 600 $\mu S\ cm^{-1}$. Lake Chivero falls within this category, as do lakes Malawi, Malombe, Victoria, Tana and George, and *Aulacoseira* has been recorded in them.

Chlorophytes represented the most diverse group in the lake with thirty-nine species. This is a common characteristic of tropical lakes (Kalff & Watson 1986). Kebede & Belay (1994) also observed that chlorophytes were the dominant group in Lake Awasa. In Lake Chivero the species that contributed most to biomass were *Coelastrum microporum, C. reticulatum, C. sphaericum* and *Gleocystis* sp. *Pediastrum simplex* and *P. duplex* were common although they did not contribute much to biomass. Rare species included *Schroederia* sp., *Kirchneriella* sp, *Actinastrum* sp., *Micractinium* sp. and *Crucigenia tetrapedia*. Falconer (1973), who did a seasonal study, did not report the occurrence of chlorophytes in 1968/1969 except for *Pediastrum boryanum*, which was rarely encountered during this study. Analysis of the 1960 and 1983 samples showed that chlorophytes have always been present in Lake Chivero but could have been considered insignificant due to their low contribution to biomass. Munro (1966) reported the presence of *Volvox* sp., *Eudorina* sp. and *Pediastrum* sp. while Robarts (1979) recorded *Actinastrum* sp., *Scenedesmus* sp., *Staurastrum* sp. and *Chlorella* sp. All these species were observed but only *Coelastrum* spp. and *Gleocystis* sp. contributed significantly to biomass. Chlorophytes appeared in the lake in late winter and become most abundant around September to December. Robarts (1979) also previously observed that species diversity in the lake increased in winter and during the dry summer period due to the appearance of several species of chlorophytes.

Two groups, cryptophytes and euglenophytes, comprised part of the phytoplankton community, but had not been reported in earlier studies. Cryptophytes were represented by two species, while mainly *Trachelomonas* sp. represented euglenophytes. Although not diverse in terms of species, they significantly contributed to biomass at specific times of the year. The low species representation by cryptophytes in Lake Chivero is probably because the lake is highly productive. Cryptophytes are associated with oligotrophic lakes (Hecky & Kling 1981, Kalff & Watson 1986) and in Lake Awassa Kebede & Belay (1994) attributed the low representation of cryptophytes to the relatively productive nature of the lake. In a similarly hyper-eutrophic system, Zeekoevlei, Harding (1996) reported a pulse occurrence of cryptophytes in winter. Lake Chivero is higher in nutrient levels than Lake Awasa but higher than Zeekoevlei (Chapter 3). Although low is species diversity, cryptophytes contributed significantly to biomass and when conditions are ideal attained very high biomasses.

Cryptophytes are generally abundant in lakes with a high concentration of dissolved organic matter plus high phosphorus concentration (Kirsten Olrik, personal communication). Their success in Lake Chivero could be partly linked to their ability to live partly heterotrophically and being less sensitive to low dissolved oxygen levels and high ammonia levels (Kirsten Olrik, personal communication).

Species of desmids observed include *Staurastrum* sp., *Closterium* sp. and *Cosmarium* sp. Munro (1966) reported the presence of *Staurastrum* sp. but later Mitchell & Marshall (1974) reported desmids as being completely absent. Desmids were rare and had insignificant contribution to biomass. They are relics of the previously riverine phytoplankton assemblage. According to Talling & Talling (1965) most desmids are characteristic of fresh waters low in total ions. Lake Chivero has increased in ionic concentration over the years. The input of high sodium and chloride content from sewage effluent into the lake has transformed the lake into a typical endorheic lake (Magadza 1997). The scarcity of desmids in the lake may be due to the high ionic concentration in the water; this has also been observed in Lake Awassa (Kebede & Belay 1994).

One species recorded during the early years that seems to have disappeared is *Ceratium hirundinella*. Falconer (1973) reported its absence in 1968 as the lake changed from riverine to lacustrine conditions. Other species that were not encountered but previously reported are *Pediastrum clathratum* (Mitchell & Marshall 1974) and *Lyngbya contorta* (Robarts 1979) that was recorded as being in large populations during May 1974 occurring together with *M. granulata*.

Eutrophic lakes are expected to have a predominance of *M. aeruginosa* in association with *Anabaena* with the exclusion of other species (Walmsley & Butty 1980). Is this still the case for Lake Chivero? It appears there has been a shift in algal species dominance and the assemblage during this study was different from that noted in previous studies. The assemblage is different to that in Hartbeespoort dam (South Africa), a hyper-eutrophic reservoir, where *M. aeruginosa* forms the main component of algae (Wicks & Thiel 1990, van Ginkel *et al.* 2000)

In other African lakes like Tanganyika (Hecky & Kling 1981), Naivasha and Oloiden (Kalff & Watson 1986), Lanao (Lewis 1987) and Awasa (Kebede & Belay 1994), 103, 143, 94, 70 and 100 species were recorded respectively. Cronberg (1997) recorded 155 species in Lake Kariba. Lake Chivero has a lower number of species compared to other lakes, probably because it is eutrophic. Shannon diversity (H′) ranged between 0.1 and 0.95. In Zeekoevlei a low diversity of between 0.8 and 2.5 was attributed to persistent disturbance by wind (Harding 1996). From an analysis of highly nutrient enriched lakes, Harding (1996) observed that diversity ranged between 0 and 4.2. Diversity recorded in Lake Chivero falls within these ranges.

4.4.5 Chlorophyll *a* and total biomass

The seasonal pattern similar to that previously observed (Falconer 1973), in the 1960s was exhibited during the clear state with three peaks, coinciding with summer, winter and a hot dry period, i.e. the three growing seasons in the lake. The February – April 2003 peak occurred when cyanophytes dominated and the October peak when chlorophytes

dominated. Previously *Microcystis* dominated during the summer chlorophyll *a* peak, *A. granulata* during the early winter peak and *Anabaena* sp. during the spring peak (Robarts *et al.* 1982). Instead of *Anabaena*, cryptophytes and chlorophytes dominated during the hot dry period, and during the clear state the early winter peak was not distinct. Chlorophyll *a* concentration decreased between April and June when bacillariophytes were dominant.

The main chlorophyll *a* peak period is variable. During the clear state the highest peak occurred in October/November 2003, whereas it has also occurred in January (Falconer 1973), June and August (Thornton 1980). Algal self-shading prevents the build-up of high chlorophyll *a* levels (Robarts 1979) comparable to those in two other hyper-eutrophic systems, Hartbeespoort dam (NIWR 1985) and Zeekoevlei (Harding 1992). The patterns exhibited by chlorophyll *a* and biomass during the clear state were comparable. The slight differences can be attributed to uncounted species, as observed by Talling (1986) and Kebede & Belay (1994). In Zeekoevlei, Harding (1996) established that levels of chlorophyll *a* do not always reflect the temporal development of the phytoplankton biomass. Harding (1996) attributed that to the presence of picophytoplankton which are usually unaccounted for. During my study species like *Aphanocapsa* sp. and *Pseudoanabaena mucicola* were not enumerated. Since they can constitute a significant portion of total phytoplankton biomass (Padisák & Dokulil 1994) their exclusion can under estimate biomass.

The seasonal successional patterns observed during the clear state was linked to changes in the physico-chemical environment, mainly temperature and the supply of nutrients. In a similar system, Hartbeespoort Dam, *M. aeruginosa* tended to be persistently abundant from mid-October until around May (Robarts & Zohary 1984) and sometimes during winter as well (NIWR 1985), while chlorophytes, mainly *Oocystis* spp., appeared in spring and *A. granulata* for short periods in winter or early spring.

4.4.6 Other possible underlying mechanisms affecting community dynamics

This study showed that cyanobacteria dominance has declined and the environment in the Lake Chivero appears favourable for cryptophytes and chlorophytes, which can successfully exploit unstable environments. According to Haffner & McNeely (1989) non-equilibrium dynamics probably favour ruderal plants while equilibrium processes would favour specialists. Thus non-equilibrium conditions in the lake are leading to dominance by ruderals and cryptophytes. The temporal changes of the physical and chemical environment result in major changes in assemblage composition and abundance.

The phytoplankton assemblage in Lake Chivero has changed from one showing a predominance of cyanophytes and bacillariophytes. Three other groups, chlorophytes, cryptophytes and euglenophytes, now contribute to biomass and species composition. Seasonal variation in algal species composition and biomass was influenced by changes in lake chemistry as it responded to changes in lake level, thereby nutrient levels. It has been observed that nutrient dynamics can influence phytoplankton assemblages by limiting rates of growth (Harris 1986) and by affecting speciation and dominance patterns (Reynolds 1988). Seasonal changes in nutrients levels affected species succession and dominance in the lake during this study.

The system is however unstable such that patterns can vary each year. In comparison to large and deep lakes that are less susceptible to external disturbances (Salmaso 2002), it appears that Lake Chivero is susceptible to the influence of lake-level changes as it responds to hydrological regimes. Water replacement time may be a major factor causing instability. Lake Chivero has a short replacement time of 0.82 years (Marshall & Falconer 1973) thus the whole water mass can be replaced within a year. This affects the water chemistry such that nutrient levels in the lake can vary from year to year and this should have an effect on phytoplankton dynamics. This may explain the decline in dominance of cyanophytes in 2003 that favour stable environmental conditions (Geraldes & Boavida 2004). Rapid flushing can interfere with physico-chemical variability patterns,

which then affects phytoplankton assemblage structure. In large and deep lakes (e.g. Lake Kariba) with longer retention times phytoplankton patterns can be more predictable and similarities occur from year to year in abundance, distribution and composition of phytoplankton (Cronberg 1997). This does not appear to be the case in Lake Chivero, where the system seems to be unstable. In 2004 a persistent bloom occurred from June to December, a situation that was different from 2003.

There is however, no long-term phytoplankton record to adequately assess the instability and response of phytoplankton to perturbations. Comparison of the qualitative phytoplankton samples for 1961, 1983 and 2003/2004 only provided insights on indications of possible changes in phytoplankton assemblages. The current data showed the increasing role of cryptophytes and chlorophytes to biomass contribution and a seasonality pattern influenced by the lake chemistry.

CHAPTER 5

SPATIAL AND TEMPORAL DYNAMICS OF AN ALGAL BLOOM: OCCURRENCE, ABUNDANCE AND LIMNOLOGICAL ASPECTS

5.1 INTRODUCTION

Harmful algal bloom species must overcome four basic impediments to bloom: a temperature threshold, chemical restraint, interspecific competition and grazing losses (Smayda 1998 cited by Hallegraeff *et al.* 2003). They succeed by breaking these constraints (O'Neill *et al.* 1986). In Lake Chivero over a 23-month period from February 2003 to December 2004 an algal bloom occurred only over an 8-month period from May to December 2004 (Chapter 4). This indicates that for the rest of the period conditions were not ideal for blooms. When the bloom developed from May 2004 the process was monitored monthly until December 2004. The objective of the study was to ascertain how the bloom developed and progressed through initiation, accumulation, stationary phase and termination and to determine whether the pattern was uniform throughout the lake.

Factors that influence bloom dynamics include (i) habitat heterogeneity and variability (physical habitat) and (ii) chemical habitat – a complex mixture of macronutrients (especially nitrogen and phosphorus) and micronutrients (iron and other trace metals) (Smayda 1998 cited by Hallegraeff *et al.* 2003). The nutrient environment is naturally variable and is subject to seasonal modifications and this influences species selection, abundance and dynamics. The factors that influence occurrence of algal blooms in Lake Chivero have not been established. In this chapter the nature of the natural occurrence of an algal bloom is discussed based on an observational study undertaken to show the sequences of multiple casual factors governing bloom development.

When tracking the dynamics of potentially harmful species during a bloom, it is recommended to also study other members of the assemblage present. Thus during this study co-occurring species and their abundances were determined. The underlying focus

of this study was to understand the conditions under which blooms developed in the lake, especially understanding the links between the physical and chemical environment and occurrence of blooms. Nutrient chemistry during the bloom was assessed to determine how it regulated the bloom, since according to Smayda (1998 cited by Hallegraeff *et al.* 2003) a change in the chemical nature of the water may be a more significant bloom stimulus than reduced turbulence. The information generated is of interest because it increases knowledge on the success and development of cyanobacteria in hyper-eutrophic waters, where they are a very important group (Sakamoto & Okino 2000, Von Rückert & Giani 2004).

The objectives of the study were:

(i) to assess the spatial and temporal dynamics and vertical distribution of algae during the bloom

(ii) to determine bloom species behaviour, variability and regulation

(iii) to determine accompanying physical and chemical habitat characteristics that favoured the bloom

5.2 MATERIALS AND METHODS

Physical, chemical and biological characteristics of a reservoir exhibit distinct longitudinal gradients linked to the reservoir's river-lake hybrid nature (Kimmel *et al.* 1990). These gradients were characterized by selecting five sampling sites (Figure 5.1) based on lake morphology, mainly depth and location. Station 1 representing the deep zone was approximately 20 m deep, while Stations 2 and 3 were in shallow creeks with a maximum depth of approximately 5 m and receiving inflows from small seasonal streams. Station 4, classified as the mid-lake station, is approximately 10 m deep while Station 5, the riverine station, was about 5 m deep and is located within the Manyame River.

Sampling was conducted monthly between May and December 2004 following the onset of the algal bloom. Further sampling was conducted in February, May and November

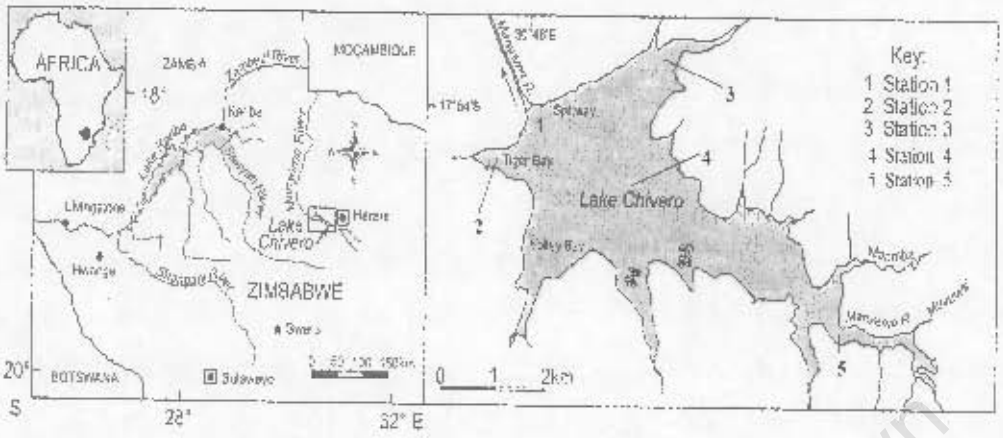


Figure 5.1 Map of Lake Chivero showing the sampling stations (Source: modified from Mhlanga *et al.* 2006)

2005 and in April 2006 after the bloom had collapsed. At stations 2, 3, 4 and 5 (Figure 5.1) water from 0, 1, 2, 3, 4 and 5 m depth intervals was collected with a Ruttner sampler and pooled together for the measurement of chlorophyll *a*, phytoplankton biomass, temperature, dissolved oxygen, pH, conductivity, total dissolved solids, turbidity, orthophosphate, total phosphorus, nitrates, ammonium and total nitrogen. At Station 1, the deepest part of the lake, samples were collected from the following depth intervals: 0, 5, 10, 15, 20 m in order to establish the vertical distribution of phytoplankton and the change in physical and chemical parameters within the water column.

Qualitative phytoplankton samples were taken with a plankton net (mesh size 10 µm) and preserved in formalin acidified with acetic acid. Water transparency was measured at each site using a Secchi disk. The euphotic zone (Z_{eu}) was calculated from the Secchi depth (Z_{SD}), where $Z_{eu} = 2.5\ Z_{SD}$: Lemmin (1995). Physical and chemical parameters and phytoplankton biomass were analysed as described in Chapters 3 and 4 respectively. Chlorophyll *a* concentration was assessed by the acetone extraction method described in Chapter 4.

5.2.1 Data analysis

The non-parametric Kruskal-Wallis ANOVA test was used to determine temporal and spatial differences of variables at Stations 2, 3, 4 and 5. A univariate ANOVA was used to test for differences of physical and chemical parameters and algal biomass with respect to depth and date of sampling at Station 1. The relationships between the variables were analysed with Spearman correlations. To examine differences in assemblage composition at the four sites (2, 3, 4, 5) Principal Components and Classification Analysis (PCCA) of phytoplankton species and environmental variables was employed. Ordination of stations/sampling dates was based on biomass of each species (natural log transformed) and the resulting correlation matrix from PCCA. All analysis was done using Statistica 7.

5.3 RESULTS

5.3.1 Characteristics of the physical variables during the bloom

Only pH was significantly different (Kruskal-Wallis ANOVA, $p < 0.05$) among the stations while temperature, dissolved oxygen, conductivity, total dissolved solids, turbidity, secchi depth and euphotic depth were not significantly different (Kruskal-Wallis ANOVA, $p > 0.05$, Table 5.1). All the physical parameters varied significantly (Kruskal-Wallis ANOVA, $p < 0.05$) between onset and end of bloom (Table 5.1). Temperature initially decreased from 22.8 °C in May to 17.2 °C in July after which it increased and reached 24.6 °C in December (Figure 5.2a). Dissolved oxygen varied between 1.6 and 11.6 mg l^{-1}, with lower levels at the onset of the bloom and highest levels by November (Figure 5.2b). The water pH was lower in May and June but increased to a maximum average of 8.8 in November at the peak of the bloom (Figure 5.2c). Conductivity varied between 396 and 546 µS cm^{-1} and increased gradually from June to December (Figure 5.2d). The concentration of total dissolved solids increased markedly from 167 mg l^{-1} in May to 213 mg l^{-1} by December (Figure 5.2e). Turbidity increased from 4.6 NTU at the onset of the bloom to 30 NTU by November (Figure 5.2f). Secchi disc transparency was 2 m in May but dropped to 0.8 m between October and

November (Figure 5.3a). The euphotic zone was deepest between May and September (mean = 3 ± 0.8 m) and decreased to about half between November and December (1.5 ± 0.2 m) (Figure 5.3b).

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Dissolved oxygen, temperature, conductivity, total dissolved solids, turbidity and pH within the water column varied significantly (ANOVA, $p < 0.05$) with depth and by month (Table 5.2). Marked changes in dissolved oxygen concentration (Figure 5.4) and pH (Figure 5.6a) occurred at 0 and 5 m depth intervals while at 10, 15 and 20 m the levels remained relatively constant. The 0 to 5 m depth zone had the highest dissolved oxygen levels (Figure 5.4) and pH throughout the bloom period that fluctuated in response to changes in algal biomass. The difference in dissolved oxygen concentrations and pH between the surface and bottom was highest between September and November, which was the period of highest chlorophyll *a* concentration (Figure 5.10) and algal biomass (Figure 5.12). In November the pH at 0 and 20 m was 9.6 and 7.2 respectively. Two zones could be distinguished down the water column: the upper 5 m with highest but constantly fluctuating dissolved oxygen concentrations and pH, and from 10 to 20 m with lower and relatively uniform changes (Figure 5.4 & Figure 5.6a respectively).

The lake was stratified for most of the period except in June, when it was isothermal (Figure 5.5) with the strongest stratification in September (surface to bottom difference T = 4.7 °C). Conductivity was relatively uniform down the water column and increased slightly with depth (Figure 5.6b). There was a gradual increase in conductivity (Figure 5.6b) and total dissolved solids (Figure 5.6c) within the water column from the onset of the bloom in May until maximum values were attained in December. Increase in conductivity at the surface occurred from 404 to 496 $\mu S\ cm^{-1}$ and at 20 m from 448 to 534 $\mu S\ cm^{-1}$ between May and December. Total dissolved solids levels at the surface increased from 165 mg l^{-1} to 203 mg l^{-1} and at the bottom from 184 to 218 mg l^{-1} respectively between May and December. Both conductivity and total dissolved solids concentration was higher at 20 m depth than at the surface. Turbidity was relatively uniform down the water column between May and September, with slightly higher levels

at 5 m (Figure 5.6d). It then increased at all depth intervals between October and December with a highest increase from 20 NTU to 75 NTU at 20 m depth.

Concurrent with the accumulation of algae in the upper surface layers (0-5 m) was a decrease in transparency at Station 1. The transparency prior to the onset of the bloom was 2.5 m but dropped to > 1m at the peak of the bloom; after the bloom collapsed transparency increased to 2 m in April 2006.

5.3.2 Characteristics of the chemical variables during the bloom

Only ammonium and total phosphorus varied significantly (Kruskal-Wallis ANOVA, $p < 0.05$) among the four stations (Table 5.1). All the chemical parameters differed significantly (Kruskal-Wallis ANOVA, $p < 0.05$) between onset and collapse of the bloom (Table 5.1).

Initially as the algal biomass increased in the lake orthophosphate concentration decreased from 0.7 mg l^{-1} in May to 0.3 mg l^{-1} in September, after which it increased to 1.2 mg l^{-1} by December (Figure 5.7a). Total phosphorus exhibited a pattern similar to that of orthophosphate, with a decline between May and September followed by a gradual increase from October (Figure 5.7b). Nitrate concentrations exhibited an interesting and marked feature (Figure 5.7c). The concentration rapidly declined between onset and end of bloom, from an average concentration of 0.9 mg l^{-1} in May to 0.3 mg l^{-1} in December (Figure 5.7c). The built-up in chlorophyll *a* concentration seemed to have been linked to the rapid decline in nitrate concentration. When a minimum average nitrate concentration of 0.1 mg l^{-1} was reached in November chlorophyll *a* had reached a maximum average concentration of 59.7 µg l^{-1}, with higher concentrations of 92.8 µg l^{-1} and 80 µg l^{-1} at Stations 4 and 9 respectively (Figure 5.10).

Table 5.1 **Kruskal-Wallis Anova test for differences in physical, chemical and biological characteristics during the bloom period at stations 2, 3, 4 and 5. These data are for the period May to December 2004. Figures marked with * are significant at $p < 0.05$ while the rest are not.**

Variable	Spatial variation among stations 2, 3, 4 and 5	Temporal variation between onset (May) and end (December) of bloom
Conductivity	H = 4.824 P = 0.185	H = 24.570 ***p = 0.0009****
Turbidity	H = 1.719 P = 0.633	H = 25.488 ***p = 0.0006****
Total dissolved solids	H = 3.919 P = 0.270	H = 25. 937 **p = 0.001***
Secchi disc transparency	H =1.432 P = 0.698	H = 20.844 ***p = 0.004****
Euphotic depth	H = 1.489 P = 0.685	H = 20.754 ***p = 0.004****
pH	H = 8.9057 ***P = 0.031****	H = 15.464 ***p = 0.030****
Dissolved oxygen	H = 2.775 P = 0.428	H = 15.272 ***p = 0.033****
Temperature	H = 0.338 P = 0.953	H = 28.662 ***p = 0.0002****
Nitrates	H = 0.326 p = 0.955	H = 28.083 ***p = 0.000****
Ammonium	H = 8.053 ***p = 0.045****	H = 17.380 ***p = 0.015****
Total nitrogen	H = 0.522 p = 0.914	H = 23.379 ***p = 0.002****
Orthophosphate	H = 5.374 p = 0.146	H = 17.884 ***p = 0.013****
Total phosphorus	H = 9.652 ***p = 0.022****	H = 15. 818 ***p = 0.027****
TN:TP ratio	H = 6.463 p = 0.0911	H = 14.488 ***p = 0.043****
Chlorophyll *a*	H = 2.534 p = 0.469	H = 20.579 ***p = 0.004****
Biomass	H = 6.178 p = 0.186	H = 20.164 ***p = 0.010****

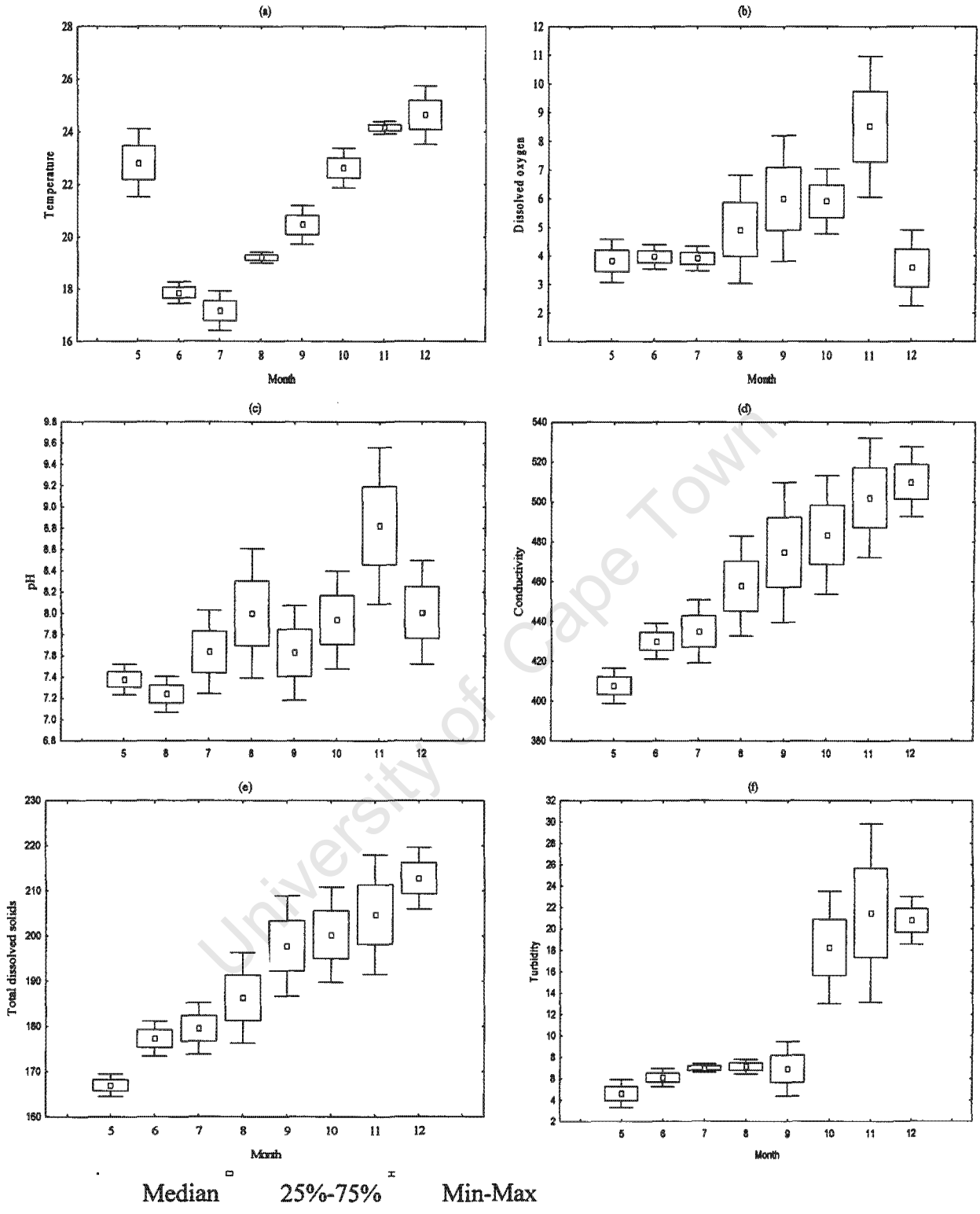


Figure 5.2 **Box–and-whisker of (a) temperature ^{o}C, (b) dissolved oxygen mg l^{-1}, (c) pH, (d) conductivity µS cm^{-1}, (e) total dissolved solids mg l^{-1} and (f) turbidity NTU during the bloom period in Lake Chivero (May – December 2004). Key (5 = May ……..12 = December). Data are mean of 4 stations, n =36. Note the different scales on Y-axis.**

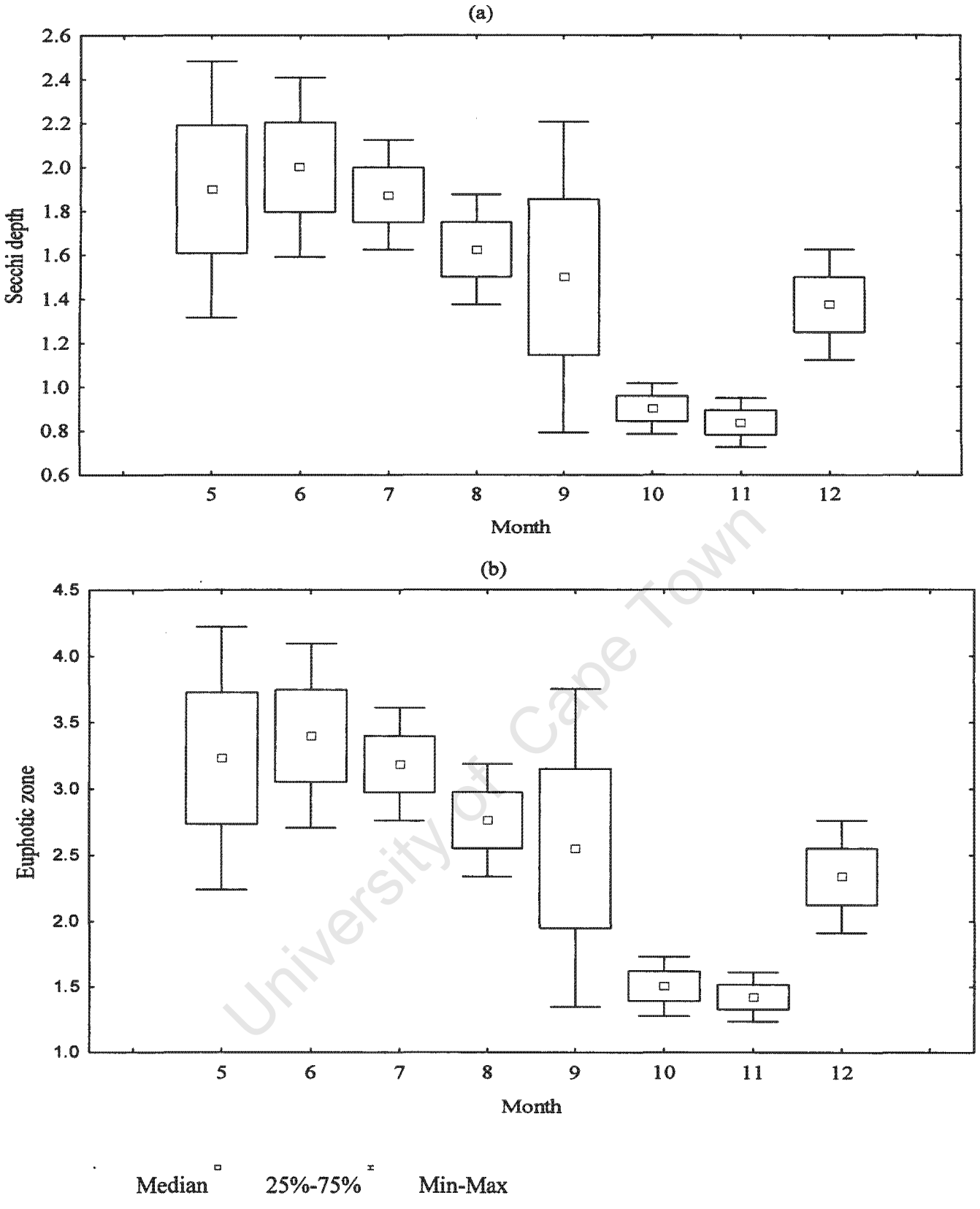


Figure 5.3 **Box-and-whisker plot of (a) Secchi depth (m) and (b) euphotic depth (m) during the bloom period in Lake Chivero (May – December 2004). Key (5 = May .....12 = December). Data are mean of 4 stations, n =36. Note the different scales on Y-axis.**

Table 5.2 **Anova test for differences in physical, chemical and biological characteristics during the bloom period down the water column at station 1. These data are for the period May to December 2004. Figures marked with * are significant at p < 0.05 while the rest are not.**

Variable	Variation with depth	Variation by month
Conductivity	F = 20.8 ***p = 0.00****	F = 139.5 ***p = 0.00****
Turbidity	F = 3.20 ***p = 0.03****	F = 8.38 ***p = 0.00****
Total dissolved solids	F = 24.5 ***p = 0.03****	F = 8.381 ***p = 0.00****
pH	F = 16.19 ***p = 0.00****	F = 4.13 ***p = 0.00****
Dissolved oxygen	F = 14.204 ***p = 0.00****	F =2.370 ***p = 0.05****
Temperature	F = 12.73 ***p = 0.00****	F = 30.94 ***p = 0.00****
Nitrates	F = 3.954 ***p = 0.01****	F 94.278 ***p = 0.00****
Ammonium	F = 6.950 ***p = 0.00****	F = 11.219 ***p = 0.00****
Total nitrogen	F = 1.17 p = 0.34	F = 8.155 ***p = 0.00****
Orthophosphate	F = 11.01 ***p = 0.00****	F = 11.66 ***p = 0.00****
Total phosphorus	F = 6.35 ***p = 0.00****	F = 9.96 ***p = 0.00****
TN:TP ratio	F = 3.448 **p = 0.021***	F = 6.971 ***p = 0.00****
Chlorophyll *a*	F = 3.79 ***p = 0.01****	F = 0.60 p = 0.75

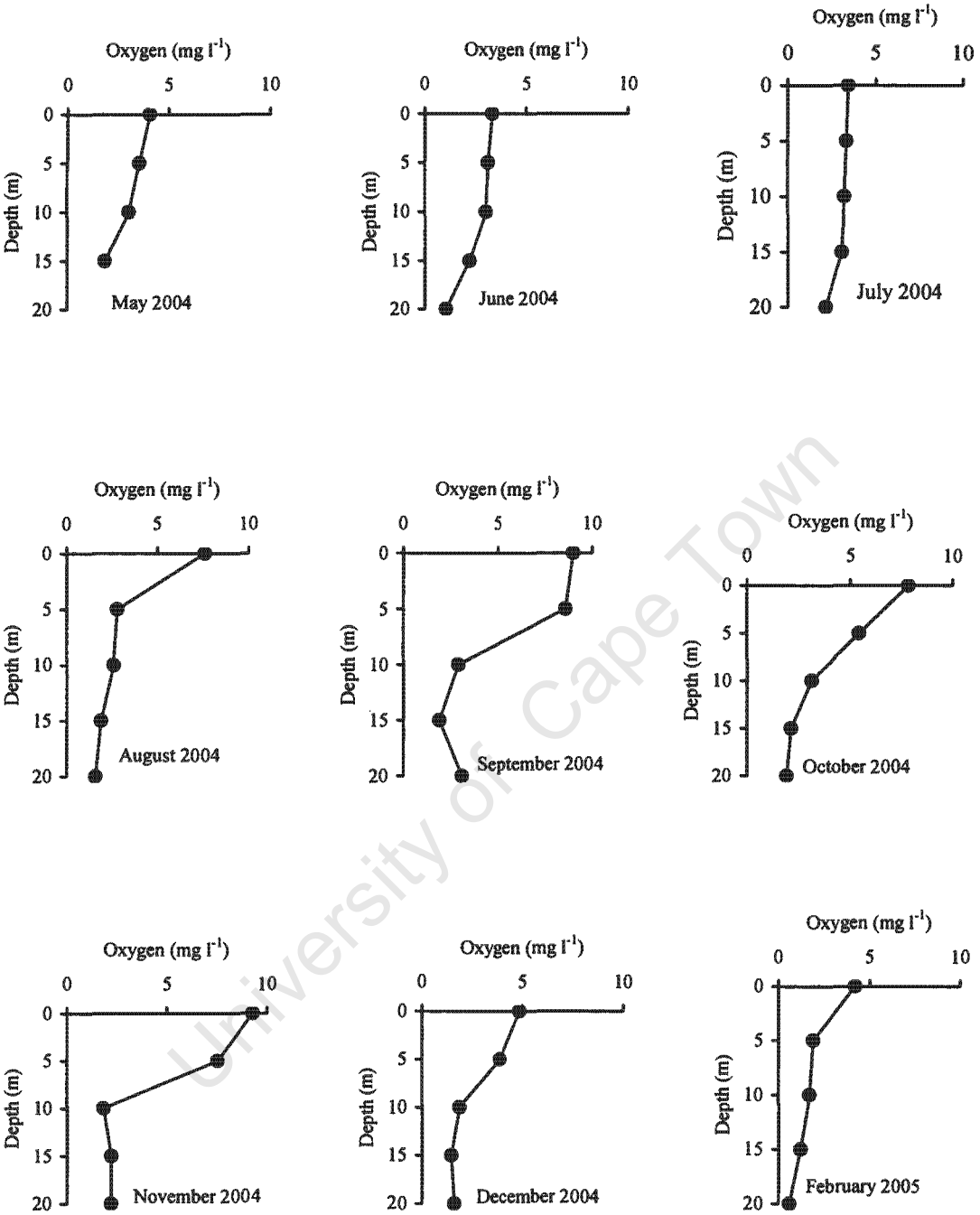


Figure 5.4 **Depth profiles of dissolved oxygen at Station 1 during the bloom period (May – December 2004) and after the bloom (February 2005).**

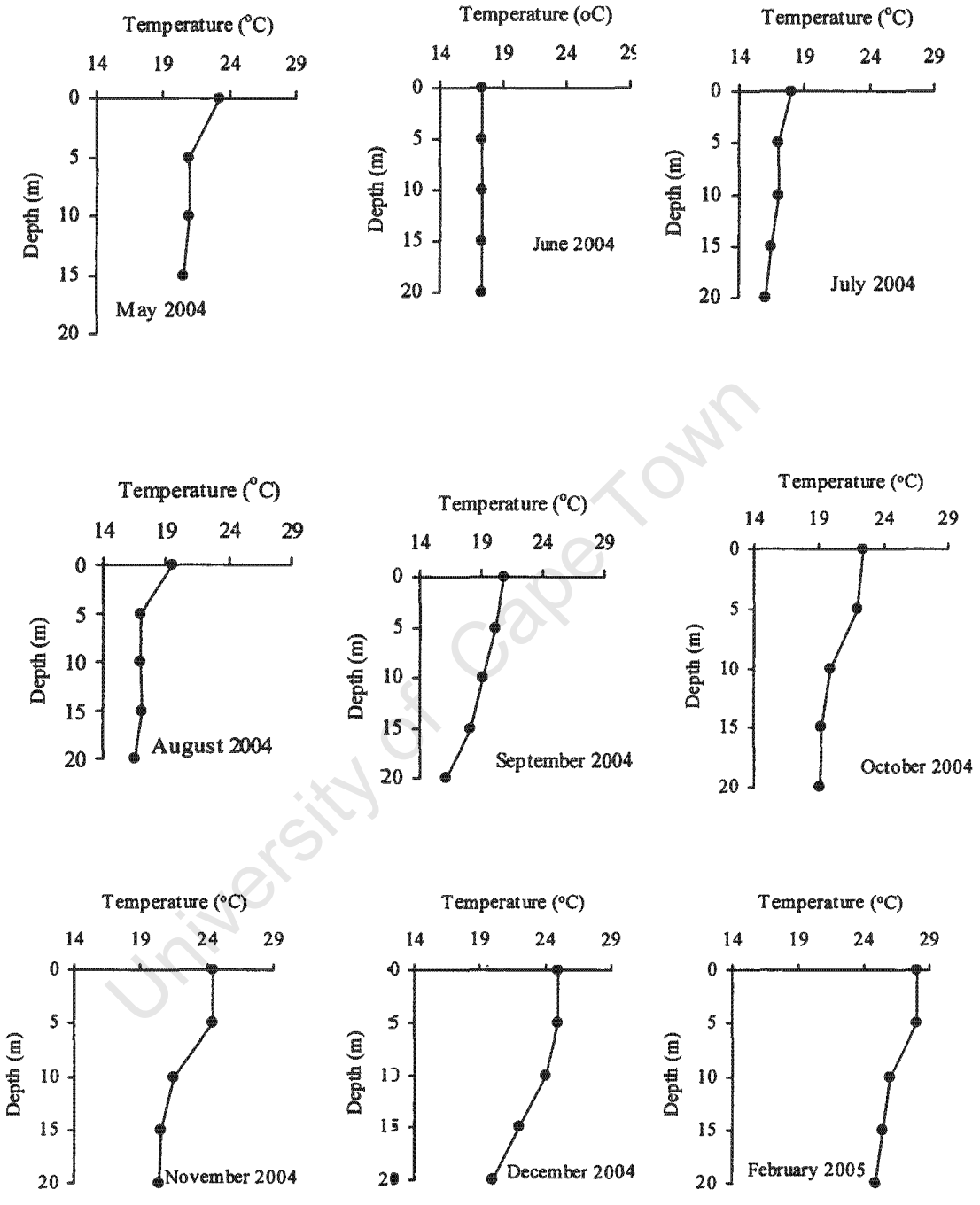


Figure 5.5 **Depth profiles of dissolved oxygen at Station 1 during May to December 2004 and after the bloom (February 2005).**

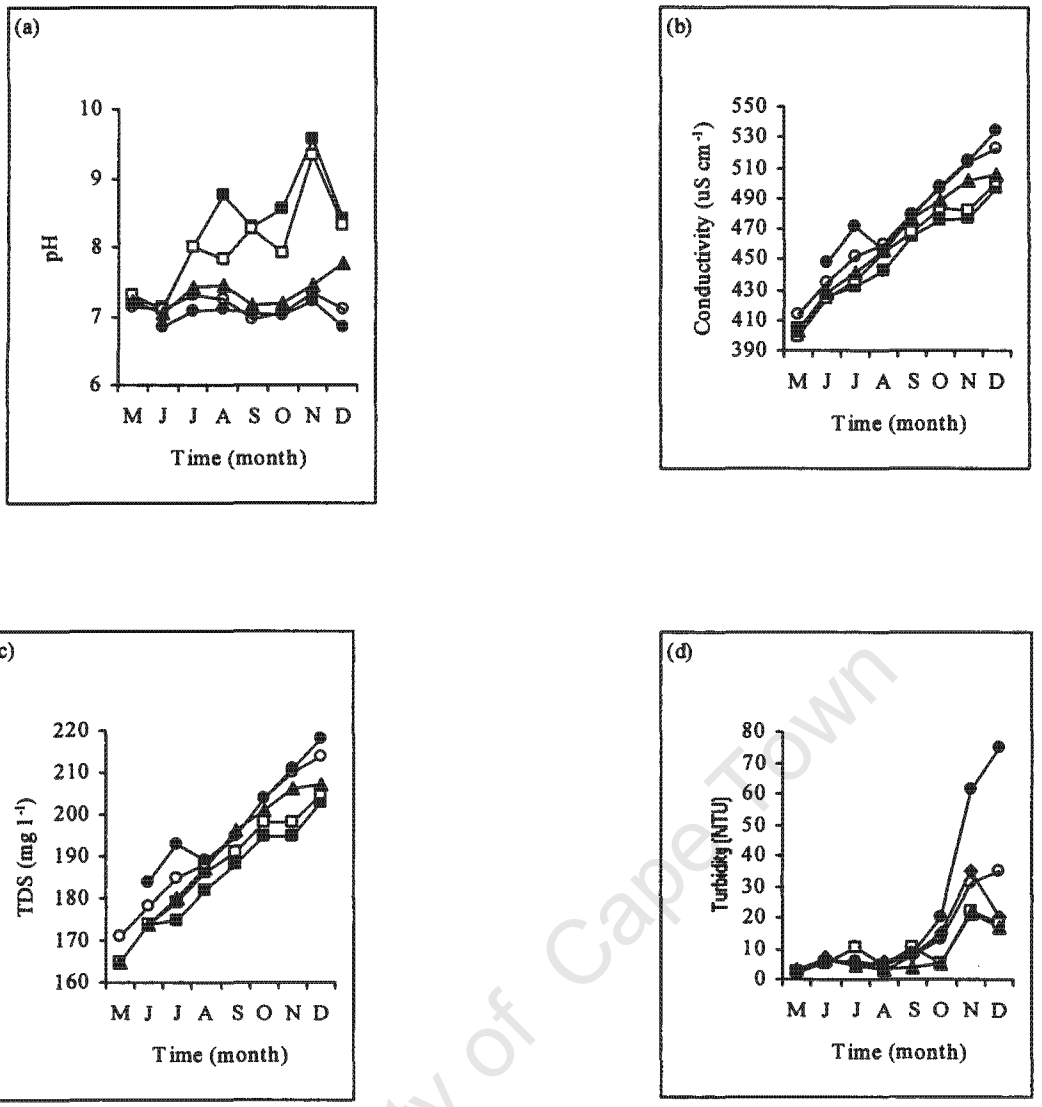


Figure 5.6 **Temporal variation in (a) pH, (b) conductivity µS cm^{-1}, (c) total dissolved solids mg l^{-1} and (d) turbidity in NTU within the water column at Station 1 in Lake Chivero during an algal bloom, May – December 2004. ■ 0 m; □ 5 m; ▲ 10 m; ○ 15 m; ● 20 m. Note the different scales on Y-axis.**

The average ammonium concentration at the onset of the bloom was 0.3 mg l^{-1} but increased and reached the highest average concentration of 2.1 mg l^{-1} by November and a maximum concentration of 4.4 mg l^{-1} at Station 5 and a minimum concentration of 0.9 mg l^{-1} at Station 4 (Figure 5.7d). Total nitrogen concentration did not exhibit a discernable pattern (Figure 5.7e) but constantly fluctuated. The average concentration of 9.2 mg l^{-1} was recorded in May and by December the average concentration was 15.2 mg l^{-1}. The concentration in the lake ranged between 5.8 mg l^{-1} and 22.1 mg l^{-1}. The TN:TP ratio ranged between 6.6 and 31.4 during the bloom period and did not exhibit a discernable pattern but fluctuated in a similar pattern to total nitrogen (Figure 5.7e). The average at the onset of the bloom was 11.1 and 12.9 in December. TN:TP was always above 10.

