Spatial and Temporal Features of Hydrodynamics and Biogeochemistry in Myponga Reservoir, South Australia

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A Thesis Submitted for the Degree of Doctor of Philosophy

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October 2011

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Abstract

Understanding hydrodynamic and biogeochemical processes in lakes is fundamentally important to the management of phytoplankton population and the improvement of water quality. Physical processes such as wind-driven surface mixing, thermal stratification and differential heating and cooling can affect the distribution of water, phytoplankton and sediments and the availability of nutrients and light. These lake processes, which are highly variable in space and time, affect phytoplankton dynamics in the field. This study aims to determine the spatial and temporal variability of phytoplankton and processes that either contribute to or override the variability in the artificially mixed Myponga Reservoir, South Australia.

A sediment survey showed that sediments underlying deep water were richer in organic matter, carbon, nitrogen and phosphorus than the sediments underlying shallow water. This may lead to different nutrient release rates between the shallow and deep areas. Both sediment resuspension and anoxic sediment nutrient release were important internal sources of nutrient to support phytoplankton growth in summer when external nutrient supplies were limited by low rainfall in the catchment.

An analysis of historical water temperature data revealed the development of micro-stratification at the sediment-water interface in summer, especially during a heatwave (air temperature > 40ºC for several consecutive days). Prolonged micro-stratification could potentially induce anoxic layers at the sediment surface, resulting in the release of nutrients. A risk assessment was conducted to predict the release of phosphorus from anoxic sediments and to evaluate the potential impact of cyanobacterial population (*Anabaena circinalis*) and the release of secondary metabolites (e.g. saxitoxin and geosmin).

Spatial variability of surface mixed layer depths exists between the side-arm and main basin. A simple light model based on the relationships of surface mixed layer depth, daily light dose and phytoplankton growth rate, was developed to estimate the potential variation of phytoplankton population in the two different light habitats (the main basin and side-arm). The model showed that phytoplankton abundance in the main basin was lower than in the side-arm. However, differential heating drove a large basin-scale convection, which circulated the water between the side-arm and main basin within

hours. This circulation overrode the time scale of days for the light-dependent growth effect between the two sites and hence there was no observable change in phytoplankton community structure.

Although no spatial variability of phytoplankton was observed at community level, significant variations of phytoplankton cellular content and stoichiometry were detected. Higher carbon cellular content in the side-arm than in the main basin was probably due to a greater exposure to light (shallower surface mixed layer in the side-arm) for photosynthesis. In the situation where nutrients were scare, higher phosphorus cellular content was found in the side-arm than in the main basin; this was possibly due to a greater exposure to resuspended nutrients from the lake bottom (shallower water in the side-arm). There was also a strong seasonal pattern in phytoplankton cellular content and stoichiometry between summer and early winter of 2009. The carbon content of phytoplankton increased over time, while the phosphorus content decreased. After the first heavy rain event (70 mm over a four-day period) in early May, carbon cellular content decreased, while phosphorus cellular content increased. These changes in phytoplankton contents were most likely related to the bio-availability of phosphorus in water.

This study reviews many complex, interactive processes driving the variability of lake physics and chemistry. The variability can yield rapid biological responses at physiological and cellular levels (e.g. F_v:F_m and cellular content), but does not necessarily appear at community levels (e.g. phytoplankton biomass, diversity). Often, conventional monitoring in lakes and reservoirs overlooks the subtle variability of phytoplankton dynamics. The relative scaling among physical, chemical and biological processes, therefore, is important to adequately describe the spatial and temporal variability in lakes and reservoirs.

Statement of Originality

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Wai Sum Chan and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Wai Sum Chan

Acknowledgements

This work has been made possible with the financial support of the Cooperative Research Centre for Water Quality and Treatment (now Water Quality Research Australia) and the School of Earth and Environmental Sciences, The University of Adelaide. Thanks also to the School for providing a comfortable working environment and general support throughout my candidature. In addition, I would like to acknowledge South Australian Water Corporation (SA Water) for providing field support and data.

I wish to take this opportunity to thank my supervisors Associate Professor Justin Brookes, Associate Professor Friedrich Recknagel and Dr David Lewis. Your knowledge, guidance, enthusiasm, support and patience have been inspirational throughout the entire course of this study. Justin, a special thanks for your weekly meeting to keep me on track!

I would like to express my gratitude to many people who have assisted me throughout the study.
Field assistance: Justin Brookes, Abigail Goodman, Mohd Yusof Ishah, Azma Hanim Ismail, Yesha Joshi, Nor Asman Kasan, Dae Heui Kim, Young Kil Kim, Tang Lei, Samuel Martin, Jeffrey Newman, Cecile Perillon, Dominic Skinner, Guowei Tan, Steven Tsang and Scotte Wedderburn from The University of Adelaide
Technical assistance: Teresa Fowles, Hasnuri Hassan, Jeffrey Hiorns, David Ladd, Daryl Mares, Debra Miller, Lyndon Palmer, Jason Peak, Colin River, Andrew Wright from The University of Adelaide; Gill Cozens, Rodrigo Gomez-Eamacho, Caroline Johnston, Julie Smith from CSIRO Land and Water; and Rod Boothey, Mike Burch, Rob Daly, Peter Hobson, Thorsten Mosisch, Greg Ingleton, Dean Newman from SA Water.
Thank you Laura Ruykys, Peter Ward, Phillip Northeast, Kane Aldridge, Deborah Furst and Pranay Sharma for being ‘harsh’ on my writing!

Many thanks to my friends Herbert Foo, Jaime Leung, Vivian Ng, The Semlers, Raymond Siu, Edward Tsang, Mei Yeh, The Zhus and many others, for providing me with the weekend getaway! A special note to ‘my’ pets and plants for cheering me up!

Most of all, I would like to thank my beloved mum, dad, sister and brother for their long-distant but closest support and loves. I love you forever! Special thanks also go to my extended family and friends back in Hong Kong and elsewhere to back me up! Without each of your support and assistance, I could not have come so far.

Finally, I would like to thank my fiancé (now husband) Guowei and his family for their understanding, patience and encouragement throughout my entire study.

From *Grace Chan*

Chapter 1 General introduction

Preamble

The purpose of this study is to explore and gain better understanding on the spatial and temporal variability of phytoplankton and processes, contributing or overriding the variability in Myponga Reservoir, South Australia. Chapter 1 introduces the variability of hydrodynamics and biogeochemistry observed in other lakes and oceans. Implications of phytoplankton variability and processes, leading to the variability are also discussed. Lastly, this chapter outlines the approaches undertaken in the following chapters.

Chapter 1 General introduction

"Lakes, far from being the homogeneous environments we might expect, offer a rich and dynamic heterogeneity at multiple spatial and temporal scales that we are just beginning to understand" (Kratz *et al.* 2005 p.329).

Lake ecosystems are controlled by biotic and abiotic features, leading to considerable variability both within and among lakes. Neighbouring lakes can be substantially different in physical, chemical and biological characteristics, even though the lakes are geographically adjacent to each other sharing similar climate, geologic setting, age, process of origin and catchment characteristics (Kratz *et al.* 2005). Within a lake, morphometric traits affect a range of physiochemical processes, which can provide diverse habitat conditions (e.g. littoral and pelagic zones) to support various biological responses. Heterogeneity may occur at different scales and small gradients are always overlaying a large-scale pattern of heterogeneity (Margalef 1958). Mullin and Brooks (1976 p.784) however argued that "*heterogeneity does not affect the dynamics of the plankton significantly because of integration over time; i.e. the average organism effectively experiences the average environment*". Nevertheless, the study of individual processes at different spatial and temporal scales and the integration of these processes are important to phytoplankton studies, providing insight into elucidation of the heterogeneous nature of phytoplankton ecology.

Phytoplankton in lakes and oceans are generally distributed unevenly (patchily) in space and time and form in clumps and clusters of all shapes and sizes (Folt and Burns 1999; Denman and Dower 2001). Phytoplankton patchiness has been observed and recorded as early as 1768 when Captain James Cook sailed around the world oceans (Bainbridge 1957). The spatial and temporal heterogeneity of phytoplankton is strongly influenced by the interactions of hydrodynamic, physical, geological, chemical and biological processes within the lake and the catchment, which also vary at different scales. Within the lake environment, spatial heterogeneity varies in vertical (e.g. surface mixed layer in the scale of centimetres to metres) and horizontal (e.g. internal wave in the scale of metres to kilometres) aspects. Processes at seasonal scale, daily scale or hourly scale have various impacts on phytoplankton physiology and, in turn, affects the growth, abundance, diversity and distribution of phytoplankton.

This study aims to gain better understanding of the impacts of some physical and geochemical processes on phytoplankton ecology at various spatial and temporal scales. Myponga Reservoir (South Australia) with its deep main basin and shallow side-arm provides a good opportunity to investigate these processes from both vertical and horizontal perspectives, even though the reservoir was subjected to seasonal treatment of copper sulphate algicide in the past and is still seasonally subjected to artificial mixing and aeration.

1.1 Importance of phytoplankton patchiness

An understanding of patchiness is fundamental to an understanding of the aquatic ecosystem as a whole (Martin 2003), as spatial and temporal heterogeneity can strongly influence the ecosystem stability, diversity, dynamics and regional productivity (Steele 1974; Hobson 1988/1989; Brentnall *et al.* 2003). These phytoplankton dynamics, in turn, may be expressed through food chains and may have implications for the global carbon budget. Consideration of phytoplankton patchiness is also important for management because the aggregation of algal blooms can influence the outcome of field monitoring regimes and the development of ecological models.

1.1.1 Food chain

Phytoplankton being primary producers are the base of the food web in aquatic systems. The complex spatial and temporal variability of the environmental factors may lead to the formation of niches for different phytoplankton species (Hillmer *et al.* 2008). Phytoplankton patchiness is particularly important to the abundance, nutrition, composition and distribution of organisms for the next trophic level (e.g. zooplankton); and this subsequently affects the diversity of other higher trophic organisms such as macro-invertebrate and fish (Hutchinson 1961; Mullin and Brooks 1976). For instance, Lake Kinneret in Israel exhibited higher phytoplankton biomass and chlorophyll concentration along the leeward shore of the lake periphery than in the lake centre (Ostrovsky *et al.* 1996). Higher rates of nutrient supply together with better light conditions at the lake periphery led to horizontal distribution of phytoplankton, which also attracted planktivorous fish to the lake periphery (Ostrovsky *et al.* 1996).

1.1.2 Carbon sequestration

Phytoplankton play a major role in global control of atmospheric carbon dioxide and oxygen levels since phytoplankton account for approximately half of the global net primary production (Field *et al.* 1998). Lakes are commonly net sources of carbon dioxide to the atmosphere, while lakes simultaneously bury large among of organic carbon in the lake sediments (Sobek *et al.* 2005; Tranvik *et al.* 2009; van de Waal *et al.* 2010). The rate of storage per unit area of carbon burial in lakes is greater than either the ocean or the terrestrial systems, especially in the time scale of millennium (Cole *et al.* 2007; Tranvik *et al.* 2009). Lakes store about 30 – 60% as much organic carbon per year as the ocean, but the total surface area of lakes are only less than 2% surface area of the sea (Cole *et al.* 2007). The higher areal rates of organic carbon storage in lakes compared to oceans are likely due to greater primary productivity and high carbon burial rates in inland waters (Dean and Gorham 1998).

Phytoplankton impact the carbon storage through effects on fluxes of inorganic and refractory organic carbon to the ocean floor and lakes bottom (Field *et al.* 1998). Diatoms, in particular, sink faster than other groups of phytoplankton due to their heavy silica cell walls and hence have a greater efficiency of carbon sequestration into the deep ocean (Smetacek 1999). Although phytoplankton uptake does not remove carbon permanently, some portions of carbon storage in lake sediments can be sequestered for 1000 to 10,000 years or more, while the carbon storage in terrestrial systems is preserved only for decades to centuries (Cole *et al.* 2007; Lal 2008). Therefore, understanding the variability of phytoplankton production and species composition is important for inferring the variability of the global carbon cycle.

1.1.3 Algal blooms

The variability of phytoplankton abundance, distribution and diversity is important to lake/reservoir authorities to manage the health and aesthetic risks associated with algal blooms, as some phytoplankton can produce toxins and odorous compounds. For instance, higher populations of phytoplankton were found at the shallower lake periphery than in the deeper lake centre due to greater nutrient fluxes from sediments (Ostrovsky *et al.* 1996; MacIntyre *et al.* 1999). These may be hotspots for the development of algal blooms and water authorities can prioritize the in-lake treatment effort in these regions. If high concentrations of localized phytoplankton die, sink and decompose at the lake bottom, a significant amount of dissolved oxygen will be

consumed in the localized areas. This may induce other management challenges such as the development of anoxic sediments in regional areas and the subsequent release of nutrients (Hupfer and Lewandowski 2008). Therefore, the localised concentration may be more important than the basin average (Hillmer *et al.* 2008), and a proper understanding of spatial information is the key for the success of an effective ecological assessment. A sampling regime capturing the heterogeneity specific to the lake can provide more reliable assessments to the composition of the lake (Dutilleul 1993; Hillmer and Imberger 2007) even though the heterogeneity is often responsible for the variability of replicate samples (Margalef 1958). This highlights the significance of understanding the spatial and temporal variability of phytoplankton and the related physiochemical processes.

1.1.4 Development of ecological models

Ecological systems are characterized by diversity, heterogeneity and complexity (Wu and David 2002) and development of ecological models helps to simplify the highly complex and interactive ecosystem into a simpler form. This helps to understand how the ecosystem works and to predict the ecological phenomena. Some theoretical approaches, however, assumed uniform distribution of populations or homogeneous biological processes and disregarded the variability found in nature, not only in large temporal cycles but also in smaller scales of spatial patchiness (Steele 1974; Mullin and Brooks 1976). Models, which are designed for one-dimensional (1D) representation, may not be suitable for a spatially heterogeneous ecosystem because the use of averaged basin values in 1D models may provide misleading results in the presence of localised patches (Hillmer *et al.* 2008). Hillmer *et al.* (2008) further explained that a 1D model may give good representation of the overall lake behaviour (e.g. vertical temperature characteristic), but may not necessarily produce satisfactory results for non-linear ecological responses (e.g. chlorophyll *a* concentration, phytoplankton composition). The discrepancy between the homogenous model outputs and the heterogeneous natural observations could increase when the model simulation is extended in space and time, leading to differences in phytoplankton abundance and composition (Hillmer *et al.* 2008). Therefore, it is important for understanding and characterising the spatial variability of a lake before application of the 1D model for ecological prediction.

1.2 Processes contributing phytoplankton patchiness

Phytoplankton are often recognized as passive particles since their distributions are largely controlled by the movement of water at a continuum of scales (Martin 2003), but yet phytoplankton display considerable patchiness (Bainbridge 1957; Cushing and Tungate 1963). The motion of water is not only responsible for the transport and distribution of phytoplankton, but also allows phytoplankton access to various light and nutrient conditions and contact with grazers, parasites and pathogens. Reynolds (1994) highlighted the spatial and temporal importance of some physical processes impacting upon phytoplankton at different levels of biological responses (Figure 1). For instance, surface irradiance can fluctuate approximately in the time scale of 10 seconds to 1 day, in the vertical space scale of 0.1 m to 50 m, and in the horizontal space scale of 1 m to 100 km. These surface irradiance fluctuations can impact biological responses of phytoplankton (e.g. nutrient uptake and assimilation) in the approximate time scale of 17 minutes to 1 day, in the vertical space scale of 0.1 m to 2 m, and in the horizontal space scale of 1 m to 500 m. Meanwhile, the surface irradiance fluctuations can affect phytoplankton photosynthesis and photoinhibition in the greater spatial and temporal variations (time scale of 1.7 minutes to 1 day, vertical space scale of 0.1 m to 5 m, and horizontal space scale of 1 m to 1000 m).

In addition to physical processes, phytoplankton patchiness becomes more apparent with the presence of a ‘seed’ heterogeneity or external ‘forcing’ such as localized nutrient flux to trigger phytoplankton growth, vertical migration via buoyancy regulation and active swimming to obtain adequate light and nutrient resources, large-scale temperature gradients, or spatial variability in growth and grazing (Martin 2003). Such external ‘forcing’ and physical processes are often accompanied with daily or seasonal cycles, contributing to the biogeochemical variability at different scales. Table 1 summarizes the temporal and spatial variability of lake processes that are discussed in the following sections.

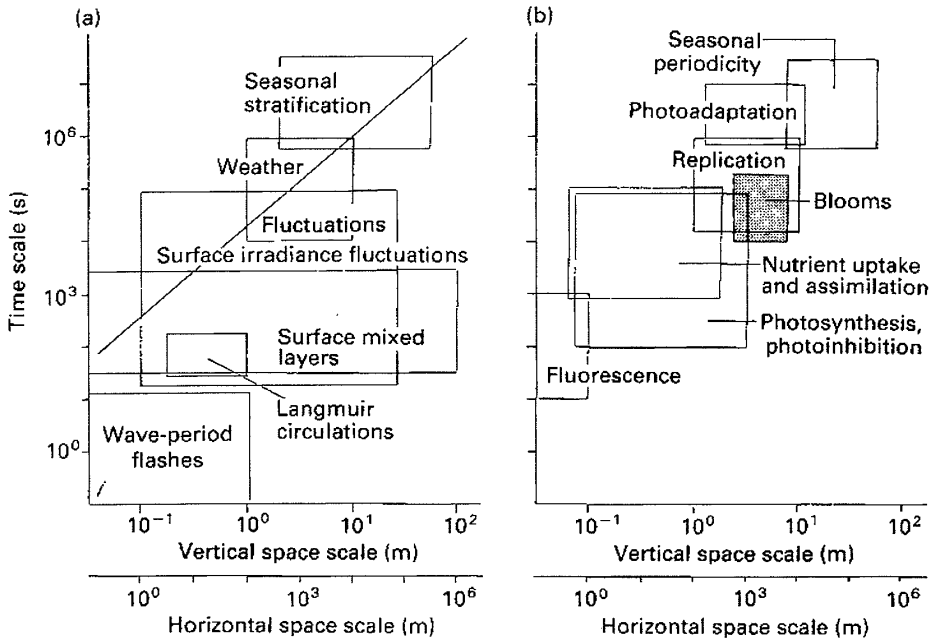

Figure 1 Temporal and spatial scales of (a) lake processes impacting upon phytoplankton and (b) the levels of biological responses (Reynolds 1994, Fig. 6.1)

Table 1 The impacts of lake processes in temporal and spatial scales

Lake processes	Impacts	Temporal scales	Spatial scales
Vertical light attenuation	- Underwater light intensity	Diurnal and seasonal, cloud cover	Surface and deep water, turbid areas
Surface mixing	- Transport of phytoplankton, hence phytoplankton light exposure	Seconds to minutes	Wind-exposed areas
River intrusion	- Quality and quantity of nutrients - Turbidity, hence light availability	Days to months, rain events	Inlets of lakes
Sediment resuspension	- Nutrient availability - Light availability	Minutes to hours, storm events	Shallow areas of lakes, littoral zones
Lateral convection	- Transport of nutrients, plankton and matters	Hours to days	Between shallow and deep areas
Thermal stratification	- Vertical exchanges of gases and nutrients	Weeks to months (seasonal)	Deep areas of lakes, pelagic zones
	- Vertical movement of plankton	Hours to days (diurnal)	Surface water of deep and shallow areas
Sediment nutrient fluxes during anoxia	- Nutrient release	Hours to weeks	Anoxic sediments
Zooplankton grazing	- Nutrient recycling	Seconds to hours	Food-abundant areas
	- Phytoplankton abundance and composition	Weeks	

1.2.1 Light

Light is the key component for the survival of photosynthetic organisms. Underwater light is strongly influenced by light attenuation and turbidity (e.g. sediments, phytoplankton cells, dissolved organic compounds), leading to a distinct vertical gradient in light intensity and spectral distribution (Wetzel 2001). Underwater light irradiance decreases logarithmically with water depth due to surface reflection, light adsorption and scattering by water and dissolved and suspended matter (Kimmel *et al.* 1990). The vertical gradient of light is important to primary productivity, as photosynthesis can only take place within an euphotic zone, which receives at least 1% surface irradiance and has a greater photosynthesis rate than respiration rate (Kalff 2002). In the broad range of the light spectrum, photosynthetic organisms utilize only a fraction of waveband from 350 to 700 nm, which is called photosynthetic active radiation (PAR). Different phytoplankton groups absorb different ranges of PAR, based on their composition of light-absorbing pigments. For instance, carotenoid and fucoxanthin pigments of diatoms mainly absorb 500 – 550 nm wavelength of light, whereas phycocyanin pigments of cyanobacteria absorb substantial light in the 600 – 650 nm waveband (Kirk 1977). Differences in the use of different light spectrum may provide opportunities for the coexistence of diverse phytoplankton species (Stomp *et al.* 2004).

The photosynthetic responses of primary producers are influenced by the time scale of change in the light field (Figure 2). Falkowski (1984) pointed out that some time scales were important to photosynthetic processes (e.g. photosynthetic oxidation-reduction reactions in the time scales of millisecond to picoseconds of the light field), but not necessarily reflected in physiological responses. The more relevant time scale of change in light fields to phytoplankton ecology starts from the order of 10^{-1} s, which is so-called 'flicker' effect induced by the wave action at water surface, affecting the photosynthetic efficiencies and assimilation of carbon (Walsh and Legendre 1983; Falkowski 1984). Light fluctuations caused by passing clouds falls into the order of 10^{0} to 10^{2} s, influencing phytoplankton chlorophyll *a* fluorescence and inorganic carbon uptake (Gallegos *et al.* 1980; Abbott *et al.* 1982). Not only phytoplankton physiology (e.g. carbon fixation, cellular pigment content, chlorophyll fluorescence, etc.) is affected by the diel time scale (~ 10^{4} s), the storm-related time scale (10^{5} – 10^{6} s) and the seasonal time scale (> 10^{6} s) of the change in light field, but also these longer time

scales alter phytoplankton productivity, species composition and succession (Falkowski 1984).

Diurnal variations of solar radiation are the important time scale in phytoplankton ecology. Diurnal variation of light is determined by the solar elevation, which rises during the day from its minimum value at dawn to its maximum value at noon and then diminishes in a symmetrical manner to its minimum value at dusk (Kirk 1983). Some motile/buoyant phytoplankton species are responsive to the diurnal change in light, such as dinoflagellate *Gymnodinium splendens* exhibited diurnal vertical migrations, swimming downward before the dark period began and upward before the end of the dark period (Cullen and Horrigan 1981); cyanobacterium *Microcystis aeruginosa* decreased buoyancy during the day and increased at night (Ibelings *et al.* 1991).

The amount of light received by phytoplankton is also largely controlled by the movement of water. Surface mixing entrains phytoplankton cells and transports them vertically along the water column (Imberger and Patterson 1990). Deep mixing can carry the entrained cells to the depth below the euphotic zone, reducing the overall light dose received by the cells. Conversely, shallow mixing retains the entrained phytoplankton cells within the productive light zone. Consequently, the degree of mixing can affect the rates of photosynthesis and growth of phytoplankton and finally the selection of phytoplankton species and community (e.g. Kirk 1983; MacIntyre 1993; Reynolds 1994). The depths of surface mixed layer can also vary spatially in different parts of a lake because of the differences in wind exposure, lake bathymetry and stratification (Spigel and Imberger 1980; Imberger and Parker 1985; Sherman *et al.* 2000). For example, variable deepening of the mixed layer was induced by a sudden increase in wind speed in the open central areas of Wellington Reservoir (Western Australia), with only minimal mixing in the sheltered parts of the lake (Imberger and Parker 1985). Phytoplankton in different parts of the lake therefore may experience different light exposure, while these changes (i.e. wind speed, mixing depth and light exposure) are at a relatively short time scale varying from minutes to seconds.

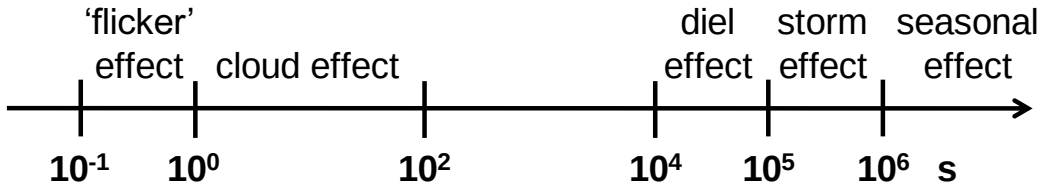

Figure 2 Time scale (in seconds) of change in quality and quantity of light fields relevant to phytoplankton physiology (adopted from Falkowski 1984)

1.2.2 Nutrients

Phytoplankton obtain a range of macro- and micro-nutrients from the surrounding environment and usually require a relatively short period of time for nutrient uptake (e.g. minutes), for carbon fixation (e.g. hours) and for a growth cycle (e.g. hours) (Reynolds 1994). Nitrogen and phosphorus (and silicon too for diatom species) are the typical limiting nutrients influencing the primary productivity (Reynolds 1984). Nutrients occur in many different forms and only bio-available forms such as nitrate, nitrite, ammonia and orthophosphate can be utilised directly by phytoplankton. Other forms of nutrients, however, can become bio-available through desorption, dissolution and biomass turnover (Scheffer 1998). Nutrients in a waterbody may originate from weathering of bedrock, atmospheric precipitation, terrestrial input, stormwater runoff, sewage effluent and agricultural discharge. Organic and inorganic nutrients settled onto lake sediments can return to the water by means of sediment resuspension, decomposition and recycling of organic matter and fluxes of nutrients under anoxic conditions. Both external and internal nutrient loadings varying in space and time significantly contribute to spatial and temporal variability of phytoplankton patchiness.

1.2.2.1 External sources of nutrients from catchment

In many lakes and reservoirs, a substantial portion of nutrients may come from the catchment and tributaries. The catchment loading affects the water quality of downstream lake in the time scale of days to months. External loading from diffuse sources is often associated with rainfall and the subsequent flow events (Linden *et al.* 2004). While loading from point sources is generally independent to the flow of river (Bowes *et al.* 2008). Typically, the concentration of point load in the river decreases with increasing river flow due to the dilution of a relatively constant input, whereas diffuse (non-point) load usually increases with river flow (Bowes *et al.* 2008; Gozzard

et al. 2011). Rivers flowing into a lake may be of different temperatures and may contain different concentrations of total dissolved solids and suspended solids (Ford 1990). The density of the inflow water regulates the depth at which the inflow water penetrates into the lake (Kratz *et al.* 2005) and can further promote vertical variability. The warm buoyant inflow water travels above the lake water, while the cold dense inflow water moves along the sediment bed (Ford 1990). If the turbulence is not strong enough to disperse the inflow water to the lake water, fine-scale layering of inflow water may form causing vertical, horizontal and temporal heterogeneity in lakes (Kratz *et al.* 2005). These horizontal density currents caused by river intrusion not only transport nutrients to different parts of the lake, but also carry heavy metals, pathogens and other contaminants (e.g. Knauer *et al.* 2000; Simek *et al.* 2001; Brookes *et al.* 2004). This is of particular concern to small drinking water reservoirs, which have relatively short water retention time. The associated risk is that pollutants from the river intrusion can potentially travel to an off-take site (Hobson *et al.* 2010) and take up to a water treatment plant increasing the health risk and treatment cost.

1.2.2.2 Internal sources of nutrients

Different physiochemical processes occurring in different parts of a lake at different time scale may contribute to spatial and temporal variation of nutrient dynamics. Sediment resuspension induced by wind and wave actions is often observed in the shallow region of the lake and at the lake boundary (e.g. Kristensen *et al.* 1992; Bloesch 1995; Ostrovsky *et al.* 1996) in the time scale of minutes to hours. The resuspension is influenced by wind speed, prevailing wind directions, surrounding topography and size, depth and morphometry of the specific lakes (Bloesch 1995). Wind-induced resuspension was observed in the shallow regions of the aquatic systems (1 – 6.5 m) when the wind speed exceeded 3 – 4 m s^{-1} (Demers *et al.* 1987; Arfi and Bouvy 1995). Sediment resuspension induced by wind and wave activities not only affects the dynamics of dissolved and particular nutrients in the water column (Martinova 1993; Niemisto *et al.* 2008), but also can temporarily alter the light field of the water column (Schallenberg and Burns 2004), leading to variations in phytoplankton biomass and composition (Carrick *et al.* 1993; Hamilton and Mitchell 1997; Ogilvie and Mitchell 1998; Schallenberg and Burns 2004).

Repetitive sediment resuspension provides regular replenishment of nutrients in the shallow areas of lakes, while lateral movement of water (e.g. convective circulation

induced by differential heating and cooling) may help to disperse the suspended matters to the deeper areas (James *et al.* 1994; MacIntyre and Melack 1995) in the time scale of hours to days. If the rates of local biogeochemical reactions outpace the rate of lateral mixing, the spatial and temporal distribution of vertical mixing events can promote ecological patchiness and, in turn, influence the lake-wide primary productivity (Besiktepe *et al.* 2003; Yeates *et al.* 2008). Sediment focusing and redistribution processes (Hilton 1985; Hilton *et al.* 1986) further contribute to spatial dynamics of geochemistry in lakes.

In the deeper region of a lake, sediment nutrient release induced by anoxia can also be an important releasing mechanism, particularly during summer stratification. Water temperature gradients offer considerable resistance to wind mixing and physically separate the lower layer of water (hypolimnion) from the upper layer of water (epilimnion), preventing the replenishment of dissolved oxygen in the hypolimnion (Mortimer 1941). Furthermore, microbial decomposition of organic matter enhances the consumption of dissolved oxygen in the hypolimnion and potentially leads to anoxia at the sediment-water interface (Kisand and Noges 2003). In temperate lakes, typical anoxic hypolimnion may take days and weeks to develop and may maintain over the summer season over a few months. While the development of an anoxic microlayer at the sediment-water interface may take a few hours and days, the anoxic microlayer may only maintain over a relatively short period of time at scales of minutes rather than days or weeks (Coloso *et al.* 2011). Under anoxic conditions, chemical reductions take place at the sediment-water interface and release the redox-sensitive nutrients from sediments such as iron-bound phosphate, ammonium ion, iron (II) ion and manganese (II) ion (e.g. Adams *et al.* 1982; Baldwin and Williams 2007). The nutrient-rich hypolimnetic water can be bought to the surface water during seasonal overturn, storm-related strong mixing, river intrusion and lateral convective circulation (Ford 1990; Davison 1993; Robarts *et al.* 1998; Wells and Sherman 2001) at various temporal and spatial scales; and the nutrients become readily available for phytoplankton uptake.

1.2.3 Grazing pressure

Zooplankton, like phytoplankton, exhibit patchiness at all scales (Martin 2003), but they are not patchy in the same way (Denman and Dower 2001). Zooplankton patchiness is not only influenced by physical processes (e.g. turbulence, currents and eddies) at large scales between 10 m and 1 km (Pinel-Alloul 1995), but also is controlled by behavioural

responses with greater ability for swimming and swarming (e.g. diel vertical migration, locating food patches, finding mates and predator avoidance) at smaller scales between 1 mm and 10 m (Folt and Burns 1999; Denman and Dower 2001). High food (phytoplankton) concentration is a strong driver of zooplankton patchiness, which in turn, can strongly influence the phytoplankton patchiness such as abundance and distributions (Therriault and Platt 1978; Denman and Dower 2001).

Filter feeding zooplankton is the major consumer of phytoplankton in freshwater systems. The filter feeders are very efficient at grazing on small, individual green algal and diatom cells, resulting in a low algal biomass and a clear-water phase in spring (Scheffer 1998). However, many cyanobacterial species form colonies such as clumps of *Microcystis*, filamentous aggregates of *Anabaena* and large filamentous benthic mats of *Oscillatoria*. These colonies are too large to be edible by zooplankton (Haney 1987; Boing *et al.* 1998) and hence phytoplankton composition can be altered over time. In addition, zooplankton grazing also releases a significant amount of unused nutrients influencing the nutrient availability for phytoplankton uptake (Sterner 1989; Sterner *et al.* 1992). Although zooplankton grazing can be an important factor, affecting the spatial and temporal variability of phytoplankton, the impact of zooplankton grazing were not included in the current study since it focused primarily on the impacts of hydrodynamic, physical and geochemical processes.

1.3 The project

Within an aquatic ecosystem, phytoplankton abundance and distribution appear to be patchy in various spatial and temporal scales. The patchiness can vary vertically along the water column from centimetres to metres and horizontally across the lake from metres to kilometres at different time scales, ranging from minutes, hours and days to weeks, seasons and years. A number of hydrodynamic, physical, geochemical and biological processes giving rise to the patchiness of phytoplankton are also highly variable in space and time and have various impacts on phytoplankton physiology, growth and diversity. Some processes may not be measurable at sufficient frequency or have detectable magnitude change above the background, but the impact of the processes may still be biologically significant. Understanding the spatial and temporal scales of changes in these processes is important to the understanding of phytoplankton dynamics.

This project aims to determine processes that may lead to phytoplankton patchiness and how these processes vary in space and time. The overall objective is to investigate the spatial and temporal variability of sediment composition, sediment nutrient flux, thermal stratification, surface mixing and underwater light climate, which could lead to the spatial and temporal dynamics of phytoplankton in Myponga Reservoir, South Australia. The spatial feature includes vertical (along the water column) and horizontal (across the reservoir) aspects, while the temporal characteristic refers to short-term (hourly, diurnally) and long-term (seasonally, yearly) fluctuations. The phytoplankton dynamics were also assessed at different levels, ranging from physiological responses of phytoplankton cells (e.g. maximal quantum yield of photosystem II, cellular stoichiometry) to community successions (e.g. phytoplankton biomass, composition and diversity).

Nutrient availability is an important factor causing spatial variability of phytoplankton. Sediment is considered as the sink and source of nutrients. An understanding of the sediment nutrient composition can therefore provide insight into the overall spatial heterogeneity in Myponga Reservoir. A sediment survey across both shallow and deep areas of the reservoir was undertaken to determine the nutrient composition, nutrient stoichiometry and particle size composition of the surface sediments (Chapter 3). A number of physiochemical and biological processes can affect the sediment nutrient composition and also the release of the sediment nutrients. For example, the onset of thermal stratification preventing vertical circulation of water can potentially lead to the depletion of dissolved oxygen at the sediment-water interface and subsequently the release of sediment nutrients. Artificial destratification systems in Myponga Reservoir helped to prevent the formation of the strong seasonal stratification (Brookes *et al.* 2000), however micro-stratification at the sediment-water interface may still be present. This leads to the detailed examination of the high resolution water temperature data collected continuously along the water column and across the reservoir in order to assess the frequency, intensity and duration of the occurrence of micro-stratification at the reservoir bed (Chapter 4). If the micro-stratification occurred long enough to cause the depletion of dissolved oxygen at the sediment-water interface, nutrient fluxes from anoxic sediments would occur. Experiments on sediment nutrient fluxes under anoxic conditions were conducted in laboratory-controlled environment. The flux rate of sediment phosphorus from the experiment was used to approximate the enhancement of cyanobacterial population due to additional phosphorus flux from anoxic sediments and

the potential impacts on the secondary productions of toxic and odorous compounds (Chapter 4).

The variations of basin morphometry and geographic setting between the side-arm (relatively shallow and sheltered) and main basin (relatively deep and open) may offer different habitat conditions (e.g. differences in surface mixed layer depths) for differential growth of phytoplankton. Modelling of the light dose received by phytoplankton under the changing surface mixed layer depths was used to estimate the phytoplankton populations for both the side-arm and main basin. *In situ* phytoplankton assemblages from the two sites were collected weekly for the warm period and monthly for the cold period over 3 years. The phytoplankton assemblages were identified and compared with the modelled population (Chapter 5). Meanwhile, the vertical mixing, leading to the potential light impact on phytoplankton population can be overcome by other hydrological processes such as lateral convective motion driven by differential heating and cooling. The water temperature profiles collected from the two sites were used to determine any lateral convective movement of water between the two sites, which may have an impact on the overall distribution of phytoplankton (Chapter 5).

Study of cellular content and stoichiometry of phytoplankton can provide information on phytoplankton nutrient status and reveal the resource availability in the ambient environment (i.e. the potential habitat variations between the side-arm and main basin). Phytoplankton cellular content and stoichiometry may also vary seasonally when the dissolved nutrients slowly deplete from the water over a growing season and without replenishment of nutrients from the catchment. To better understand the spatial and temporal dynamics of phytoplankton, a biweekly monitoring survey was conducted to collect phytoplankton assemblages at the side-arm and main basin over the summer, autumn and early winter periods (January – July) and to measure the cellular carbon, nitrogen, phosphorus and chlorophyll contents of the phytoplankton assemblages (Chapter 6).

Underwater light and nutrient conditions can rapidly vary in vertical and horizontal scales within the reservoir, while biological responses of phytoplankton can also display differently in physiology and growth. Bioassays using *in situ* phytoplankton assemblages were conducted in the shallow and deep sites to determine any spatial variations in nutrient conditions. This was achieved by suspending the semi-permeable

membrane bottles in the two sites over a period of days. Concurrently, the sealed bottle experiments were conducted with different nutrient conditions (added nitrogen, added phosphorus, added nitrogen and phosphorus and no additional nutrients). Both semi-permeable membrane bottle and sealed bottle experiments were suspended at various depths of the water column to achieve different underwater light conditions (80%, 50% and 10% surface irradiance). The measurements of phytoplankton photosynthetic activity and phytoplankton biomass may reveal any minor fluctuation of nutrients between the shallow and deep sites and the effects of phytoplankton physiology and growth on light- and nutrient-limiting conditions (Chapter 7).

Chapter 2 Study site

Preamble

Chapter 2 provides relevant information on the study area, Myponga Reservoir and the surrounding catchment. This chapter also highlights the past water quality and the management history of the reservoir. The historical water quality data were kindly provided by SA Water.

Chapter 2 Study site

2.1 Myponga catchment

Myponga Reservoir water is entirely fed by rivers and creeks from its own catchment area of approximately 124 km^2 (Figure 3). About 96% total catchment water, which is predominantly from the Myponga River, contributes to the elongated side-arm of the north-eastern part of the reservoir. The remaining 4% catchment water from the surrounding creeks flows directly into the main basin of the reservoir (Linden *et al.* 2004). Under the prevailing Mediterranean climate, the area receives mainly winter rainfall with an annual average of 742 mm, which forms approximately 332 mm of runoff and 420 mm of evaporation per year (Australian Natural Resources Atlas 2007). The catchment area varies from a high peak (Myponga Hill at 440 m), to steep slopes and broad flat floodplains (Thomas *et al.* 1999).

Approximately 90% native vegetation has been cleared in the catchment since European settlement. A recent land use survey in the Myponga catchment suggested 48.7% land is used for broadscale grazing and 20.7% for intensive grazing (Bradford *et al.* 2008). The unfenced livestock grazing activities significantly increase erosion on hill slopes, streambanks and gullies, leading to high sediment deposition (1482 t y^{-1}) into the watercourse (Australian Natural Resources Atlas 2007). Paddock runoff not only transports a large quantity of loose soil, but also a substantial amount of nutrients, organic matter, pathogens and animal faeces into the water and continuously degrades the water quality (Bradford *et al.* 2008). These are the sources of non-point diffuse pollution into the watercourse.

Hill slope, streambank and gully erosions as well as paddock runoff that transport a quantity of sediments, organic matters and animal faeces into the Myponga River continuously degrade the river water quality. In fact, the major pathway of nutrients introduced to the Myponga River is the throughflow (horizontal movement of water) in the soil subsurface. A detailed study of the movement of phosphorus (P), nitrate (NO_3) and dissolved organic carbon (DOC) in Myponga catchment soils concluded that the soil type, the soil texture contrast, the soil porosity and the quantity of clay were the determining factors for the water movement in soil along with P, NO_3 and DOC movement (Stevens *et al.* 1999).

Stevens *et al.* (1999) found that there was a strong texture contrast between the surface and subsurface soils in Myponga catchment. The A-horizon soil (40 – 65 cm deep) was comprised of coarse loamy sand or sandy loam with a high hydraulic conductivity of 2.6 m d^{-1}. On the contrary, the underneath B-horizon soil (60 – 140 cm deep) was heavy clay sodic soil with a very low hydraulic conductivity of 3.3×10^{-5} m d^{-1} (Stevens *et al.* 1999). The hydraulic conductivity provides a strong evidence that the rain/irrigated water leached down quickly to the sandy subsurface. The preferred flow path of the water in the soil column of Myponga catchment would be throughflow from sandy A-horizon, then clayey B-horizon and little overland flow (Stevens *et al.* 1999). The soil water movement is of particular concern in terms of the nutrient transport from land to water.

DOC and NO_3 are readily dissolved in water and leached through the soil profile and along the preferred pathway of A-horizon and B-horizon respectively. Stevens *et al.* (1999) also discovered little dissolved phosphorus (0.01 – 0.02 mg L^{-1}) in the overland and subsurface flows in Myponga catchment. But larger amount of phosphorus was transported as particulate phosphorus due to the dispersive characteristics of sodic soil in B-horizon. The dissolved and particulate nutrients from overland runoffs and subsurface flows of the catchment moved to the downstream creeks and rivers. The relatively nutrient-enriched water (0.134 – 0.31 mg TP L^{-1}) eventually reached the Myponga Reservoir, affecting the raw water quality (Stevens *et al.* 1999). This was also observed and recorded by Linden *et al*. (2004), suggesting that average annual reservoir loads of total phosphorus (TP) and total Kjeldahl nitrogen (TKN) were highly correlated to the annual creek flow.

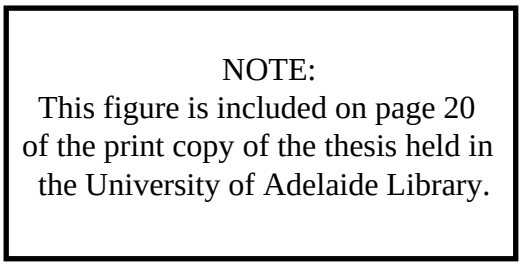

Figure 3 A map of Myponga Reservoir catchment (Bradford *et al.* 2008)

2.2 Myponga Reservoir

Myponga Reservoir (S 35°24', E 138°25') is located on the Fleurieu Peninsula approximately 60 km south of Adelaide, South Australia (Figure 3). The construction of Myponga Reservoir started in 1957 and completed in 1962 and it is now the main water supply to about 50,000 people in the region of central and southern Fleurieu Peninsula (Bradford *et al.* 2008). In the northwest of the reservoir, the concrete arch dam wall with a ski-jump spillway is 49 m tall and 226 m long. The reservoir stores up to 26,800 ML of water with a surface area of 280 ha at its full capacity. It has a maximum depth of 36 m near the dam wall and 12 m in the side-arm. The average water retention time is approximately three years, based on the extraction of water from the dam wall off-take and the reservoir storage capacity (Lewis *et al.* 2002; Brookes *et al.* 2004).

The water quality of Myponga Reservoir has been monitored regularly by the water authority and many of these physiochemical parameters display seasonal fluctuations (Table 2 and Figure 4). The surface water temperature usually rises above 20ºC in summer and falls just below 10ºC in winter. The surface water is well oxygenated with a higher dissolved oxygen level in the cold water and a relatively lower level in the

warm water. Myponga water is highly discoloured (brown) due to the relatively high concentrations of dissolved organic carbon that originates from catchment (~ 12 mg L^{-1} on average), but is relatively low in turbidity (~ 3 NTU on average). Occasionally, turbidity levels can increase up to 30 NTU, often coinciding with the outbreaks of *Anabaena* blooms. The total and particulate nutrient levels peak in response to the winter rain and fall gradually over summer.

Figure 4 Historical surface water quality data at Loc 1 (main basin) of Myponga Reservoir (source of data: SA Water)

Table 2 General characteristics and limnological variables of Myponga Reservoir (source of data: SA Water)

General characteristics of Myponga Reservoir	
Position	S 35˚24´, 138˚25´E
Trophic status	Mesotrophic
Surface area (km^2)	2.8
Volume (ML)	26 800
Mean depth (m)	15
Maximum depth (m)	36 (main basin), 12 (side-arm)
Water retention time (years)	3
Catchment area (km^2)	124
Climate	Mediterranean
Average annual rainfall (mm)	742
Average annual runoff (mm)	332
Average annual evaporation (mm)	420

Limnological variables of Myponga Reservoir		
Surface measurements collected at Loc 1 (main basin)	**Mean**	**Min / Max**
Water temperature, WT (°C) 7/1/2002 – 29/6/2009	17.4	7 / 26
Dissolved oxygen, DO (mg L^{-1}) 7/1/2002 – 22/6/2009	9.9	4.5 / 15
Turbidity, Turb (NTU) 7/1/2002 – 22/6/2009	3	0.3 / 32
Secchi depth, SD (m) 22/5/2006 – 30/3/2009	2.2	0.5 / 4
Dissolved organic carbon, DOC (mg L^{-1}) 15/12/1997 – 14/12/2009	11.92	9 / 16.8
Ammonia, NH_3 (mg L^{-1}) 23/8/1984 – 29/12/2009	0.032	0.005 / 0.256
Nitrate and Nitrite, NO_x (mg L^{-1}) 23/8/1984 – 29/12/2009	0.104	0.005 / 0.38
Total Kjeldahl nitrogen, TKN (mg L^{-1}) 23/8/1984 – 14/12/2009	1.047	0.05 / 3.26
Total nitrogen (NO_x+TKN), TN (mg L^{-1}) 23/8/1984 – 14/12/2009	1.144	0.126 / 3.268
Filterable reactive phosphorus, FRP (mg L^{-1}) 23/8/1984 – 29/12/2009	0.02	0.005 / 0.203
Total phosphorus, TP (mg L^{-1}) 23/8/1984 – 29/12/2009	0.057	0.005 / 0.43
Chlorophyll *a*, Chl*a* (μg L^{-1}) 28/11/1997 – 29/12/2009	7.8	0.4 / 138
Anabaena circinalis (cell mL^{-1}) 1/1/2000 – 16/3/2009	4241	0 / 288000

2.3 Water quality management in Myponga Reservoir

During the warm season, occasional blooms of *Anabaena circinalis* degrade water quality by the release of cyanotoxins and odorous compounds requiring additional water treatment. There is a range of lake management strategies in Myponga Reservoir to combat the excessive growth of phytoplankton and also to control the release of

contaminants from the sediments. The use of algicide has a long history to control the outbreaks of algal blooms in Australian reservoirs. The algicide copper sulphate ($CuSO_4$) at a dose rate of 2 mg L^{-1} was used as in-lake management since 1963 (Welk 2007). The dosing frequency was on an as-needed basis before the concentration of *Anabaena circinalis* reached 1000 – 2000 cell mL^{-1}.

Along with the routine practice of copper sulphate dosing for almost every summer, a 200 m multidiffuser aerator with 160 diffusion outlets was installed at the reservoir bottom near the dam wall in June 1993. It ejects air bubbles at a rate of 120 L s^{-1} supplying oxygen to the deep reservoir bottom (Lewis *et al.* 2003). At the end of 1999, two giant surface mixers (4.9 m diameter of mixer blades and 13 m long of draft tube) were installed in the deep sites of the main basin. Each mixer pumps the surface water down to 14 m at 3500 L s^{-1} (Lewis *et al.* 2003). Both aerator and mixers operate throughout late spring and early autumn (September – March), but not during the period of algicide treatment, when the deep parts of the reservoir are susceptible to thermal stratification.

The artificial destratification units have been shown to break down the strong seasonal thermocline in the deep parts of Myponga Reservoir, where the aerator and mixers operate and to reduce the release of iron and manganese from sediments (Brookes *et al.* 2000). One surface mixer near the dam wall experienced technical problems and stopped functioning since March 2006. At the same time, the water authority has eased the use of algicide in Myponga Reservoir. Only the bubble aerator and one mixer are currently in place over the warm seasons as the in-lake management to control the release of contaminants from sediments and to certain extent to minimize the growth of nuisance cyanobacteria. In addition, the control of the depth of the off-take is a reactive strategy to selectively avoid the in-take of the surface scum to the water treatment plant. Other long-term lake restoration practices such as fencing and revegetation along the watercourses of the catchment were reported to reduce the sediment loads (thus nutrients) by 12% each year (Bradford *et al.* 2008) and may reduce the frequency and intensity of algal blooms.

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Chapter 3 Spatial dynamics of sediment nutrient content and stoichiometry (TC:TN:TP)

Preamble

Chapter 3 presents a survey of spatial distribution of sediment characteristics and nutrient composition in the surface sediments of Myponga Reservoir and discusses the potential causes, leading to the spatial variability. In the context of the study, the nutrient dynamics of sediments are highly interactive with the nutrient dynamics of the overlying water, which can impact the dynamics of phytoplankton.

