Impact of destratification on the treat-ability of natural organic matter in drinking water reservoirs

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Abstract

Natural organic matter (NOM) in drinking water reservoirs affects the cost of potable water production by exerting coagulant and disinfectant demand. It also affects product water quality by acting as a disinfection by-product precursor and imparting colour and taste if not removed satisfactorily. Therefore, processes that determine the concentration and character of NOM and its transport and formation in reservoirs are of interest and concern to catchment, reservoir and water utility managers. Thermal stratification, caused by solar heating of the reservoir surface, plays a major role in determining water quality in reservoirs. A number of biological and chemical processes that result in reduced water quality, such as release of soluble metals or the growth of cyanobacteria, result after periods of persistent stratification. Artificial destratification is widely implemented to manage these water quality problems in reservoirs by breaking down thermal stratification using bubble plumes or submerged impellers. However little is known about the effect of artificial destratification on NOM. Inflow events, where increased river flow causes a riverine intrusion into the reservoir, may compromise water quality from the perspective of turbidity and pathogens. Changes in organic matter concentration and character may also occur during such an event, but has not been investigated from the perspective of potable water production.

The aim of this study was to describe the potential impact of stratification, and therefore destratification, and inflow hydrodynamics on the raw water quality in drinking water reservoirs, from the perspective of NOM. Investigations of the changes in the concentration, character and removal of NOM by conventional treatment processes during inflow hydrodynamics and thermal stratification were performed using observational and manipulative experiments and empirical and process based modelling. Further conceptual

models were developed to place NOM within the existing frameworks of reservoir management from the perspective of other water quality hazards.

The potential for short term fluctuations (hours to days) in concentration and character of NOM due to flood inflow hydrodynamics was demonstrated. A coagulant dose model (mEnCo) predicted increased coagulant dose requirements for this changed raw water quality, 40% of this increased coagulant dose was attributable to changes in the NOM. The hazard presented by NOM may co-occur or be temporally displaced from other hazards such as pathogens and turbidity. The use of a selective withdrawal strategy at the water treatment plant (WTP) offtake would allow the avoidance of these changes in raw water quality.

The effect of the inflow on the microbial activity in the reservoir was pronounced, indicating a significant influence on the labile organic matter, especially in the particulate fraction. Community level physiological profiling, using BIOLOG^{TM} Ecoplates, implied microbial communities imported from the catchment were able to utilise a greater number of substrates than reservoir communities indicating that catchment soil communities may be an important source of metabolic diversity.

The concentration of dissolved organic carbon (DOC) in Myponga River was predominantly described (~70% of the variance) by a nonlinear relationship with flow. Monitoring of the organic carbon concentration-flow hysteresis effects for a single winter indicated that the potential risks to water quality were greater in the late winter due to the hydrologic and organic character parameters (catchment saturation etc); however the hydrologic characteristics are likely to be more important in determining the risk. Therefore current hydrology monitoring systems such as online rainfall and gauge height sensors should be sufficient to inform management response to large inflow events, given sufficient understanding of the NOM dynamics. The annual allochthonous organic carbon load (240 ± 110 tonnes) was estimated to be 4 times greater than the autochthonous carbon

load (mean 1999 - 2003; 60 ± 34 tonnes). In 2002, a year of very low rainfall, the estimated autochthonous and allochthonous load were similar. Over 70% of the consumption of this organic matter could be attributed to microbial activity, and of this, 85 to 95% to the pelagic microbial population.

Changes in the apparent optical properties and molecular weight distribution (MWD) measured with high performance size exclusion chromatography (HPSEC), were observed in the field and in simulated water columns. However little change in the proportion of non-removable organic matter was observed (0.7%). The observations made during a mesocosm experiment were at least partially attributed to the pH of the sampled experimental water columns. Analysis of MWD with HPSEC with DOC detection suggested that UV transparent HMW OM had accumulated in the surface mesocosms, which was not completely removed during water treatment.

The operational response of reservoir offtake management should consider organic matter to ensure the best quality raw water is supplied to WTPs. Inflow events represent the greatest potential risk for short-term fluctuations in the organic matter character and concentration. The decreased temporal variability in organic matter properties that would occur under artificially destratified conditions would probably provide a more consistent raw water quality than that from a stratified reservoir. The benefit of this consistency in raw water quality is likely to outweigh any changes in the treat-ability of natural organic matter under stratified conditions. The decision to employ artificial destratification should always consider system specific knowledge and conditions.