Nitrate, ammonium, orthophosphate, total phosphorus and TN:TP ratio differed significantly (ANOVA, $p < 0.05$, Table 5.2) with depth while total nitrogen was relatively uniform down the water column. A decrease of orthophosphate and total phosphorus concentration occurred within the water column from highest average concentrations in May at the onset of the bloom until August when an increase occurred reaching a peak in November for orthophosphate and December for total phosphorus (Figure 5.8a, Figure 5.8b respectively). Total phosphorus was highest at 20 m between May and August, after which higher levels were recorded at 15 m. Orthophosphate was also generally higher at 20 m depth than at the surface. Nitrate decreased sharply within the water column following the onset of the bloom, from a highest average concentration of 2 mg l^{-1} in July to the lowest average level of 0.3 mg l^{-1} in December (Figure 5.8c). Nitrate concentration was slightly higher within the 0 m to 5 m depth and lowest at 20 m depth. Ammonium constantly fluctuated within the water column (Figure 5.8d). It was higher between 10 and 20 m than between 0 and 5 m. Ammonium concentration increased at all depth intervals during the course of the bloom. The highest increase from 1.3 mg l^{-1} in June to 4.4 mg l^{-1} in November occurred at 20 m depth after which ammonium levels markedly dropped down the water column. A summary of the condition in the lake during the bloom period is shown in Table 5.3.

5.3.3 Spatial and temporal variation of chlorophyll *a* during and after the bloom

A gradual built-up of chlorophyll *a* occurred until a maximum concentration was attained in November (Figure 5.10). The built-up of chlorophyll *a* was relatively uniform in the lake except for the marked variability in October and November (Figure 5.10). In November spatial variability in chlorophyll *a* concentration occurred as follows: Station 2 > Station 5 > Station 4 with the lowest concentration at station 3. The average concentration during the bloom period was 20.3 $\mu g\ l^{-1}$. The maximum concentrations attained in November varied significantly (Kruskal-Wallis ANOVA, $p < 0.05$) among the stations, with a highest concentration of 92.8 $\mu g\ l^{-1}$ at station 2 and a lowest concentration of 17.8 $\mu g\ l^{-1}$ at station 3 while concentrations at stations 4 and 5 then were 48.1 $\mu g\ l^{-1}$ and 80 $\mu g\ l^{-1}$ respectively.

Table 5.3 The summary of physical, chemical and biological variables measured in Lake Chivero during an algal bloom, May – December 2004. (n for each variable = 32). (Values are means of 4 stations ±sd)

Variable	Maximum value	Minimum value	Average ± sd
Water temperature (°C)	25.5	16.2	21.1 ± 2.8
Conductivity ($\mu S\ cm^{-1}$)	546	396	463 ± 40
Secchi disk (m)	2.5	0.75	1.5 ± 0.54
Turbidity (NTU)	30	3.1	11.5 ± 7.6
Total dissolved solids ($mg\ l^{-1}$)	224	164	191 ± 17
Dissolved oxygen ($mg\ l^{-1}$)	11.62	1.61	5.1 ± 2.1
pH	9.67	7	7.8 ± 0.6
Nitrate ($mg\ l^{-1}$)	1.12	0.04	0.59 ± 0.34
Ammonium ($mg\ l^{-1}$)	4.40	0.02	0.68 ± 0.08
Total nitrogen ($mg\ l^{-1}$)	22.1	5.8	13.43 ± 3.52
Orthophosphate ($mg\ l^{-1}$)	1.21	0.29	0.62 ± 0.21
Total phosphorus ($mg\ l^{-1}$)	1.59	0.5	0.84 ± 0.28
TN:TP	31.4	6.58	17.08 ± 5.97
Chlorophyll a ($\mu g\ l^{-1}$)	92.8	1	20.49 ± 21.56

There was a significant difference (Kruskal-Wallis ANOVA, $p < 0.05$) in chlorophyll *a* concentration between onset and collapse of the bloom (Table 5.1).

Chlorophyll *a* concentration significantly correlated with pH ($r = 0.44$, $n = 32$, $p < 0.05$), dissolved oxygen ($r = 0.672$, $n = 32$, $p < 0.05$) and TN:TP ratio ($r = 0.348$, $n = 32$, $p < 0.05$) and negatively correlated with nitrate concentrations ($r = -0.366$, $p < 0.05$, $n = 32$). The vertical profiles of chlorophyll *a* concentration exhibited a close relation with the changes in dissolved oxygen ($r = 0.48$, $p < 0.05$, $n = 40$) and pH ($r = 0.47$, $p < 0.05$, $n = 40$) and correlated negatively with orthophosphate ($r = -0.363$, $p < 0.05$, $n = 40$). The highest chlorophyll *a* concentration occurred between the 0 and 5 m depth zone. Chlorophyll *a* concentration was notably higher at the surface (Figure 5.11) between July and October. As the surface concentration built-up during this period, a decline occurred at other depths intervals. A marked "deep" was observed at other depths, especially at 5 m, when a peak occurred at 0 m in August. Chlorophyll *a* occurred down the whole water column indicating that phytoplankton was present down the whole water column. Chlorophyll *a* concentration differed significantly (ANOVA, $F = 3.786$ $p < 0.05$) with depth.

The vertical distribution of chlorophyll *a* at Station 1 between May and December 2004 is shown in Figure 5.11. Chlorophyll *a* displayed a similar pattern to algal biomass. The concentrations during the bloom period were highest between 0-5 m and decreased with depth. The highest surface chlorophyll *a* concentration was attained in August (55.7 mg l^{-1}) at station 1 and at this stage most of the phytoplankton biomass had accumulated at the surface since from 5 m the concentrations were very low. After the collapse of the bloom chlorophyll *a* concentration was relatively uniformly distributed down the water column although levels were higher at the surface. This was particularly so in May 2005 where the concentration was very high and uniform down the water profile, being distinctly different from the other months.

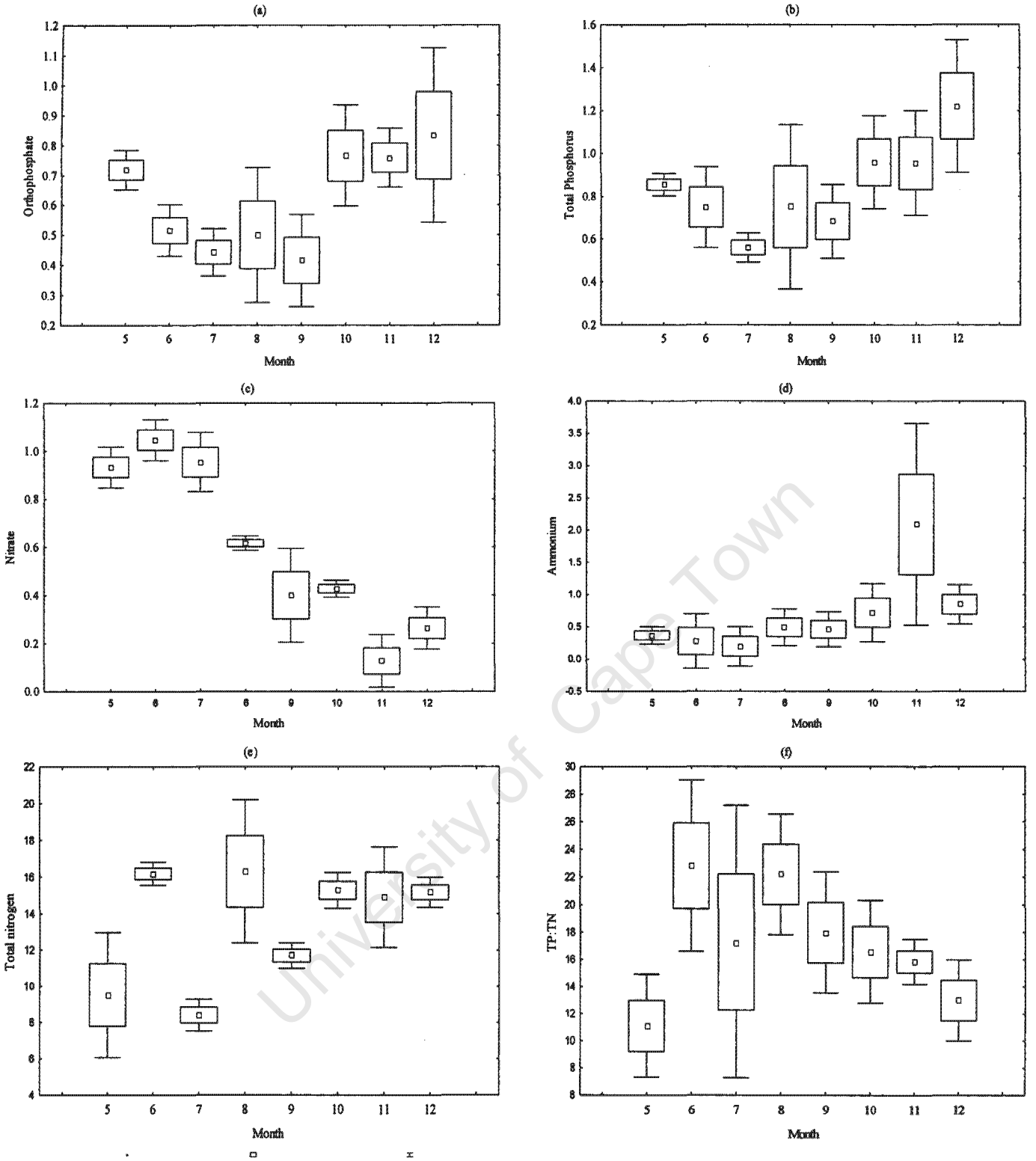


Figure 5.7 **Box-and-whisker plot of (a) orthophosphate mg l^{-1} (b) total phosphorus mg l^{-1} (c) nitrate mg l^{-1} (d) ammonium mg l^{-1} (e) total nitrogen mg l^{-1} and (f) TN:TP ratio during the bloom period in Lake Chivero (May – December 2004). Key (5 = May ……..12 = December). Data are mean of 4 stations, n =36. Note the different scales on Y-axis.**

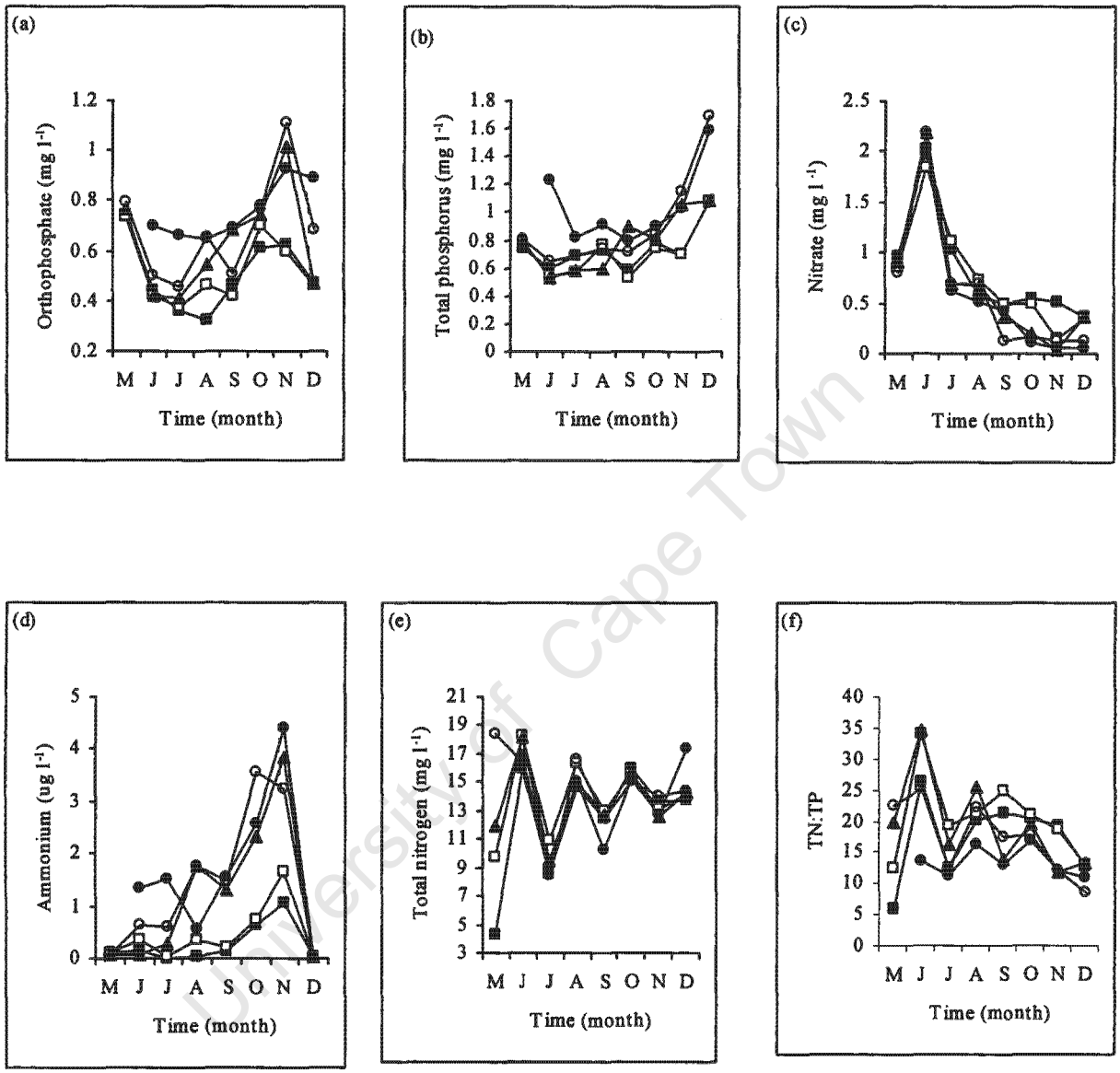


Figure 5.8 **Temporal variation of (a) orthophosphate mg l^{-1} (b) total phosphorus mg l^{-1} (c) nitrate mg l^{-1} (d) ammonium mg l^{-1} (e) total nitrogen mg l^{-1} and (f) TN:TP ratio within the water profile at Station 1 in Lake Chivero during an algal bloom, May – December 2004. ■ 0 m; □ 5 m; ▲ 10 m; ○ 15 m; ● 20 m. Note the different scales on Y-axis.**

5.3.4 Structure of the phytoplankton assemblage during the bloom

The temporal dynamics of the phytoplankton biomass during the bloom period is shown in Figure 5.12. There were no significant differences in biomass among the stations (Kruskal-Wallis Anova, $p > 0.05$, Table 5.1) but the biomass varied significantly between (Kruskal-Wallis Anova, $p < 0.05$, Table 5.1) onset and end of bloom. Biomass was lowest (1.1 mg l^{-1}) in May at the commencement of the bloom but increased and attained an average biomass of 6.7 mg l^{-1} in October (Figure 5.12). Diatoms dominated the phytoplankton assemblage in May and June after which *Microcystis* started to increase in dominance (Figure 5.13).

The pattern of phytoplankton biomass increase and the change in the algal assemblage was uniform and similar at all stations (Figure 5.13) except in November when the phytoplankton assemblage at stations 2 and 3 comprised only *Microcystis*. Marked differences in phytoplankton biomass were observed in November with the highest (11.3 mg l^{-1}) at station 2.

There were significant differences in biomass distribution with depth (ANOVA, $F = 10.4$, $p < 0.05$) with high biomass concentrated within 0-5 m depth and the lowest biomass at 20 m (Figure 5.14). This pattern was similar throughout the bloom period. During the cold dry winter period (May to August) the highest biomass contribution within the water column was by two diatoms, *A. granulata* and *Cyclotella* sp., which contributed over 80% of the total biomass at all depth intervals. In August *Coelastrum* attained a high biomass of 7.8 mg l^{-1} at 0 m depth. The importance of diatoms declined from August after which *Cryptomonas*, *Microcystis*, *Coelastrum* and *Gleocystis* started to increase in importance. Highest *Microcystis* biomass at 0 m was attained in September, after which it remained the dominant species until December. In September *Microcystis* had attained a biomass of 6.7 mg l^{-1} while *Cryptomonas* had a biomass of 1.5 mg l^{-1}. *Microcystis* and *Cryptomonas* were dominant within 0-5 m depth. In November and December *Microcystis* was dominant within 0-5 m depth while other taxa were negligible. *Microcystis* also constituted the scant biomass recorded from 10 to 20 m.

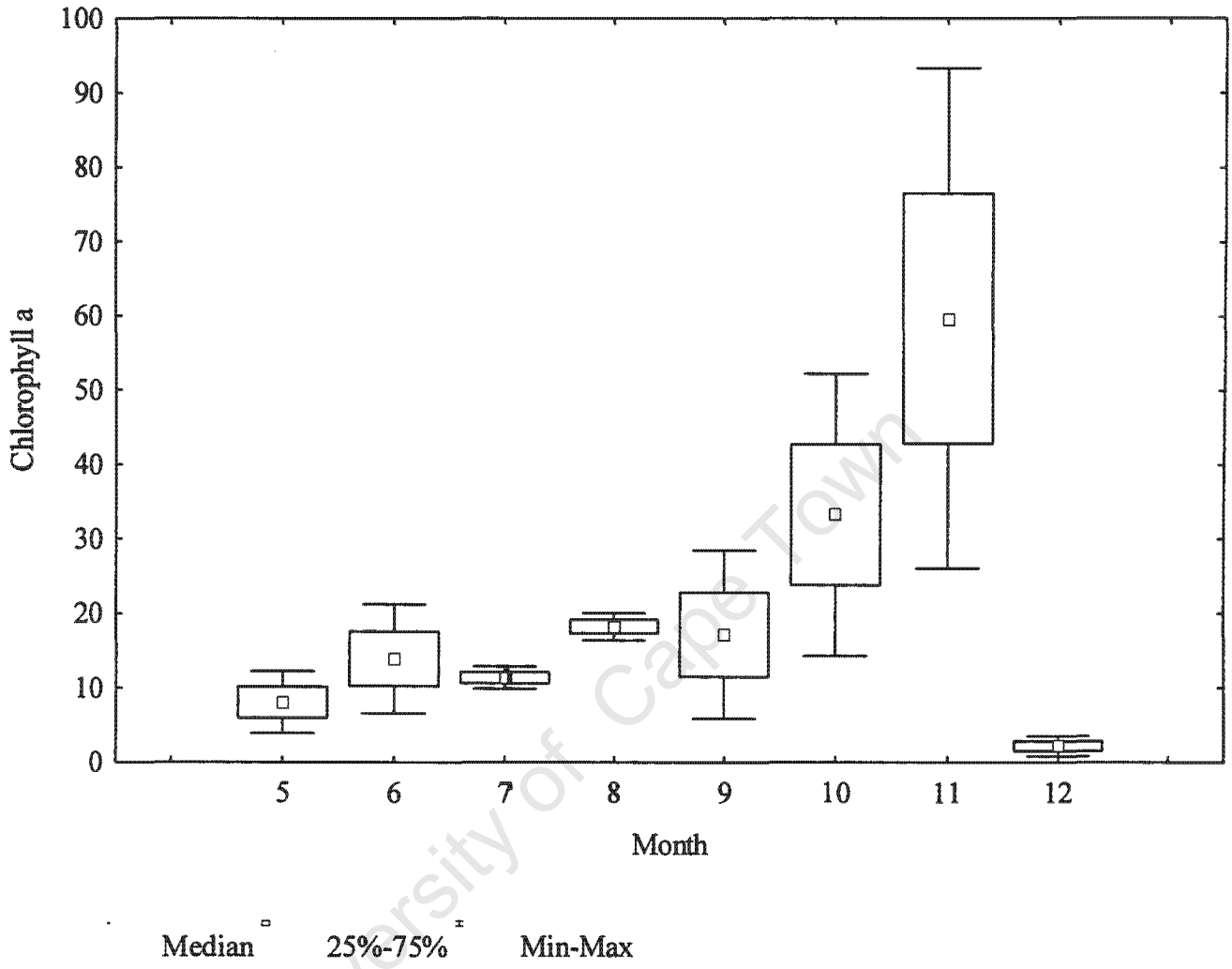


Figure 5.10 **Variation of chlorophyll *a* concentration in Lake Chivero during an algal bloom, May – December 2004. (5 = May ……..12 = December). Data are mean of 4 stations.**

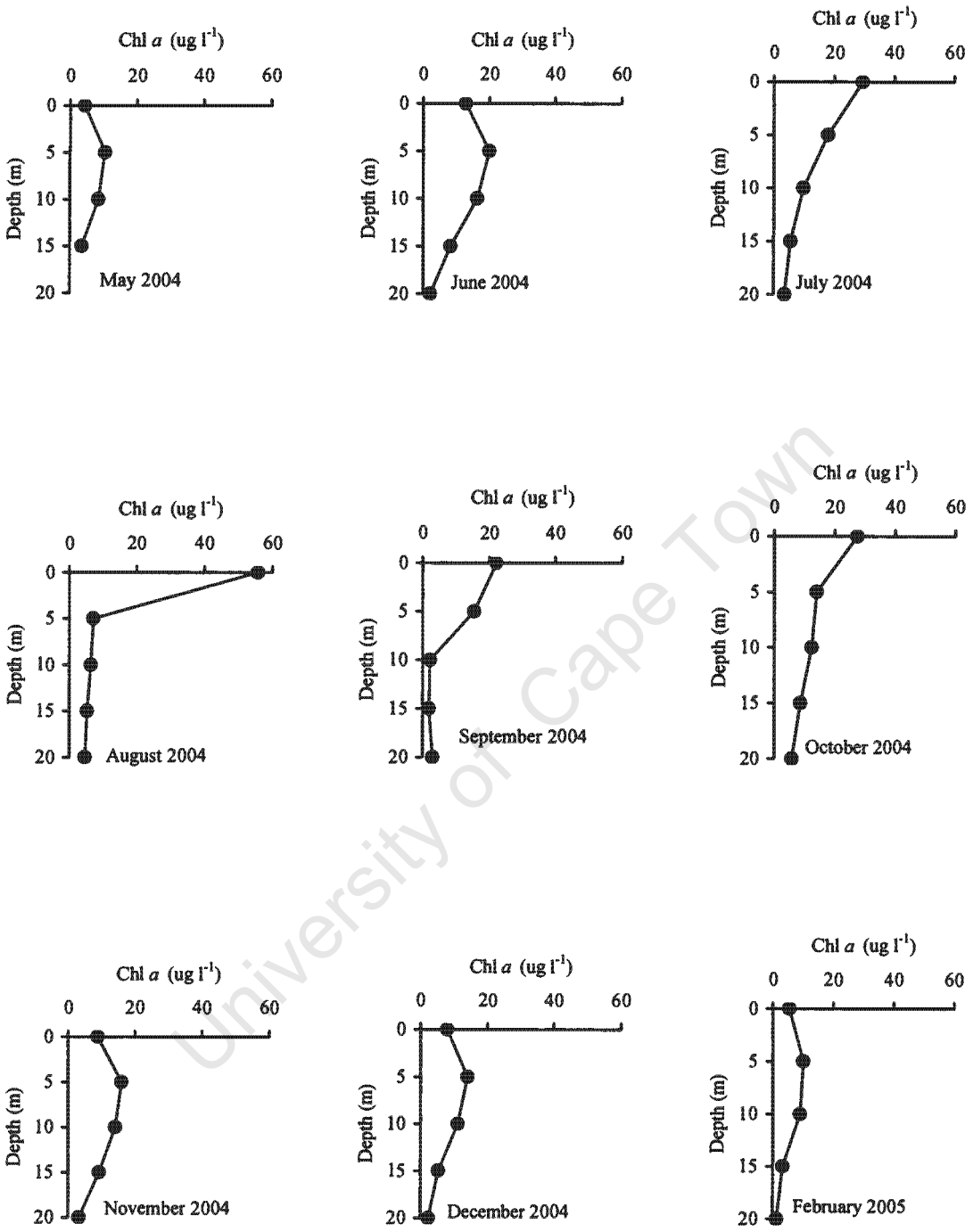


Figure 5.11 Depth profiles of chlorophyll *a* at Station 1 during (May – December 2004) and after the bloom (February 2005).

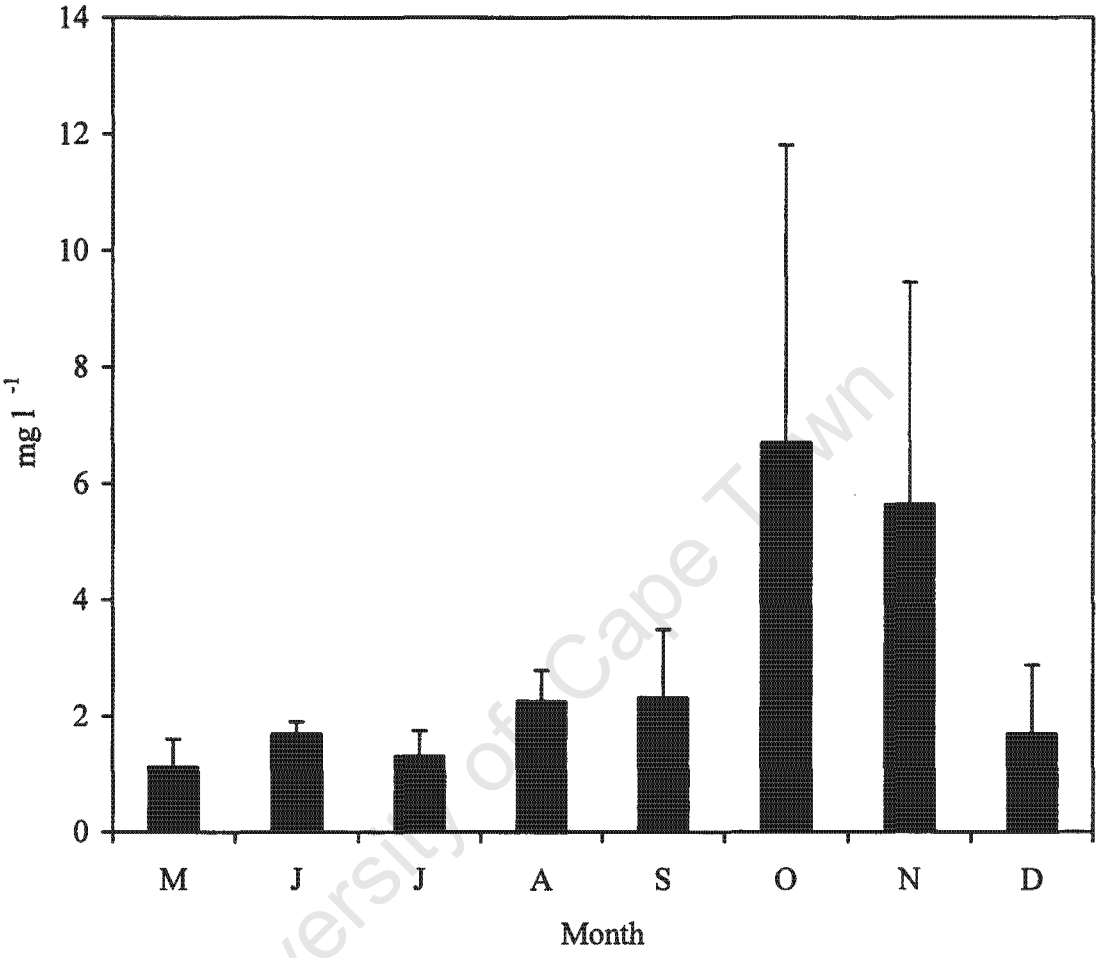


Figure 5.12 **Temporal variation of phytoplankton biomass in Lake Chivero during an algal bloom, May – December 2004. Data are mean of integrated samples (0-5 m) at stations 2, 3, 4 and 5.**

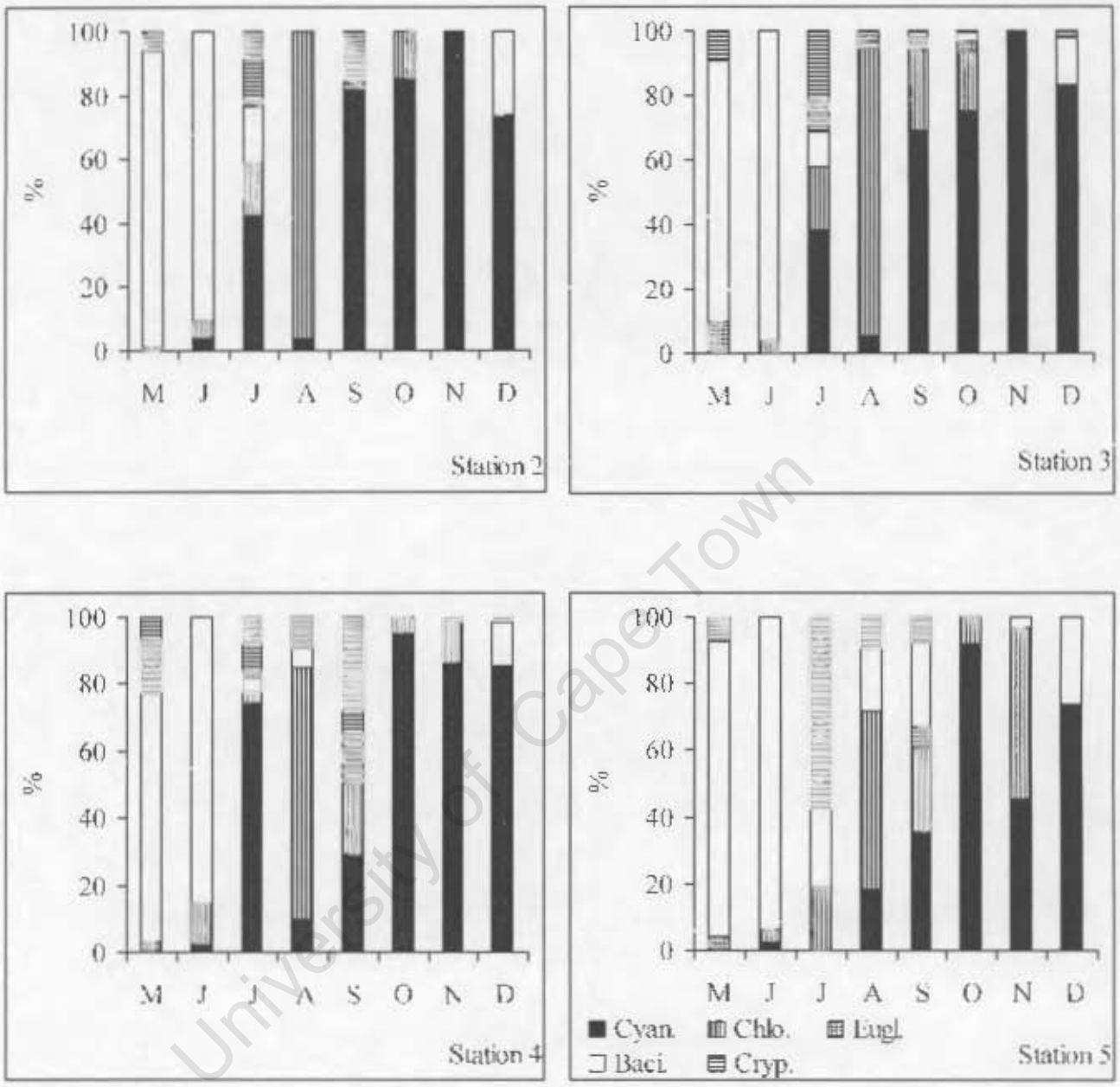


Figure 5.13 **Changes in the phytoplankton community composition (percentage of total biomass) at stations 2, 3, 4 and 5 in Lake Chivero during an algal the bloom, May– December 2004. (integrated samples 0-5 m) Key: Cyan. = Cyanophyceae, Chlo. = Chlorophyceae, Eugl. = Euglenophyceae, Baci. = Bacillariophyceae, Cryp. = Cryptophyceae.**

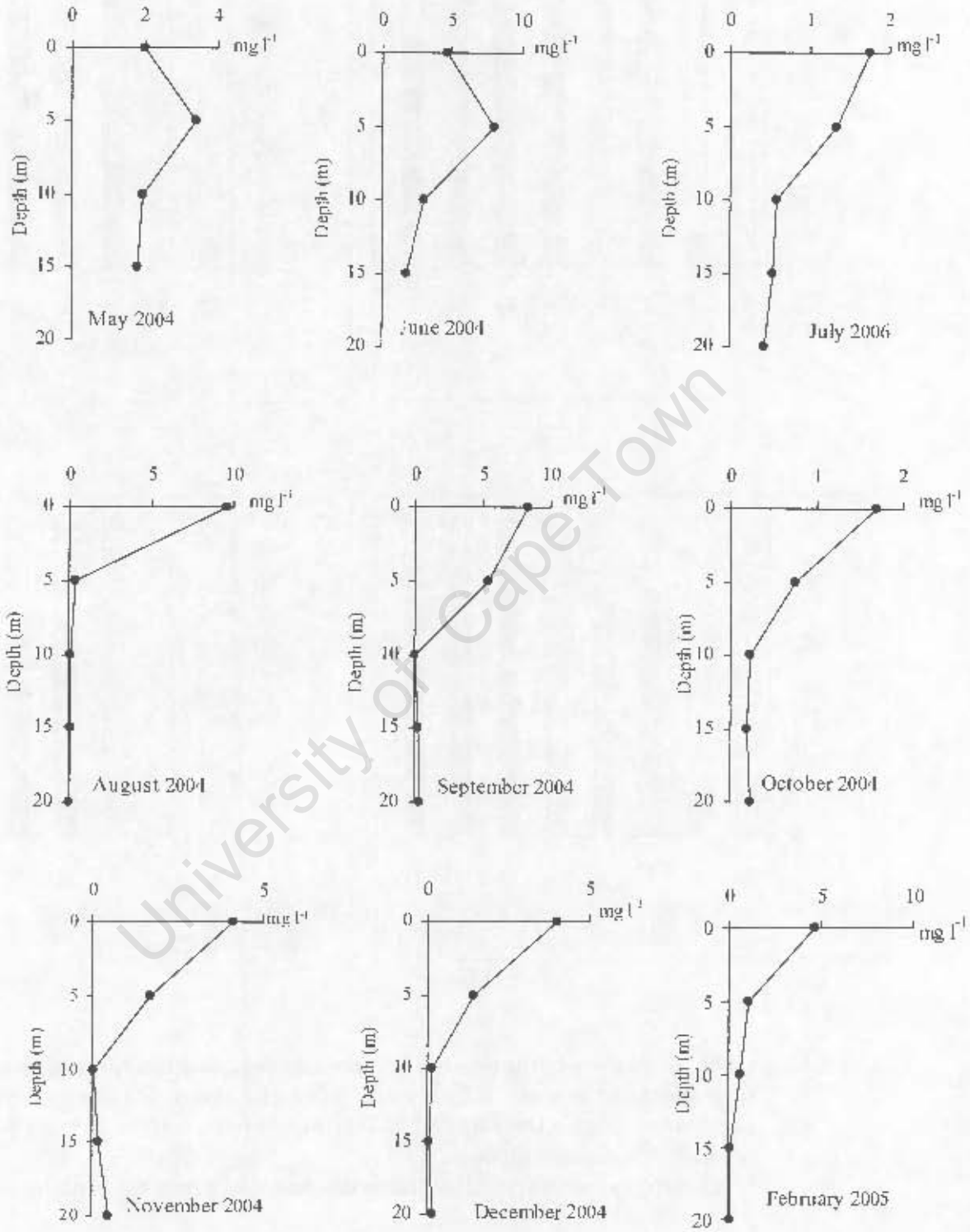


Figure 5.14 Vertical distribution of algal biomass at station 1 in Lake Chivero during (May – December 2004) and after the bloom (February 2005).

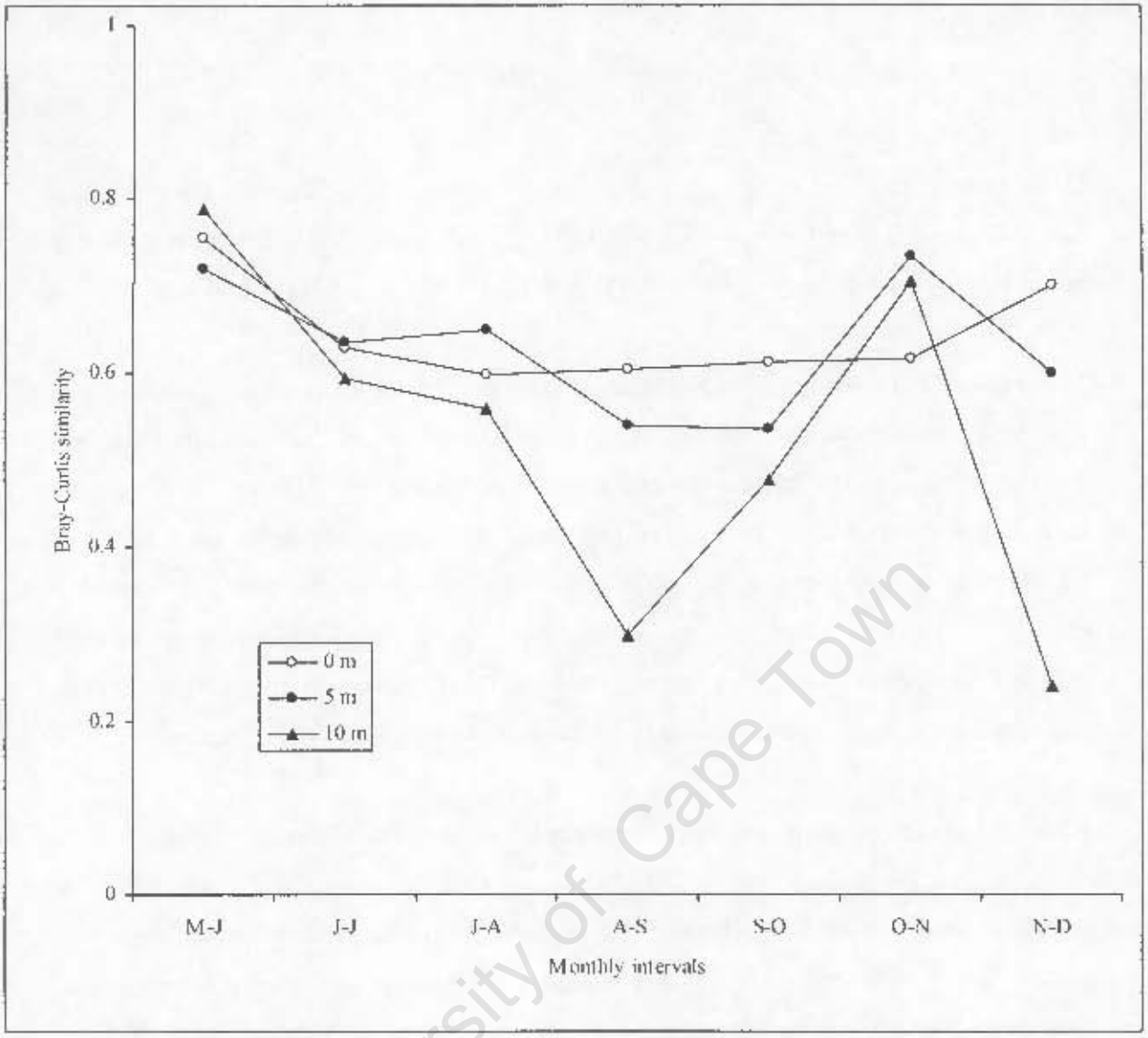


Figure 5.15 **Change of individual species biomass in phytoplankton as similarity by Bray-Curtis between successive monthly at Station 1 in Lake Chivero during an algal bloom period, May – December 2004.**

Phytoplankton biomass significantly correlated with pH ($r = 0.529$, $n = 32$, $p < 0.05$), dissolved oxygen ($r = 0.636$, $n = 32$, $p < 0.05$), TN:TP ratio ($r = 0.418$, $n = 32$, $p < 0.05$) and chlorophyll *a* concentration ($r = 0.871$, $n = 32$, $p < 0.05$).