To the best of my knowledge, this detailed sediment survey is the first time conducted in Myponga Reservoir. With guidance from supervisors, I was responsible for the design of the survey, the data collection, the chemical analysis as stated in the methods, the statistical analysis and the data interpretation.

Chapter 3 Spatial dynamics of sediment nutrient content and stoichiometry (TC:TN:TP)

3.1 Introduction

Lake sediment is an important component in the lake ecosystem acting as a sink and/or source of nutrients. Seasonal riverine intrusions bring a variety of contaminants such as sediments, fertilizers, pesticides, manures, terrestrial vegetation and domestic and industrial wastes from upstream catchments to downstream lakes. Sediments can act as a buffering system for the overlying water, retaining allochthonous and autochthonous nutrients. Sediments in Lake Taihu, China, for example, accumulated about 65% total phosphorus input via adsorption, precipitation and physical sedimentation of particulate matter (Kelderman *et al.* 2005). Similarly, significant amounts of organic matter produced by phytoplankton were reported to settle on the sediment surface of Lake Arreskov, Demark, where phosphorus and nitrogen sedimentations were respectively 2 – 5 and 1.5 – 3 times the annual external loading (Andersen and Jensen 1992) .

There are many sediment distribution processes, giving rise to spatial variability of sediment and nutrient composition. Hilton *et al.* (1986) summarised many of these processes causing sediment focussing, which describes the occurrence of greater sediment accumulation in the deep rather than shallow part of lakes (Likens and Davis 1975). In particular, seasonal riverine intrusion processes such as riverine delta formation and riverine plume sedimentation cause the accumulation of large and heavy sediment particles at the mouth of a river, where energy is gradually lost through mixing of river water with lake water and the transport of fine suspending sediment particles (Hilton *et al.* 1986). Other processes such as slumping and sliding on slopes and peripheral wave action are likely to influence the sediment distribution throughout the year.

Sediment resuspension is governed by waves and currents, water content, particle size and bioturbation (Bloesch 1995; Noges and Kisand 1999). Wind-wave resuspension is the dominant mechanism for nutrient release from shallow aerobic sediments and these resuspended nutrients directly supply the euphotic zone (Hamilton and Mitchell 1997). In laboratory simulations of sediment resuspension, large amount of soluble reactive phosphate was released from sediments; such release was primarily dependent on the

equilibrium conditions of the soluble reactive phosphate in the water column but basically independent of the increase in suspended solids and the duration of resuspension (Sondergaard *et al.* 1992). Hamilton and Mitchell (1997) also observed that *in situ* resuspension events increase the concentration of suspended solids in shallow lakes, resulting in a decrease of the total nitrogen to total phosphorus ratio in water. However, such events had little influence on the concentration of dissolved nutrients in water. The resuspended bio-available phosphorus may be rapidly taken up by phytoplankton and also re-adsorbed onto the resuspended fine-grained particles (Hamilton and Mitchell 1997).

The release of sediment nutrients is also associated with oxygen conditions near the water-sediment interface. Low redox potential (below +200 mV) and low dissolved oxygen (below 4 mg L^{-1}) can be induced by high organic matter decomposition on the sediments and strong thermal stratification in the water column, which prohibits gas transfer to the sediments to meet the oxygen demand (Mattson and Likens 1993). Mobilization of sorbed phosphorus from iron (III) complex occurs under anoxic conditions, which reduce iron (III) compounds to iron (II) ions and release phosphate (Sondergaard *et al.* 2003). Concurrently, anoxic sediments enhance the reduction of nitrate to nitrogen gas via denitrification (Andersen and Jensen 1992) and the degradation of organic matter to methane gas via methanogenesis (Segers 1998; Wetzel 2001; Laanbroek 2009). The fluxes of nutrients in the nutrient-rich bottom water, however, require a physical mechanism (e.g. autumn turnover) to transport to the surface water.

Both nutrient fluxes from sediment resuspension and anoxic sediments are important sources of internal nutrient load, especially when external nutrient load is minimal. In many lakes, the persistent internal loading of phosphorus from sediments delays the restoration of eutrophication after the reduction of external phosphorus loading (Marsden 1989; Kleeberg and Kozerski 1997). For instance, the net internal phosphorus loading in shallow eutrophic Danish lakes was estimated to persist for 15 years after reduced external phosphorus loading (Sondergaard *et al.* 1999). The release of sediment nutrients and the recycling of nutrients in epilimnetic water are able to support a substantial growth of phytoplankton when other conditions (e.g. light and temperature) are met. Since different parts of the lake (i.e. shallow and deep) are subject to different

release mechanisms, the nutrient fluxes are expected to vary across the lake, which in turn, may contribute to the variability of phytoplankton nutrient content.

The processes of sediment nutrient recycling have been reported in many individual shallow and deep lakes (e.g. Lebo and Reuter 1995; Kleeberg and Kozerski 1997; Eckert *et al.* 2003; Krogerus and Ekholm 2003). However, although many lakes have both shallow and deep regions, relatively few reports discuss the relative composition of nutrients and the contribution to the water column from both shallow and deep regions of a single lake (e.g. Jones and Simon 1981; Kumke *et al.* 2005). Furthermore, most studies mainly focus on sediment phosphorus dynamics (Noges and Kisand 1999; Shilla *et al.* 2009) and occasionally include sediment nitrogen regeneration (Hamilton and Mitchell 1997; Niemisto *et al.* 2008). Relatively little has been reported on the composition of carbon, nitrogen and phosphorus in sediments although there are works on this topic (Andersen and Jensen 1992; Trolle *et al.* 2009). The aim of the current study was to examine the relative proportions of sediment carbon, nitrogen and phosphorus across shallow and deep regions of Myponga Reservoir, South Australia and to provide some possible explanations for the spatial variation, based on the existing knowledge from individual shallow and deep lakes.

3.2 Methods

3.2.1 Sediment sampling and analysis

Lake sediment samples (triplicates) were collected on 18 – 19 August 2008 along a transect of 8 sites across both shallow and deep areas of Myponga Reservoir (Figure 5 and Table 3). The sediment cores had an average depth of 6 cm. The depths of the cores depended on the sediment composition at the particular site; coarse sandy sediments were more difficult to recover than fine organic mud. A modified Hongve sampler (Wright 1990), made of heavy stainless steel tube with an internal diameter of 59 mm and an external diameter of 61 mm was used to sample sediments. The degree of sediment disturbance caused by the Hongve sampler is estimated by the Hvorslev ratio (Terzaghi and Peck 1967). To prevent excessive compaction of the sediments during coring, the cross-sectional area of the sampler wall should be less than 20% cross-sectional sampling area (Terzaghi and Peck 1967). In the case of our sampler, this calculation suggested a minimal disturbance (7%) to the sediment cores retrieved. The sampler, attached to a rope, was dropped vertically from a boat. When the sampler sank into the sediments, the rope was jerked to allow a stopper to seal the sampler and create a vacuum, which held the sediments within the sampler. Before retrieving the sampler

from the water, the end of the sampler was covered to avoid sample loss (Wright 1990). The sediment cores were then transferred to polycarbonate tubes with rubber seals and stored in the dark at 4ºC for transportation. The sediment cores were processed within 96 hours of collection.

Each core was gently slipped out of its polycarbonate tube and sliced at 2 cm intervals. The top layer (0 – 2 cm) of the wet sediments was homogenized, weighed for fresh weight moisture content and then dried in an oven at 105°C until a constant weight was reached (approximately 48 hours). The moisture content of the sediments was determined by the difference between the fresh and dry weights and expressed as a percentage. All glassware and plastic containers used for nutrient analysis were pre-washed with diluted hydrochloric acid (2%) to remove chemical residues. For the analyses of organic matter (OM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), total iron (Fe), total aluminium (Al) and total calcium (Ca), finely ground (< 2 mm) oven-dried sediments were used. The reported concentrations of all nutrients were expressed as mg kg^{-1} of dry sediments.

Organic matter (OM) in the sediments was estimated by the 'loss-on-ignition' (LOI) method 2540G (APHA 2005). In this method, oven-dried samples were combusted in a muffle furnace at 550°C until a constant weight was reached (approximately one hour) and cooled in a desiccator in order to determine the weight loss. The percentage of OM can be used to estimate the percentage of organic carbon (OC) in the sediments by an empirical factor of two i.e. % OM = 2 × % OC (Nelson and Sommers 1982). For TC and TN, approximately 0.2 g of oven-dried fine sediments were analysed using a LECO TruSpec C/H/N Elemental Determinator (LECO, St. Joseph, USA) with blank and EDTA calibrations. The sediment samples were combusted at a maximum temperature of 950°C. To determine TP, Fe, Al and Ca, about 0.1 g of oven-dried fine sediments were analysed by an acid dissolution method in a 1:3 mixture of concentrated nitric acid (65%) and concentrated hydrochloric acid (37%) at 140°C (Nieuwenhuize *et al.* 1991; Zarcinas *et al.* 1996). The digested solutions about 1% acidic condition were diluted with 20 mL of deionized water and then sent to Waite Analytical Services, The University of Adelaide (Urrbrae, South Australia) for the analyses of TP, Fe, Al and Ca using Inductively Coupled Plasma Atomic Emission Spectrometry.

Phosphorus fractions were also determined on wet sediments (~ 0.3 g) by a sequential chemical extraction. An end-to-end shaker was used to extract loosely bound or labile-P by 1 M ammonium chloride (NH_4Cl) solution at pH 7 for two hours (Hieltjes and Lijklema 1980). The remaining sediments were further extracted for one hour by a mixture of 0.11 M sodium dithionite ($Na_2S_2O_4$) and 0.11 M sodium bicarbonate ($NaHCO_3$) solutions in order to release iron-bounded phosphorus (Fe-P) from sediment particles (Rydin and Welch 1998). The supernatants for both labile-P and Fe-P extractions were analysed by the Ascorbic Acid Reduction method (Murphy and Riley 1962) using a Unicam S.P. 600 spectrophotometer with a 1 cm cell. The reported concentrations of labile-P and Fe-P, which were analysed using wet sediments, were adjusted using the corresponding moisture content. Since the concentrations of labile-P were very low (< 0.5 mg kg^{-1}) relative to other nutrient concentrations, the results of labile-P were not reported further.

The particle size distribution of wet sediments (~ 0.2 g) was measured by a Laser In-Situ Scattering and Transmissometry Particle Size Analyzer (LISST-100X Type C, Sequoia Scientific, Bellevue, USA). The LISST is a laser diffraction device, which emits a solid-state laser at a wavelength of 670 nm (visible red). The refractions from the particles are received by 32 ring-detectors, which measure the concentrations of suspended particles in a logarithmical range from 2.5 to 500 μm (Sequoia Scientific 2008). Based on the Wentworth Classification (1922), grain size below 3.9 μm was considered clay particles, 3.9 – 62.5 μm as silt particles and 62.5 – 500 μm as very fine/medium sand particles.

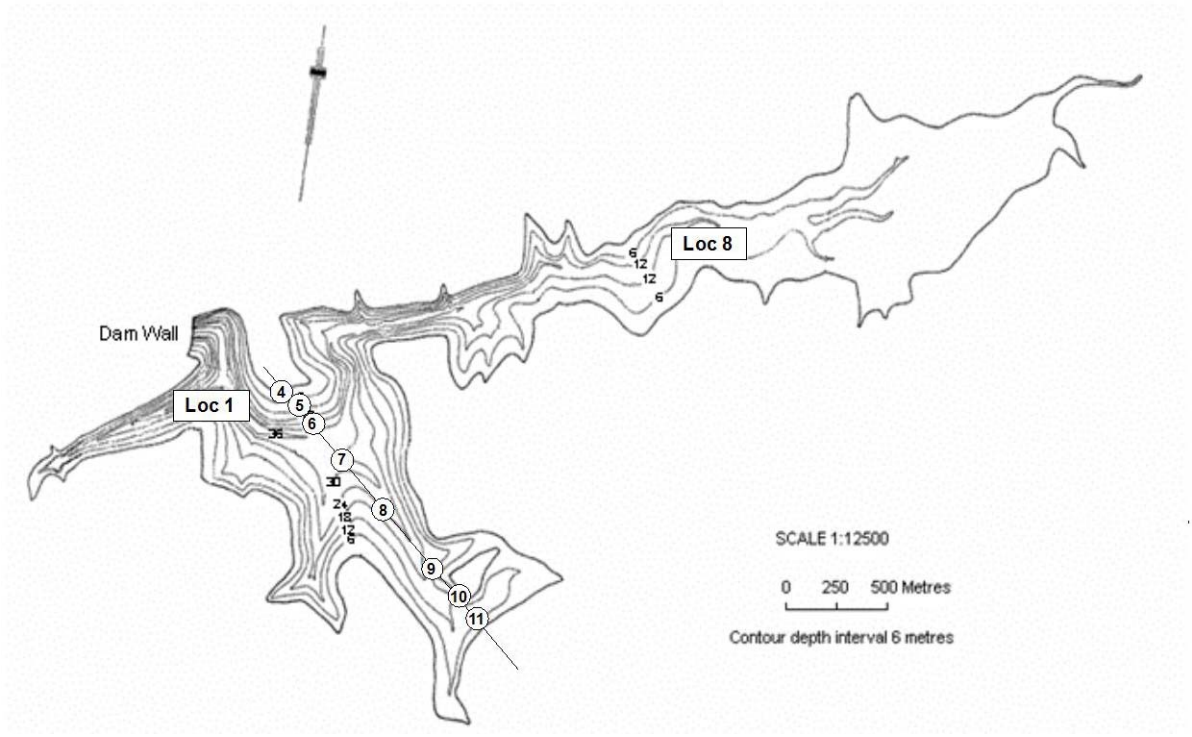

Figure 5 Sediment sampling sites (Sites 4 – 11) and phytoplankton sampling locations (Loc 1 in the main basin and Loc 8 in the side-arm) in Myponga Reservoir

Table 3 Location, depth of overlaying water (m), shortest distance to the shore (m), slope (%) and water content (%) for each sampling site in Myponga Reservoir

Site	Longitude	Latitude	Water depth (m)	Distance (m)	Slope (%)	Water content (%)
4	E138.25.533	S35.24.135	2	20	10.0	33.4
5	E138.25.549	S35.24.146	6	90	6.7	44.1
6	E138.25.566	S35.24.177	20	200	10.0	69.2
7	E138.25.617	S35.24.221	30	260	11.5	72.9
8	E138.25.807	S35.24.340	24	190	12.6	71.9
9	E138.25.996	S35.24.458	13	150	8.7	68.0
10	E138.26.133	S35.24.582	7	190	3.7	50.4
11	E138.26.205	S35.24.664	2.5	60	4.2	54.9

3.2.2 Phytoplankton sampling and analysis

Surface seston (triplicates) were sampled weekly between January and July 2009 for two sites near the inlet (Loc 8) and off-take (Loc 1) of Myponga Reservoir (Figure 5). Plankton nets (20 μm mesh size, 30 cm diameter) were towed on the sides of a boat for about 100 m to collect sufficient quantity of samples for TC, TN and TP analysis. All samples were stored in 300 mL polyethylene terephthalate (PET) bottles on ice in the dark. The detailed methods for phytoplankton sampling and analysis are described in Chapter 6.

Upon return to the laboratory, the seston samples were immediately filtered through 88 µm mesh to exclude zooplankton, plant debris and sediment particles. The filtered samples were scanned under a light microscope (Olympus BX40F4, Japan) revealing that the filtered samples (20 – 88 µm) mainly contained phytoplankton cells. Consequently, the filtered seston samples were hereafter called phytoplankton samples. The wet phytoplankton samples were kept in a freezer at -80ºC and then dried using a vacuum freeze-drier. The dried phytoplankton samples were homogenized and sent for TC and TN analysis at the Analytical Services Unit, CSIRO Land and Water (Urrbrae, South Australia). Using the method described by Matejovic (1997), TC and TN were determined by high temperature combustion (~ 1000°C) in an atmosphere of oxygen using a LECO CNS-2000 (LECO, St. Joseph, USA). Carbon was converted to carbon dioxide gas and determined by infrared detection, while nitrogen was determined as nitrogen gas by thermal conductivity detection. For TP analysis, a sub-sample of the dried phytoplankton was digested using nitric acid and hydrogen peroxide, followed by the measurement of Inductively Coupled Plasma Atomic Emission Spectrometry by Waite Analytical Services, The University of Adelaide (Urrbrae, South Australia).

3.2.3 Water sampling and analysis

Historical water quality data such as dissolved organic carbon (DOC), total nitrogen (TN) and total phosphorus (TP) were provided by the South Australian Water Corporation (SA Water) for the two locations (Loc 1 and Loc 8, Figure 5) between 27th August 2007 and 30th June 2008 and for Myponga Creek from 1985 – 1997. A brief summary of the nutrient analysis for carbon, nitrogen and phosphorus is presented in Table 4. Although the sediments, phytoplankton and water samples were collected during different periods, the stoichiometric ratios could still provide insight into the nutrient recycling found in these components.

Table 4 Brief descriptions of the nutrient analysis in the study

	Analysis	Methods	Detection limits	Service providers
Sediment	Total carbon, TC (mg kg^{-1})	Combust oven-dried sample using LECO TruSpec CHN	50	Own
	Total nitrogen, TN (mg kg^{-1})	Combust oven-dried sample using LECO TruSpec CHN	80	Own
	Total phosphorus, TP (mg kg^{-1})	Digest oven-dried sample with hydrochloric acid and nitric acid; measure by ICPAES	0.0256	WAS[1]
Phytoplankton	Total carbon, TC (µg g^{-1})	Combust freeze-dried sample using LECO CNS-2000	-	ASU[2]
	Total nitrogen, TN (µg g^{-1})	Combust freeze-dried sample using LECO CNS-2000	-	ASU
	Total phosphorus, TP (µg g^{-1})	Digest freeze-dried sample with hydrochloric acid and nitric acid; measure by ICPAES	0.0256	WAS
Water	Nitrate and nitrite, NO_x (mg L^{-1})	Filter through 0.22 µm membrane; measure oxides of nitrogen by automated flow colorimetry	0.005	AWQC[3]
	Ammonia, NH_3 (mg L^{-1})	Filter through 0.22 µm membrane; measure ammonia-N by automated flow colorimetry	0.005	AWQC
	Total Kjeldahl nitrogen, TKN (mg L^{-1})	Digest with sulphuric acid and potassium sulphate; measure ammonia-N by automated flow colorimetry	0.05	AWQC
	Filterable reactive phosphorus, FRP (mg L^{-1})	Filter through 0.22 µm membrane; then same procedures as the analysis of TP for water	0.005	AWQC
	Total phosphorus, TP (mg L^{-1})	Digest with sulphuric acid and potassium sulphate; measure orthophosphate by automated flow colorimetry	0.005	AWQC

1 WAS = Waite Analytical Services, The University of Adelaide, Urrbrae, South Australia
2 ASU = Analytical Services Unit, CSIRO Land and Water, Urrbrae, South Australia
3 AWQC = Australian Water Quality Centre, SA Water, Adelaide, South Australia

3.2.4 Statistical analyses

Differences among sites were examined statistically using SPSS 15.0 for Windows. Using the statistical approach of Coakes *et al*. (2009), data were first checked for homogeneity of variances (Levene's test). When data were normally distributed and had equal variances (*p*-value of Levene's test > 0.05), one-way analysis of variance (ANOVA) was performed. If samples failed the Levene's test, (*p*-value < 0.05), a robust Welch ANOVA was used to examine the equality of means, suggesting a difference between the variances in the population.

Scatterplots were first used to explore the relationship between two variables (e.g. checking for outliers, inspecting the distribution of data points and determining the direction of the relationship between the variables) before calculating correlations

(Pallant 2007). Scatterplot matrix was presented rather than individual scatterplots due to the vast combinations of relationships. Parametric Pearson Product-moment Correlation was then used to describe the linear relationship between two variables if there were no violation of the assumptions of normality, linearity and homogeneity of variance. Kolmogorov-Smirnov statistic or Shapiro-Wilk statistic can be used to assess the normality of the population distribution (the significant value > 0.05). In the case of violation of the assumption, non-parametric Spearman's Rank Order Correlation (rho) was used to calculate the strength of the relationship between two variables (Pallant 2007). In addition, Partial Correlation, which statistically controls the influence of the confounding variable on the relationship of interest, was used to determine any linear relationship between two variables. The two variables are neither dependent upon each other, nor cause-and-effect relationship (Zar 1999). The correlation coefficient (r) was used to describe the strength and direction of the relationships. If the r is close to 1, the relationship between variables is very strong. In contrast, there is no relationship between variables when the r is close to zero. The positive and negative signs for the r represent a positive correlation and a negative correlation respectively (Zar 1999; Coakes *et al.* 2009). A perfect correlation of 1 or –1 indicates that the value of one variable can be determined exactly by knowing the value on the other variable.

3.3 Results

3.3.1 Nutrient distribution in sediments

The concentrations of OM, TC, TN, TP, Fe-P, Fe, Al and Ca plotted along a bathymetric gradient transect in the main basin of the reservoir are presented in Figure 6. The water depth of the shallow sites varied from 2 to 7 m, while the water depth of the deep sites varied from 13 to 30 m. All plots showed similar trends with lower concentrations of nutrients in the shallow parts of the reservoir (Sites 4, 5, 10 and 11) and higher concentrations in the deep areas (Sites 6, 7, 8 and 9). Nutrient concentrations in the shallow sites were all significantly different from the deep sites (all ANOVA tests, $p \leq 0.01$, $n = 24$, Table 5). The concentrations of OM, TN, TP, Fe, Al and Ca were significantly different among the shallow sites. In contrast, only the concentrations of OM, TN and Al were statistically different among the deep sites. All of these differences were statistically significant (all ANOVA tests, $p \leq 0.05$, $n = 12$, Table 5).

It is also interesting to note that the nutrient concentrations between the shallow and deep sites varied for OM, TC, TN and TP (Figure 6 and Table 6). The mean OM content in the deep areas (132,564 mg kg^{-1} or 13.2%) was double that of the shallow areas (62,103 mg kg^{-1} or 6.2%). The mean concentration of TC at the deep sites (58,911 mg kg^{-1} or 5.9%) was 1.6 times higher than at the shallow sites (36,721 mg kg^{-1} or 3.7%). The mean concentration of TN at the deep sites (4,811 mg kg^{-1} or 0.48%) was 2.4 times higher than at the shallow sites (2,079 mg kg^{-1} or 0.21%). The mean concentration of TP tripled at the deep sites (1,298 mg kg^{-1} or 0.13%) when compared to the shallow sites (420 mg kg^{-1} or 0.04%).

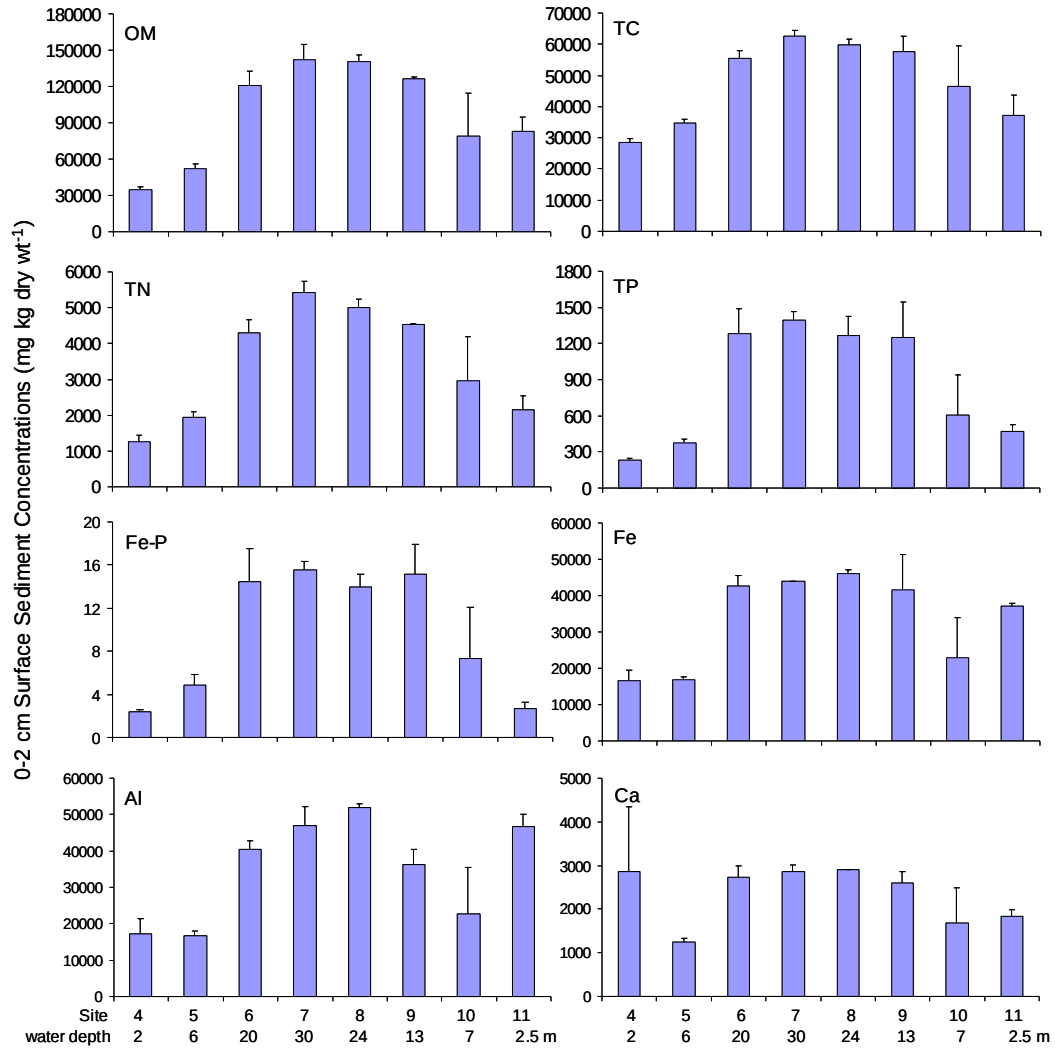

Figure 6 Organic matter (OM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), iron-bounded phosphorus (Fe-P), iron (Fe), aluminium (Al) and calcium (Ca) concentrations (± standard deviation) of the surface sediments (0 – 2 cm) across a bathymetric gradient transect of Myponga Reservoir. All units are expressed as mg kg dry $weight^{-1}$

Table 5 Statistical significance (*p*-value) of sediment particles and sediment nutrients using One-way or Welch ANOVA

One-way/Welch ANOVA significance level	*p*-values		
	***n* = 12**	***n* = 12**	***n* = 24**
Sediment characteristics	**Among shallow sites (4, 5, 10, 11)**	**Among deep sites (6, 7, 8, 9)**	**Between shallow and deep sites**
Clay	0.047*	0.759	0.000***
Silt	0.404	0.229	0.504
Sand	0.440	0.150	0.000***
OM	0.008**	0.049*	0.000***
TC	0.105	0.083	0.001***
TN	0.042*	0.004***	0.000***
TP	0.006**	0.821	0.000***
Fe	0.008**	0.753	0.000***
Al	0.002***	0.003***	0.001***
Ca	0.032*	0.302	0.009**

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

Table 6 Comparisons of total carbon (TC), total nitrogen (TN) and total phosphorus (TP) contents and the corresponding ratios in surface sediments, in phytoplankton cells, in marine seston and in water at Myponga Reservoir and Myponga Creek. Dissolved organic carbon (DOC) is presented when the measurements of TC are not available

		TC	**TN**	**TP**	**TC:TN**	**TC:TP**	**TN:TP**
		(%)			**(By dry weight)**		
Loss from sediment flux	**Mean**	2.1×10^{-3}	1.9×10^{-4}	6.8×10^{-6}	9	316	35
Sediments (0 – 2 cm)	Shallow sites	3.7*	0.21*	0.04*	19*	97*	5*
	Deep sites	5.9	0.48	0.13	12	46	4
	Mean	**4.8**	**0.34**	**0.09**	**16**	**72**	**4**
Phytoplankton cells	Loc 8	33.9*	2.17*	0.31*	17*	141*	7.9*
	Loc 1	26.5	2.28	0.28	12	112	8.9
	Mean	**30.1**	**2.23**	**0.295**	**14**	**126**	**8.4**
Marine seston	Redfield ratios				5.7	40	7
		DOC	**TN**	**TP**	**DOC:TN**	**DOC:TP**	**TN:TP**
		(mg L^{-1})			**(By concentration)**		
Reservoir water	Loc 8	11.5	1.03	0.04*	12	309*	29*
	Loc 1	11.4	0.98	0.03	12	413	34
	Mean	**11.4**	**0.98**	**0.03**	**12**	**355**	**30**
Myponga Creek	**Mean**	**10.1**	**0.90**	**0.04**	**11**	**247**	**23**

* Variables between the two locations were statistically different in One-way or Welch ANOVA tests ($p < 0.005$)

3.3.2 Stoichiometric ratios in sediments and phytoplankton cells

The ratio of organic matter to total carbon (OM:TC) in Myponga sediments (Figure 7) was very similar to a conversion factor of organic matter to organic carbon suggested by Nelson and Sommers (1982). This implies that the total carbon content in the sediments was largely made up of organic carbon. Inorganic carbon such as carbonates from the background sediments contributed little to the total carbon pool.

The TC:TN, TC:TP and TN:TP ratios (Figure 7) show a relationship between the shallow and deep areas, which is inverse to that seen for the concentration data (Figure 6). For the shallow sites, the TC and TN concentrations were low and the TC:TN ratio was high (19:1). In contrast, high TC and TN concentrations in deep areas yielded a lower TC:TN ratio (12:1). This pattern is more pronounced for the TC:TP ratio, where the shallow sites (97:1) doubled the ratio for the deep sites (46:1). For the TN:TP ratio, the mean of 5:1 in the shallow areas was slightly higher than the mean of 4:1 in the deep areas (Table 6). These differences between the shallow and deep sites were highly significant in ANOVA tests ($p < 0.001$, $n = 12$).

The results of sediment stoichiometry were compared with the Redfield ratios, which are the average C:N:P ratios of 106:16:1 by atom (Redfield *et al.* 1963) or 40:7:1 by dry weight (Vallentyne 1974) observed in healthy marine plankton. The Redfield ratio comparison showed that the TC:TN ratio in the surface sediments of Myponga was substantially higher than the Redfield ratio of 5.7 (Table 6). The TC:TN ratio in the deep sites was in fact double the Redfield ratio and in the shallow sites, triple. Similarly, the TC:TP ratio in the surface sediments of the shallow regions was 2.4 times higher than the Redfield ratio (40:1), but the ratio was maintained for the deep regions. In contrast, the Redfield N:P ratio (7:1) was higher than the sediment TN:TP ratio for both shallow and deep areas.

In addition to the comparisons between sediment and Redfield ratios (Table 6), it is relevant to compare the sediment stoichiometry with *in situ* phytoplankton stoichiometry. This is because the sediment nutrients can be largely regulated by the quality of settling organic compounds in the water column. There was no statistical difference between the mean TC:TN ratio for sediments and phytoplankton cells (ANOVA test, $p = 0.29$, $n = 124$). However, both the TC:TP and TN:TP ratios for phytoplankton were nearly double that of sediments (Welch test, $p < 0.001$, $n = 124$).

This difference implied a great loss of TC from lake sediments and/or a substantial gain of TP in lake sediments.

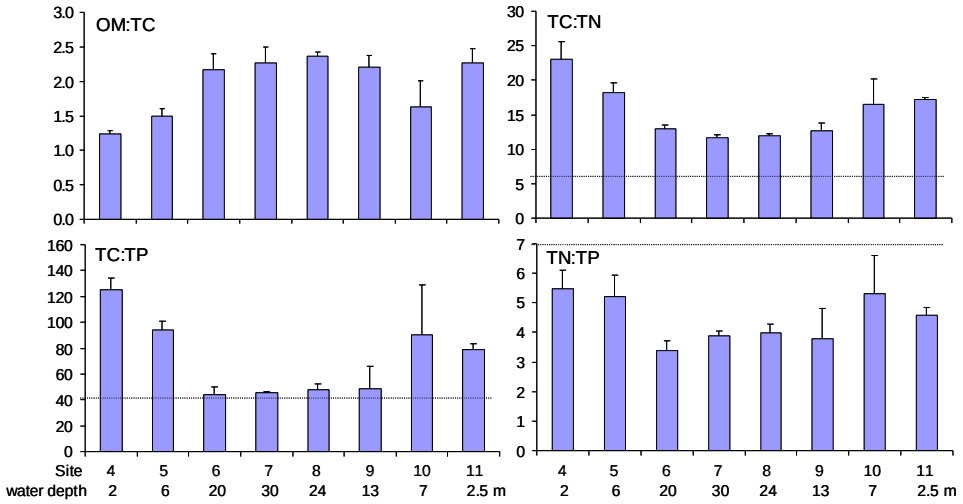

Figure 7 Ratios of organic matter: total carbon, total carbon: total nitrogen, total carbon: total phosphorus and total nitrogen: total phosphorus (± standard deviation) of the surface sediments (0 – 2 cm) across a bathymetric gradient transect of Myponga Reservoir. The dashed lines denote the Redfield ratios by dry weight, which are 5.7 for C:N, 40 for C:P and 7 for N:P (Redfield *et al.* 1963; Vallentyne 1974)

3.3.3 Particle Size Composition

The surface sediments categorised into three particle size groups i.e. clay, silt and sand particles are presented in Figure 8. Silt particles comprised approximately 55% sediments and were the major particle component of Myponga sediments. The silt particles were evenly distributed across the reservoir bed as suggested by ANOVA test ($p = 0.284$, $n = 24$). In contrast, clay and sand compositions were different between the four shallow and four deep sites. The clay content was significantly higher ($p < 0.001$, $n = 24$) in the deep regions (33%) than the shallow regions (21%), while the sand content was much lower ($p < 0.001$, $n = 24$) in the deep region (13%) than the shallow region (23%).

Scatterplot matrix (Figure 9) was first used to explore relationships between sediment particles and nutrient content before calculating correlations. Two correlations (Table 7 and Table 8) were then used to determine the relationships between sediment particles

and nutrient content with and without the influence of water depth. Pearson Product-moment Correlation/ Spearman's Rank Order Correlation suggested a strong linear relationship between water depth and all types of nutrients and between water depth and all types of sediment particles, except silt (Table 7). The sediment particles (e.g. clay and sand) correlated well to most nutrients. Meanwhile, the nutrients themselves were associated with each other. However, these strong relationships could be misleading since the previous statistical tests suggested significant differences between shallow and deep sites (Table 5).

Furthermore, Partial Correlation further revealed a linear relationship between sediment particles and nutrient content without the influence of water depth (Table 8). This time, the results were quite different. For instance, no correlation was found between sediment particles and sediment nutrients. The positive correlation was still strong among OM, TC, TN and TP, but weaker relationships for Fe and Al with other nutrients. There was no correlation between Ca and any other nutrients using the Partial Correlation; however in Pearson Product-moment Correlation/ Spearman's Rank Order Correlation, there was a significant relationship. Coefficient of determination (r^2) for these relationships suggested that OM explained about 73% variance in TC, about 82% variance in TN and about 50% variance in TP. In addition, TP was strongly correlated to Fe ($r = 0.761$, $p < 0.005$, $n = 24$) and moderately correlated to Al ($r = 0.444$, $p < 0.05$, $n = 24$), indicating the potential release of redox-sensitive P and sorptive-bound P respectively.

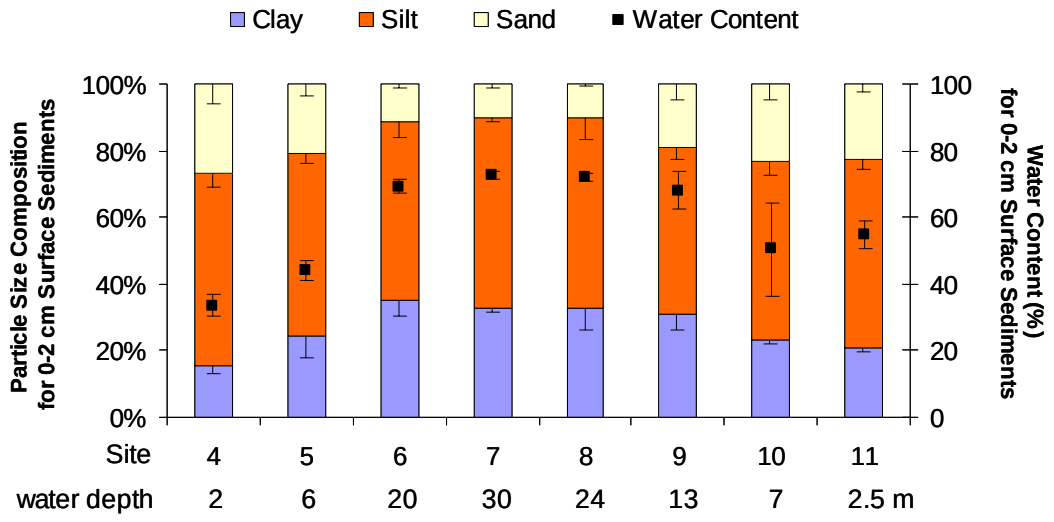

Figure 8 Particle size composition (%) for clay (<3.9 µm), silt (3.9 – 62.5 µm) and sand (62.5 – 500 µm) and water content (%) of the surface sediments (0 – 2 cm) across a bathymetric gradient transect of Myponga Reservoir. Standard deviations are included

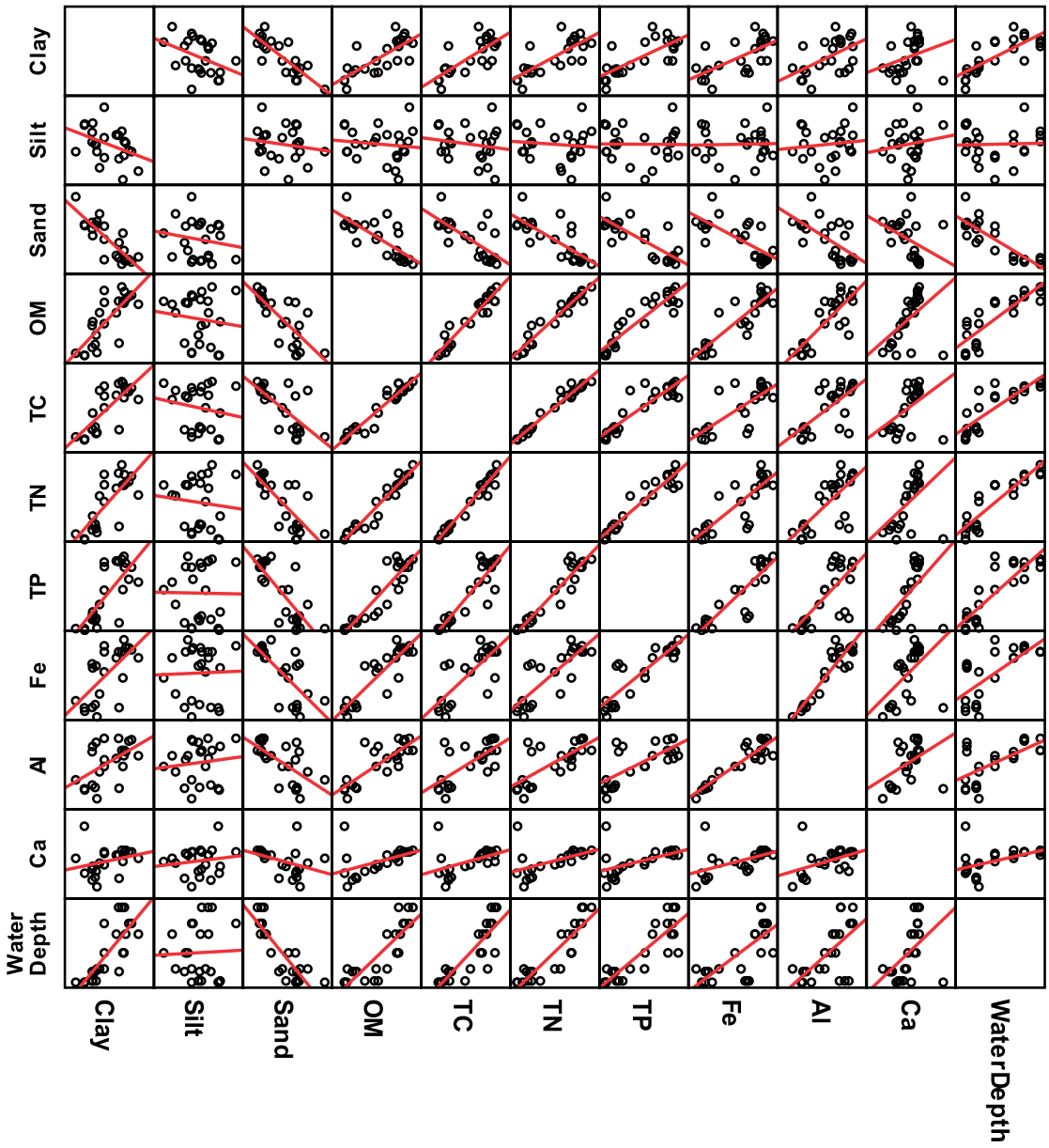

Figure 9 Scatterplot matrix for correlations of particle size composition, nutrient content and water depth of sediments in Myponga Reservoir. Linear line is fitted in each scatterplot

Table 7 Pearson Product-moment Correlation/ Spearman's Rank Order Correlation (n = 24) for particle size composition, nutrient content and water depth of sediments in Myponga Reservoir

Pearson/ Spearman's Correlation	Correlation coefficient (r)										
	Clay	**Silt**	**Sand**	**OM**	**TC**	**TN**	**TP**	**Fe**	**Al**	**Ca**	**Water depth**
Clay	-	-0.397	-0.840***	0.776***	0.761***	0.778***	0.757***	0.660***	0.530**	0.280	0.780***
Silt		-	-0.164	-0.123	-0.172	-0.104	-0.012	0.029	0.119	0.158	0.035
Sand			-	-0.761***	-0.716***	-0.775***	-0.806***	-0.726***	-0.639***	-0.395	-0.859***
OM				-	0.958***	0.971***	0.920***	0.894***	0.815***	0.485*	0.843***
TC					-	0.981***	0.905***	0.771***	0.686***	0.456*	0.845***
TN						-	0.955***	0.833***	0.727***	0.498*	0.898***
TP							-	0.886***	0.724***	0.531**	0.866***
Fe								-	0.925***	0.509*	0.717***
Al									-	0.430*	0.638***
Ca										-	0.466*
Water depth											-

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

Table 8 Partial Correlation (n = 24) for particle size composition and nutrient content of sediments in Myponga Reservoir. A confounding variable, water depth, was controlled statistically to prevent its influence on the relationships of sediment particles and nutrients

Partial Correlation	Correlation coefficient (r)									
	Clay	**Silt**	**Sand**	**OM**	**TC**	**TN**	**TP**	**Fe**	**Al**	**Ca**
Clay	-	-0.678***	-0.531***	0.353	0.302	0.283	0.260	0.231	0.066	-0.150
Silt		-	-0.262	-0.285	-0.379	-0.309	-0.085	0.005	0.126	0.161
Sand			-	-0.136	0.040	-0.016	-0.243	-0.310	-0.232	0.012
OM				-	0.853***	0.905***	0.706***	0.772***	0.669***	0.195
TC					-	0.944***	0.648***	0.443*	0.356	0.132
TN						-	0.804***	0.619***	0.454*	0.204
TP							-	0.761***	0.444*	0.288
Fe								-	0.871***	0.285
Al									-	0.195
Ca										-

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

3.4 Discussion

3.4.1 Spatial dynamics of nutrients and sediment particles

Results suggest that the high accumulation of OM and nutrients occurred in the deeper parts of Myponga Reservoir (Sites 6, 7, 8 and 9), but there were relatively low levels of OM and nutrients in the shallow parts (Sites 4, 5, 10 and 11). Similar observations have been recorded in other lakes, which showed increased organic content, nitrogen and phosphorus with increasing water depth (e.g. Carignan and Lean 1991; Lebo and Reuter 1995; Shilla *et al.* 2006). This has been attributed to the gravitational movement of benthic particles over a declined sediment bottom (Shilla *et al.* 2006). In fact, this is a common sediment redistribution process, termed sediment focussing, which involves greater sediment accumulation taking place in the deeper rather than the shallower parts of lakes (Likens and Davis 1975).

Since the reservoir bed of Myponga Reservoir is relatively steep, sediment sliding and slumping on slopes, a common mechanism of sediment focussing (Hilton 1985), may partially explain the distribution of organic matter and nutrient content. Sediments tend to accumulate on a slope below 3.8%, but the process gradually decreases with increasing inclination (Hakanson 1977). For example, few fine-grained sediments have been found on slopes greater than 7.5% for large lakes and 15% for small lakes (Rowan *et al.* 1992). In Myponga Reservoir, the slopes of the reservoir bed were estimated from the bathymetry (Figure 5), ranging from 3.7 to 12.6% (Table 3). These inclinations are likely to cause sediment sliding and slumping and consequently, concentrating particles on the flatter, deeper reservoir bed.

The water content of surface sediments, plus particle size, can be used to distinguish the zones of sediment erosion, transportation and accumulation (Hakanson 1977). Water content less than 50%, such as that found at shallow part (Sites 4 and 5) at Myponga Reservoir, is associated with coarse-grained, non-cohesive sediments in the erosion zone. In contrast, the shallow (Sites 10 and 11) and deep (Sites 6 – 9) regions, which have water content of 50% to 75%, were considered to be within the transportation zone. This zone has discontinuous sedimentation influenced by periodic resuspension from wave activities (Blais and Kalff 1995). In the current study, sediment particle size was also well described by the content of water in the surface sediments (Figure 8 and Table 3) and as expected, showed an inverse relationship between sediment particle size and sediment water content (Hakanson 1977; Rowan *et al.* 1992).

Different linear relationships between sediment particles and nutrient content were also revealed in scatterplot matrix (Figure 9) and using Pearson Product-moment Correlation/ Spearman's Rank Order Correlation (Table 7) and Partial Correlation (Table 8). The observed relationship between particles and nutrients was due solely to the influence of water depth. As the water depth (a confounding variable) showed an impact on sediment nutrient content (Figure 6), Partial Correlation rather than classical Pearson Product-moment Correlation/ Spearman's Rank Order Correlation was more appropriate to explore the relationships because it excluded the possible effect of this confounding variable.

Sediment resuspension near the shore is commonly observed in shallow lakes (Niemisto *et al.* 2008; Trolle *et al.* 2009) and shallow parts of deep lakes (Likens and Davis 1975). Wind-induced resuspension of particulate organic matter in the shallow water (< 6 m) can be initiated by wind speeds of 2 to 4 m s^{-1} (Demers *et al.* 1987; Arfi and Bouvy 1995; Selig 2003). However, if the wind speed reaches about 6 m s^{-1}, no further increase is generally observed in the concentration of particulate organic matter in the water column. This is because most deposited organic matter has been resuspended (Demers *et al.* 1987). As such, wind-induced mixing and turbulence constantly stir up sediment particles in shallow sites and particles are resuspended a number of times before a long-term settlement (Ekholm *et al.* 1997). Hakanson (1977) suggested that fine materials are unlikely to deposit in high-energy environment e.g. low water depth relative to a large wind fetch. This seems to correspond to our observations, with fine particles in the deep parts of the reservoir and coarse particles in the shallow areas (Figure 8). This can likely be explained by the fact that resuspended coarse heavy sand particles tend to settle faster and closer to the original resuspended areas through the process of gravity, while fine light clay materials remain in suspension longer and can be carried further away by water currents and deposited in the deeper regions of the reservoir (Cyr 1998). Deep sites of Myponga Reservoir (13 – 30 m) are also less susceptible to wind disturbance, allowing continuous particle sedimentation and nutrient accumulation.

Sediment particle composition is also important in determining the nutrient binding capacity. Fine clay particles are known to have a higher phosphorus binding capacity than coarse sand particles in soils and sediments (Knosche 2006), as clay compounds form Fe, Al and Ca complexes, which sorb phosphorus. For example, DePinto *et al.* (1981) reported that the P-bioavailability was strongly correlated to the presence of Fe

and Al. However at Myponga Reservoir, P was strongly correlated to Fe, less so but still significant with Al and none with Ca when Partial Correlation was used to exclude the confounding effect of water depth. The Fe-P in Figure 6 further suggested higher concentrations in the deeper Myponga sediments. Fe-P may be released at high pH due to anion competitions, where hydroxyl ions (OH^-) and silicate ions (SiO_4^{4-}) compete more efficiently and displace phosphate ions (PO_4^-) for the iron-sorption sites (Hingston *et al.* 1967). This can be an important P-releasing mechanism in the littoral zone, where sediments containing Fe-P are resuspended to the algal productive surface layer with higher pH (Drake and Heaney 1987), leading to the low concentration of Fe-P in the shallow sites.