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 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.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Signed

Leon Gareth Linden

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Abbreviations

PET	poly ethylene terathalate
PVC	poly vinyl chloride
LDPE	low density poly ethylene
PS	poly styrene
VHA	very hydrophobic acid
SHA	slightly hydrophobic acid
CHA	charged hydrophilic
NEU	neutral hydrophilic
QC	quality control
DOC	dissolved organic carbon (mg L^{-1})
UVA	ultraviolet absorbance at 254 nm (cm^{-1} or m^{-1})
HU	true colour, hazen units, determined at 456 nm
BRP	bacterial regrowth potential
SUVA	specific ultraviolet absorbance (m^2 g^{-1})
SpHU	specific colour (HU (mg $L)^{-1}$)
AOC	assimilable organic carbon
BDOC	biodegradable organic carbon
MPD	model predicted dose

Chapter 1. Introduction

1.1. Natural Organic Matter

Natural Organic Matter (NOM) is a complex, heterogeneous residue of decomposing biomass. It is ubiquitous in aquatic systems and consequently it plays a number of significant roles in aquatic ecology and the in the use of water resources by human society. It has been identified as having UV protective properties, being a food source and having direct inhibitory and stimulatory roles in aquatic ecology (Steinberg 2003). To the drinking water industry, it contributes to the cost of water treatment by exerting a coagulant demand, acts as a precursor for the production of potentially carcinogenic by-products during chemical disinfection and is an aesthetic problem to customers if present in product water at a high enough concentration (visual and taste). Consequently, the factors that determine NOM concentration and character in raw water storage reservoirs, and the potential to manage this integral part of the water supply chain is of significant interest to the water industry.

NOM is variable in source, composition and character, its complex nature has demanded research employing chemical, physical and biological methods (Frimmel 1998, Jones and Bryan 1998, Hem and Efraimsen 2001, Janos 2003, Abbt-Braun *et al.* 2004). The physical properties of NOM include particulate, colloidal and dissolved states, with the greatest proportion being found in the dissolved fraction (here and in the majority of scientific literature defined as < 0.45 μm) in freshwater environments. The size range of organic matter extends from the dissolved state through the colloidal phase and into the solid phase where it can be considered as suspended particles (Figure 1.1 from Von der Kammer *et al.* 2004). While it may be dissolved, low molecular weight NOM may also functionally act as suspended material by adsorbing to suspended solids (e.g. clay particles, Clapp and Hayes 1999).

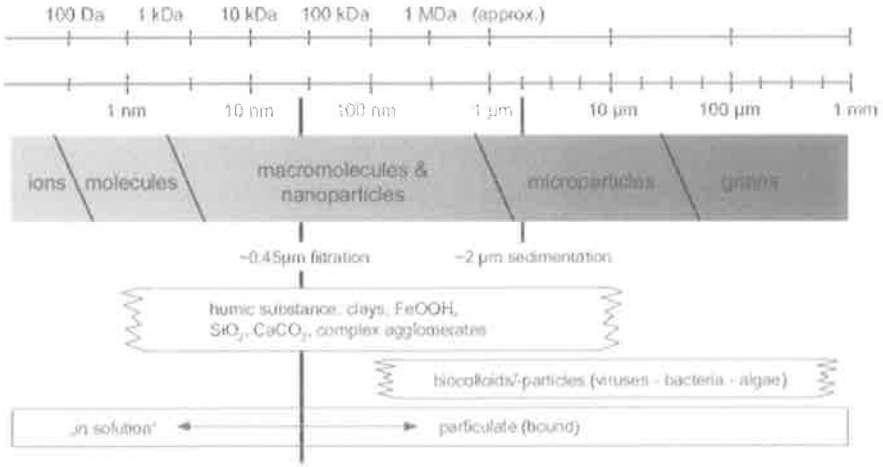


Figure 1.1. Size domains of natural colloidal particles. Values of molecular mass (kDa) are approximate as the spatial extent of organic matter depends on molecular structure, polarity and the matrix characteristics (e.g. ionic strength). Operationally defined cut-offs are given for classical laboratory filtration (0.45 µm) and settling (2 µm). (From Von der Kammer *et al.* 2004).