The change of species in the phytoplankton assemblage during the bloom period at 0, 5 and 10 m was evaluated by the Bray-Curtis similarity between two successive monthly samples (Figure 5.15). Greatest similarity was recorded between May and June samples indicating that the share of biomass of individual species was similar in May and June. Similarity gradually dropped from July until September indicating that phytoplankton species composition was not stable during that period. The drop was most marked between August and September samples at 10 m depth. Similarity then increased until high similarities were measured at all levels between October and November.

5.3.5 Structure of the phytoplankton assemblage after the bloom

After the algal bloom had collapsed the phytoplankton biomass and assemblage was determined in February, May and November 2005 representing the rainy season (summer), cold dry season (winter) and hot dry season respectively and in April 2006 (Figure 5.16). When the bloom crashed there was a pronounced species shift to a dominance by *Cryptomonas* and *Cyclotella* (Figure 5.16). *Cryptomonas* co-occurring with *Cyclotella* was markedly dominant at all stations. *Microcystis* was scarce in samples collected in February 2005 and absent in the other samples. *Cryptomonas* was also dominant within the 0-5 m depth although it occurred down the profile. Two *Scenedesmus* species (*S. denticulatus* and *S. acuminatus*) had also colonised the phytoplankton. Notable in the samples after the collapse of the bloom was the presence of *Anabaena* sp. and *A. tanganyike* although in negligible quantities. In May 2005 algal biomass was high right down the water column up to 20 m. The notable observation after the collapse of the bloom was the decline in dominance of *Microcystis*, increasing dominance of *Cryptomonas*, appearance of two species of *Scenedesmus* (*S. denticulatus* and *S. acuminatus* although in negligible quantities) and appearance of *Anabaena* and *A. tanganyike* although in low numbers.

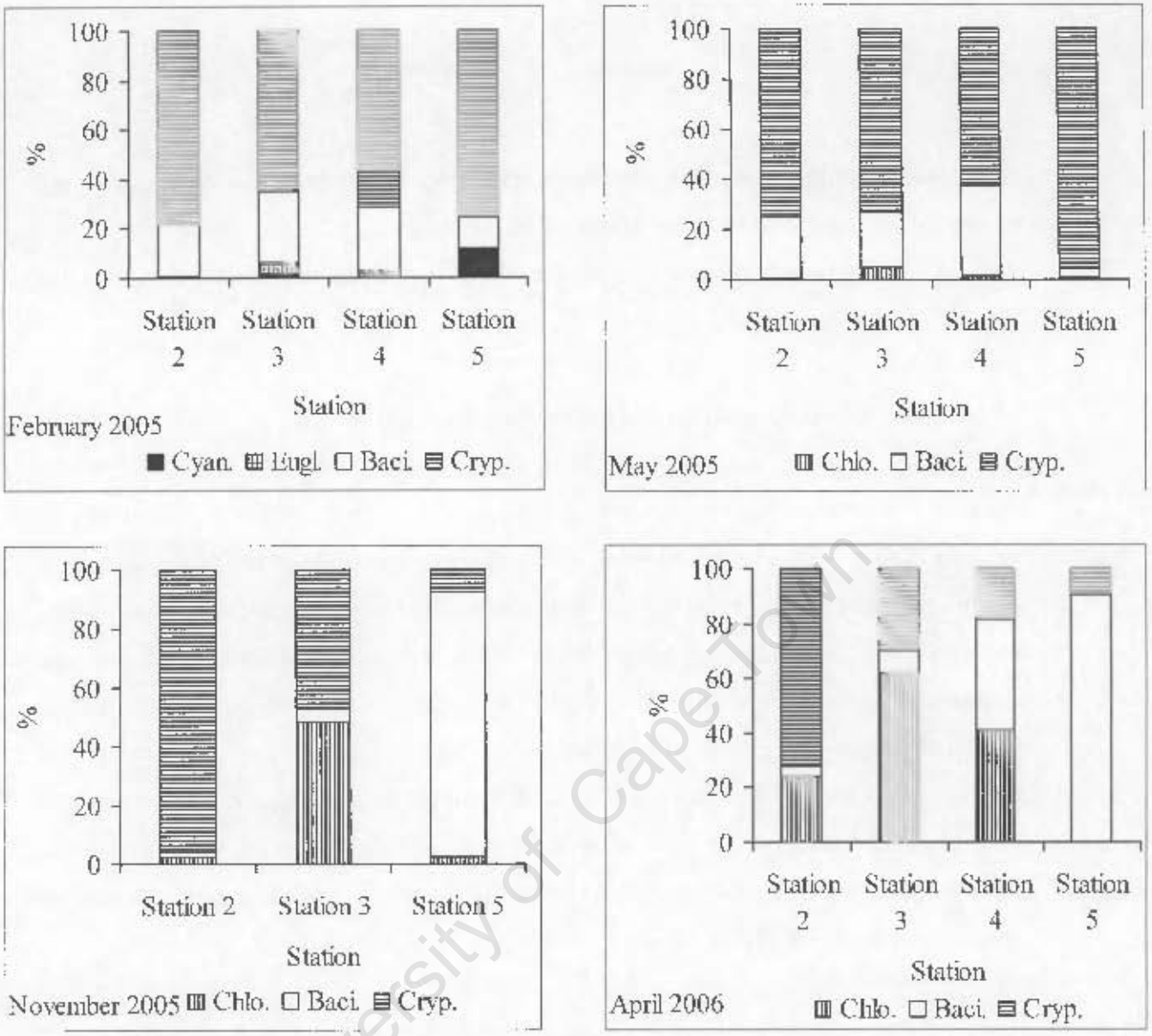


Figure 5.16 **Phytoplankton community composition (percentage of total biomass) at stations 2, 3, 4 and 5 in Lake Chivero after the bloom. (integrated samples 0-5 m) Key: Cyan. = Cyanophyceae, Chlo. = Chlorophyceae, Eugl. = Euglenophyceae, Baci. = Bacillariophyceae, Cryp. = Cryptophyceae.**

There was variability in dominance patterns at the stations. Diatoms mainly *Cyclotella* sp. tended to be more dominant at station 5 in November 2005 and April 2006. Algal biomass was also markedly higher after the bloom especially in May (11.8 mg l^{-1}) and November (20.1 mg l^{-1}).

5.3.6 Analysis using multivariate exploratory techniques

The first three eigenvalues from the PCCA analysis explained 50% of the variability of the data (Table 5.3). The relationship of the environmental variables to the species is shown on Figure 5.17. Factor 1 was highly correlated with temperature, conductivity, turbidity, total dissolved solids, secchi depth, nitrate and total phosphorus ($r > 7$) although ammonium and orthophosphate were also important ($r > 5$). Factor 2 was highly correlated with *Microcystis* ($r > 7$) and also with *Rhodomonas* sp. and *Gleocystis* sp. ($r \leq 5$). *Microcystis* was associated with TN:TP ratio and nitrate. *Coelastrum* sp., *Aulacoseira granulata* and *Gleocystis* sp. showed an association with temperature, total phosphorus and orthophosphate. *Cryptomonas* sp., *Cyclotella* sp. and *Trachelomonas* sp. showed an association with ammonium, dissolved oxygen, turbidity and total dissolved solids.

The similarity among the sampling stations during the bloom period is shown in Figure 5.18. Factor 1 depicted the temporal development of the bloom with respect to changes in the phytoplankton assemblage. The period from May to September when the community was still mixed is on the positive side of Factor 1 while the period from October to December when *M. aeruginosa* became dominant is on the negative side.

Table 5.3 **Summary of the PCCA analysis for the relationship between phytoplankton and environmental factors in Lake Chivero between February 2003 and December 2004.**

Value no.	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	7.09	28.39	7.09	28.39
2	2.84	11.37	9.94	39.76
3	2.64	10.56	12.58	50.33
4	2.08	8.34	14.66	58.67
5	1.67	6.68	16.34	65.36
6	1.52	6.09	17.86	71.45
7	1.36	5.44	19.22	76.89
8	1.03	4.12	20.25	81.02

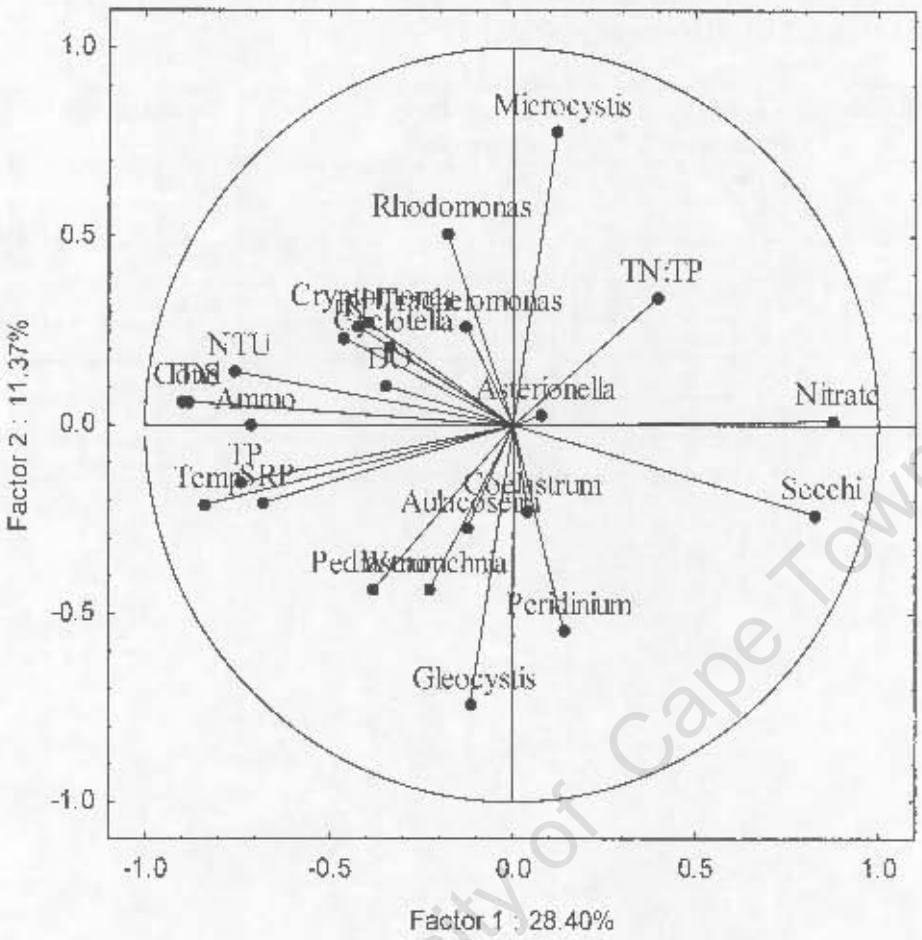


Figure 5.17 **A biplot of the relationship between physical and chemical parameters and abundant phytoplankton taxa. The abbreviations used for physical and chemical parameters area: Cond = Conductivity, Ammo = Ammonium, NTU = Turbidity, DO = Dissolved oxygen, TN:TP = TN:TP ratio, SRP = Orthophosphate, Secchi = Secchi depth, TP = Total phosphorus.**

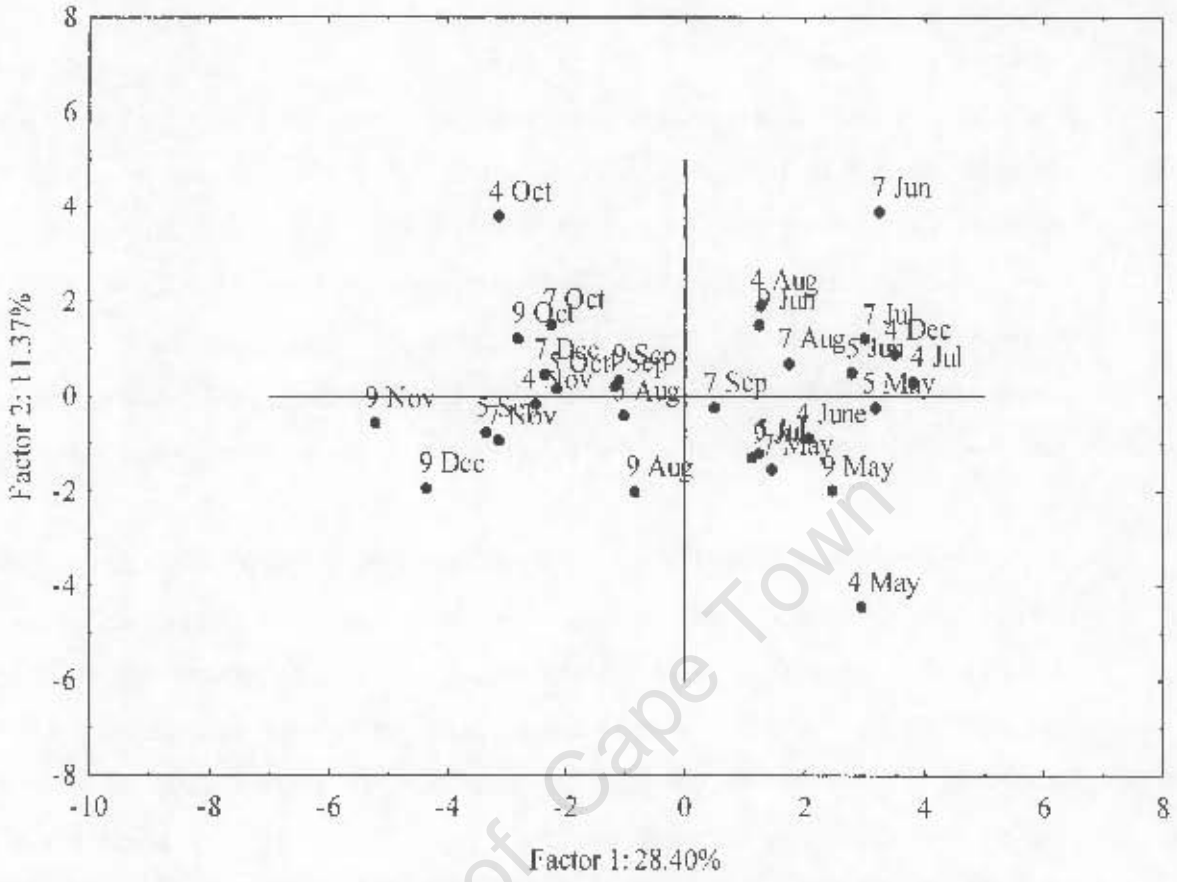


Figure 5.18 **A biplot of the relationship between sampling stations (4, 5, 7, 9) during the bloom period. Number preceding each sampling months denotes the station.**

5.4 DISCUSSION

5.4.1 Phytoplankton species composition and succession during and after the bloom

This study provided a detailed timeline of the waxing and waning of an algal bloom in Lake Chivero in relation to the physical and chemical environment. Following onset of the bloom, the algal assemblage shifted towards an equilibrium stage, very close to competitive exclusion when *M. aeruginosa* assumed dominance. Initially all the species

increased in biomass, but at the end a single species, *M. aeruginosa,* constituted more than 83% of the phytoplankton biomass. Bacillariophytes expected in winter and chlorophytes and cryptophytes expected during the hot dry season (Chapter 4) were initially present but a gradual shift in species dominance occurred as *Microcystis* replaced them. Competitive exclusion with single dominance of *Microcystis* occurred in November 2004 at station 2 and 3. At this stage equilibrium according to a definition by Sommer *et al.* (1993) (Chapter 1 Section 1.5) had been attained. This general pattern occurred at all stations indicating spatial uniformity in the development of the bloom.

Mechanisms whereby *Microcystis* control growth of other species are linked to control of light penetration in the water column. The periods of *Microcystis* domination in the phytoplankton assemblage had the lowest Secchi depth and euphotic depth. As turbidity and total dissolved solids increased light penetration was severely reduced and Secchi depth and Z_{eu} decreased such that the abundance of non-buoyant species declined. Competitive exclusion seemed to have been the major influence involved in phytoplankton dynamics then. The distribution of *Microcystis* in the water column showed that it had accumulated in the upper (0-5 m) creating conditions of light limitation for non-buoyant species. Under conditions of light deprivation, algae capable of adjusting their position in the water column can develop a competitive advantage over species relying solely on water movements to overcome gravitational force (Reynolds & Walsby 1975). Thus dense surface accumulations (0-5 m) of *Microcystis* could have controlled underwater light climate by preventing access to light by the subsurface plankton like *Cryptomonas, Cyclotella* and *Coelastrum*, which were gradually competitively excluded. Decline in light availability should have been the main factor that influenced loss of other species although other factors like interspecies competition, availability of vitamins and trace elements could have also triggered species switches. In Hartbeespoort Dam, Hambright & Zohary (2000) also observed that *Microcystis* controlled growth in other species *via* its control over light penetration into the water column.

Equilibrium with domination by *Microcystis* was short-lived because by February 2005 the bloom had collapsed which resulted in an increase of Z_{eu} and the re-establishment of a *Cryptomonas*- and *Cyclotella*-dominated phytoplankton assemblage. According to Hokmann (1993), equilibria with dominance by cyanobacteria tend to be single-species dominated and show stable seasonal dynamics. This was not the case in Lake Chivero since the period of "equilibrium" was short. Immediately upon decline of *Microcystis* domination; *Cryptomonas* and *Cyclotella* attained high biomasses. Their immediate establishment appears not to have been related to nutrient changes but to decline in the density of *M. aeruginosa*. Sant' Anna *et al.* (1997 cited in Crossetti & Bicudo 2005) also reported that after the decline of *M. aeruginosa,* chlorophytes and cryptophytes immediately established in a eutrophic reservoir in South-eastern Brazil.

The presence of *Microcystis* was a major determinant of the success of other species. Hambright & Zohary (2000) also observed proliferation of cryptophytes and chlorophytes under non-bloom conditions in Hartbeespoort Dam when *Microcystis* failed to bloom. This occurred after the disruption of the *Microcystis*-dominated phytoplankton assemblage through repeated flushing of *Microcystis* scum.

It is known that severe disturbances may cause a "shift" in the successional process to a new successional outcome, while minor disturbances can lead to a "reversion" to an earlier stage of the same eventual successional outcome (Reynolds 1983). During this study the collapse of the bloom acted as a disturbance that reset phytoplankton assemblage to the previous state. As the bloom developed phytoplankton succession proceeded with decreasing species diversity towards a climax (equilibrium) stage, although this was interrupted and reset to an earlier succession stage. In hyper-eutrophic systems collapse of a bloom is an indicator of instability (Chapter 1 Section 1.2) that is preceded by a build-up of high phytoplankton biomasses.

According to Scheffer *et al.* (2003) the time-course of change in the community remains unpredictable. In Lake Chivero, I had predicted predominance by cyanobacteria, which is contrary to these findings, and which indicates instability within the system. Stefaniak *et*

al. (2005) noted that we could never precisely determine the current state of a system since it constantly changes in response to the slightest perturbations.

The phytoplankton assemblage after the collapse of the bloom was similar to that which occurred prior to the bloom. The slight increase in the abundance of two nitrogen-fixing species, *Anabaena* sp. and *Anabaeniopsis* sp., after the decline in nitrate indicates that they could be favoured under nitrate-limiting conditions. Generally these species have declined in Lake Chivero (Chapter 5), although due to their ability to fix nitrogen they may again increase when the TN:TP ratio is low and nitrogen limiting. Appearance after the bloom collapsed of two *Scenedesmus* species, *S. denticulatus* and *S. acuminatus,* which are pioneer species, showed that the lake had reverted to the initial stages of the successional process.

5.4.2 Effect of environmental variables on bloom initiation and collapse

Physical and chemical factors controlling cyanobacterial bloom potentials are numerous and complex (Chapter 1 Section 1.3) and determine which genera and species become established and dominant in specific ecosystems (Paerl 1996). During this study high nutrient levels *per se* seem not have been the main factor because concentrations have been high even during the clear state (Chapter 3). The key question is what was peculiar during the bloom period with respect to physical and chemical characteristics?

Lake Chivero is supersaturated with nutrients (both N & P), such that the relations between the availability of P and N seemed to have been of importance in influencing the development and subsequent collapse of the dominance by *Microcytis*. The influence of nitrate as the likely trigger that shifted the phytoplankton assemblage to dominance by *Microcystis* has been discussed (Chapter 3 Section 4 and Chapter 4). Among phytoplankton species, cyanobacteria are known to be better than eukaryotic algae competitors for nitrogen (Tilman *et al.* 1986, Michard *et al.* 1996). This probably explains the observation that as orthophosphate increased and nitrate declined chlorophytes and cryptophytes were successionally replaced by *M. aeruginosa.* The

increase in dominance by *M. aeruginosa* showed that it had a competitive advantage over *Cryptomonas* and *Coelastrum* in relatively low nitrogen conditions. This finding is in contrast to the observations of Jensen *et al.* (1994) that sufficient external addition of nitrate could induce the dominance of the bloom by Chlorophyceae and a decrease in the abundance of cyanobacteria.

Opposite relationships between cyanobacterial blooms and nitrate have been observed elsewhere. Goodwin (1977 cited by Von Rückert & Giani 2004) detected a negative correlation between nitrate concentrations and cyanobacterial density in Pampulha reservoir (Brazil). In spring when temperature increases, the system becomes stratified and nitrate becomes depleted from the euphotic zone and at this moment cyanobacterial blooms occur (Giani 1994, Goodwin & Giani 1998). In Pampulha reservoir, cyanobacterial blooms occurred when ammonium concentrations were very high and nitrate not detected. Although ammonium has been hypothesized to influence cyanobacteria dominance (Blomqvist *et al.* 1994) it seemed to have not influenced/initiated bloom dynamics during my study.

Nitrate and ammonium are both used as inorganic sources of nitrogen by cyanobacteria (Von Rückert & Giani 2004) but nitrate seemed to have been the main nitrogen source during this study. The decline in levels of nitrate within the water column during the bloom indicated that it was readily utilized. It has been observed in other freshwater systems that nitrate becomes depleted after periods of intensive algal growth, especially when thermal stability retains nutrients in the hypolimnion (Reynolds 1984). Ammonium is known to interfere with nitrate uptake through its inhibition of nitrate reductase (Syrett 1981). Although not assessed, that appears not to have been the case during the bloom in Lake Chivero, where opposite trends occurred, with nitrate levels decreasing and ammonium increasing.

When the bloom attained highest biomass, ammonium levels had increased in the lake. The highest concentration occurred at the river station in November, probably indicating an external source from sewage although contributions could have also come from the re-

suspension of sediments or nitrogen metabolism by microorganisms. Decomposition could have been a major source since ammonium levels were higher (4.4 mg l^{-1} at 20 m in November) in bottom than in surface waters.

Studies on algal bloom development in both tropical and temperate areas have attributed bloom development to high concentrations of nutrients and light availability, together with optimal surface temperatures (White *et al.* 2003). While in this study it appears that nitrate availability might have been the main primary factor, the contributory role of other factors cannot be excluded. Fabbro (1999) noted that the effect or role of a single environmental factor on algal bloom initiation varies depending on the range of morphologies and physiologies of the genera present and on their preferred optimal growth conditions. Thus a particular genus will dominate only if appropriate conditions are provided. *Microcystis* is favoured in an environment with diel cycles of stratification and mixis (Reynolds 1994) and lengthy periods of physical stability, i.e. stable climatic and hydrological conditions are a prerequisite for the development of bloom populations (Reynolds & Walsby 1975).

Lake Chivero was stratified for the whole bloom period except in June, and this could have enhanced the accumulation of *Microcystis* at the surface because it can control its vertical buoyancy (Reynolds 1972). Large-scale vertical mixing counteracts near-surface accumulations of buoyant bloom populations and forces competition for light and nutrients with more 'desirable', non-buoyant eukaryotic taxa (Paerl 1996). In Lake Chivero, *Microcystis* could have been competing with *Cryptomonas*, *Cyclotella* and *Coelastrum*, which assumed dominance during the clear state (Chapter 4) and after the bloom had collapsed. During the period of non-*Microcystis* domination regular mixis due to windy conditions could have caused frequent mixing of algal cells within the euphotic zone, thereby counteracting the effects of self-shading (Harding 1996) and favouring eukaryotic algae.

Absence of any relationship between orthophosphate concentration and phytoplankton biomass suggests that cyanobacterial dominance within the phytoplankton assemblage

was limited mainly by nitrogen while the other taxa were limited by light as shown by their decline after *Microcystis* dominated. Total phosphorus and orthophosphate concentrations did not exhibit clearly discernable relationships to the development of the algal biomass and chlorophyll *a* during the bloom period. Phosphorus is high in the lake and there is a constant external supply through incoming sewage effluent (Nhapi 2004). The gradually decline that occurred between May and September 2004 should have been due to utilization by algae. Concentrations did not fall below limiting levels however, and in fact increased from October, until the bloom collapsed in December. The source could have been partly external because the increase was most apparent at the river station.

Bloom phenomena have also been linked with localised nutrient enrichment whereby favourable physical conditions must act synergistically with localised nutrient enrichment for nuisance-bloom formation (Fogg 1969, Reynolds & Walsby 1975). The contributory role of nutrients in bloom development in Lake Chivero can be inferred by comparing nutrient levels in 2003 and 2004 during the same period (Chapter 3). Nitrate, TN:TP ratio and total nitrogen were higher in 2004 by several orders of magnitude than in 2003. Blooms started appearing in May when nitrates and total nitrogen concentrations were 0.9 mg l^{-1} and 7 mg l^{-1} respectively, probably indicating these as near-optimum nitrogen conditions for *Microcystis* bloom formations at prevailing physical and chemical conditions. This further shows that the bloom only started when nitrates had reached a "critical" concentration. As nitrates got depleted orthophosphate increased. It has been observed that high concentrations of nitrate can suppress phosphate liberation from the sediments (Bostrom & Petterson 1982 cited by Sakamoto & Okino 2000) while in some cases nitrate enhances phosphorous mobilization in the sediments by stimulating microbial activity (Boström *et al.* 1988).

The data from Lake Chivero suggest that after reaching a "critical" nitrate concentration a release of phosphorus was stimulated from the sediment, leading to an increase in phosphorus concentration in the lake. The drop in dissolved oxygen between 10 and 20 m as the bloom developed could have further stimulated phosphorus release from sediments. Phosphorus increased at all stations as nitrogen and dissolved oxygen

declined. Sakamoto & Okino (2000) suggested a similar phenomenon in Lake Suwa in Japan.

This study could not explain why nitrates were higher in 2004 than in 2003. The likely source of nutrients in winter is from turnover, which cannot explain the differences. Higher levels of nitrates could have come in through sewage effluent. A gradation with highest levels at the river station and lowest levels at station 1 was not observed, indicating that the source of nutrients was more likely to be from the sediments than from the inflowing river. In fact except for March, nitrates were higher at station 1 than at station 5.

With respect to nitrate, which seemed to be the main influencing factor, no differences in concentration were observed among the stations. Other physical and chemical parameters were also uniform at the four stations. The lesser accumulation of chlorophyll *a* and algal biomass at station 3, especially in November, could have been an influence of wind. Otherwise there was no significant spatial variability.

The cause of the collapse of the bloom was not determined. When the bloom collapsed, however, orthophosphate concentration had increased while ammonium and nitrate concentrations had declined. Temperature was high and optimal for cyanobacteria growth, pH was high and the lake was still stratified. Since cyanobacteria require either ammonium or nitrate as nitrogen sources (Von Rückert & Giani 2004), the most likely reason could have been nitrogen limitation. According to Reynolds (1984) algal growth may be limited, saturated or in some cases inhibited by one particular nutrient, which could have been the case during this study. It is also possible that the maximum biomass that the system can accumulate had been attained causing the bloom to collapse and thereby re-setting the system into a new successional process.

This study provided additional insights towards understanding the factors controlling cyanobacterial dominance and growth in hyper-eutrophic systems. It showed that cyanobacterial blooms can exhibit profound sensitivity to minor shifts in environmental

conditions (Paerl 1988), in this case nitrate. Understanding the dynamics of the nutrient environment, especially "critical concentrations" may be a useful concept in timing bloom development in hyper-eutrophic lakes thereby assisting in improving the success rate of management and control of blooms (Carpenter 1989).

CHAPTER 6

RESPONSES OF PHYTOPLANKTON ASSEMBLAGES ISOLATED OVER SHORT PERIODS OF TIME: ENCLOSURE EXPERIMENTS

6.1 INTRODUCTION

Composition, structure and species succession of phytoplankton assemblages represent the combined effects of allogenic, autogenic and sequential factors (Reynolds 1983, Sommer *et al.* 1986, Haffner & McNeely 1989). Effects of changes driven by allogenic (external) factors weaken while autogenic (internal) factors strengthen the organization of the assemblage (Reynolds 1980). Allogenic, autogenic and sequential factors (hydrographic disturbances) do not operate exclusively. The quantity of available nutrients, physical variables such as light, temperature and mixing in the water column and biological factors such as grazing and competition operate simultaneously, which makes it technically difficult to separately quantify their influences on phytoplankton assemblages (Kalf & Knoechel 1978, Dos Santos & Calijuri 1997).

The interactive influence of these factors is complex but overally determine the structure of the phytoplankton assemblage. It is generally accepted that in Lake Chivero, the increase in nutrient levels through eutrophication has had the major impact on the phytoplankton assemblage since the lake was formed (Marshall 2005). Allogenic disturbances (e.g. wind-induced mixing and storms) should play a role in changes observed in phytoplankton dynamics in Lake Chivero (Chapter 4). Wind-induced mixing act as superimposed disturbances on successional events. In severe circumstances, wind and storms can cause a "shift" in the succession process resulting in a new successional outcome (Reynolds 1993).

Turbulence (wind induced irregular stirring and mixing), for instance, could be a significant factor limiting the dominance of cyanobacteria in Lake Chivero. According to

Steinberg & Hartman (1988), water column stability becomes an important physical factor influencing cyanobacterial development above a threshold of 10 $\mu g\ l^{-1}$ total phosphorus. They reckon that by characterizing different forms of turbulence, the presence or absence of cyanobacteria in lakes can be predicted. This is based on the observation that cyanobacteria tend to build up dense populations when turbulence is rather low, while when turbulence is high, especially in circumstances when the wind-mixing depth is greater than the euphotic depth, or when the mixing pattern is irregular, cyanobacteria tend to be outcompeted.

Turbulence is a quasi-resource (Harris *et al.* 1980) comparable to nutrients or light, which is exploited differently by different phytoplankters (Steinberg & Hartmann 1988). In Lake Chivero during this study period there was a marked decline in the dominance of *M. aeruginosa*, a specialists according Reynolds (1996). Dominance by specialists indicates a state of equilibrium because phytoplankton succession proceeds with decreasing species diversity towards a climax (equilibrium) stage (Reynolds 1996). The decline of specialists in Lake Chivero therefore indicates that the system is currently in a state of instability.

Despite the fact that nutrient levels were above the limiting levels for cyanobacteria (Chapter 3), specialists were not dominant in Lake Chivero during parts of the study period (Chapter 4 and Chapter 5). Instead conditions were favourable for *Cryptomonas* and *Cyclotella*. This indicates that there might have been at least one factor overriding the effect of nutrients in determining *Microcystis* growth. This could include grazing and the actual light conditions in the water column (Kirsten Olrik, personal communication). Christian *et al* (1986) reckon that turbulence is an important factor since an unstable water column is a major deterrent factor in the development of cyanobacterial blooms (Reynolds & Walsby 1975). There could have been a "continuous disturbance" – an event that persistently interrupts the progression of the phytoplankton succession to equilibrium by constantly resetting it to an earlier succession stage where growth strategists dominate (Sommer *et al.* 1993). This could be contrary to a situation in Zeekoevlei where *Microcystis* dominated partly because it is constantly wind-mixed and

turbulent (Harding 1996). In Zeekoevlei, Harding (1996) observed that *M. aeruginosa* has adapted to continuous but stable turbulence experienced by the lake thereby out-competing normally dominant ruderal plants in a system with low TN:TP ratio, high water temperature, pH > 9 and low light availability.

During my study when turbulence was low, algal cells would migrate to the surface but sudden turbulence would prevent build up of high biomasses. I hypothesised that continuously turbid conditions were disrupting the build-up of biomass of *Microcystis* (and other species) in the euphotic zone and influencing the species dominance pattern. This chapter reports on the influence of turbulence (and other associated factors) on species composition and phytoplankton biomass, evaluated by assessing the variations in the composition of isolated phytoplankton assemblages that occur over short periods in enclosures.

When observations are being made to evaluate how environmental variability influences assemblage structure, Dos Santos & Calijuri (1997) recommend that the scale of observation should fit as closely as possible to the scale of organism response or generation time. The average generation time for phytoplankton is between 1 and 2 days (Dos Santos & Calijuri 1997). Nine to eleven days was therefore considered as an appropriate duration since it has also been observed (Reynolds & Reynolds 1985, Harris 1986) that changes in the stability of the water column, at intervals of about 10 days are responsible for changes in the composition and maintenance of species diversity and that the biomass and taxonomic composition of the phytoplankton assemblage can change in a few days in response to changes in the mixing layer. The other assumption was that nutrients would not decline to limiting levels in enclosures within a period of 11 days, allowing me to make inferences on the effect of forcing factors other than nutrients on the phytoplankton assemblage.

The objectives of the study were:

(i) to evaluate and compare the variations in the composition of isolated phytoplankton assemblages that occur over short periods of time to the lake assemblage, during three times of the year in a tropical hyper-eutrophic lake

(ii) to compare changes in the physical and chemical parameters in the enclosures and in the lake and infer the factors that predominantly influence the structure of the phytoplankton assemblage

6.2 MATERIALS AND METHODS

The algal assemblage was isolated into *in situ* enclosures on three occasions representing summer (21 February to 2 March 2005), winter (21 to 31 May 2005) and end of winter (15 to 25 August 2005). The enclosures (1 X 1 X 1 m) were made of 0.2-mm reinforced polyethylene. Each enclosure had a capacity of $1m^3$, was closed at the bottom and open to the atmosphere. A special support system enabled the enclosures to float above the water surface in order to avoid entry of lake water into the enclosure. A water pump was used to pump lake water into 3 replicate enclosures on each occasion. The enclosures were incubated for a maximum of 11 days and a minimum of 9 days in a bay in the lake where the maximum depth was about 3 m. The bay is located in the south bank of Lake Chivero, near the Parks and Wildlife Management Authority lodges (Volley Bay see Figure 3.1).

Samples from the three replicate enclosures and from the lake were removed every second day for analysis. Prior to sample collection the water within the enclosures was thoroughly mixed with a stirrer and then collected with a Ruttner sampler. Water was collected from the lake from the euphotic zone (within 1 m depth) and from the enclosures between 0900 and 1100 hours on each sampling occasion.

Temperature, conductivity, turbidity, pH and dissolved oxygen and total dissolved solids were measured as described in Chapter 3. Chemical analysis for total nitrogen, ammonium, nitrates, total phosphorus and soluble reactive phosphorus were carried out following the methods in Golterman *et al.* (1978) described in Chapter 3. Phytoplankton samples were preserved in Lugol's iodine solution. Utermöhl's sedimentation method (Chapter 4) was used to identify and enumerate phytoplankton (Utermöhl 1958, Cronberg

1982). The concentration of chlorophyll *a* was determined by the acetone extraction method (see details in Chapter 4).

6.2.1 Statistical analysis

Differences between the lake and the enclosures were tested with Repeated Measures Analysis of Variance (ANOVA), testing for the between-treatment effect of "isolation" and the within-treatment effects of "Time" (One-way ANOVA for February, May and August data sets). The hypothesis that isolating the algal assemblage had no effect on chlorophyll *a* concentration, phytoplankton biomass, phytoplankton dominance patterns, physical and chemical parameters was tested. The statistical analyses were performed using STASTICA 7. Shannon's diversity index, H′, was computed using natural logarithms of species biomass on the programme Primer 6 version 6.1.5 (Chapter 4).

6.3 RESULTS

The three periods were characterized as summer (February), winter (May) and end of winter (August).

6.3.1 Physical characteristics

Temperature fluctuations in the enclosures and in the lake were similar and not significantly different in the lake and in the enclosures in February (Figure 6.1a, $F(5,12) = 1.4$ $p > 0.05$) and August (Figure 6.1c, $F(1,10) = 0.3$ $p > 0.05$) while enclosure temperature was significantly lower than lake temperature in May (Figure 6.1b, $F(5,12) = 4.3$ $p < 0.05$). Temperature was higher in February (27.4 °C enclosure, 27.3 °C lake) than in May (19.9 °C enclosure, 20.3 °C lake) and August (19.9 °C enclosure, 20 °C lake). There was a marked variation in temperature of up to 4 °C between start and end of the experiment in all experiments.

The pH was significantly higher in enclosures probable due to carbon limitation in February (Figure 6.1d, $F(5,12) = 15.5$ $p < 0.05$), May (Figure 6.1e, $F(5,12) = 6.1$ $p < 0.05$) and August (Figure 6.1f, $F(4,10) = 27.4$ $p < 0.05$) than in the lake. It exhibited a steady increase especially in August from 7.5 to 9.2 by day 9 (Figure 6.1 d-f) while in the lake it rose from 7.8 to 8.4 during the same period. Conductivity in the enclosures and in the lake was similar at day 1 (502 $\mu S\ cm^{-1}$ in February, 529 $\mu S\ cm^{-1}$ in May and 554 $\mu S\ cm^{-1}$ in August), dropped by day 3 and then increased. Conductivity was significantly higher in the lake than in the enclosure in February (Figure 6.1g, $F(5,12) = 18.4$ $p < 0.05$), May (Figure 6.1h, $F(5,12) = 43.8$ $p < 0.05$) and August (Figure 6.1i, $F(4,10) = 16.8$ $p < 0.05$). Turbidity was significantly higher in enclosures than in the lake in February (Figure 6.1a, $F(5,12) = 10.9$ $p < 0.05$) and in May (Figure 6.1b, $F(5,12) = 4.2$ $p < 0.05$) while in August turbidity in the lake was significantly higher than in the enclosure in August (Figure 6.2c, $F(4,10) = 5.9$ $p < 0.05$).

Dissolved oxygen increased in enclosures to reach a maximum concentration at day 5 on all occasions: 9.7 mg l^{-1} in February, 6.2 mg l^{-1} in May and 10.9 mg l^{-1} in August, after which it declined except in February when it remained relatively constant (Figure 6.2 d-f). Dissolved oxygen was significantly higher in enclosures than in the lake in February (Figure 6.2d, $F(5,12) = 16.7$ $p < 0.05$), May (Figure 6.2e, $F(5,12) = 35.1$ $p < 0.05$) and August (Figure 6.2f, $F(4,10) = 40.8$ $p < 0.05$).

The concentration of total dissolved solids was significantly higher in the lake than in the enclosures in February (Figure 6.2g, $F(5,12) = 34.4$ $p < 0.05$), May (Figure 6.2h, $F(5,12) = 8.6$ $p < 0.05$) and August (Figure 6.2i, $F(4,10) = 31.7$ $p < 0.05$). This could be explained by re-circulation in the lake and uptake by cryptophytes in the enclosure. It was above 200 mg l^{-1} on all occasions (Figure 6.2 g-i). In enclosures a marked drop occurred initially but it gradually increased from day 3 (Figure 6.2 g-i).

Isolation in enclosures resulted in an increase in pH, dissolved oxygen concentration and a decrease in conductivity and total dissolved solids.

6.3.2 Chemical characteristics

Ammonium concentration was significantly higher in the lake than in the enclosures in February (Figure 6.3a, $F(5,12) = 17.7$ $p < 0.05$) and May (6.3b, $F(5,12) = 6.8$ $p < 0.05$) while in August there was no significant difference (Figure 6.3c, $F(4,10) = 2.2$ $p > 0.05$). A decrease in ammonium concentration occurred in the enclosures between day 1 and 5 (Figure 6.3 a-c) indicating utilization by algae. The levels dropped from 0.3 to 0.1 mg l^{-1}, 0.8 to 0.3 mg l^{-1} and 0.7 to 0.4 mg l^{-1} in February, May and August respectively.

Orthophosphate exhibited a different pattern on the 3 sampling occasions (Figure 6.3 d-f). The concentrations in the lake and the enclosures were not significantly different in February (Figure 6.3d, $F(5,12) = 1.6$ $p > 0.05$) and August (Figure 6.3f, $F(4,10) = 1.7$ $p > 0.05$) while in May enclosure concentration was significantly lower than lake concentration (Figure 6.3e, $F(5,12) = 4.5$ $p < 0.05$). A drop occurred in enclosures from 1.2 to 0.2 mg l^{-1} in February while on other occasions levels remained above 1 mg l^{-1}. In May orthophosphate concentration increased in enclosures from 1.3 mg l^{-1} at day 1 to 1.7 mg l^{-1} at day 11 while in the lake it fluctuated between 1.3 and 1.4 mg l^{-1}. Total phosphorus followed the pattern of orthophosphate (Figure 6.3 g-I) and was not significantly different between the enclosure and the lake in February (Figure 6.3g, $F(5,12) = 0.5$ $p > 0.05$), May (Figure 6.3h, $F(5,12) = 1.1$ $p > 0.05$) and August (Figure 6.3i, $F(4,10) = 2.6$ $p > 0.05$).

Nitrate concentrations were significantly higher and relatively constant in the lake in February (Figure 6.3j, $F(5,12) = 75$ $p < 0.05$), May (Figure 6.3k, $F(5,12) = 20.2$ $p < 0.05$) and August (Figure 6.3l, $F(4,10) = 14.6$ $p < 0.05$). In February and August nitrate concentration decreased from 0.8 to 0.2 mg l^{-1} and from 1.4 to 0.5 mg l^{-1} respectively in enclosures between day 1 and 5 after which a stable level was maintained while in May an increase occurred in the enclosures from a concentration of 0.4 mg l^{-1} at day 5 to 1.9 mg l^{-1} at day 11 (Figure 6.3 j-l). This coincided with an increase in biomass two nitrogen fixing cyanobacteria, *Anabaena* sp. and *A. tanganyike* in the enclosures (Figure 6.6b).