Fine particles also have a large surface area, providing more binding sites for phosphorus adsorption (Blais and Kalff 1995; Lukkari *et al.* 2009). This may partially explain the high nutrient content in deep sites of Myponga Reservoir, where higher clay, Fe and Al contents were observed. In contrast, Selig (2003) showed the opposite relationship between particle size and nutrient content with a higher nutrient content in sand rather than silt and clay particles in a German lake. The sediment particles above 63 μm (sand) contained 69% organic C, 71% TN and 61% TP, whereas the sediment grains smaller than 63 μm (clay and silt) only retained 31% organic C, 29% TN and 39% TP (Selig 2003). This implies that the nutrient binding capacity for the same sediment particle sizes can vary, affecting the sediment nutrient retention.

3.4.2 Stoichiometric ratios as indicators of sediment nutrient recycling

The previous section highlights some possible mechanisms, leading to the low OM, TC, TN and TP concentrations in the shallow sites comparative to the deep sites (Figure 6). However, the relative difference between the shallow and deep sites varied for each nutrient. For example, TP in the deep areas was three times higher than in the shallow areas, but TC in the deep areas was only 1.6 times higher than in the shallow areas. This implies that an accumulation or loss rate of one nutrient is higher than another across the shallow and deep sites. The elemental ratios for each nutrient (Figure 7) provided insight to help to unravel underlying environmental processes that may lead to these differences.

If the TC:TN:TP ratio is compared with other reference point such as Redfield ratios (Table 6), the relative proportion of the nutrients in the ratio suggests that there is

enrichment of TC in Myponga sediments and/or the loss of TN and TP from the sediments (e.g. Lukkari *et al.* 2009). This may be due to large preferential removal of nitrogen and phosphorus, relative to carbon from the diagenesis of organic matter at the time of, or prior to burial in sediments (Krom and Berner 1981). The regenerated inorganic N and P from sediments or the water column are often absorbed and assimilated rapidly by autotrophic and heterotrophic microbes and stored as organic matter. In contrast, autotrophic organisms can obtain C for photosynthesis via the ionization of atmospheric carbon dioxide to soluble bicarbonate or carbonate ions and are therefore less dependent on recycled C from sediments. Thus, N and P may be reused more often than C, leading to the accumulation of C in the sediments.

As suggested by the OM data (Figure 7), the measured TC in the surface lake sediments of Myponga Reservoir was mostly composed of organic carbon from the sedimentation of biological particulates and detritus. Mineral carbonates would have had little influence on the TC measurements because the samples were combusted below 1000ºC (Matejovic 1997). Nutrient content of phytoplankton particulate matter had a particularly strong impact on sediment nutrient composition. The mean TC:TN:TP ratio for phytoplankton was about 126:8:1 (by dry weight) over a growing season of summer and autumn in Myponga Reservoir (Table 6). This ratio was almost double the mean TC:TN:TP ratio of Myponga surface sediments (72:4:1). The sedimentation of high carbon input from phytoplankton materials could contribute to the relatively high TC:TN and TC:TP ratios in sediments. In addition, sinking of zooplankton faeces or faecal pellets can be significant sources of nutrients for sediments. The stoichiometry of the zooplankton faeces is influenced by the elemental needs of zooplankton (Sterner and Elser 2002). Herbivorous zooplankton grazes on phytoplankton with highly variable C:N:P ratios, while assimilating a relatively constant C:N:P ratio and releasing the difference between the two (Sterner 1989). Copepods, with their high body N:P ratios, produce egestion with a relatively lower N content, while cladocerans, especially *Daphnia* with their low body N:P ratios, have high demands of P from food and egest low P faecal matter (Andersen and Hessen 1991; Sterner *et al.* 1992). No release of P from *Daphnia pulex* was observed when the food quality (algal content) was below 6 – 8 µg P mg^{-1} C, because all the ingested phosphorus was used for growth and reproduction of the daphnids (Olsen *et al.* 1986). Thus, the sedimentation of zooplankton faecal production can disproportionately affect the sediment stoichiometry over time. However, the study of zooplankton stoichiometry in Myponga Reservoir was

beyond the scope of this study. Further research is needed to quantify the relative contributions of zooplankton and the faecal matter to the sediment nutrient composition.

A substantial amount of carbon is also known to be introduced from Myponga catchment to the reservoir in the form of dissolved organic carbon (DOC). Increased rainfall and storm events are likely to increase runoff of DOC from terrestrial systems to lakes (Hinton *et al.* 1997). Small lakes such as Myponga Reservoir are more susceptible to allochthonous DOC inputs than large lakes (Sterner *et al.* 1997). An estimation of annual DOC input from Myponga catchment was about 240 tonnes (Linden 2007); such a large input of DOC would have an indirect effect on sediment C accumulation via microbial uptake and subsequent sedimentation. Linden (2007) further explained that the DOC input was likely originated from the native vegetation in the Myponga catchment. In addition, an extensive pine plantation around Myponga Reservoir may also contribute to the DOC inputs, as reported in other South Australian reservoir (Naidu *et al.* 1993). They (1993) reported that the decomposition of large amounts (10 cm thick) of pine leaf litter and organic matter on the soil surface was responsible for the high DOC input to the surrounding reservoir (Naidu *et al.* 1993). Although the high input of carbon from the catchment appears mostly in dissolved form, certain proportions of the DOC input may increase particulate C either via flocculation or via microbial uptake, leading to a higher seston C:P ratio (Brock and Clyne 1984; Sterner *et al.* 1997). The phytoplankton with high C:P ratio are then consumed by other organisms through the food chain and some organisms will eventually die and settle onto the reservoir bottom, contributing indirectly to the TC pool in sediments.

3.4.3 Possible mechanisms contributed to the nutrient loss from sediments

Both TC:TN and TC:TP ratios of the sediments were higher than the Redfield ratios, suggesting the enrichment of TC in Myponga sediments and/or the loss of TN and TP from the sediments. In contrast, the TN:TP ratio was lower than the Redfield ratio implying the loss of TN from the sediments and/or the accumulation of TP in the sediments. Mechanisms, causing sediment nutrient loss are discussed as followed and they provide possible explanations for the observations in Myponga sediments.

In sediments, complex organic matter is decomposed and mineralized to simple molecules such as carbon dioxide (CO_2), methane (CH_4), ammonia (NH_3) and orthophosphate (PO_4^-) by a range of aerobic and anaerobic bacteria. Tezuka (1989)

suggested that the C:N:P ratio of phytoplankton determined the relative amounts of dissolved inorganic nitrogen and phosphorus released during aerobic decomposition. For example, when aerobic bacteria decomposed P-limited algae with high C:P and low C:N ratios, the bacteria tended to retain the limiting P for their growth and release the additional inorganic N. On the contrary, when the detritus was derived from the N-limited algae with high C:N and low C:P ratios, bacteria were likely to retain more N and remineralize more inorganic P during aerobic decomposition. In the case of Myponga Reservoir, the mean phytoplankton over the summer/autumn seasons tended to be P-limited (TN:TP = 8), while the mean sediments had a lower TN:TP ratio of 4 (Table 6). This seems to match well with the findings of Tezuka (1989), where phytoplankton stoichiometry affected the nutrient recycling by bacterial decomposition and, in turn, could modify the sediment stoichiometry over time.

During the process of mineralization, only C and N can actually leave the aquatic system and escape into the atmosphere during respiration, methanogenesis and denitrification. In contrast, P is retained in the system (Schindler *et al.* 1976; Hecky *et al.* 1993). The flux rates of these nutrients and the corresponding nutrient loss are presented in Table 9. The CO_2 flux from sediments to water and then to the atmosphere is a significant mechanism of C loss from the aquatic system. The CO_2 flux rate from Myponga sediments, in relation to microbial respiration, was calculated from its sediment oxygen demand (SOD) over a six-hour incubation study in August 2008 (Chapter 4). Based on the simplified respiration equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$, the average rate of SOD (298.63 mg O_2 m^{-2} d^{-1}) was converted to the average flux rate of CO_2 (224.11 mg C m^{-2} d^{-1}). Therefore, C can be lost via CO_2 flux during mineralization of organic C in sediments.

During anoxic decomposition, methane is produced mainly from the methyl carbon of acetate by the fermentation of complex organic compounds and partly from the reduction of CO_2 (Wetzel 2001). The CH_4 emission from Myponga sediments was estimated using the average CH_4 flux rate (2.52 – 3.72 mg C m^{-2} d^{-1}) from sediments of Lake Washington, USA (Kuivila *et al.* 1988). The reason this rate was used is that Lake Washington is a deep, mesotrophic freshwater lake with a well-oxygenated layer of hypolimnion throughout the year (Kuivila *et al.* 1988); so the conditions are similar to Myponga Reservoir. Methane gas is produced in the deep anoxic sediments and passed through the oxic layer of surface sediments, where methane oxidation takes place to

convert CH_4 gas to CO_2 gas (methanogenesis). Kuivila *et al.* (1988) observed in Lake Washington sediments that about half of the methane production was oxidized and released as CO_2 during the upward movement between the oxic sediment-water interfaces.

Along with the decomposition of organic matter, nitrogen recycling also takes place in sediments. Organic N (e.g. proteins, amino acids, urea, etc) is degraded and remineralized into ammonia by heterotrophic bacteria. Ammonia is also released by aquatic animals as an excretory product, but this is at low quantity in comparison to the ammonia generated by bacterial decomposition (Wetzel 2001). Under anaerobic conditions, Myponga sediments had an average NH_3 release rate of 5.1 mg N m^{-2} d^{-1} (Chapter 4). However, the flux of NH_3 was not included into the total loss of sediment N because the NH_3 is part of the flux of total Kjeldahl nitrogen (TKN). The flux of TKN, which is the sum of organic nitrogen, NH_3 and ammonium, was about 9.62 mg N m^{-2} d^{-1} from the anoxic Myponga sediments (Chapter 4). Another major pathway for sediment N loss is through denitrification. A series of biochemical reduction of oxidized nitrogen anions, NO_3^- → NO_2^- → nitrous oxide (N_2O) → N_2 takes place under anoxic conditions. As a result, N is lost from the aquatic systems to the atmosphere as N_2 gas, which can re-enter the ecosystem via nitrogen fixation. As reported by Seitzinger (1988), the N_2 flux via denitrification for oligo-mesotrophic lake sediments can range from 1.5 – 19 mg N m^{-2} d^{-1}.

Much of the planktonic organic P is recycled rapidly within the epilimnetic water. The P turnover time varies from minutes to weeks dependent upon the lake productivity and the season (Wetzel 2001). This may suggest that a phosphorus atom can be reused by phytoplankton for a few times over a growing season (Andersen and Jensen 1992; Reynolds 1996a) and can fix carbon atoms several times before sedimentation. Settled organic P is degraded and released as bio-available PO_4^-, which soon becomes unavailable by assimilation with surrounding microorganisms and by adsorption onto substances like iron oxides, humics, carbonates and clays (van Eck 1982). In addition, some portions of Fe-P and immobile P (e.g. Al- and Ca-bound P and refractory organic P) could be buried permanently in the sediments and removed from the nutrient cycle (Slomp *et al.* 1996; Lukkari *et al.* 2009). In the case of sediments in Myponga Reservoir, the average flux rate of TP (0.72 mg P m^{-2} d^{-1}) was measured under anoxic conditions (Chapter 4).

A number of possible pathways for nutrient loss from sediments were discussed. As seen in Table 9, there was more TC loss from sediments (~ 21 mg kg^{-1}) than the TN loss (1.05 – 2.69 mg kg^{-1}) and the TP loss from sediments (0.07 mg kg^{-1}). Since different nutrient flux rates lead to different nutrient loss, the relative proportions of nutrients in sediments may change over time. However, such change in sediment nutrient loss is negligible in comparison to the changes in sediment nutrient gain from phytoplankton sedimentation. There were differences of a few orders of magnitude between the sediment nutrient loss and gain. The TC content for phytoplankton was about 30%, while the TC loss from sediments was only 0.0021% (Table 6). The differences in the rates of sediment nutrient loss could not help to explain the variations between sediment stoichiometry and phytoplankton stoichiometry in Myponga Reservoir. However, the discrepancy between the two stoichiometries could be explained by the timescale of sediment sampling and phytoplankton sampling, since sediment is an integrator over time.

Table 9 Potential nutrient loss from Myponga sediments: the sum of fluxes of carbon dioxide gas (CO_2) and methane gas (CH_4) from sediments expressed as total carbon (TC) flux from sediments; the sum of fluxes of nitrogen gas (N_2), ammonia gas (NH_3) and total Kjeldahl nitrogen (TKN) from sediments expressed as total nitrogen (TN) flux from sediments; and total phosphorus (TP) flux from sediments. Nutrient flux rate is expressed as mg m^{-2} d^{-1} while nutrient loss is expressed as mg kg^{-1} d^{-1}

	Average flux rate from sediments (mg m^{-2} d^{-1})	* Nutrient loss from sediments (mg kg^{-1} d^{-1})	Source for flux rates
CO_2 flux as C	224.11	21.1	Chapter 4
CH_4 flux as C	2.52 – 3.72	0.24 – 0.35	(Kuivila *et al.* 1988)
TC flux	**226.63 – 227 .83**	**21.34 – 21.45**	
N_2 flux as N	1.5 – 19	0.14 – 1.79	(Seitzinger 1988)
NH_3 flux as N	5.11	0.48	Chapter 4
TKN flux as N	9.62	0.91	Chapter 4
TN flux ^	**11.12 – 28.62**	**1.05 – 2.69**	
TP flux	**0.72**	**0.07**	Chapter 4

* The radius of the cylindrical sampling core is 0.0295 m and the corresponding surface area is **0.002734 m^2**
On average, the mass of dry sediments in the core is **0.029037 kg**
Nutrient loss = flux rate x surface area of core / mass of dry sediments of core

^ TN flux did not include the NH_3 flux because TNK flux has already included the NH_3 flux

3.5 Conclusion

This study gave insight into the spatial variability of surface sediment nutrients and sediment particle distribution between the shallow and deep sites in Myponga Reservoir. Sediments in the deep part of the reservoir were generally richer in organic matter, carbon, nitrogen and phosphorus compared with the sediments in the shallow region. More fine particles (clay) were found in the deep sites, while more coarse particles (sand) were observed in the shallow sites. These observations in Myponga Reservoir may be explained by several sediment redistribution processes and different chemical binding capacities between clay and sand particles. The comparison between sediment stoichiometry and Redfield ratio implied that the accumulation of carbon in Myponga sediments is probably due to sedimentation of carbon-rich phytoplankton and high external DOC loads from the catchment. Sedimentation of zooplankton and the faecal matter may also impact the sediment stoichiometry, however no data from Myponga Reservoir were available to support this.

A number of possible pathways for nutrient loss from sediments were discussed. During aerobic decomposition, bacteria may retain more P and recycle more N if the organic matter being decomposed was P-limited. Only C and N could actually leave the aquatic system to the atmosphere during respiration, methanogenesis and denitrification, while P appears to be recycled within the system. However, the actual quantity of sediment nutrient loss through these processes was small when compared to the quantity of sediment nutrient gain from phytoplankton sedimentation.

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Chapter 4 Potential nutrient release from anoxic sediments and implication on cyanobacterial blooms

Preamble

With an aid of artificial destratification unit operated seasonally in the main basin of Myponga Reservoir, strong seasonal thermal stratification is unlikely to occur. However, thermal micro-stratification may still occur at the sediment-water interface, leading to sediment nutrient flux under anoxic conditions. These internal nutrient loads could be biologically significant to phytoplankton growth, especially in low concentrations of bio-available nutrients during summer.

Chapter 4 first describes the likelihood of the occurrence of thermal micro-stratification over time and space. Laboratory experiments were conducted to determine the potential nutrient fluxes under anoxic sediments. Finally, a risk assessment was conducted to evaluate the potential risks associated with anoxic fluxes of nutrients to promote cyanobacterial blooms.

Apart from the water temperature data provided by SA Water, I was responsible for all field samplings, laboratory experiments, data collection, data analysis and interpretation, under guidance from supervisors.

Chapter 4 Potential nutrient release from anoxic sediments and implication on cyanobacterial blooms

4.1 Introduction

External nutrient loads to drinking water reservoirs are largely controlled by the land use of surrounding catchments (Ulrich 1997) and the intensity, duration and timing of seasonal and annual rainfall patterns (Linden *et al.* 2004). Since Myponga Reservoir experiences a Mediterranean climate, allochthonous nutrient loads typically dominate during the high rainfall season (winter and spring), whereas autochthonous and internal sources of nutrients become more important during the dry summer.

External sources of nutrients undergo a series of biochemical reactions when passing through a storage system (e.g. lakes, reservoirs and wetlands); and some nutrients are partially retained in the system. Nutrients attached to coarse soil particles are often deposited at inlet sites of a lake when the energy of flow is dissipated into the lake water (Hilton *et al.* 1986). Fine particulate nutrients remain in suspension and gradually settle, as the energy is lost by mixing the river water with the lake water. Whilst particulate nutrients (e.g. organic matter, nutrients adsorb onto sediment particles) are not bio-available, soluble and diffusible nutrients (e.g. orthophosphate, nitrate, ammonium) can readily be absorbed by phytoplankton and macrophytes through semi-permeable cell membranes (Reynolds 1984). If the lake experiences thermal stratification in summer, anoxic conditions may occur at the sediment-water interface (Davison 1993). This may lead to the release of redox-sensitive nutrients from sediments such as iron-bound phosphorus, ammonium, iron (II) and manganese (II) ions, contributing to the pool of bio-available nutrients (Adams *et al.* 1982; Christophoridis and Fytianos 2006).

Thermal stratification is typical for deep lakes and reservoirs and results from the formation of a warm, low density surface layer (the epilimnion) when exposed to high solar radiation over summer (Golterman 1976). Due to the density gradient, the epilimnion is physically separated from the cooler denser hypolimnion that has no access to oxygen from the atmosphere (Wetzel 2001). Seasonal thermal stratification in Myponga Reservoir has been prevented since the installation of an aerator and two surface mixers in the early 1990s (Brookes *et al.* 2000; Lewis 2004). However, micro-stratification (fine-scale temperature differences) may still occur within the vicinity of

destratification, while stronger thermal stratification can also develop in the regions of the lake not reached by artificial mixing. In the regions, where stratification may occur, microbial decomposition of organic matter at the sediment-water interface could create anoxic microlayers, leading to the release of nutrients and metals from the sediments, as observed in shallow lakes (Lofgren and Bostrom 1989; Kisand and Noges 2003).

The mechanisms of the release of phosphate (PO_4^-), ammonium (NH_4^+), iron (Fe^{2+}) and manganese (Mn^{2+}) from anoxic sediments have been observed and studied intensively over many decades (e.g. Mortimer 1941; Adams *et al.* 1982; Gelda *et al.* 1995; Baldwin and Williams 2007). The current study takes these mechanisms a step further by approximating the cyanobacterial risk associated with the release of nutrients from sediments. The phosphorus flux rate from the incubation of anoxic sediments was used to calculate the risk associated with the growth of *Anabaena circinalis*. *Anabaena circinalis* is a cyanotoxin-producing and odorous-causing cyanobacterium seasonally abundant in many Australian freshwater systems (May and McBarron 1973; Bowmer *et al.* 1992). The risk assessment was conducted based on the empirical relationship between phosphorus cellular content and actual cell quotas of saxitoxin and geosmin measured from natural *Anabaena circinalis* populations in Australia (Reynolds 1984; Burch 2010).

The objectives of the study were: (i) to determine the degree of thermal stratification (frequency, intensity and duration) in various locations of Myponga Reservoir with and without the impact of artificial destratification; (ii) to assess the likelihood of the development of anoxic conditions near the sediment-water interface; (iii) to determine the flux rates of nutrients under anoxic incubations; and (iv) to conduct a risk assessment for the estimated population of *Anabaena circinalis* and the associated productions of toxin and odorous compounds under different stratification and phosphorus flux scenarios. The study will also examine the importance of the internal nutrient loads under thermal stratification, which provides nutrients in additional to external loads that may promote the proliferation of toxic cyanobacterial blooms.

4.2 Methods

4.2.1 Water temperature profile

Since the 1980s, SA Water monitored temperature profiles in the water column of Myponga Reservoir at various locations and with different resolution (Figure 10). In the

1980s and 1990s, water temperature was measured weekly at different depths (0.2, 10 and 30 m) near the Dam Wall of the reservoir by SA Water. Between 1999 and 2003, two thermistor chains (BetaTHERM Sensors, Ireland) with a number of temperature sensors through the water column were installed in the main basin (MET 1) and side-arm (MET 2) of the reservoir. These sensors provided measurements of temperature at 10-minute intervals from the water surface to the reservoir bottom. An additional thermistor chain (MEA2173 precision temperature sensors, Measurement Engineering Australia, South Australia) was deployed in the main basin (MET 1) in 2007 to detect fine-scale stratification, with a higher measurement resolution of ±0.003°C at 10-minute intervals.

High resolution water temperature data allow detailed assessments (Table 10 – Table 14) of the strength, duration and frequency of micro-stratification across different parts of the reservoir (dam wall, main basin and side-arm) with various management approaches (no mixing, aerator, surface mixer) over time (1984 – 2003). Although water temperature was recorded continuously throughout the day and year, the assessments only utilised data between 1st September and 1st May each year, when thermal stratification was most likely to occur during the warmer seasons. In the assessments, the percentage of time of the occurrence of stratification was with reference to this period (September – May). In the absence of dissolved oxygen measurements in the field, high resolution water temperature data can be used to determine the degree, duration and frequency of stratification, which then can infer to the likelihood of the occurrence of anoxic conditions at the sediment-water interface.

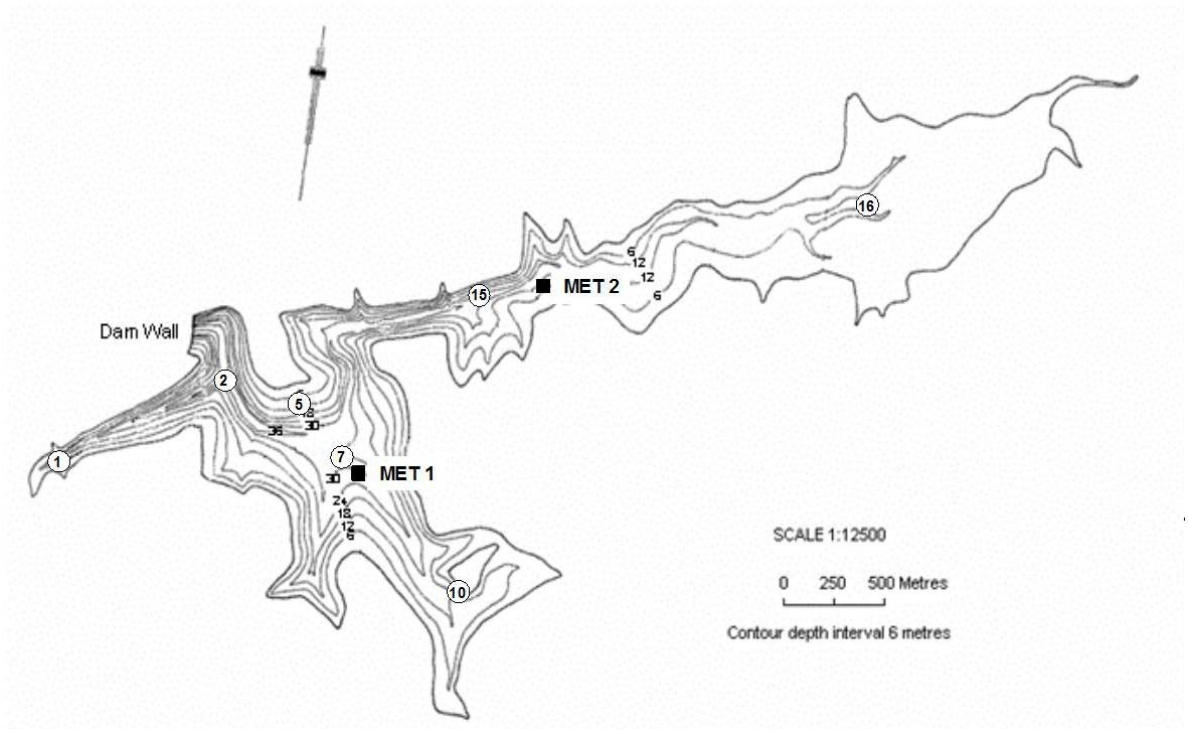


Figure 10 Meteorological stations (MET 1 and MET 2) for water temperature profiling at 10-minute intervals and sediment sampling sites in August 2008 (Sites 1, 2, 7, 10 15 and 16) and in May 2010 (Sites 5 and 7) for sediment oxygen demand and nutrient flux experiments in Myponga Reservoir

4.2.2 Measurements of oxygen demand

In the context of the present study, two experiments were conducted in August 2008 and May 2010 to determine the sediment oxygen demand and sediment nutrient flux. During the August 2008 experiment, six sediment cores were collected across the reservoir (Sites 1, 2, 7, 10, 15 and 16) without replicates for each site. However, Chapter 3 revealed a significant difference in the nutrient content of sediment between shallow and deep parts of the reservoir. Consequently, a repeated experiment with triplicates was carried out to investigate potential site variations in nutrient flux rates (Site 5 for shallow region and Site 7 for deep region). Figure 10 presents the sampling sites for the two experiments.

A modified Hongve sampler with an internal diameter of 5.9 cm was used to retrieve an approximately 7 cm of sediment (Wright 1990). Sediment cores were kept in sealed polycarbonate tubes (surface area ~ 0.00255 m^2) in the dark. About 10 L of surface water was also collected. Upon return to the laboratory, the overlying water of each

sediment tube was carefully removed to minimize sediment disturbance. The tubes were then filled up with reservoir water filtered with Whatman GF/C filters (~ 0.6 L). The tubes were sealed by dissolved oxygen sensors (TPS WP-82Y, Queensland, Australia) inserted into the screwed caps. The sediment cores were allowed to sit undisturbed for 15 minutes before the dissolved oxygen measurements commenced in order to minimize the effect of sediment resuspension (Doyle and Lynch 2005). A magnetic stirrer was attached to the dissolved oxygen sensor and suspended in the water (Figure 11). The tubes were placed around a magnetic plate, which regulated the movement of the magnetic stirrers. The stirrers provided a degree of mixing in the tubes, but were not strong enough to resuspend sediments. Control treatment representing the water oxygen demand was established in the same manner, except the tube contained filtered water but no sediment.

The concentrations of dissolved oxygen (DO) were measured every hour only for the initial stage of incubation (approximately 7 hours) in the first experiment due to the failure of the DO sensors. In the second experiment, DO was recorded every 30 minutes for the 5.5-day incubation period. The oxygen consumption rate was calculated by multiplying the rate of change of DO concentration by the volume of water and dividing by the sediment surface area in the core (Beutel 2003). Only the first 24 hours of data was used to calculate the rate of change of DO concentration, as suggested by Nielsen *et al*. (2000), with approximately 20% of the DO remaining after this time. All oxygen consumption rates are expressed as dissolved oxygen (mass) per unit surface area per unit time (mg m^{-2} d^{-1}).

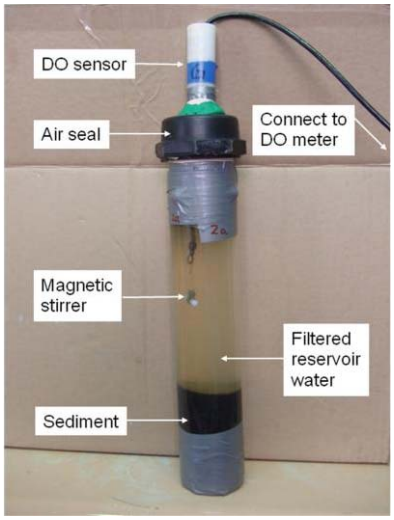


Figure 11 Illustration for the set up of sediment oxygen demand experiment

4.2.3 Sediment nutrient flux and concentration

The sediment tubes were also used to estimate the potential nutrient release from the anoxic sediments. The nutrient flux rates were estimated by the change in nutrient concentrations in the filtered water at the start and completion of the experiment, assuming linearity in fluxes over time. The estimated nutrient flux rates were expressed as nutrient (mass) per unit surface area per unit time (mg m^{-2} d^{-1}). The incubations for both the August 2008 and May 20010 experiments were conducted in the dark at 20°C (a typical temperature in the bottom of Myponga Reservoir in summer) for about 4.5 days and 5.5 days respectively. The nutrients of the interest for which flux was measured were ammonia (NH_3), nitrate and nitrite (NO_x), total Kjeldahl nitrogen (TKN), filterable reactive phosphorus (FRP), total phosphorus (TP), total manganese (Mn) and total iron (Fe). Total nitrogen (TN) was the sum of NO_x and TKN. Chemical analysis was undertaken by Australian Water Quality Centre (Adelaide, South Australia) for the August 2008 experiment and by Analytical Services Unit of CSIRO Land and Water (Urrbrae, South Australia) for the May 2010 experiment.

After the August 2008 incubation, the sediments were harvested and the sediment nutrient concentrations of organic matter (OM), total carbon (TC), total nitrogen (TN), TP, Mn and Fe were determined. Approximately 0.2 g of oven-dried fine sediments were used to measure the TN concentration using a LECO TruSpec C/H/N Elemental Determinator (LECO, St. Joseph, USA) with blank and EDTA calibrations. The sediment samples were combusted at a maximum temperature of 950°C. For determination of TP, Fe and Mn concentrations, approximately 0.1 g of oven-dried fine sediments were analysed by an acid dissolution method in a 1:3 mixture of 65% nitric acid and 37% hydrochloric acid at 140°C (Zarcinas *et al.* 1996). The digested solutions with 1% acid were diluted with 20 mL of deionised water and then were sent to Waite Analytical Services (Urrbrae, South Australia) for the analyses of TP, Fe and Mn using Inductively Coupled Plasma Optical Emission Spectrometry.

4.2.4 Risk assessment for cyanobacteria in relation to sediment phosphorus flux

A risk assessment methodology was used to evaluate the potential impact of sediment phosphorus flux on a population of cyanobacterium and the following assumptions were made: (i) phosphorus was the only limiting factor for the growth of *Anabaena circinalis*, the problematic toxin- and odour-producing cyanobacterium in Myponga Reservoir; other factors (e.g. light, temperature, grazing, etc) were assumed to have no

impact on the growth of *Anabaena circinalis*; (ii) to maximize the possible risk, the entire amount of TP flux was assumed to be bio-available and was taken up by the one species *Anabaena circinalis*; (iii) 0.8 µg of TP could produce 1 µg of chlorophyll *a* (Chl*a*) of *Anabaena circinalis* obtained from the measured data of TP:Chl*a* in Chapter 6; (iv) a healthy cell of *Anabaena circinalis* contained 0.6 – 0.9 pg of chlorophyll *a* (Reynolds 1984); and (v) the *Anabaena* cells in the water column floated and accumulated into 1 m of water surface.

Using the relationships between phosphorus cellular content and *Anabaena circinalis* cell concentration from literature and experimental data, the risk assessment can approximate *Anabaena* cell numbers in relation to the degree of stratification and the release of sediment phosphorus under anoxic conditions. The *Anabaena* cell numbers were then used to predict the production of the problematic metabolites, saxitoxin and geosmin. This was based on *Anabaena* cell numbers and the saxitoxin and geosmin contents of *Anabaena* cells in natural populations of Australian freshwater systems. The risk assessment for the potential release of saxitoxin and geosmin was approximated using the data of Burch (2010), where 0.33 µg L^{-1} of extracellular saxitoxins was produced by every 5000 cells of *Anabaena circinalis*, 50 – 150 ng of intracellular geosmin was produced by every microgram of chlorophyll *a* of *Anabaena circinalis* and 15% – 40% of the intracellular geosmin could be released to water (extracellular geosmin).

4.2.5 Statistical analyses

All results were examined to determine any significant difference using Statistical Package for the Social Sciences (SPSS) 15.0 for Windows. Using the statistical approach of Coakes *et al.* (2009), data were first checked for homogeneity of the variance test (Levene's test). When a sample of data was normally distributed and had equal variances amongst the pool of samples (p-value of Levene's test > 0.05), one-way analysis of variance (ANOVA) was performed. When samples failed the Levene's test (p-value < 0.05), a robust Welch ANOVA test was used to examine the equality of means.

4.3 Results

4.3.1 Thermal gradients between surface and bottom water

Prior to the installation of aerators, Myponga Reservoir exhibited a distinctive thermal stratification in the deep parts of the reservoir in summer (Brookes *et al.* 2000). The weekly profile of water temperature revealed the temperature difference between the water surface and the reservoir bottom (Figure 12). The maximum water temperature difference recorded on 2nd February 1993 was as great as 12°C between the surface water (25°C) and the bottom water (13°C). Table 10 shows the details of the duration and the intensity of the seasonal stratification observed in the natural condition. In most years, the stratification lasted for weeks to months. In the most severe incident, a stable stratification with a temperature gradient of 7°C was maintained over four months during the 1985-1986 summer. Since an aerator has been installed at Dam Wall in June 1993, the distinctive thermal stratification has been weakened and slowly dissipated over time (Figure 12 and Table 10). The statistical analysis of the temperature data displayed the temperature differences with and without the aerator/mixers in Table 11 (One-way/Welch ANOVA test, $p < 0.05$).

For both the main basin (MET 1) and side-arm (MET 2), the surface water temperature was greater than the bottom water temperature by 1ºC for about 50% of the time (Table 11). There were also various occasions, where the surface temperature was greater than the bottom water temperature by 3ºC. The distinct temperature gradient implies that there was little mixing between the surface and bottom water. Hence, the replenishment of oxygen from the atmosphere to the bottom water was limited during these periods. The data in Table 11 also shows a significant difference of water temperature between sites (One-way/Welch ANOVA, $p > 0.05$, $n = 10$). The percentage of time, where the surface water temperature differed from the bottom water temperature by 0.5 - 5°C was higher in the side-arm than the main basin. Stratification occurred more often in the side-arm than the main basin, probably due to the influence of the mixers and greater wind fetch.

Apart from the frequency and intensity of stratification, it is also important for knowing the duration of stratification in order to evaluate the potential impact of water quality. For most stratification events, the temperature gradient between surface and bottom water ceased within 24 hours (Table 12). However, there were a number of occasions,

where the stratification was maintained over 5 days. The longer the duration of stratification the higher the chance of anoxia develops in the sediment-water interface. A good example to illustrate the potential formation of a persistent thermal stratification is the continuous extreme hot weather. In the last few years, South Australia has experienced a number of record-breaking heatwaves (National Climate Centre 2009). A period of six consecutive days of air temperatures above 40°C resulted in a persistent thermal stratification in Myponga Reservoir between 27th January and 1st February 2009 (Figure 13). During the heatwave period, the epilimnetic water (22°C – 26°C) did not mix with the hypolimnetic water (21°C – 22°C) with a distinct thermocline at about 6 m. Without mixing between epilimnetic and hypolimnetic water, there would be minimal replenishment of oxygen to the lake bottom. Consequently, the consumption of dissolved oxygen by microbial decomposition may have led to anoxia at the sediment-water interface.

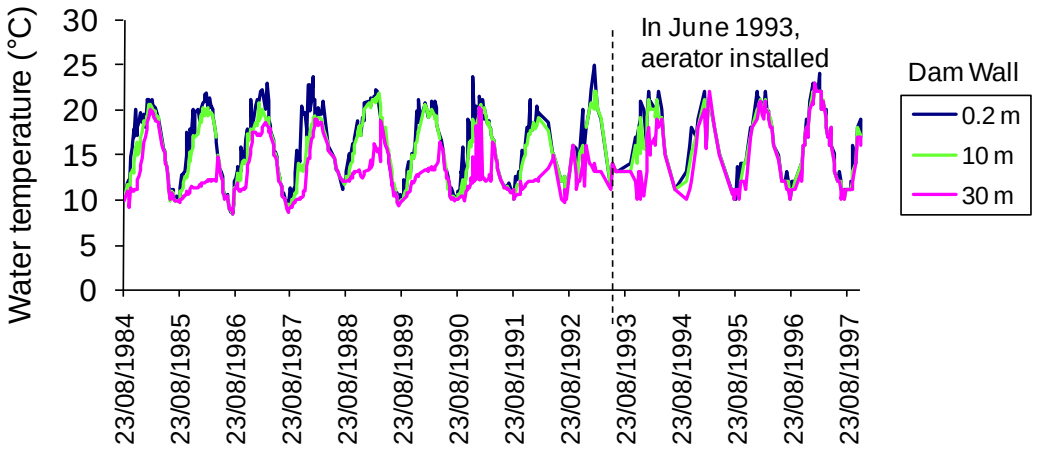


Figure 12 Weekly water temperature profiles (°C) were recorded near the dam wall of Myponga Reservoir between 23 August 1984 and 23 August 1997. The dashed line represents the installation of aerator in June 1993

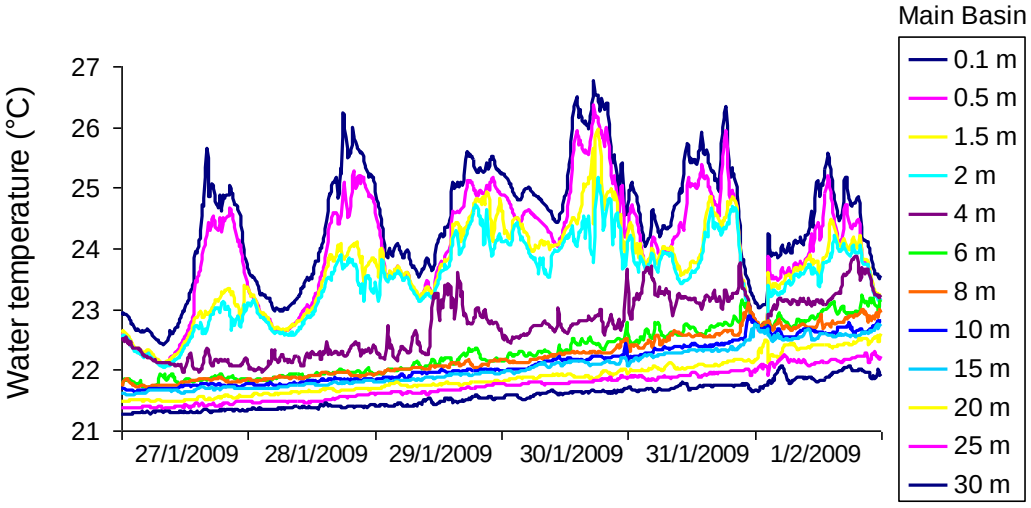


Figure 13 Water temperature profiles (°C) in the main basin (MET 1) of Myponga Reservoir during the heatwave in 2009 summer

Table 10

A comparison of the maximum number of days before aerator installation (1984 – 1993) and after installation (1993 – 1997) near the Dam Wall of Myponga Reservoir, where water temperature at the surface (0.2 m) differed from temperature at the bottom (30 m) by various temperature gradients over time (modified from Brookes *et al.* 2000)

Table 11 A comparison of the percentage of time before aerator installation (1984 – 1993) and after installation (1993 – 1997) near the Dam Wall and between main basin (MET 1) and side-arm (MET 2) of Myponga Reservoir, where water temperature at the surface (0.2 m) differed from temperature at the bottom (30 m for Dam Wall and MET 1 and 15 m for MET 2) by various temperature gradients over time. One-way/Welch ANOVA was used to determine any significance between sites for each temperature difference

Site	Influence by aerator/mixer	Years (1st Sep – 1st May)	Percentage of time, where water temperature at water surface differed from temperature at reservoir bottom by θ °C									
			θ ≤ 0	> 0.1	> 0.5	> 1	> 2	> 3	> 4	> 5	> 6	≥ 7
	One-way/Welch ANOVA: dam wall No aerator vs With aerator		NS	NS	*	**	NS	***	*	**	*	NS
Dam wall	No	1984 – 1985	0.00	100.00	87.89	72.20	50.22	43.95	31.39	28.25	12.56	6.28
		1985 – 1986	3.36	96.64	96.64	96.64	93.70	87.82	87.82	76.47	70.59	55.04
		1986 – 1987	0.00	100.00	89.36	86.38	74.47	44.26	26.81	5.96	0.00	0.00
		1987 – 1988	0.00	100.00	79.31	70.26	64.22	57.33	42.24	28.02	21.98	18.97
		1988 – 1989	0.00	100.00	91.07	91.07	87.95	84.82	72.32	53.13	34.38	21.88
		1989 – 1990	0.00	100.00	100.00	93.94	85.28	76.19	64.94	57.58	51.52	27.27
		1990 – 1991	2.52	94.54	79.83	75.21	75.21	63.45	60.92	55.04	38.66	29.83
		1991 – 1992	0.00	100.00	100.00	100.00	93.20	85.44	77.18	40.78	32.04	13.59
		1992 – 1993	3.85	96.15	96.15	96.15	88.46	69.23	69.23	65.38	29.12	21.43
	Aerator	1993 – 1994	0.00	100.00	100.00	96.15	92.31	65.38	53.85	19.23	19.23	15.38
		1994 – 1995	29.63	70.37	66.67	44.44	18.52	18.52	18.52	7.41	0.00	0.00
		1995 – 1996	30.30	66.67	63.64	36.36	15.15	0.00	0.00	0.00	0.00	0.00
		1996 – 1997	49.36	50.64	44.68	12.77	0.00	0.00	0.00	0.00	0.00	0.00
	One-way/Welch ANOVA: Main basin vs Side-arm		NS	NS	*	**	***	**	***	*	NS	NS
Main basin (MET 1)	Mixer	1999 – 2000	6.25	88.79	66.14	44.17	19.95	7.55	1.88	0.26	0.00	0.00
		2000 – 2001	13.02	82.18	62.76	44.67	21.25	9.43	3.49	1.04	0.23	0.03
		2001 – 2002	5.10	91.00	69.57	43.27	16.77	7.43	1.07	0.16	0.02	0.01
		2002 – 2003	5.24	88.28	60.36	34.59	10.73	3.40	1.09	0.39	0.11	0.01
		2007 – 2008	1.01	91.15	66.70	45.85	20.96	9.22	3.29	0.97	0.18	0.01
		2008 – 2009	0.40	97.38	81.67	54.06	17.34	4.01	0.61	0.01	0.00	0.00
Side-arm (MET 2)	No	1999 – 2000	3.32	94.03	78.90	58.82	30.71	15.43	6.64	2.68	0.85	0.16
		2000 – 2001	4.72	92.40	76.77	61.42	35.01	17.57	7.99	2.73	0.91	0.37
		2001 – 2002	3.25	94.52	81.16	61.54	29.49	13.06	4.65	1.41	0.17	0.01
		2002 – 2003	0.51	93.85	75.40	50.72	24.19	9.42	3.73	1.47	0.49	0.22

Significant difference at different temperature gradients

NS Not significant * $p < 0.05$

** $p < 0.01$ *** $p < 0.005$

Table 12 A comparison of the frequency of the numbers of hours between main basin (MET 1) and side-arm (MET 2) of Myponga Reservoir, where water temperature at the surface (0.2 m) differed from temperature at the bottom (30 m for MET 1 and 15 m for MET 2) by various temperature gradients over time

Site	Years (1st Sep – 1st May)	Temperature gradient θ (°C)	Frequency of numbers of hours, where water temperature at water surface exceeds temperature at reservoir bottom by θ °C								
			≤ 3	3 - 6	6 - 12	12 - 24	24 - 48	48 - 72	72 - 96	96 - 120	≥ 120
Main basin (MET 1)	1999 – 2000	0.5	139	16	25	23	6	2	5	4	6
		1	120	31	30	20	8	3	0	1	5
		2	104	16	23	20	6	1	2	0	0
		3	79	12	15	7	0	1	0	0	0
	2000 – 2001	0.5	143	15	24	26	9	3	3	4	4
		1	129	25	38	29	5	6	1	0	3
		2	118	27	20	19	4	1	0	0	2
		3	81	11	9	9	3	0	0	0	1
	2001 – 2002	0.5	113	10	17	20	10	2	2	1	2
		1	111	22	20	26	2	3	0	0	1
		2	85	14	14	1	1	1	0	0	1
		3	68	5	7	1	0	0	1	0	0
	2002 – 2003	0.5	170	24	30	36	7	6	2	1	5
		1	123	22	38	28	9	3	1	0	2
		2	87	15	19	17	1	0	0	0	0
		3	56	3	7	3	1	0	0	0	0
	2007 – 2008	0.5	68	10	14	21	5	1	1	1	4
		1	62	13	18	19	2	2	0	1	4
		2	49	10	14	16	0	2	0	0	1
		3	52	11	7	5	4	0	0	0	0
	2008 – 2009	0.5	41	5	11	17	7	3	1	2	9
		1	67	21	28	30	7	6	1	2	3
		2	81	15	19	15	3	2	0	0	0
		3	57	4	9	3	0	0	0	0	0
Side-arm (MET 2)	1999 – 2000	0.5	161	8	23	38	9	5	5	1	8
		1	138	8	32	42	14	4	2	1	5
		2	118	12	33	26	11	3	0	1	1
		3	1	0	0	0	0	0	0	0	0
	2000 – 2001	0.5	151	12	17	41	8	1	3	3	4
		1	116	16	19	31	9	4	4	4	5
		2	158	16	32	37	5	4	0	0	3
		3	136	17	31	21	4	0	0	1	0
	2001 – 2002	0.5	83	4	13	15	5	8	2	2	9
		1	93	11	14	34	7	7	3	1	3
		2	123	17	31	24	2	2	0	0	1
		3	125	22	15	7	2	1	0	0	0
	2002 – 2003	0.5	140	7	17	38	12	8	8	2	8
		1	97	22	25	44	6	7	3	2	3
		2	106	23	36	27	6	2	1	0	0
		3	114	14	20	9	2	0	0	0	0

4.3.2 Thermal gradients in the bottom 5 m

Detailed assessments of the duration of individual micro-stratification were conducted across sites, between years and at different temperature gradients. In the main basin (MET 1), the percentage of time, where water temperature differed between the bottom 5 m by 0.1°C varied from 8% to 47% (Table 13). When the temperature difference between 25 m and 30 m exceeded 0.3°C, the percentage of time dropped to 0.88% – 10%. A similar trend was also observed in the side-arm (MET 2). However, the percentage of time, where water temperature differed between the bottom 5 m was significantly higher in the side-arm than the main basin when the temperature differences varied between 0.1°C and 0.5°C (One-way/Welch ANOVA, $p > 0.05$, $n = 10$). This suggests that there is a higher chance for the side-arm to develop anoxic conditions in the bottom water. However, the temperature difference between the bottom water in most cases lasted for less than three hours and disappeared within 24 hours (Table 14). On a few occasions, the temperature difference lasted for a few days, which increased the chance of oxygen depletion between the bottom 5 m layers of water. Table 14 shows the details of the frequency and duration of the temperature difference.