Chemically, NOM consists of a mixture of distinct compounds and heterogeneous macromolecules which contain a range of ionisable functional groups. Consequently, NOM is often classified based upon solubility at different pH. Much of the nomenclature of NOM has evolved as the process of characterising this complex entity progressed within the field of soil science. This nomenclature has subsequently been applied to aquatic NOM (a history of soil organic matter nomenclature is provided by Tan 2003). A modern nomenclature of soil organic matter (SOM), by Stevenson (1994) is detailed in Table 1.1. The terminology used to describe or characterise NOM should be interpreted in the context of the isolation methods as they represent functional divisions based on the average properties of the isolated molecules. In this nomenclature, which is based on solubility in various solvents, the divisions is the result of the sum of their functional groups rather than a distinct difference in their structure or composition. The concentrations and proportions of these different fractions within a given sample of NOM will depend upon the source of the organic matter and the processes that acted upon it prior to its collection (Thurman 1985, Gaffney *et al.* 1996).

The biological activity of NOM is closely linked to its chemical composition and as such, ranges from very labile biochemically distinct compounds (non-humic) to biologically refractory compounds (humic and fulvic acids) (Steinberg 2003). Some components of NOM can act as electron receptors, either in a terminal role during microbial respiration or in an antagonistic role, directly interfering in the electron transport chain in photosynthesis. Paradoxically perhaps, it is those compounds that have classically been considered to be biologically refractory that play these roles as electron acceptors.

Table 1.1. Nomenclature and definitions used for NOM (Stevenson 1994).

Term	*Definition*
Litter	Macro-organic matter that lies on the soil surface
Light Fraction	Undecayed plant and animal tissues and their partial decomposition products that occur within the soil proper and that can be recovered by flotation with a liquid of high density
Soil Biomass	Organic matter present as live microbial tissue
Humus	Total of the organic compounds in soil exclusive of undecayed plant and animal tissues, their partial decomposition products and the soil biomass
Humic Substances	A series of relatively high molecular weight, yellow to black coloured substances formed by secondary synthesis reactions. The term is used as a generic name to describe the coloured material or its fractions obtained on the basis of solubility characteristics. These materials are distinctive to the soil or sediment environment in that they are dissimilar to the biopolymers of micro-organisms and plants
Non-humic Substances	Compounds belonging to known classes of biochemistry such as amino acids, carbohydrates, fats, waxes, resins, organic acids, etc.
Humin	The alkali insoluble fraction of humus
Humic Acid	The dark coloured organic that is extractable by weak alkali and is insoluble in weak acid
Hymatomelanic Acid	The alcohol soluble fraction of humic acid
Fulvic Acid	Fraction of humus that is soluble in both weak alkali and weak acid

1.1.1. Composition of NOM

While the non-humic fraction can readily be described in terms of its biochemical composition, the humic substances are polydispersed electrolytes with a high degree of molecular irregularity and heterogeneity (Steinberg 2003). Consequently a descriptive approach of their average properties (such as solubility) has been necessary in the pursuit of the structure of humic substances (Hayes *et al.* 1989). The structures and substructures that are found in humic substances encompass the range of biochemicals found in living

tissue and includes peptides and amino acids (Jahnel and Frimmel 1995, Grøn *et al.* 1996), glucosamine (Jahnel and Frimmel 1996), carbohydrates (Watt *et al.* 1996, Jahnel *et al.* 1998) and fatty acids (Stevenson 1994). Of note is the observation of the strong correlation between the proportional abundance of sugars and amino acids found in humic substances under acid hydrolysis (Watt *et al.* 1996) and that fulvic acids contain lower concentrations of these residues than do humic substances. This reflects the theory that fulvic acids, as being more oxidised and containing fewer ionisable functional groups are in a more advanced state of diagenesis than humic acids (Steinberg 2003).

The secondary and tertiary structures of humic substances have the potential to affect their role in biogeochemical and ecological processes. The main determinants of its secondary and tertiary structure are the pH environment, the presence, concentration and type of multivalent cations and the concentration of the humic substances themselves. X-ray and transmission electron microscopy indicate that humic substances form aggregates of up to several micrometers diameter, given the appropriate conditions (Lead *et al.* 1999, Myneni *et al.* 1999, Thieme *et al.* 2002). The exact nature of the secondary and tertiary structures of humic substances continues to be a matter of contention and a number of models, which all partly satisfy the observed properties of humic substances, have been proposed. The most relevant modern models include "protein-like macromolecules" (Ghosh and Schnitzer 1980), "Micelles" (Wershaw 1989) and supramolecular associations (Piccolo 2001). Further progress has been made using molecular modelling (Schulten and Leinweber 1996, 2000), however the predicted structure and properties remain to be confirmed by chemical methods. Recent work using low molecular weight organic acids to disrupt the tertiary structure of humic substances (Piccolo *et al.* 2005) and the use of small angle neutron scattering (Diallo *et al.* 2005) lend support to the supramolecular model of humic substance tertiary structure.