Isolation caused a decrease in nutrient levels except for an increase in nitrate and phosphorus that occurred in May.

6.3.3 Chlorophyll *a* concentration

Chlorophyll *a* concentrations were enhanced by isolation on all the 3 occasions (Figure 6.4). The starting concentration was only similar in August. An increase in chlorophyll *a* concentration occurred in enclosures between day 1 and 3 in May (123.9 to 167.7 $\mu g\ l^{-1}$) and August (20 to 91.6 $\mu g\ l^{-1}$) after which the concentrations remained above levels recorded at day 1. In February although the concentration at Day 3 was higher in the enclosure than in the lake, the highest concentration in the enclosure (54.3$\mu g\ l^{-1}$) was recorded at day 1. In the lake chlorophyll *a* concentrations fluctuated with no clear pattern; between 13 and 59.1 $\mu g\ l^{-1}$ in February, between 34 and 103 $\mu g\ l^{-1}$ in May and between 19.9 and 65.5 $\mu g\ l^{-1}$ in August. In February (Figure 6.4a, $F(5,12) = 3.9\ p < 0.05$) and August (Figure 6.4c, $F(4,10) = 13\ p < 0.05$) although chlorophyll *a* concentration increased significantly with isolation, the temporal variability was not significantly different between enclosure and lake ($F(5,12) = 1.9\ p > 0.05$, $F(4,10) = 0.4\ p > 0.05$ respectively). However in May chlorophyll *a* concentration was significantly higher in enclosure than in the lake over time (Figure 6.4b, $F(5,12) = 3.3\ p < 0.05$).

Chlorophyll *a* concentrations in the lake fluctuated by up to 50% and more. The days when concentrations were lowest in the lake contrast markedly with the higher concentrations in the enclosures on the same day. This shows that differences in physical perturbations can have marked effect on chlorophyll *a* concentration under the same ambient conditions. Higher chlorophyll *a* concentrations in the enclosures were linked to

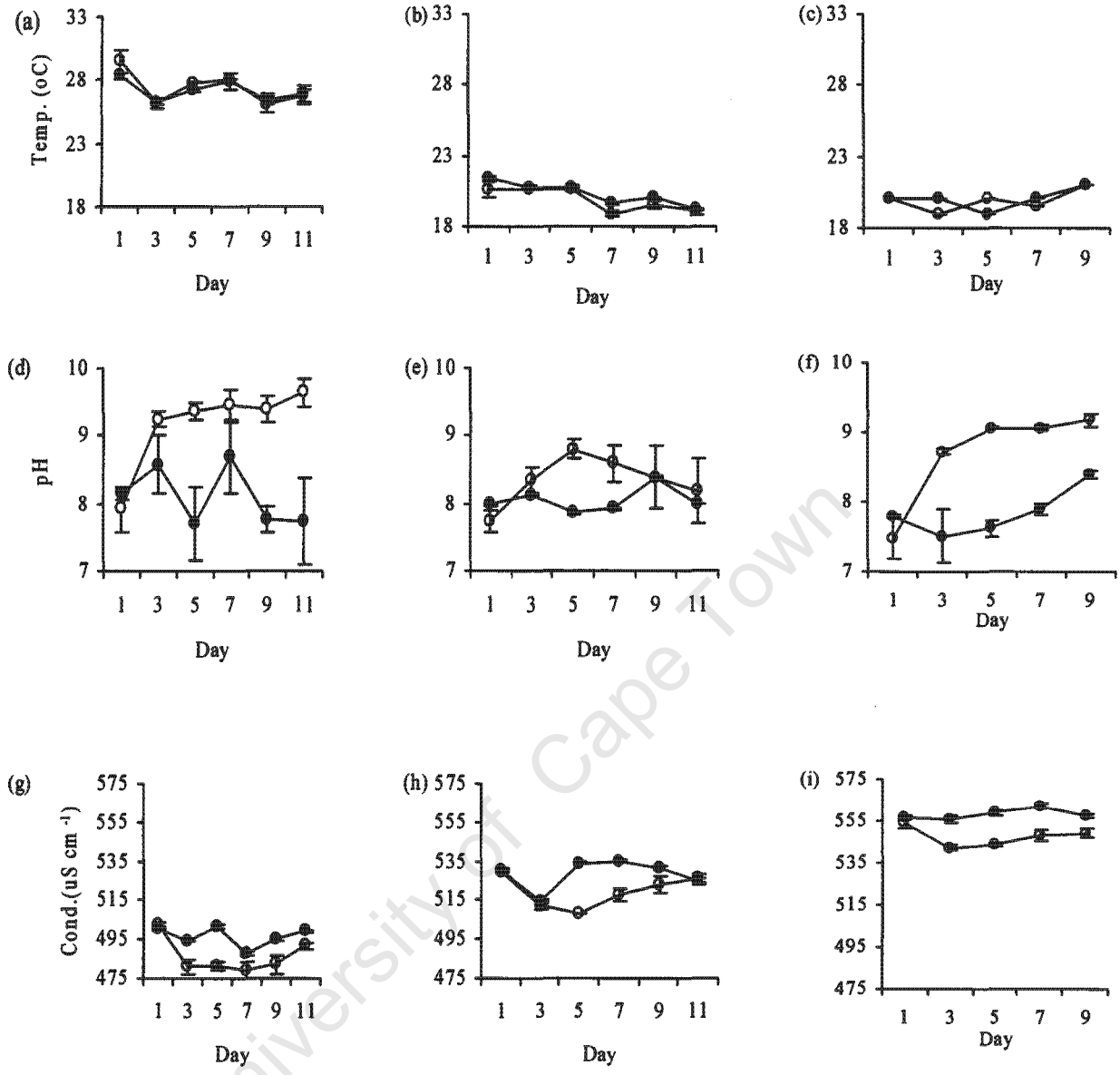


Figure 6.1 **Variation of physical parameters (mean ± std) in enclosures (o) and the lake (•) over short periods [(a), (d) and (g) = February; (b), (e) and (h) = May; (c), (f) and (i) = August]. Enclosure data are means of three replicates ± SD (bars that are not visible are hidden by symbols) while "lake" is an integrated sample over 0–1 m. [temp. = temperature, cond. = conductivity].**

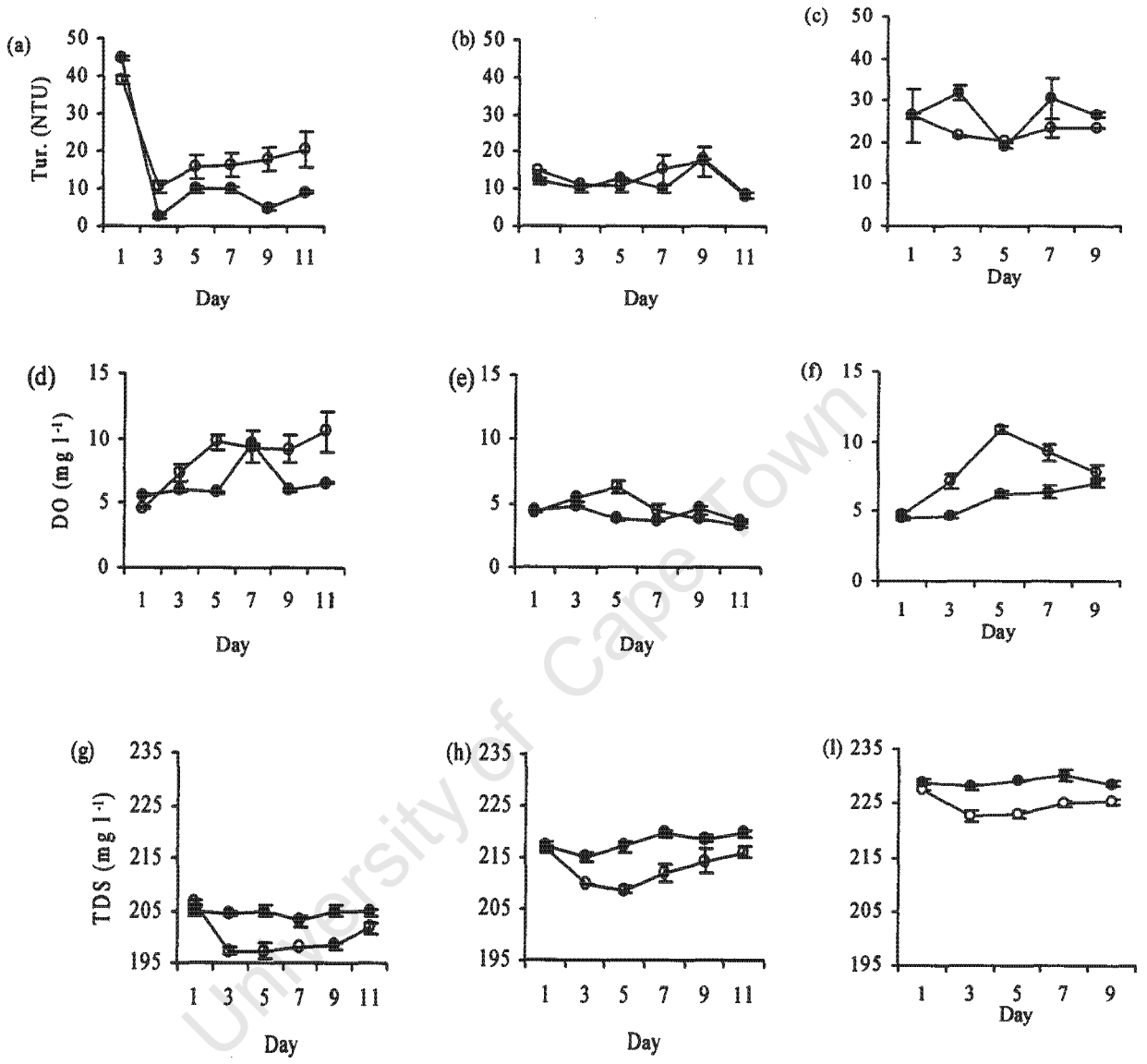


Figure 6.2 **Variation of physical parameters (mean ± std) in enclosures (o) and the lake (•) over short periods [(a), (d) and (g) = February; (b), (e) and (h) = May; (c), (f) and (i) = August]. Enclosure data are means of three replicates ± SD (bars that are not visible are hidden by symbols) while lake is integrated sample 0–1 m. [Tur. = Turbidity, DO = Dissolved oxygen, TDS = Total dissolved solids].**

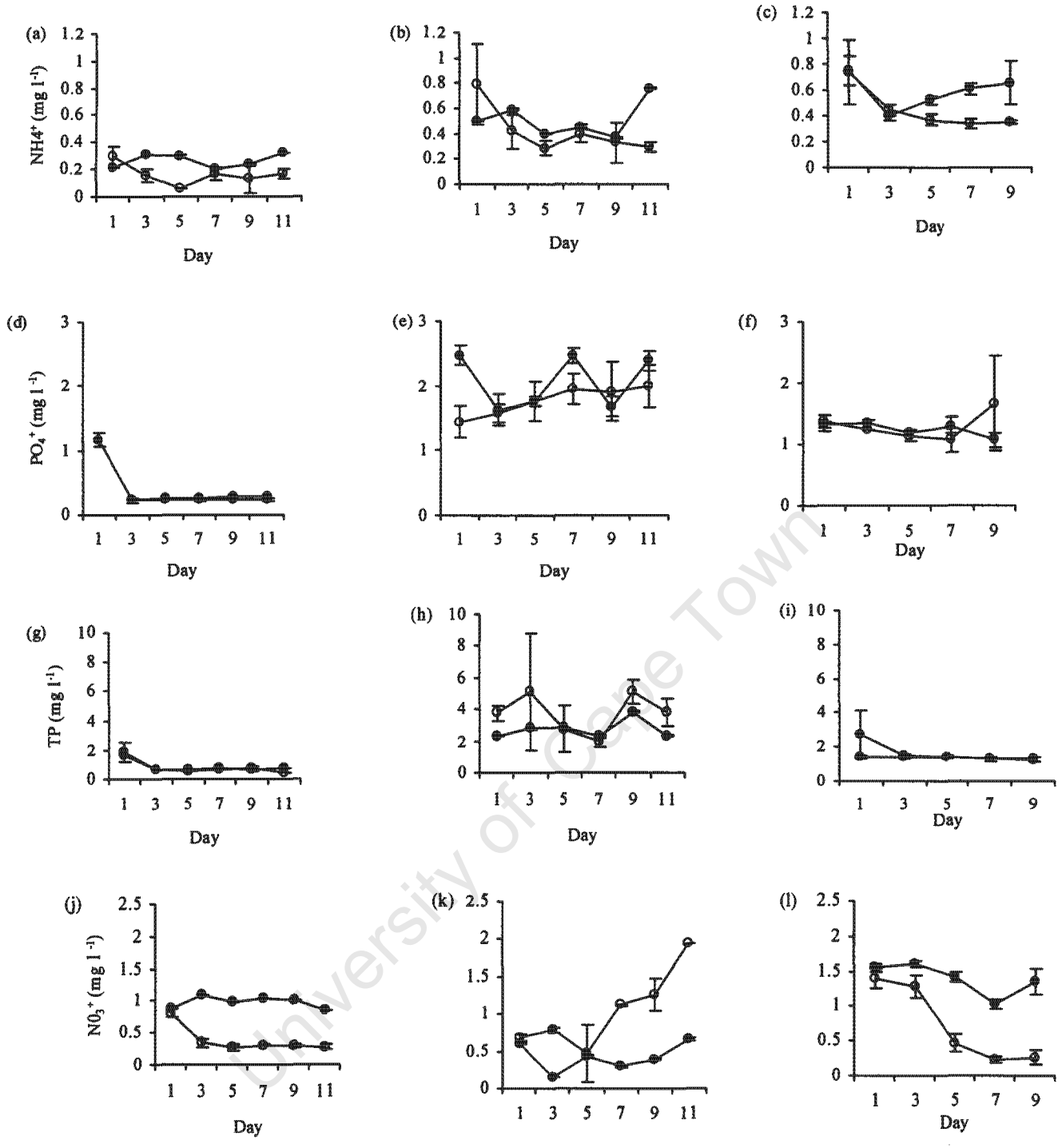


Figure 6.3 **Variation of chemical parameters (mean ± std) in enclosures (o) and the lake (•) over short periods [(a), (d), (g) and (j) = February; (b), (e), (h) and (k) = May; (c), (f), (i) and (l) = August]. Enclosure data are means of three replicates ± SD (bars that are not visible are hidden by symbols) while lake is integrated sample 0–1 m. [NH_4^+ = Ammonium, PO_4^+ = orthophosphate, TP = total phosphorus, NO_3^+ = nitrate].**

high dissolved oxygen and pH indicating that phytoplankton biomass was comparatively higher in the enclosure than in the lake.

6.3.4 Phytoplankton assemblage and biomass

The variation in the relative abundances of the dominant species in the lake and in the enclosure during summer (February) is shown in Figure 6.5. During the summer period the phytoplankton assemblage in the euphotic zone of the lake over the 9-day sampling period comprised of *Cryptomonas* with a relative biomass > 65% and together with *Cyclotella* comprised > 90% of the total biomass (Figure 6.5a). Two cyanobacterial species, *M. aeruginosa* and *Anabaena* sp., contributed > 8% of the total biomass in the lake over the same period. Rare species included *P. duplex* and *Trachelomonas* sp.

In the enclosure at the beginning of the experiment *Cryptomonas* and *Cyclotella* co-dominated with a relative biomass contribution of >90% while cyanobacteria comprised only 8% (Figure 6.5b). All species, but especially *Cyclotella*, increased in biomass by day 3 following isolation. *Cyclotella* declined from day 5, however, and was absent in enclosures on days 7 and 9. *Microcystis aeruginosa* continued to increase from day 3 and had assumed 69% of the total biomass at day 9 (Figure 6.5b). *Cryptomonas* remained dominant up to day 5, after which it declined to 22% of the relative biomass by day 9. Isolation in summer resulted in the exclusion of *Cyclotella*, a decline in *Cryptomonas* and an increase in *M. aeruginosa* and *Anabaena* sp. During summer total biomass increased between day 1 and 5 after isolation (Figure 6.7a) due to increase in the population of *Cryptomonas* but decreased from day 7 when a switch from *Cryptomonas/Cyclotella* co-dominance to dominance by *M. aeruginosa* occurred. Total biomass however was not significantly different between the lake and the enclosure over time (Figure 6.7a. $F(5,9) = 0.5$ $p > 0.05$).

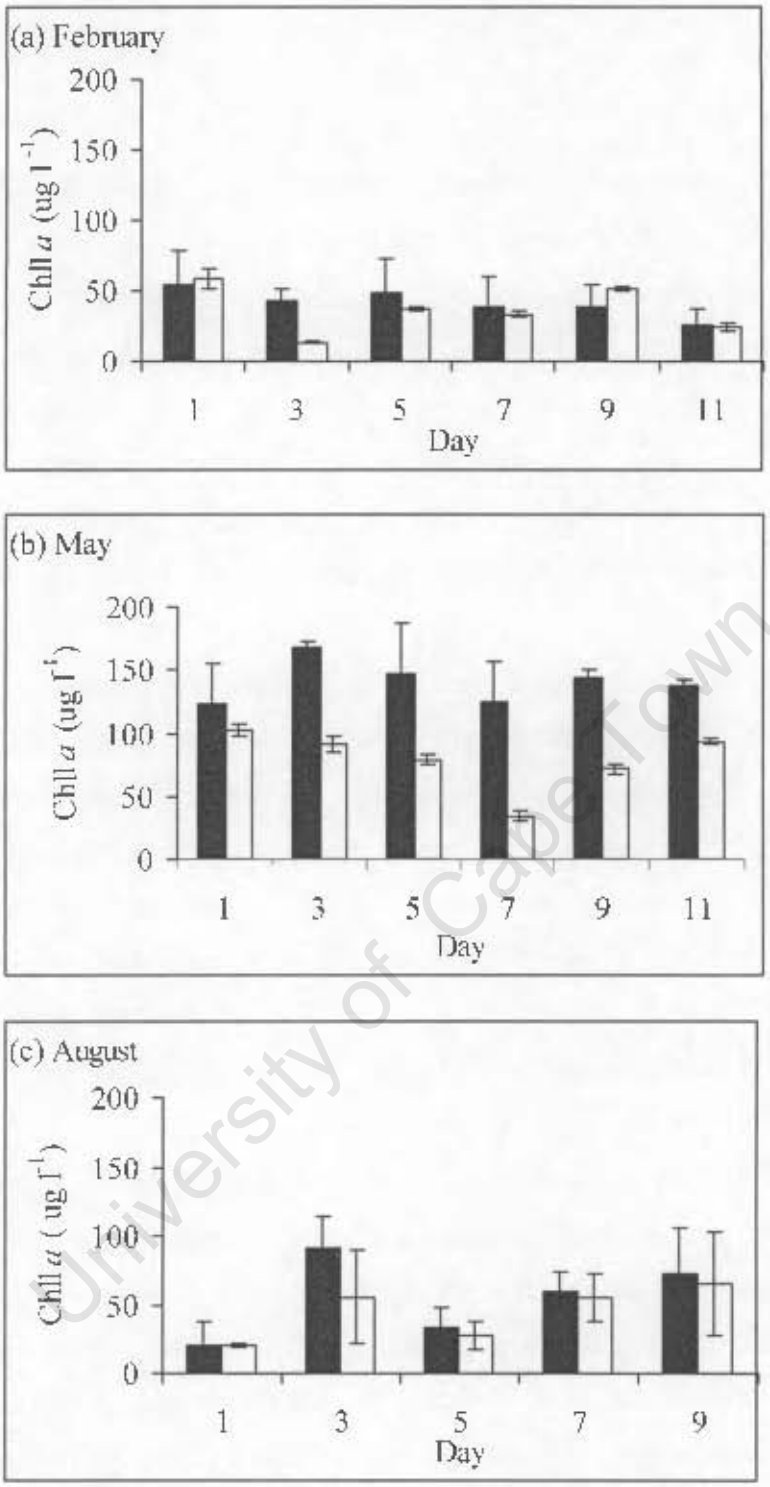


Figure 6.4 **Temporal variation in chlorophyll *a* concentration in enclosures (■) and the lake (□) in (a) February (b) May and (c) August. Enclosure data are means of three replicates ± SD while the lake value is from an integrated sample 0–1 m.**

The variation in the relative abundances of the dominant species in the lake and in the enclosure during winter (May) is shown in Figure 6.5c. During winter *Cryptomonas* sp. and *Cylotella* sp. were dominant in the lake over the whole period, comprising > 95% of the total biomass, with cyanobacteria comprising of *Anabaena* sp. and *A. tanganyike* making <2% (Figure 6.5c). Chlorophytes (*P. duplex* and *Coelastrum* spp.) and euglenophytes were rare. *Cryptomonas* and *Cyclotella* co-dominated in the enclosure with relative biomass > 90% until day 5 (Figure 6.5d). As *Cryptomonas* biomass continued to increase in enclosures *Cylotella* declined.

Cryptomonas had assumed 93% of the total biomass by day 9. *Anabaena*/*Anabaeniopsis* and chlorophytes also increased slightly in the enclosures. Isolation in winter resulted in an increase in biomass of *Cryptomonas* and a decline in *Cyclotella*. The biomass of *Cryptomonas* sp. in enclosures during winter increased markedly (Figure 6.7b). The mean enclosure total biomass was significantly higher than the mean lake total biomass ($F(1,12) = 26.2$ $p < 0.005$) however variability over time was not significantly different ($F(5,12) = 0.3$ $p > 0.05$) because of the high overlaps of the 0.95 confidence intervals. Generally total biomass was higher in May than in February and August.

Variations in the relative abundances of the dominant species in the lake and in the enclosure at the end of winter (August) are shown in Figure 6.6. The phytoplankton assemblage in the lake comprised of *Cryptomonas* and chlorophytes (three *Coelastrum* species and three *Scenedesmus* species). *Cryptomonas* was dominant in the lake comprising > 88% of the total biomass throughout the period (Figure 6.6a). Total biomass in the lake increased gradually over the period of 9 days (Figure 6.7c). In the enclosure the phytoplankton assemblage was dominated by *Cryptomonas* until day 7 when a switch occurred to dominance by *Coelastrum* spp (Figure 6.6b). Maximum biomass in the enclosures was attained at day 3 (Figure 6.7c). Isolation at the end of winter resulted in an initial increase in biomass of *Cryptomonas* that latter declined following a switch to dominance by chlorophytes. In August mean lake biomass was not significantly different from the mean enclosure biomass (Figure 6.7c, $F(1,10) = 0.9$ $p >$

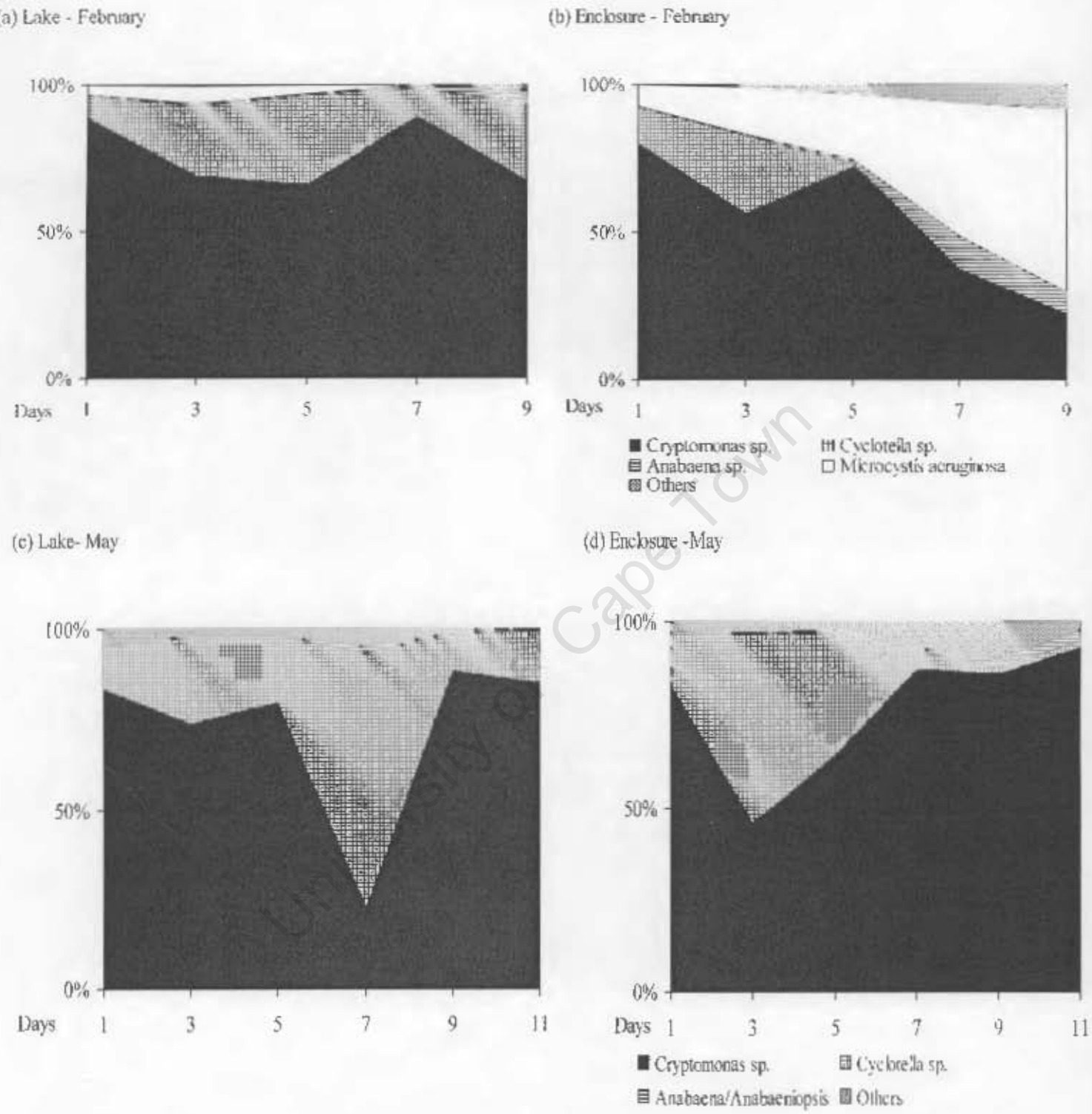


Figure 6.5 **Temporal variation of the relative abundance of the most abundant species in the lake and the enclosure during summer (a & b) and winter (c & d).**

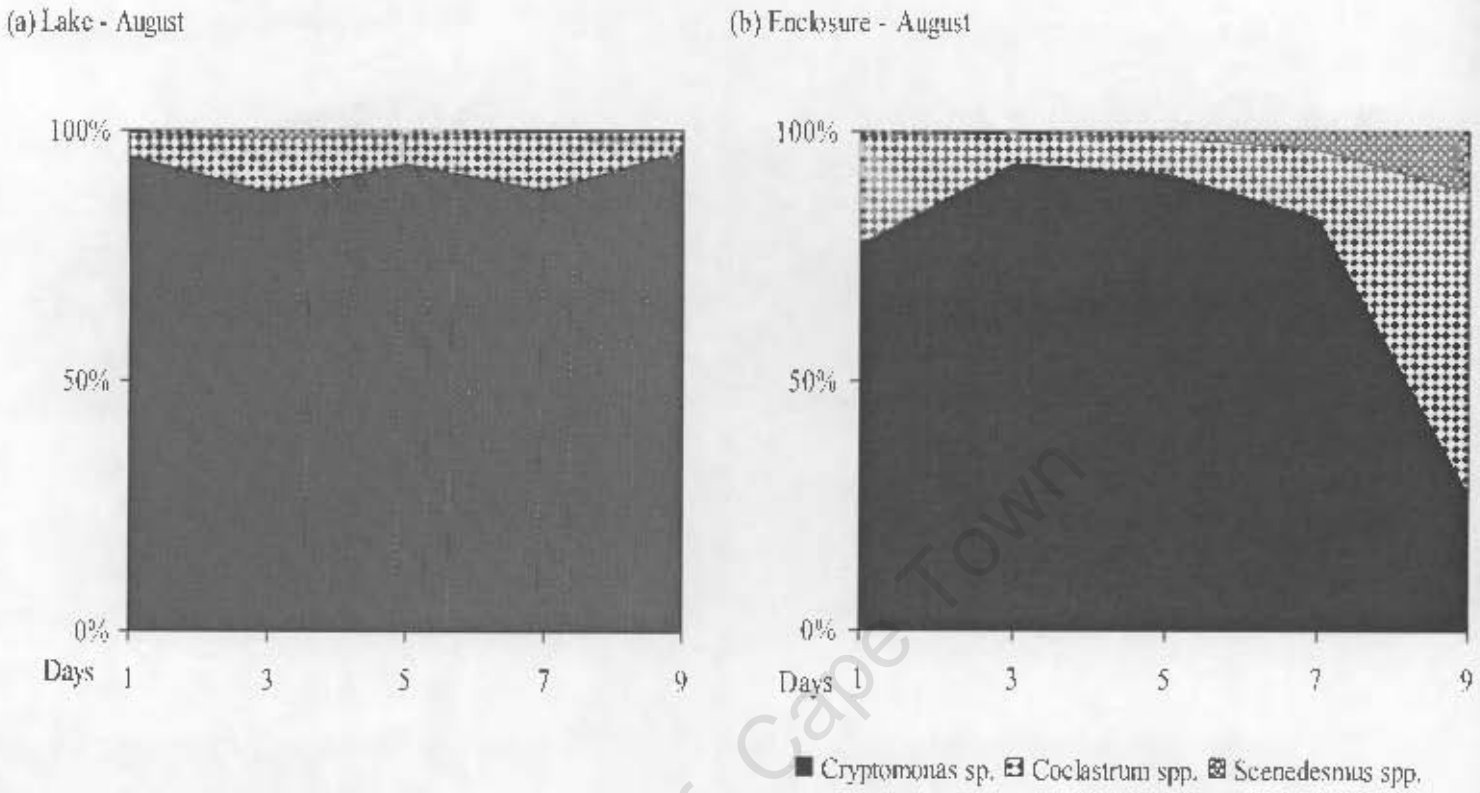


Figure 6.6 **Temporal variation of the relative abundance of the most abundant species in the lake and the enclosure at the end of winter.**

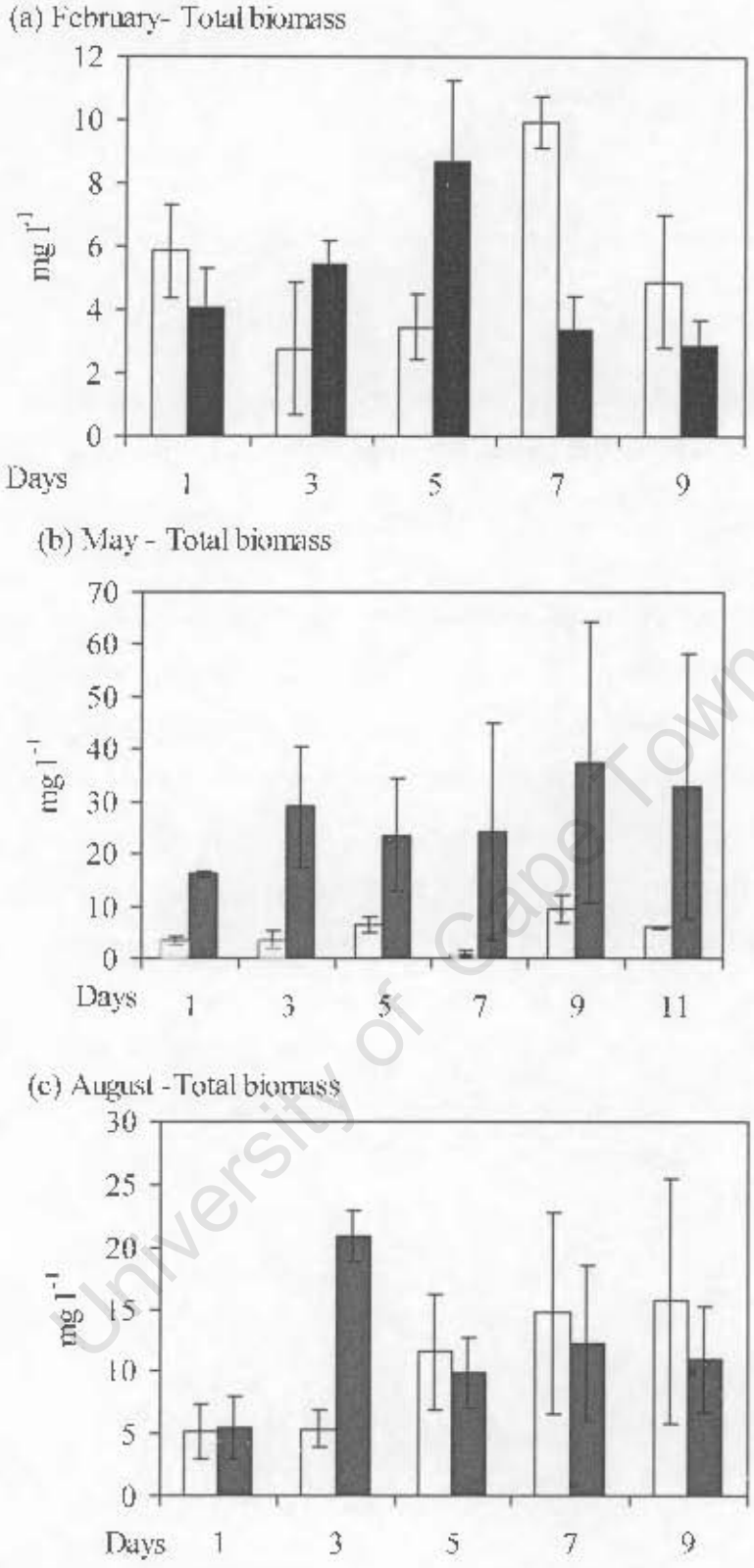


Figure 6.7 **Variation in total biomass in enclosures (■) and the lake (□) in (a) February (b) May and (c) August. Enclosure data are means of three replicates ± SD while the lake value is from an integrated sample 0–1 m. Note different Y-axis scale.**

0.05) although enclosure and lake biomasses varied significantly with time (Figure 6.7c F (4,10) =5.7 $p < 0.05$).

The phytoplankton assemblage in the lake during all the three periods was dominated by *Cryptomonas* sp., which had numerical superiority over the other species, with an average relative abundance > 65 % and together with *Cryptomonas* > 90%.

Variations in species diversity, calculated by the Shannon-Weaver diversity index in February, May and August, are shown in Figure 6.8. The mean species diversity was generally similar over the three sampling periods. In February although the mean species diversity in the enclosure was significantly higher than the mean species diversity in the lake (F (1,9) = 7.3 $p < 0.05$), temporal variability was similar (Figure 6.8b, F(5,9) = 0.1 $p > 0.05$). In May diversity in the lake and the enclosure was similar (Figure 6.8b, F(5,12) = 2.6 $p > 0.05$) although in the lake diversity fluctuated but consistently dropped in the enclosure. In August diversity in the lake and the enclosure was similar between day 1 and 5, after which diversity in enclosures increased. In August mean diversity in the enclosure was significantly higher in the lake (Figure 6.8c, F (4,10) = 31.7 $p < 0.05$) and was also significantly higher over time in the enclosure (F (4,10) = 9.3 $p < 0.05$).

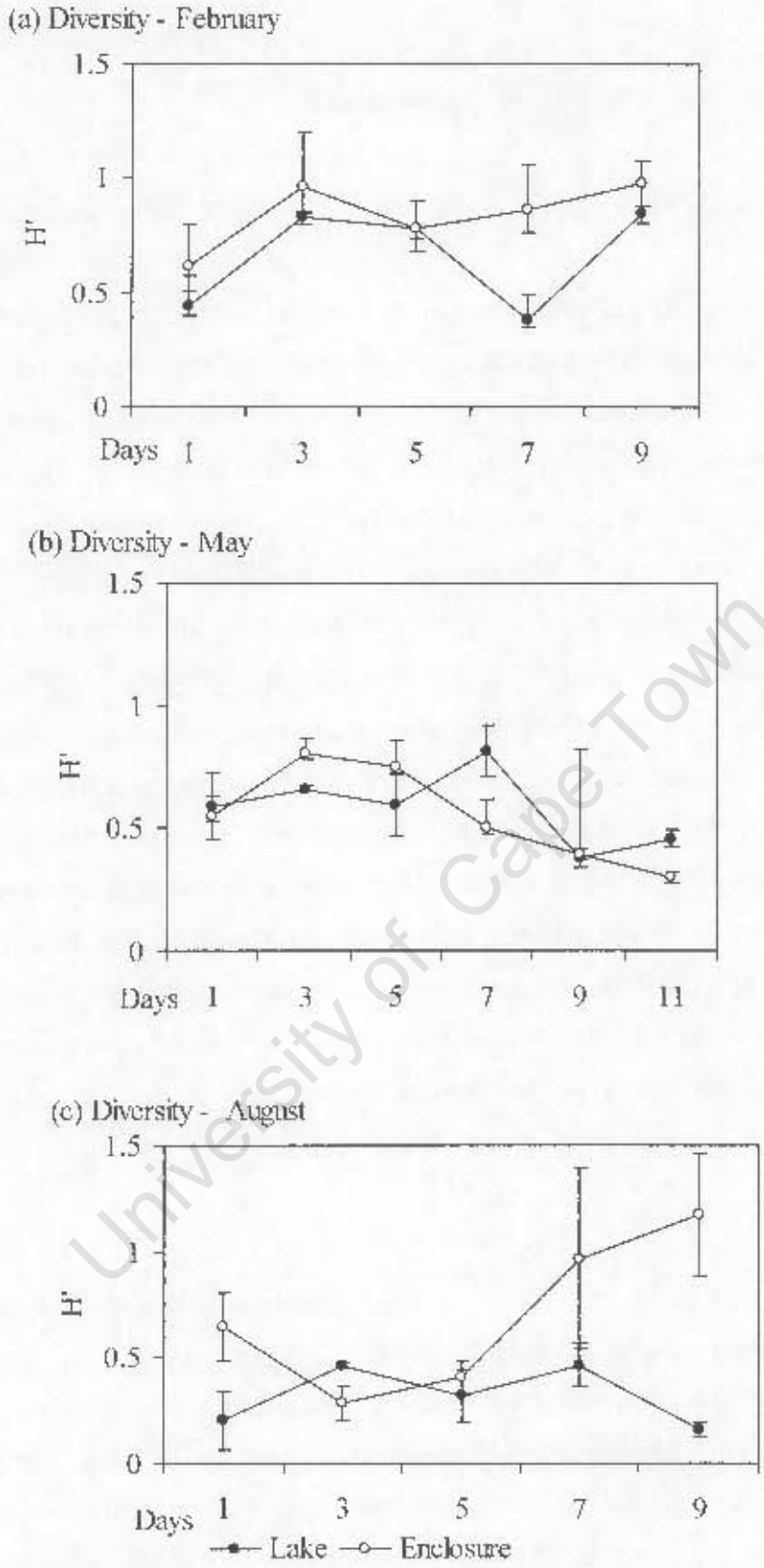


Figure 6.8 **Temporal variation of diversity (Shannon-Weaver Index) during the three sampling periods.**

6.4 DISCUSSION

Biomass was generally enhanced by isolation. Although the temporal change was not significant, an increase in biomass in enclosures was apparent between day 1 and day 5. The higher pH and dissolved oxygen levels in enclosures during all three periods indicate that primary productivity was higher in enclosures than in the lake. This in turn is an indication that small-scale random events (perturbations) could be important in regulating phytoplankton biomass in Lake Chivero. The marked fluctuations of chlorophyll *a* concentration and biomass at 2-day intervals in the lake are an indication of the effect of physical perturbations. According to Haffner & McNeely (1989) small-scale perturbations can shift a phytoplankton assemblage from being regulated by autogenic factors such as buoyancy control or nutrient competition to being regulated by allogenic factors such as turbulence or temperature. The increase in biomass just by isolation shows that turbulence could be an importance factor since nutrients were either lower in enclosures or not different between enclosures and the lake. The assumption is that turbulence was reduced in the enclosures when water was isolated. It is also possible that light conditions in the enclosure would probably be better than in the lake and therefore enhance the phytoplankton primary production whereas in the lake the algae were circulated down into dim light several times during the day (Kirsten Olrik, personal communication).

In Thau lagoon, Millet & Cecchi (1992) established that 1-to13-day physical perturbations were major constraints regulating dynamics of the phytoplankton assemblages, which comprised centric diatoms, nanoflagellates and to a lesser extent dinoflagellates. They observed that drastic perturbations by wind at 2-week intervals induced a recurring reinitialization of succession such that succession never continued beyond dominance by opportunist species. It appears that in Lake Chivero physical disturbances could be a major temporal limitation of the development of algal succession from ruderals plants and cryptophytes to specialists because according to Padisák *et al.* (1988), a minimum calm period of 5-7 days is necessary for a shift from ruderal plants to specialists – from physical to biological control.

The effect of physical disturbances was illustrated by the pattern of species replacement after isolating different phytoplankton assemblages in summer, winter and end of winter. In summer, isolation in enclosures of a phytoplankton assemblage comprising both *Cryptomonas* sp. and *Cyclotella* sp. and specialists (*M. aeruginosa* and *Anabaena* sp.) resulted in the gradual competitive decline of *Cryptomonas*/*Cyclotella* after 7-9 days. After nine days *Microcystis* and *Anabaena* predominated, *Cyclotella* was excluded while *Cryptomonas* had declined. In winter when the isolated phytoplankton assemblage did not include significant numbers of *Microcystis*, *Cryptomonas* remained dominant, although the build-up in biomass of *Cryptomonas* after seven days resulted in a decline in *Cyclotella*. At the end of winter the isolation of a phytoplankton assemblage that comprised of *Cryptomonas* and chlorophytes resulted in decrease in *Cryptomonas* as the biomass of *Coelastrum* spp. increased. Comparatively in the lake both *Cyclotella* and *Cryptomonas* predominated in summer and winter while *Cryptomonas* and *Coelastrum* spp. predominated at the end of winter.