Table 13 A comparison of the percentage of time between main basin (MET 1) and side-arm (MET 2) of Myponga Reservoir, where water temperature differed between the bottom 5 m (25 - 30 m for MET 1 and 10 - 15 m for MET 2) by various temperature gradients over time. One-way/Welch ANOVA was used to determine any significance between sites for each temperature difference (n = 10)

Site	Years (1st Sep – 1st May)	Percentage of time, where water temperature differed between the bottom 5 m by θ °C									
		θ ≤ 0	> 0.01	> 0.05	> 0.075	> 0.1	> 0.15	> 0.2	> 0.3	> 0.5	≥ 1
		NS	NS	NS	*	*	***	***	***	**	-
Main basin (MET 1)	1999 – 2000	17.65	80.01	64.22	53.96	46.31	32.08	21.66	10.01	1.12	0.00
	2000 – 2001	23.17	73.22	52.97	38.33	28.03	14.24	5.99	1.47	0.00	0.00
	2001 – 2002	53.25	41.50	20.35	12.15	8.34	5.18	3.74	1.66	0.10	0.00
	2002 – 2003	16.92	86.11	51.41	35.45	24.49	12.26	4.55	1.28	0.06	0.00
	2007 – 2008	0.06	99.62	82.10	63.41	46.59	22.56	9.51	2.14	0.07	0.00
	2008 – 2009	2.64	94.48	79.00	62.90	45.38	21.01	5.83	0.88	0.00	0.00
Side-arm (MET 2)	1999 – 2000	5.32	93.96	85.83	77.85	70.72	55.57	42.65	25.77	7.93	0.44
	2000 – 2001	4.82	93.85	83.49	74.51	66.36	46.93	33.38	20.23	6.99	0.65
	2001 – 2002	13.88	83.65	66.94	55.95	46.80	31.60	22.11	11.46	3.72	0.11
	2002 – 2003	0.28	99.28	90.31	80.49	72.12	56.02	41.69	23.66	7.19	0.03

Significant difference between MET 1 and MET 2 at different temperature gradients

NS	Not significant
*	$p < 0.05$
**	$p < 0.01$
***	$p < 0.005$
-	Statistical tests cannot be performed because at least one group has zero variance

Table 14 A comparison of the frequency of the numbers of hours between main basin (MET 1) and side-arm (MET 2) of Myponga Reservoir, where water temperature differed between the bottom 5 m (30 m for MET 1 and 15 m for MET 2) by various temperature gradients over time

Site	Years (1st Sep – 1st May)	Temperature gradient θ (°C)	Frequency of numbers of hours, where water temperature at the bottom 5 m exceeds temperature at reservoir bottom by θ °C								
			≤ 3	3 - 6	6 - 12	12 - 24	24 - 48	48 - 72	72 - 96	96 - 120	≥ 120
Main basin (MET 1)	1999 – 2000	0.05	1044	65	43	34	12	5	1	3	2
		0.075	939	69	37	26	12	5	2	1	2
		0.1	953	47	36	29	9	6	0	0	1
		0.15	676	55	37	13	4	3	0	0	1
	2000 – 2001	0.05	1049	87	57	40	9	5	3	0	0
		0.075	1012	79	37	26	10	2	1	0	0
		0.1	837	60	37	15	7	1	1	0	0
		0.15	562	34	20	5	2	1	0	0	0
	2001 – 2002	0.05	727	21	11	4	3	0	0	0	0
		0.075	527	12	6	1	2	0	0	0	0
		0.1	421	9	3	1	1	0	0	0	0
		0.15	316	4	5	1	0	0	0	0	0
	2002 – 2003	0.05	1303	122	51	18	7	3	0	0	1
		0.075	1021	64	30	14	4	1	0	0	1
		0.1	715	48	19	9	2	1	0	0	1
		0.15	349	18	8	4	1	1	0	0	1
	2007 – 2008	0.05	298	56	31	32	9	9	2	1	3
		0.075	387	62	36	29	12	2	3	0	2
		0.1	343	56	30	31	5	2	0	1	1
		0.15	224	43	23	13	2	0	0	0	0
	2008 – 2009	0.05	353	58	48	42	22	11	3	0	3
		0.075	508	82	51	48	21	6	0	0	1
		0.1	534	73	52	25	10	4	0	0	1
		0.15	316	38	29	17	4	0	0	0	0
Side-arm (MET 2)	1999 – 2000	0.05	488	46	33	34	25	11	4	1	6
		0.075	585	40	52	36	30	9	3	1	2
		0.1	721	49	54	46	24	6	1	0	2
		0.15	701	58	56	41	13	2	0	1	2
	2000 – 2001	0.05	680	48	33	48	24	9	11	3	1
		0.075	830	61	46	54	19	8	8	0	1
		0.1	1004	73	53	58	12	7	3	1	1
		0.15	1163	59	41	37	10	3	1	0	1
	2001 – 2002	0.05	858	50	31	33	13	5	3	1	1
		0.075	821	39	35	37	13	2	0	2	0
		0.1	799	43	44	28	10	2	1	0	0
		0.15	637	36	29	19	7	1	0	0	0
	2002 – 2003	0.05	626	26	34	28	16	14	14	4	4
		0.075	729	39	30	37	29	6	9	3	3
		0.1	733	45	49	42	21	5	8	2	2
		0.15	832	71	42	50	17	3	1	2	0

4.3.3 Oxygen demand for water and sediments

Oxygen demand for Myponga water and sediments was determined using the oxygen consumption rate over the first few hours of the August 2008 incubation due to the malfunction of the sensors. The DO concentrations over a longer incubation period were projected based on the initial exponential decline as presented in the equations of Figure 14a. The projection provided a rough estimation of the incubation time required for the depletion of DO, which can be compared with the measurements of the May 2010 experiment. Total oxygen demand (water + sediment oxygen demand) for Myponga sediments varied from 58 to 678 mg m^{-2} d^{-1} with an average of 299 mg m^{-2} d^{-1} (Table 15). The water oxygen demand was as high as 271 mg m^{-2} d^{-1}. The measured oxygen demand curve during May 2010 incubation showed a rapid decline in dissolved oxygen concentration during the first 24 hours to approximately 20% of the air saturation (Figure 14b). This is followed by a steady decrease toward 0% over the remaining time. The uncorrected sediment oxygen demand for the May 2010 incubation varied from 7.25 to 12.07 mg m^{-2} d^{-1}, whereas the water oxygen demand from the control treatment varied from 8.25 to 20.31 mg m^{-2} d^{-1} (Table 15). There was no significant difference for the average oxygen demand between the shallow (9.51 mg m^{-2} d^{-1}) and deep (10.81 mg m^{-2} d^{-1}) sites, based on the ANOVA test ($p > 0.05$).

Table 15 Oxygen consumption rates (mg m^{-2} d^{-1}) calculated from the linear slope (mg L^{-1} d^{-1}) of the specific incubation time (hour) and for Myponga water (control) and sediments for the August 2008 and May 2010 incubations (± standard deviation)

August 2008	Incubation time (hour)	Lineal slope (mg L^{-1} d^{-1})	Oxygen consumption rate (mg m^{-2} d^{-1})
Water (control)	7	0.264	**271**
Water + Sediment (average of sites)	5.5	0.293	**299**
Site 1	3	0.329	**632**
Site 2	6	0.378	**348**
Site 7	9	0.100	**58**
Site 10	5	0.521	**575**
Site 15	7	0.260	**214**
Site 16	3	0.353	**678**
May 2010			
Water (average for control)	24	0.048 ± 0.03	**14.28** ± 8.53
Water + Sediment (average for shallow Site 5)	24	0.038 ± 0.01	**9.51** ± 2.00
Water + Sediment (average for deep Site 7)	24	0.056 ± 0.01	**10.81** ± 1.67

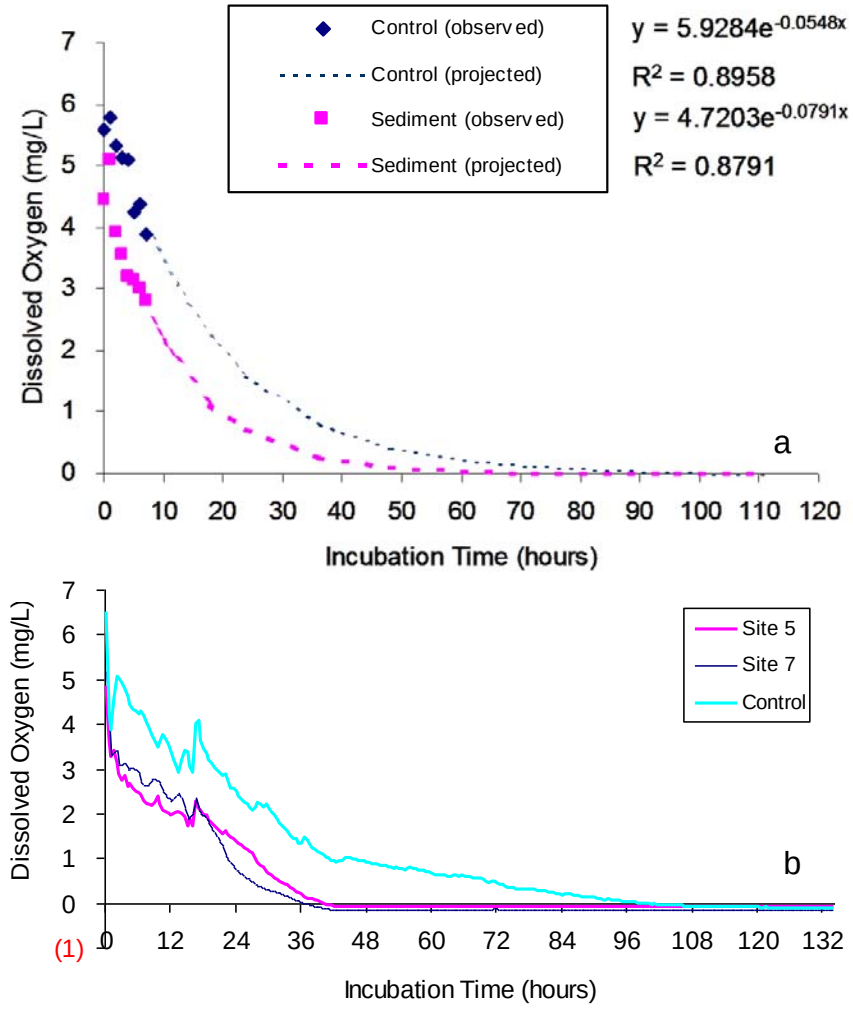


Figure 14 Oxygen demand curves measured from the two incubation experiments: (a) average oxygen demand for Control (water only) and Sediment (water plus sediment) for the August 2008 experiment. Exponential equations from the initial observed data (makers) were used for projection of dissolved oxygen concentrations (dashed lines) over longer incubation time, (b) average oxygen demand for Site 5 (shallow), Site 7 (deep) and Control (water only) for the May 2010 experiment

4.3.4 Sediment nutrient flux

The concentrations of OM, TC, TN, TP, Mn and Fe for Myponga sediments collected during August 2008 were measured and expressed as milligrams per kilograms of dry sediments (Table 16; Chapter 3 for details). The sediment cores were used for the incubation studies and the nutrient concentrations of the overlying water were analysed before and after the incubation. In general, nutrient fluxes after anoxic incubations were quite similar between the August 2008 and May 2010 experiments (Table 17). For both experiments, the concentrations of NH_3, TN, TP, Mn and Fe increased greatly and the NO_x concentration decreased during the incubations. The FRP concentration in the water decreased after the anoxic incubation during the August 2008 experiment, but increased during the May 2010 experiment. However, the nutrient concentrations in the overlying water varied depending upon the volume of water in the sediment cores. The nutrient concentrations in the water, therefore, were converted to a rate of nutrient flux per unit area of sediment per unit of time for appropriate comparison.

The nutrient flux rates calculated from the differences of initial and final concentrations of the overlying water were presented in Table 18. The majority of nutrients were released from the sediments (e.g. NH_3, TN, FRP, Mn and Fe), as the flux rates of sediments (plus water) were higher than the flux rates of water (control). The negative flux rates of NO_x and FRP corresponded to the decrease in nutrient concentrations after the incubation. The bio-available nutrients were likely to be taken up by microbes and transformed to organic forms, which was reflected in the high flux rates of TN (4.2 – 12.6 mg m^{-2} d^{-1}) and TP (0.4 – 3.1 mg m^{-2} d^{-1}).

Table 16 Concentrations of organic matter (OM), total carbon (TC), total nitrogen (TN), total phosphorus (TP), total manganese (Mn) and total iron (Fe) in Myponga sediments collected in August 2008. All units are expressed as mg kg^{-1}

	Sediment nutrient concentrations (mg kg^{-1})*					
August 2008	**OM**	**TC**	**TN**	**TP**	**Mn**	**Fe**
Sediment (average of sites)	**113518**	**52476**	**4069**	**967**	**567**	**32833**
Site 1	76923	38127	2669	410	390	25000
Site 2	156250	63391	5428	1720	770	42000
Site 7	153226	64200	5790	1450	550	44000
Site 10	89109	47886	3500	910	960	34000
Site 15	100840	49435	3265	480	500	29000
Site 16	104762	51818	3763	830	230	23000

* To convert mg kg^{-1} to %, divide the value by 10,000

Table 17 Nutrient concentrations (mg L^{-1}) of the overlying water (± standard deviation) before and after the anoxic incubations

		Nutrient concentrations in overlying water (mg L^{-1})						
August 2008		**NH_3**	**NO_x**	**TN**	**FRP**	**TP**	**Mn**	**Fe**
Before incubation (initial)		<0.005	0.153	0.923	0.027	0.046	0.005	0.066
After incubation	Water (control)	0.036	0.039	1.019	0.012	0.047	0.017	0.488
	Water + Sediment (average of sites)	0.511	0.052	1.703	0.006	0.112	0.825	0.886
	Site 1	0.546	0.040	1.850	<0.005	0.167	0.906	0.708
	Site 2	0.361	0.069	1.499	0.005	0.082	0.652	0.634
	Site 7	0.850	0.027	2.017	0.006	0.101	0.747	0.991
	Site 10	0.314	0.053	1.513	0.008	0.094	1.410	1.157
	Site 15	0.242	0.057	1.297	<0.005	0.086	0.888	0.895
	Site 16	0.755	0.063	2.043	<0.005	0.143	0.348	0.931
May 2010								
Before incubation (initial)		0.008 ± 0.00	0.111 ± 0.00	1.367 ± 0.04	0.019 ± 0.00	<0.100	<0.030	0.032 ± 0.01
After incubation	Water (average for control)	0.128 ± 0.09	0.068 ± 0.05	2.100 ± 0.34	0.041 ± 0.05	0.126 ± 0.05	0.030 ± 0.00	0.073 ± 0.03
	Water + Sediment (average for shallow Site 5)	0.686 ± 0.26	0.011 ± 0.00	2.740 ± 0.28	0.046 ± 0.03	0.402 ± 0.21	1.083 ± 0.04	6.231 ± 1.84
	Water + Sediment (average for deep Site 7)	0.863 ± 0.10	0.010 ± 0.00	2.823 ± 0.57	0.076 ± 0.08	0.624 ± 0.45	1.289 ± 0.37	9.929 ± 4.66

Table 18 Nutrient flux rates (mg m^{-2} d^{-1}) from Myponga water (control) and sediments to the overlying water (± standard deviation) after the anoxic incubations

	Nutrient flux rates (mg m^{-2} d^{-1})						
August 2008	**NH_3**	**NO_x**	**TN**	**FRP**	**TP**	**Mn**	**Fe**
Water (control)	0.435	-1.596	1.349	-0.208	0.013	0.169	5.916
Water + Sediment (average of sites)	5.111	-1.105	8.511	-0.232	0.721	8.945	8.945
Site 1	6.071	-1.266	10.399	-0.249	1.360	10.109	7.203
Site 2	3.829	-0.905	6.191	-0.239	0.388	6.957	6.107
Site 7	8.691	-1.294	11.250	-0.214	0.566	7.630	9.514
Site 10	3.073	-1.074	6.346	-0.204	0.517	15.108	11.731
Site 15	2.460	-1.079	4.198	-0.249	0.446	9.906	9.299
Site 16	7.784	-1.012	12.568	-0.249	1.090	3.850	9.704
May 2010							
Water (average for control)	1.153 ± 0.91	-0.413 ± 0.49	7.057 ± 3.26	0.215 ± 0.45	0.253 ± 0.44	0.000 ± 0.00	0.395 ± 0.30
Water + Sediment (average for shallow Site 5)	5.442 ± 2.25	-0.803 ± 0.08	11.067 ± 2.94	0.230 ± 0.26	2.519 ± 2.00	8.447 ± 0.97	49.928 ± 17.20
Water + Sediment (average for deep Site 7)	5.370 ± 1.30	-0.629 ± 0.12	8.794 ± 2.80	0.343 ± 0.51	3.061 ± 2.67	7.620 ± 1.24	59.444 ± 24.82

4.3.5 Risk assessment for cyanobacteria in relation to sediment phosphorus flux

The average TP flux rate of Myponga sediments from the August 2008 incubation study, which was 0.721 mg m^{-2} d^{-1} (Table 18), was used for the risk assessment because most of the FRP was probably incorporated into bacterial biomass (Brookes and Deegan 2008). If the micro-stratification just above the sediments occurred and lasted for about a day, the dissolved oxygen levels at the sediment-water interface would become very low as observed in the incubation study. In the risk assessment, four scenarios of the TP flux for 1, 2, 3 and 4 days from sediments are presented in Table 19. These fluxes of TP at the sediment boundary were mixed to the adjacent 1 m of water. Potentially, the TP in the bottom water could be mixed through and diluted into the water column (i.e. 5, 15 and 30 m), with the aids of artificial mixing, seasonal overturn, storm-related strong mixing, river intrusion and lateral convective circulation (Ford 1990; Davison 1993; Robarts *et al.* 1998; Wells and Sherman 2001). Little variation of water temperature throughout the water column can be an indicator of the presence of water column mixing. The average TP concentration, mixed through 30 m water column, varied from 0.024 µg L^{-1} for the 1-day flux to 0.096 µg L^{-1} for the 4-day flux.

Assuming the release of TP from sediments was all absorbed by *Anabaena circinalis* cells, the chlorophyll *a* concentration could be estimated using the measured TP:Chl*a* ratio of 0.8 in Chapter 6. The calculated chlorophyll *a* concentration mixing through 30 m water column varied from 0.03 µg L^{-1} for the 1-day flux to 0.12 µg L^{-1} for the 4-day flux (Table 20). The population of *Anabaena circinalis* was estimated from the typical chlorophyll *a* content (0.6 – 0.9 pg Chl*a* per cell). The resultant cell concentrations appeared low (33 – 200 cell mL^{-1}) when they were evenly diluted and mixed through the water column. However, *Anabaena circinalis* cells are capable of migrating up and down with an aid of buoyancy regulation during calm periods (e.g. heatwaves) (Westwood and Ganf 2004). If all the cells floated and concentrated at the water surface to acquire light, the cell concentrations could easily increase to the alarmed levels of 1000 cell mL^{-1} for the 1-day flux of TP and up to 6000 cell mL^{-1} for the 4-day flux.

Table 19 Total phosphorus (TP) concentrations from the flux of Myponga anoxic sediments into 1 m of bottom water for different durations of flux (1 – 4 days) and different levels of water mixing (5, 15 and 30 m)

		TP flux rate from August 2008 experiment (mg m^{-2} d^{-1})	TP concentration due to flux in 1 m of water above sediments (mg m^{-3} or μg L^{-1})	TP concentration mixed through bottom (μg L^{-1})		
				5 m water column	15 m water column	30 m water column
1-day flux	**Water (control)**	0.013	0.013	0.003	0.001	0.000
	Water + Sediment (average of sites)	**0.721**	**0.721**	**0.144**	**0.048**	**0.024**
	Site 1	1.360	1.360	0.272	0.091	0.045
	Site 2	0.388	0.388	0.078	0.026	0.013
	Site 7	0.566	0.566	0.113	0.038	0.019
	Site 10	0.517	0.517	0.103	0.034	0.017
	Site 15	0.446	0.446	0.089	0.030	0.015
	Site 16	1.090	1.090	0.218	0.073	0.036
2-day flux	**Water (control)**	0.013	0.026	0.005	0.002	0.001
	Water + Sediment (average of sites)	**0.721**	**1.443**	**0.289**	**0.096**	**0.048**
	Site 1	1.360	2.719	0.544	0.181	0.091
	Site 2	0.388	0.776	0.155	0.052	0.026
	Site 7	0.566	1.132	0.226	0.075	0.038
	Site 10	0.517	1.034	0.207	0.069	0.034
	Site 15	0.446	0.893	0.179	0.060	0.030
	Site 16	1.090	2.179	0.436	0.145	0.073
3-day flux	**Water (control)**	0.013	0.039	0.008	0.003	0.001
	Water + Sediment (average of sites)	**0.721**	**2.164**	**0.433**	**0.144**	**0.072**
	Site 1	1.360	4.079	0.816	0.272	0.136
	Site 2	0.388	1.164	0.233	0.078	0.039
	Site 7	0.566	1.698	0.340	0.113	0.057
	Site 10	0.517	1.552	0.310	0.103	0.052
	Site 15	0.446	1.339	0.268	0.089	0.045
	Site 16	1.090	3.269	0.654	0.218	0.109
4-day flux	**Water (control)**	0.013	0.052	0.010	0.003	0.002
	Water + Sediment (average of sites)	**0.721**	**2.886**	**0.577**	**0.192**	**0.096**
	Site 1	1.360	5.438	1.088	0.363	0.181
	Site 2	0.388	1.552	0.310	0.103	0.052
	Site 7	0.566	2.264	0.453	0.151	0.075
	Site 10	0.517	2.069	0.414	0.138	0.069
	Site 15	0.446	1.785	0.357	0.119	0.060
	Site 16	1.090	4.359	0.872	0.291	0.145

Table 20 Risk assessment for potential populations of *Anabaena circinalis* supported by TP flux from Myponga anoxic sediments (based on August 2008 experiment) for different durations of flux (1 – 4 days). These estimations were based on assumptions of: (i) measured TP:Chl*a* in Myponga Reservoir = 0.8 ± 0.5 SD; (ii) a healthy *Anabaena circinalis* cell contained chlorophyll *a* in a range of 0.6 – 0.9 pg; and (iii) all *Anabaena* cells floated toward and accumulated in the water surface

		Estimated chlorophyll *a* mixed through 30 m water column ($\mu g\ L^{-1}$)	Estimated *Anabaena circinalis* mixed through 30 m water column (cell mL^{-1})		Estimated *Anabaena circinalis* floating into 1 m water surface (cell mL^{-1})	
			0.8:1 TP:Chl*a*			
			0.6 pg Chl*a* $cell^{-1}$	0.9 pg Chl*a* $cell^{-1}$	0.6 pg Chl*a* $cell^{-1}$	0.9 pg Chl*a* $cell^{-1}$
1-day flux	**Water (control)**	0.001	1	1	27	18
	Water + Sediment (average of sites)	**0.030**	**50**	**33**	**1503**	**1002**
	Site 1	0.057	94	63	2832	1888
	Site 2	0.016	27	18	808	539
	Site 7	0.024	39	26	1179	786
	Site 10	0.022	36	24	1077	718
	Site 15	0.019	31	21	930	620
	Site 16	0.045	76	50	2270	1514
2-day flux	**Water (control)**	0.001	2	1	54	36
	Water + Sediment (average of sites)	**0.060**	**100**	**67**	**3006**	**2004**
	Site 1	0.113	189	126	5665	3777
	Site 2	0.032	54	36	1616	1077
	Site 7	0.047	79	52	2359	1572
	Site 10	0.043	72	48	2155	1437
	Site 15	0.037	62	41	1859	1240
	Site 16	0.091	151	101	4541	3027
3-day flux	**Water (control)**	0.002	3	2	81	54
	Water + Sediment (average of sites)	**0.090**	**150**	**100**	**4509**	**3006**
	Site 1	0.170	283	189	8497	5665
	Site 2	0.048	81	54	2424	1616
	Site 7	0.071	118	79	3538	2359
	Site 10	0.065	108	72	3232	2155
	Site 15	0.056	93	62	2789	1859
	Site 16	0.136	227	151	6811	4541
4-day flux	**Water (control)**	0.002	4	2	108	72
	Water + Sediment (average of sites)	**0.120**	**200**	**134**	**6012**	**4008**
	Site 1	0.227	378	252	11330	7553
	Site 2	0.065	108	72	3232	2155
	Site 7	0.094	157	105	4717	3145
	Site 10	0.086	144	96	4310	2873
	Site 15	0.074	124	83	3719	2479
	Site 16	0.182	303	202	9081	6054

4.4 Discussion

4.4.1 Stratification and destratification

Thermal stratification is commonly observed in deep lakes in regions with Mediterranean and temperate climates from late spring to early autumn (Sundaram and Rehm 1973). Myponga Reservoir developed stable stratification with some annual variations of the intensity and duration before artificial mixing had been implemented in 1993, which was clearly shown in the early temperature monitoring program by SA Water (Figure 12). The resulting density gradient prevented the mixing between the warm epilimnion water and cool hypolimnion water and limited the replenishment of oxygen in the hypolimnion water. Gradual oxygen depletion in the hypolimnion could lead to the release of nutrients and metals from sediments, degrading the quality of water. In the years 1985-1986, Myponga Reservoir experienced persistent stratification over 124 days, where the mean daily water temperature at the surface water was higher than at the reservoir bottom by 7ºC. The prolonged period of thermal stratification gave rise to the maximum iron and manganese concentrations of 2.55 mg L^{-1} and 1.8 mg L^{-1} respectively (Brookes *et al.* 2000). By means of an aerator and surface mixers installed in 1993, the release of iron and manganese from anoxic sediments have been significantly reduced (Brookes *et al.* 2000). However, areas outside the effective zone of artificial mixing may still be subject to a certain degree of stratification, since the process of artificial destratification cannot completely mix the entire reservoir (Sherman *et al.* 2000). The duration and intensity of stratification at MET 2, which had no influence of artificial mixing, were significantly higher than at MET 1, which was subject to the artificial destratification (Table 11 and Table 12).

A gradient of water temperature is a good indication for layering of water. When the water persistently exists in two different temperatures, there is virtually no mixing (e.g. water and chemical exchange) between the two layers. This also applies to fine-scale of temperature gradients, provided that the temperature sensors are sensitive enough to capture minor temperature differences. The temperature sensors for this study were highly sensitive to the temperature change with a high measurement resolution of ±0.003°C. If the temperature gradient between water layers was greater than 0.1°C, the temperature difference was most likely due to the real temperature change rather than an artefact due to the sensitivity of the sensors. Therefore, the temperature gradient of 0.1°C was used as the threshold to determine the presence or absence of micro-stratification between the bottom 5 m layers of the reservoir water.

Advanced sensing technology provides opportunities to detect any fine-scale stratification. Analysis of collected data suggested that the fine-scale stratification was dominant across the bottom of Myponga Reservoir (Table 13 and Table 14). The temperature gradients between the bottom 5 m of water column were small, however the two layers of water were physically separated by the density difference. Temperature differences as small as a few hundredths of a degree can prevent mixing between water layers (Kratz *et al.* 2005). Small temperature gradients such as 0.04ºC could avoid downward movement of buoyant phytoplankton (Sherman *et al.* 2000) and dissolved compounds.

At the side-arm (MET 2), where there was no influence of artificial mixing, the micro-stratification occurred for 64% of the potential stratification time (1st September – 1st May), with a 0.1ºC temperature gradient from sediment-water interface to 5 m above the sediment surface. Under the influence of artificial mixing, the micro-stratification in the main basin (MET 1) was only observed approximately 33% of the potential stratification time. Within these periods of stratification, there were many occasions, where the stratification was maintained for 48 hours or above. The duration of the stratification could further be enhanced by the exceptional and prolonged hot weather. The heatwave (maximum daily air temperature above 35°C) in Adelaide lasted for eight consecutive days in the first half of March 2008 and for nine consecutive days during late January and early February 2009 (National Climate Centre 2009). These hot summer days exacerbated the development of anoxia near the sediments. This has also been observed in deep, thermally stratified lakes in Switzerland, where the extremely high degree of thermal stability resulted in extraordinarily strong hypolimnetic oxygen depletion during the 2003 European heatwave (Jankowski *et al.* 2006).

4.4.2 Oxygen demand and nutrient flux under anoxia

In the isolated bottom water, microbial respiration and chemical oxidation of reduced chemical species continuously consume oxygen until depletion (Adams *et al.* 1982; Doyle and Lynch 2005). The reported oxygen demand of this study represents the biological and chemical oxygen demand without the influence of photosynthesis. The sediment oxygen demand was corrected by the water oxygen demand (control), which is usually low and negligible relative to the sediment oxygen demand (Gelda *et al.* 1995). However, for Myponga water oxygen demand was occasionally similar to, or even higher, than the total oxygen demand, suggesting little oxygen consumption by

sediments in the particular samples. High water oxygen demand may be explained by the oxidation of naturally-occurring reduced chemicals (e.g. Fe^{2+}, Mn^{2+}, NH^{4+}, HS^{-} and CH_4) (Gelda *et al.* 1995) and the oxidative decomposition of abundant dissolved organic matter in water (Adams *et al.* 1982). Since the total oxygen demand (water + sediment) in this study was not corrected by water oxygen demand, careful interpretation was required when comparing with other studies.

The incubation study of undisturbed sediment cores (surface area ~ 0.00255 m^2) from Myponga Reservoir showed that the isolated water (volume ~ 0.6 L) became anoxic (< 1 mg O_2 L^{-1}) approximately within one day. For the August 2008 experiment, the oxygen demand for Myponga sediments at the initial incubation period (equipment breakdown) varied from 58 to 678 mg m^{-2} d^{-1}, with an average of 299 mg m^{-2} d^{-1}. These values were comparable to the oligotrophic stratified Lake Superior, where values ranged from 106 to 240 mg m^{-2} d^{-1} (Carlton *et al.* 1989) and the mesotrophic stratified Lake Erie with an average sediment oxygen demand of 300 mg m^{-2} d^{-1} (Adams *et al.* 1982). Lakes with high productivity and large hypolimnetic areas give rise to high sediment oxygen demand (Beutel 2003). For example, Onondaga Lake in New York, which is hypereutrophic and stratified, has a high sediment oxygen demand, ranging from 1750 to 6150 mg O_2 m^{-2} d^{-1} (Gelda *et al.* 1995). The nutrient release rates from the undisturbed laboratory incubation, however tended to be lower than *in situ* release rates (Nurnberg *et al.* 1986), as currents near the sediment-water interface can create a greater diffusional driving force, resulting in a higher sediment oxygen demand (Beutel 2003).

During the second experiment in May 2010, much lower sediment oxygen demand (7.25 – 12.07 mg m^{-2} d^{-1}) were found because the oxygen consumption rates were taken from different stages of the oxygen demand curves (i.e. first few hours for August 2008 experiment and first 24 hours for May 2010 experiment). Some researchers used the slope of the first three hours of the dissolved oxygen consumption curves to calculate the sediment oxygen demand (Doyle and Lynch 2005), whereas others have utilised the slope of the whole incubation period, ranging from 2 to 8 days (Adams *et al.* 1982; Beutel 2003). Nevertheless, it is important to note that the oxygen demand for a long incubation time was probably limited by the oxygen availability in the confined incubation tube, not by the exhaustion of substrate that consumes oxygen (Brookes and Aldridge 2007). The presentation of the oxygen demand for both August 2008 and May

2010 experiments was included to highlight potential variations, arising from the duration of incubation time.

The low oxygen level of water appeared to initiate a range of chemical reductions at the sediment-water interface. At the end of the incubation study, the release of nutrients was high; the highest concentrations of NH_3, Mn and Fe in water were 0.9, 1.4 and 9.9 mg L^{-1} respectively. These concentrations far exceeded the acceptable levels for aesthetic considerations in the Australian Drinking Water Guidelines, which are 0.5 mg L^{-1} for NH_3, 0.1 mg L^{-1} for Mn and 0.3 mg L^{-1} for Fe (NHMRC 2004). The concentrations of NH_3, TN, TP, Mn and Fe in the overlying water increased significantly, while the concentrations of NO_x and FRP reduced. The trend and magnitude of these results were similar to the study in Lake Hume, Australia, where the sediments were a net source of NH_3 and Fe and a net sink of NO_x in the anoxic mesocosms (Baldwin and Williams 2007). Microbial activities could utilise small proportions of NO_x, but the majority of NO_x was most likely reduced to nitrogen gas via denitrification under anoxic conditions (Mitchell and Baldwin 1999). The formation of NH_3 from anoxic sediments may partly come from the microbially mediated dissimilatory nitrate reduction to ammonia (Rysgaard *et al.* 1993) or from the mineralization of organic matter via ammonification. The average NH_3 flux rate (5.111 mg m^{-2} d^{-1}) from the anoxic sediments of Myponga Reservoir was comparable to the flux rate of other meso/eutrophic systems in the United States ranging from 5 to 10 mg m^{-2} d^{-1} (Beutel 2006). For oligo/mesotrophic and eutrophic/hypereutrophic reservoirs, the release rates for NH_3 were <5 mg m^{-2} d^{-1} and >15 mg m^{-2} d^{-1}, respectively (Beutel 2006). Baldwin and Williams (2007) explained that the process of dissimilatory nitrate reduction to ammonia was negligible since the loss rate of NO_x was much slower than the production rate of NH_3; that is similar to the observation in Myponga Reservoir.

The result of Baldwin and Williams (2007) also corresponded to one of the current FRP results, which did not have the expected increase of FRP when Fe(II) ions were reduced under anoxia. However, the TP concentration in the overlying water increased dramatically at the end of the August 2008 incubation study. This is probably because the bio-available FRP released from the sediments was rapidly taken up by phosphorus-deficient cells (Reynolds 1984) and transformed into organic phosphorus that was part of the TP measurement. An alternative explanation is that the FRP desorbed through the Fe(III) to Fe(II) reduction and may re-adsorb onto non-reduced iron complexes, clay,

organic matter, etc (Baldwin *et al.* 2002). The average flux rate of TP from anoxic Myponga sediments (0.7 mg m^{-2} d^{-1}) was considerably low, similar to that in oligotrophic lakes (< 1 mg m^{-2} d^{-1}), whereas the TP flux rate from anoxic sediments was recorded up to 50 mg m^{-2} d^{-1} for hypertrophic lakes (Nurnberg 1988).

4.4.3 Estimated internal nutrient loads

The internal nutrient loads from anoxic sediments of Myponga Reservoir were estimated from the nutrient flux rates of the August 2008 incubation study (Table 18) and the surface area of the potential anoxic sediments (Auer *et al.* 1993). It was assumed that approximately 70% of the total surface area of the reservoir at its full capacity (2,000,000 m^2) could potentially develop an anoxic microlayer at the sediment-water interface. For the external load of nutrients, the annual catchment load data from Linden *et al.* (2004) was used. Myponga Reservoir receives large external nutrient loads from the catchment compared with the internal loads induced by anoxia. The external TP load was 4.8 – 10.4 times higher than the internal TP load induced by anoxic sediments (Table 21). However, the external load was mainly associated with the winter rainfall, while the internal load was associated with the summer stratification. In winter, high daily flows from the catchment (> 25 ML) gave rise to a greater percentage of TP load per volume under a non-linear flow to load relationship (Linden *et al.* 2004). Little or no external load of nutrients is expected in dry summer. Even though there may be episodic heavy rains in summer, the rain is most likely to be absorbed by the dry soil in the catchment. Little rainfall will contribute to the river and the reservoir. Rain events in Myponga regions started as early as in March, however the rainfall neither increased inflow to the reservoir nor nutrient levels in the reservoir until July when the soil profile was saturated (Chapter 6). Without the external input of nutrients in summer, the internal load of nutrients becomes the dominant source of nutrients to the water column, controlling the primary productivity.

Table 21 A comparison of the internal nutrient loads from anoxic sediments of Myponga Reservoir (August 2008 experimental data) and the external nutrient loads from catchment (modified from Linden *et al.* 2004). All units are expressed as kg d^{-1}

	Internal nutrient loads from anoxic sediments (kg d^{-1})						
August 2008	**NH_3**	**NO_x**	**TN**	**FRP**	**TP**	**Mn**	**Fe**
Water (control)	**0.87**	**-3.19**	**2.70**	**-0.42**	**0.03**	**0.34**	**11.83**
Water + Sediment (average of sites)	**10.22**	**-2.21**	**17.02**	**-0.46**	**1.44**	**17.89**	**17.89**
Site 1	12.14	-2.53	20.80	-0.50	2.72	20.22	14.41
Site 2	7.66	-1.81	12.38	-0.48	0.78	13.91	12.21
Site 7	17.38	-2.59	22.50	-0.43	1.13	15.26	19.03
Site 10	6.15	-2.15	12.69	-0.41	1.03	30.22	23.46
Site 15	4.92	-2.16	8.40	-0.50	0.89	19.81	18.60
Site 16	15.57	-2.02	25.14	-0.50	2.18	7.70	19.41

	External nutrient loads from catchment (kg d^{-1})						
Year	**NH_3**	**NO_x**	**TN**	**FRP**	**TP**	**Mn**	**Fe**
1999	**1.50**	**3.70**	**35.25**	**3.52**	**6.90**	**-**	**-**
2000	**3.01**	**3.27**	**81.79**	**6.32**	**15.03**	**-**	**-**

4.4.4 Cyanobacterial risk assessment and management implication

The use of fundamental, theoretical relationships between TP, chlorophyll *a* and *Anabaena circinalis* concentrations provided an opportunity to undertake a cyanobacterial risk assessment in Myponga Reservoir. The risk assessment is associated with the duration of thermal stratification immediately above the sediments and the flux of phosphorus from the anoxic sediments. The flux of phosphorus can promote the formation of *Anabaena* blooms, leading to the release of saxitoxin and geosmin in drinking water reservoirs. This increases the potential health and aesthetic risk to humans. Therefore, the risk assessment becomes an important tool for water authorities to estimate the impacts of *Anabaena* blooms. However, the risk assessment should be used with cautions because all released TP from sediments was assumed to be taken up solely by one species *Anabaena circinalis*. Also, the entire amount of TP flux from sediments was assumed to be bioavailable and this entire portion was assumed to reach the euphotic zone for phytoplankton uptake.

The risk assessment indicated that the 1-day flux of phosphorus from Myponga sediments (2-day stratification, leading to 1-day depletion of oxygen at the sediment-water interface) could promote approximately 1000 – 1500 *Anabaena* cells per millilitre of water, while the 4-day flux could lead to 4000 – 6000 cell mL^{-1} (Table 20). The estimations were related to cell floating and accumulating into the 1 m of water surface

for light harvesting in the area not affected by mixing. These cell concentrations trigger the low alert (500 – 2000 cell mL^{-1}) and high alert (≥ 5000 cell mL^{-1}) levels of the Alert Levels Framework for Cyanobacteria in Drinking Water (Burch 2006). The corresponding operational actions for the low alert are to collect weekly samples and cell counts and to regularly inspect water surface for algal blooms near the reservoir offtake. For the high alert actions, health risk assessments, including toxin monitoring and effectiveness of toxin treatment are required in addition to the low alert actions (Burch 2006).

The relationships among *Anabaena circinalis*, saxitoxin and geosmin were obtained from extensive measurements of natural cyanobacterial populations in Australia. Burch (2010) suggested that every 5000 *Anabaena circinalis* cells could produce about 0.33 µg L^{-1} of saxitoxins. The estimation of geosmin production was more complicated due to variations in the geosmin content in *Anabaena circinalis* cells and the release of extracellular geosmin into water. For the best case scenario in the risk assessment, 50 ng of geosmin was present in every microgram of chlorophyll *a* in *Anabaena circinalis*; 100 ng of geosmin for the most likely scenario; and 150 ng of geosmin for the worst case scenario. Based on this, geosmin in *Anabaena* cells (intracellular) could release 15%, 25% and 40% of geosmin to the water (extracellular) under the best case, most likely and worst case scenarios respectively.

The *Anabaena* cell concentrations induced from the sediment phosphorus flux could produce the high levels of saxitoxin and geosmin, based on the empirical relationships of the risk assessment (Table 22). The single day flux of TP from sediments could trigger the formation of *Anabaena* cells (1000 cell mL^{-1}) and the resultant release of intracellular saxitoxin (0.07 µg L^{-1}), intracellular geosmin (36 ng L^{-1}) and extracellular geosmin (5 ng L^{-1}) for the best case scenario. For the worst case scenario, the single day flux of sediment TP could induce 1500 cell mL^{-1} of *Anabaena*, 0.1 µg L^{-1} of saxitoxin, 162 ng L^{-1} of intracellular geosmin and 65 ng L^{-1} of extracellular geosmin. The release of saxitoxin may not exceed the provisional guidelines for drinking water (3 µg L^{-1}), however the release of geosmin can lead to consumer complaints of ‘smelly’ water. These resultant metabolites from *Anabaena circinalis* blooms therefore significantly increase the water treatment cost to provide palatable quality of drinking water since conventional water treatment (e.g. coagulation, sedimentation and filtration) is only effective for the removal of these compounds within cells. Additional treatments (e.g.

activated carbon and ozone) are needed for dissolved compounds and often involve high operational costs.

The estimated cost for the reduction of extracellular geosmin to the acceptable level (10 ng L^{-1}) using powdered activated coconut carbon is presented in Figure 15. Newcombe and Cook (2002) estimated that 20 ng L^{-1} of geosmin in water for two weeks would cost about $5000 worth of activated carbon to reduce the geosmin concentration to an acceptable level. If the initial concentration was higher (100 ng L^{-1}) and lasted longer (30 days), the estimated cost for the use of activated carbon would be much higher (AU$60,000 – AU$80,000) in order to maintain the quality of water. The high and long-lasting concentrations of geosmin are the key factors for the high treatment cost.

Figure 15 Cost estimation of powdered activated coconut carbon dosing for the reduction of geosmin concentration to 10 ng L^{-1} for a prolonged period at an Adelaide water treatment plant (adapted from Newcombe and Cook 2002)

Table 22 Relationships among the concentrations of *Anabaena circinalis* (cell mL^{-1}), saxitoxin ($\mu g\ L^{-1}$) and geosmin ($ng\ L^{-1}$) (Burch 2010)

Scenarios	*Anabaena circinalis* (cell mL^{-1})	Intracellular saxitoxin ($\mu g\ L^{-1}$)	Intracellular geosmin ($ng\ L^{-1}$)	Extracellular geosmin ($ng\ L^{-1}$)
Best case	1000	0.07	36	5
	2000	0.13	72	11
	3000	0.20	108	16
	4000	0.26	144	22
	5000	0.33	180	27
	6000	0.40	216	32
Most likely	1000	0.07	72	18
	2000	0.13	144	36
	3000	0.20	216	54
	4000	0.26	288	72
	5000	0.33	360	90
	6000	0.40	432	108
Worst case	1000	0.07	108	43
	2000	0.13	216	86
	3000	0.20	324	130
	4000	0.26	432	173
	5000	0.33	540	216
	6000	0.40	648	259

4.5 Conclusion

Nutrient fluxes from anoxic sediments can be an important source of nutrients for the development of cyanobacterial blooms in lakes and reservoirs, especially in the dry summer when the external nutrient sources are limited. Although the operation of artificial destratification reduces the intensity of thermal stratification, the historical water temperature suggested that the bottom reservoir water could still experience a degree of stratification and isolation from the atmosphere in a few occasions every summer. The fine-scale stratification, which isolates water layers from the atmospheric oxygen, became more pronounced during intense heatwaves.

The laboratory incubation of sediment cores suggested that the dissolved oxygen near the sediment-water interface can be depleted within 24 hours of isolation. Consequently, nutrients and metals are released under the reduced conditions, degrading the quality of water. The release rate of TP from anoxic sediments was used to approximate the potential population of *Anabaena circinalis* and the associated toxic and odorous impacts. The risk assessment indicated that a single day flux of sediment phosphorus induced by the 2-day stratification could lead to a cell density of *Anabaena circinalis* of 1000 – 1500 cell mL^{-1}, an intracellular saxitoxin concentration of 0.07 $\mu g\ L^{-1}$ and an extracellular geosmin concentration of 5 – 43 $ng\ L^{-1}$. These concentrations could trigger

the low alert level of the Alert Levels Framework for Cyanobacteria in Drinking Water and the subsequent management actions. This situation is most likely to occur when micro-stratification takes place at the sediment-water interface (i.e. a few times a year as suggested by historical data). However, the risk assessment is most likely predicting the maximum threshold of *Anabaena* population induced by anoxic sediment nutrient fluxes due to the underlying assumptions. Although the risk assessment only focused on the nutrient release from anoxic sediments, the same approach to assess phytoplankton abundance can be used for the external nutrient loads and for other problematic bloom-forming phytoplankton in other aquatic environments.

Chapter 5 Influences of hydrodynamics on phytoplankton distribution

Preamble

Chapter 5 explores the potential impact of different light habitats in Myponga Reservoir on a phytoplankton population using a light model. This chapter also examines other hydrodynamic processes such as convection, giving rise to the phytoplankton variability, based on a 3-year monitoring survey, which revealed the spatial and temporal variability of the phytoplankton community composition.

The field data of the 3-year monitoring survey were collected by a team of researchers from the Cooperative Centre for Water Quality and Treatment and SA Water. Following their research directions, I further explored and investigated the historical data at different temporal and spatial scales. Under guidance from supervisors, I was responsible for all data analysis and interpretation in the light model, in the phytoplankton C-S-R strategic classification and in the convection study.

Chapter 5 Influences of hydrodynamics on phytoplankton distribution

5.1 Introduction

Phytoplankton ecology and community composition is determined by the rate of resource delivery and the rate of habitat change. If the major resource requirements for phytoplankton are light and nutrients and the habitat is governed by the degree of mixing relative to light penetration, then the magnitude and composition of the phytoplankton community can be determined by meteorological and physical features that vary with space and time. Processes that occur at scales ranging from microhabitat to global processes all influence phytoplankton habitat and physiology. Similarly, processes may impact the phytoplankton physiology instantaneously or they may take a period of weeks for the impact to be observed on the phytoplankton community. Therefore, the context of scale is important in phytoplankton ecology.

With respect to habitat change in a lake or reservoir, the relevant physical process is wind-induced mixing, which deepens the surface mixed layer (SML) and transports phytoplankton cells vertically (Imberger and Patterson 1990). Some phytoplankton cells may be circulated to a depth, where the rate of photosynthesis is limited or is insufficient to compensate for the rate of respiration (Reynolds 1988). So, the mixing has the ability to modify the underwater light climate received by the phytoplankton. This can affect the rate of photosynthesis, the rate of growth, the frequency of cell division and ultimately the selection of species (Kirk 1983; MacIntyre 1993; Reynolds 1994). However, lateral transport processes, such as convection induced by differential heating and cooling and wind-driven circulation, can transport phytoplankton cells and dissolved substances between site-specific habitats (e.g. Monismith *et al.* 1990; James *et al.* 1994; MacIntyre and Melack 1995; Wells and Sherman 2001). Processes, which result in basin scale circulation and significant flux of phytoplankton between sites, would cause the phytoplankton community to appear homogeneous throughout the reservoir.

If there are differences in thermal stratification, nutrient supply or inoculum at different sites within a reservoir, these may result in different species being favoured and lead to “patchiness” in the phytoplankton community. In general, phytoplankton species can be

classified into three main adaptive strategies (C-S-R) with respect to energy availability (light), resource availability (nutrient) and disturbance (mixing) as summarised in Table 23 (Reynolds 1988, 1997). C-strategists (e.g. *Monoraphidium*, *Chlorella*, etc) are invasive *competitors* characterised by small cell size, high surface area to volume ratio, high growth rate and high metabolic activity over a wide range of temperatures, but are sensitive to low light dose. They are more susceptible to grazing but less to sedimentation. S-strategists (e.g. *Peridinium*) have acquisitive *stress* tolerance with large cell size, low surface area to volume ratio, low growth rate and low metabolic activity, particularly at low temperatures. They are more resistant to sinking and grazing losses due to self-regulation of their vertical movement. R-strategists (e.g. *Nitzschia*, *Scenedesmus*, etc) are attuning or acclimating *ruderal* species, which have intermediate to large cell size, high surface area to volume ratio, potentially high growth rate and high metabolic rate, even at low temperatures. They generally rely on turbulent mixing to remain in suspension and are relatively resistant to grazing due to their large cell size.

Patterns of phytoplankton succession may be explained by the C-S-R strategies (Reynolds 1996b). In the situation, where energy and nutrient resources are plentiful, fast-growing and fast-reproducing C-strategists will invade and exploit most effectively. When the resources are consumed, the competitive advantage moves toward larger, slower-growing, self-regulating, resource-conserving S-strategists. However, the hydraulic interferences (e.g. wind induced mixing, storm, convectional overturn, etc) disrupt the calm and stable habitat structure. The frequent turbulent mixing through the light gradient favours the R-strategists. The identification of the phytoplankton life-style strategies helps to understand the general habitat patterns at the continuously changing lake conditions.

The aim of this study was to investigate features of a reservoir, at the scales of site, season and year, which may give rise to differences in the phytoplankton populations. A hypothetical model, based on the relationships among surface mixed layer depth, daily light dose and phytoplankton growth rate, was developed to estimate the potential variation of phytoplankton population in the two different habitats (the main basin and side-arm at Myponga Reservoir) over time. Additionally, the physical processes that transport nutrients and phytoplankton between sites were investigated. Furthermore, to gain an appreciation of spatial and temporal variability, spatial differences in the phytoplankton communities present in the side-arm and main basin and the inter-annual

variability in the species composition and magnitude of the phytoplankton community, were examined.

Table 23 A summary of the characteristics of planktonic C-S-R strategies (Reynolds 1988)

5.2 Methods

5.2.1 Thermistor and meteorological data

The intensive field program started in October 1998 and concluded in April 2001. This sampling was conducted by a team of researchers from Cooperative Centre for Water Quality and Treatment and SA Water. Throughout this period, an aerator and surface mixers in the main basin were operated annually from October to April. The phytoplankton sampling locations and meteorological stations are shown in Figure 16. Water temperature (ºC) was measured at 20 depths with a thermistor chain (BetaTHERM Sensors, Ireland) adjacent to each meteorological station. The sensors at MET 1 and MET 2 provided measurements of water temperature at 10-minute intervals from the water surface to the reservoir bottom. Air temperature (ºC) and wind speed (m s^{-1}) were also recorded every 10 minutes at MET 1.