1.1.2. Source of NOM in the Aquatic Environment

The NOM found in the aquatic environment is a mixture of organic matter derived from allochthonous and autochthonous sources. It may have been photosynthetically fixed in the catchment and then transported, dissolved or suspended in water into the aquatic system (allochthonous), or have been produced within the water body by autotrophic production by aquatic macrophytes, phytoplankton, chemo-autotrophic bacterioplankton, periphyton or microphytobenthic organisms (autochthonous). The relative contribution of these sources varies between different aquatic systems and is affected by many factors and processes including vegetation type, soil type, topography, hydrodynamics, trophic state and climate. The composition of NOM at any given time is a function of its original chemical structure and the changes that have occurred to its original state, and thus it state of diagenesis.

1.1.2.1. Formation of Refractory Organic Matter

The traditional view of humification proposes two pathways; the degradative pathway and the condensation polymerisation pathway (Hatcher and Spiker 1988, Steinberg 2003). Humic substance formation via the degradative pathway occurs via the partial degradation of biopolymers, such as cellulose and lignin, and is considered to be the predominant process in the humification of the biomass of terrestrial vegetation in the soil and litter layers and the biomass of macrophytes in the littoral zone. At least three different condensation polymerisation models have been proposed, the polyphenol model, the melanoidin model and the polyunsaturated structure model (Steinberg 2003). These processes were originally proposed to occur in the humification of autochthonous organic matter, when the formation of refractory substances with properties similar to humic substances were observed to develop in bacteria free algal cultures (Stabel 1977), in seawater (Marxsen and Fiebig 1993, Lara and Thomas 1995) and in freshwaters (Tranvik 1992).

The polyphenol model of humification proposes that the reaction of quinones with amines and ammonia is a significant polymerisation reaction. The available quinones are potentially produced via the degradation of lignin, which produces phenols, and the subsequent production of quinones. The polymerisation rate is accelerated by the presence of transition metal oxides, cations of Mn^{2+} and Fe^{3+} and clay or mineral particles. The oxidation of phenols to the quinone precursors for reaction with reduced nitrogen containing compounds is facilitated by photosensitisation or enzymatic activity, or alternately autoxidation of some phenolics may occur (Steinberg 2003).

The Maillard reaction, between reducing sugars and amino acids, is the major process of condensation in the melaniodin model of humification (Stuermer and Harvey 1974). The production of humic like molecules by this pathway has been experimentally confirmed to occur in laboratory experiments (Yamamoto and Ishiwatari 1989). Field based evidence for the occurrence of the 'protein-based melanoidin model' has also been presented (Yamamoto and Ishiwatari 1992). However the melanoidin model alone is unable to account for the total amount of aliphatic structures in humic substances (Steinberg 2003). The polyunsaturated structure model describes the humification process from poly-unsaturated compounds such as fatty acids and carotenoids. This model relies on the UV cross linking of polyunsaturated structures (Harvey *et al.* 1984), however this process fails to explain the high nitrogen content of humic substances (Steinberg 2003).

The genesis of humic substances is probably a mixture of all these processes occurring in tandem, depending on the availability of the appropriate precursors and environmental conditions. These polymerisation processes will all be more efficient at higher reactant concentrations and consequently interactions at interfaces are important. The concentration of organic and inorganic nutrients will be higher at the surface of decaying biomass, there is dynamic interaction with the medium around suspended particles and many important precursors may be particulate or colloidal.

A more general model of humic substance formation that incorporates these humification models is given by Ziechmann (1996, in Steinberg 2003). This model divides the humification process into the metabolic, radical and conformational phases. The metabolic phase consists of biochemical reactions such as biosynthesis of aromatic and non-aromatic biomolecules and the enzymatic degradation of biomolecules into aliphatic and aromatic fragments. These fragments can enter the radical phase where non-specific chemical reactions (*sensu* condensation models) form humic acid precursors. Humic acid precursors and non-humic substances enter the conformational phase where intra and inter molecular interactions determine the final tertiary structure of the humic system. The resulting conceptual model suggests any chemical member of the system (a compound) may react with practically any other compound and consequently no clearly defined chemistry or composition for a humic substance can occur (Ziechmann 1996, in Steinberg 2003).