Physical mixing and turbulence can play major roles in preventing one species from completely dominating the other (Margalef 1978, Harris 1983), which may partly explain the decline in dominance by *M. aeruginosa* in Lake Chivero. The phytoplankton assemblage may be frequently adjusting to persistent turbulence, resulting in *M. aeruginosa* being out-competed because mixing will be counteracting near-surface accumulations of buoyant *M. aeruginosa,* thereby forcing competition for light and nutrients with non-buoyant eukaryotic taxa.

Weather fluctuations strongly determine the physical environment of the water column at a daily scale, which in turn strongly influences phytoplankton dynamics. Since the influence of wind could not be measured directly, just isolation of three distinct phytoplankton assemblages in enclosures indirectly showed that weather and physical variables may account for the large part of the observed natural variability in phytoplankton dynamics (succession) in Lake Chivero, especially in the absence of nutrient limitation.

Chlorophyll *a* concentrations in May were double the concentrations recorded in February and August. This was due to a high biomass of *Cryptomonas* sp. Orthophosphate, total phosphorus and ammonium concentrations were highest in the lake during winter, probably arising from overturn. This could have provided ideal conditions for *Cryptomonas* sp. to increase in biomass. Elsewhere cryptophytes have been observed to establish maximal populations during or immediately after a redistribution of nutrients in the water column through turbulence (Klaveness 1988, Pautova *et al.* 1989, Istaványovics *et al.* 1994). Their marked increase is attributed to their small size and high area/volume ratio that enables them to attain high rates of growth and respiration (Dos Santos & Calijuri 1998). Irruptions of high biomasses of *Cryptomonas* sp. were also observed during the clear state (Chapter 4) when conditions became ideal.

In Lake Chivero, *Cryptomonas* sp. is now a dominant species (Chapter 4) as also shown by its predominance in summer, winter and end of winter during this study. Reynolds (1996) proposed a sequential dominance in phytoplankton succession of (i) R- ruderal plants predominating during mixing (ii) C-growth specialists at the beginning of stratification and (iii) S-specialists at the end of the stratification period and at the end of succession. This pattern was not exhibited in Lake Chivero; instead cryptophytes and C-strategists (baccillariophytes) comprised the algal assemblage in summer, winter and end of winter.

Temperature was higher in February (summer) and lower in May and August (winter and end of winter respectively). Although temperature influences the physical structure of ecosystems (Do Santos & Calijuri 1998) it is unlikely to have influenced the predominance of cyanobacteria. *M. aeruginosa* only occurred in February in the lake, probably because it was the period immediately after collapse of the cyanobacterial bloom, which provided the inoculum (Chapter 5). During the clear state *M. aeruginosa* was abundant around February but it also occurred during the turbid state when temperatures were low (Chapter 5). *Microcystis aeruginosa* was not limited by nutrients because ammonium, nitrates, orthophosphate and total phosphorus were high in winter. Other species predominated instead. The applicability of hypotheses that influence

cyanobacterial dominance in Lake Chivero, as discussed in Chapters 4 and 5, showed that generally the conditions were not favourable for the presence of *M. aeruginosa,* a specialist and an indicator of structural stability of lakes (Reynolds 1988). The increase of *M. aeruginosa* in enclosures in February showed that a physically stable environment favours it.

Except for nitrates that increased in May, nutrients declined in the enclosures as they were utilized by phytoplankton. This coincided with an increase in the abundance of two nitrogen-fixing cyanobacterial species, *Anabaena* sp. and *A. tanganyike,* in the enclosures. It is not apparent why there was a slight increase of these two species in winter compared to the other periods. The two species were also observed in the lake, but in the enclosures there was an increase of the population by the end of the experiment. The increase in these species in the enclosures seems to be related to the physical stability, which contrasted with the constantly perturbed state in the lake. The increase in nitrate could be an evidence of nitrogen fixation or an oxygenation that helps the shift from NH_4 to NO_3, which occurred since nitrate also increased in the lake from day 7 although it lagged behind levels in the enclosure probably because it was constantly dispersed. Decline in ammonium and nitrate in enclosures shows that both were used as sources of nitrogen by phytoplankton. An increase in orthophosphate in enclosures in May could have resulted from contamination of the enclosure water by bird feacal matter. Many birds are present on Lake Chivero.

The lake phytoplankton assemblage was generally similar in all three seasons with respect to dominance by *Cryptomonas* - except for the presence of *M. aeruginosa* in summer and *Anabaena* and *A. tanganyike* in winter and a slight increase of chlorophytes at the end of winter. The community in the lake typified a state of non-equilibrium with the predominance of species that grow rapidly (R-selective or C-strategists). *Cryptomonas* sp., which occurred together with *Cyclotella* sp., has been reported to survive in a large variety of environmental conditions, either in the mixing period or during stratification (Reynolds 1982, 1983, 1996).

The predominance of *Cryptomonas* sp. in Lake Chivero confirms that its survival strategy is intermediate between those of the growth strategists (C) and the species that are tolerant of disturbances (Ruderal plants) as observed by Do Santos & Calijuri (1998). It is successfully managing to out-compete *M. aeruginosa* in Lake Chivero. *Cyclotella* sp. was also dominant, especially in winter, although it sometimes occurred with *A. granulata* (Chapter 4). Diatoms are growth strategists and ruderal plants and predominate during periods of circulation with high availability of nutrients, especially nitrogen, and good light conditions (Sommer 1988). In winter when *Cyclotella* was abundant, the lake was isothermal and dissolved oxygen was 2 mg l^{-1} at 20 m (Chapter 5) indicating that mixing had occurred.

During this study *A. granulata* was not present in the lake although it was recorded between 2003 and 2004 (Chapter 4). The shift from a predominance by *A. granulata* to *Cyclotella* sp. shows that diatoms can exhibit a wide spectrum of responses and survival strategies in relation to nutrient availability, light and competition. The clear state in Lake Chivero (Chapter 4) seem to have favourably selected for *Cyclotella* sp. a diatom that seems to have more successively adapted to the existing conditions than *A. granulata.* The growth of diatoms is mainly related to the ratio of silica to phosphorus and availability of light (Reynolds 1983, Do Santos & Calijuri 1998) of which phosphorus and light were high in Lake Chivero in winter, but silica was not measured.

During winter (May and June) *Cyclotella* sp. comprised over 80% of the total biomass and assumed absolute dominance at stations 3, 4, and 5 in June (Chapter 5). Favourable conditions in winter included (i) high availability of nutrients from circulation (ii) good light conditions since the lake was in a clear state most of the time (Chapter 4) and (iii) high concentration of nitrates in May and June (Chapter 5), conditions of which are preferred by diatoms (Sommer 1988).

Cyclotella sp. and *Cryptomonas* sp. are benefiting from the decline of *M. aeruginosa* population density in Lake Chivero. They now successively occupy niches that were freed by *M. aeruginosa* because of their morphological structure and reproductive

processes. As observed during the turbid state and in enclosures in February, excessive growth of *M. aeruginosa* completely suppressed these two species, confirming the observation by Murphy & Lean (1976).

Conditions in the lake were not favourable for the predominance of *M. aeruginosa* but *Cyclotella* and *Cryptomonas*, which are typical of the initial phases of succession. *Microcystis aeruginosa* was dominant only during the turbid state when the lake was stratified and nitrate levels were high (Chapter 5). *Microcystis* only occurs in water with oxygen (Kirsten Olrik, personal communication) thereby high nitrate concentration. It has been observed in subtropical environments that *M. aeruginosa* is numerically superior in summer during periods of stratification (Reynolds *et al.* 1981) while during mixing it remains in sediments (Bell & Ahlgren 1987). Although it occurred in summer during this study it was not numerically superior over other taxa, which further supports an observation that there has been a shift in the phytoplankton assemblage in Lake Chivero (Chapter 4). Cryptophytes and growth strategists dominated in the lake and enclosures, showing the predominance of a community in a state of non-equilibrium. According to Reynolds (1984) ruderal plants favour unstable environmental conditions with strong water mixing and high availability of P and N resources.

Although these observations were undertaken over a few days, they showed that other than nutrient availability, turbulence from wind mixing prevents accumulation of high algal biomasses in Lake Chivero, including that of cyanobacteria. In Chapter 4 seasonal variation in phytoplankton dynamics was linked to nutrient fluctuations, which can be presupposed to be the principal forcing function in the lake. This study however, showed that events with shorter periods, such as weeks or days, might be relevant to the dynamics of a reservoir (Fonseca 1997). The decline of *M. aeruginosa* indicates the importance of the role played by allogenic factors in regulating composition and abundance of cryptomonads over short-term scales (Haffner & McNeely 1989).

The Shannon-Weaver index showed that diversity was similar in the lake during the three periods and it also did not vary markedly with isolation. The increase in diversity

observed in enclosures in August was due to increase in rapidly growing *Scenedesmus* and *Coelastrum* species. Diversity is reported to increase with occurrence of disturbances and diminishes with stability (Connell 1978, Sommer *et al.* 1993). Isolation over a longer duration could have reflected this.

This study showed that increase in stability in enclosures led to an increase in chlorophyll *a* concentration and biomass of all species including cyanobacteria. Confinement of the phytoplankton assemblage in enclosures enhanced cyanobacteria dominance but when *M. aeruginosa* was absent isolation perpetuated the predominance of *Cryptomonas*. The non-equilibrium state in Lake Chivero caused by changes in physical processes (turbulence) presents an ideal niche for species with higher growth rate. It appears that allogenic processes might be regulating the phytoplankton assemblage leading to an increase of species richness and diversity in the algal assemblage of Lake Chivero rather than the predominance of *M. aeruginosa.* The study provides information on responses and survival strategies of the phytoplankton assemblage in Lake Chivero. Although experimental findings do not exactly match what happens in the natural environment they are useful to explore the influence of key factors on a complex biological assemblage. Further investigation will be required to test the applicability of Intermediate Disturbance Hypothesis (IDH) to the phytoplankton assemblage in Lake Chivero at finer sampling intervals of weekly or bi-weekly for at least one year.

CHAPTER 7

NITRATE-INDUCED CHANGES AND EFFECT OF VARYING NITROGEN: PHOSPHORUS RATIOS ON THE PHYTOPLANKTON ASSEMBLAGE IN LAKE CHIVERO: MICROCOSM EXPERIMENTS

7.1 INTRODUCTION

Historically phosphorus has been considered to be the primary nutrient limiting phytoplankton growth in freshwater ecosystems, which has led to management efforts being focused on controlling phosphorus loading (Dzialowski *et al.* 2005). Lake Chivero, which receives high loadings of nitrogen and phosphorus (Nhapi 2004), has a relatively low TN:TP ratio (Chapter 3), perhaps attributable to the low TN:TP ratio of sewage effluent (Robarts 1981), which is the main source of nutrients in the lake. The nutrient-loading ratio of the sewage effluent into Lake Chivero is in the order of 1:2 TN:TP and has produced a system that is nitrogen-limited (Robarts 1981). Globally, nitrogen limitation now occurs more commonly than previously thought (Dzialowski *et al.* 2005). Even co-limitation by both P and N has also been observed to be common in European lakes where 63% of 30 lakes were co-limited by N and P while P limited only 24% (Maberly *et al.* 2002). Previously Robarts & Southall (1975, 1977), using bioassay cultures with *Selenastrum capricornutum,* established that nitrogen was potentially the primary growth-limiting nutrient in Lake Chivero.

Nutrient ratios influence the growth, physiological state and assemblage structure of phytoplankton in lakes (Cuvin-Aralar *et al.* 2004). In algal studies one of the ways that is used to determine the influence of specific factors on phytoplankton assemblages is to isolate whole phytoplankton assemblages either in microcosms (<1 m^3), mesocosms (between 1 and 10 m^3) or macrocosms (>10 m^3) (Lalli 1990). These are then incubated after varying a specific parameter while maintaining other conditions as close to natural as possible. In order to determine the influence of nitrate additions and the consequent change in TN:TP ratio, microcosms studies were carried out in Lake Chivero. The

advantage of using natural enclosure bioassays, according to Takahashi (1990), is that they capture and maintain natural ecosystems having multispecies with multitrophic levels that are partly self-controlled by feedback mechanisms within the microcosm. However it is also noted that experimental enclosures may also exclude important ecological processes, such as mixing and nutrient availability.

Through laboratory cultures it has been observed that optimum molar ratios for freshwater phytoplankton lie between 7 and 87 (Rhee & Gotham 1982, Hecky & Kilham 1988). However Schöllhorn & Granéli (1997) reported that less is known about TN:TP ratios and phytoplankton species composition *in situ*. This information is not available for Lake Chivero. The water quality data in Chapter 3 showed that the levels of nitrogen and phosphorus varied both seasonally and annually. From an applied point of view it is interesting to determine how varying nitrate loadings into the lake will influence phytoplankton species composition and biomass. It is, furthermore, of management interest to be able to maintain optimal ratios that can support socially and ecologically preferable chlorophytes and cryptomonads rather than dominance by undesirable cyanobacteria.

During this study the proportion of nitrogen to phosphorus (TN:TP ratio) in the lake was low (below 30) (Chapter 3). Although such a ratio in other water bodies has been observed to favour dominance by cyanobacteria (Smith 1983), this was not the case in Lake Chivero during this study (Chapter 4). Instead the phytoplankton assemblage comprised of diatoms, cryptomonads, chlorophytes and euglenophytes. This observation was used to manipulate TN:TP ratios by changing nitrate concentrations in microcosms in order to determine how this will affect phytoplankton dynamics.

The development of a cyanobacterial bloom in Lake Chivero from May to December 2005 coincided with an increase in nitrate and total nitrogen concentration - thus a slightly higher TN:TP ratio than those that had prevailed in the lake during the clear state (Chapter 3). When the bloom collapsed the nitrate and TN:TP ratio had dropped. From this observation, I hypothesized that increasing nitrate concentration and the resulting

increase in the TN:TP ratio must be a major factor influencing species composition change and probably the development of cyanobacterial blooms in the lake. During these experiments I tested whether varying TN:TP ratio had effect on phytoplankton dynamics.

The objectives of this study were:

(i) to determine the effect of increasing nitrate concentration and the connected increase of TN:TP ratio on phytoplankton species composition, biomass, succession, and dominance patterns

(ii) to determine species-specific differences in phytoplankton responses to the changes of TN:TP ratio

7.2 MATERIALS AND METHODS

7.2.1 Experimental design

Two *in situ* microcosm experiments of 9 days each (Experiment 1: end of winter, from 15 to 24 August 2005 and Experiment 2: end of the rainy season, from 4 to 12 April 2006) were run in a small sheltered bay where the depth was about 3 m. There were five treatments each replicated twelve times. Water from the lake with natural populations of phytoplankton was collected into five 25-litre plastic buckets. The first bucket was used as a control with no treatment added. The other 4 buckets were spiked with nitrate ($NaNO_3$) and phosphate (K_2HPO_4) at four different concentrations as shown in Table 7.1. The ambient nitrate concentration was increased in the following ratios: 1X, 20X, 50X and 100X. One phosphate concentration was used. In the control no nutrient adjustments were made. The spiked water was thoroughly mixed with a stirrer. From each bucket a sample of 1 litre was collected for analysis of physical and chemical variables and phytoplankton. The remaining water in each respective treatment was then put in 12 2-litre cylindrical plastic bottles made from polyethylene making a total of 60 microcosms. The bottles were filled to 1.5 litres, closed at the top with caps and were then suspended

on a floater in the lake and incubated for ten days. The natural zooplankton community was included in all treatments.

Table 7.1: Nitrate and phosphate added at the beginning of the experiments

Treatment	Nitrate	Phosphate
Lake	-	-
Control	-	-
NP1	0.09 g	0.05 g
NP20	1.80 g	0.05 g
NP50	4.51 g	0.05 g
NP100	9.02 g	0.05 g

7.2.2 Sampling and sample analysis

Physical and chemical variables were measured at 2-day intervals. Three replicate bottles from each series were removed every other day for analysis (Day 1, Day 3, Day 5, Day 7, Day 9). Sampling of surface water in the lake was also carried out every other day. After collection phytoplankton samples were immediately preserved in Lugol's solution. Cell counts were made with an inverted microscope according to Utermöhl (1958) and Cronberg (1982) (Chapter 4). Conductivity, pH, dissolved oxygen, total dissolved solids, temperature and turbidity were measured with field meters (Chapter 3). Nutrient analysis was carried out according to the methods outlined in Chapter 3.

7.2.3 Statistical analysis

Within each experiment, differences among treatments in physical and chemical parameters and phytoplankton biomass were tested using Repeated Measures Analysis of Variance (RM-ANOVA) for the 9 sampling days. For each experimental run a separate RM-ANOVA was conducted using the programme Statistica 7. When significant treatment effects were observed Tukey's Honestly Significant Different tests were used to determine which treatments were significantly different from each other. Multivariate descriptive analyses on physical, chemical data and phytoplankton data were undertaken

using Principal Components and Classification Analysis (PCCA) using the programme Statistica 7.

7.3 RESULTS

The six microcosm treatments were exposed to similar ambient conditions during each experimental period.

7.3.1 Physical variables and nutrients

The water temperature was not significantly different among the six treatments during the first experiment ($F = 2.0$ $p > 0.05$, Figure 7.1a) but was significantly different during the second experiment ($F = 4.3$ $p < 0.05$, Figure 7.2b). The average temperature at the beginning and end of the experiments were 19.8 °C and 23 °C during experiment 1 and 22.2 °C and 26.2 °C during experiment 2.

After spiking there was marked differences in conductivity in the treatments with highest levels ranging as follows: NP100 > NP50 > NP20 (Figure 7.1c & Figure 7.1 d). Conductivity varied significantly among treatments (Experiment 1 $F = 702.6$ $p < 0.05$; Experiment 2 $F = 127$ $p < 0.05$). Conductivity level in the lake, control and NP1 was similar during both experiments while other treatments were significantly different (Figure 7.1c, Figure 7.1d). Total dissolved solids exhibited a pattern similar to conductivity (Figure 7.1e & Figure 7.1f) and both parameters did not decline during the experiment period. Total dissolved solids concentration was significantly different among treatments (Experiment 1 $F = 613.7$ $p < 0.05$; Experiment 2 $F = 126.9$ $p < 0.05$). The lake concentration was similar to control and NP1 while the other treatments were significantly different (Figure 7.1e & Figure 7.1f).

Average dissolved oxygen concentration in microcosms increased from 3.3 to 12.3 mg l^{-1} during experiment 1 and from 6.4 to 11.6 mg l^{-1} during experiment 2. The levels were significantly different among treatments (Figure 7.2a & Figure 7.2b, Experiment 1 F =

3.8 $p < 0.05$; Experiment 2 $F = 16.5$ $p < 0.05$). The lake dissolved oxygen concentration was significantly lower than all microcosm treatments (Figure 7.2a & Figure 7.2b) during both experiments. The average microcosm pH increased from 7.2 to 10.5 (Figure 7.2c) and from 7.9 to 10.8 (Figure 7.2d) during experiment 1 and 2 respectively. The lake pH was significantly lower than that in the microcosms (Experiment 1 $F = 21.2$ $p < 0.05$; Experiment 2 $F = 13.3$ $p < 0.05$) while the pH levels in all other treatments was similar (Figure 7.2c & Figure 7.2d). Turbidity was significantly different among treatments (Experiment 1 $F = 6.4$ $p < 0.05$; Experiment 2 $F = 16.6$ $p < 0.05$) being higher in all microcosm treatments than in the lake (Figure 7.2e & Figure 7.2f). Average turbidity in microcosms increased from 34.3 to 46.1 mg l^{-1} during experiment 1 (Figure 7.2e) and from 8.2 to 13.7 mg l^{-1} during experiment 2 (Figure 7.2f).

Ammonium concentration was generally high in the lake, but there were no significant differences among treatments (Experiment 1 $F = 1.1$ $p > 0.05$; Experiment 2 $F = 0.5$ $p > 0.05$, Figure 7.3a & Figure 7.3b). After spiking, nitrate and total nitrogen concentrations in treatments ranged as follows: NP100 > NP50 > NP20 while there were no significant differences among the lake, control and NP1 during both experiments (Figure 7.3c & Figure 7.3d). Nitrate concentration was significantly different among treatments during both experiments (Experiment 1 $F = 107.6$ $p < 0.05$; Experiment 2 $F = 241.4$ $p < 0.05$, Figure 7.3c & Figure 7.3d). Nitrate levels remained high throughout the experimental period during both experiments. Total nitrogen was significantly different among treatments (Experiment 1 $F = 50.8$ $p < 0.05$; Experiment 2 $F = 1792.2$ $p < 0.05$, Figure 7.3e & Figure 7.3f) in a similar way to the pattern shown by nitrate.

During experiment 1, orthophosphate was depleted in all treatments by day 9 while during experiment 2 levels declined. There were significant differences among treatments during both experiments (Experiment 1 $F = 4.6$ $p < 0.05$; Experiment 2 $F = 3.1$ $p < 0.05$, Figure 7.4a, Figure 7.4b). There were no significant differences among treatments for total phosphorus during experiment 1 ($F = 0.9$ $p > 0.05$, Figure 7.4c) while during experiment 2 differences among treatments were significant ($F = 12.5$ $p < 0.05$, Figure 7.4d). The TN:TP ratio remained constant and the ranges in each treatment are

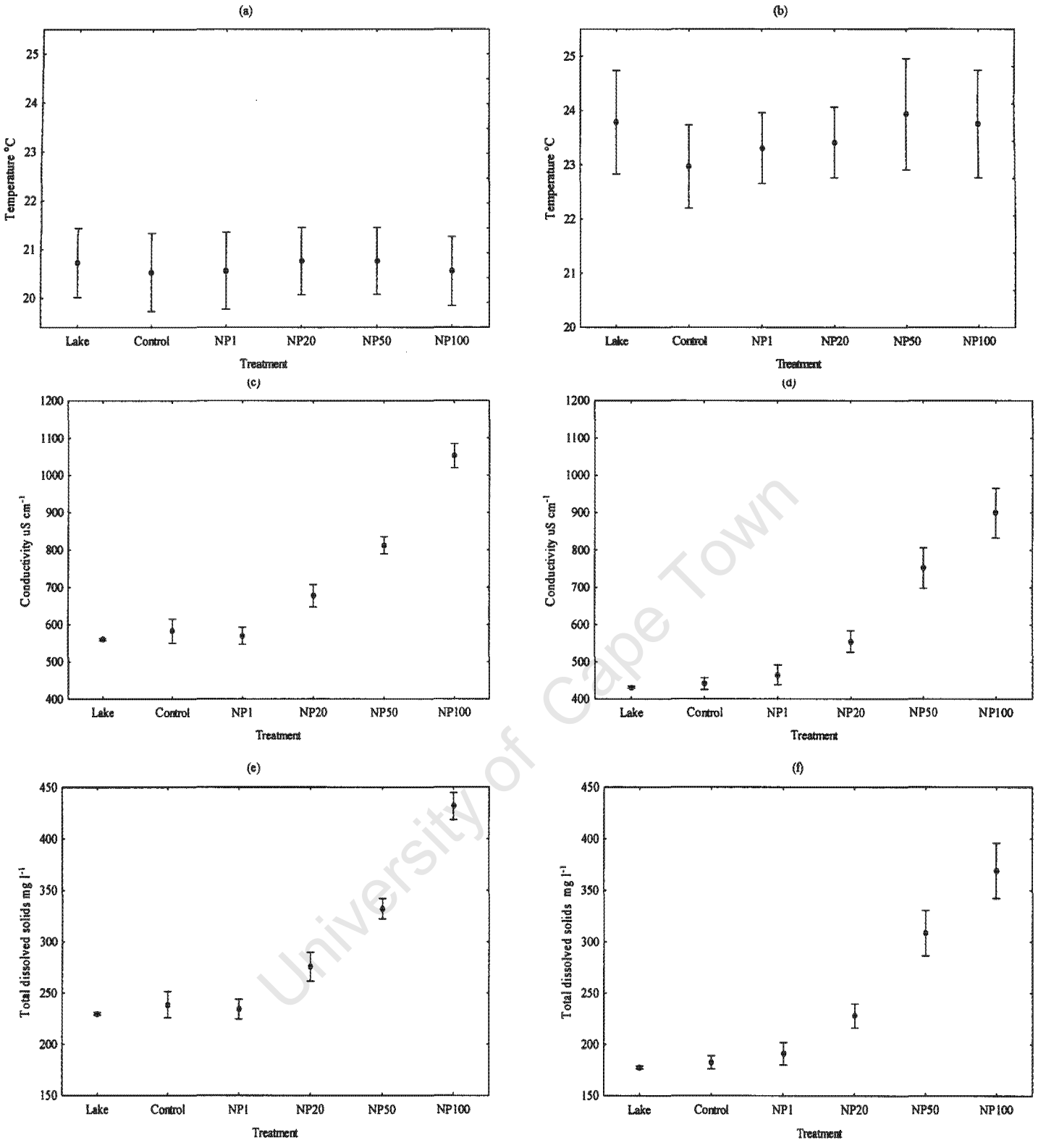


Figure 7.1: **Whisker plots (mean; n =12) of temperature, conductivity and total dissolved solids in six treatments during the experimental period. Vertical bars denotes 0.95 confidence interval. (Experiment 1 = a, c, e; Experiment 2 = b, d, f).**

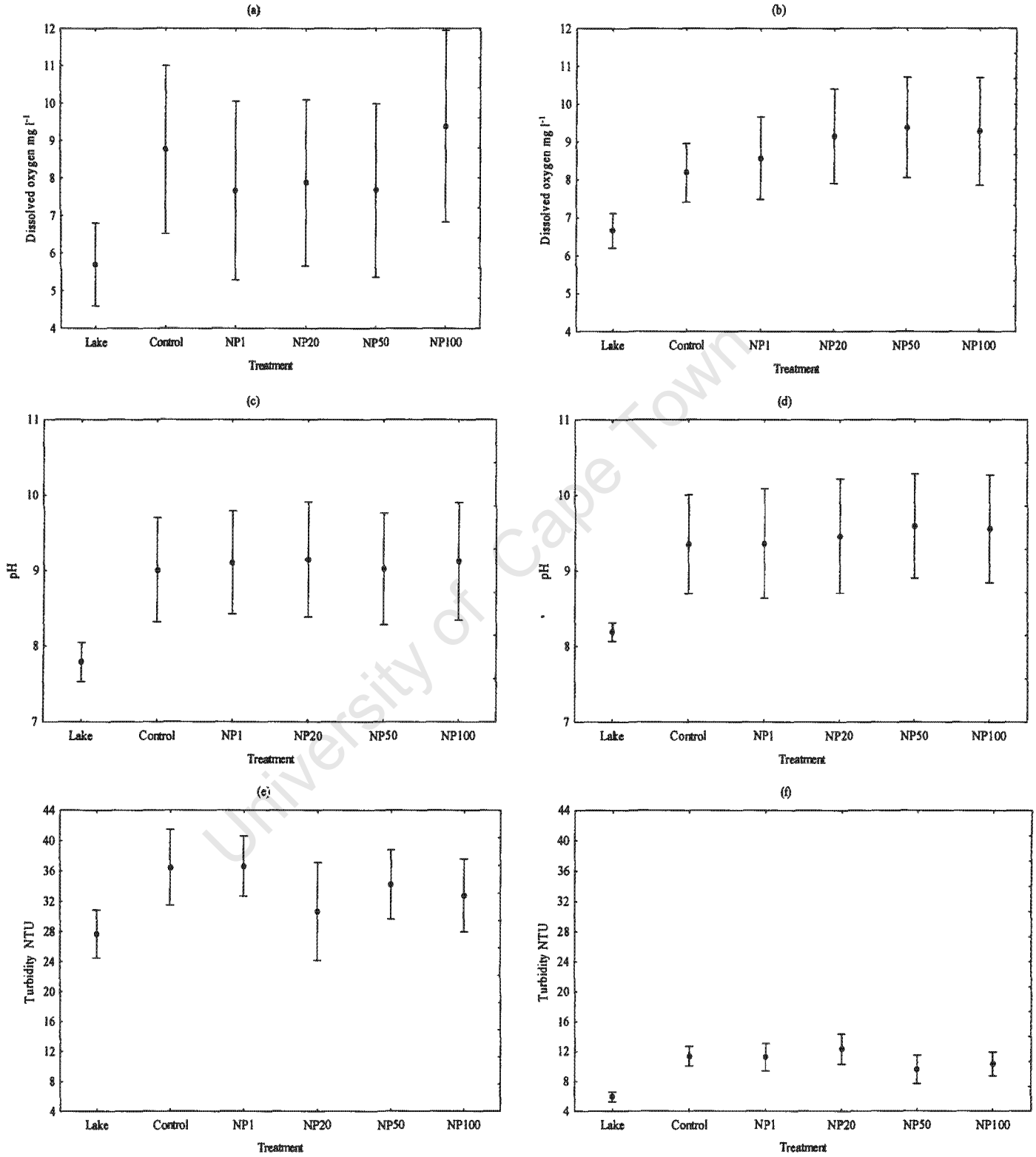


Figure 7.2: Whisker plots (mean, n = 12) of dissolved oxygen, pH and turbidity in six treatments during the experimental period. Vertical bars denotes 0.95 confidence interval. (Experiment 1 = a, c, e; Experiment 2 = b, d, f).

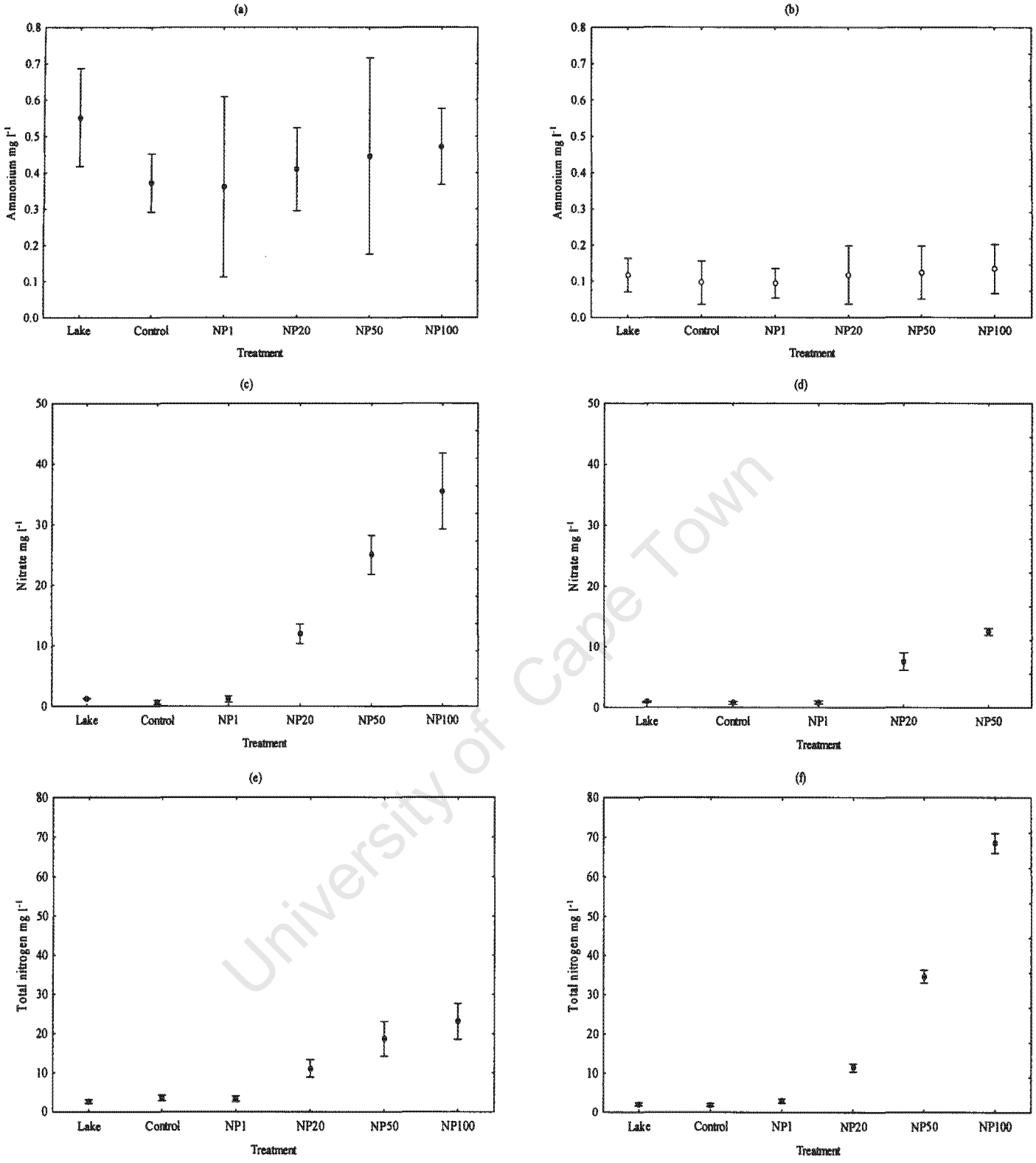


Figure 7.3: **Whisker plots (mean, n = 12) of ammonium, nitrates, total nitrogen in six treatments during the experimental period. Vertical bars denotes 0.95 confidence interval. (Experiment 1 = a, c, e; Experiment 2 = b, d, f).**

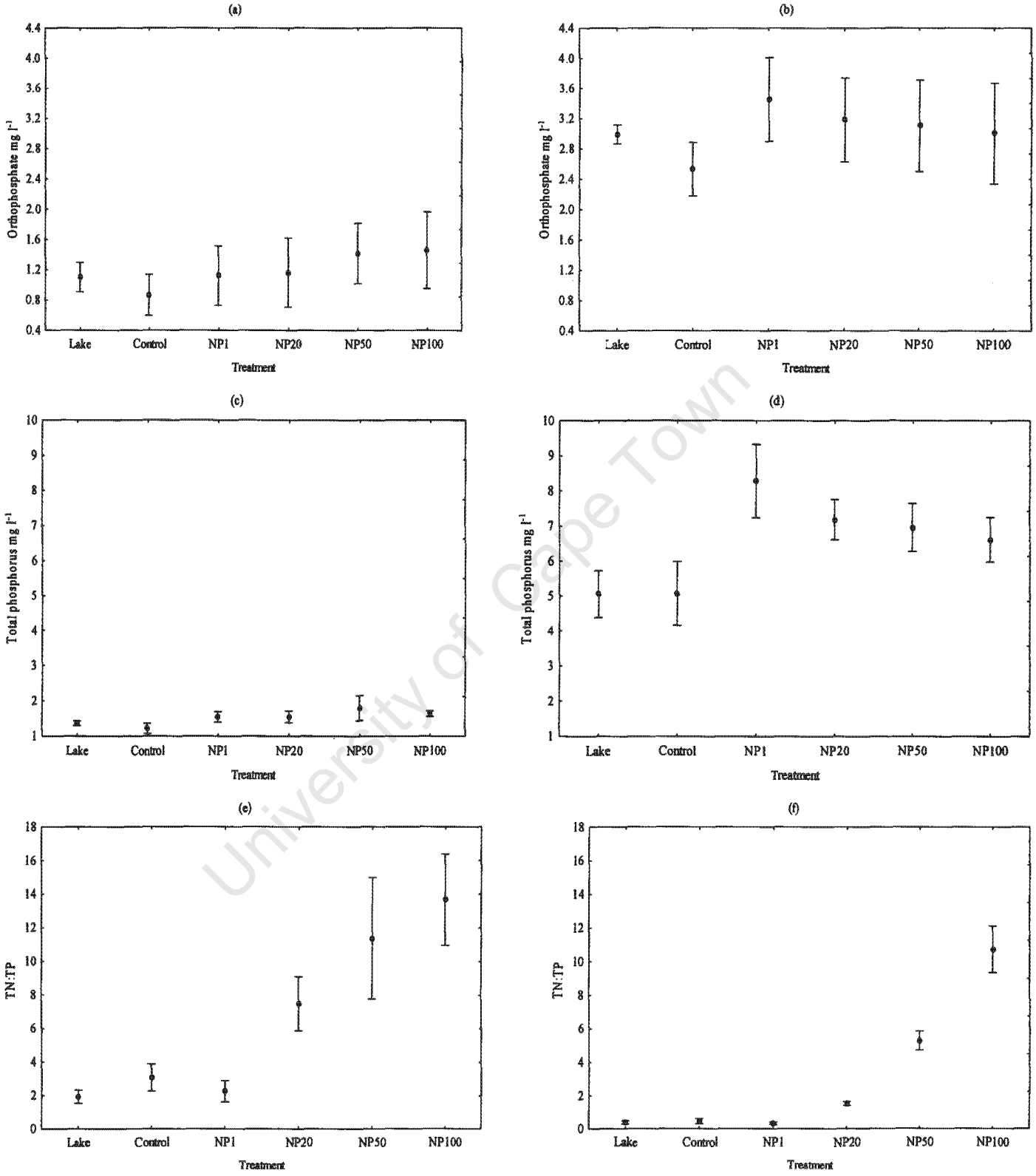


Figure 7.4: **Whisker plots (mean, n = 12) of orthophosphate, total phosphorus and TN:TP ratio in six treatments during the experimental period. Vertical bars denotes 0.95 confidence interval. (Experiment 1 = a, c, e; Experiment 2 = b, d, f).**

shown in Table 7.2. There were significant differences among treatments for TN:TP ratio (Experiment 1 $F = 32.5$ $p < 0.05$; Experiment 2 $F = 214.6$ $p < 0.05$). As with nitrate and total nitrogen the lake TN:TP ratio was similar to control and NP1 but significantly different from other treatments (Figure 7.4e & Figure 7.4f).

Table 7.2: TN:TP ratios in six treatments during the experiments

Treatment	Experiment 1	Experiment 2
Lake	1:0.53	1:2.53
Control	1:0.34	1:2.83
NP1	1:0.44	1:2.90
NP20	1:0.14	1:0.63
NP50	1:0.09	1:0.20
NP100	1:0.07	1:0.10

7.3.2 Growth of microalgal groups and species responses

The variation in total algal biomass as the biological response of the phytoplankton assemblage to nitrate addition (= TN:TP ratio) is shown in Figure 7.5. Total algal biomass in the lake fluctuated between 2.8 and 13. 4 mg l^{-1} and between 1.6 and 15.8 mg l^{-1} during experiment 1 and experiment 2 respectively. An increase in algal biomass occurred in all treatments. During experiment 1 the highest biomasses were attained at day 7 in all treatments with a maximum of 24.2 mg l^{-1} in NP20. The trend in total biomass increase was generally similar in all treatments including the control. Only the Control and NP1 had significantly higher biomasses than the lake while the rest were similar (Figure 7.5b). Total biomass in NP100 was significantly lower than in the control and in NP1 (Figure 7.5b).

During experiment 2 highest biomasses were attained at day 5 in all treatments except NP100 where highest biomass occurred at day 7. Only in NP50 and NP100 was total biomass significantly ($p < 0.05$) higher than in the lake while the rest of the treatments were similar (Figure 7.5d). The total biomass in NP100 was significantly ($p < 0.05$) higher than in NP1.

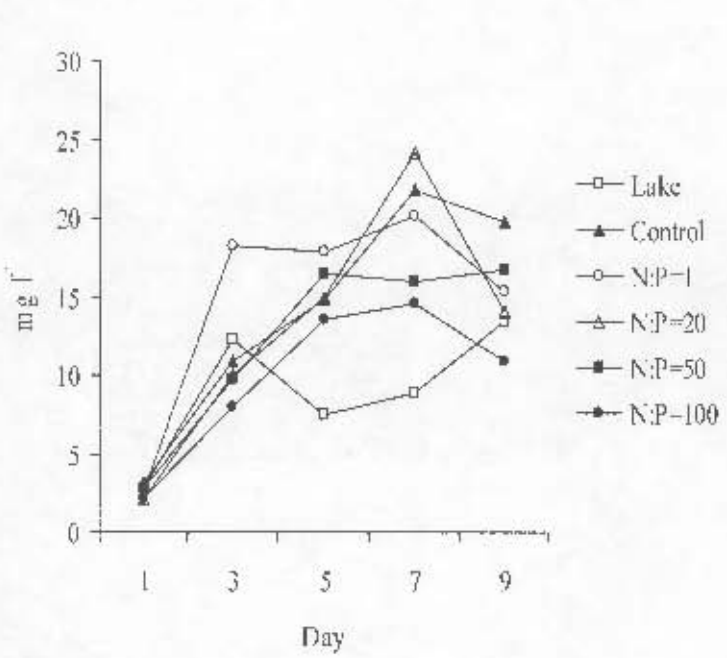
During experiment 1 the phytoplankton assemblage in the lake over the 9-day period was dominated by *Cryptomonas* (Figure 7.6). Addition of nitrate and consequent increase in TN:TP ratio shifted the assemblage from a dominance by *Cryptomonas* to an assemblage dominated by *Coelastrum* occurring with *Scenedesmus* and *Pediastrum* in all treatments (Figure 7.6). *Cryptomonas* markedly declined with increase in TN:TP ratio and totally disappeared at day 7 and day 5 in NP50 and NP100 respectively (Figure 7.6). All the treatments showed the same pattern and regardless of a lack of addition of nitrate in the control a shift to dominance by chlorophytes also occurred. Generally these results showed that nitrate (and consequent change in TN:TP ratio) and isolation from physical factors (wind turbulence) shifted the *Cryptomonas*-dominated phytoplankton assemblage to a chlorophyte-dominated algal assemblage. The conditions within the microcosms favoured the development of chlorophytes (*Scenedesmus*, *Coelastrum* and *Pediastrum*).