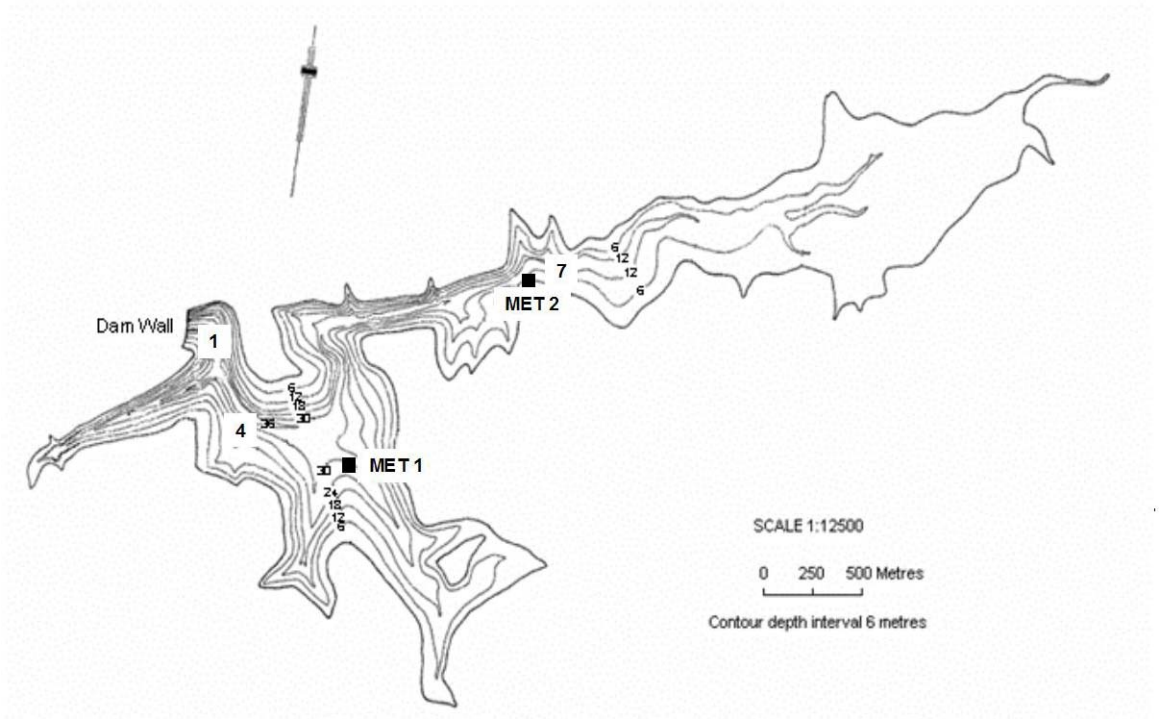


Figure 16 Phytoplankton sampling locations (1, 4 and 7) between October 1998 and April 2001 and meteorological stations (MET 1 and MET 2) in Myponga Reservoir

5.2.2 Surface mixed layer

The surface mixed layer (SML) also referred to as Z_{mix} was calculated as the shallowest depth, where the water temperature of the uppermost thermistor was at least 0.1^{o}C greater than the temperature of the thermistor down the chain. Water temperature at dawn (06:00) was used to calculate the SML depths for the main basin (MET 1) and side-arm (MET 2), as the maximum SML depth typically occurred before dawn in the end of nocturnal cooling (Sherman *et al.* 2000).

5.2.3 Light attenuation and euphotic depth

Downwelling irradiance was measured at 10 cm intervals through the euphotic zone with an underwater light meter (LiCor UQW 3946) at the same time as phytoplankton sampling. Irradiance was logarithmically transformed (Wetzel 2001) and the attenuation coefficient calculated by linear regression with respect to depth (Kirk 1983):

$$I_z = I_0 e^{-K_d z}$$

where I_z is the irradiance at depth z, I_0 is the surface irradiance and K_d is the light attenuation coefficient. The euphotic depth (Z_{eu}) is defined as the depth, at which 1% surface irradiance remains. The coloured nature of the water due to tannin in Myponga Reservoir leads to a shallow euphotic depth.

5.2.4 Phytoplankton sampling and analysis

Integrated samples over a depth of 0 to 5 m were collected from three locations (1, 4 and 7) using a 5 m hosepipe. For phytoplankton identification and enumeration, the integrated samples were preserved with Lugol's iodine and enumerated using a Sedgewick-Rafter chamber. Cell counts were performed weekly between October and April and monthly between May and September during the sampling periods between 1998 and 2001. Algal taxa were identified to at least genera and species where possible; however, for brevity, only genera are presented in this study. The number of taxa was used to determine phytoplankton richness. Biovolumes of phytoplankton taxa were then calculated according to the geometric equations suggested by Hillebrand *et al.*(1999).

Chlorophyll *a* concentrations were determined by filtering one litre of a sample through GF/C filters and extracting the pigment in cold 95% ethanol (-20 ºC) in the dark, for a minimum of 24 hours. Absorption at 665 nm and 750 nm was measured using a Varian Cary 1 Spectrophotometer and chlorophyll *a* concentration calculated using the equation in Lorenzen (1967).

Cluster analysis using PC-ORD (McCune and Mefford 1999) was used to determine the spatial variability of phytoplankton from sampling Locations 1, 4 and 7. The analysis involved grouping the sampling locations, based on Species Composition Analysis. Group averages were calculated using the linkage method and the Sørensen distance measure. An indicator species analysis using the methods of Dufrene and Legendre (1997) was used in conjunction with a multi response permutation procedure (MRPP) (McCune and Mefford 1999) to determine which phytoplankton genera were significantly contributing to the observed differences in sampling dates ($p < 0.05$). The phytoplankton taxa were also classified into planktonic C-S-R strategists, based on morphometric characteristics (Reynolds 1996b). Phytoplankton taxa were then rearranged on y-axis of surface area to volume (s/v) ratio against x-axis of maximum dimension of s/v (Reynolds 1996b) in order to illustrate the morphometric features of C-S-R strategies.

Phytoplankton diversity (H'') was calculated using the Shannon-weaver index incorporating phytoplankton biovolume as an estimation of phytoplankton biomass in Pielou (1975):

$$H'' = \sum_{i=1}^{s} \frac{b_i}{B} \log_2 \frac{b_i}{B}$$

where B is the total biovolume, b_i is the biovolume of the ith species and s is the number of species. The diversity index can be a useful tool to analyse spatial heterogeneity since the changes in the diversity index are descriptive of changes over time in the structure of populations (Margalef 1958).

The Summed Difference Index (SDI) was used to assess change in phytoplankton composition following Lewis (1978):

$$SDI = \frac{\sum_{i=1}^{s} \left\{ \left| \left[\frac{b_i(t_1)}{B(t_1)} \right] - \left[\frac{b_i(t_2)}{B(t_2)} \right] \right| \right\}}{(t_2 - t_1)}$$

where $bi(t_1)$ is the biovolume of the ith species and $B(t)$ the total biovolume at time t. Rates of less than 0.1 d^{-1} defined stable periods in the phytoplankton community, while rates exceeding 0.1 d^{-1} indicate periods of more rapid structural change in the community (Reynolds 1980).

5.2.5 Simple model relating *Anabaena circinalis* growth to mixing and light

To determine whether site variability in the physical habitat would significantly affect the phytoplankton population, a simple model was constructed in relation to SML depth, light dose in the water column and specific growth rate of phytoplankton species. In the model, a diurnal variation of surface irradiance for the maximum annual daylength (usually on 22nd December at this latitude 35.4 S) was calculated, based on Kirk's equation (1983):

$$I(t) = I_m \sin(\pi t/DL)$$

where I(t) is the instantaneous solar irradiance at time t hours after sunrise, I_m is the maximum irradiance at solar noon (1750 µmol m^{-2} s^{-1}) and DL is the daylength (14.5 hours). Figure 17 presents the diurnal variation of the surface irradiance from 05:00 sunrise to 19:00 sunset (without correction on daylight saving). The sum of I(t) calculated at 5-minute increments gives a total daily irradiance between wavelengths of 400 – 700 nm (photosynthetically active radiation). This is, hereafter, referred to a daily light dose at the water surface (58.15 mol m^{-2} d^{-1}).

This daily light dose at the water surface can reflect the underwater light condition if corrected for the reflectance at the water surface of solar elevation and wind roughness (Kirk 1983; Walsby 1997). The equations from Walsby (1997) were used to determine the amount of light reflected from the water surface dependent upon the solar elevation and the corresponding sub-surface irradiance. The solar elevation is a function of the day of year, the time of day, zenith angle and the geographical latitude. Since the Walsby's equations were set up for data from the northern hemisphere, it is important to adjust the latitude to negative for data from the southern hemisphere (e.g. -35.4 for Myponga, South Australia). Further corrections for reflectance can be made to account for wind roughening of the water surface (Walsby 1997). However, a smooth water reflectance was considered satisfactory for this model of light dose in different Z_{eu}/Z_{mix} conditions. Having accounted for reflection from the water surface, the average sub-surface daily light dose for January at Myponga was 55.16 mol m^{-2} d^{-1}.

The daily light dose received by phytoplankton circulating through the SML is also determined by the mixing characteristics of the water body and can be calculated using the equation in Riley (1957):

$$I_{avg} = I_0(1 - e^{-K_d Z_{mix}})/K_d Z_{mix}$$

where I_{avg} is the average amount of light in the water column, I_0 is the sub-surface irradiance of 55.16 mol m^{-2} d^{-1}, K_d is the light attenuation coefficient and Z_{mix} is the SML depth. The daily light dose under different physical conditions of light penetration and SML depth were then calculated. Euphotic depth (Z_{eu} = 3.77 m) and light attenuation (K_d = 1.22) measured on 9th January 2000 were used in conjunction with the SML depth to estimate the daily light dose for the prediction of algal population in the main basin and side-arm of Myponga Reservoir.

A growth-irradiance curve was used to calculate a population of toxic cyanobacterium, *Anabaena circinalis*. The curve was constructed based on the laboratory culture of *Anabaena circinalis* strain (Ana330E isolated by Peter Baker of SA Water from River Murray, Renmark, South Australia) at a range of light intensities on a 12:12 light: dark cycle (100 µmol m^{-2} s^{-2}) using WC media at 25ºC (Brookes 1997). The measured data were fitted with a hyperbolic tangent function (Jassby and Platt 1976), which adequately describes the relationship of photosynthesis and light for phytoplankton with a high R^2 of 0.97 (Figure 18):

$$P = P_m \tanh(\alpha I/P_m)$$

where P is the instantaneous production rate of phytoplankton, P_m is the specific production rate at optimal light intensity (0.59), α is the initial slope of the light-saturation curve in the linear range (0.0888) and I is the light intensity. The growth rate (r') was used instead of the production rate in this context.

Finally, the initial concentration of *Anabaena circinalis* (50 cell mL^{-1}) was modelled over a week for both the main basin and side-arm, based on the exponential growth equation (Reynolds 1984):

$$N_t = N_{t-1}e^{r'}$$

where N is the concentration of *Anabaena circinalis* (cell mL^{-1}), r' is the growth rate of *Anabaena circinalis* and t is the time for cell multiplication (1 day).

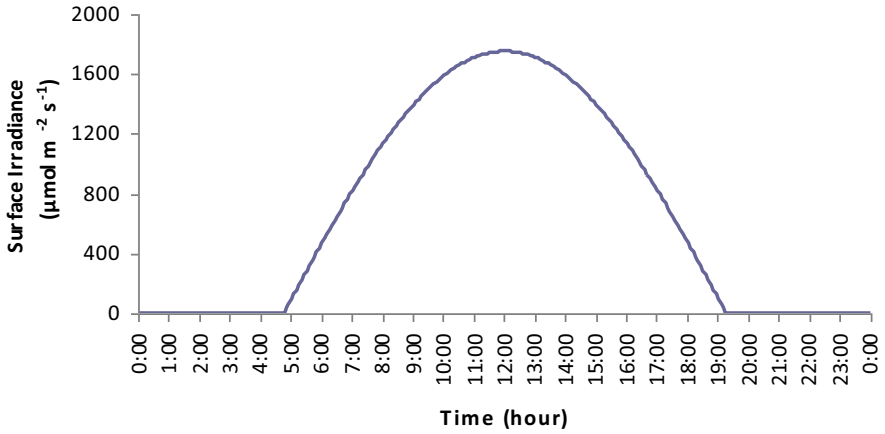


Figure 17 Diurnal variation of surface irradiance (µmol m^{-2} s^{-1}) estimated from the maximum irradiance of 1750 µmol m^{-2} s^{-1} and the maximum annual daylength of 14.5 hours in Adelaide, South Australia (without correction for daylight saving time). The estimated daily light dose was 58.15 mol m^{-2} d^{-1} with no or constant cloud cover and no correction for solar elevation

Figure 18 Growth rate of *Anabaena circinalis* (d^{-1}) with different light doses (mol m^{-2} d^{-1}) under optimal laboratory conditions adapted from Brookes *et al.* (2008)

5.2.6 Convective motion of water

Convective motion, driven by differential heating and cooling, may help to create a basin-scale circulation, which transports dissolved and particulate substances from one site to another. The convective motion in lakes can be described in the following equations (Monismith *et al.* 1990):

$$U \sim (\alpha \Delta\theta g H)^{1/2}$$

$$T = UL/\alpha g \Delta\theta H$$

where U is the velocity for convective motion (m s^{-1}), α is the thermal expansivity of water (1.4 x 10^{-4} °C^{-1}), Δθ is the longitudinal temperature gradient (°C), g is the gravitational acceleration (9.8 m s^{-2}), H is the depth for convective motion (1 m), T is the time for spin-up of convective motion (s) and L is the distance between the main basin and side-arm of Myponga Reservoir (1300 m). The actual water temperature difference, the duration of the temperature difference and the depth of water experiencing the temperature difference are important for determining the size, velocity and spin-up time (turnover time) for convective motion. A significant temperature gradient between the main basin and side-arm was observed on 10th January 2000, this was used to illustrate the convective motion in Myponga Reservoir.

5.3 Results

5.3.1 Spatial variation of surface mixed layer and underwater light condition

The objective of the study was to investigate the potential site variation (the main basin and side-arm) in phytoplankton populations driven by hydrological processes. SML depths were used to infer the vertical circulation of phytoplankton through the water column. Wind speed and temperature appeared to influence the SML depth (Figure 19). A relatively shallow SML was observed with a relatively slow wind speed (3 – 4 m s^{-1}), high air temperature (20 – 30°C) and high water temperature (20 – 27°C). The deeper SML was detected on the windy (6 – 7 m s^{-1} of wind speed) and cool (15 – 20°C of air temperature and 19 – 21°C of water temperature) days. It is important to note that the SML and the surface temperature varied diurnally, as the wind effect was never the same on any two given days (Monismith *et al.* 1990).

The SML depth was maintained below the euphotic depth for most of the study period. Occasionally, the SML depth stayed within the euphotic depth in summer when extensive insolation occurred and calm conditions persisted. On the windy days, the SML also varied between the main basin (MET 1) and side-arm (MET 2). The surface

water can mix down to the bottom of the main basin (30 m) and of the side-arm (15 m). Since the phytoplankton circulated vertically within the SML, the difference in SML depth between the two locations provided different light exposure to phytoplankton. The light model was developed to evaluate the potential variation of phytoplankton population between locations, based on the relationships of SML depth, euphotic depth, daily light dose received by phytoplankton and growth rate of phytoplankton.

The daily light dose phytoplankton would receive, whilst travelling through a water column, was calculated for different SML depth and euphotic depth scenarios (Table 24). When the SML depth was equal to the euphotic depth ($Z_{eu}/Z_{mix} = 1$), phytoplankton would receive 11.86 mol m^{-2} d^{-1} of light dose. The light dose decreased with decreasing Z_{eu}/Z_{mix} ratios and increased with increasing Z_{eu}/Z_{mix} ratios. The monthly measurement of euphotic depth in Myponga Reservoir varied from 2 m to 4 m during the sampling period (Figure 20). The euphotic depth of 3.77 m recorded on 9th January 2000 and the changing SML depth were used to estimate the daily light dose received by phytoplankton. Since the light dose varied on a daily basis, the growth rate of *Anabaena circinalis* altered correspondingly (Figure 18). According to the model prediction, the concentration of *Anabaena circinalis* was significantly different between the sites over a period of seven days (Table 25). If the concentration of *Anabaena circinalis* on 3rd January 2000 was 50 cell mL^{-1}, one week later on 10th January 2000 the concentration of *Anabaena circinalis* in the side-arm (1643 cell mL^{-1}) would be four times higher than that in the main basin (404 cell mL^{-1}). The spatial variation in *Anabaena* population was attributed to the differences in SML depth, daily light dose and *Anabaena* growth rate between sites.

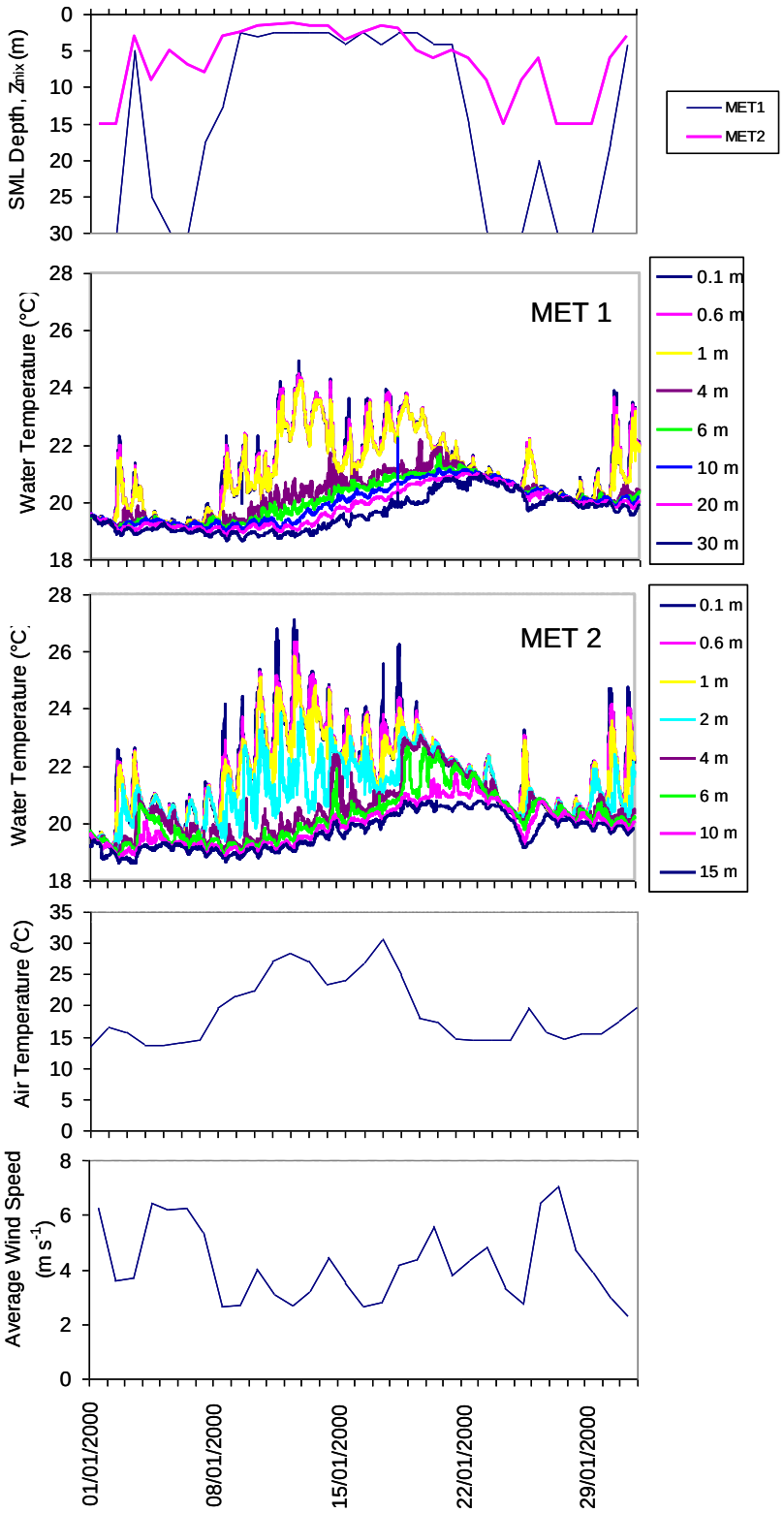


Figure 19 Surface mixed layer (SML) depth (m) and water temperature profiles (°C) recorded at dawn (06:00) at the meteorological stations in the main basin (MET 1) and in the side-arm (MET 2) of Myponga Reservoir in January 2000. Daily average air temperature (°C) and daily average wind speed (m s^{-1}) were only recorded at MET 1

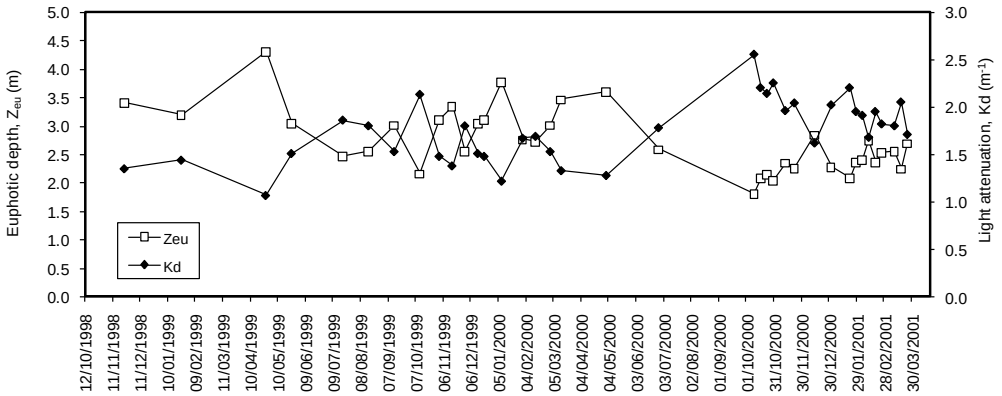


Figure 20 Water column transparency as measured by light attenuation, K_d (m^{-1}) and euphotic depth, Z_{eu} (m) between November 1998 and April 2001 in Myponga Reservoir

Table 24 Modelled mean daily light dose (mol m^{-2} d^{-1}) experienced by phytoplankton circulating through various surface mixed layer depths (m) in the water column with different euphotic depths. A sub-surface daily light dose of 55.16 mol m^{-2} d^{-1} was used to calculate the daily light dose

Light attenuation coefficient, K_d	4.61	2.30	1.54	1.15	0.92
Euphotic depth, Z_{eu} (m)	1	2	3	4	5
Surface mixed layer depth, Z_{mix} (m)	Modelled mean daily light dose (mol m^{-2} d^{-1})				
1	11.86	21.56	28.19	32.76	36.05
2	5.99	11.86	17.13	21.56	25.20
3	3.99	7.98	11.86	15.47	18.70
4	2.99	5.99	8.96	11.86	14.60
5	2.40	4.79	7.18	9.55	11.86
6	2.00	3.99	5.99	7.98	9.94
7	1.71	3.42	5.13	6.84	8.54
8	1.50	2.99	4.49	5.99	7.48
9	1.33	2.66	3.99	5.32	6.65
10	1.20	2.40	3.59	4.79	5.99
11	1.09	2.18	3.27	4.36	5.44
12	1.00	2.00	2.99	3.99	4.99
13	0.92	1.84	2.76	3.69	4.61
14	0.86	1.71	2.57	3.42	4.28
15	0.80	1.60	2.40	3.19	3.99
16	0.75	1.50	2.25	2.99	3.74
17	0.70	1.41	2.11	2.82	3.52
18	0.67	1.33	2.00	2.66	3.33
19	0.63	1.26	1.89	2.52	3.15
20	0.60	1.20	1.80	2.40	2.99
21	0.57	1.14	1.71	2.28	2.85
22	0.54	1.09	1.63	2.18	2.72
23	0.52	1.04	1.56	2.08	2.60
24	0.50	1.00	1.50	2.00	2.50
25	0.48	0.96	1.44	1.92	2.40
26	0.46	0.92	1.38	1.84	2.30
27	0.44	0.89	1.33	1.77	2.22
28	0.43	0.86	1.28	1.71	2.14
29	0.41	0.83	1.24	1.65	2.07
30	0.40	0.80	1.20	1.60	2.00

Table 25 Modelled growth rate (d^{-1}) and concentration of *Anabaena circinalis* (cell mL^{-1}) in the main basin (MET 1) and side-arm (MET 2) at Myponga Reservoir using the euphotic depth (Z_{eu} = 3.77 m) and light attenuation (K_d = 1.22) measured on 9th January 2000

	Date	SML depth, Z_{mix} (m)	Ratio of Z_{eu}/Z_{mix}	Daily light dose, I (mol m^{-2} d^{-1})	Growth rate, r' (d^{-1})	Concentration, N (cell mL^{-1})
Main basin (MET 1)	03/01/2000	5	0.75	9.02	0.52	50
	04/01/2000	25	0.15	1.81	0.16	58
	05/01/2000	30	0.13	1.51	0.13	67
	06/01/2000	30	0.13	1.51	0.13	76
	07/01/2000	17.5	0.22	2.58	0.22	95
	08/01/2000	12.5	0.30	3.62	0.29	127
	09/01/2000	2.5	1.51	17.23	0.58	227
	10/01/2000	3	1.26	14.68	0.58	404
Side-arm (MET 2)	03/01/2000	3	1.26	14.68	0.58	50
	04/01/2000	9	0.42	5.02	0.38	73
	05/01/2000	5	0.75	9.02	0.52	122
	06/01/2000	7	0.54	6.46	0.44	190
	07/01/2000	8	0.47	5.65	0.41	286
	08/01/2000	3	1.26	14.68	0.58	509
	09/01/2000	2.5	1.51	17.23	0.58	912
	10/01/2000	1.6	2.36	24.24	0.59	1643

5.3.2 *In situ* variability of phytoplankton composition in space and time

Field measurements of a phytoplankton population and diversity were collected between October 1998 and April 2001 in three locations (1, 4 and 7) across Myponga Reservoir. During the sampling periods, four applications of copper sulphate ($CuSO_4$) algicide at a dose rate of 2 mg L^{-1} were recorded in Myponga Reservoir on 6th January 1999, 11th January 2000, 16th December 2000 and 31st January 2001. The chlorophyll *a* concentration and total biovolume did not vary substantially between locations (Figure 21). The chlorophyll *a* concentration varied between 1 and 34 µg L^{-1}, while total biovolume varied between 4.2 x 10^4 and 6.6 x 10^6 μm^3. Maximum biomass was generally recorded in the period of January to March, which corresponded to high water temperatures.

Using Species Composition Analysis, the three locations (1, 4 and 7) were grouped together for the majority of months in the sampling period and the months were clustered into groups presented in a dendrogram (Figure 22). This indicated that all three locations supported similar phytoplankton species and spatial variability was minor across the reservoir. It should be noted that, with several exceptions, at least two locations were always grouped together for each month. The exceptions for the Species

Composition Analysis were October 1998, February 2000, March 2000, May 2000 and September 2000.

Indicator Species Analysis with MRPP was used to determine the significant indicator phytoplankton species for the groups clustered by Species Composition Analysis. Each group represented different sampling dates with specific indicator species, demonstrating diverse temporal variability of phytoplankton in Myponga Reservoir during the 3-year sampling period (Table 26). The Indicator Species Analysis also revealed the inter-annual variability of phytoplankton composition. The three consecutive January sampling periods were divided into two groups. Group 4 (January 1999 and 2000) was mostly dominated by C-strategists with a mixture of cyanobacteria and green algae, whereas Group 28 (January 2001) consisted of a combination of C-S-R strategists with 15 indicator species of cyanobacteria, green algae, diatoms and dinoflagellates. However, little inter-annual difference in phytoplankton composition was found in late summer periods (March and April), as all three sampling years were clustered into Group 6, comprising the same indicator species. No indicator species was identified for Group 1 and Group 2, which were mostly associated with the autumn-winter-spring sampling periods.

These indicator species of phytoplankton showed their adaptive strategies (C-S-R) to the changing environmental conditions in Myponga Reservoir, based on their morphometry (Figure 23). In Myponga Reservoir, some phytoplankton displayed the typical characteristics of their adaptive strategies, including green alga *Chlorella* as C-strategist, dinoflagellate *Peridinium* as S-strategist and diatom *Melosira* as R-strategist. However, a number of phytoplankton taxa in Myponga Reservoir did not show the typical C-S-R features as described by Reynolds (1988). For instance, cyanobacteria *Aphanocapsa* and *Phormidium* and green alga *Dictyosphaerium* were classified as competitive C-strategists by Reynolds (1988), but they appeared to be disturbance-tolerant R-strategists in Myponga Reservoir. On the other hand, diatoms *Cyclotella* and *Fragilaria* were generally classified as R-strategists by Reynolds (1988), but they showed the morphometric characteristics of C-strategists in Myponga Reservoir.

Temporal variability of phytoplankton composition was presented for Location 1 only (Figure 24), as the above Species Composition Analysis demonstrated that phytoplankton patchiness was small (Figure 22). The dominant phytoplankton groups in

terms of biovolume were diatoms and green algae during the periods of January and March. Yellow-brown algae were generally present at low biovolume throughout the year. There were a couple of occasions in August 1999 and November 2000, where the biovolume of yellow-brown algae was high, whilst other phytoplankton groups were low in biovolume. Dinoflagellate volume was small and was only recorded during the period of November 2000 and March 2001. Cyanobacteria were found all year round at low biovolume, but increased briefly in summer and peaked in January 1999 and 2000.

During the 3-year sampling period, the numbers of taxa tended to increase in each spring and stay constant throughout the remaining year (Figure 25a). In the 1998-1999 summer, there were approximately 10 taxa recorded in Myponga Reservoir, 15 taxa in the 1999/2000 summer and 20 taxa in 2000-2001 summer. However, minor variations in the taxa numbers were observed among Locations 1, 4 and 7. Phytoplankton diversity as measured by the Shannon-Weaver Index was highly variable over the sampling period, ranging from 0.15 to 3, but was relatively similar across sampling locations (Figure 25b). Although the index oscillated in summer, higher diversity tended to be recorded in November-January. This was consistent with an increase in algal growth associated with higher temperatures and sufficient nutrients. The Summed Difference Index also varied over the season (Figure 25c). In December-January the index tended to be above 0.1 d^{-1}, indicating that the phytoplankton community was undergoing significant change in composition, consistent with the high diversity index. During April-October, the summed difference index was less than 0.1 d^{-1}, indicating that the phytoplankton community reached a stable period over winter.

The earlier light model results (Table 25) suggested a significant change in phytoplankton population between the main basin and side-arm due to different SML depth, leading to different light exposure and growth rate for phytoplankton. However, there was no observable difference in phytoplankton population and diversity from the field sampling program. This implies that there may be other lake processes, contributing to the spatial homogeneity of phytoplankton, as discussed next.

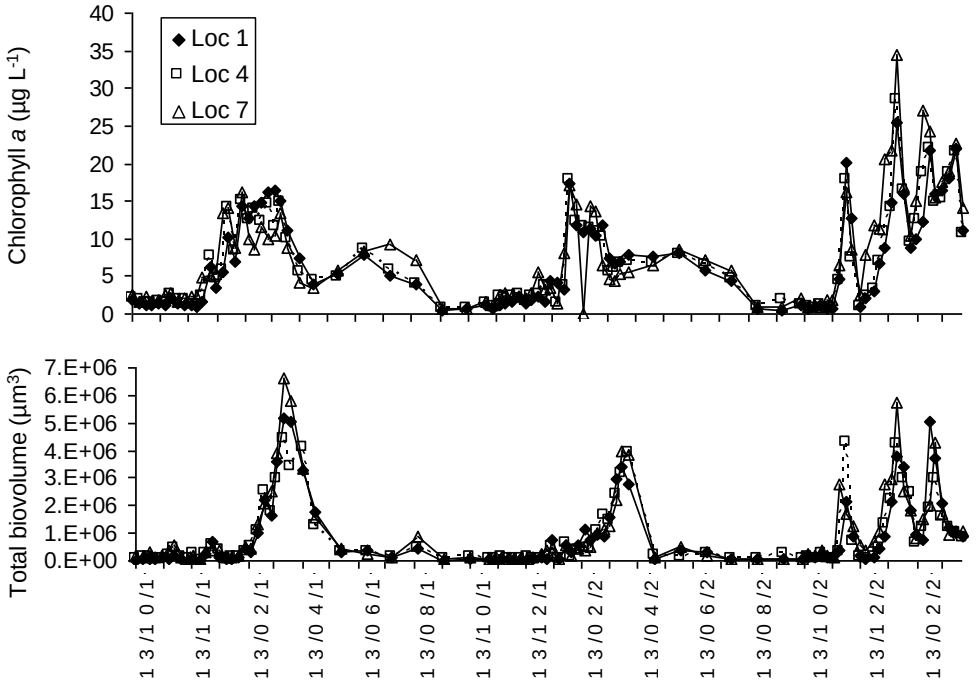


Figure 21 Chlorophyll *a* concentration (µg L^{-1}) and total biovolume of phytoplankton (µm^3) at Location 1 (dam wall), Location 4 (main basin) and Location 7 (side-arm) in Myponga Reservoir measured between October 1998 and April 2001

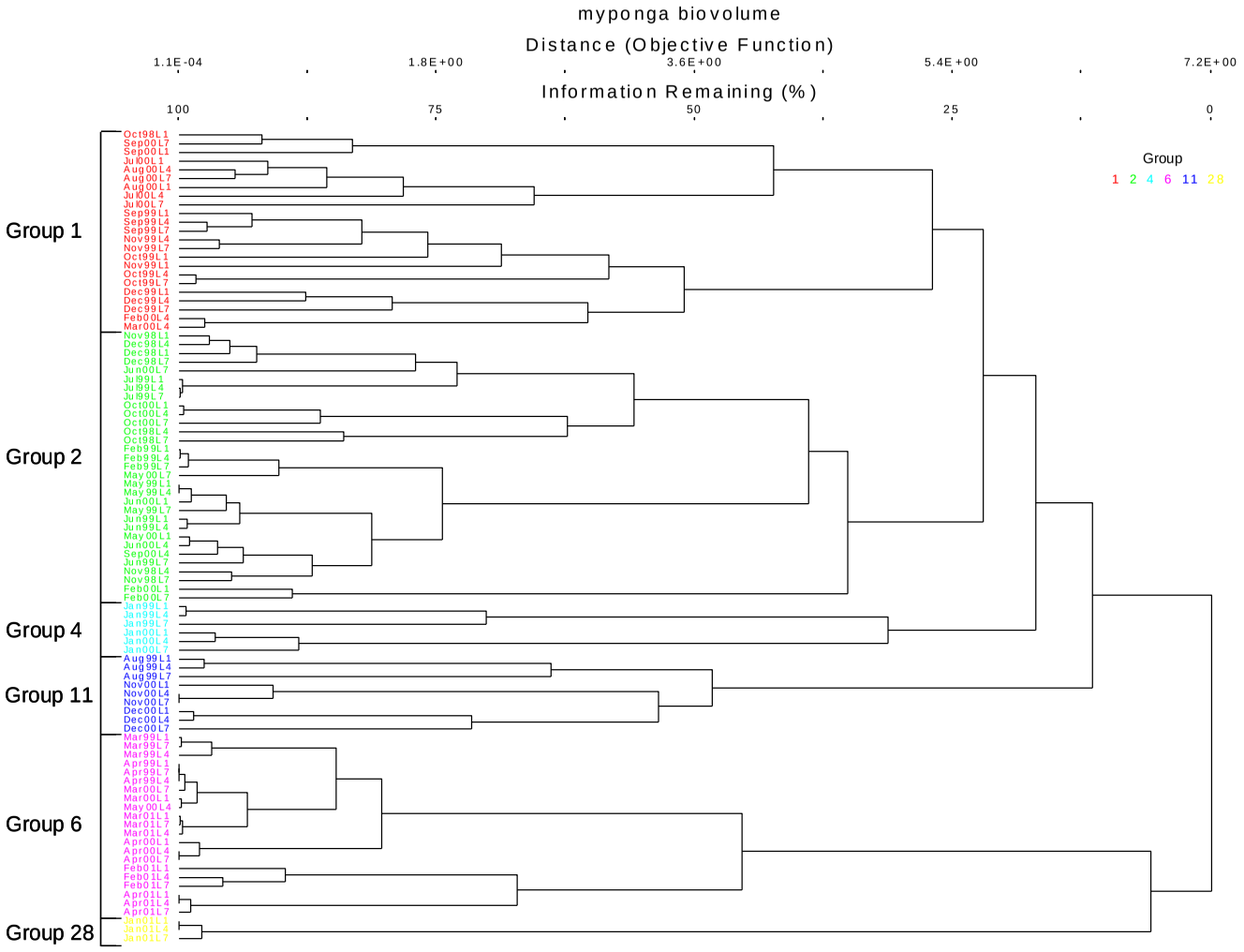
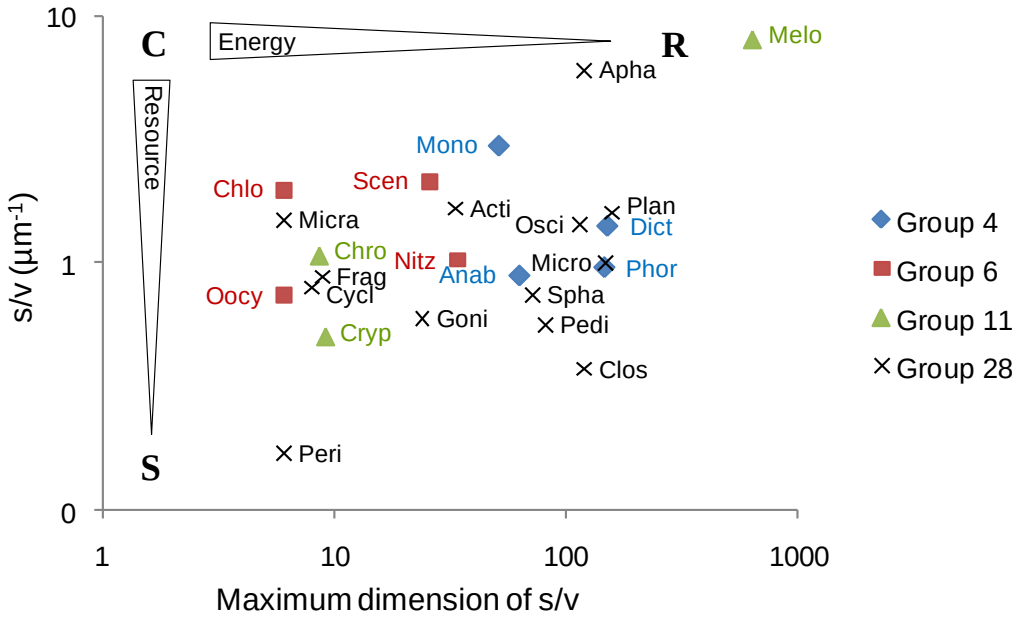


Figure 22 Dendrogram of sampling dates (October 1998 – April 2001) and locations (1, 4 and 7) using Species Composition Analysis



Note: Anab = *Anabaena circinalis*, Dict = *Dictyosphaerium*, Mono = *Monoraphidium*, Phor = *Phormidium*, Chlo = *Chlorella*, Nitz = *Nitzschia*, Oocy = *Oocystis*, Scen = *Scenedesmus*, Chro = *Chroomonas*, Cryp = *Cryptomonas*, Melo = *Melosira*, Acti = *Actinastrum*, Apha = *Aphanocapsa*, Clos = *Closterium*, Cycl = *Cyclotella*, Frag = *Fragilaria*, Goni = *Gonium*, Micra = *Micractinium*, Micro = *Microcystis aeruginosa*, Osci = *Oscillatoria*, Pedi = *Pediastrum*, Peri = *Peridinium*, Plan = *Planktothrix*, Spha = *Sphaerocystis*

Figure 23 Classification into planktonic C-S-R adaptive strategies of phytoplankton taxa, collected from Myponga Reservoir between October 1998 and April 2001. Based on the morphometric characteristics (surface area: volume ratio, s/v, μm^{-1}), phytoplankton were classified into *Competitors* (C-), *stress tolerant* (S-) and *ruderals* (R-) strategists. Each strategists favour different resource (nutrient) and energy (light) levels. Each phytoplankton genus was also grouped according to the Species Composition Analysis

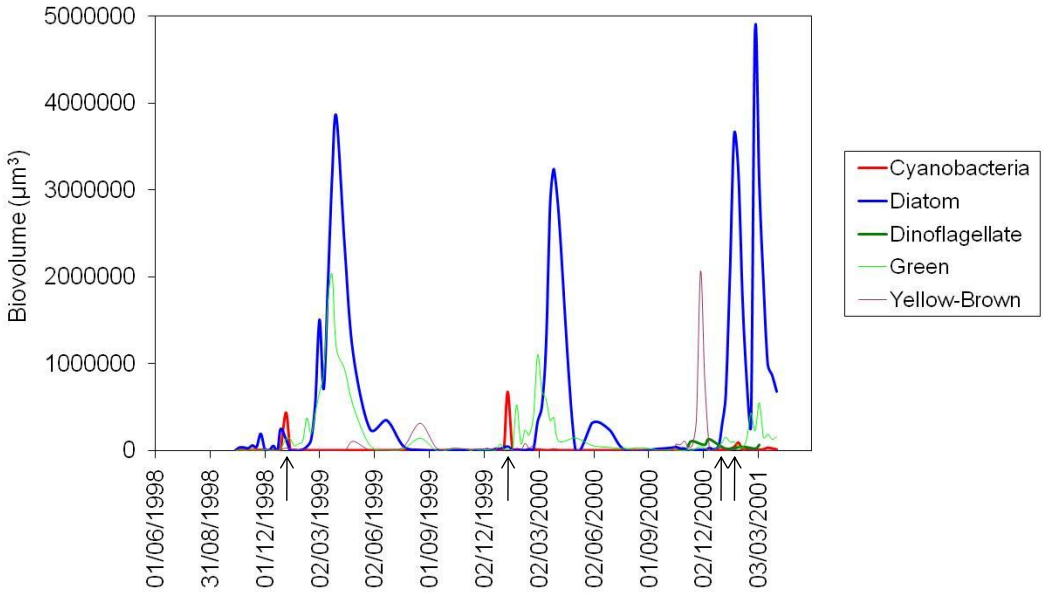


Figure 24 Temporal changes in biovolume (μm^3) for different phytoplankton composition in Location 1 of Myponga Reservoir. The black arrows on the x-axis represent the application of copper sulphate algicide on 6th January 1999, 11th January 2000, 16th December 2000 and 31st January 2001

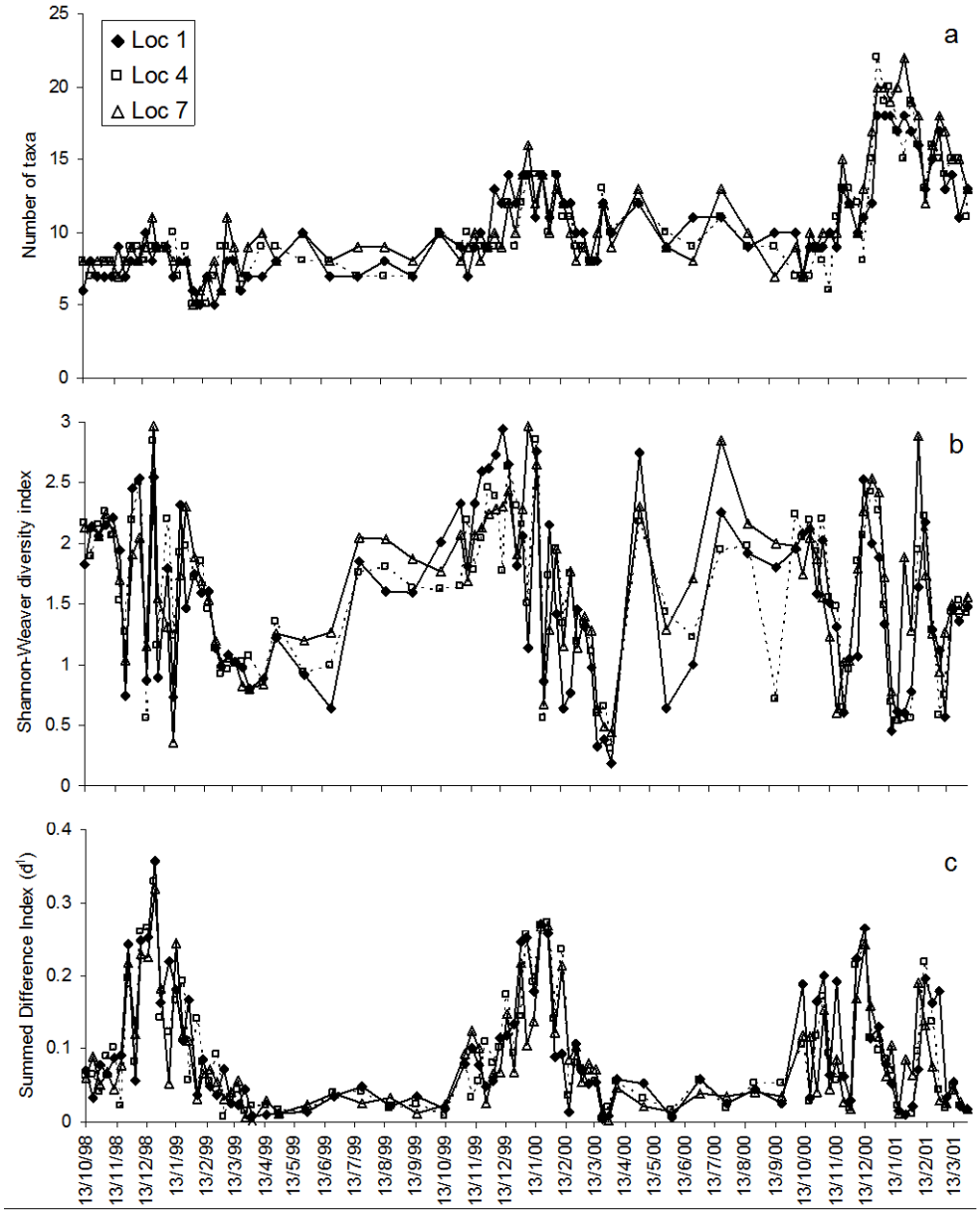


Figure 25 Temporal and spatial changes in (a) number of taxa, (b) Shannon-Weaver diversity Index and (c) Summed Difference Index (d^{-1}) in Myponga Reservoir between October 1998 and April 2001

Table 26 Indicator species of phytoplankton taxa for the groups clustered by Species Composition Analysis. Each taxa was classified into C-S-R adaptive strategies

Groups from Species Composition Analysis (Figure 22)	Phytoplankton genera	Phytoplankton groups	*p*-value for Indicator Species Analysis	s/v (μm^{-1})	Unit volume (μm^3)	C-, S-, R-strategist (Reynolds 1988; Hart 2006)
Group 4 Jan 1999 Jan 2000	*Anabaena circinalis*	Cyanobacteria	0.001	0.90	160.9	C-S
	Dictyosphaerium	Green	0.005	1.43	38.8	C
	Monoraphidium	Green	0.031	3.02	17.8	C
	Phormidium	Cyanobacteria	0.010	0.97	192.4	C
Group 6 Mar, Apr 1999 Mar, Apr 2000 Feb, Mar, Apr 2001	*Chlorella*	Green	0.004	2.00	14.1	C
	Nitzschia	Diatom	0.001	1.04	423.0	R
	Oocystis	Green	0.009	0.75	268.1	C-S
	Scenedesmus	Green	0.001	2.17	21.3	R-C
Group 11 Aug 1999 Nov, Dec 2000	*Chroomonas*	Yellow-Brown	0.003	1.08	104.7	C
	Cryptomonas	Yellow-Brown	0.001	0.51	1039.1	R-C, C-S
	Melosira	Diatom	0.021	8.01	392.0	R
Group 28 Jan 2001	*Actinastrum*	Green	0.001	1.67	39.3	C
	Aphanocapsa	Cyanobacteria	0.037	6.00	0.52	C
	Cladophora	Green	0.032	-	-	-
	Closterium	Green	0.001	0.38	10723.3	R
	Crucigenia	Green	0.001	-	113.1	C
	Cyclotella	Diatom	0.001	0.80	392.7	C-R
	Fragilaria	Diatom	0.002	0.88	141.4	R
	Gonium	Green	0.001	0.60	523.6	C-R
	Micractinium	Green	0.002	1.50	33.5	C
	Microcystis aeruginosa	Cyanobacteria	0.001	1.00	113.1	S
	Oscillatoria	Cyanobacteria	0.001	1.44	127.2	R
	Pediastrum	Green	0.001	0.57	628.3	C-R
	Peridinium	Dinoflagellate	0.001	0.17	22449.3	S
	Planktothrix	Cyanobacteria	0.001	1.60	49.1	R, R-S
	Sphaerocystis	Green	0.004	0.75	268.1	C

5.3.3 Differential heating and cooling driving convective motion of water

Differential heating was clearly observed at Myponga Reservoir, especially in a hot summer. The water temperature difference between the main basin and side-arm over a 24-hour period is illustrated clearly in Figure 26, providing evidence of the differential heating, and hence convective circulation. On 10th January 2000, the surface water temperature in the main basin increased after sunrise and reached its maximum

temperature of 22.3ºC at 10:30. Once the destratification systems were operated, the water temperature in the main basin dropped gradually. The destratification systems in the main basin were then turned off at 16:00 and the water temperature started to increase by the remaining heat. In contrast, the surface water temperature in the side-arm increased, since the sunrise. The side-arm reached its maximum surface temperature of 25.4 ºC at 16:50 due to the absence of artificial destratification.