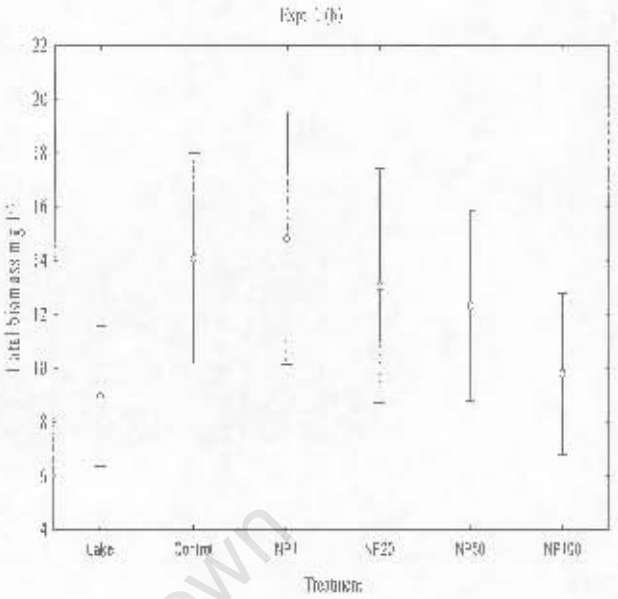
A similar response was observed during experiment 2 (Figure 7.7). The lake and the inoculum in all treatments were dominated by *Cryptomonas* and two species of diatom, *Cyclotella* and *Aulacoseira* (= *Melosira*) and to a lesser extent chlorophytes (*Pediastrum*, *Coelastrum*, *Scenedesmus*) and *Microcystis*. *Cryptomonas* markedly declined especially in NP20, NP50 and NP100 where by day 3 it was negligible in the culture. *Cryptomonas* also declined in the control and NP1 but remained in the culture up to day 9. The diatoms *Cyclotella* and *Aulacoseira* (= *Melosira*) also decreased and were very low in all treatments by day 9. Chlorophytes *Coelastrum*, *Pediastrum* and *Scenedesmus* became dominant from day 5 in all treatments, including the control. Favourable growth for *Pediastrum* occurred in NP1 and NP20. *Microcystis* only slightly increased in NP1.

7.3.3 Integrated analysis of abiotic and biological variables

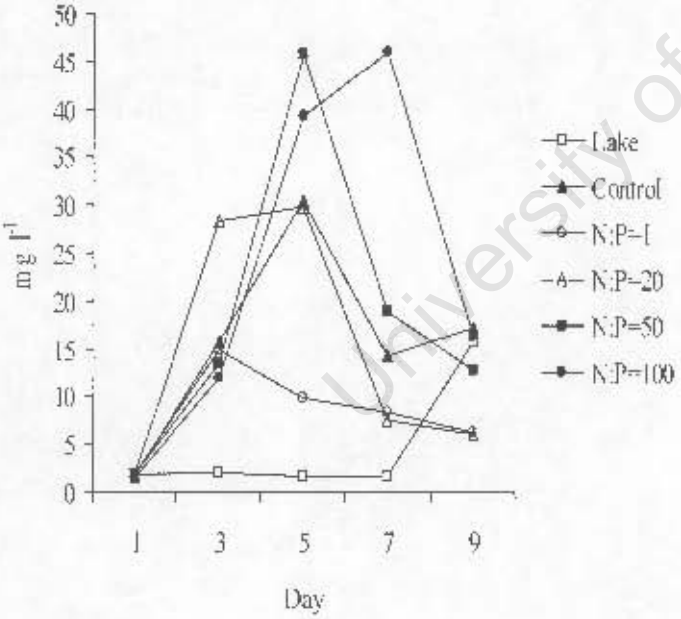
Ordination of data from the 6 treatments using Principal Components and Classification analysis (PCCA) for experiment 1 explained 64.7% of the variability of biological data in the first 2 axes (Table 7.3, Figure 7.8a & b). Factor-variable correlations showed that pH and dissolved oxygen ($r > 0.7$) were the most important variables for factor 1 while conductivity, total nitrogen and nitrates ($r > 0.7$) were the most important variables on

Expt. 1





Expt. 2



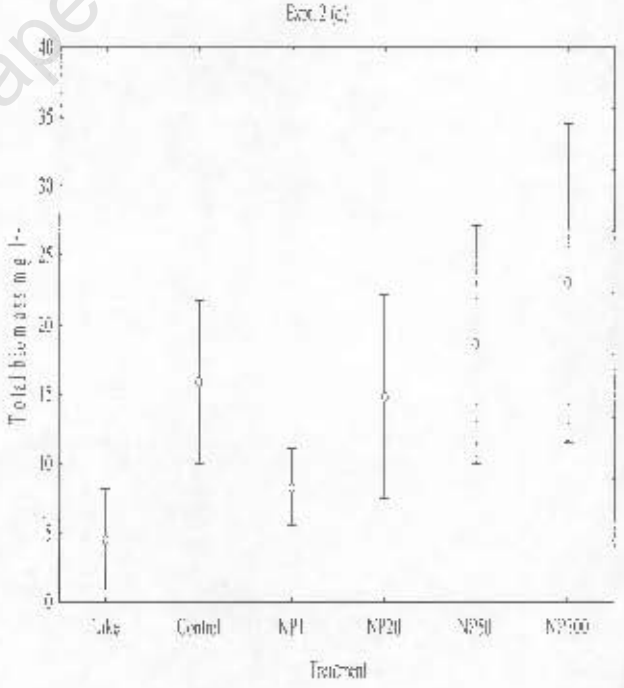


Figure 7.5 **Temporal variation of total phytoplankton biomass in six treatments during the experiment period.**

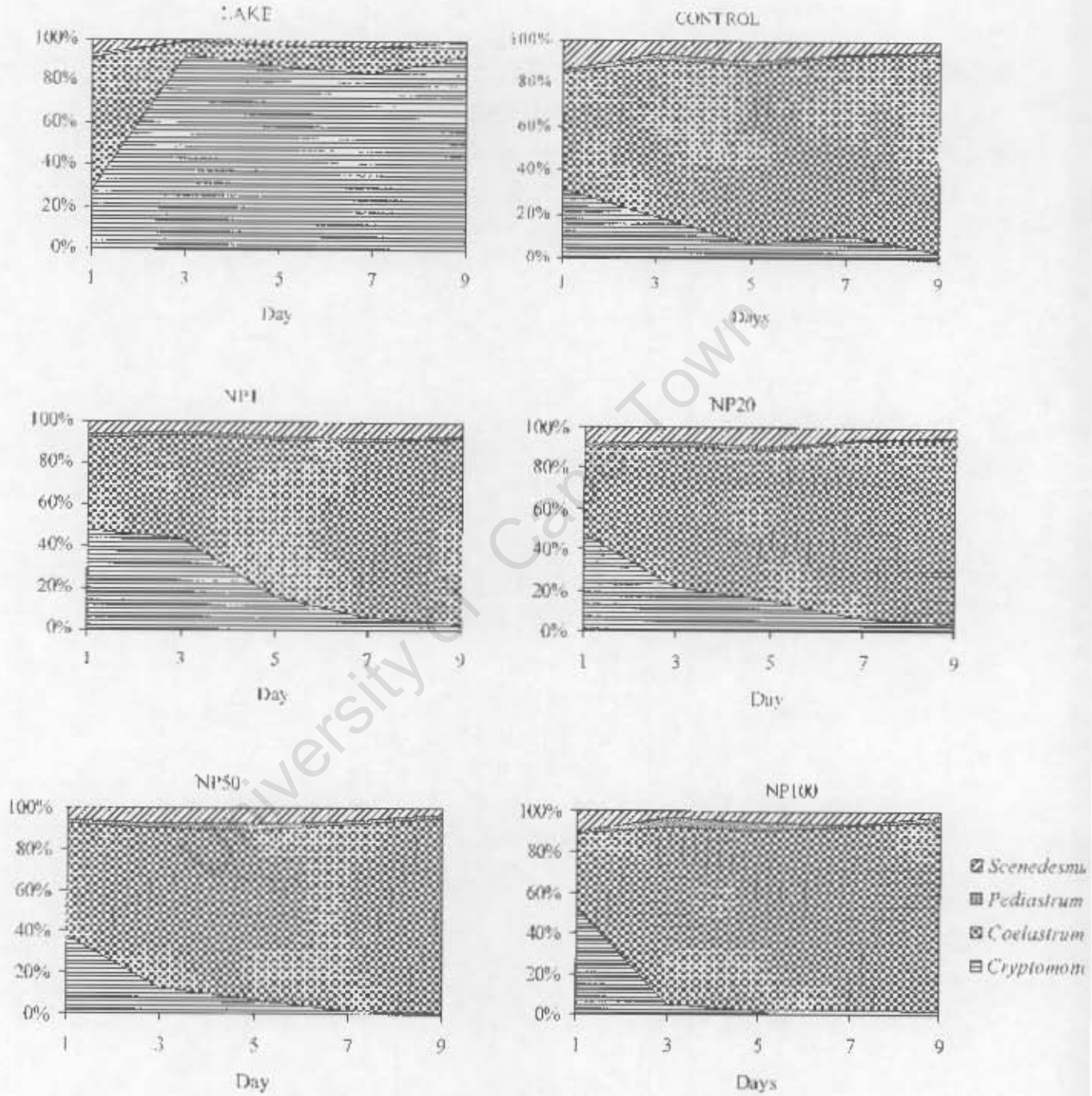


Figure 7.6 : Succession of dominant genera during experiment 1 showing the mean percentages of the 4 dominant species in each treatment in terms of biomass.

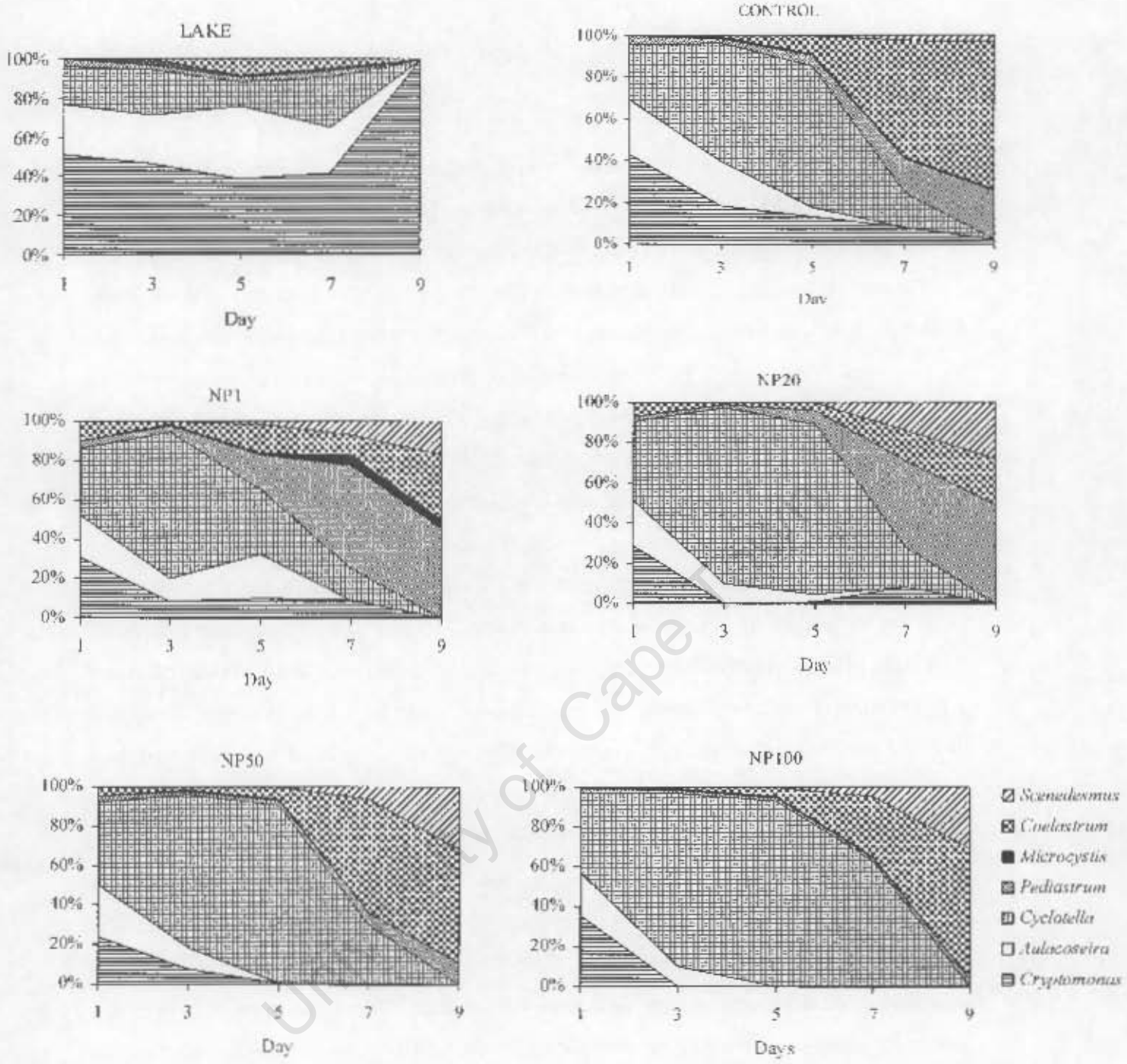


Figure 7.7 **Succession of dominant genera during experiment 2 showing the Mean percentages of the 4 dominant species in each treatment in terms of biomass.**

axis 2 (Table 7.4). PCCA depicted the trophic gradient and the temporal variation of the samples (Figure 7.8b). On Factor 1, lake, control and NP1 samples were grouped together on the positive side of Factor 1, 20NP samples occupied the middle while 50NP and 100NP samples were grouped together on the positive side of Factor 2 (Figure 7.8b). Chlorophytes *Pediastrum*, *Coelastrum* and *Scenedesmus* were associated with high levels of dissolved oxygen, pH, turbidity and chlorophyll *a*, which typified samples of all treatments during day 5 and 7 of the experiment. *Cryptomonas* was associated with day 1 and day 3 samples from all treatments, a period when it was dominant in the presence of high orthophosphate and total phosphorus levels (Figure 7.8b).

For experiment 2, ordination of data from the six treatments using PCCA explained 54.8 % of the variability of biological data in the first 2 axes (Table 7.3, Figure 7.8c & d). Turbidity, pH and dissolved oxygen ($r > 0.7$) were the most important variables on factor 1 in association with *Coelastrum* and *Pediastrum* (Table 7.4). This grouping comprised of day 7 and 9 samples from all treatments (Figure 7.9d). The most important variables on factor 2 were total nitrogen and TN:TP ratio ($r > 0.7$) (Table 7.4). *Cryptomonas* was associated with all lake samples and day 1 and 3 samples from all the treatments in which it was dominant (Figure 7.8d). Diatoms *Cyclotella* and *Aulacoseira* were associated with temperature and are located towards the middle of the ordination in association with day 5 samples, a period when they became most dominant in the treatments. *Scenedesmus* was associated with ammonium and total phosphorus. As in experiment 1, Factor 1 separated samples according to trophic gradient while Factor 2 separated samples according to temporal variation (Figure 7.8d).

Table 7.3 PCCA synthesis for the six treatments' data for experiment 1 and 2

	Experiment 1		Experiment 2	
Result synthesis	**Factor 1**	**Factor 2**	**Factor 1**	**Factor 2**
Eigen values	6.40	4.954	6.628	4.324
% of variance explained	35.5	29.1	33.1	21.6
Cumulative % explained	35.5	64.7	33.1	54.8

Table 7.4 Abiotic and biotic variables correlations (n = 72) with factors 1 and 2

Variables	Experiment 1		Experiment 2	
	Factor 1	Factor 2	Factor 1	Factor 2
Temperature	-0.683	-0.280	0.292	-0.186
pH	-0.850	-0.381	0.836	-0.444
Conductivity	-0.598	0.764	0.664	0.688
Turbidity	-0.494	-0.222	0.785	-0.438
Dissolved oxygen	-0.861	-0.350	0.886	-0.320
Total dissolved solids	-0.597	-0.351	0.662	0.691
Ammonium	-0.423	0.015	0.003	0.272
Orthophosphate	0.616	0.639	-0.442	0.561
Total phosphorus	0.365	0.692	0.227	0.261
Total nitrogen	-0.519	0.760	0.683	0.755
Nitrates	-0.555	0.754	0.614	0.638
TN:TP ratio	-0.668	0.622	0.618	0.710
Chlorophyll *a*	-0.432	-0.262	0.552	-0.398
Cryptomonas	0.655	-0.269	-0.280	-0.369
Coelastrum	-0.662	-0.502	0.737	-0.373
Pediastrum	-0.587	-0.281	0.768	-0.502
Scenedesmus	-0.199	0.709	0.205	0.179
Aulacoseira	-	-	0.417	-0.270
Cyclotella	-	-	0.471	-0.081
Microcystis	-	-	0.365	-0.326

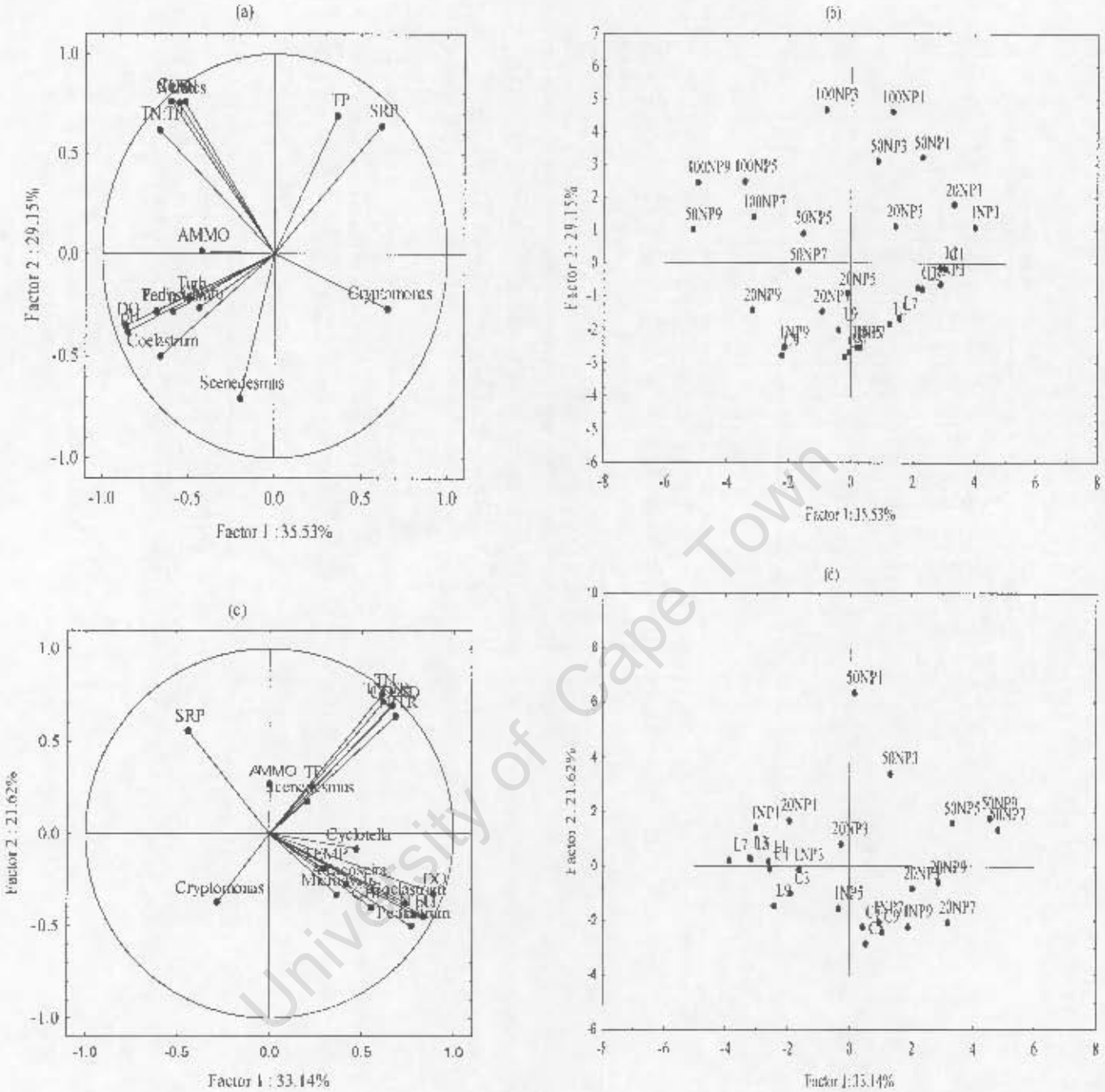


Figure 7.8 **PCCA biplot of mean values of abiotic and biological variables of the six treatments during the experiment period. Abbreviations: Cond = conductivity, DO = dissolved oxygen, TN = total nitrogen, pH = pH, TP = total phosphorus, SRP = orthophosphate, AMMO = ammonium, NITR = nitrate, L = lake, C = control, 1NP =NP1, 20NP = NP20, 50NP = NP50, 100NP = NP100. Numbers following abbreviations indicate day of experiment. (a, b = expt. 1; c,d = expt. 2)**

7.4 DISCUSSION

Increasing nitrate availability during the two experiments resulted in chlorophytes out-competing (a) *Cryptomonas* and (b) *Cryptomonas* and diatoms during experiments 1 and 2 respectively. It appears that the environment in Lake Chivero selectively favoured *Cryptomonas* sp. *Cryptomonas* sp. was dominant in Lake Chivero during the study period (Chapters 4, 5 & 6). As proposed by Levich *et al.* (1993), a species is favoured when both absolute concentrations of nitrogen and phosphorus and their ratio in the environment conforms to that particular species' requirements.

The succession patterns that *Cryptomonas* exhibited in all the treatments showed that its favourable TN:TP ratio during the experiments ranged between 1-10. According to Healy & Henzel (1980), the optimum TN:TP ratio for *Cryptomonas erosa* is 39. Schöllhorn & Granelli (1997) observed an increase of *Cryptomonas* spp and *Rhodomonas* in TN:TP ratios of 16, 40 and 100 during the first half of their experiment and a decrease thereafter. In their experiment high cell densities were maintained longer in the TN:TP ratios of 40 and 100 than in 16, indicating that the optimum ratio might be above 16. The patterns observed during the experiments and field observations (Chapter 4 & 5) showed that the species in Lake Chivero tolerated a TN:TP ratio of up to 10.

The decline of *Cryptomonas* in the control shows that other than nutrient ratios, it can tolerate a turbid environment because when confined with chlorophytes it was out-competed. An increase in chlorophytes and decline of *Cryptomonas* was also observed in untreated enclosures at the end of winter (Chapter 6).

At the end of the experiments the assemblage was dominated by chlorophytes (*Coelastrum*, *Pediastrum* and *Scenedesmus*). Increase of chlorophytes after addition of nitrogen in enclosure experiments has been reported (Pick 1989, Tilman *et al.* 1986). Orthophosphate had declined but nitrate concentration was still high in microcosms. The genus *Scenedesmus* comprises of fast-growing opportunistic species. According to the resource-competition theories, small chlorophytes are favoured over larger phytoplankton

under the conditions found in the microcosms because they have a higher nutrient affinity resulting from their small size and higher ratios of surface to volume (Raven 1998). The increase of *Scenedesmus* was most notable during experiment 2 in NP50 and NP100. As such chlorophytes had out-competed *Cryptomonas* sp. at the end of the experiments.

Levich & Bulgakov (1993) also observed a replacement of all species by the chlorophyte *Scenedesmus quadricauda* at a TN:TP ratio of 20 . High ratios in nutrient medium (20-50) stimulated growth of chlorophytes but the favourable ratios were near 20 (Levich *et al.* 1993). Levich *et al.* 1993 observed that although chlorophytes remain dominant at higher ratios (50 and 100) their biomass does not increase. This was also observed during these experiments since increasing the TN:TP ratio above 10 did not markedly increase total biomass but just favoured dominance by chlorophytes. Generally after enrichment chlorophytes benefited from *Cryptomonas* decrease and their establishment seemed to have dependent on their survival "strategies".

Influence of light has to be established further. In the microcosms the light conditions of the phytoplankton are better than those in the lake where the phytoplankton species are circulated in the light for a shorter period and remain down in dim light for most of the day (Kirsten Olrik, personal communication). Consequently in the microcosms small fast growing C-species (small diatoms and chlorophytes) will dominate and the more shade-adapted cryptophytes will decline, whereas in the lake, the more shade-adapted cryptophytes will dominate (Kirsten Olrik, personal communication).

During the experiment 2, the diatom *Cyclotella* initially responded fast to added nitrate and became the dominating species in all treatments by day 3. *Cyclotella* sp. made up to 77% of the total biomass in all treatments by day 3, after which it declined. In contrast *Aulacoseira* immediately declined upon nitrate addition and in the control. This was most apparent at NP50 and NP100, where it had disappeared in the culture by day 5. From other enclosure experiments (Lagus *et al.* 2004), centric diatoms are known for their fast growth responses to nutrient enrichment, as exhibited by *Cyclotella.* Its later decline could have been due to limitation by trace elements, especially availability of

silica (Conley *et al.* 1993). The initial high growth rate of *Cyclotella* indicates that silica may not have limited phytoplankton growth in the experiments – while the later decline could be that it was competitively excluded by chlorophytes. In microcosms, Si would quickly become a limiting factor for small diatom C-species, resulting in their decline (Kirsten Olrik, personal communication). *Cyclotella* was more competitively superior to *Aulacoseira* under nitrate addition. Favourable TN:TP ratio for diatoms is between TN:TP ratio of 5 and 20 (Levich *et al.* 1993), a condition that prevailed in Lake Chivero.

Tilman's resource competition theory states that under nutrient limitation in equilibrium conditions, those species that have either the lowest requirements for the limited resources, or the highest ability to utilize it, will succeed in competition (Tilman *et al.* 1982). Observations from these experiments and field observations (Chapter 4) support this theory. Under nitrogen limitation, smaller species with the highest abilities to utilize N over *M. aeruginosa* seems to be now competitively successful in Lake Chivero.

The resource-ratio hypothesis predicts that the relative abundances of coexisting species depend on the ratio of the limiting resources, not on the absolute concentrations (Tilman *et al.* 1982). In Lake Chivero although both N and P are high, their ratio seems to have marked influence on assemblage dynamics. Cryptophytes seemed to have been superior competitors at the prevailing TN:TP ratio range of 2-22, favouring particularly a range of 1-10 (See Chapter 3 clear state) – which should be their optimal resource ratio in Lake Chivero. This resource ratio prevailed for the greater part of the study period in Lake Chivero thereby explaining their dominance. Increase in nitrate concentrations as observed during this experiment would competitively exclude them, leading to dominance by chlorophytes favoured at such nitrate concentration. This is in accordance with the ideas of Tilman (1982) who stated that superior competitors are expected to be dominant at their optimal resource ratios and to be succeeded by others with different optimal resource ratios, if the resource ratios in the environment change.

Although it has generally been observed that cyanobacterial dominance is mainly attributed to high loadings of P and N, the decline in Lake Chivero shows that the

resource ratios could have a role in their recent decline probably through competitive exclusion by species favoured by the existing TN:TP resource ratios. It was not possible in either experiment to separate the effect of nitrate-induced change from that of isolation since similar changes occurred in both the nitrate-added treatments and the control. The effect of nitrate can be illustrated by grouping the treatments into 3 groups: (i) lake (ii) control + NP1 + NP20 and (iii) NP50 + NP100. The pattern in the lake was markedly different from group (ii) and (iii) since *Cryptomonas* dominated throughout. In the second group *Cryptomonas* occurred until day 9 only in low concentration while in the third group it drastically declined and was absent in culture by day 5. It can be inferred that at > 50X nitrate addition the decline was accelerated by nitrate addition while below that the observed changes could be linked to isolation only.

Increasing nitrate concentration on the phytoplankton assemblage of Lake Chivero increased phytoplankton biomass. Phosphorus concentration is high in Lake Chivero (Chapter 3) indicating hyper-eutrophic conditions. Nitrogen limitation of phytoplankton growth is known to occur when P concentrations are high (Dzialowski *et al.* 2005). However increase in total biomass in the control indicates that, other than nitrate addition, isolation also enhanced biomass accumulation (see chapter 6) or that ambient nutrient concentration in the confined assemblage was adequate to induce change particularly so since nutrients and physical conditions in lake, control and NP1 were similar. The phytoplankton assemblage was N-limited up to between day 5 and 7. After attaining maximum biomass other factors became limiting. The most likely limiting factor then could have been light. Phosphorus could have also been limiting because further additions of nitrate above 20X for experiment 1 was not accompanied by an increase in biomass. However during experiment 2 addition of nitrate above 50X enhanced biomass because total biomass only at NP50 and N100 was significantly higher than in the lake. Probably due to differences in ambient nitrogen concentrations during the two experimental periods phosphorus became limiting at different levels of nitrate addition.

Since the period of high nitrate levels coincided with the shift to dominance by *M. aeruginosa* (Chapters 3, 4 and 5) I expected that by increasing nitrate in the microcosms *Microcystis* would assume dominance. This was not so in August 2005 and April 2006 – most probably because *Cryptomonas* dominated the lake phytoplankton assemblage. *Microcystis* was not seen (but could still have been there in small numbers) in August 2005 but occurred in very low levels in April 2006. Dominance of *Microcystis*, even though the nutrient levels were high in microcosms, could have been limited by the small quantity of "seed" in the inocolum.

This study showed it may be possible to manage the distribution of phytoplankton in the natural algal communities in Lake Chivero by varying nitrate concentrations and nutrient ratios in order to optimize the dominance of cryptophytes, bacillariopytes, chlorophytes and to decrease cyanobacteria. Further understanding however will be required of the influence of predator-grazer pressure on the phytoplankton and its effect on the phytoplankton assemblages and the eutrophication of the lake. However other physical factors like isolation also have a marked effect on species dominance. Maintaining either cryptomonad or chlorophyte dominance would lead to a socially and ecologically acceptable lake.

CHAPTER 8

GENERAL DISCUSSION

8.1 INTRODUCTION

Hyper-eutrophic lakes are highly productive, disturbed and unstable ecosystems (Barica 1980, Barica 1993, UNEP 2000, Robarts *et al.* 2005) characterized by a high standing biomass of phytoplankton, mainly cyanobacteria (Pitois *et al.* 2000). Phytoplankton biomass can exceed 400 $\mu g\ l^{-1}$ as chlorophyll *a* (Barica 1980) and chlorophyll *a* concentrations as high as 1 000 $\mu g\ l^{-1}$ have been reported for Hartbeespoort dam (NIWR 1985). High phytoplankton biomasses are linked to high and uncontrolled nutrient inputs. Hyper-eutrophy is considered to be a nuisance due to the persistence of noxious cyanobacteria blooms, which may have devastating ecological effects (Smith *et. al.* 2002, Pitois *et al.* 2000). Many cyanobacterial species produce toxins that are toxic to other aquatic organisms and also to humans (Codd *et al.* 1997, Börner & Dittmann 2005, Zurawell *et al.* 2005); they may produce objectionable tastes and odours (Wang *et al.* 2005) and make water purification difficult (Nhapi & Tirivarombo 2004), while the collapse of blooms has been linked to massive fish kills (Mhlanga *et al.* 2006).

Persistence of seasonal blooms of *M. aeruginosa,* which first appeared in 1960 and later became established as a permanent bloom in 1963, has been a management challenge in Lake Chivero (Marshall 1997). Magadza (1994) reported a short-lived clear-water state ("metastable mesotrophic condition") following nutrient reduction in 1979, when effluent was diverted through irrigated pastureland but the lake reverted to a turbid state as nutrient levels increased again and the lake has never switched back to a clear-water state. As such the improvement in the clarity of the lake waters, resulting in a clear state in 2003, raised questions on the ecological status of the lake with respect to the existing algal assemblages and related environmental factors. The key question addressed in this thesis was, "has there been a shift in the algal assemblage in Lake Chivero, if so, and is this related to changes in nutrient status?" To answer this question, I divided my research into two parts: the first parts comprises field observational investigations where I

assessed (i) the spatial and temporal variation in physical and chemical characteristics (chapter 3), (ii) algal dynamics (chapter 4) and (iii) occurrence, abundance and limnological aspects linked to the development of a bloom (chapter 5), whereas in the second part I used enclosures to study responses of isolated phytoplankton assemblages over short periods of time in order to infer the influence of perturbation (Chapter 6) and lastly in microcosms I determined nitrate-induced changes and the effect of varying nitrogen : phosphorus ratios on the phytoplankton assemblage (Chapter 7).

8.2 Physical and chemical characteristics and their influence on algal dynamics

Results in Chapter 3 show that the degree of eutrophication has not reduced compared to that reported in previous studies. I show the spatial and temporal variation in physical and chemical characteristics during the study period. The noteworthy feature of temporal variation over a 23-month period was the distinction between clear and turbid states with respect to physical and chemical characteristics. This was clearly shown on Figures 3.6 and 3.7 where 'turbid state' samples with little transparency and a euphotic zone of no more than 0.9 m were associated with high nitrate, total nitrogen and TN:TP ratios while 'clear state' samples were associated with high levels of orthophosphate and total phosphorus, high transparency and a euphotic zone of up to 6 m.

These findings show that cyanobacterial dominance was limited by a relative lack of nitrogen during the clear state (assuming phosphorus levels were ideal) and only developed during the turbid state when nitrates and total nitrogen had reached "critical levels" (meaning a concentration when the switch/shift occurred). Limitation of phytoplankton abundance as a result of nutrient availability is relatively well studied in tropical lakes, where nitrogen seems to be more often limiting than phosphorus (Payne 1986).

Temporal variations in limnochemistry were not stable (unpredictable) as shown by the distinctiveness of the clear and the turbid state but dissolved oxygen seemed to be predictable although they would be considerably lower at night. Levels of nutrients (N

and P) in particular, and also physical variables (dissolved oxygen and pH) were not perpetually high in the lake but constantly fluctuated. This was particularly notable with respect to total nitrogen and total phosphorus. For total nitrogen two periods occurred during the clear state: February to July 2003 with high concentrations (average 9.2 mg l^{-1}) and August to December 2003 with lower concentration (average 3.9 mg l^{-1}). Total nitrogen remained high during the turbid state with marked fluctuations of between 8.2 and 14.8 mg l^{-1}. Limnochemistry was, however, spatially uniform and uniformity among the sites can be attributed to thorough mixing by wind.

8.3 Algal dynamics

In Chapter 4 the development of the algal assemblage in relation to environmental variables is discussed and the differences in phytoplankton assemblages during the clear and the turbid states is characterized. In the clear and turbid states, algal assemblages reflected two distinct periods of temporal niche separation. A typical successional pattern was exhibited during the clear state with cyanobacteria dominating during the hot rainy season (February – March 2003), bacillariophytes during the cool winter period (April to July 2003) and cryptophytes and chlorophytes during the hot period (September-November/December 2003). The turbid state, which was relatively shorter, started with a mixed assemblage but a gradual shift finally resulted in dominance of *M. aeruginosa,* almost assuming equilibrium prior to the collapse of the bloom.

The study intended to establish the extent to which the algal assemblage in Lake Chivero has changed 51 years after impoundment. As shown in Chapter 4, characterization of the phytoplankton assemblage and its periodicity showed that cyanobacteria were not, as previously supposed, singly dominant in this hyper-eutrophic lake. Instead an unanticipated shift between a clear and a turbid state occurred. Algal species dominance and composition have changed and the change is linked not to improvement in water quality but probably to nutrient balance. The physical and chemical results suggests that dominance by *Microcystis* could have been linked to "nitrate/nitrogen" levels in the lake, indicating that the balance between N and P could be more important than their absolute

concentrations. The findings suggest that the shift between the two states was linked to increased concentrations of nitrate in the lake. The dynamics of nitrates (in relation to existing ambient physical and chemical factors) appeared to have been one of the major factors that triggered the shift between the two states. The critical range in nitrate concentration, where the lake is likely to shift to a turbid state, was 0.3 - 1.7 mg l^{-1}, assuming that other relevant conditions are favourable.

The species composition of the phytoplankton assemblages present in Lake Chivero during this study was not similar (except for a brief period during the turbid state) to that described in two other hyper-eutrophic systems in southern Africa, Zeekoevlei and Hartbeespoort dam. The latter two are dominated by *M. aeruginosa*, while during this study there was a dominance of cryptomonads, chlorophytes and bacillariopytes in Lake Chivero. Since the three systems are hyper-eutrophic one would have assumed them to support a similar assemblage. This thesis demonstrates that alternate stable states can occur in hyper-eutrophic lakes although this could be a rare phenomenon that has not been rigorously demonstrated in similar systems.

8.4 Applicability of models of cyanobacterial dominance

A wide range of models has been proposed to explain the dominance of cyanobacteria in aquatic ecosystems (Paerl 1988, Shapiro 1990). The development of the algal assemblage during this study deviated from that proposed by most models attempting to understand cyanobacterial dominance in hyper-eutrophic lakes (see Chapter 1, Section 1.3) except for the low light (Zevenboom & Mur 1980, Smith 1986, Mur & Scheurs 1995) and buoyancy (Mur *et al.* 1999, Mitrovic *et al.* 2001) models. The patterns observed in species diversity, abundance and changes in Chapter 4 deviated from those expected in a hyper-eutrophic lake. The lake did not exhibit a perpetual dominance of cyanobacteria, as would be expected in a hyper-eutrophic lake. A selective advantage was not imparted to cyanobacteria because of low TN:TP ratios (<29), high temperatures (25 – 35 °C) and high pH/low $C0_2$, despite the fact that these conditions prevailed in the lake.

According to the low-light model cyanobacteria are have adapted to grow at low light conditions (Zevenboom & Mur 1980) because they have low light energy requirements and can regulate their position within the water column as described by the buoyancy model (Reynolds *et al.* 1987). The low-light and buoyancy models might have operated during the turbid state (i.e. low light conditions), as illustrated by the observations made during the waxing and waning of the algal bloom reported in chapter 5. My findings show that almost equilibrium conditions were achieved for a short time during the turbid state when *Microcystis* almost achieved absolute dominance. During that short period competitive exclusion, evidenced by the decline of other species, occurred. *Microcystis* migrated (according to the buoyancy theory) and accumulated in 0-5 m depth zone, thereby limiting light penetration in the water column and thus competitively excluding other species.

According to the "inorganic nitrogen hypothesis" (Blomqvist *et al.* 1994) high levels of ammonium favour the development of non-nitrogen-fixing cyanobacteria, whereas nitrate-nitrogen favours the development of eukaryotic plankton. The opposite was observed in Lake Chivero, since *Microcystis* became dominant under elevated nitrate levels (Chapter 3). The only aspect that relates to this hypothesis is the notable decline of *Anabena* sp. and *A. tanganyike,* events that are probably linked to increases in nitrogen in the lake. The "trace element hypothesis" (Rueter & Petersen 1987) states that cyanobacterial dominance under conditions of high nitrogen and phosphorus loading is limited by the deficiency of trace elements such as iron (Paerl 1996, Nagai *et al.* 2005) while the grazing resistance hypothesis (Haney 1987, Webster & Peters 1987, DeMott & Moxter 1991) considers that the size, low nutritional value, grazing-resistant coverings and toxicity associated with certain species of cyanobacteria would deter feeding by zooplankton. Inferences cannot be made on the applicability of these models to the situation that prevailed in Lake Chivero during the study period. This is a subject of further research. Algal toxins (allelopathy model: Suikkanen *et al.* 2006) are unlikely to have contributed, however, because the levels detected in the lake were very low (Mhlanga *et al.* 2006).

Generally the findings reported in Chapter 4 show that the decline in dominance of cyanobacteria and the increasing predominance by cryptomonads and chlorophytes under conditions of high pH, low TN:TP ratio and hyper-eutrophy is contrary to some of the proposed models on cyanobacterial dominance. Of the discussed nine models on cyanobacterial dominance only the (i) low- light hypothesis and (ii) the buoyancy hypothesis (Chapter 5) seemed applicable. I propose that a different mechanism may have been at play: the "critical nitrate concentration" and related high dissolved oxygen levels (Kirsten Olrik, personal communcation) or nutrient balance between P and N seems to have been the major factor influencing the development of the phytoplankton assemblages during the study period.

Sakamoto & Okino (2000) noted that there is limited applicability of the generally accepted models on factors responsible for cyanobacterial dominance in natural waters. They reckon that cyanobacterial dominance in natural waters is an integral effect of several factors rather than a "single factor". General applicability of these models would thus be expected to vary from one water body to another. In the case of Zeekoevlei, Harding (1996) observed that low light, buoyancy and, to a lesser extent, elevated water temperature influenced cyanobacterial dominance. In Lake Chivero, although my study has shown that nitrogen uptake by the phytoplankton assemblage could have been the main factor determining the composition of the phytoplankton assemblage, especially the shift between the clear and the turbid state, other factors, such as availability of trace elements, might have played a contributory role. Strong mixing episodes (high wind velocity) in Lake Bourget (France), for instance, contributed to the decrease in phytoplankton population resulting in a clear state (Vinçon-Leite *et al.* 2002). Wind could be a major forcing factor in Lake Chivero. This study has shown, however, that cyanobacteria are not necessarily dominant at low N:P ratios.

8.5 Alternate stable states: bi-stability in aquatic ecosystems

A switch from a state dominated by eukaryotic algae to one dominated by cyanobacteria occurred within the algal assemblage of hyper-eutrophic Lake Chivero. It was of

ecological and theoretical interest that a prolonged clear state of 15 months occurred in a hyper-eutrophic lake where algal blooms had previously been considered to be a permanent feature (Munro 1966, Robarts 1979, Magadza 2003). The turbid state lasted for only 8 months, after which the lake reverted back to a clear state with dominance by eukaryotic algae. I propose that these findings indicate that two different states can exist in a hyper-eutrophic lake. This also is the first report in Lake Chivero of a possibility of the existence of two states under hyper-eutrophic conditions, a phenomenon that is, however, common in shallow lakes.

The alternative stable state model requires (e.g. Scheffer *et al.* 1993, Scheffer *et al.* 2001, Okey 2004) that each biologically distinct state must be capable of existing under similar abiotic environmental conditions and implies that switches between states are usually triggered by a catastrophic disturbance event (Connell & Sousa 1983). The situation in Lake Chivero was not triggered by a catastrophic event but I propose that it is possible for such a switch to occur in a hyper-eutrophic lake in response to changes to ambient nutrient balances. Such a change in nutrient balances is in fact 'catastrophic' from the point of view of the phytoplankton; we think of 'catastrophes' being things like floods and droughts but this isn't necessarily so. The catastrophe theory defines catastrophes as sudden shifts to a radically different community (Zeeman 1976, Saunders 1980). This is what happened in Lake Chivero – where there was relatively rapid movement between states. Over the 15 months period when the lake was in a clear state the algal assemblage was in state dominated by eukaryotic algae, which were favoured by the prevailing ambient nutrient levels. The change to higher nitrate/nitrogen levels presumably triggered a "catastrophic response" thereby resulting into a shift/movement to a " radically different assemblage" dominated by *Microcystis*. This aspect requires to be investigated further, especially by means of long-term monitoring.