At 16:50, the maximum temperature difference between the main basin and side-arm at the surface 0.1 m water was 5.4ºC (Figure 26). Based on the equations of Monismith *et al.* (1990), this differential heating could induce a convective motion between the two locations with a spin-up time of about 4 hours and 22 minutes (Table 28). However, the convection at this temperature gradient (5.4ºC) was only maintained for 10 – 20 minutes (Table 27).

As the temperature gradient decreased from 4°C to 1°C, the estimated duration of the gradient increased from 3 hours 30 minutes to 13 hours 20 minutes respectively. Besides, the duration of the gradient decreased with the increasing water depth. It is important to note that the duration of the temperature gradient was rather similar between the depths of 0.1 m and 1 m. This 1 m surface water was, therefore, defined as an active layer for convective circulation in this incident. For the depth below 4 m, the water temperature difference between the main basin and side-arm was small (< 1°C).

Convective motion of water occurred when there was a substantial temperature difference between locations. The velocity and spin-up time for convective motion were calculated for different temperature gradients (Table 28). The estimated velocity varied from 0.037 m s^{-1} to 0.083 m s^{-1} for temperature gradients of 1°C to 5°C respectively. This estimation means that the water at a 5°C temperature gradient would take about 4 hours and 22 minutes to travel laterally within the 1 m layer of surface water between the main basin and side-arm with a distance of 1300 m. However, the field measurement showed that the 5°C temperature gradient was only maintained for 10 to 20 minutes (Table 27). Hence, the convective motion for the 5°C gradient vanished before the water could reach the other side of the reservoir. On the other hand, the 2°C temperature gradient was maintained for 11 hours and 10 minutes, while the spin-up time for the convective motion was only 6 hours and 54 minutes (velocity = 0.052 m s^{-1}).

This implied that the water could move from the main basin to the side-arm and return half-way before the convective motion stopped at this velocity.

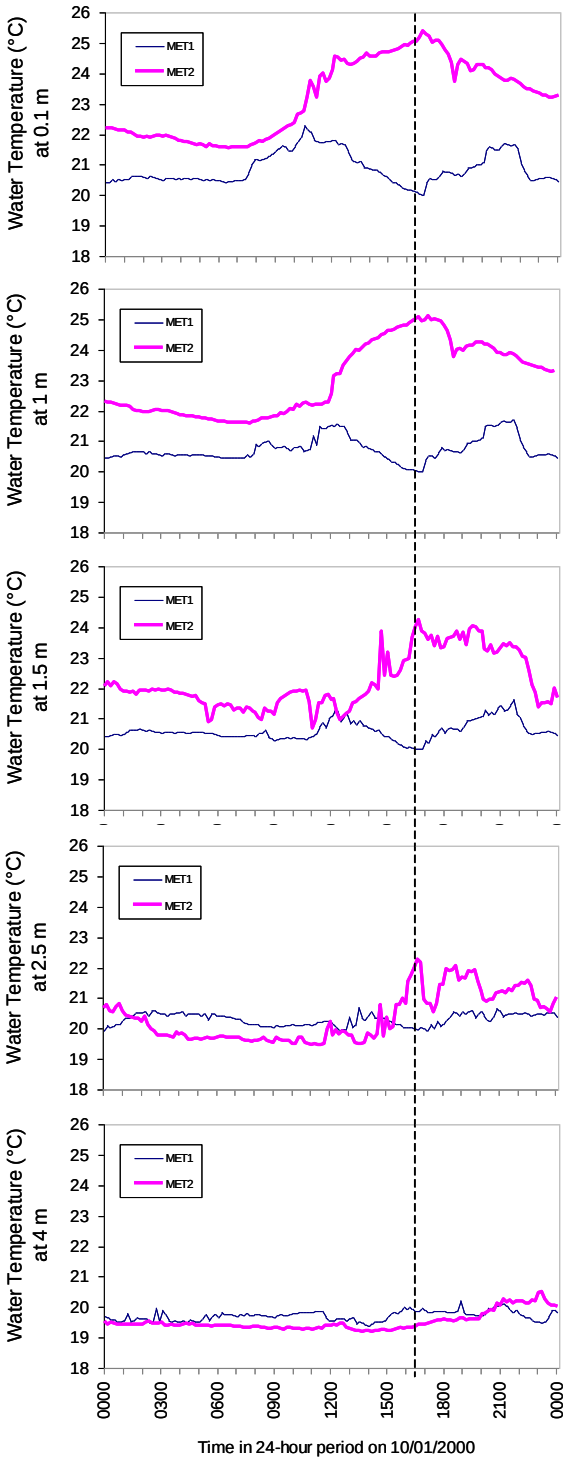


Figure 26 Water temperature (°C) difference between the main basin (MET 1) and side-arm (MET 2) at various depths over a period of 24 hours on 10th January 2000. The maximum temperature difference at the water surface was recorded at 16:40 (dashed line)

Table 27 Duration of the water temperature difference (°C) between the main basin (MET 1) and side-arm (MET 2) at various depths of water (m). The measurements were based on one continuous period of thermal stratification on 10th January 2000 starting from 10:40 until mid-night

Water temperature difference between MET 1 and MET 2	Duration of the water temperature difference at various depths of water on 10th January 2000				
	0.1 m	1 m	1.5 m	2.5 m	4 m
1°C	13h 20m	12h 30m	9h 50m	3h 20m	0
2°C	12h 30m	11h 10m	7 hrs	20 mins	0
3°C	7h 10m	6h 40m	2 hrs	0	0
4°C	3h 30m	3h 20m	10 mins	0	0
5°C	20 mins	10 mins	0	0	0

Table 28 Velocity (m s^{-1}) and spin-up time (s) for convective motion of water travelling between the main basin (MET 1) and side-arm (MET 2) at different temperature gradients (°C)

Difference in water temperature, $\Delta\theta$	Velocity scale for convective motion, U (m s^{-1})	Time scale for spin-up of convective motion, T (s)
1°C	0.037	35097s → 9h 45 m
2°C	0.052	24817s → 6h 54m
3°C	0.064	20263s → 5h 38m
4°C	0.074	17548s → 4h 52m
5°C	0.083	15696s → 4h 22m

Equations from Monismith *et al.* (1990):

$U \sim (\alpha \Delta\theta g H)^{1/2}$

$T = UL/\alpha g \Delta\theta H$

Assuming: Thermal expansivity of water, $\alpha = 1.4 \times 10^{-4}$ °C^{-1}

Gravitational acceleration, $g = 9.8$ m s^{-2}

Depth length-scale for convective motion, $H = 1$ m

Length scale between MET 1 and MET 2, $L = 1300$ m

5.4 Discussion

5.4.1 Temporal variability in phytoplankton community

Chlorophyll *a* concentration and phytoplankton biovolume did not vary substantially between the three locations, but strong seasonal changes (Figure 21) and inter-annual variability of phytoplankton composition in January (Table 26) were observed. The observed seasonal community in Myponga Reservoir shifted from cyanobacteria (e.g. *Anabaena* and *Phormidium*) to green algae (e.g. *Chlorella*, *Oocystis*, *Scenedesmus*) and then diatom (e.g. *Nitzschia*) in summer-autumn periods. In a typically hot summer, surface stratification creates calm and stable conditions and the light and nutrient resources are likely to become vertically segregated. These conditions favour the S-strategists, which can adjust their vertical positions to exploit light and nutrient resources in the water column (Raven and Richardson 1984; Reynolds 1988). If the conditions are maintained, buoyant cyanobacteria can outcompete non-motile green algae and diatoms over time (Viner 1985). However, the water authority of Myponga Reservoir applied copper sulphate algicide around January when *Anabaena* started to grow (copper sulphate application has eased since March 2006). The algicide was often applied at relatively low cell counts between 1000 – 2000 cell mL^{-1} to control further development of *Anabaena* blooms (Kelly 1998). For the 2000-2001 summer, two applications of algicide were made within two months (i.e. 16th December 2000 and 31st January 2001) and no artificial mixing was carried out during the period of algicide treatment. This could significantly disturb the abundance, composition and succession of phytoplankton (Welk 2007) differentiating January 1999 and 2000 (Group 4) from January 2001 (Group 28).

Copper sulphate is a non species-specific algicide, affecting all phytoplankton species. The reestablishment of phytoplankton communities such as green algae and diatoms was observed after such drastic disturbance (Schrader *et al.* 2000). Fast growing phytoplankton species (C- and R-strategists) would first emerge to exploit the bio-available nutrients released during cell lysis, while the water column stability would determine the dominant strategist. The periodic deep mixing would favour disturbance-tolerant R-strategists, whereas calm stratified periods would favour competitive C-strategists (Reynolds 1988). However, the repeated application of algicide (disturbance) within a relatively short time frame meant that the phytoplankton communities could not fully utilise their adaptive strategies to exploit the environments before a reset by algicide treatments. Therefore, the adaptive strategies were indistinct for a number of

indicator species in January (Group 4 and 28 in Table 26), whereas indicator species from other groups (other sampling periods without the use of algicide) showed similar C-S-R strategies as suggested by Reynolds (1988). The significant change in phytoplankton composition was also noted in the high Summed Difference Index (> 0.1 d^{-1}) in December-January periods (frequent disturbance by algicide) just prior to the greatest increase in chlorophyll and biovolume in March-April periods (Figure 25).

Myponga Reservoir has been mixed artificially throughout every summer in order to reduce the intensity of thermal stratification and the release of internal nutrients (Brookes *et al.* 2000). Phytoplankton, circulated through the variable surface mixed layer depths, would probably be flexible in their physiology in order to cope with the rapidly changing light climate. Otherwise, rapid growers could exploit the favourable conditions when they occurred. However, it was also evident that many species were present to capitalise on the conditions, which best suited their adaptations. During April-October, the Summed Difference Index was less than 0.1 d^{-1}, indicating that the community was not undergoing significant change in composition. For this time period, the community biovolume was also reasonably constant. The apparent coexistence of numerous species did not necessary mean that they were all exploiting the same habitat. There are numerous habitats in reservoirs that might favour growth of one species over another, but the basin scale circulation could homogenise the populations and contribute to a diverse community in Myponga Reservoir.

5.4.2 Basin-scale circulation contributing to a spatial homogeneity in lakes

The complex morphometry of Myponga Reservoir offers multiple habitats that could support different phytoplankton taxa or allow different growth rates. Under calm and stable conditions, uneven vertical distribution of phytoplankton is often observed. Some phytoplankton (e.g. dinoflagellates and cyanobacteria) may float and swim towards the water surface and grow more rapidly at high light intensity, while non-motile diatoms and green algae sink gradually (Walsby 1997; Huisman *et al.* 2004). A diatom, *Aulacoseira*, could sink out of the euphotic zone at a speed of 0.95 m d^{-1} under thermally stratified conditions (Sherman *et al.* 1998). However, the active movement of phytoplankton is easily overcome by the motion of water, which has a strong effect on the distribution of phytoplankton (Martin 2003). Water movement also influences phytoplankton exposure to light, access to nutrients and contacts with grazers, parasites and pathogens (Reynolds 1994).

Surface mixing allowed the entrainment of phytoplankton and carried them up and down the surface mixed layer. The SML depth represented the region, where mobile and non-mobile phytoplankton were circulated (Sherman *et al.* 2000). A relatively shallow SML was observed during the calm and hot weather, while the deeper SML was measured on the windy and cool days. The maximum SML depth varied significantly between the two sites, primarily due to the limitation of mixing depth by the reservoir bottom (Sherman *et al.* 2000). The daily light dose received by phytoplankton varied between sites dependent upon the SML depth. MacIntyre (1993) reported that the entrained phytoplankton could travel from a 90% to 5% light level in 3 – 4 minutes and sometimes could circulate below the euphotic depth, reducing the light exposure. Since the daily light exposure affects the relative growth rate of phytoplankton, variations in phytoplankton population over time were modelled.

The light model estimated that the population of *Anabaena circinalis* was as much as four times higher in the shallow side-arm than the deep main basin over a 7-day period. This estimation was only based on the light variation scenario and did not take into account any potential nutrient variation between sites. As suggested by Ostrovsky *et al.* (1996), average phytoplankton biomass and productivity increased at the lake periphery, where hydrological processes (e.g. internal seiche, turbulence, resuspension, etc) enhanced nutrient supply to the epilimnion. However, the morphometric complexity also drives differential heating and cooling, which generates basin scale circulation transporting phytoplankton between sites. Field measurements of chlorophyll *a* concentration, phytoplankton biovolume, species composition, number of taxa, species diversity and amount of community change (SDI) showed homogenous distribution amongst Locations 1, 4 and 7; this is rather different from the modelled phytoplankton dynamics.

The difference in bathymetry between the relatively shallow side-arm and the relatively deep main basin suggested that the side-arm with a smaller thermal mass would heat and cool at a faster rate than the main basin with a larger thermal mass. This lateral thermal stratification driving significant convective motion can only occur when a surface area of a shallow part of a reservoir were greater than that of a deep part of a reservoir (ratio of deep areas to shallow areas < 1) and when a depth of a deep part of a reservoir was 50% greater than that of a shallow part of a reservoir (ratio of shallow

depth to deep depth < 0.5) (Wells and Sherman 2001). Myponga Reservoir has these areal and depth criteria.

The convection induced by differential heating and cooling is particularly important to lakes with substantial shallow areas (e.g. side-arm), where littoral water in the side-arm of the lake exchanges laterally with the surface pelagic water in the centre of the lake (Monismith *et al.* 1990; Sturman *et al.* 1999; Imberger 2001). Upwelling and downwelling periodically mix water, which has been in contact with the sediment bottom at some time (Monismith *et al.* 1990). The flow allows the transport of solutes, particles and organisms (e.g. dissolved gases, nutrients, pollutants, phytoplankton, etc) between littoral and pelagic zones and between hypolimnetic and epilimnetic water (James *et al.* 1994; MacIntyre and Melack 1995; Sturman *et al.* 1999). For instance, significant phosphorus fluxes (0.2 – 1.8 mg TP m^{-2} d^{-1}) from the littoral to the pelagic zone via convective circulation were observed in Eau Galle Reservoir of Wisconsin (James and Barko 1991).

Previous drogue studies in Myponga Reservoir gave a qualitative representation of basin exchange, demonstrating the occurrence of large-scale circulation patterns and a significant exchange of water between the side-arm and main basin (Lewis 2004). The velocities for the drogue movement varied from 0.01 to 0.08 m s^{-1} (Lewis 2004). At these velocities, the time taken for travelling through the side-arm in either direction would vary from approximately 2 to 23 hours, indicating that water exchanges between the two locations can occur in a short time scale. It is, therefore, plausible that phytoplankton responses to site-specific habitat differences were not observed at Myponga Reservoir, because the large-scale lateral water movement might overcome the heterogeneity between sites.

Differential heating was clearly evident at Myponga Reservoir in summer, but no significant differential cooling was observed in the study period of January 2000. Differential cooling, which often contributes to the overturning of the water column, may form during winter (Wells and Sherman 2001), but the cold weather has less impact on phytoplankton biomass and diversity (Figure 21 and Figure 25). Hence, convective motion driven by differential cooling will not be discussed further (see Horsch and Stefan (1988), James and Barko (1991) and Wells and Sherman (2001) for details).

Water temperature differences between the shallow side-arm and deep main basin occur when solar heat is distributed evenly over a water surface with different water depths. Hence, the rate of change in temperature would be greater in the shallower side-arm than the deeper main basin (Patterson 1984). In addition, the destratification systems in Myponga Reservoir aided in heat dissipation and the surface temperatures in the main basin were generally lower than the side-arm. When the destratification systems were turned off at 16:00 on 10th January 2000, the water temperature in the main basin started to increase. The maximum temperature gradient between the main basin and side-arm reached as large as 5.4ºC at 16:50. This differential heating could induce convective motion between the two locations with a spin-up time of about 4 hours and 22 minutes, but the convection at this temperature gradient was only maintained for 10 – 20 minutes, as previously described. This implies that a deeper mixed layer may be formed when the gravitational current either has not developed fully or cannot reach the deep region before the pressure forcing is over (Wells and Sherman 2001). Imberger (2001) emphasized that an effective lateral mixing is highly time-dependent, driven by the diurnal cycle of the lake.

In the case of Myponga Reservoir, convective motion induced by temperature difference below 3ºC seems to be more realistic. This provided longer time to form the convection, allowing the complete circulation of water from one site to another. The estimated spin-up time and velocity of convection in this study were very similar to the measured results from the previous drogue studies in Myponga Reservoir, South Australia (Lewis 2004) and in Wellington Reservoir, Western Australia (Monismith *et al.* 1990). The velocity of convection and the resulting water exchange between the side-arm and main basin can be fast simply by natural convection (Monismith *et al.* 1990). In this study, the time scale for the temperature difference and convective motion (minutes – hours), however, was very short relative to the time for significant growth to be observed in the phytoplankton community (many hours – days).

Other physical/hydrological processes, such as intrusions from river flow and destratification systems, can also be contributing to the relative homogeneity in phytoplankton distribution (Fischer *et al.* 1979). However, Myponga River does not generally flow during summers. Hence, the inflow intrusion from the river can be excluded as a mechanism, contributing to homogeneity of the phytoplankton population in Myponga Reservoir.

Artificial mixing would be manifested in the development of radial intrusion flow that would theoretically penetrate until a physical barrier (e.g. the shoreline) was encountered. Therefore, it could be expected that the radial intrusions generated by the surface mixers and aerator in Myponga Reservoir could travel up to the side-arm. Disregarding entrainment, the velocities in the intrusions were inversely proportional to radial distance from the source of the intrusion. Intuitively, the intrusion flows travelling through the side-arm entrance would be orders of magnitude less than that near the mixers. Therefore, the intrusions would not be expected to cause any significant mixing along the side-arm. Nevertheless, the near field intrusions around the mixing devices would deepen the surface mixed layer, creating horizontal instability caused by density differences across the reservoir surface. This would result in horizontal mixing and cause the side-arm to interact with the main basin.

5.5 Conclusion

In this study, the potential impact on the phytoplankton population was modelled under different underwater light exposures, influenced by variations in surface mixed layer depths between the shallow and deep regions of Myponga Reservoir. The variability in the physical and chemical environment can lead to patchiness in the phytoplankton community, but in the case of Myponga Reservoir the phytoplankton patchiness is most likely overcome by basin-scale circulation induced by thermal gradients between the shallow and deep regions. Further to this, other motion of water can also transport cells between sites. Lake morphometry and local meteorological conditions are all likely to have contributed to phytoplankton patchiness or relative homogeneity.

Chapter 6 Temporal and spatial dynamics of phytoplankton cellular content and stoichiometry (TC:TN:TP:Chl*a*)

Preamble

In the previous chapters, I have shown different lake processes controlling the nutrient and light conditions, which may be contributing factors to determine phytoplankton variability. Chapter 6 details a temporal and spatial survey of the phytoplankton biochemistry during a typical South Australian summer. It is believed to be the first study of the phytoplankton cellular content and stoichiometry at Myponga Reservoir in such an intensive spatial and temporal manner.

Under guidance from supervisors, I was responsible for the whole phytoplankton survey, sample processing, data analysis and interpretation. The water quality data (e.g. nutrients and phytoplankton cell counts) and meteorological/hydrological data (e.g. rainfall and inflow) were kindly provided by SA Water.

Chapter 6 Temporal and spatial dynamics of phytoplankton cellular content and stoichiometry (TC:TN:TP:Chla)

6.1 Introduction

South Australia has a Mediterranean climate and Myponga Reservoir receives external nutrient loadings primarily from its catchment during the wet season (winter) and only occasionally by thunderstorms in the dry summer. Historical data of Myponga Reservoir clearly show the seasonal water nutrient dynamics with relatively high nutrient concentrations in winter and relatively low in summer (Figure 27). Phytoplankton utilise available nutrients in the warm growing season and sometimes reach a high abundance.

The density of the phytoplankton community measured as chlorophyll *a* in Myponga Reservoir typically increases in spring and remains steady throughout summer (Figure 27). The community structure of phytoplankton at Myponga Reservoir is often dominated by diatoms, yellow-brown algae and green algae (Lewis 2004). Occasional cyanobacterial blooms of *Anabaena circinalis* have been observed during periods with high irradiance, low wind velocity and prolonged thermal stratification, whereby excessive growth of *Anabaena circinalis* has been successfully inhibited by algicide dosing (Lewis 2004). During summers, the bio-available nutrient concentrations in the water column that support primary production decrease slowly and sometimes fall below the minimum level of detection (i.e. nitrate and nitrite, $NO_x < 0.005$ mg L^{-1} and filterable reactive phosphorus, FRP < 0.005 mg L^{-1}). If there is no further external input of nutrients in the summer, the entire nutrient supply for phytoplankton growth may rely on the internal recycling of nutrients within the system (Caraco *et al.* 1992).

In addition, the low concentrations of bio-available nutrients, often below the minimum level of detection, does not necessarily mean that the nutrient is limiting the growth of phytoplankton, as some phytoplankton populations (e.g. *Asterionella*) are capable of storing nutrients in excess of immediate demands (Mackereth 1953; Reynolds 1984). The analysis of phytoplankton stoichiometry will provide better understanding of the phytoplankton nutrient requirements and also reflect the barely detectable nutrient levels

in the ambient water. Finkel *et al.* (2010) also suggested that the elemental stoichiometry is a promising ecophysiological trait for tracking changes in phytoplankton community structure in response to climate change. Phytoplankton stoichiometry is relatively flexible when compared to the narrow stoichiometric ranges in zooplankton and fish (van de Waal *et al.* 2010). It is because phytoplankton are capable of fixing carbon and taking up nutrients separately; hence phytoplankton stoichiometry can vary over large ranges and generally reflect the light availability and inorganic nutrient supply in their aquatic environment (Sterner and Elser 2002; van de Waal *et al.* 2010). For instance, when the concentration of bio-available P is high in the water, phytoplankton cells are often C-poor and P-rich, leading to relatively low intracellular C:P ratios (van de Waal *et al.* 2010). In contrast, when light energy and inorganic C are high relative to nutrient availability, phytoplankton are comparatively rich in C and poor in P, resulting in high intracellular C:P ratios (Sterner *et al.* 1997).

Phytoplankton stoichiometry not only provides insight into the phytoplankton nutrient status and constraints and the resource availability in the ambient environment, but also reveals the quality of food available for next trophic organisms. Heterotrophic organisms (e.g. zooplankton) obtain carbon and nutrients simultaneously from their food (e.g. phytoplankton); if the nutritional value of the food quality is low (i.e. high C:nutrient ratios in phytoplankton), the relatively low nutrient-demanding species (e.g. cladoceran *Daphnia* and rotifer *Brachionus*) may be favoured (Urabe *et al.* 2002; Jensen and Verschoor 2004). The change in nutritional value of phytoplankton can lead to the shift in species composition of zooplankton, which in turn, may influence the structure and functioning of entire aquatic food webs (van de Waal *et al.* 2010).

Discussions on the importance of elemental composition of phytoplankton have recently been extended to the influence of the production of phytoplankton secondary metabolites. Van de Waal *et al*. (2009) found that the environmental availability of inorganic C and N affects the intracellular C:N ratio of a cyanobacterium *Microcystis aeruginosa* and the production and composition of its toxic metabolites, microcystins, which have at least 89 different variants with different amounts of nitrogen content (Welker and von Dohren 2006). In a low inorganic N environment, *Microcystis* cells with low cellular N:C ratios (high C:N ratios) produced low concentrations of the N-rich variant of microcystin-RR (Van de Waal *et al.* 2009). If *Microcystis* cells were exposed to the excess supply of both inorganic C and N but with light limitation, the

cellular N:C ratios would become high (i.e. low C:N ratios) favouring the production of microcystin-RR (Van de Waal *et al.* 2009). Therefore, they warned that rising atmospheric CO_2 levels in eutrophic aquatic systems would favour cyanobacterial growth as well as toxin productions.

The classical Redfield ratio of $carbon_{106}$:$nitrogen_{16}$:$phosphorus_1$ by atom (Redfield *et al.* 1963) or C_{40}:N_7:P_1 by dry weight (Vallentyne 1974) was used as a benchmark to compare the nutrient status with the observed phytoplankton stoichiometry in Myponga Reservoir. Although the Redfield ratio referred to the marine particulate matter (seston) at the nutrient-sufficient status and the near-optimal growth rates (Goldman *et al.* 1979), it was generally believed that the C:N:P ratio for lacustrine particulate matter was similar to those in the marine environment (Stumm 1983; Harris 1986). However, variations were found between small lakes and large lakes as well as freshwater seston and marine seston (Hecky *et al.* 1993; Elser and Hassett 1994; Sterner *et al.* 2008) and therefore "*the Redfield ratios are the exception rather than the rule in freshwater*" (Hecky *et al.* 1993 p. 709). Sterner *et al.* (2008) even suggested to replace the constant Redfield ratio to a more general power function model to describe biogeochemical cycling at regional or smaller scales of aquatic systems. Nevertheless, the Redfield ratio is still appropriate at large spatiotemporal scales (Sterner *et al.* 2008) and is relevant for general comparison.

There are some hydrological and hydrodynamic processes, which potentially provide alternative sources of nutrients to maintain the high population of phytoplankton during the period of low or undetectable nutrients. The in-lake processes include the nutrient recycling from organic matter, detritus and phytoplankton itself, the nutrient release from anaerobic sediments and the nutrient resuspension in the shallow water. Occasionally, heavy summer rains may provide additional influx of nutrients from the catchment. The nutrient release rates from these processes may vary from shallow to deep areas and from inflow to outflow areas within the water body. It is reasonable to consider the stoichiometric change in phytoplankton in two different habitat conditions: a relatively shallow inlet (the side-arm) and a relatively deep outlet (the main basin) of Myponga Reservoir.

In Mediterranean lakes and reservoirs, external nutrient loads from catchment are often limited by the dry summers, while bio-available nutrients may deplete slowly in the

water column to support phytoplankton productions. Nutrient content within the phytoplankton cells may also change with the decreasing nutrient levels in the water column, leading to temporal heterogeneity of phytoplankton stoichiometry. The aim of the study was to investigate any seasonal change in nutrient content and stoichiometry of a natural phytoplankton community in Myponga Reservoir of South Australia from January to July 2009. The study also aimed to determine whether the phytoplankton community was limited by the availability of nutrients (i.e. nitrogen and phosphorus). Concurrently, spatial differences of phytoplankton population and stoichiometry were explored in the shallow and deep areas of the reservoir, where the potential variability of light and nutrient may give rise to the spatial variation. This study could provide evidence on how much variability of phytoplankton biochemistry there is over time and space.

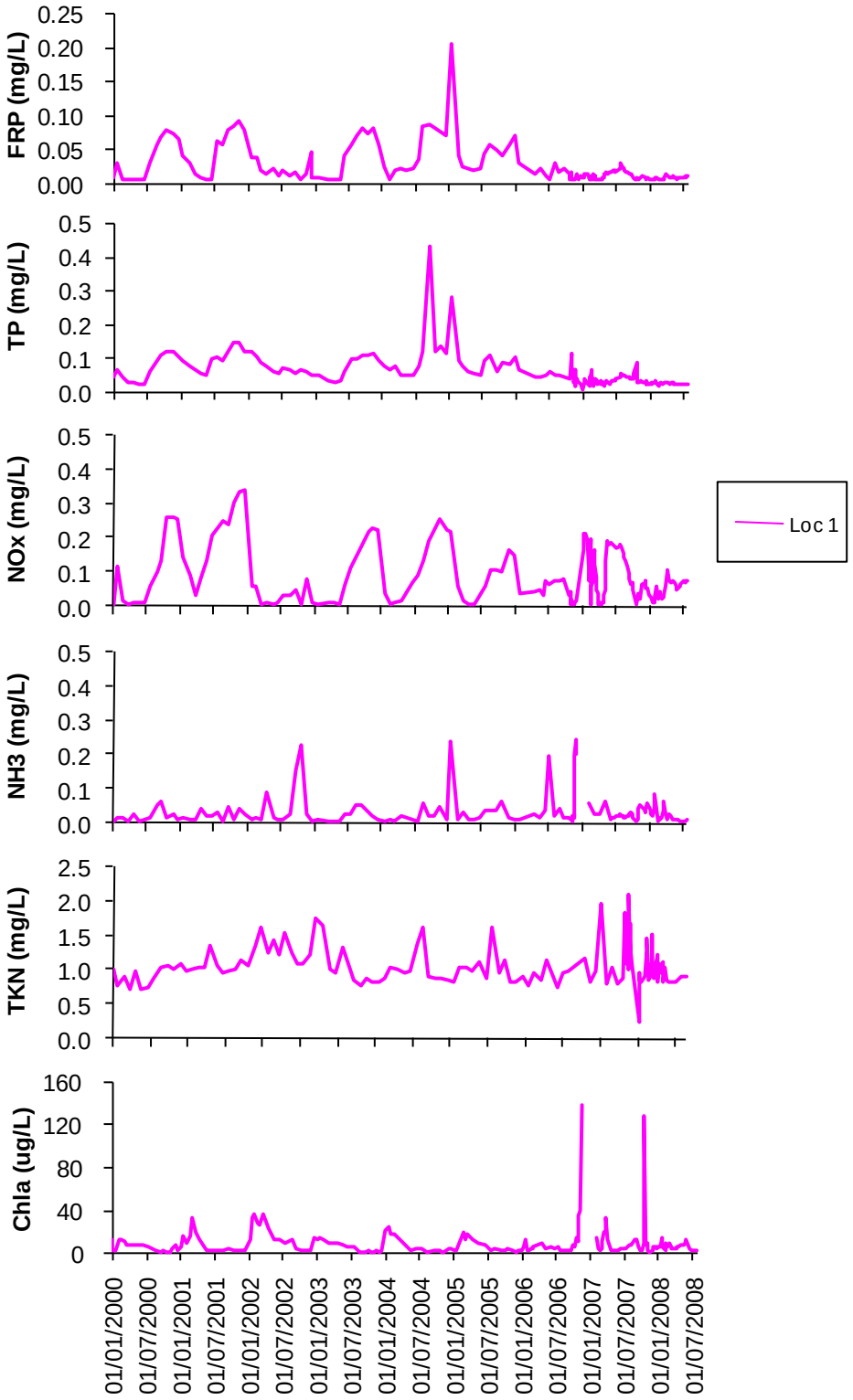


Figure 27 Historical data for filterable reactive phosphorus FRP (mg L^{-1}), total phosphorus TP (mg L^{-1}), nitrate and nitrite NO_x (mg L^{-1}), ammonia NH_3 (mg L^{-1}), total Kjeldahl nitrogen TKN (mg L^{-1}) and chlorophyll *a* (µg L^{-1}) concentrations in the surface water of Myponga Reservoir (Loc 1 in the main basin)

6.2 Methods

6.2.1 Measurements of hydrological and water quality variables

Rainfall at Myponga Reservoir and inflow from Myponga River were collected daily by SA Water between January and July 2009. Meanwhile, water quality measurements, including filterable reactive phosphorus (FRP), total phosphorus (TP), nitrate and nitrite (NO_x), ammonia (NH_3), total nitrogen (TN) and chlorophyll *a* (Chl*a*) were also collected weekly by SA Water in the two locations (Figure 28): main basin (Loc 1) and side-arm (Loc 8). Detailed methods for nutrient analysis are referred to Table 4 in Chapter 3. Phytoplankton identification and cell count to genus level were conducted and provided by SA Water for one location (Loc 1 in the main basin) throughout the study.

6.2.2 Phytoplankton measurements

Natural phytoplankton assemblages in the surface water were sampled weekly in two locations of Myponga Reservoir between January and July 2009 (Figure 28). Loc 8 in the side-arm of the reservoir is sited within the shallower inlet region, whereas Loc 1 in the main basin is relatively deep and further away from the inlet. Three replicate samples were taken at each site using plankton nets with a pore size of 20 µm mesh. Horizontal net tows from a boat were performed for 2 minutes at a speed of about 2 knots per hour (total distance about 100 m). Net tows were used in preference to discrete samples, as concentrated samples were required for elemental analysis of the phytoplankton. The samples were stored in clean polyethylene terephthalate (PET) bottles on ice, in the dark for transport back to laboratory (approximately 2 hours duration).

Upon return to the laboratory, the phytoplankton samples were immediately filtered through a 88 µm mesh to remove zooplankton, sediment particles and plant materials. Under microscopic examination, only a small proportion of zooplankton material remained in the filtered samples. The majority of the filtered material was phytoplankton (Figure 29). Nevertheless, the samples would contain microscopic assemblages of detritus, bacteria, protozoa and small metazoan zooplankton with overlapping cell sizes (Sterner and Elser 2002). Furthermore, some larger phytoplankton species such as *Closterium aciculare* (about 150 µm long) were filtered out of the samples due to their elongated shape. Therefore, the samples only represented

the natural population of phytoplankton in the size range between 20 and 88 µm. All filtered samples were kept in –80ºC freezer and dried using a vacuum freeze-dryer to avoid cell rupture.

Cellular concentrations of total carbon (TC), total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (Chl*a*) were measured and expressed as µg g^{-1} of dry phytoplankton materials. The dried samples were homogenized and sent for TC and TN analysis at the Analytical Services Unit in CSIRO Land and Water (Urrbrae, South Australia). TC and TN were determined by high temperature combustion (~1000°C) in an atmosphere of oxygen using a LECO CNS-2000 (St. Joseph, USA) following the methods described in Matejovic (1997). Carbon was converted to carbon dioxide gas and determined by infrared detection, while nitrogen was determined as nitrogen gas by thermal conductivity detection. For TP analysis, a sub-sample of the dried phytoplankton was digested using nitric acid and hydrogen peroxide, followed by the measurement of Inductively Coupled Plasma Atomic Emission Spectrometry at Waite Analytical Services of The University of Adelaide (Urrbrae, South Australia). For Chl*a* content in phytoplankton cells, a known volume of well-mixed concentrated sample was filtered through GF/C filters and extracted in methanol for 24 hours in the dark. The solvent was then measured by Hitachi Spectrophotometer U-1000 (Tokyo, Japan) at 665 nm wavelength and corrected by the absorbance at 750 nm wavelength (Golterman *et al.* 1978).

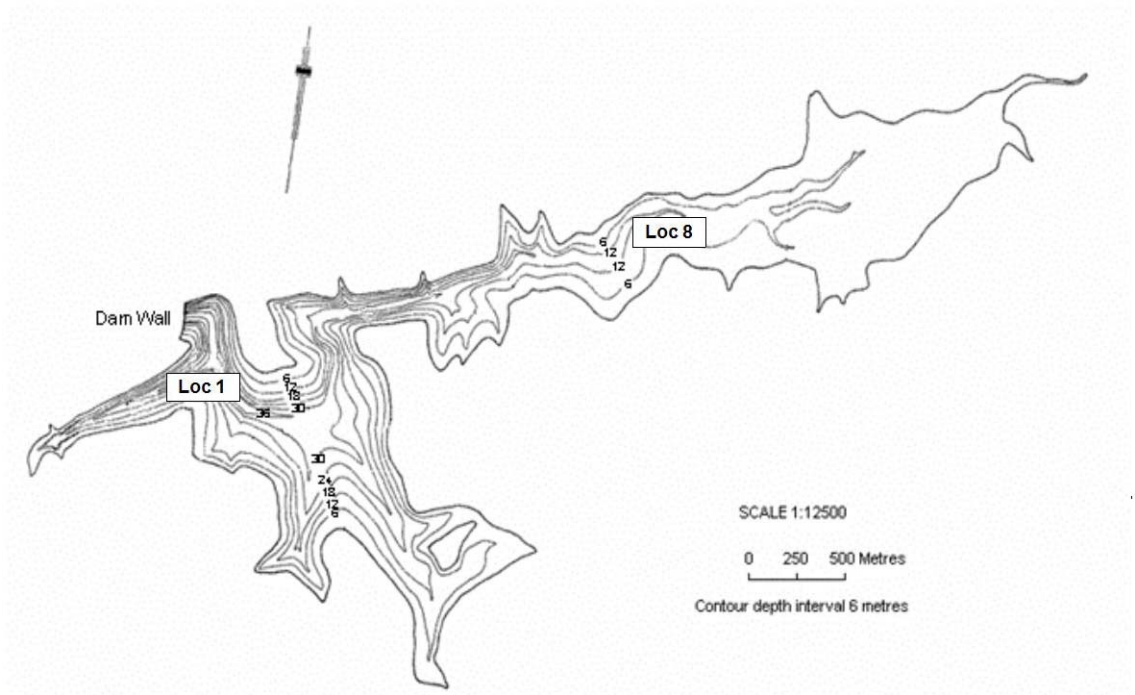


Figure 28 Phytoplankton and water sampling sites in Myponga Reservoir: Loc 1 (main basin) and Loc 8 (side-arm)

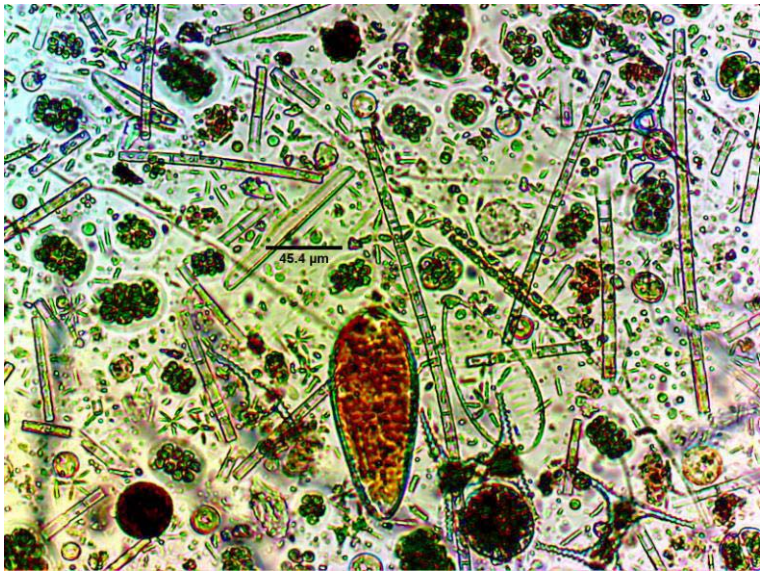


Figure 29 Microscopic photograph of phytoplankton assemblages in Myponga Reservoir collected by net towing

6.2.3 Statistical analyses

All statistical tests, including repeated-measures analysis of variance (ANOVA) and Pearson Product-moment Correlation/ Spearman's Rank Order Correlation were conducted using SPSS 15.0 for Windows. The analysis of repeated-measures ANOVA was used to test the equality of means for the *location* effect, the *time* effect and the interactive effect between location and time. There are four assumptions for the repeated-measures ANOVA: (i) random selection of samples from the population of interest; (ii) normal distribution for each population; (iii) homogeneous variances for different populations; and (iv) sphericity – equal variance of the population difference at repeated measures (Coakes *et al.* 2009). The first three assumptions are similar to other ANOVA tests, while the last assumption validated by Mauchly's sphericity test is specific to the repeated-measures ANOVA. If the significance of Mauchly's test is small ($p < 0.05$), the assumption of sphericity will be violated and the results from the repeated-measures ANOVA may be in error. Correction factors from the Greenhouse-Geisser and Huynh-Feldt epsilon (estimates) can be used to adjust the degrees of freedom for the calculations of *F*- and *p*-values. The conservative Greenhouse-Geisser epsilon is recommended for the adjustment when the epsilon is less than 0.75. If the Greenhouse-Geisser epsilon is greater than 0.75, the Huynh-Feldt epsilon is used for the correction (Girden 1992).

Once the adjustment is made for the violation of the sphericity assumption, it is possible to properly evaluate the results of the repeated-measures ANOVA as suggested by Corston and Colman (2003). If the *p*-value for the between-subjects *location* effect is smaller than 0.05, there is a significant difference between the two locations (the side-arm and main basin). If the *p*-value for the within-subjects *time* effect is smaller than 0.05, there is a significant difference over the study period between January and July 2010. If the *p*-value for the within-subjects *time*location interactive* effect is smaller than 0.05, it suggests that the difference over time depends on the location.

Scatterplots were first used to explore the relationship between two variables (e.g. checking for outliers, inspecting the distribution of data points and determining the direction of the relationship between the variables) before calculating correlations (Pallant 2007). Scatterplot matrix was presented rather than individual scatterplots due to the vast combinations of relationships. Due to the violation of assumptions of normality, linearity and homogeneity of variance for a parametric test (i.e. Pearson

Product-moment Correlation), a non-parametric test (i.e. Spearman's Rank Order Correlation) was used to calculate the strength of the relationship between two variables (Pallant 2007). The two variables are neither dependent upon each other nor cause-and-effect relationship (Zar 1999). Correlation coefficient (r) was used to describe the strength and direction of the relationships. If the r is close to 1, the relationship between variables is very strong. In contrast, there is no relationship between variables when the r is zero. The signs of the r represent a positive or a negative correlation (Zar 1999; Coakes *et al.* 2009).

6.3 Results

6.3.1 External nutrient loadings from catchment

The nutrient concentrations in Myponga Reservoir are largely affected by the external nutrient inputs from the catchment along with the dynamics of rainfall and inflow (Linden *et al.* 2004). Daily rainfall within the region and daily inflow into Myponga Reservoir from its main river systems were measured during the study period between January and July 2009 (Figure 30). There was no rain in the dry summer months of January and February. Occasional rains started to develop in March with a monthly rainfall of 23 mm and a daily rainfall, ranging from 0.4 to 9.4 mm. The rains became more frequent and heavier from April onward, with a monthly rainfall of about 80 mm between April and June. However, the inflow of the reservoir remained consistently low between January and June. A significant inflow from the catchment to the reservoir was only recorded from mid July, after the heavy rainfall over 100 mm across 8 days. It is apparent that the earlier rainfall (March to June) received from the catchment area did not immediately transfer to the riverine system and then to the reservoir. Instead, most of the rainwater was absorbed by the dry soil between March and June and this showed a noticeable time lag of four months before a significant amount of runoff reached the downstream reservoir in July (Figure 30).

The rainfall and inflow data were reflected the influx of nutrients from the catchment to the reservoir. The bio-available nutrient concentrations such as FRP and NO_x in the reservoir water were below the minimum level of detection (< 0.005 mg L^{-1}) in the dry months (Figure 31). A small increase of the nutrient concentrations was detected in May when the rainy season started. The nutrient levels in the reservoir increased dramatically and concurrently with the inflow in July. The nutrient concentrations in the side-arm

(Loc 8) increased earlier and higher than in the main basin (Loc 1), as the nutrient-rich inflow water passed through the side-arm first and then mixed with the water in the main basin.

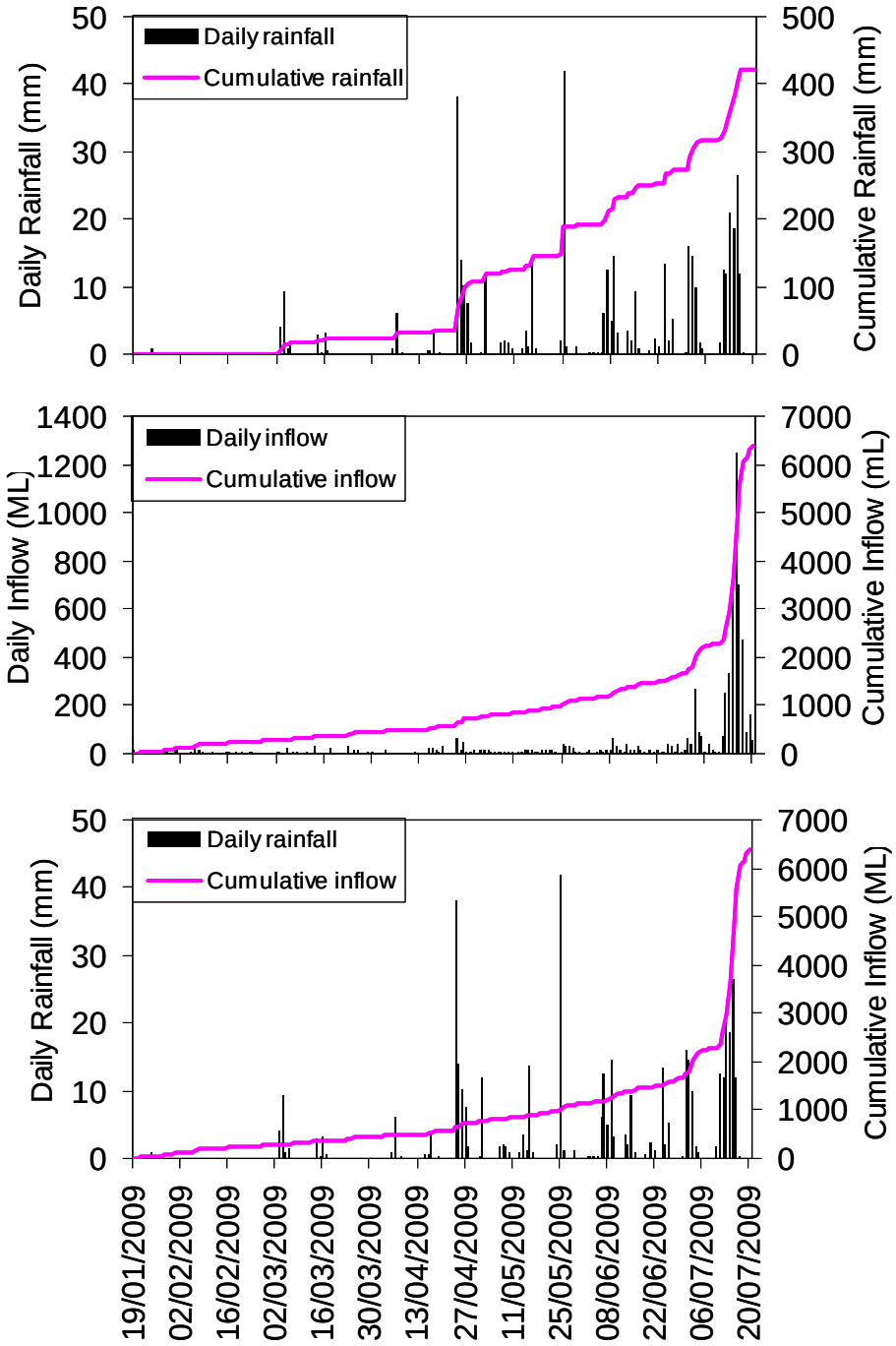


Figure 30 Daily and cumulative rainfall (mm) in the Myponga region and daily and cumulative inflow (ML) from Myponga River collected between January and July 2009

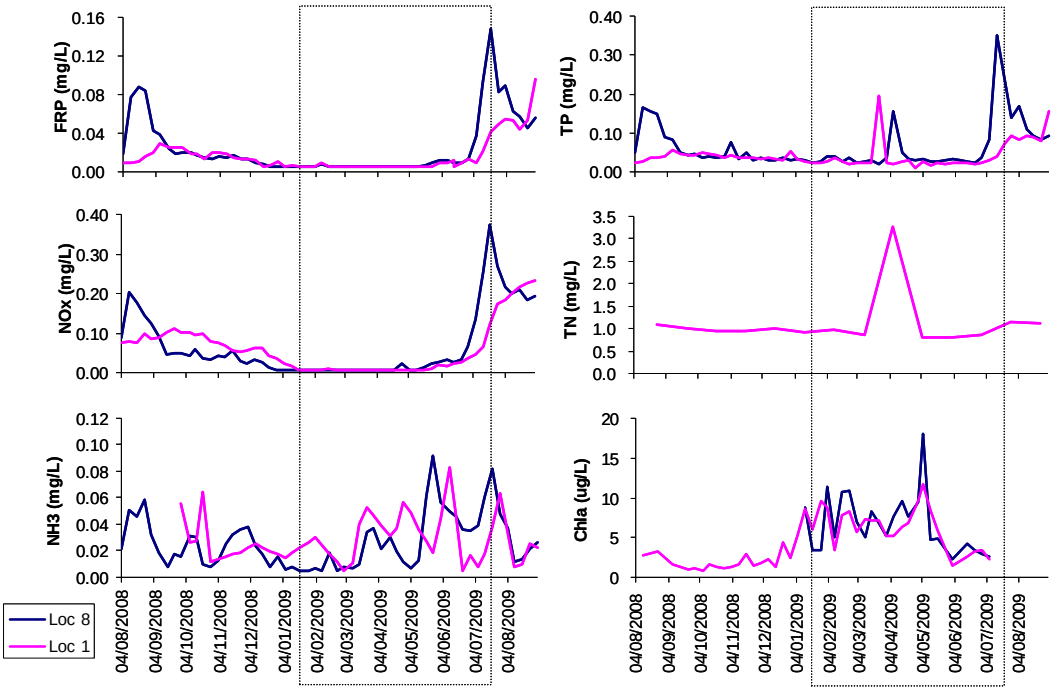


Figure 31 Concentrations of filterable reactive phosphorus FRP (mg L^{-1}), nitrate and nitrite NO_x (mg L^{-1}), ammonia NH_3 (mg L^{-1}), total phosphorus TP (mg L^{-1}), total nitrogen TN (mg L^{-1}) and chlorophyll *a* (µg L^{-1}) in the water column of Myponga Reservoir collected in two locations. The dashed frames represent the current study period between January and July 2009

6.3.2 Phytoplankton cellular contents

Cellular contents (TC, TN, TP and Chl*a*) of phytoplankton assemblages were measured over a growing season in two locations within Myponga Reservoir (Figure 32). The investigation ended in July when there were large amounts of terrestrial material, such as pine pollen that could not be separated from the phytoplankton cells due to size similarity. The repeated-measures analysis of variance was used to determine the effects of location, time and their interaction for each cellular content (Table 29). The TC, TN, TP and Chl*a* contents in dry phytoplankton biomass were significantly different between the two locations (repeated-measures ANOVA, $p < 0.01$) even though the standard deviations for a few samples were relatively large. The mean carbon (TC) and phosphorus (TP) contents of phytoplankton were statistically higher in the side-arm (Loc 8), whereas the mean TN and Chl*a* contents were higher in the main basin (Loc 1). Scatterplot matrix (Figure 33) and Pearson Product-moment Correlation/ Spearman's

Rank Order Correlation (Table 30) further reviewed these relationships linearly. For both locations, there was a negative correlation between TC and TP and a positive correlation between TN and TP. The negative correlation means that the TC increased, while the TP decreased and vice versa. For the positive correlation, the TN increased/decreased with the TP. It is important to point out that the correlation does not imply any causal relationship. Apart from a positive relationship between TC and Chl*a* in Loc 8, no other relationship was detected; neither between TC and TN nor between nutrient and Chl*a*.