8.6 Equilibrium, stability and steady states

The clear state lasted for the longest part of the study period but a steady-state phytoplankton assemblage did not develop then. The clear state was, however, more

stable than the turbid state where total fresh-weight biomass constantly fluctuated as *M. aeruginosa* assumed dominance. There were limited periods of equilibrium in which 1-3 of the species were in excess of 80% of the total fresh weight biomass for a continuous period. Lake Chivero exhibited fluctuations in concentrations of dissolved oxygen, nutrients, chlorophyll *a* concentration and phytoplankton biomass – indicating instability and non-equilibrium dynamics as exhibited in hyper-eutrophic lakes. It is known that ecological stability is low and periodic crashes of populations and cyclic anoxia generally help re-establish equilibrium and steady-state conditions in hyper-eutrophic lakes (Barica 1980). Lake Chivero was not an exception to this, but also exhibited instability and a bloom crash.

8.7 Survival strategies and functional classification

Findings in Chapter 4 show increasing dominance of cryptomonads and the decline of *M. aeruginosa*, a specialist, during the study period. Specialists indicate a state of equilibrium while the predominance by species that grow rapidly (C-growth strategists) is an indicator of instability. I assumed that instability would be due to persistent wind induced perturbations so (Chapter 6) three distinct algal communities were isolated in enclosures to test whether wind-induced perturbations (assuming reduced wind intensity in enclosures) could explain, short-term (1-11 days) trends in the variability of phytoplankton biomass and species dominance patterns in Lake Chivero.

During experiment 1 (in summer), isolating a phytoplankton assemblage comprising both *Cryptomonas* sp. and *Cyclotella* sp. and specialists (*M. aeruginosa* and *Anabaena* sp.) resulted in the competitive decline of *Cryptomonas* and *Cyclotella* after 7-9 days with specialists assuming dominance. *Microcystis* and *Anabaena* were dominant and *Cyclotella* was excluded while *Cryptomonas* had declined by the end of the experiment. The findings of experiment 1 indicate that small-scale perturbations favour the predominance of species that grow rapidly; this partly explains the decline in cyanobacteria and the predominance by chlorophytes and cryptophytes in the lake. In the enclosure where conditions were relatively "physically stable", cyanobacteria had

competitively excluded other species and comprised over 60% of the total biomass by day 9.

Isolation of a winter phytoplankton assemblage with insignificant numbers of *Microcystis*, and a predominance of *Cryptomonas* and *Cyclotella,* resulted in a decrease of *Cyclotella* as the biomass of *Cryptomonas* increased. In the third experiment (end of winter) isolation of a phytoplankton assemblage that comprised *Cryptomonas* and chlorophytes resulted in a decrease in *Cryptomonas* as the biomass of *Coelastrum* spp increased. The second experiment show that when *Cryptomonas* and *Cyclotella* occur together the former will be favoured while the latter is competitively excluded. When *Cryptomonas* and *Coelastrum* spp. occur together as in the third experiment it is the latter that is favoured. Notably, during the three experimental runs, comparatively in the lake *Cyclotella* and *Cryptomonas* predominated in summer and winter while *Cryptomonas* and *Coelastrum* spp. predominated at the end of winter. The lake environment that is more "physically perturbed" is a favourable environment for *Cryptomonas*. On all three occasions the effect of isolation was most apparent with regard to succession but not to total biomass or chlorophyll *a*, which only increased slightly.

The effect of perturbations is embodied in the Intermediate Disturbance Hypothesis (Connell 1978), a hypothesis that this study could not investigate but an aspect that should be investigated further. The findings in Chapter 6 just indicate that "isolation thereby physical stability" in enclosures led to competitive exclusion of *Cryptomonas* by cyanobacteria during summer, while in winter *Cyclotella* was competitively excluded by *Cryptomonas* and at the end of winter *Coelastrum* competitively excluded *Cryptomonas*.

In Chapter 4 I show that coherent phytoplankton associations that can be ascribed to trait-separated functional groups according to Reynolds *et al.* (2002) occurred during the study period. The six dominant phytoplankton genera recorded in Lake Chivero during this study were ascribed to functional class assignments as follows: (i) Ruderals plants (R) = *Cyclotella* and *Aulacoseira*; (ii) growth strategists (C) = *Coelastrum*, *Scenedesmus* (iii) Specialists (S) = *Microcystis* while *Cryptomonas* borders between R and C. The clear

and the turbid state were easily reflected in the "strategies" (C, S, R) and the composition of the phytoplankton (Reynolds *et al.* 2002). During the clear state the seasonal shift of cyanophytes → bacillariopytes → cryptophytes → chlorophytes could be represented as M → B → Y → J, but generally the Y functional group predominated. The alphanumeric codes are as defined by (Reynolds *et al.* 2002).

8.8 Nitrate-induced changes in the phytoplankton assemblage and effect of TN:TP ratio

Two experiments run (Chapter 7) to determine the effect of increasing nitrate concentration and the connected increase of TN:TP ratio on phytoplankton resulted in chlorophytes out-competing *Cryptomonas*. *Cryptomonas* also declined in the control. Inferences could be made after grouping treatments into three groups constituting the (i) lake (ii) control + NP1 + NP20 and (iii) NP50 + NP100. In the lake *Cryptomonas* dominated throughout while *Cryptomonas* occurred until day 9 in the second group and only in low concentrations. In the third group it drastically declined and was absent in culture by day 5. The main inference from these experiments is that nitrate addition accelerated *Cryptomonas* decline at > 50X nitrate addition while below that the observed changes could be linked to isolation only. The experiments could not separate the effect of isolation from that of nitrate addition.

The marked decline of *Cryptomonas* in treatments with > 50X nitrate addition (TN:TP > 10) interesting can be related to the situation observed in the lake. In the lake *Cryptomonas* dominated during the clear state when TN:TP ranged between 1-10 but declined during the turbid state when it ranged between 11-22. An inference can be made by linking experimental results to variation of species dominance observed in the lake in relation to water column TN:TP ratio. Cryptophytes were favoured in lake at a TN:TP ratio of 1-10 (see Chapter 3 clear state) while increases in nitrate concentration (and consequently in TN:TP ratios > 10) competitively excluded them, leading to dominance by chlorophytes. This is in accordance with the resource-ratio hypothesis of Tilman *et al.* (1982), which predicts that the relative abundances of coexisting species depend on the ratio of the limiting resources and not only on absolute concentrations. Except for a slight

increase during experiment 2, the abundance of *Microcystis* was not enhanced by nitrate addition. This could have been due to the low biomass of *Microcystis* in the water column. It has also been reported elsewhere (Leonardson & Ripl 1980) that chlorophyte numbers are enhanced by nitrate addition.

8.9 Management implications

Cyanobacteria are the foci of lake management (Carpenter 1989) and for Lake Chivero this centre on the fact that the lake is the city of Harare's main water supply source for drinking water. Cyanobacteria decline will consequently alleviate three main management challenges in Lake Chivero, namely (i) fish kills that occur following collapse of blooms (Appendix 1) and (ii) possibility of passing on LPS endotoxins and microcystins to consumers (Appendix 2) and problems of filtration and purification.

The main recommendation from this study centres on the need to maintain the lake in a "clear state". As proposed by Scheffer *et al.* (2001) " *building and maintaining resilience of desired ecosystems state is likely to be the most pragmatic and effective way to manage ecosystems in the face of increasing environmental change*". Efforts to reduce nitrogen (and phosphorus) loading in the lake should be run con-currently with in-lake monitoring of phytoplankton assemblages. Adjustments can then be made until critical levels of N and P are reached at which the lake is "held" in a state not dominated by cyanobacteria.

The existing water quality monitoring system on Lake Chivero is based on monitoring physical and chemical parameters, which provides information on the concentration and levels only of the parameters monitored. What is important, as observed during this study, is to be able to detect subtle responses of the biota, in this case phytoplankton, to their physical and chemical environment. Traditional physical and chemical evaluations of water quality are now recognised as being inadequate (perhaps "insufficient') (Barbour *et al.* 1996 cited in Dallas 2002). The use of organisms as biomonitoring agents is considered as being more informative since organisms are sensitive to alterations in the water body (Dallas 2002). The responses seen in the phytoplankton assemblage during

this study, for instance, could be more useful in formulation of management strategies than physical and chemical data *per se*.

It is proposed to develop a strategy that will enhance domination of cryptophytes, chlorophytes and bacillariophytes in Lake Chivero while decreasing the proportion of cyanobacteria. The shift between clear and turbid states observed during the 26-month monitoring programme is attributed to low nitrogen levels limiting cyanobacterial growth. Water column TN:TP ratios ranged between 2 and 22 and the system was nitrogen-limited to cyanobacteria growth during the clear state when the water column TN:TP ratio ranged between 2 and 10. It became phosphorus-limited during the turbid phase when the ratio ranged between 11 and 22. TN:TP ratio can be an effective tool indicating nutrients as potential limiting factors for cyanobacteria in Lake Chivero.

Overall, findings from this study suggest that management efforts should focus on controlling nitrogen inputs in the lake in order to reduce incidences of cyanobacterial blooms and to control phytoplankton dominance patterns. Furthermore, the water column TN:TP ratio can be used as an indicator of potential nutrient limitation and to predict cyanobacterial blooms in Lake Chivero.

8.9 Conclusion

In summary, the situation in the Lake Chivero during the study period with respect to development of algal assemblages was different from expectations in a hyper-eutrophic lake. Somehow the lake exhibited "bistability" with distinct clear and turbid states. The observation in Lake Chivero provides a basis of beginning to understand possible existence of two states within the algal assemblage under hyper-eutrophic conditions.

8.10 Aspects for further research

(1) The importance of the predator-grazer pressure on the phytoplankton and its effect on the phytoplankton assemblages and the eutrophication of the lake

(2) Factors enhancing the metabolic clear-water states with nutrient concentrations like a sewage pond

(3) Applicability of the Intermediate Disturbance Hypothesis (IDH) to the phytoplankton assemblage in Lake Chivero

(4) Establishing whether the Nile Tilapia (*Oreochromis niloticus*) has reached an effective grazing level thereby forcing the lake to switch to a clear state after 40 years of turbid state with cyanopytes

(5) Influence of key selective factors namely light, carbon, oxygen depletion at night, mixing zone ($Z_{euphotic}$:Z_{mixing}) and Si limitation on the algal assemblage

APPENDIX 1

PAPER I: Observations on limonological conditions associated with a fish kill of *Oreochromis niloticus* in Lake Chivero following collapse of an algal bloom

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APPENDIX 2

PAPER II: Cyanobacteria and cyanotoxins in the source water from Lake Chivero, Harare, Zimbabwe, and the presence of cyanotoxins in drinking water

Observations on limnological conditions associated with a fish kill of *Oreochromis niloticus* in Lake Chivero following collapse of an algal bloom

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Abstract

Possible causes of deaths of *Oreochromis niloticus* in Lake Chivero were examined in relation to changes in limnological conditions monitored over a 25-month period. The fish deaths coincided with the collapse of an algal bloom that had developed and built up in the lake for 8 months. Chlorophyll *a* and dissolved oxygen increased to average concentrations of 42.4 $\mu g\ l^{-1}$ and 10.9 $mg\ l^{-1}$ respectively prior to the collapse of the bloom. Dissolved oxygen decreased when the bloom started to die off and coincided with the fish deaths when the average surface dissolved oxygen concentration in the lake was 3.9 $mg\ l^{-1}$ and was at a depth of 5 m <2 $mg\ l^{-1}$. Mortality probably resulted from depressed oxygen levels caused by the high oxygen demand from the massive algal die-off and released algal toxins. This is the first time that die-off of algae has been linked to fish-kills in Lake Chivero as occurs in other hypereutrophic systems.

Key words: algal bloom, fish kill, *Oreochromis niloticus*

Résumé

Les possibles causes de la mortalité de l' *Oreochromis niloticus* dans le Lac Chivero furent examinées par rapport aux changements dans les conditions limnologiques contrôlées sur une période de 25 mois. La mort des poissons coïncida avec la chute d'une prolifération (bloom) des algues qui avait développé et accumulé dans le lac au cours de huit mois. Le taux de chlorophyll *a* et d'oxygène dissolu atteignit les concentrations moyennes de 42,4 $\mu g\ l^{-1}$ et 10,9 $mg\ l^{-1}$ respectivement avant la chute de la prolifération. L'oxygène dissolu diminua quand la prolifération commença à mourir et coïncida avec la mort des poissons quand la concentration moyenne d'oxygène dissolu à la surface du lac fut 3,9 $mg\ l^{-1}$ et <2 $mg\ l^{-1}$ à une profondeur de 5 m. En toute probabilité, la mortalité résultait de niveaux bas d'oxygène dus à la demande élevée par la mortalité massive des algues et les toxines algiques déchargées. Ceci est la première fois que la mortalité des algues a été liée à la mortalité de poissons dans le Lac Chivero, comme dans les systèmes hypereutrophes.

Introduction

Hypereutrophic systems characterized by mean phosphorus concentration of approximately 100 $\mu g\ l^{-1}$, and mean and maximum chlorophyll *a* levels of 25 and 75 $\mu g\ l^{-1}$ respectively [Organization for Economic Cooperation and Development (OECD), 1982] are highly productive and tend to be ecologically unstable (Barica, 1981). They experience periods of rapid phytoplankton development followed by population crashes when production becomes unsustainably high (Barica, 1981). Periodic crashes of algal populations or huge oxygen demands from anaerobic hypolimnia during turnover can cause anoxia in hypereutrophic systems and this can result in fish mortalities (Robarts, 1985).

Lake Chivero in Zimbabwe is hypereutrophic (Magadza, 2003). Unchecked eutrophication occurs primarily as a result of discharge of nutrient-rich sewage and industrial effluents. Eutrophication first became evident in the form of algal blooms in 1960 (Munro, 1966) when the city of Harare began discharging insufficiently treated sewage into the lake (Marshall, 1997). During this period conductivity was 100 $\mu S\ cm^{-1}$, soluble reactive phosphorus 0.04 $mg\ l^{-1}$ and ammonia 0.1 $mg\ l^{-1}$ (Marshall, 2005). The lake became hypereutrophic in the mid-late 1960s (Magadza,

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2003). Conductivity then had increased to 170 $\mu S\ cm^{-1}$, soluble reactive phosphorus to 0.22 mg l^{-1} and ammonia to 0.4 mg l^{-1} (Marshall, 2005) and algal blooms became a permanent feature in the lake (Marshall, 1997). Algal blooms declined in response to reduction in nutrient loading between 1970 and 1980 when sewage effluent was diverted through irrigated pastureland. Conductivity decreased to 126 $\mu S\ cm^{-1}$, soluble reactive phosphorus to 0.04 mg l^{-1} and ammonia to 0.04 mg l^{-1} (Marshall, 2005).

An extensive outbreak of water hyacinth (*Eichhornia crassipes*) between 1985 and 1991 that covered about 35% of the lake's surface (Chikwenhere & Phiri, 1999) indicated a further deterioration in water quality (Marshall, 1997). The infestation by water hyacinth temporarily masked deterioration in water quality because the weeds removed nutrients from the water, as shown by the decrease in the concentrations of soluble reactive phosphorus to 0.03 mg l^{-1} and ammonia to 0.06 mg l^{-1} (Marshall, 2005). Destruction of weed mats using 2–4D in 1990 led to an increase in soluble reactive phosphorus to 0.1 mg l^{-1} and ammonia to 0.13 mg l^{-1} because nutrients were released into the water from decomposing plants. This resulted in the reappearance of continuous algal blooms, mostly of unpalatable and often toxic species (Rommens *et al.*, 2003).

Fish kills of *Oreochromis macrochir* have been reported in Lake Chivero from as early as 1971 (Burke & Thornton, 1982), when the lake became hypereutrophic (Moyo, 1997). Fish kills were caused by sudden turnovers that occurred between February and March, bringing deoxygenated bottom waters to the surface and causing anoxia. Moyo (1997) linked a fish kill of approximately 100 tonnes of *O. macrochir* in the lake in March/April 1996 to deoxygenation that was compounded by ammonia toxicity which was unacceptably high at 3.5 mg l^{-1}. Frequent fish-kills now characterize Lake Chivero, especially between November and April (Magadza, 2003) although the cause has not previously been established. Fish-kills occurred in the lake during the 1999–2002 period starting in January and ending in March (C. Phiri, pers. comm.).

The link between collapse of an algal bloom and fish kills has not been reported for Lake Chivero. During a 25-month study period between February 2003 and February 2005 a fish kill was observed during February and March 2005. This paper reports the limnological conditions leading up to the fish-kill and discusses possible causes in relation to the development of an algal bloom and changes in dissolved oxygen, ammonia, pH and temperature, which have previously been implicated in fish deaths in the lake.

Materials and methods

Study area

Lake Chivero (formerly Lake McIlwaine) is a manmade lake that is situated on the Manyame River and is located 37 km to the southwest of Harare, the capital city of Zimbabwe at an altitude of 1363 m a.s.l. It is located at latitude 17°54′S and longitude 30°48′E (Fig. 1). It is a warm monomictic lake (Munro, 1966). At full supply level it has an area of 26.3 km^2, a volume of 250.4 × $10^6 m^3$ and a mean depth of 9.4 m. Table 1 lists the main hydrological and morphometric features of the lake (Burke & Thornton, 1982). The deepest part with a maximum depth of 27.4 m occurs near the lake spillway (Munro, 1966) but because of siltation this has changed and the maximum depth recorded during this study was 20 m. Station 1, located near the spillway, was the deepest while the maximum depth at station 2 was 3 m and at station 3 was 3.5 m.

When the lake was constructed in 1952 its main function was to provide water to the city of Harare but it now also supports a fishery and is used for recreational activities. Twenty-nine species of fish have been recorded in the lake but the most successful have been the cichlids, which are adapted to living in standing waters (Marshall, 2005). Two introduced species, *O. macrochir* and *Oreochromis niloticus*, dominate the fishery. *Oreochromis macrochir* accounted for 70% of the commercial catch until the 1990s (Marshall, 1978) while *O. niloticus* is now dominant.

Sewage and industrial effluent are discharged into the lake through the Mukuvisi and Marimba Rivers (Marshall, 1997). Mukuvisi River receives approximately 36 million litres of treated effluent daily (Mathuthu *et al.*, 1995) and contributes the highest nutrient load to the lake (Thornton, 1980). About 80% of the total inflow to the lake is from Manyame River. Abstraction for the city of Harare and downstream users take 60% while evaporation account for 30% of the outflow (Ballinger & Thornton, 1982).

Sampling procedures

Chlorophyll *a*, dissolved oxygen, ammonium, pH and temperature were monitored monthly at three stations (Fig. 1) between February 2003 and February 2005 except for January 2005. Water samples were collected using a Ruttner sampler from the following integrated depth intervals: 0–2, 2–4 and 4–6 m at station 1, 0– 2 and 2–3 m at station 2 and 0–2 and 2–3.5 m at station 3

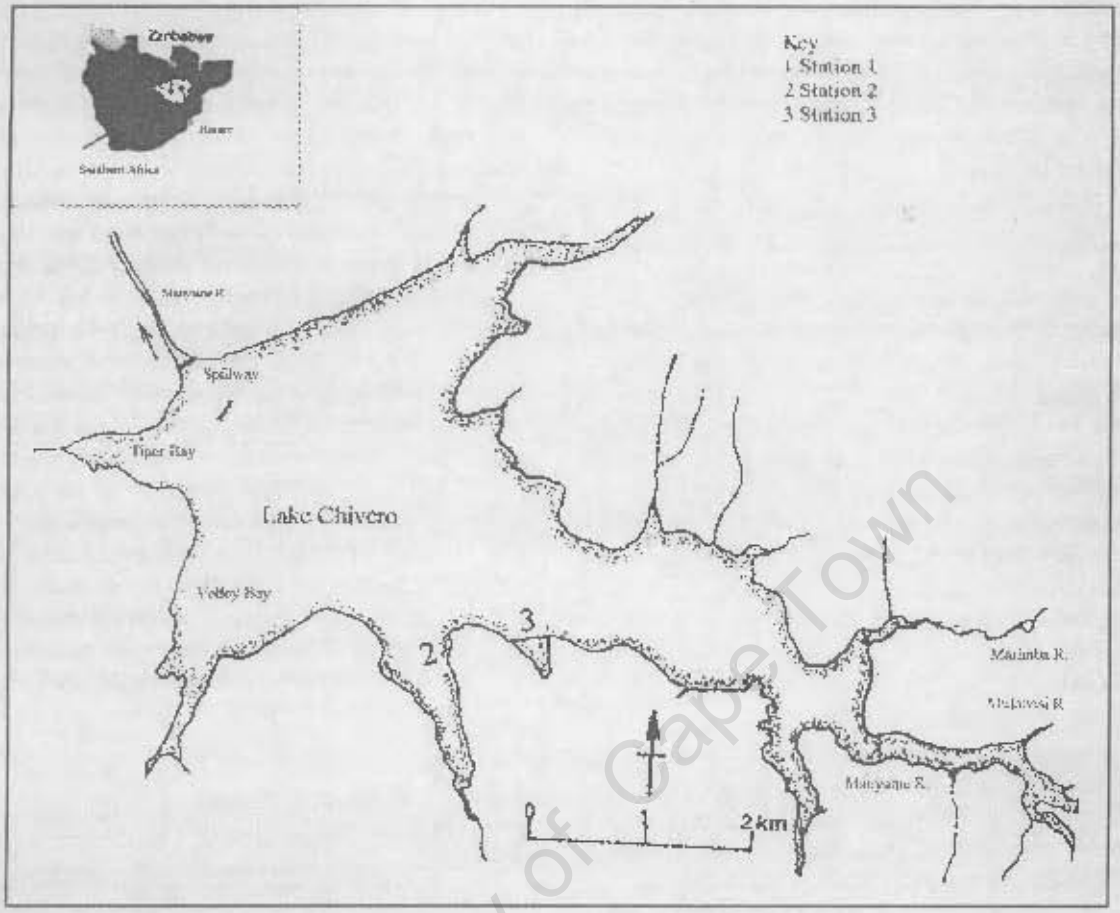

Fig 1 Location map of Lake Chivero showing the sampling stations (1–3)

Table 1 Hydrological and morphometric features of Lake Chivero

Characteristic	
Full supply volume ($\times 10^6$ m^3)	250
Full supply surface area (km^2)	26.30
Catchment area (km^2)	2227
Shoreline length (km)	74
Maximum depth (m)	27.43
Mean depth (m)	9.4
Maximum breadth (km)	8.0
Mean breadth (km)	1.68
Length (km)	15.7
Renewal time (years)	0.82

(Fig. 1). Following the onset of the bloom in May 2004 samples were taken down vertical profiles at 5-m depth intervals at station 1 to ascertain the vertical distribution of the same variables during the bloom period.

Temperature (°C), pH and dissolved oxygen (mg l^{-1}) were measured with a mercury thermometer, a WTW 330i pH meter (Geotech Environmental Equipment, Inc., Denver, Colorado, USA) and a WTW Oxi 330 oxygen meter (Geotech Environmental Equipment, Inc., Denver, Colorado, USA) respectively. The concentration of ammonium (mg l^{-1}) was determined by the indo-phenol blue method (Golterman, Clymo & Ohnstad, 1978) and chlorophyll *a*(μg l^{-1}) by the acetone extraction method (Golterman *et al.*, 1978). Chlorophyll *a* concentration was used as an estimate of phytoplankton biomass.

Fish are reported to have started dying around the first week of February 2005. Fish that had just died or were gulping for air on the water surface were collected using a scoop net. A total of 55 fish were collected between 24th February and 1st March 2005. The total length, standard length and weight of each fish were measured and all

specimens were identified. The condition factor was calculated in order to determine the health status of the fish. The condition factor (c.f.) was calculated using the equation $c = W \times 100/l^3$ (Pauly, 1984) where W = weight in grams (ungutted weight) and l = length in centimeters (standard length).

Results

Over the whole sampling period from February 2003 to February 2005, a fish-kill occurred only around February/March 2005 and members of only one species, *O. niloticus*, died. Many dead fish were observed along the shoreline during two lake-wide surveys undertaken on the 24th and 27th February. Fish dead or gulping for air on the surface were observed within the lake suggesting that the fish kill was lake-wide and not localized. Waves washed dead fish ashore. The mean total length of the fish was 25 ± 1.6 cm (range 21–33 cm). The mean standard length was 21 ± 1.44 cm (range 17–27 cm) and average weight was 282 ± 60 g (range 161–530 g) while the mean condition factor (c.f.) of the fish was 3.2 ± 0.48 (range 2.44–4.66).

An algal bloom was present in Lake Chivero from May until December 2004, during which time surface scums covered the whole lake. By February/March, when the fish-kill occurred, the bloom had crashed. Comparison of algal development in Lake Chivero from February 2003 to February 2005, using chlorophyll *a* as a measure of algal biomass (Fig. 2a), suggests a link between the development and subsequent collapse of the algal bloom and the fish-kill. A mild bloom developed in February 2003, with an average chlorophyll *a* concentration of 18.5 ± 7.8 $\mu g\ l^{-1}$ (Fig. 2a). Algal cells were visible on the water surface but the bloom did not develop further and fish did not die. In February 2004 the chlorophyll *a* concentration was low (average concentration 7.5 ± 1.3 $\mu g\ l^{-1}$). A gradual build-up in algal biomass occurred from May 2004 (chlorophyll *a* = 7.1 ± 0.1 $\mu g\ l^{-1}$) when another bloom started developing and reached a maximum chlorophyll *a* concentration of 42.2 ± 2.4 $\mu g\ l^{-1}$ in November. The bloom persisted in the lake until December. It must have crashed between January and February, probably because it had reached an unsustainable maximum level of productivity. When the fish started dying in February 2005, the average

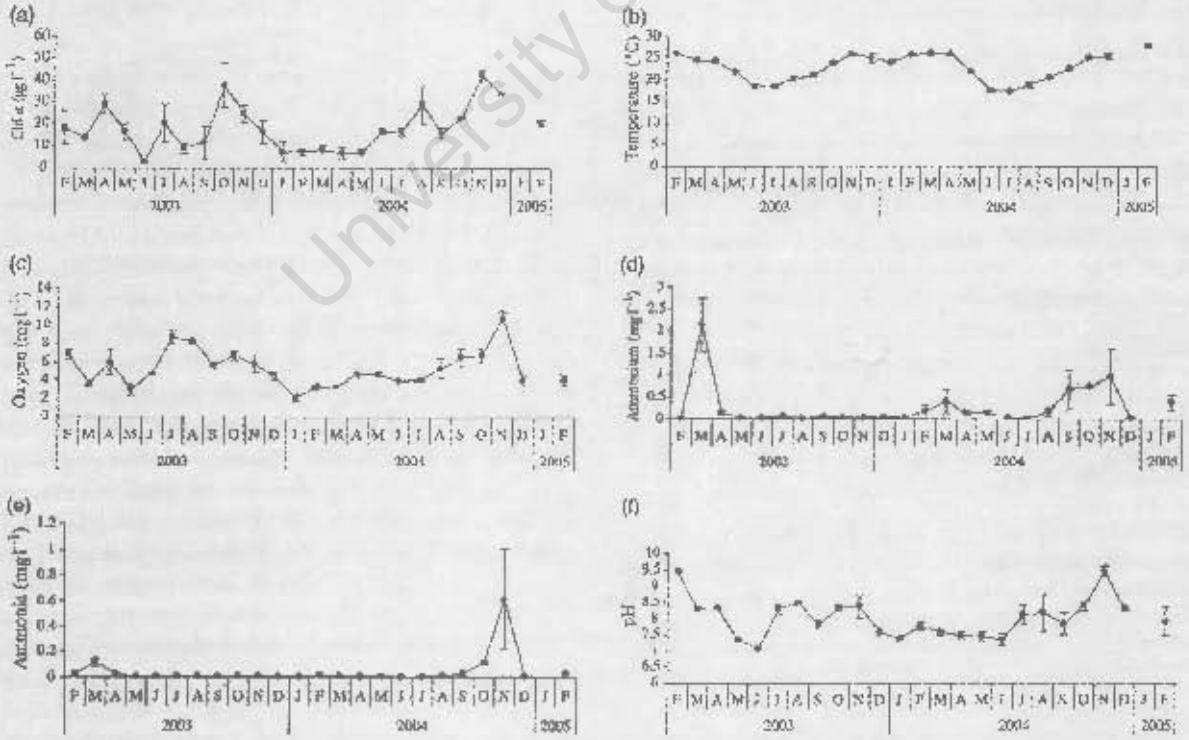

Fig. 2 Temporal change in (a) chlorophyll *a*, (b) temperature, (c) dissolved oxygen, (d) ammonium, (e) ammonia and (f) pH (mean of three stations) in Lake Chivero from February 2003 to February 2005

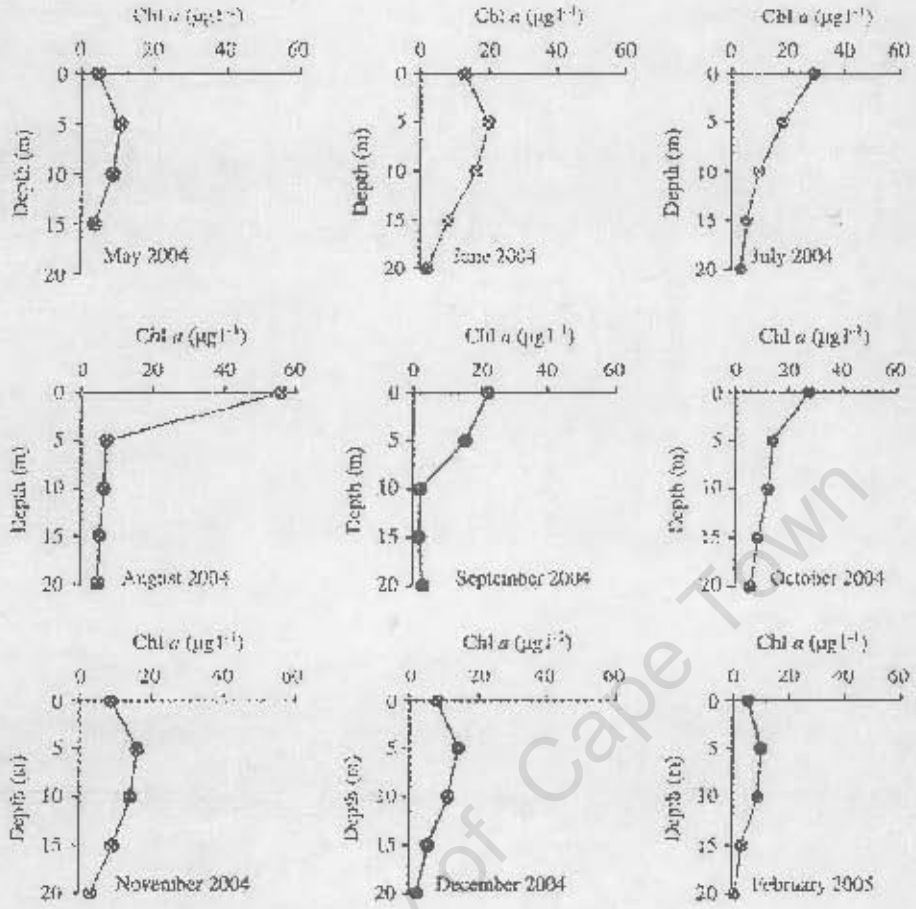

Fig 3 Depth profiles of chlorophyll *a* at station 1 before and during the fish kill

chlorophyll *a* concentration was 20.7 ± 1.4 µg l^{-1}. Depth profiles of chlorophyll *a* are shown in Fig. 3; concentrations decreased from surface to bottom and were highest between 0 and 10 m.

The period around February to March was when surface temperatures were highest in the lake (Fig. 2b). The average surface water temperature was lowest (22.1 ± 0.04°C) when the bloom started developing in May 2004, rising to a maximum of 28.1 ± 0.3°C in February 2005 (Fig. 3b). Temperature profiles during the period May 2004 to February 2005 are presented in Fig. 4. Surface water temperature varied between 17.4°C and 28°C and the bottom temperature between 16°C and 20.5°C. The lake was stratified during the fish kill in February, temperatures at the surface and the bottom being 28°C and 25°C respectively (Fig. 4). In June the lake was isothermal while for the rest of the time the lake was stratified (Fig. 4).

The temporal changes in the concentrations of dissolved oxygen between February 2003 and February 2005 are shown in Fig. 2c. During the period when there was no bloom, concentrations ranged between 1.8 and 9.5 mg l^{-1}. Dissolved oxygen positively correlated with chlorophyll *a* ($r = 0.51$) and pH ($r = 0.52$) (Fig. 2a,c,d). Following the onset of the bloom in May 2004, dissolved oxygen increased to a maximum concentration of 10.9 ±0.6 mg l^{-1} in November, after which it dropped to an average concentration of 3.9 ± 0.6 mg l^{-1} in February 2005 when the bloom had crashed, a concentration similar to that in December 2004, yet fish kills occurred only in February/March 2005. Figure 2c shows that the period around January to March (except for February 2003, when a mild bloom occurred) was characterized generally by the lowest dissolved oxygen concentrations in the lake during the study period. Ammonium and temperature

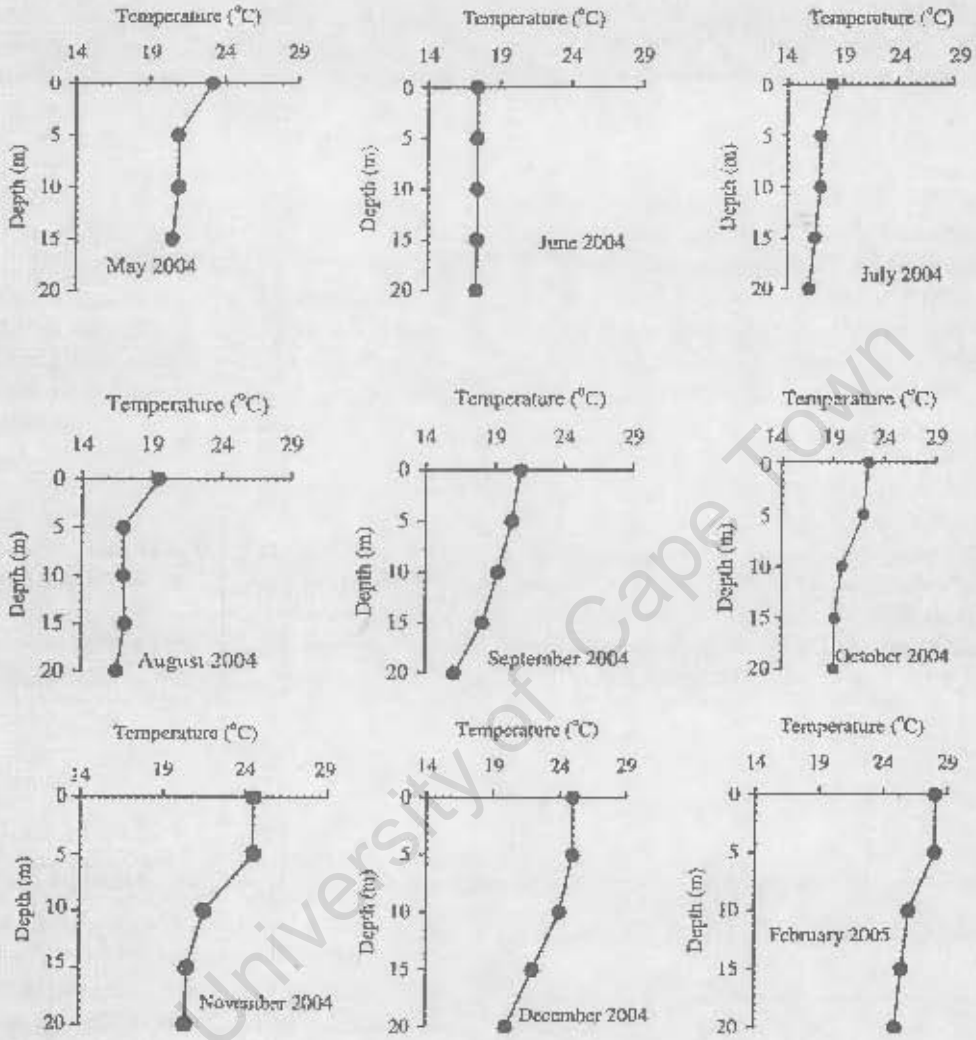

Fig 4 Depth profiles of temperature at station 1 before and during the fish kill

levels were also highest during this period (Fig. 2d,b). Changes in dissolved oxygen concentration down the water column from May 2004 to February 2005 are shown in Fig. 5. At the onset of the bloom in May the dissolved oxygen concentrations at the surface and bottom were 4.1 mg l^{-1} and 1.9 mg l^{-1}, respectively (Fig. 5). A slight decline in surface values occurred in June and July, after which the concentrations increased to a maximum of 9.3 mg l^{-1} in November. Concentrations at the bottom ranged from 1 to 3.1 mg l^{-1} prior to the collapse of the bloom, the maximum occurring in September. After the bloom collapsed, dissolved oxygen levels dropped to 4.2 mg l^{-1} at the surface and 0.6 mg l^{-1} at the bottom. From 5 m to 20 m dissolved oxygen levels ranged from 1.9 to 0.6 mg l^{-1}.

As ammonia toxicity has been implicated in fish kills in Lake Chivero, ammonium levels were monitored and ammonia calculated using a conversion factor (Dallas & Day, 2004). Temporal changes in ammonium and ammonia concentrations in the lake between February 2003 and February 2005 are shown in Fig. 2d,e respectively. Except for the peak in March 2003, concentrations were low (<0.5 mg l^{-1}) between February 2003 and January 2004. A slight increase occurred in March 2004, after which levels declined then increased again from August to November 2004 but not above the March 2003 concen-

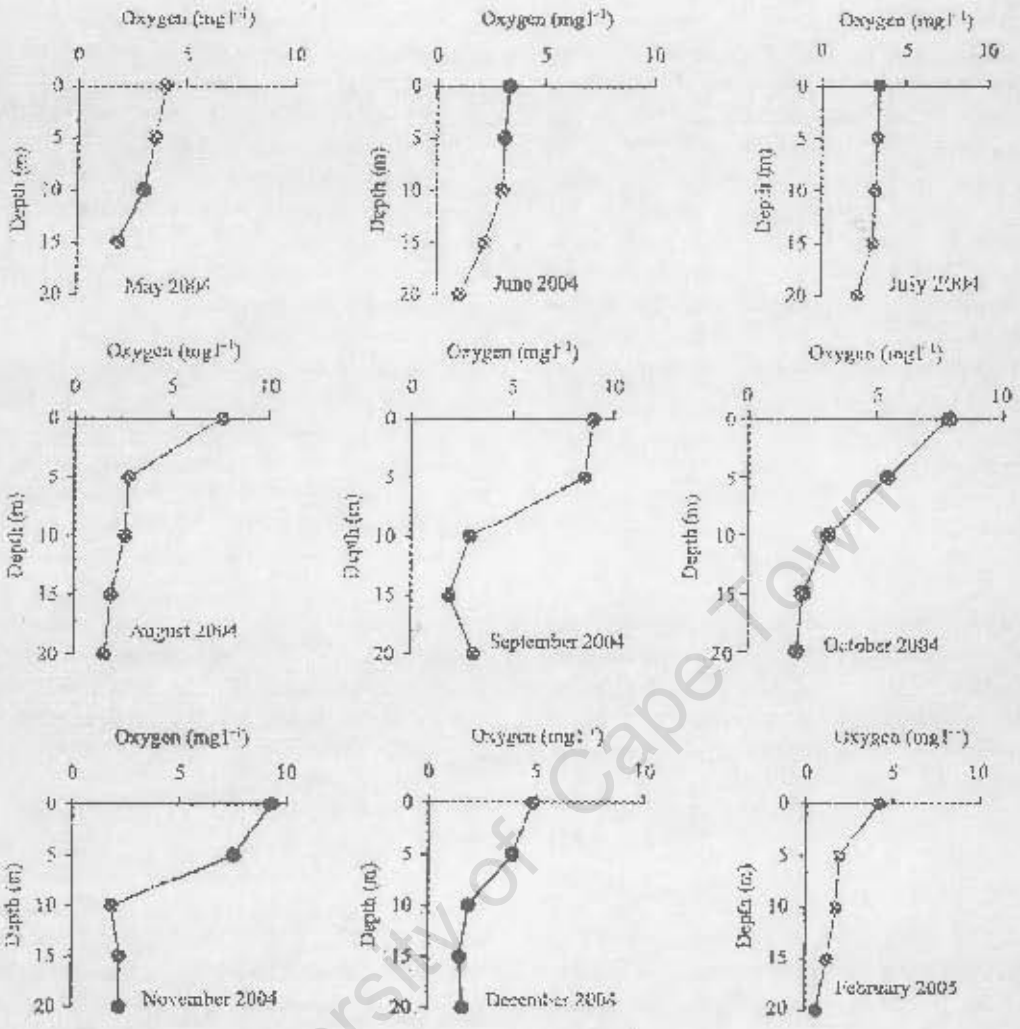

Fig. 5 Depth profiles of dissolved oxygen at station 1 before and during the fish kill

trations. Changes in ammonium profiles during the development of the bloom are shown in Fig. 6. Except in May, concentrations were highest in bottom waters and increased as the bloom developed. Highest concentration occurred in November. The concentrations at the surface and the bottom in November were 1.1 and 4.4 mg l^{-1}, respectively, while the surface and bottom concentrations in February during the fish kill were 0.2 and 4.1 mg l^{-1}, respectively (Fig. 6).

Changes in pH between February 2003 and February 2005 are shown in Fig. 2f. Patterns differed during the bloom and no-bloom periods. As the bloom developed, pH increased to reach the highest average level of 9.5 ± 0.1 in November 2004 (Fig. 2f). Change in pH was positively correlated with chlorophyll *a* ($r = 0.52$) and dissolved oxygen ($r = 0.70$). The pH was highest at the surface and lowest at the bottom between May and February. In February, when the fish-kill occured, the pH at the surface was 7.6 and at the bottom 6.6.