The three nutrient contents (TC, TN and TP) for the phytoplankton communities changed significantly over the study period (Figure 32). Marked changes of nutrient contents were observed between 16th February and 4th May, where TP content declined gradually from 5450 µg g^{-1} (0.55%) to 1595 µg g^{-1} (0.16%), but TC content increased steadily from 276,133 µg g^{-1} (27.6%) to 362,150 µg g^{-1} (36.2%). This trend was also revealed by the negative correlation between TC and TP contents (Table 30). The variation in TN content (1.4 – 4%) over time appeared stable relative to the variations in TC content (15.4 – 44.5%) and in TP content (0.1 – 0.6%). Nevertheless, a significant time effect for TN content was found (repeated-measures ANOVA, $p < 0.01$, Table 29), this may be linked to the highest TN cellular content (3.2 – 4%) observed on 16th February 2009 (two weeks after the severe heatwave). The highest TP cellular content (0.6%) was also recorded on the same sampling date. Unlike Chl*a* content, the effect of time was not detected by the repeated-measures ANOVA ($p > 0.05$), indicating a consistent Chl*a* content in phytoplankton community over time.

There were significant interaction between time and location for TC, TN and TP contents in phytoplankton cells (repeated-measures ANOVA, $p < 0.05$, Table 29). This implied that the nutrient contents in the phytoplankton communities were changing over time, but were changing in different ways; therefore the trends representing the two locations in Figure 32 were not parallel. Interestingly, the trends for Chl*a* content at Locations 1 and 8 were also not parallel (Figure 32), while no time and location interaction for Chl*a* content was detected by the repeated-measures ANOVA ($p > 0.05$) in Table 29. This may be explained by the fact that Chl*a* content did not display any time effect (repeated-measures ANOVA, $p > 0.05$, Table 29).

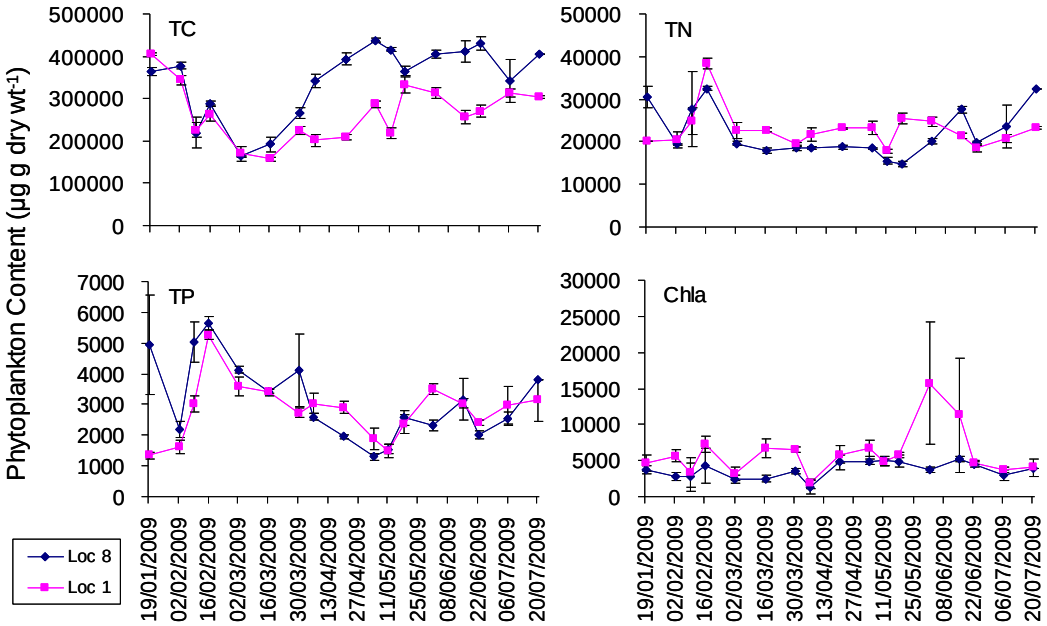


Figure 32 Total carbon (TC), total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (Chla) contents (± standard deviation) in phytoplankton cells collected in two locations (Loc 1 in the main basin and Loc 8 in the side-arm) of Myponga Reservoir between January and July 2009. All units are expressed as µg g dry weight^{-1}

Table 29 Repeated-measures analysis of variance: between-subject factor location within-subject factor time and interaction time*location for phytoplankton contents, phytoplankton stoichiometric ratios and water quality parameters collected between January and July 2009

Measurements		*Location*	*Time*	*Time * Location*
Phytoplankton	TC	$F(1, 4) = 543.98$ $p < 0.005$ ***	$F(15, 60) = 100.19$ $p < 0.005$ ***	$F(15, 60) = 31.95$ $p < 0.005$ ***
	TN	$F(1, 4) = 22.90$ $p < 0.01$ **	$F(15, 60) = 24.20$ $p < 0.01$ **	$F(15, 60) = 8.67$ $p < 0.05$ *
	TP	$F(1, 4) = 58.69$ $p < 0.005$ ***	$F(15, 60) = 28.30$ $p < 0.005$ ***	$F(15, 60) = 10.29$ $p < 0.05$ *
	Chl*a*	$F(1, 4) = 657.92$ $p < 0.005$ ***	$F(15, 60) = 4.34$ $p > 0.05$	$F(15, 60) = 2.58$ $p > 0.05$
	TC:TN	$F(1, 4) = 576.18$ $p < 0.005$ ***	$F(15, 60) = 160.92$ $p < 0.005$ ***	$F(15, 60) = 86.37$ $p < 0.005$ ***
	TC:TP	$F(1, 4) = 62.79$ $p < 0.005$ ***	$F(15, 60) = 65.76$ $p < 0.005$ ***	$F(15, 60) = 37.00$ $p < 0.005$ ***
	TN:TP	$F(1, 4) = 28.79$ $p < 0.01$ **	$F(15, 60) = 23.59$ $p < 0.005$ ***	$F(15, 60) = 11.34$ $p < 0.05$ *
	TC:Chl*a*	$F(1, 4) = 16.10$ $p < 0.05$ *	$F(15, 60) = 4.77$ $p > 0.05$	$F(15, 60) = 1.44$ $p > 0.05$
	TN:Chl*a*	$F(1, 4) = 5.38$ $p > 0.05$	$F(15, 60) = 4.01$ $p > 0.05$	$F(15, 60) = 0.63$ $p > 0.05$
	TP:Chl*a*	$F(1, 4) = 7.18$ $p > 0.05$	$F(15, 60) = 4.24$ $p > 0.05$	$F(15, 60) = 1.02$ $p > 0.05$
Water	Chl*a*	$F(1, 6) = 35.01$ $p < 0.005$ ***	$F(15, 90) = 55.19$ $p < 0.005$ ***	$F(15, 90) = 6.79$ $p < 0.05$ *

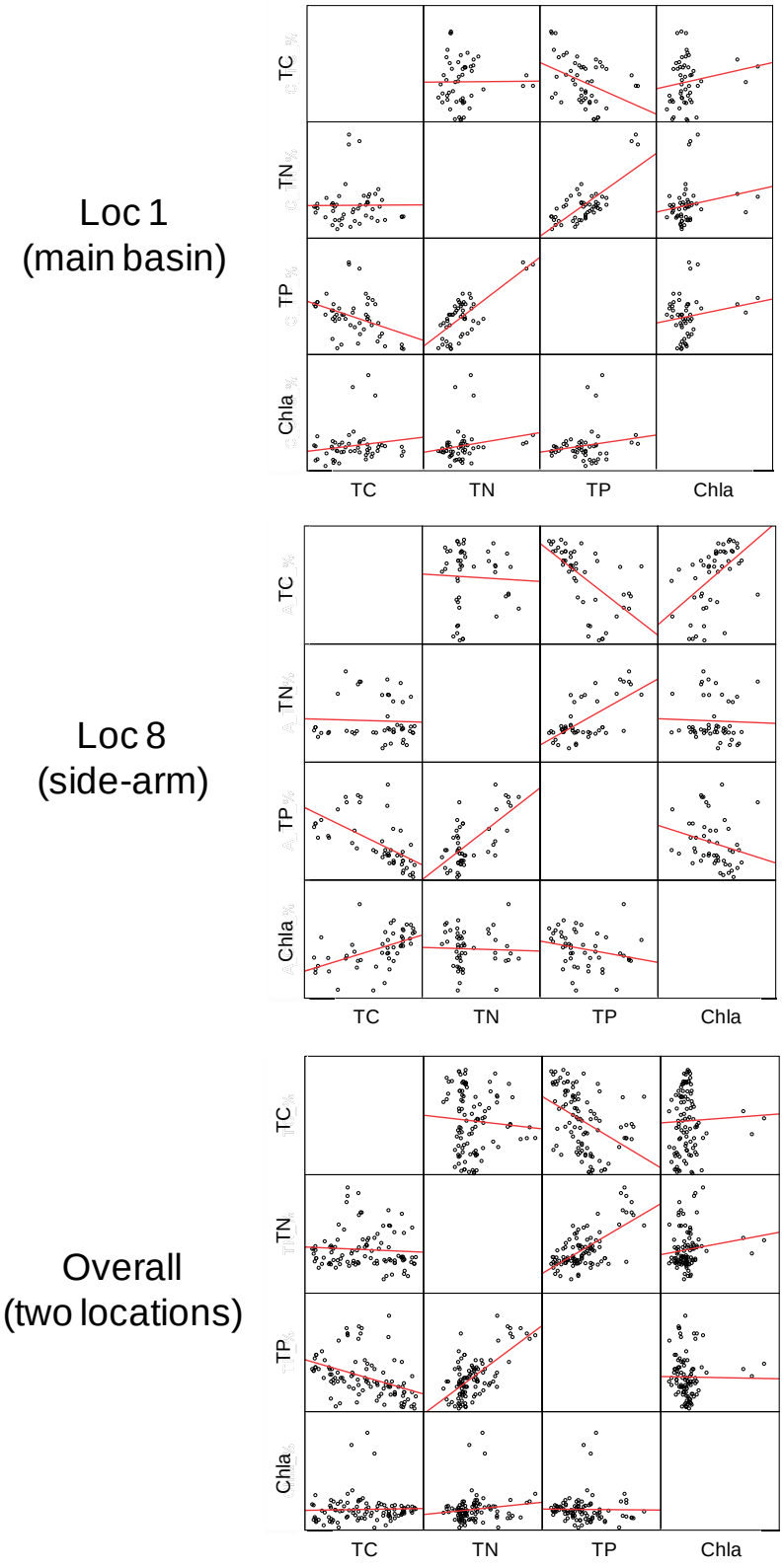


Figure 33 Scatterplot matrix for correlations of total carbon (TC), total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (Chla) contents in phytoplankton cells collected in two locations of Myponga Reservoir between January and July 2009. Linear line is fitted in each scatterplot

Table 30 Pearson Product-moment Correlation/ Spearman's Rank Order Correlation for total carbon (TC), total nitrogen (TN), total phosphorus (TP) and chlorophyll *a* (Chla) contents in phytoplankton cells collected in two locations of Myponga Reservoir between January and July 2009

Pearson/ Spearman's Correlation		TC	TN	TP	Chl*a*
Loc 1 (main basin) (*n* = 51)	**TC**	-	0.064	-0.414***	0.148
	TN		-	0.541***	0.322*
	TP			-	0.082
	Chl*a*				-
Loc 8 (side-arm) (*n* = 49)	**TC**	-	-0.024	-0.764***	0.632***
	TN		-	0.459***	-0.169
	TP			-	-0.376**
	Chl*a*				-
Overall (two locations) (*n* = 100)	**TC**	-	-0.089	-0.541***	0.093
	TN		-	0.498***	0.185
	TP			-	-0.170
	Chl*a*				-

* $p < 0.05$
** $p < 0.01$
*** $p < 0.005$

6.3.3 Phytoplankton stoichiometry

Phytoplankton stoichiometry was calculated from the cellular contents of TC, TN, TP and Chl*a* in a basis of dry weight (Figure 34). At the beginning of the sampling period (19th January – 9th February 2009), there were sharp decreases in the stoichiometric ratios of TC:TN, TC:TP and TN:TP in the phytoplankton assemblages. The TC:TN ratio dropped from approximately 20 to 9 for the main basin (Loc 1) and side-arm (Loc 8); the TC:TP decreased from about 300 to 70 for Loc 1 and from about 160 to 40 for Loc 8; the TN:TP ratio declined from approximately 15 to 8 for Loc 1 and from approximately 9 to 5. These ratios became stable over the next few weeks (16th February – 2nd March) and were very close to the Redfield ratios (C:N:P ~ 40:7:1, by weight) for healthy marine plankton. The ratios gradually increased at different rates and peaked at about the same time in early May (i.e. TC:TN ~ 13, TC:TP ~ 150 and TN:TP ~ 12 at Loc 1; TC:TN ~ 27, TC:TP ~ 340 and TN:TP ~ 14 at Loc 8) and followed by the declines toward winter. These dramatic changes in phytoplankton

stoichiometry over time were highly significant (Table 29). Meanwhile, the repeated-measure ANOVA also revealed strong differences for TC:TN, TC:TP and TN:TP ratios between the two locations (Table 29). The mean TC:TN and TC:TP ratios were higher in the side-arm, while the mean TN:TP ratio was higher in the main basin. The effects of time and location interaction were also prominent, indicating the ratios in the two locations exhibited differently over the study period.

The TC:Chl*a*, TN:Chl*a* and TP:Chl*a* ratios (hereafter known as nutrient:Chl*a* ratios) were relatively stable over the growing season and across the two sites when compared with the dynamic TC:TN, TC:TP and TN:TP ratios. No significant time and location effects were found in the nutrient:Chl*a* ratios, except TC:Chl*a* slightly varied between the two sites (Table 29). The constant ratios of nutrient:Chl*a* over the changing time and various locations suggested that the ratios of TC:TN, TC:TP and TN:TP were better indicators than the ratios of TC:Chl*a*, TN:Chl*a* and TP:Chl*a* to describe the relative nutrient status in phytoplankton population.

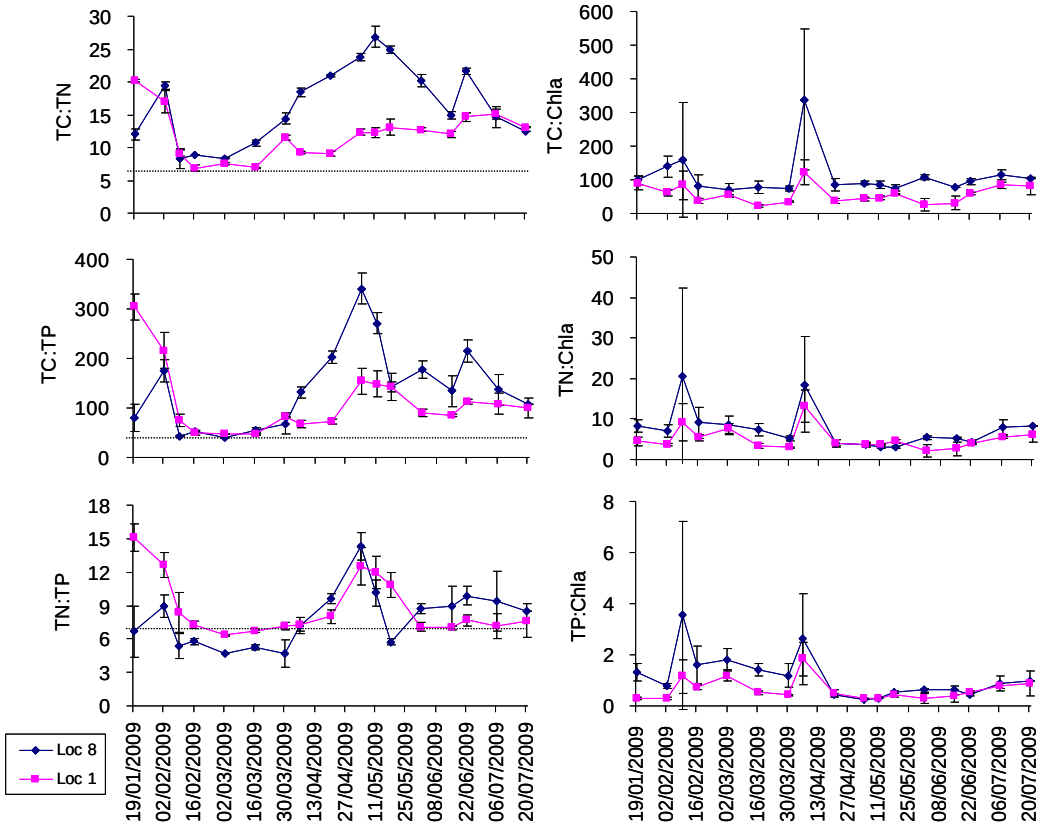


Figure 34 Stoichiometric ratios (by weight) of total carbon, total nitrogen, total phosphorus and chlorophyll *a* (± standard deviation) in phytoplankton cells collected in two locations of Myponga Reservoir between January and July 2009. The dashed lines denote the Redfield ratios by dry weight, which are 5.7 for C:N, 40 for C:P and 7 for N:P (Redfield *et al.* 1963; Vallentyne 1974)

6.3.4 Phytoplankton composition

Phytoplankton composition in the surface water of the main basin (Loc 1) was identified and the major phytoplankton genera of the functional groups were counted weekly over the growing season. There was a shift in the dominant phytoplankton functional groups over time (Figure 35). Blue-green algae (*Anabaena* and *Aphanocapsa*) and cryptophyta (*Chroomonas*) appeared and reached their highest concentrations of 900, 2000 and 800 cell mL^{-1} respectively, in February and then the concentrations declined rapidly in March. Green algae (*Aulacoseira*) and diatoms (*Cyclotella*) emerged from March to June, while other genera of green algae and cryptophytes co-dominated in July. There were a few different green algae (e.g. *Actinastrum*, *Ankistrodesmus*, *Chlorella*, *Dictyosphaerium* and *Oocystis*) that dominated at different times. *Oocystis* and

Actinastrum were found nearly throughout the entire study period. Typically, when *Oocystis is found* at a lower concentration, *Actinastrum* is measured at a higher concentration. Autumn appeared to be more favourable for other green algae (e.g. *Dictyosphaerium*, *Ankistrodesmus* and *Chlorella*). Other common phytoplankton, for example *Closterium*, *Staurastrum*, *Ceratium*, *Melosira* and *Cryptomonas* were found in Myponga Reservoir, but were not presented in the results due to their low concentrations.

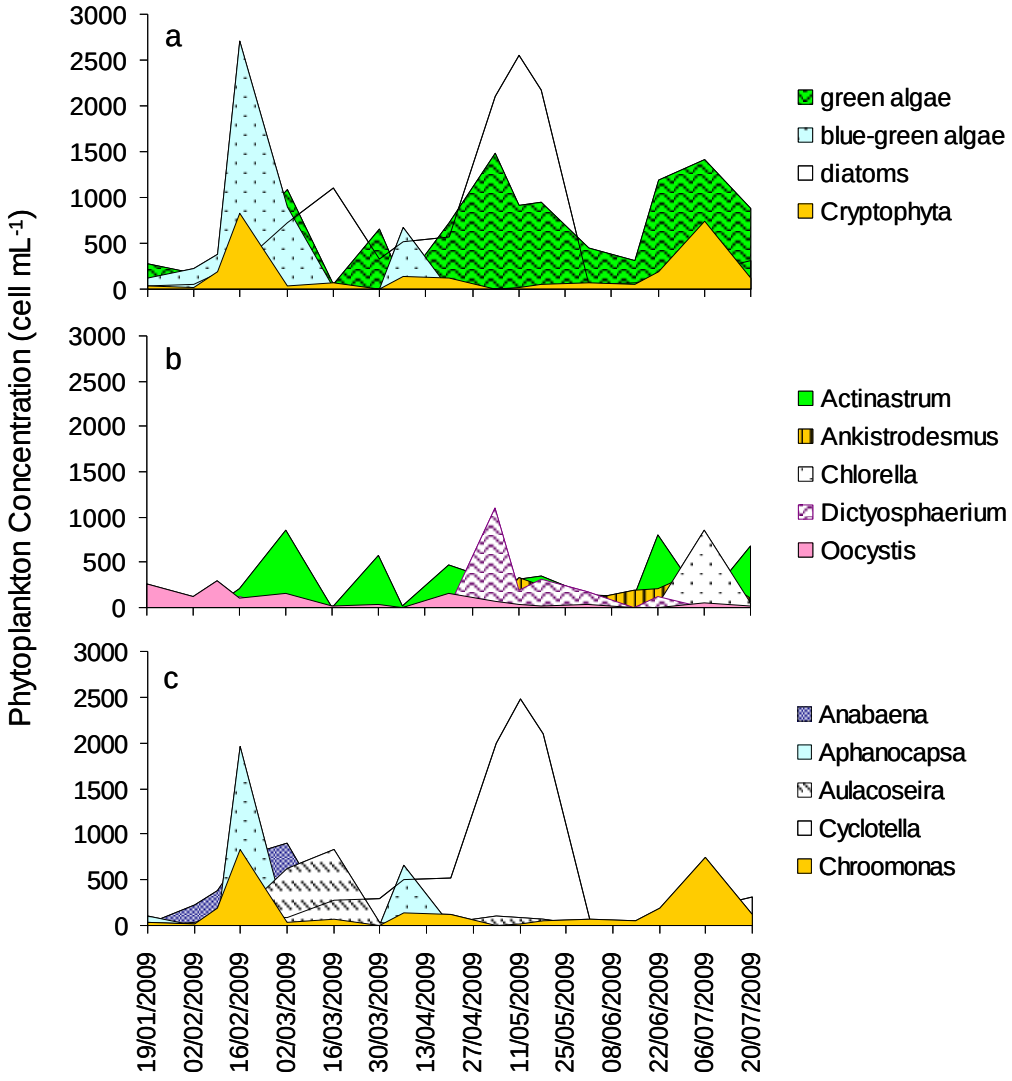


Figure 35 Phytoplankton compositions (cell mL^{-1}) for the surface water of Loc 1 (main basin) in Myponga Reservoir presented in functional groups (a) and at genus levels (b and c)

6.4 Discussion

6.4.1 Temporal dynamics of phytoplankton cellular content and stoichiometry

The changes of phytoplankton cellular contents can be regulated by ambient conditions in the aquatic system (e.g. light climate and bio-available nutrients) and internal metabolism (Reynolds 1984). At the beginning of the study in January 2009, the bio-available nutrients in the water of Myponga Reservoir were very low, approaching or falling below the minimum level of detection (i.e. FRP and NO_x < 0.005 mg L^{-1}). Meanwhile, phytoplankton cellular content of TC was relatively high and the cellular content of TP was moderately low. These data suggested that the phytoplankton populations in Myponga Reservoir appeared to be nutrient-limited when the investigation began. During the period between 27th January and 1st February 2009, South Australia experienced a record-breaking heatwave with six consecutive days of air temperatures above 40ºC (National Climate Centre 2009). After the heatwave, the phytoplankton TC content decreased gradually, while TN and TP contents increased significantly over the following few weeks. Two possible explanations for these cellular changes were the release of sediment nutrients under anoxic conditions and nutrient recycling from cell lysis.

As discussed in Chapter 4, the heatwave may have promoted the development of extended thermal stratification in Myponga Reservoir isolating the lower water layer (hypolimnion) from the atmosphere (Figure 13). This may have led to the oxygen depletion at the sediment-water interface, triggering the release of nitrogen and phosphorus from the sediment. The destratification unit in the reservoir could help to transport the relative nutrient-rich hypolimnetic water to the relative nutrient-poor surface water for phytoplankton uptake. These small amounts of sediment nutrient release may not have been detectable during the water sampling, however the bio-available nutrients can be assimilated rapidly with the N- and P-limiting phytoplankton cells (Hochstadter 2000). It is evident that a slight increase of FRP in the water was observed from 0.005 (detection limit) to 0.009 mg L^{-1} (Figure 31), but there were noticeable increases in phytoplankton TN contents from 2% to 4% and phytoplankton TP contents from 0.2 to 0.5% shortly after the heatwave (Figure 32).

The intense prolonged solar input during the heatwave may also damage the D1 protein of the photosystem II reaction centres, leading to the reduction of photosynthetic rates and carbon fixation of phytoplankton. Consequently, a reduction of carbon content

relative to nitrogen and/or phosphorus can be seen (Franklin *et al.* 2003). In many cases, damage from photoinhibition can be recovered by the repair or replacement of damaged DNA and proteins (Bouchard *et al.* 2006). However, extreme UV exposure can cause severe damage to cells, leading to cell death, cell lysis and nutrient release in addition to the natural recycling process. This may also reduce phytoplankton photosynthesis rates and carbon productions; and hence resulting in lower carbon content relative to other nutrient contents in cells. In addition, the recycled N and P in the epilimnetic water can be incorporated rapidly within nutrient-depleted phytoplankton (Hochstadter 2000), leading to the increases of TN and TP cellular contents. The increasing cellular contents of TN and TP led to decreasing TC:TN and TC:TP ratios. The recycled nutrients (at low levels) induced by the heatwave were not detectable in the water, but were clearly reflected at the cellular level, where TN and TP content of phytoplankton increased. The affinity of nutrient uptake by phytoplankton was high when the internal nutrient content was low (Jansson 1988). P-starved phytoplankton can absorb phosphorus 10 – 100 times faster than non-starved phytoplankton (Jansson 1988).

While the internal sources of nutrients may have become available after a heatwave, the high TC:TN, TC:TP and TN:TP ratios restored close to the classical Redfield ratios of 5.7, 40 and 7 (by weight) respectively, even though the lake seston generally yielded higher C:N:P ratios than the marine seston (Hecky *et al.* 1993; Elser and Hassett 1994). Phytoplankton stoichiometric ratios in Myponga Reservoir were maintained for a few weeks and then started to deviate from the Redfield ratios toward the end of summer. These ratios implied that the phytoplankton populations once again experienced nutrient limitation. Rapid recycling of nutrients by zooplankton even at small scales could help to maintain the growth of phytoplankton (Abbott 1993), while the ambient nutrient concentrations remained low or undetectable. When grazing P-limited algae, zooplankton may recycle nutrients at higher N:P ratio, which could further accentuate P-limitation in surviving algae, leading to even higher algal N:P (Sterner and Elser 2002). The recovery of nutrient-limited cells would probably require the injection of nutrients from the catchment and/or from the lake sediments.

During the sampling period between January and July 2009, the TC content of phytoplankton increased over time and reached the highest content, while TP content decreased toward the lowest point at the beginning of May 2009. Subsequently, both cellular contents then changed inversely (i.e. TC content decreased, while TP increased

gently). Coincidentally, these changes occurred immediately after the first heavy rain event on 24th April 2009 (70 mm over four days). Although there was no observable increase of inflow from Myponga River (Figure 30) or nutrients in the reservoir water (Figure 31) after this rain event, it is believed that the localised runoff provided an external source of nutrients to relieve the P-depleted phytoplankton cells in the reservoir. External nutrient loadings were highly dependent on the amount of rainfall received in the catchment area (Linden *et al.* 2004), yet the rainfall did not exactly reflect the amount of water received in the downstream reservoir when the landscape was dry. This is probably because much of the rainwater from small episodic rain events was absorbed by the dry soil before the appearance of significant runoff water in the reservoir. Nevertheless, the nutrient limitations of phytoplankton slowly eased when the rainfall, inflow and external nutrient loads increased toward winter.

The severe phosphorus deficiency in water and phytoplankton in summer was probably the driving cause for the high TC cellular content in Myponga Reservoir. High C:nutrient ratios in phytoplankton can be driven by high light energy relative to nutrient availability (Sterner *et al.* 1997). High light intensity promotes higher growth rates of phytoplankton, while low nutrient supply prevents phytoplankton from keeping up with the nutrient demand, resulting in relative low growth rate and more incorporated C per unit nutrient (Sterner and Elser 2002). At a given growth rate, increasing light intensity leads to increasing C:N and C:P ratios, but has little effect on N:P ratio of phytoplankton (Healey 1985; Sterner and Elser 2002).

The phytoplankton cellular contents of TC, TN, TP and Chl*a* in Myponga Reservoir were comparable to some classical studies observed in laboratory cultures and in field samplings. Phytoplankton TC content in Myponga Reservoir varied from 15.4% to 44.5% dry weight (Figure 32). Under the optimal laboratory conditions (i.e. light, temperature and nutrients) conducted by Ketchum and Redfield (1949), about 51 – 56% TC in a basis of ash-free dry weight were observed in five species of green algae and one marine diatom. However, Round (1965) suggested that the TC content could vary from 35% (when insufficient light or inorganic carbon sources) to 70% (when growth limitation by other nutrients) of ash-free dry weight. It is important to note that a cellular content at an ash-free dry weight basis would give a higher value than a cellular content at a total dry weight basis. This can be adjusted based on Nalewajko (1966) that green algae could contain 10.2% ash content, whereas diatoms could make up of 41.4%

ash due to the heavy siliceous wall. Phytoplankton cellular contents of TN, TP and Chl*a* in Myponga Reservoir also appears in the same order of magnitude to the phytoplankton observed in other aquatic systems. In Myponga Reservoir, elemental cellular content of phytoplankton in the dry weight basis varied from 1.4% to 4% with an average of 2.2% for TN, from 0.1% to 0.6% with an average of 0.3% for TP and from 0.1 to 2.3% with an average of 0.5% for Chl*a* (Figure 32). Other natural phytoplankton populations comprised of 4 – 9%, 0.03 – 0.8% and 0.9 – 3.9% of ash-free dry weight for TN, TP and Chl*a* respectively (Round 1965).

6.4.2 Spatial dynamics of phytoplankton cellular content and stoichiometry

The cellular content and stoichiometry of phytoplankton communities in Myponga Reservoir varied significantly between the two morphometrically different locations. The side-arm (Loc 8) represented the shallower area (maximum water depth of 12 m) near the major inflow from Myponga River, whereas the main basin (Loc1) represented the deeper water habitat (~30 m) near the outlet of the reservoir. The inflow, however, had little influence on the nutrient concentrations in the reservoir water until the winter rainy season (July). The spatial variation of phytoplankton TC content may be due to the differences of surface mixed layers (SML) depths between the two sites, where the SML could reach the bottom of the main basin (30 m) as well as the bottom of the side-arm (15 m). Phytoplankton circulating at various SML depths between the two locations would experience different light exposures. Under high light condition, the rate of carbon fixation would exceed the rate of nutrient assimilation; thus, the excess of carbon derived from photosynthesis can be stored as C-rich polysaccharides or lipids (Konopka and Schnur 1980; Maranon and Gonzalez 1997; Madariaga 2002), leading to the enhancement of C:N and C:P ratios in the side-arm. Conversely, the lower level of light exposures in the main basin can reduce the photosynthesis rate of phytoplankton and the production of cellular TC, leading to the depression in the C:N and C:P ratios (Sterner and Elser 2002). The change in elemental composition of phytoplankton cells is attributed to the change in biochemical materials in the cells influenced by the ambient light and nutrient environment.

TP content of phytoplankton also appeared higher in the side-arm than in the main basin. This may be due to the nutrients from inflow and from sediment resuspension. Since the water chemistry in the reservoir is highly associated with the inflow water (Linden *et al.* 2004), it is not surprising that phytoplankton in the side-arm could utilise

the nutrients from the inflow water first due to the geographic advantage over the main basin. The small amount of nutrient from the inflow in summer did not seem to affect the nutrient concentration in water, but was displayed in the cellular nutrient content of phytoplankton when the ambient nutrients were quickly incorporated into biomass. During the high winter inflow, the higher nutrient concentrations in water were detected first in the side-arm, then in the main basin.

Nutrient release from sediment resuspension in the shallow part of the reservoir has also contributed to the variability of TP content in phytoplankton. In the shallow site, the opportunity for phytoplankton to capture nutrients is enhanced due to closer proximity to sediments. The surface mixed layer also displayed the likelihood of the wind-driven mixing reaching the bottom of the side-arm (Chapter 5). The mixing may have resuspend the surface layer of sediments, leading to the release of labile-P from the pore water and loosely adsorbed P on the particle surface (e.g. Sondergaard *et al.* 1992; Shinohara and Isobe 2010) and hence increasing phytoplankton production (Ogilvie and Mitchell 1998).

6.4.3 Seasonal fluctuation of phytoplankton composition

There was a clear shift in phytoplankton composition over time. The opportunistic cyanobacteria bloomed at the same time as the high phytoplankton cellular contents of TN and TP in mid-February, which may be related to the sediment nutrient flux and phytoplankton recycling after the heatwave. Diatoms reached the highest concentration coincidently with the highest TC content. It was unknown why this result was different from the general findings that diatoms had lower carbon per cell volume than other phytoplankton groups such as green algae, dinoflagellate and golden algae (Strathmann 1967; Sicko-Goad *et al.* 1984). In general, Chl*a* content varied with different functional groups of phytoplankton. Green algae generally have higher Chl*a* content than diatoms and blue-green algae, as Chl*a* is the major photosynthetic pigment for green algae. On the contrary, diatoms and blue-green algae contain high content of other photosynthetic pigments (e.g. carotenoids and xanthophylls in diatoms and phycobilins in blue-green algae), apart from chlorophyll pigments (Bailey-Watts 1978; Reynolds 1984). However, the seasonal fluctuation of phytoplankton assemblages did not have observable difference in the Chl*a* content in the study (no statistical difference for Chl*a* content over time). This may be due to the co-dominancy of green algae with other algal groups.

The change in Chl*a* content might be more significant if only one algal group dominated at a time.

6.5 Conclusion

This study investigated the temporal and spatial dynamics of phytoplankton cellular content and stoichiometry and discussed potential causes of these changes. The TC:TN:TP ratios clearly revealed the nutrient status of phytoplankton and these ratios were good indicators for the nutrient limitation status in the aquatic environment, especially when both NO_x and FRP concentrations in water were low and hardly detectable in summer. In this study, nitrogen did not appear to be the major limiting nutrient as the TN content in the phytoplankton cells (about 2.2%) were relatively consistent over time. In contrast, phosphorus could be the limiting nutrient during the study period as the TP cellular content decreased significantly from 0.6% to 0.1%, leading to the increase of TN:TP ratio. Meanwhile, the TC cellular content increased from 15% to 45% during the P deficiency, resulting in the raise of TC:TN and TC:TP ratios. These stoichiometric ratios deviated from the average values (i.e. Redfield ratios) toward the end of the dry season. When the catchment runoff started to replenish nutrients in the reservoir during the rainy season, the phytoplankton cellular content of TC dropped and the TP cellular content rose slowly, leading to the restoration of stoichiometric ratios toward the Redfield ratios.

The spatial variations of phytoplankton cellular content and stoichiometry were also observed in the two different habitats of Myponga Reservoir. Higher TC and TP cellular contents were found in the side-arm than in the main basin, probably due to the bathymetrical (shallower) and geographical (close to inflow) advantages for the side-arm. The spatial and temporal variability of phytoplankton cellular content and stoichiometry in Myponga Reservoir were likely driven by the nutrient deficiency in the ambient environment as well as the site differences in light availability due to different SML depths. The elemental variations between sites implied the possible variations of growth between sites, which may potentially lead to spatial variability in phytoplankton community. However, Chapter 5 concluded that the potential spatial variability of phytoplankton abundance, composition and diversity can be overcome by rapid lateral transport of water. The time scale of physiochemical processes is therefore important when determining the biological responses at different time scales.

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Chapter 7 Phytoplankton responses to variable light and nutrient conditions

Preamble

The data presented in previous chapters suggested that spatial variability of phytoplankton dynamics can vary at different biological scales (i.e. phytoplankton variability was observed at cellular level, but not at community level). This chapter further assesses various phytoplankton responses (physiology and growth) to variable light and nutrient conditions. I was responsible for the experimental design, data collection, statistical analysis and data interpretation with guidance from supervisors.

Chapter 7 Phytoplankton responses to variable light and nutrient conditions

7.1 Introduction

Spatial variability of phytoplankton in lakes is driven by a number of hydrological, physical and chemical processes. Near-shore wave action and wind-induced turbulence create different environmental conditions between shallow and deep water (e.g. MacIntyre *et al.* 1999; Schallenberg and Burns 2004). It was believed that sediment resuspension in the shallow area and near the lake perimeter could trigger the pulse of dissolved inorganic nutrients into the water column and promote phytoplankton growth (e.g. Hansen *et al.* 1997). However, nutrients associated with sediment resuspension are not necessarily available for phytoplankton uptake. For example, particles resuspended by wind shear were strongly related to total nutrients rather than dissolved inorganic nutrients (Hamilton and Mitchell 1997). In addition, resuspended sediments can also adsorb dissolved inorganic phosphorus, reducing the bio-availability of this nutrient in the water (Olila and Reddy 1993). Nevertheless, if the resuspended total nutrients consisted of pH sensitive components e.g. iron- and aluminium-bound phosphorus, these bio-available nutrients can be released when mixing with the algal productive surface layer with higher pH (Drake and Heaney 1987). Therefore, it is apparent that shallow areas are subject to regular fluxes of bio-available nutrients from sediment resuspension. These localised physiochemical variations can be biologically significant to phytoplankton growth, especially in the situation, where the concentrations of bio-available nutrients are low in water without the replenishment of external nutrients.

The responses of phytoplankton to changing light and nutrient conditions vary at different scales from cell physiology to population dynamics. The relative time scale of the physiological responses included the uptake of sufficient phosphorus (~ 7 min) and the fixation of sufficient carbon (~ 4.4 h) to complete a growth cycle (~ 9.05 h) by a freshwater *Chlorella* cell (Reynolds 1990), while the time scale of phytoplankton community responses fall into 1 – 10 weeks (Reynolds 1994). As the current study carried out experiments for a few days, the biological measurements should be able to capture the biological changes. Two relatively short responses of phytoplankton (chlorophyll *a* and maximal quantum yield of photosystem II) were used to evaluate the effects of different nutrient and light on physiology and growth. The concentration of

chlorophyll *a* (Chl*a*) was used as an indicator of phytoplankton biomass, as Chl*a* is a common photosynthetic pigment present in all freshwater phytoplankton and is relatively simple to measure (Reynolds 1984). However, the Chl*a* pigment content in cells could vary under different light conditions (Beardall and Morris 1976). Therefore, the experimental design included treatments with various light intensity in order to differentiate phytoplankton responses due to light variation or physiological adaptation.

Another parameter to assess phytoplankton responses was a maximal quantum yield of photosystem II (F_v:F_m), based on the fundamental relationship between fluorescence yield and photochemical quantum yield (Schreiber *et al.* 1995). Autotrophs absorb light energy to undergo a series of photochemical processes during photosynthesis. Some of the absorbed energy re-emit as fluorescence with a longer wavelength and the remaining energy is dissipated as heat under the first law of thermodynamics (Heinz Walz GmbH 2003):

Fluorescence + Photochemistry + Heat = 1

In short, the F_v:F_m ratio provided the photosynthetic activity of phytoplankton, which inferred to phytoplankton physiological status (healthy or stress). Phytoplankton under optimal laboratory conditions yielded a F_v:F_m ratio of about 0.65, which was relatively constant across numerous phytoplankton species (Kolber and Falkowski 1993). Several authors suggested that the decline in the F_v:F_m ratio represented a physiological stress of photosynthetic apparatus in phytoplankton cells, mostly resulting from nutrient limitation, photoinhibition of phytoplankton and/or change in species composition of phytoplankton community (Fuchs *et al.* 2002; DiTullio *et al.* 2005; Goto *et al.* 2008). Photosynthetic efficiency of phytoplankton decreased at high irradiances, exhibiting a midday depression in F_v:F_m up to 80% decrease, but recovered completely at night (Bergmann *et al.* 2002). The F_v:F_m ratio also varied with a single or combined effects of light, macro- and micro-nutrients, biocides, water temperature, diel cell cycle and phytoplankton species (West *et al.* 2003; Devilla *et al.* 2005; Holeton *et al.* 2005; Goto *et al.* 2008). Nevertheless, the comparison of the F_v:F_m ratio before and after the experiments was essential to assess phytoplankton physiology due to the particular impacts of light and nutrient treatments.

To test for the site differences, a natural phytoplankton population from Myponga Reservoir was kept in semi-permeable membrane bottles suspending in the shallow and deep water. The semi-permeable membrane allowed dissolved substances (< 0.45 μm)

to diffuse freely into and out of the bottles; hence the phytoplankton population inside the bottles was subjected to the changing quality of the ambient water (Owens *et al.* 1977; Westwood 2003). One advantage for the use of semi-permeable membrane to conduct bioassays is to minimize artificial nutrient limitation observed in closed bottle bioassays (Carpenter 1996; Posselt *et al.* 2009). Another advantage for the membrane bottles is that the membrane is an effective barrier to prohibit zooplankton grazing on the experimental phytoplankton community. However, many delicate membrane bottles were destroyed by the unexpected strong turbulence in the Shallow Site and no conclusion could be drawn for the potential site variations. Meanwhile, robust enclosed plastic bottles were deployed to determine the impacts of additional nutrients (nitrate and phosphate) on phytoplankton and to identify any limiting nutrient. The influence of underwater light climate on phytoplankton was also investigated in the study.

7.2 Methods

7.2.1 Experimental design

Three *in situ* bottle experiments were conducted in Myponga Reservoir to determine how the phytoplankton respond to different nutrient levels, light conditions and various locations subject to sediment nutrient flux. Initial and Final treatments represented the phytoplankton responses in the ambient water before and after the experiment. The location difference was specifically targeted to the potential variation of resuspended sediment nutrients between the Shallow and Deep Sites. Treatments for the Shallow and Deep Sites represented phytoplankton responses in the membrane bottles, which suspended in the surface water of the shallow region (2 m depth) and deep region (30 m depth). Control, N+, P+ and N+P+ treatments represented phytoplankton responses in the sealed bottles with modified nutrient levels in the water (i.e. no added nutrient for Control, added nitrogen for N+, added phosphorus for P+ and added nitrogen and phosphorus for N+P+). For each site and nutrient treatment, there were three extra treatments on light intensity (i.e. about 80%, 50% and 10% surface irradiance). Every treatment had three to five replicates and the three incubation experiments were carried out during 18th – 21st December 2007, 1st – 6th February 2008 and 20th – 26th March 2008. The three experiments were considered as repeated measurements; hence no time effect was included in the data analysis. A summary of the experimental design and a schematic illustration of the experimental design are presented in Table 31 and Figure 36 respectively.

Water quality sondes (Hydrolab DS5X, Colorado, USA) were used to measure water temperature, pH, specific conductivity and dissolved oxygen in the surface water near the Deep Site of Myponga Reservoir at the end of each experiment. Total Kjeldahl nitrogen (TKN), nitrate+nitrite (NO_x), filterable reactive phosphorus (FRP) and total phosphorus (TP) were collected in the surface water near the Deep Site of Myponga Reservoir prior to each experiment. Wind speed at 2 m above the water surface (Climatronics WM-111, New York, USA) was also measured every 10 minutes in the main basin of Myponga Reservoir. The nutrient and wind speed data were kindly provided by SA Water.

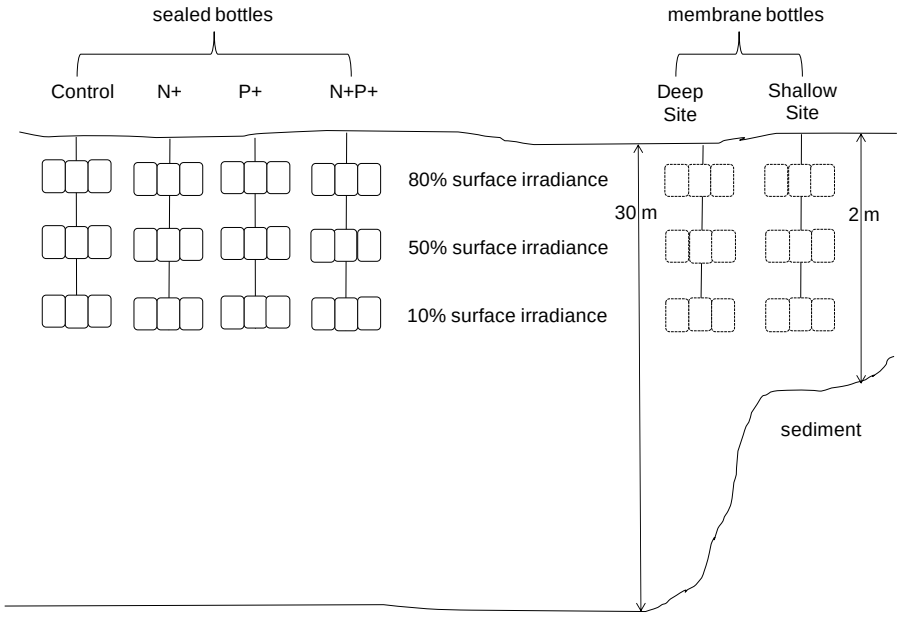

Figure 36 Schematic illustration of the experimental design

Table 31 Experimental design for the three incubations in Myponga Reservoir

Experimental date	**18 – 21 Dec 2007**	**1 – 6 Feb 2008**	**20 – 26 Mar 2008**
Surface irradiance (I_0)	80%, 50%, 10%	80%, 50%, 10%	80%, 50%, 10%
Site treatments (0.45 μm membrane bottle)			
Shallow Site	×, ✓, ✓	×, ×, ✓	×, ×, ×
Deep Site	✓, ✓, ✓	✓, ✓, ✓	×, ✓, ✓
Nutrient treatments (sealed bottle)			
Control (N- P-)	✓, ✓, ✓	✓, ✓, ✓	✓, ✓, ✓
N+		✓, ✓, ✓	✓, ✓, ✓
P+		✓, ✓, ✓	✓, ✓, ✓
N+ P+	✓, ✓, ✓	✓, ✓, ✓	✓, ✓, ✓
Light treatments (bottles were suspended at different depths of water to receive different levels of surface irradiance)			
80% of I_0	77% SI at 0.10 m	81% SI at 0.125 m	80% SI at 0.15 m
50% of I_0	52% SI at 0.25 m	56% SI at 0.35 m	52% SI at 0.45 m
10% of I_0	9% SI at 0.90 m	10% SI at 1.35 m	10% SI at 1.55 m

× denotes broken membrane bottles, hence no result was available for the treatment

7.2.2 Site and nutrient treatment

Surface samples of reservoir water were taken and filtered through 105 μm mesh to eliminate the effect of zooplankton grazing in the experiments. The filtered water was then filled into the membrane bottles and transparent polyethylene terephthalate (PET) bottles (Figure 37). A semi-permeable membrane bottle was constructed by a transparent polycarbonate tube with a polysulfone filter (0.45 μm pore size and 47 mm diameter, Tuffryn HT-450, Pall Corporation, Michigan, USA) tightened by a perfectly fitted ring cap at both ends. The same design of the membrane bottle was successfully deployed in the River Murray of South Australia (Westwood and Ganf 2004). The membrane filters allow dissolved substances (e.g. nitrate, phosphate, carbon dioxide and oxygen) to move freely into and out of the bottles at a rapid diffusion rate of 0.315 μg phosphate per hour under static conditions (Westwood 2003). This design aimed to determine any significant difference in phytoplankton responses between the Shallow and Deep Sites. Unfortunately, many membranes in the Shallow Site were damaged by the unexpected strong turbulence in the three attempts with modifications of bottle designs. Hence, there were insufficient data to testify the hypothesis of phytoplankton responses on site variation.

For nutrient treatments, no chemical was added to the filtered water in the sealed bottles for Control treatment. However, exceptionally high concentrations of nitrate and phosphate were added to the sealed bottles for N+, P+ and N+P+ treatments, based on the recipe of BG-11 culture medium (Andersen 2005). Sodium nitrate ($NaNO_3$) and dipotassium hydrogen phosphate (K_2HPO_4) were used at concentrations of 1.09 g NO_3 L^{-1} and 16.6 mg PO_4 L^{-1}. For the December and February experiments, additional nutrients that were used to make up the BG-11 medium were also added to the N+, P+ and N+P+ treatments. For the March experiment, only $NaNO_3$ and K_2HPO_4 were used in the corresponding treatments.

Figure 37 Semi-permeable membrane bottles for Shallow and Deep treatments (left) and sealed bottles for Control, N+, P+ and N+P+ treatments (right)

7.2.3 Light treatment

Different underwater light intensities were also considered in the study. The membrane and sealed bottles from every site and nutrient treatment were suspended in the water at three depths (i.e. 80%, 50% and 10% surface irradiance). The relationship between water depth and underwater light is explained by the Beer-Lambert Law (Wetzel 2001):

$$I_z = I_0 e^{(-K_d z)}$$

where I_z = irradiance at water depth *z*, µmol m^{-2} s^{-1}; I_0 = surface irradiance, µmol m^{-2} s^{-1}; K_d = vertical light attenuation coefficient, m^{-1}; and z = depth where bottles were suspended, m. The average K_d was estimated from a series of light intensity measurements at 0.25 m intervals for the surface 2 m of the water column using an underwater quantum sensor (LI-COR 1000, Nebraska, USA). The average K_d was then used to calculate the corresponding depths for light treatments. For example, 0.15 m below the water surface received approximately 80% I_0 in the March experiment, 50% I_0 at 0.45 m and 10% I_0 at 1.55 m. This depth-light relationship was obtained on the initial day of each experiment.

7.2.4 Measurements of phytoplankton responses

The concentration of Chl*a* was used as an estimation of biomass for a phytoplankton population in response to the nutrient and light treatments. To determine the Chl*a* concentration, a known amount of water sample was filtered through a glass fibre filter (GF/C), which was immersed in 5 mL of methanol for 24 hours in the dark. The extracts were measured using Hitachi Spectrophotometer U-1000 (Tokyo, Japan) at 665 nm

wavelength in a 1 cm cell and corrected by the absorbance at 750 nm wavelength. The Chl*a* concentration was calculated, based on the equation in Golterman *et al.* (1978):

$$x = 13.9*E_1$$

where x = concentration of Chl*a* equivalent in the solvent, µg mL^{-1}, E_1 = absorbance at 665 nm in 1 cm cell and 13.9 is a factor suggested by Talling and Driver (1963, cited in Golterman *et al*. 1978). The concentration of Chl*a* equivalent in the solvent multiplied by the volume of solvent and divided by the volume of filtered water will give the total Chl*a* concentration in µg L^{-1}.

The quantum yield of photosystem II (F_v:F_m) ratio was used to determine the physiological state of phytoplankton in response to the nutrient and light treatments. The F_v:F_m ratio for phytoplankton ranges from 0 to a theoretical laboratory limit of 0.65; the ratio lower than 0.3 is generally considered low (DiTullio *et al.* 2005). The decline in the F_v:F_m ratio is indicative of stress response for phytoplankton and the reduction of photosynthetic efficiency (DiTullio *et al.* 2005). The F_v:F_m was obtained based on the assumptions that all photosystem II reaction centres were closed by a short pulse of saturating light (hence the photochemistry is zero) and the ratio between fluorescence and heat were not changed before and during the saturating light pulse. After a dark adaptation for the phytoplankton samples, the minimal fluorescence yield (F_o) was measured before the pulse of saturating light; and the maximal fluorescence yield (F_m) was determined during the pulse. The variable fluorescence yield (F_v) is the difference between F_m and F_o ($F_m - F_o$). The fluorescence yields were measured on the day of collection using PHYTO-PAM Phytoplankton Analyzer System (Heinz Walz GmbH, Effeltrich, Germany). The water samples collected from the field were kept in the dark at room temperature until the analysis and no samples were refrigerated. For each sample, a small amount of water was filtered through GF/C filter and the filtrate was used to offset the background fluorescence. Hence, the fluorescence results were more representative to the phytoplankton cells.

7.2.5 Statistical analyses

All results were examined statistically using SPSS 15.0 for Windows. Using the statistical approach of Coakes *et al* (2009), data were first checked for homogeneity of the variance test (Levene's test). When a sample of data was normally distributed and had equal variances amongst the pool of samples (*P*-value of Levene's test > 0.05), one-way analysis of variance (ANOVA) was performed. When samples failed the Levene's

test (*P*-value < 0.05) meaning that there is a difference between the variances in the population, a robust Welch ANOVA was used to examine the equality of means. This was followed by a post hoc test, Least Significant Difference (LSD) at a significance level of 0.05. The LSD test allowed multiple comparisons of the actual difference between each group means. Since the LSD tests also revealed the similarity and difference between treatments, the results of ANOVA tests were not displayed.

7.3 Results

7.3.1 Nutrient effect on phytoplankton biomass and photosynthetic activity

Physiological responses of natural phytoplankton populations in Myponga Reservoir were investigated under four nutrient treatments (no added nutrient, added N, added P and added both N and P) and three light treatments (high, medium and low light intensity) over a 4-day incubation (Figure 38), a 6-day incubation (Figure 39) and a 7-day incubation (Figure 40). Samples of the filtered water were taken for the measurements of Chl*a* and F_v:F_m at the beginning of each experiment and these Initial samples were the basis for comparisons with other treatment samples.

The Chl*a* results suggested no statistical difference between Initial and Control treatments in most cases (LSD test, $p > 0.05$), except for the two cases at 10% I_0 where the Chl*a* concentrations increased significantly from 4.6 µg L^{-1} to 11.2 µg L^{-1} in the February experiment and from 2.9 µg L^{-1} to 15.4 µg L^{-1} in the March experiment. Interestingly, most of the F_v:F_m results in the three experiments showed a significant decline (LSD test, $p < 0.05$) for the Control treatment (0.54 – 0.55) when compared with the Initial treatment (0.34 – 0.44).

For the N+P+ treatment, there was a substantial increase in Chl*a* concentration after the incubation; this was found in all levels of light intensities (LSD test, $p < 0.05$). The greatest increase of Chl*a* concentration was recorded in the February experiment at 80% I_0; the Chl*a* concentration was 29 times higher on the last day of the experiment (134.1 µg L^{-1}) than on the first day (4.6 µg L^{-1}). While there was a strong increase in phytoplankton biomass with added N and P treatment, the F_v:F_m ratio showed some interesting physiological responses of phytoplankton. The F_v:F_m ratios for the N+P+ treatment increased to approximately 0.63 from the Initial value of 0.55 during the December experiment at all light intensities (LSD test, $p < 0.05$). This suggested that the addition of nutrients has improved the overall phytoplankton health. During the

February experiment, the addition of N and P did not affect the F_v:F_m ratio at 50% and 80% I_0, but did increase the F_v:F_m ratio at 10% I_0. The F_v:F_m ratios for the March experiment were rather different from the other experiments. The addition of N and P showed the impairment of phytoplankton health (lower F_v:F_m compared with Initial treatment), but yet increased the phytoplankton biomass (higher Chl*a*). It seems that the F_v:F_m ratio revealed some important physiological information of phytoplankton that the Chl*a* measurement could not display.

When only nitrate was added to the water, no difference of the Chl*a* concentration and the F_v:F_m ratio was observed between the Initial and N+ treatments during the February experiment at all light intensities (LSD test, $p > 0.05$). On the other hand, a significant increase of the Chl*a* concentration at all light intensities was recorded in the March experiment from 2.9 µg L^{-1} to approximately 13.8 µg L^{-1}. However, the F_v:F_m ratio of the N+ treatment decreased from 0.54 to approximately 0.45 across the light treatment, indicating a stress response for phytoplankton.

The Chl*a* results of the P+ treatment were consistent in both the February and March experiments; the Chl*a* concentrations on the first day of the experiment were the same as that on the last day for both 80% and 50% surface irradiance. In these light conditions, the F_v:F_m ratio remained the same in the February experiment (approximately 0.57) and reduced in the March experiment (from 0.54 to approximately 0.44). On the other hand, increased Chl*a* concentrations and decreased F_v:F_m ratio were observed at 10% surface irradiance for both February and March experiments (LSD test, $p < 0.05$).

The limited results from the Shallow and Deep Sites could not draw a valid conclusion for the spatial impact on phytoplankton physiology and biomass. However, the available results could provide some insight into how the phytoplankton responded to the ambient water in the semi-permeable membrane bottles in comparison to the Control sealed bottles. The Chl*a* concentrations either increased or was maintained in the Deep Site, whereas the F_v:F_m ratios were mostly the same before and after the incubations. Except on one occasion (10% I_0 in the March experiment, Figure 40), the depression in the F_v:F_m ratio was found in the Deep Site. For the remaining intact membrane bottles of the Shallow Site, the Chl*a* concentrations and the F_v:F_m ratios at the end of the incubations were mostly the same as the Initial measurements. Nevertheless, these

results cannot provide sufficient information to determine whether the site difference could affect the physiology and/or growth of phytoplankton. Yet, some membrane bottles (Deep and Shallow Sites) showed higher Chl*a* concentrations and F_v:F_m ratios than the sealed bottles (Control treatment) and some remained the same. This implies that the replenishment of nutrients through the membrane into the bottles allowed some growth of phytoplankton, while physiological stress was displayed in the confined bottles.

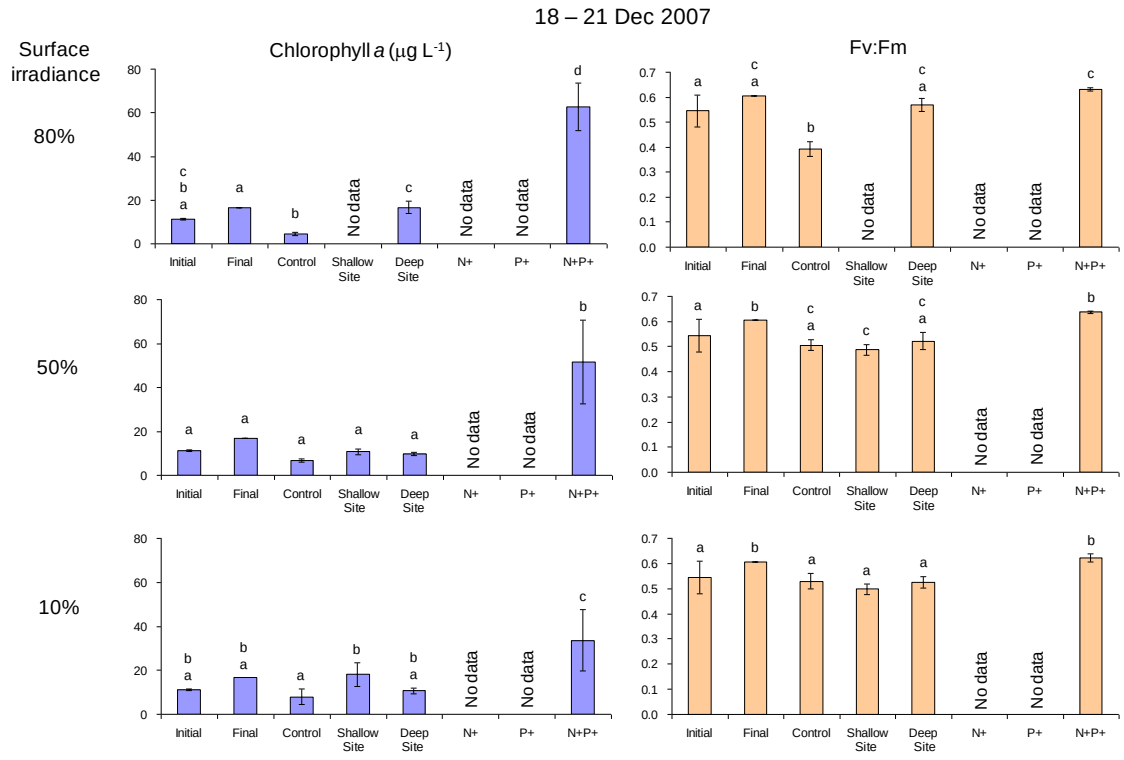

Figure 38 Chlorophyll *a* concentration (µg L^{-1}) and maximal quantum yield of photosystem II (F_v:F_m) of a natural population of phytoplankton in Myponga Reservoir were measured at different levels of surface irradiance under various site and nutrient treatments on 18 – 21 December 2007. The letter above each bar represents the statistical similarity or difference among treatments in the same graph, based on Least Significant Difference (LSD) test at a 0.05 level of statistical significance

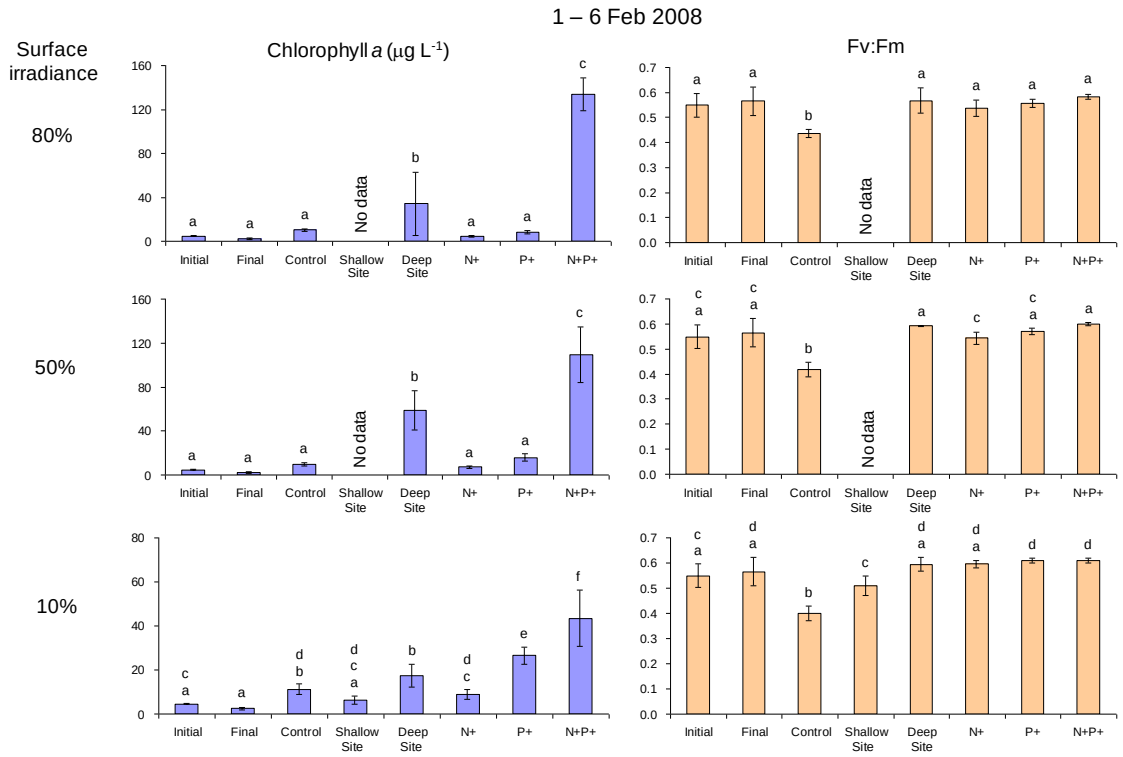

Figure 39 Chlorophyll *a* concentration (µg L^{-1}) and maximal quantum yield of photosystem II (F_v:F_m) of a natural population of phytoplankton in Myponga Reservoir were measured at different levels of surface irradiance under various site and nutrient treatments on 1 – 6 February 2008. The letter above each bar represents the statistical similarity or difference among treatments in the same graph, based on Least Significant Difference (LSD) test at a 0.05 level of statistical significance

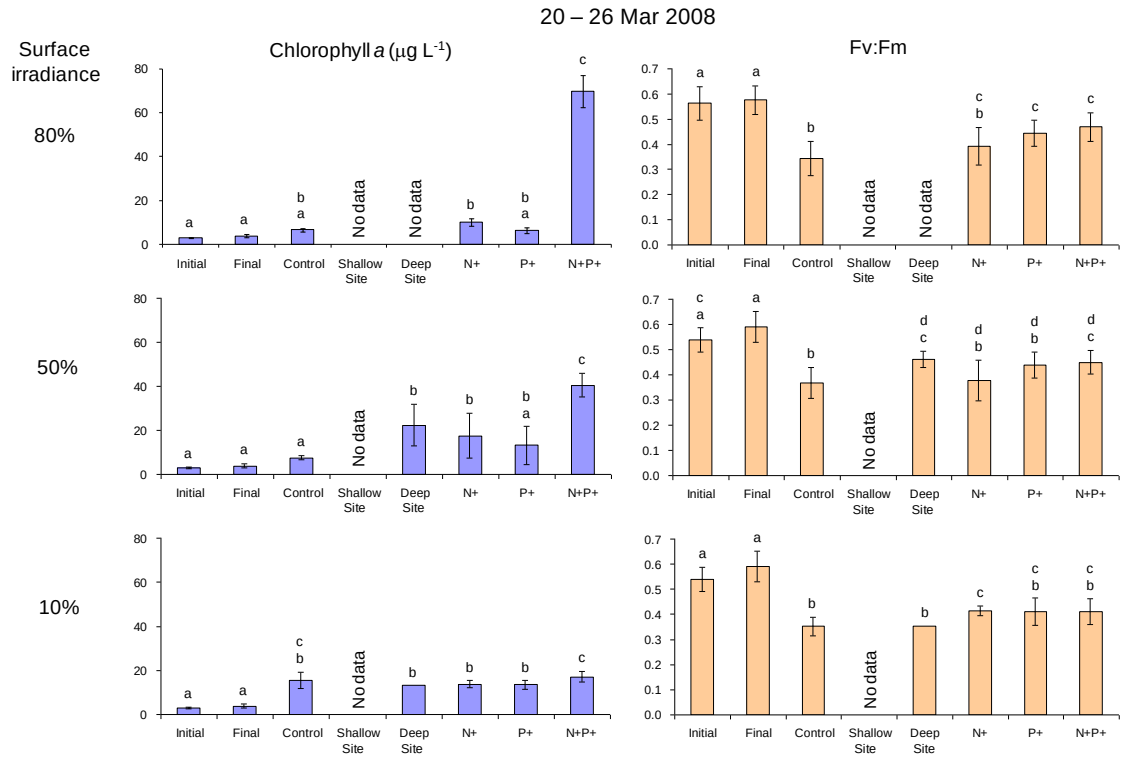

Figure 40 Chlorophyll *a* concentration (µg L^{-1}) and maximal quantum yield of photosystem II (F_v:F_m) of a natural population of phytoplankton in Myponga Reservoir were measured at different levels of surface irradiance under various site and nutrient treatments on 20 –26 March 2008. The letter above each bar represents the statistical similarity or difference among treatments in the same graph, based on Least Significant Difference (LSD) test at a 0.05 level of statistical significance

7.3.2 Light effect on phytoplankton biomass and photosynthetic activity

The phytoplankton responses to light availability were also thoroughly compared within each nutrient treatment by the LSD test (Table 32). For the Control, N+ and P+ treatments, the Chl*a* concentrations were usually two times higher at the low light intensity (10% I_0) than at the high light intensity (80% I_0). In the case of P+ treatment during the February experiment, the Chl*a* concentration at low irradiance (26.6 µg L^{-1}) was three times higher than at high irradiance (8.1 µg L^{-1}). In contrast to the N+P+ treatment, the Chl*a* concentrations at the high irradiance were three to four times higher than at the low irradiance. Nevertheless, there were a few occasions where no significant light effect was observed.

In many cases, the F_v:F_m ratios did not display any significant difference between high and low light intensities. For those treatments that showed the light effect, the F_v:F_m ratios tended to be lower at 80% I_0 and higher at 10% I_0, except for the Control treatment in the February experiment, where higher light intensity yielded higher a

F_v:F_m ratio. These implied that the phytoplankton under low light intensities were healthier and more photosynthetically active than the phytoplankton under high light intensities.

Table 32 A comparison of the light effects on the mean chlorophyll *a* and the mean maximal quantum yield of photosystem II (F_v:F_m) in Myponga Reservoir. The letters represent any statistical similarity or difference among different surface irradiances for one treatment, based on Least Significant Difference (LSD) test at a 0.05 level of statistical significance

Site/Nutrient treatments	Surface irradiance	Mean Chl*a* (µg L^{-1})			Mean F_v:F_m		
		Dec 07	Feb 08	Mar 08	Dec 07	Feb 08	Mar 08
Initial	-	11.1	4.6	2.9	0.55	0.55	0.54
Final	-	16.7	2.4	3.8	0.61	0.57	0.59
Control	80%	4.4 a	10.3 a	6.6 a	0.39 a	0.44 a	0.34 a
	50%	6.7 a	9.6 a	7.6 a	0.51 b	0.42 ab	0.37 a
	10%	7.9 a	11.2 a	15.4 b	0.53 b	0.40 b	0.35 a
Shallow *	80%	-	-	-	-	-	-
Site	50%	10.7	-	-	0.49	-	-
	10%	18.1	6.4	-	0.50	-	-
Deep *	80%	16.7 a	34.2 ab		0.57 a	0.57 a	
Site	50%	9.7 b	58.5 a	22.4	0.52 a	0.59 a	0.46
	10%	10.7 b	17.4 b	13.2	0.53 a	0.60 a	0.35
N+	80%	-	4.5 a	10.1 a	-	0.54 a	0.39 a
	50%	-	7.3 ab	17.5 a	-	0.54 a	0.38 a
	10%	-	8.9 b	13.8 a	-	0.60 b	0.41 a
P+	80%	-	8.1 a	6.2 a	-	0.56 a	0.44 a
	50%	-	15.8 b	13.3 ab	-	0.57 a	0.44 a
	10%	-	26.6 c	13.5 b	-	0.61 b	0.41 a
N+P+	80%	62.8 a	134.1 a	70.0 a	0.63 a	0.58 a	0.47 a
	50%	51.7 a	109.5 a	40.4 b	0.64 a	0.60 b	0.45 a
	10%	33.6 a	43.4 b	17.0 c	0.62 a	0.61 b	0.41 a

* No letter was presented next to the mean values for some Shallow and Deep treatments, as the LSD test cannot be carried out due to insufficient data

7.3.3 Water quality

During the three experiments, some physical and chemical variables of water quality were recorded (Table 33). The water quality measurements during the experiments displayed the typical summer characteristics of Myponga Reservoir with warm (~ 20ºC) and well-oxygenated (~ 7.7 mg L^{-1}) surface water. The bio-available nutrients in the water were generally low (0.011 – 0.054 mg NO_x L^{-1} and < 0.006 mg L^{-1}) in the three experimental periods and sometimes fell below the detection limits of the equipment. The water was reasonably clear with low turbidity, except during outbreaks of algal blooms. The surface water was generally subject to a light breeze from the ocean, but high wind speed up to 15 m s^{-1} was recorded during the experiments (Figure 41).

Table 33 Water quality parameters collected in Myponga Reservoir during the three experimental periods, which were 18 – 21 December, 1 – 6 February 2008 and 20 – 26 March 2008

	18 – 21 Dec 2007	1 – 6 Feb 2008	20 –26 Mar 2008
Water temperature (°C)	19.81	21.80	19.90
Average wind speed (m s^{-1})	3.05	4.31	3.12
Maximum wind speed (m s^{-1})	15.13	15.13	14.43
Light attenuation coefficient, K_d	2.63	1.68	1.46
Euphotic depth (m)	1.75	2.75	3.15
pH	8.12	7.84	7.77
Specific conductivity (µS cm^{-1})	696.92	744.41	763.06
Dissolved oxygen (mg L^{-1})	8.76	7.69	6.74
Turbidity (NTU)	2.5	1.1	1.5
Total Kjeldahl nitrogen, TKN (mg L^{-1})	0.92	1.01	1.12
Nitrate + nitrite, NO_x (mg L^{-1})	0.054	0.011	0.028
Filterable reactive phosphorus, FRP (mg L^{-1})	< 0.005	< 0.005	0.006
Total phosphorus, TP (mg L^{-1})	0.035	0.026	0.028

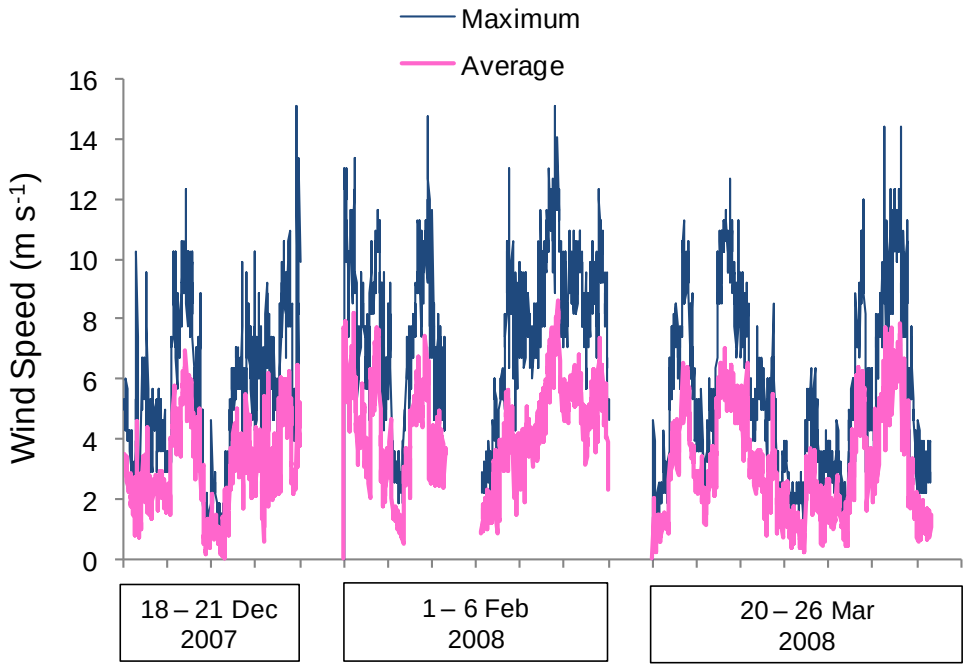

Figure 41 Average and maximum wind speeds (m s^{-1}) in Myponga Reservoir during the three experimental periods, which were 18 – 21 December, 1 – 6 February 2008 and 20 – 26 March 2008

7.4 Discussion

The *in situ* experiments in Myponga Reservoir were designed to investigate the impacts of nitrate and phosphate on phytoplankton biomass and photosynthetic activity and the vertical spatial variability of phytoplankton at different water depths with various underwater light conditions. The LSD test was then used to determine the similarities and differences of phytoplankton responses before and after the incubation with various nutrient and light conditions.

In general, this experimental study showed the consistency of the findings by Elser *et al.* (1990) that combined N+P+ enrichment enhanced phytoplankton growth much more substantially than did addition of N+ or P+ separately. On average, the phytoplankton response in terms of Chl*a* concentration did not differ substantially between the N+ and P+ enrichment bioassays; and this result also corresponded well with the findings of Elser *et al.* (1990). The Chl*a* concentration increased with increasing light intensity when both nitrate and phosphate were plentiful in the water. Under these favourable conditions, the photosynthetic activity of phytoplankton (expressed in F_v:F_m) increased, or was maintained at the initial level. However, depressions in the F_v:F_m ratio in the March experiment inferred the development of unhealthy phytoplankton even with abundant N, P and light supply. This suggested that other factors such as lack of carbon dioxide supply in the confined bottles might inhibit the photosynthetic activity (Dromgoole 1978). Other possible explanations also included the exhaustion of dissolved oxygen for respiration (Littler 1979) and other essential nutrients for growth (Buesa 1977) and the damage caused by concentrated algal excretes (Findenegg 1965). These adverse effects became more prominent after long incubation periods. A longer incubation period in the March experiment (7 days) compared to other shorter incubations (4 and 6 days) means that there was a higher chance for an essential substrate to deplete or an algal by-product to accumulate in the experimental bottle (Littler 1979), leading to the decline in the F_v:F_m ratio.

The Chl*a* concentration is widely used as the indicator of phytoplankton biomass, however it is an imperfect measure as the photosynthetic pigment content may vary under different light irradiances (Beardall and Morris 1976). This was shown in the results of Control, N+ and P+ treatments (Table 32). The increase in phytoplankton biomass (expressed in Chl*a* concentration) was expected to be minimal under the deficiency of one or both nutrients, regardless of the variation in light supply. However,

some of the Chl*a* concentrations actually increased under low light conditions. The increased Chl*a* content is one of the phytoplankton photoadaptation mechanisms (e.g. increasing cell sizes and surface area per unit mass and producing accessory photosynthetic pigments) to promote light-harvesting at low light intensity (Beardall and Morris 1976; Falkowski and Owens 1980; Falkowski 1984). Some algal species are able to maintain lower cell-specific rates of respiration in order to survive in the prolonged darkness (Reynolds 1994). Therefore, the increase in Chl*a* concentration at 10% I_0 was likely related to the physiological adaptation of phytoplankton rather than the increase in phytoplankton biomass.

On the other hand, no significant change of the Chl*a* concentration at the high and medium light intensities in the Control, N+ and P+ treatments was likely due to the nutrient deficiency in water, not caused by photoinhibition. Harris (1984) and Aalderink and Jovin (1997) explained that photoinhibition was commonly observed in the *in situ* experimental bottles just below the water surface. It is because the suspended bottles were incubated at a fixed depth, where phytoplankton were exposed to prolonged solar radiation. Unlike in the free-floating water, phytoplankton could move across the water column into and out of the euphotic zone, experiencing a wide range of light intensities. Nonetheless, the high F_v:F_m ratio in the nutrient-depleted treatments showed a strong physiological evidence of 'healthy' phytoplankton.

Another aspect of this experiment was to determine whether N or P was the limiting nutrient for phytoplankton growth. The results of N+ and P+ treatments were generally pointing toward the conclusion that both N and P were limiting the growth during the February experiment, as no significant increase in phytoplankton biomass was observed in the N+ and P+ treatments. Co-limitation of nutrients in lakes was more common than limitation by either phosphorus or nitrogen alone (Elser *et al.* 1990). The water quality analysis for Myponga Reservoir also showed very low concentrations of nitrate (0.011 mg L^{-1}) and phosphate (< 0.005 mg L^{-1}) present in the water during the February experiment. Besides, the addition of nitrate seemed to promote the Chl*a* concentration slightly, but it does suggest that N would become limiting. The minor increase in phytoplankton biomass was not only triggered by the addition of nitrate, but also required the presence of phosphate in water or in phytoplankton cells. The water quality analysis reported a small amount of FRP (0.006 mg L^{-1}) available in the reservoir water during the March experiment. In addition, luxury uptake and storage of phosphorus (e.g.

polyphosphate granules) in excess of immediate demands by phytoplankton helped to delay the onset of external nutrient limitation (Reynolds 1984; Reynolds 1988). Internal phosphorus storages can sustain phytoplankton growth for several generations in the complete absence of external phosphorus sources (Jansson 1988). For instance, the cellular phosphorus content of a freshwater diatom *Asterionella formosa* during the luxury uptake was sufficient to sustain four full divisions of a cell growing in phosphorus-free medium (Mackereth 1953). The stored phosphorus in phytoplankton cells was used as a reserve to maintain phytoplankton growth in the short term. When both internal and external P were depleted in the experimental bottles, the stress response of phytoplankton may display (e.g. a decrease in the F_v:F_m ratio).

One of the original intentions of the *in situ* experiments was to examine the lateral spatial variability of phytoplankton between Shallow and Deep Sites. However, many fragile membrane bottles were destroyed by strong wind, turbulence and wave activities, especially in the shallow nearshore area. Little information could be retrieved from the Shallow treatment. However, in the deeper region away from the near-shore wave actions, the successful rates for the deployment of the membrane bottles were much higher. The results generally indicated higher phytoplankton population (higher Chl*a* concentration) and better phytoplankton health (higher F_v:F_m ratio) in the membrane bottles (Deep and Shallow Sites) than in the confined bottles without additional nutrients (Control treatment), as the semi-permeable membrane allows dissolved substrates to move continually into the membrane bottles and replenish nutrients.

Last but not least, the study suggested that the changes in environmental conditions could affect phytoplankton biology, but not necessarily display the impacts at all levels of responses. As seen in Figure 40, at high levels of light and nutrients the Chl*a* concentration increased, while the F_v:F_m ratio decreased after the 7-day incubation. The F_v:F_m ratio showed the immediate physiological impairment of phytoplankton, while the Chl*a* concentration required a longer time to respond to the stress in biomass. In other words, other factors started to affect the health of phytoplankton, but the adverse health effect has not reflected in the biomass of phytoplankton. It is similar to the findings of Chapters 5 and 6, where the horizontal spatial variations were detected in the rapid biological responses (e.g. phytoplankton cellular content and stoichiometry), but not in the biological responses, which require a longer time to display the impact (e.g. Chl*a* concentration, phytoplankton biovolume, species composition and phytoplankton

diversity). Hence, the impact of the environmental variability depends upon the scales of biological interests, which also exhibit in various time scales.

7.5 Conclusion

This study has achieved the overall aim to determine the variability of phytoplankton under different light and nutrient conditions. Although the apparatus for the site treatment was destroyed by vigorous weather conditions, the *in situ* light and nutrient treatments revealed some important information about the physiological responses for a natural phytoplankton population. The Chl*a* concentration (phytoplankton biomass) increased with light intensity when sufficient nutrients were available. Under nutrient deficiency, the Chl*a* concentrations were higher in the low irradiance than in the high irradiance. This may imply the change in physiological adaptation of phytoplankton to produce more light-harvesting pigments under low light intensity rather than the increase in phytoplankton biomass. Both N and P seemed to be the limiting nutrient for phytoplankton growth, as a significant increase in Chl*a* concentration was observed in nutrient-sufficient treatment (N+P+). No/minor increase in Chl*a* concentration was recorded in both P-depleted and N-depleted treatments and the decline in these F_v:F_m ratios provided evident to disclose the unhealthy status of phytoplankton. The F_v:F_m ratio showed the immediate physiological impairment of phytoplankton, while the Chl*a* concentration reflected a longer response of stress in biomass.

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Chapter 8 General discussion and conclusion

Preamble

The unique basin morphometry of Myponga Reservoir (relatively shallow, elongated side-arm near the major inflow and relatively deep main basin near the outflow) provides a range of habitat conditions with different hydrological and geochemical settings. These hydrological and geochemical processes affect the nutrient and underwater light conditions within the reservoir. These processes and conditions both vary at temporal scales of season, daily or hourly and over spatial scales from localised to basin levels. These, in turn, impact on phytoplankton physiology, growth, abundance, diversity and distribution. The spatial and temporal dynamics in Myponga Reservoir were demonstrated in modelling and field studies in Chapters 3 – 7. The current chapter highlights the interrelationship of these processes and conditions and how they affect phytoplankton variability over space and time.

Chapter 8 General discussion and conclusion

8.1 Spatial variability of nutrients in Myponga Reservoir

Sediments are considered to be important sources of internal nutrient loads in lakes and reservoirs (e.g. Ekholm *et al.* 1997; Sondergaard *et al.* 2003), but little is known about the sediment nutrient compositions and the nutrient release rates in Myponga Reservoir, South Australia. Chapter 3 filled this knowledge gap by assessing the spatial heterogeneity of sediments and the geochemistry between the shallow and deep regions of Myponga Reservoir. Surface sediments in the deep sites were characterized by higher organic matter, higher nutrient content and a higher proportion of fine particles relative to the sediments in the shallow sites. A number of lake processes may help to explain these variations, including sediment focussing and redistribution (Hakanson 1977; Hilton 1985), nutrient binding capacity of sediment particles (DePinto *et al.* 1981), sedimentation of phytoplankton, zooplankton carcasses and faecal pellets (Sterner and Elser 2002), differential loss of sediment nutrients (Hecky *et al.* 1993) and external nutrient load from the catchment (Linden 2007). It is important to understand the possible causes for the spatial variability of sediment nutrients, because the implications of sediment nutrient variations (e.g. sediment nutrient recycling) to phytoplankton dynamics can be significant when the sediment nutrients are released and become bio-available.

Strong seasonal thermal stratification in Myponga Reservoir is being prevented by an artificial destratification system that operates in summer (Brookes *et al.* 2000; Lewis 2004). However, Chapter 4 provided evidence for the occurrence of thermal micro-stratification at the sediment-water interface, which could lead to sediment nutrient releases under anoxic conditions. The high resolution water temperature data revealed that the frequency and intensity of the micro-stratification at the side-arm of Myponga Reservoir was stronger than in the main basin. This is probably because the use of surface mixers in the main basin helps to reduce the frequency and intensity of stratification (Brookes *et al.* 2000). The localised fluxes of sediment nutrients may not necessarily be detected by the routine chemical analysis. However, the variations in the localised fluxes could be of biological significance, causing the spatial variability of phytoplankton between the main basin and side-arm. This is particularly true when the bio-available nutrient levels are low in the water. Modelling of the population of toxic

cyanobacteria (*Anabaena circinalis*) induced by the sediment nutrient flux (Chapter 4) suggested that 1 day of total phosphorus flux could promote the growth of an *Anabaena* population (1002 – 1503 cell mL^{-1}) to an 'alarming' level, based on the *Alert Levels Framework for Cyanobacteria in Drinking Water* (Burch 2006). However, it is likely to predict the maximum threshold of *Anabaena* population induced by anoxic sediment nutrient fluxes because of the assumptions made in the model. The modelling of *Anabaena* abundance can be further complicated by the spatial variability of sediment nutrient content and the frequency, intensity and duration of micro-stratification, which can be intensified during heatwaves.

8.2 Spatial variability of light in Myponga Reservoir

The basin morphometry of Myponga Reservoir may contribute to the horizontal spatial variability of light within the reservoir. Chapter 5 demonstrated the spatial variability of surface mixed layer depths between the relatively deep main basin and the relatively shallow side-arm. Phytoplankton circulating in a deep SML depth (in the main basin) would receive a lower daily light dose when compared to the phytoplankton circulating in the shallower SML depth (in the side-arm). If light quantity is the limiting factor for phytoplankton growth, the daily light dose could affect the growth rate of phytoplankton and, in turn, phytoplankton abundance. The abundance of *Anabaena circinalis* was modelled based on the measured SML depths between the side-arm and main basin of Myponga Reservoir, the daily light dose received by phytoplankton and the growth rates of *Anabaena* at the corresponding light doses. The model suggested that if there was the same starting abundance of *Anabaena*, after 7 days, the final abundance of *Anabaena* in the shallower side-arm would be four times higher than in the deeper main basin (Chapter 5). Therefore, the light variability due to the spatial variations of SML depths could provide different light habitat conditions for differential growth of phytoplankton. In turn, this would lead to the observed spatial variability of phytoplankton dynamics.

8.3 Nutrient and light variability leading to phytoplankton variability?

As seen in the field experiments of this study, phytoplankton due to the impacts of variable nutrient and light conditions vary at different biological time scales. Chapter 7 used two phytoplankton responses, chlorophyll *a* (Chl*a*) and maximal quantum yield of photosystem II (F_v:F_m), at different time scales to show the impact of nutrient and light on overall phytoplankton growth (Chl*a*) and phytoplankton physiological status (F_v:F_m). The results highlighted that the impact of nutrients and light may not necessarily be evident at all levels of phytoplankton responses at the time of measurements. For instance, the Chl*a* concentration increased after the 7-day incubation at high light and nutrient levels, while the F_v:F_m ratio decreased. The F_v:F_m ratio could be used to detect the immediate physiological impairment of phytoplankton. However, the Chl*a* concentration displayed the cumulative effect of past responses and probably required a longer time to display the stress in phytoplankton biomass. Therefore, the different scales of biological responses can be important for determining the impacts of environmental changes.

In the variable nutrient and light conditions in the field, phytoplankton in Myponga Reservoir displayed significant spatial variability at the cellular level (Chapter 6), but not at community level (Chapter 5). In Chapter 6, phytoplankton assemblages collected in the shallower side-arm showed higher average TC and TP content than those collected in the deeper main basin. Due to the shallower surface mixed layer depth, phytoplankton in the shallower side-arm would probably receive higher light doses (Chapter 5) and hence accumulate greater TC within the phytoplankton cells. The higher phytoplankton TP content in the side-arm may be related to the greater sediment P availability. This is probably due to more occurrences of micro-stratification at the sediment surface of the side-arm. Therefore, there would be higher chances of sediment phosphorus release under anoxic conditions in the hot and calm period (Chapter 4). Although there was strong evidence for the spatial differences in phytoplankton nutrient content (Chapter 6), the phytoplankton data from the 3-year monitoring survey showed homogenous distribution of phytoplankton across the reservoir in terms of biomass, species composition, species diversity and community change (Chapter 5).

8.4 Processes that may overcome the spatial variability of phytoplankton

Many processes that affect light and nutrient variability can contribute to the spatial variability of phytoplankton, while other hydrological and physical processes at the basin-scale can also overcome the localized spatial variability of phytoplankton. For instance, convective motion of water driven by differential heating can induce rapid lateral circulation of surface water within a few hours. The effect of this was evident in the difference between the side-arm and main basin of Myponga Reservoir (Chapter 5). Convective motion between the shallow and deep water become faster with increasing temperature gradient. The time scale of the convective water exchange (minutes – hours) was relatively short when compared to the time required to change the phytoplankton abundance (hours – days) and the community composition (weeks – months). This may help to explain the homogeneity of phytoplankton abundance, composition and diversity between sites observed in the 3-year field monitoring data (Chapter 5). In addition, wind and wind-driven currents can significantly influence the horizontal distribution of phytoplankton (e.g. George 1981; Verhagen 1994; Webster and Hutchinson 1994). In particular, buoyant cyanobacteria tend to concentrate toward the downwind side of the lake (George and Edwards 1976; Hutchinson and Webster 1994), while other phytoplankton groups remain uniformly distributed (Baker and Baker 1976). Lateral movement of water, therefore, can help to transport phytoplankton cells across various nutrient and light habitats and to overcome the spatial variability of phytoplankton at community level. However, at the cellular level, phytoplankton still display substantial variability over space.

8.5 Temporal variability of lake processes in Myponga Reservoir

Various environmental processes are time-dependent (Reynolds 1994) and several physical and geochemical processes that occur in a relatively short time scale were discussed in this study. The entrained phytoplankton under the rapid changing mixing environment (from minutes to days) would receive variable light energy over a short time scale. Micro-stratification at the sediment-water interface can maintain for hours and days. This may result in the development of anoxic sediments, which can lead to sediment nutrient fluxes over a period of anoxia (usually hours – days). The short-term change in light and nutrient conditions showed a significant impact on phytoplankton physiology (F_v:F_m, elemental content and stoichiometry), which also operates in a short

time scale (minutes – days). The change in phytoplankton elemental content (e.g. C, N and P) may influence the species composition of herbivorous zooplankton (Urabe *et al.* 2002; Jensen and Verschoor 2004) as well as the production of phytoplankton toxic metabolites (Van de Waal *et al.* 2009).

These short-term localised processes could be overridden by other short-term basin-scale processes. One of these basin-scale processes is convection induced by differential heating, which can circulate the water laterally between the side-arm and main basin within several hours. This rapid circulation can homogenize the potential species diversity between sites. On the other hand, the relatively long-term hydrological processes, including seasonal fluctuations in water temperature, rainfall, and external nutrient load can affect long-term biological processes such as phytoplankton abundance, composition, diversity. These long-term processes require weeks or months to become evident. An understanding of the time scale of biological processes is, therefore, critical to understanding time scale of environmental processes.

8.6 Conclusion

One may argue that Myponga Reservoir, which is seasonally mixed by artificial aerators and mixers, would be a homogeneous system. This study provides evidence that Myponga Reservoir is *"far from being the homogeneous environments we might expect and offers a rich and dynamic heterogeneity at multiple spatial and temporal scales that we are just beginning to understand"* (Kratz *et al.* 2005 p.329).

(1) In general, thermal stratification is not expected in an artificially aerated and mixed lake. However, for Myponga Reservoir thermal micro-stratification was detected in the artificially destratified parts of the reservoir. The frequency and intensity of micro-stratification were even more severe in the other sides of the reservoir, which were not influenced by the artificial destratification. In addition, the micro-stratification was exacerbated during the prolonged heatwave, potentially leading to the development of anoxia at the sediment-water interface and the release of sediment nutrients.

(2) In general, internal nutrient loading is not expected in an artificially aerated and mixed lake. However, internal nutrient load has most likely occurred in conjunction with the presence of thermal micro-stratification in Myponga

Reservoir. The potential sediment nutrient fluxes derived from laboratory experiments can be used to approximate the additional population of phytoplankton.

(3) In general, homogeneous distribution of phytoplankton is expected in an artificially aerated and mixed lake. In Myponga Reservoir, the monitoring survey ascertained that the phytoplankton populations were distributed relatively homogeneous. However, the phytoplankton stoichiometry showed distinctive spatial differences. The spatial variations in phytoplankton stoichiometry were probably related to the spatial variations in light and nutrients.

The current research focuses on numbers of hydrodynamic and geochemical processes affecting phytoplankton dynamics at different spatial and temporal scales. Other lake processes (e.g. zooplankton grazing, nutrient recycling, phytoplankton metabolism, etc) are also important to phytoplankton dynamics, but were beyond the scope of this study. Nevertheless, this study improves our understanding of the hydrodynamic and geochemical processes that affect phytoplankton dynamics, ranging from the shorter time scales of phytoplankton physiology up to the longer time scales of phytoplankton community structure. The rapid changes in nutrient and light conditions (e.g. localised changes within hours and days) can affect the short-term dynamics of phytoplankton at physiological and cellular levels. However, these rapid localised changes may not necessarily be evident in phytoplankton population and community structure at the time of measurements. Nevertheless, the relatively long-term seasonal and interannual dynamics of phytoplankton abundance and diversity usually coincide with prolonged changes in nutrient and light conditions at seasonal and annual scales.

This study provides important scientific knowledge on the variability and complexity of the limnological processes impacting phytoplankton ecology. By gaining a better understanding of the spatial and temporal variability of lake processes, water authorities can better tailor and improve the management of such water quality issues, as the origin and extent of algal blooms and the release of contaminants from sediments. Specifically, this project provides some important baseline information for the management of the water quality for Myponga Reservoir, including: (i) the compositions of nutrients (e.g. C, N, P) and metals (e.g. Mn, Fe) across the surface sediments of Myponga Reservoir and its implications; (ii) the likelihood of the

occurrence of micro-stratification at the sediment-water interface, which may lead to the release of nutrients and metals under anoxic conditions, with the associated risk of cyanobacterial blooms; (iii) the various responses of natural phytoplankton population (e.g. F_v:F_m, stoichiometry, Chl*a*, abundance, composition, diversity, etc) to ambient light and nutrient conditions; and (iv) the physical and hydrodynamic processes that may override some biological responses.

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