Discussion

The fish kill coincided with the collapse of an algal bloom suggesting that the dead algae were the likely causative factor. Decreased algal productivity and subsequent breakdown of algal material by aerobic bacteria alter the

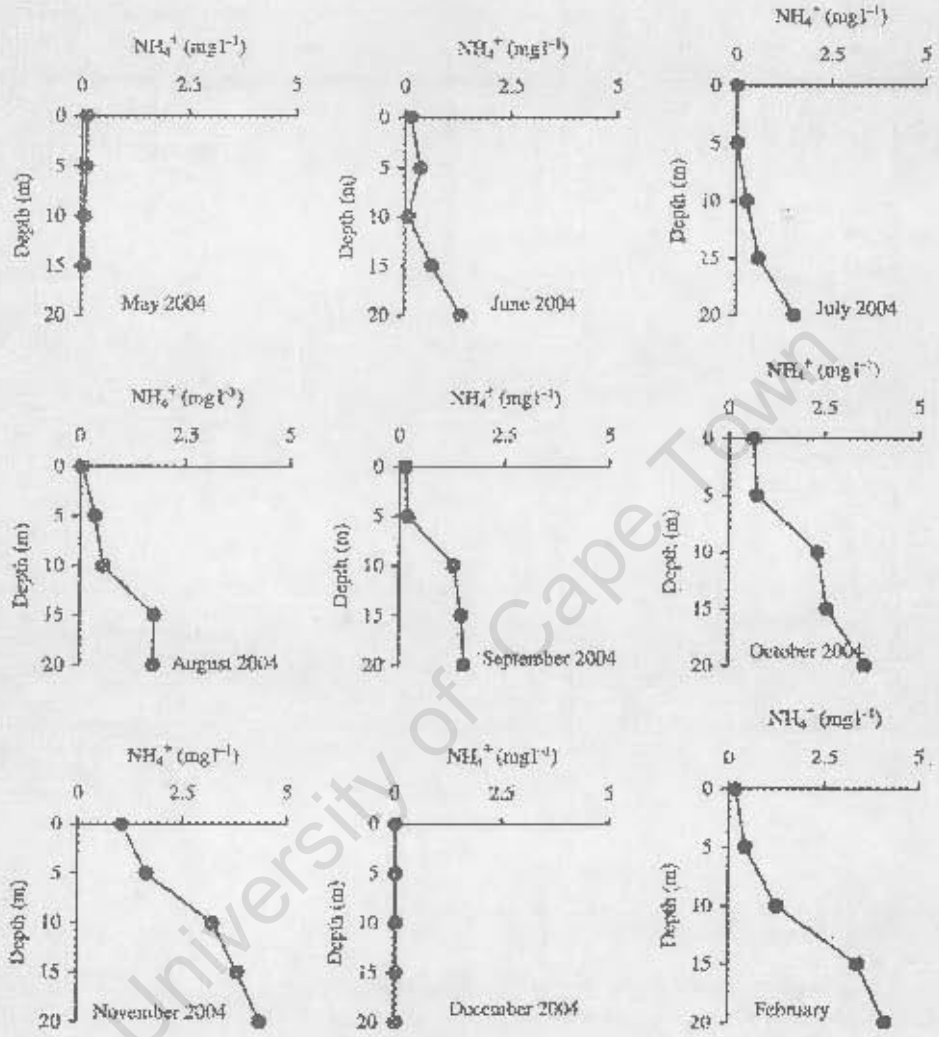

Fig 6 Depth profiles of ammonium at station 1 before and during the fish kill

blooms collapsed could have depressed dissolved oxygen to below 1.9 mg l^{-1} from 5 m depth, a level at which cichlid fish become sensitive (Mugadza, 1997). Low dissolved oxygen concentrations probably contributed to fish mortality as observed in the Nyanza gulf of Lake Victoria, where fish kills of *O. niloticus* occurred when levels dropped to 3.2–4.8 mg l^{-1} following collapse of an algal bloom (Ochumba, 1990). Oxygen levels could have been as low as 0.4 mg l^{-1}, as in the *O. macrochir* fish kill (Moyo, 1997) such that fish could have been affected mainly at night.

Interestingly, fish did not die in March and May 2003, between January and March 2004 and in December 2004 when dissolved oxygen concentrations were as low as during the fish kills. This indicates that factors other than low dissolved oxygen concentrations probably algal toxins released following the collapse of the algal bloom (Carmichael & Falconer, 1993; Chorus & Bartram, 1999), could have also contributed to fish deaths. Microcystins have been reported to be toxic to fish (Andersen *et al.*, 1993) and *Microcystis aeruginosa*, a common producer of microcystin (Falconer *et al.*, 1994) and dominant in Lake Chivero during blooms (Mitchell & Marshall, 1974; Roberts & Southall, 1977), could have been a source of toxins. Previously an enlargement of fish livers of dead *O. macrochir*

had been attributed to possible algal toxicity (Moyo, 1997) such that the contributory role of algal toxicity cannot be excluded.

Lowest dissolved oxygen levels occurred around January – March, similar to the period of severe oxygen deficiency indicated by Magadza (1997). From information now available it is probable that high levels of dissolved oxygen occur as a result of high photosynthetic rates when algal biomasses are large. As the algae die off around February to March, dissolved oxygen declines in the lake, sometimes to a point at which fish-kills occur, as observed by Barica (1981) in other hypereutrophic lakes. In such systems oxygen regimes are often unbalanced, with periods of supersaturation followed by complete anoxia as large populations of phytoplankton collapse (Barica, 1981). The variations in oxygen concentrations from May 2004 to February 2005 illustrate this phenomenon and the variations in chlorophyll *a* link them to changes in phytoplankton biomass in the lake.

Previous fish kills in Lake Chivero have been attributed to deoxygenation of the water during turnover when oxygen deficient, ammonia-rich water is brought to the surface (Burke & Thornton, 1982; Moyo, 1997). Sudden overturns between February and March are the major cause of fish kills in Lake Chivero (Moyo, 1997). In February 2005, when the fish died, the lake was stratified so fish deaths are unlikely to have been because of turnover unless a sudden turnover was not detected.

Ammonia poisoning has been implicated in fish deaths in Lake Chivero (Magadza, 1997; Moyo, 1997) and massive mortality of tilapia is reported to occur when concentrations exceed 2 mg l^{-1} (Popma & Masser, 1999). The concentrations during this study were below the level toxic to fish. Low levels of ammonia in surface water also indicate that turnover had not occurred because ammonia would be expected to be high after turnover (Marshall, 1997).

The pH of the water has not been directly implicated in fish kills, its influence being mainly through shifting the equilibrium from nontoxic ammonium to un-ionized ammonia, which is toxic (Magadza, 1997). According to Dallas & Day (2004) at a pH of 9 and a temperature of 25°C, slightly more than a third of the ammonium ions in water are converted to toxic un-ionized ammonia. The pH during the fish kill did not exceed a range 5–10, within which tilapia can survive (Popma & Masser, 1999) and is unlikely to have contributed to fish deaths.

All previously recorded fish kills in Lake Chivero have been of *O. macrochir* (Burke & Thornton, 1982; Moyo, 1997). Another observation regarding fish kills in Lake Chivero is the differential size mortality. During this kill, the only fish to be found dead ranged in standard length between 17 and 27 cm. The specimens of *O. macrochir* that died in 1996 ranged in length from 12 to 26 cm. It is not known why in this case *O. niloticus* of a particular size died, instead of *O. macrochir*. In trying to explain why *O. macrochir* was the only species to die during the massive 1996 fish-kill, Moyo (1997) suggested that it might have been the species most susceptible to deoxygenation and ammonia toxicity but it has yet to be established why the fish kills are species- and size- specific (Marshall, 2005) and why this particular genus is affected. The average condition factor for this size class was 3.2 and it ranged from 2.44 to 4.66. The fish were in good condition as the average condition factor was within the range reported for *O. niloticus* (Abubakar, 1988). This indicates that external factors might have caused fish mortality.

Although a link between fish kills of *O. niloticus* with the breakdown of an algal bloom is reported for the first time in Lake Chivero, elsewhere this phenomenon is well documented (Ochumba, 1985, 1987, 1990). This is an issue of concern which requires the institution of appropriate management measures because as the severity of algal blooms in the lake increases more kills may occur when the blooms crash.

Acknowledgements

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Cyanobacteria and cyanotoxins in the source water from Lake Chivero, Harare, Zimbabwe, and the presence of cyanotoxins in drinking water

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The phytoplankton community and cyanotoxins in Lake Chivero (formerly Lake McIlwaine) and the presence of cyanotoxins in treated drinking water were investigated between 2003 and 2004. A typical seasonal succession of Cyanobacteria species occurred from January to April, Bacillariophyta from May to July, and Cryptophyta and Chlorophyta from August to December. *Microcystis aeruginosa* and *M. wesenbergii*, known producers of the toxin microcystin, and the non-toxic cyanobacterium *M. novacekii* dominated during summer. The highest concentrations of microcystins and lipopolysaccharide endotoxins occurred when cyanobacterial biomass was highest. Lipopolysaccharide endotoxin concentrations in the lake ranged between 8 and 3 200 Endotoxin Units (EU) ml^{-1}. Microcystin concentrations in treated water were below the recommended safe limit for drinking water. Lipopolysaccharide endotoxin concentrations in treated water ranged from 0.15 to 11 EU ml^{-1}. The phytoplankton community comprised non-microcystin-producing species for the greater part of the study period.

Keywords: cyanotoxins, dominant organisms, endotoxins, LPS, microcystins, phytoplankton

Introduction

Lake Chivero (formerly Lake McIlwaine) is a eutrophic artificial impoundment in tropical Africa, built between 1952 and 1953 on the Manyame River 37km south-west of the city of Harare. It lies in the same catchment as, and is downstream of, the city it supplies, and thus has received sewage effluent since 1952 (Marshall and Falconer 1973) and is now hyper-eutrophic (Magadza 2003).

Eutrophication is manifested in the presence of cyanobacterial blooms and recurrent infestations by water hyacinth, *Eichhornia crassipes*. Blooms started appearing on the lake as it became eutrophic in 1960 and, by 1963, had become a permanent feature (Munro 1966). The blooms declined in the 1970s as water quality improved but, when conditions deteriorated again in 1980 (Marshall 1997), two cyanobacterial species — *Microcystis aeruginosa* and *Anabaena* sp. — formed dense blooms.

The first outbreak of water hyacinth occurred soon after construction of the dam wall but was controlled by spraying with 2,4-D between 1953 and 1958 (Marshall 2005). Physical removal and chemical control using 2,4-D also successfully controlled another outbreak that occurred in 1970 when a drop in lake level, following a drought in 1967/1968, exposed dormant water hyacinth seeds. The most severe outbreak, which covered approximately 35% of the lake surface, occurred in 1985 (Chikwenhere and Phiri 1999). This was brought under control in 1992 by spraying with 2,4-D, after which biological control with the weevil *Neochetina eichhorniae* was instituted. Biological control has been successful and by 2000 weed coverage was reduced to 3–5% of the total area of the lake (Chikwenhere 2001).

Since Lake Chivero is the main source of drinking water for Harare, cyanobacterial blooms pose a potential health risk to consumers. The major concern resulting from massive growths of cyanobacteria in reservoirs is the production of toxic compounds that can be a significant threat to human health (Carmichael and Falconer 1993, Chorus and Bartram 1999). This danger was recognized in Lake Chivero in the 1960s when Zilberg (1966) observed that children living in areas of the city supplied from Lake Chivero developed gastroenteritis each year, at times when natural blooms of *Microcystis aeruginosa* were decaying in the reservoir. Marshall (1991) later established a correlation between incidences of gastroenteritis and toxic cyanobacterial blooms in Lake Chivero. Despite the potential health concerns, very little work has been done in the lake on the ecology of cyanobacteria or on the toxins that they might produce. The only sustained study of phytoplankton ecology in the lake was carried out in 1968–1969 (Falconer 1973), but this work remains unpublished and has been largely overlooked. Similarly, studies on the toxins are circumstantial (Zilberg 1966, Marshall 1991).

Magadza (2003) suggested that drinking water from Lake Chivero could pose potentially serious health risks to consumers. The common group of algal toxins that could be a potential source of human injury are the microcystins, which are hepatotoxins that poison the liver and promote tumours (Falconer 1998). They have also been linked to skin rashes, hayfever-like symptoms, gastroenteritis and toxic hepatitis (Hawkins *et al.* 1985), as well as to the death of dialysis patients (Pouria *et al.* 1998). High levels of lipopolysaccharide (LPS) endotoxins from the outer cell membranes of most gram-negative bacteria (Sykora and Keleti 1988) and cyanobacteria (Weckesser *et al.* 1979) may also cause human illnesses when inhaled from drinking water (Muittari *et al.* 1980). These endotoxins are highly toxic and inflammatory (Razuddin *et al.* 1983) causing acute respiratory illness, inhalation fever, gastrointestinal disorders and inflammation at the alveolar level after exposure to them in water and in water-derived aerosols (Muittari *et al.* 1980). The only previous investigation of LPS endotoxins in Lake Chivero (Annadotter *et al.* 2005) showed that in October 1988 the phytoplankton community was dominated by *Microcystis aeruginosa* and *M. botrys* and that endotoxins ranged from 1 000–7 750 EU ml^{-1}, while endotoxins in Harare tap water, collected at a hotel in Harare, ranged from 60–205 EU ml^{-1}. The high levels in tap water were associated with a transient influenza-like reaction upon inhalation of aerosols.

This paper reports on the phytoplankton community and cyanotoxins in Lake Chivero from 2003–2004. In addition, the presence of cyanotoxins in treated water and selected physico-chemical data are discussed.

Methods

Sampling

From February 2003–February 2004 phytoplankton, microcystins, LPS endotoxins and physico-chemical parameters were monitored monthly at three stations (Figure 1). Water depth at Station 1, located near the water intake tower, is approximately 20m, while Stations 2 and 3 were in the shallow zone (maximum depth approximately 5m). Water samples for phytoplankton and microcystin analyses were collected using a Ruttner sampler from the following integrated depth intervals: 0–2m, 2–4m and 4–6m at Station 1; 0–2m and 2–3.5m at Station 2; and 0–2m and 2–3m at Station 3. Phytoplankton and microcystin were measured in the samples from each depth and a mean was calculated for the euphotic zone for each site.

Samples for LPS endotoxin analysis were collected from the surface directly into pyrogen-free plastic vials and for microcystin into microcentrifuge tubes. Samples were kept cool during transportation and, upon arrival in the laboratory, immediately frozen at –20°C. Phytoplankton samples were preserved in Lugol's solution. Samples of treated drinking water were collected monthly between April 2003 and January 2004 from the Morton Jaffray Waterworks Plant, which supplies treated water to Harare.

Analyses

Temperature, pH, conductivity, turbidity and dissolved oxygen were measured with a mercury thermometer, a WTW pH 330i meter, a WTW Cond 330i meter, a Hach Field Turbidimeter and a WTW Oximeter 330, respectively. Transparency was determined by means of a Secchi disc. The concentrations of orthophosphate, total phosphorus, nitrate and total nitrogen were determined according to Golterman *et al.* (1978). Orthophosphate and total phosphorus were determined colourimetrically. Nitrate was determined by the cadmium-copper reduction method and total nitrogen by the alkaline persulphate method. Chlorophyll *a* was determined by the acetone extraction method (Golterman *et al.* 1978).

Utermöhl's sedimentation method was used to identify and enumerate phytoplankton (Utermöhl 1958, Cronberg 1982). Approximately 60–100 cells of the dominant species were counted under 400X magnification using differential interference-contrast optics. Cell volumes were estimated from the mean cell dimensions and cellular shape of each species. For the calculation of fresh weight, the specific density of phytoplankton cells was assumed to be 1.0 (Cronberg 1997).

LPS endotoxin was analysed quantitatively by an accredited laboratory at the Department of Clinical Bacteriology, Gothenburg University, using the *Limulus* Amebocyte Lysate Endochrome assay (Levin and Bang 1968, Levin 1987) from Charles River Endosafe. The analyses were carried out according to the instructions provided by the manufacturer. Endotoxin quantities were expressed as Endotoxin Units, EU ml^{-1}. The endotoxin assay measures the total endotoxin activity in the water samples, i.e. endotoxins from cyanobacteria and gram-negative bacteria. Amounts of microcystins were analysed with a commercially available Enzyme Linked Immuno Sorbent Assay (ELISA) Plate kit, (Envirogard®), specific for microcystin-LR, -RR, -YR and nodularin. The kit uses test tubes coated with antibodies, which bind either to microcystins or form microcystin-enzyme conjugates. Samples were freeze-thawed three times in order to break the cells and to allow intracellular toxins to leak into the water and were sonicated for 5min to dissolve the cell-bound hepatotoxins into the water. The toxins were analysed spectrophotometrically with a Microreader Hyperion 3 (450nm detection). The detection limit of the microcystin/nodularin concentration was 0.1µg l^{-1}.

Results

Physico-chemical conditions

Secchi disc transparency ranged between 1 and 2.75m, while pH varied between 7 and 9.5. The highest pH of 9.5 occurred in February 2003, after which it decreased to 7 in June 2003 and thereafter fluctuated between 7.5 and 8.5 (Figure 2a). Water temperature was highest in summer (26.1°C, December–April) and spring (26.1°C, September–November), and lowest in winter (18.6°C, May–August) (Figure 2b). Conductivity, turbidity and dissolved oxygen ranged from 323–445µS cm^{-1}, 2.7–15.5mg l^{-1} and 1.8–9.5mg l^{-1} respectively.

Two periods could be distinguished with respect to nitrogen and phosphorus concentrations in the lake.

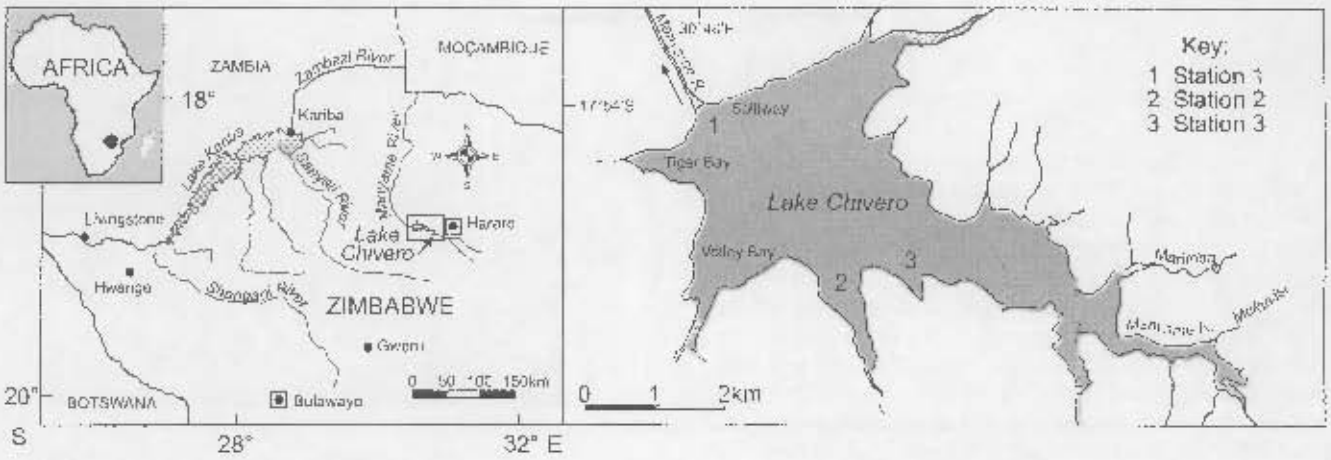

Figure 1: Map of Lake Chivero, showing the sampling stations

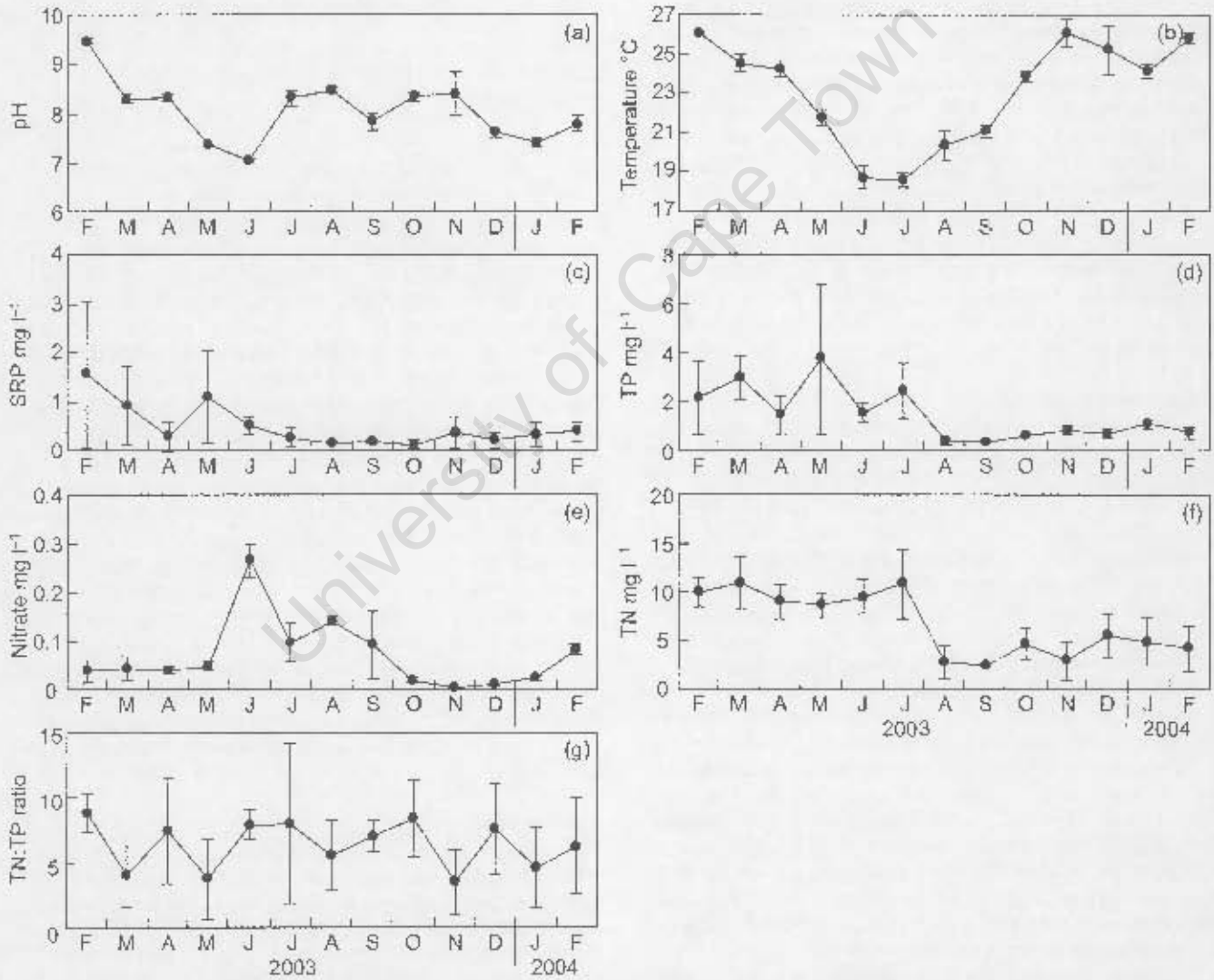

Figure 2: Temporal variation of (a) pH, (b) temperature, (c) orthophosphate (SRP), (d) total phosphorus (TP), (e) nitrate, (f) total nitrogen (TN) and (g) TN:TP ratio in Lake Chivero from February 2003 to February 2004. Values are means of three stations (refer Figure 1); error bars = standard deviation

Concentrations were highest from February–July 2003 and lowest from August 2003–February 2004 (Figure 2d, 2f). The highest average orthophosphate concentration of 0.9mg l^{-1} occurred between February and June 2003; a decrease in July to a lower average concentration of 0.3mg l^{-1} was maintained until February 2004 (Figure 2c). A high average total phosphorus concentration of 2.4mg l^{-1} and large fluctuations (range 0.7–3.9mg l^{-1}) occurred from February to July 2003 (Figure 2d). This was followed by a decrease in August, after which concentrations remained at a fairly average uniform concentration of 0.7mg l^{-1} (Figure 2d).

The concentrations of nitrate in the lake were fairly uniform between February and May 2003 (Figure 2e). This was followed by a sudden increase in nitrate to an average concentration of 0.3mg l^{-1} in June, after which levels dropped in July to an average concentration of 0.1mg l^{-1}. A slight increase occurred in August, thereafter declining to a lowest average level of 38µg l^{-1} in November and remaining low at all stations with a slight increase in February 2004. Total nitrogen concentrations fluctuated widely between 2 and 14.7mg l^{-1} at all stations (Figure 2f). Concentrations averaged about 9.2mg l^{-1} between February and July 2003, declining sharply to 2mg l^{-1} in August and averaging 3.9mg l^{-1} between August 2003 and February 2004. The TN:TP ratio was below 20 and fluctuated between 1.9 and 10 throughout most of the study period (Figure 2g).

Phytoplankton community

The phytoplankton community was represented by five dominant taxonomic groups: cyanobacteria, bacillariophytes, chlorophytes, cryptophytes and euglenophytes. A total of 64 phytoplankton species was identified. Chlorophytes comprised 61% (39 species) of the taxa, cyanobacteria 16% (10 species), bacillariophytes 9%, cryptophytes 3% and euglenophytes 7%. The dominant cyanobacteria were *Microcystis aeruginosa*, *M. wesenbergii* and *M. novacekii*. Other cyanobacteria occurring at very low densities were *Microcystis botrys*, *Aphanocapsa* cf. *incerta*, *Planktothrix agardhii* and *Woronichnia* sp. *Anabaena* sp. occurred at a very low frequency and was only observed in samples collected between December and January. Cyanobacteria — mainly *M. aeruginosa*, *M. wesenbergii*, and *M. novacekii* — dominated between February and March. In February 2003 cyanobacteria contributed 82% of the total biomass (Figure 3). The water was clear for most of the study period except for the high cyanobacterial biomass in February 2003, when the average biomass reached 18.1mg l^{-1} (Table 1). Cyanobacterial biomass declined from June until December.

Bacillariophytes occurred throughout the study period, but were most abundant in winter (April–July) when the phytoplankton community was dominated by two diatoms, *Aulacoseira granulata* and *Cyclotella* sp. The percentage contribution of bacillariophytes to total biomass during this period ranged from 13.1–87.7% (Figure 3). This was the period of lowest biomass in the lake: average 1.1mg l^{-1} (range 0.4–2.4mg l^{-1}) (Figure 4). *Aulacoseira granulata* was the dominant diatom and

Figure 3: Temporal changes of percentage algal biomass contribution in Lake Chivero from February 2003 to February 2004. Cyano. = Cyanophyceae, Chlor. = Chlorophyceae, Eugle. = Euglenophyceae, Bacil. = Bacillariophyceae, Crypt. = Cryptophyceae

occurred throughout the study period while the density of *Cyclotella* was sometimes very low.

At the end of the cold period the community became dominated by cryptophytes and chlorophytes from July until the beginning of the rainy season in November/December (Figure 3). The favourable growing period for the cryptophytes was between July and October, when their biomass reached a maximum and their percentage contribution to total biomass ranged from 28.8–65.4%. The dominance of cryptophytes overlapped with chlorophytes, which became dominant between September and November (Figure 3). A marked increase in biomass of chlorophytes occurred in November with a bloom of *Coelastrum microporum*, *C. reticulatum* var. *cubanum* and *C. sphaericum* having a mean biomass of 8.9mg l^{-1}, which was 97% of the total biomass. Euglenophytes — represented mainly by *Trachelomonas* spp., with rare encounters of *Phacus* sp. and *Euglena* sp. — did not exhibit clear seasonal patterns (Figure 3). Total phytoplankton biomass in the lake ranged from 0.1–36.9mg l^{-1} with peaks in February, August and November (Figure 4). Chlorophyll *a* concentration ranged from 2–48.9µg l^{-1}, with a mean of 16.1 (±8.62) µg l^{-1} (Figure 4). Three peaks were exhibited: between February and April, in July, and between October and November.

Cyanotoxins

Microcystin concentrations were highest in February 2003, when cyanobacterial biomass was highest, and thereafter decreased until February 2004 (Table 1). The mean surface concentration in February 2003 was 2.4µg l^{-1}, while the mean concentration in the water column was 1.6µg l^{-1}. Microcystin concentrations in the lake varied between 0.2 and 4.2µg l^{-1} (Table 1). Treated water from the water treatment works had microcystin concentrations of <0.1µg l^{-1}, except in July when levels of 0.1 and 0.2µg l^{-1} were detected (Table 2). Treated water from Morton Jaffray Waterworks had LPS endotoxin concentrations ranging from 0.15–11 EU ml^{-1} (Table 2). LPS endotoxin concentrations were highest during the period when cyanobacterial biomass was highest (Table 1). The mean LPS endotoxin

Table 1: Temporal changes of cyanobacterial biomass, microcystins and LPS endotoxins in Lake Chivero from February 2003–February 2004. Values are the mean of three stations ± standard deviation

Month	Cyanobacterial biomass (mg^{-1})	Microcystins ($\mu g\ l^{-1}$)	LPS endotoxins (EU ml^{-1})
February 2003	18.06 ± 16.26	1.62 ± 0.60	907 ± 1 061
March 2003	1.00 ± 1.65	0.55 ± 0.14	1 225 ± 1 720
April 2003	0.58 ± 0.54	0.26 ± 0.02	293 ± 102
May 2003	0.06 ± 0.08	0.22 ± 0.03	360 ± 44
June 2003	0.04 ± 0.04	0.27 ± 0.13	26 ± 9
July 2003	0.02 ± 0.02	0.17 ± 0.02	23 ± 15
August 2003	0.03 ± 0.05	0.15 ± 0.03	64 ± 37
September 2003	0.001 ± 0.003	0.15 ± 0.04	40 ± 17
October 2003	0.11 ± 0.10	0.29 ± 0.07	92 ± 12
November 2003	0.009 ± 0.01	0.11 ± 0.03	68 ± 24
December 2003	0.005 ± 0.005	0.10 ± 0.05	69 ± 50
January 2004	0.08 ± 0.05	0.09 ± 0.05	47 ± 16
February 2004	0.21 ± 0.21	0.14 ± 0.011	60 ± 24

concentration in the lake was 251 EU ml^{-1} and values varied between 8 and 3 200 EU ml^{-1}.

Discussion

Phytoplankton community and influencing factors

The phytoplankton community in Lake Chivero, as in other eutrophic systems in southern Africa, has previously been dominated by cyanobacteria, mainly *M. aeruginosa*, *Anabaena flos-aquae* and *Anabaenopsis tanganyike* (Munro 1966, Falconer 1973, Marshall and Falconer 1973, Mitchell and Marshall 1974, Robarts and Southall 1977, Robarts 1979). The sampling period reported on here seems to have coincided with a period of reduced cyanobacterial abundance, since cyanobacterial blooms were not permanent or persistently abundant. There was an unusual decline in *M. aeruginosa*, while *Anabaena* sp. — also a potential producer of microcystins (Carmichael 1992) — was rare.

Dense cyanobacterial blooms appeared in the lake immediately after its construction, when the lake became eutrophic, and became a permanent feature as the water quality continued to deteriorate (Munro 1966). Although data are not available for the period since then, visual observations over the years have indicated that blooms are the norm. Anomalously low concentrations of cyanobacteria were encountered during the present study, however, with *M. wesenbergii*, *M. novacekii* and *M. aeruginosa* being dominant only between January and April. Nutrient loading into the lake has not improved (see Table 3) and therefore the decline in cyanobacterial dominance was not due to an improvement in water quality.

Microcystis novacekii and *M. wesenbergii*, which co-occurred with *M. aeruginosa*, were not reported in former studies on Lake Chivero, but are common in eutrophic systems and have been described in Lake Kariba by Cronberg (1997). These are all new records in Lake Chivero.

The most remarkable difference between the cyanobacterial assemblage in the 1960s and 1970s and that recorded in the present study was the decline in the dominance of *Anabaena* sp. and *A. tanganyike*, two heterocystous species previously reported by Mitchell and Marshall (1974) as being amongst the dominant phytoplankton in 1970–1971. At that time *A. flos-aquae* was reported as co-dominating with *M. aeruginosa*, particularly during winter

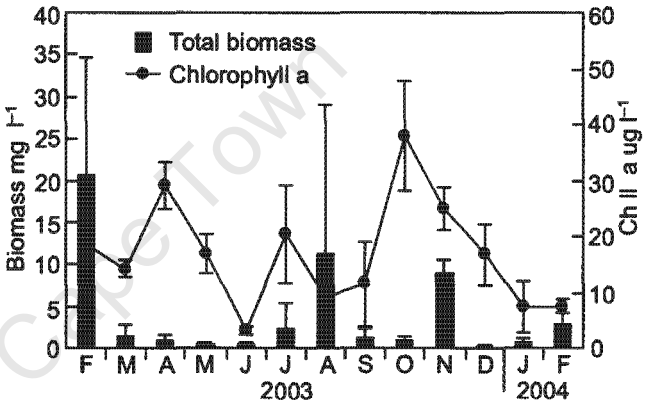

Figure 4: Temporal changes in total biomass and chlorophyll *a* in Lake Chivero from February 2003 to February 2004. Values are the mean of three stations; error bars = standard deviation

Table 2: Levels of LPS endotoxins and microcystin in treated water from Morton Jaffray Waterworks Plant between April 2003 and January 2004

Date	Microcystin ($\mu g\ l^{-1}$)	LPS endotoxins (EU ml^{-1})
9 April	<0.10	11.00
28 April	<0.10	3.00
5 May	<0.10	0.15
23 June	<0.10	0.55
9 July	<0.10	0.38
23 July	0.12	0.68
23 July	0.16	1.00
28 August	<0.10	1.90
29 September	<0.10	2.90
5 November	<0.10	5.60
29 December	<0.10	3.20
30 January	<0.10	2.90

Table 3: Comparison of water chemistry parameters (ranges of concentrations) in Lake Chivero from 1957–2004. Data from 1957–1980 are from the mid-lake station (Thornton and Nduku 1982); those from the present study are from Station 1, near the water intake tower

Years	1957–1958	1968–1969	1976–1977	1979–1980	2003–2004
Conductivity (µS cm^{-1})	82–102	123–232	46–320	90–150	327–498
Secchi disc (m)	1.50–2.25	–	0.50–4.00	1.50–2.00	1.20–2.75
pH	7.5–8.0	8.0–9.6	6.4–9.8	6.5–9.1	7.1–9.5
Nitrates (mg l^{-1})	–	tr–0.47	0.03–0.68	tr–0.20	0.004–0.23
Orthophosphate (mg l^{-1})	–	tr–0.80	tr–0.30	tr–0.08	0.18–1.36
Chlorophyll a (µg l^{-1})	–	50–150	tr–146	2–29	2–26

(around July) when oxygen tensions in the epilimnetic waters were lowest (Munro 1966). Falconer (1973) observed *A. flos-aquae* to form an almost monoculture in July 1969, whereas only a few filaments of *Anabaena* sp. were occasionally encountered in the present study. *Anabaenopsis tanganyike* was not recorded during this study, although it was previously reported from the epilimnitic waters, mainly between February and April (1969), while for the rest of the year it occurred only in low numbers, if at all (Falconer 1973).

According to Blomqvist *et al.* (1994), nitrogen scarcity favours the development of nitrogen-fixing species. *Anabaena* sp. and *A. tanganyike* are expected to dominate when nitrogen is limiting (i.e. at low N:P ratios) because they are capable of nitrogen fixation (Gallon 2001). Nitrogen was the primary phytoplankton growth-limiting nutrient in Lake Chivero during the early years (Robarts and Southall 1977); it seems that, at that time, low nitrogen levels favoured the occurrence of nitrogen-fixing cyanobacteria to the extent that they were dominant. According to Reynolds (1998), phytoplankton dominance is the result of a stochastic combination of environmental variables. *Anabaena* favours nitrate depletion (Sakamoto and Okino 2000), stable environmental conditions such as the absence of water turbulence, long water retention times (Reynolds *et al.* 2002) and high irradiance (Ahn *et al.* 2002). The average nitrogen load received by the lake is now four times higher than the levels estimated in 1996 (Nhapi 2004), so nitrate levels are now higher than the levels recorded when the dominance of *Anabaena* was reported. The lake has always been subject to turbulence and has a short water-retention time of 0.82 years (Marshall and Falconer 1973), so high nitrate levels are the most likely factor limiting the dominance of *Anabaena*. However, since *Anabaena* is present in low numbers, it might be expected to increase if the levels of nitrate were to decrease, although generally *Anabaena* spp. are also not common in southern African lakes and reservoirs.

As previously observed (Falconer 1973), in the 1960s chlorophyll *a* exhibited three peaks, coinciding with summer, winter and spring, i.e. the three growing seasons in the lake. The February–April peak occurred when cyanobacteria dominated and the October peak when chlorophytes dominated. Previously, *Microcystis* dominated during the summer chlorophyll *a* peak, *A. granulata* during the early winter peak, and *Anabaena* sp. during the spring peak (Robarts *et al.* 1982). Instead of *Anabaena*, cryptophytes and chlorophytes dominated in spring, and during this study the early winter peak was not distinct. Chlorophyll *a* concentration decreased between April and June when bacillariophytes were dominant. The main chlorophyll *a* peak period is variable. During this study the highest peak occurred in October/November, whereas it has also occurred in January (Falconer 1973), June and August (Thornton 1980). Algal self-shading prevents the build-up of high chlorophyll *a* levels (Robarts 1979) comparable to those in two other eutrophic systems, Hartbeespoort Dam (NIWR 1985) and Zeekoevlei (Harding 1992). The patterns exhibited by chlorophyll *a* and biomass were comparable. The slight differences can be attributed to uncounted species, as observed by Talling (1986) and Kebede and Belay (1994).

The seasonal successional pattern observed in the present study was linked to changes in the physico-chemical environment, mainly temperature and the supply of nutrients. In a similar system (Hartbeespoort Dam) *M. aeruginosa* tended to be persistently abundant from mid-October until around May (Robarts and Zohary 1984) and sometimes during winter as well (NIWR 1985), while chlorophytes, mainly *Oocystis* spp., appeared in spring and *A. granulata* for short periods in winter or early spring. In the case of Lake Chivero, the shift from a predominance of cyanobacteria to a mixed community with a pronounced seasonal species successional pattern is appropriate with respect to toxin production, since high toxin levels would be expected only in summer.

Several factors influence the dominance of cyanobacteria in phytoplankton communities (Ballot *et al.* 2005). As a eutrophic lake, Lake Chivero is characterised by high conductivity, nitrogen and phosphorus levels and pH >8, which should favour the dominance of cyanobacteria (Shapiro 1990). The pH in Lake Chivero fell in the range 6.3–9.8 during the period reported by Thornton and Nduku (1982), while values between 8.4 and 9 were recorded by Rommens *et al.* (2003). The major determinant of pH is photosynthesis.

Temperature and nutrient loading have been considered as important environmental factors influencing cyanobacterial dominance (Paerl 1996). Growth rates of cyanobacteria are optimal within a range of 25–35°C (Ganf 1974). Temperatures measured during this study, except between May and August, were near the optimal range for cyanobacteria and fell within the range 14–25°C, as was previously recorded by Thornton and Nduku (1982). Orthophosphate concentration has previously been reported to range from 0.27–0.49mg l^{-1} (Magadza 1997) and more recently from

0.91–1.22mg l^{-1} (Rommens *et al.* 2003). Concentrations were lower in the 1960s and 1970s (Table 3). During this study a higher maximum concentration of 1.89mg l^{-1} was recorded, indicating further enrichment, as also shown by the increase in conductivity (Table 3).

Besides conductivity and nutrients, other factors possibly influence and are also affected by the dominance of cyanobacteria. Robarts (1979) recorded low transparencies (0.6–1.6m) caused by high cyanobacteria densities, which caused self-shading. The high Secchi disc visibilities, up to 2.5m, recorded during the present study can be explained by the decline and lesser dominance of cyanobacteria within the phytoplankton community during the study period. Secchi disc transparency was correlated with algal biomass, as Robarts (1979) observed during the 1970s. High cyanobacterial biomasses previously caused permanent oxygen supersaturation in the epilimnion (Marshall and Falconer 1973) but, during the present study, high levels of dissolved oxygen occurred only when phytoplankton biomasses were high.

Low TN:TP ratios (<29) have been suggested as a major factor favouring the dominance of cyanobacteria (Smith 1983). Although the TN:TP ratios were below 20 during the entire study, cyanobacteria were not persistently dominant. TN:TP ratios are said to become insignificant if the nutrient concentrations exceed those limiting cyanobacteria growth (Reynolds 1992), and during this study nitrogen and phosphorus levels in the lake were not limiting for cyanobacteria. Jensen *et al.* (1994) made a similar observation for shallow Danish lakes.

Cyanotoxins

Microcystins and LPS endotoxins were detected both in the lake and in drinking water derived from the lake. The highest microcystin concentrations were recorded in February 2003 when *M. aeruginosa*, a common producer of microcystin (Falconer *et al.* 1994), was present together with *M. wesenbergii* and *M. novacekii*. *Microcystis wesenbergii* is potentially toxic (Cronberg pers. comm.) and *M. novacekii* is non-toxic, so it is likely that the microcystins were produced by *M. aeruginosa* and *M. wesenbergii*. A decline in microcystin levels from April–February 2004 coincided with a successional replacement of cyanobacteria by bacillariophytes and chlorophytes, taxa that do not produce toxins. The microcystin levels can therefore be attributed to high cyanobacterial biomasses. Microcystin concentrations in the treated water were below the guideline value of 1µg l^{-1} 'tolerable daily intake for microcystins for lifetime exposure' (WHO 1998). High levels were not expected in drinking water during this study because there were no blooms in the lake and cyanobacterial biomasses were low for most of the time.

It is interesting to compare the levels of LPS endotoxins detected in raw and drinking water to values recorded in studies elsewhere. Investigations in Finnish freshwater lakes have reported mean levels of 1 400 (range 2 000–3 800) EU ml^{-1} during blooms (Rapala *et al.* 2002). Concentrations of 1 050–1 350 EU ml^{-1} for raw water have been reported for South African and Namibian waters (Burger *et al.* 1989), while Mwaura *et al.* (2004) detected LPS endotoxin levels ranging from 68–4 269 EU ml^{-1} in two small lakes in Kenya. Treated water in South Africa and Namibia had levels of 5–71 EU ml^{-1} (Burger *et al.* 1989) and 14 EU ml^{-1} in Finland. Since the levels detected in Harare drinking water during this study were well below such values, indications are that they should not be of concern as regards human health.

The major conclusion is that the phytoplankton community of Lake Chivero was not dominated by cyanobacteria during the study period, but showed a typical successional pattern. *Microcystis aeruginosa*, a potential microcystin producer, was dominant only in February, whereas *Anabaena* was scarce. Microcystin levels in drinking water were low and LPS endotoxins were below levels reported elsewhere, indicating that their potential risk to humans was minimal during the study period. In order to assess the long-term risk to human health it will be necessary, however, to institute a routine monitoring programme linked to the production of drinking water.

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