1.1.2.2. Allochthonous

Allochthonous organic carbon, photosynthetically produced in the catchment, consists of organic matter derived from biopolymers of higher plants, including cellulose and lignin, and a smaller contribution of proteins, waxes, tannins, gums (generally complex carbohydrates), fats, essential oils and pigments (e.g. chlorophyll). This material is degraded and humified in the terrestrial environment before it is transported into the aquatic environment, often in the organic rich O and A soil horizons (Neff and Asner 2001). Allochthonous inputs to the aquatic environment may be in the particulate, colloidal or dissolved form (Cole *et al.* 1989, Thomas 1997, Kim *et al.* 2000, Yoshioka *et al.* 2000, Assemi *et al.* 2004, McCallister *et al.* 2004). Particulate inputs to reservoirs include gross particulate matter that falls directly into the water body and transport of suspended organic matter by streams and rivers that drain into the water body. The majority of dissolved inputs are generated by rainfall infiltrating and leaching organic matter from the soil profile. The flux of organic matter to a reservoir is therefore determined by the flux of

organic matter from the catchment into the system of waterways draining the river basin and the processes that act upon it during transport. Therefore, the catchment characteristics and the prevailing hydrology determine the organic carbon flux.

It can be difficult to separate the influence of physical catchment properties and hydrological characteristics as they interact to determine the export of organic carbon from a catchment. Physical factors at catchment and field/slope scale are important contributors as they influence the mobilization of particles and solutes through the processes of detachment and dissolution (Nash *et al.* 2002). Detachment commences with the mobilization of sediments by physical processes such as raindrop impact and water flow and physico-chemical processes like slaking and dispersion (Leeper and Uren 1997). These materials are transported at a rate proportional to the kinetic energy of the flowing water (Shainberg *et al.* 1994) and therefore factors that increase flow rate or turbulence will increase the rate of detachment and transport. The factors that determine the transport of substances in the aqueous phase include the solubility of the compound, its sorption characteristics and the presence or absence of other substances that modify the interactions with the sorption sites. The most important factor for dissolution is the residence time within the catchment and hence the amount of time available for soil water interaction (Kirkby *et al.* 1997). The residence time is determined by the flow path of the water, which is in turn determined by the characteristics of the catchment and the hydrology of the flow event in question.

The flow path of water through the catchment has been identified as a very important factor in the transport of organic matter (Edwards and Cresser 1987, Billett and Cresser 1992) and is modulated by soil type and structure (Cronan and Aiken 1985, Stevens *et al.* 1999, Cox and Pitman 2001). The flow paths of water within the landscape can be divided into surface pathways and subsurface pathways. Overland flow (a surface pathway) occurs either when the infiltration rate of the soil is exceeded (due to the rates of water addition

and surface infiltration and the subsoil hydraulic conductivity) or the soil profile becomes saturated, preventing further infiltration (Emmett 1978). Infiltration excess overland flow occurs when the infiltration rate is negligible or much less than the rate of water addition at the soil surface. Often this is due to the poor hydraulic conductivity of the substrata, such as when the permeable A-horizon is overlying a clay rich, less permeable B-horizon (Chittleborough 1992, Cox and McFarlane 1995). Saturation excess overland flow also occurs in regions where interflow or ground water rises close to the surface, for example proximal to stream channels and at the break of the slope. Interflow (or throughflow) occurs when water infiltrates into the soil and then travels laterally before being discharged as overland flow. A significant proportion of the lateral flow may occur in the top 15 - 20 cm of the soil profile (Bishop *et al.* 1990) and this may lead to the reduced adsorption of organic matter by avoiding available adsorption sites lower in the soil profile (Moore 1989). This phenomena may be the result of the greater lateral hydraulic conductivity compared to the vertical hydraulic conductivity found in some soils (Ward 1984). While macropores within the soil have been proposed to play a role in this movement they are generally more important in the rapid movement of water from the vadose zone (unsaturated zone) to the phreatic zone (saturated zone) (Davie 2002) and for water movement within the riparian zone (Fiebig *et al.* 1990). Interflow often occurs at the interface of two soil horizons and is referred to as A/B or B/C interflows (Chittleborough *et al.* 1992, Stevens *et al.* 1999).

The first hydrological determinant of DOC export is the nature of the precipitation. Concentrations of DOC in precipitation may be as high as 10 mg L^{-1} in throughfall in forested catchments (Likens *et al.* 1983). While this may seem quite high it is generally small compared to internal input, it may however be significant in some systems (Schreiber and Duffy 1982, McDowell and Likens 1988). The concentration and character of the DOC changes significantly as it passes through the canopy, litter and organic rich soil

horizons (McDowell and Wood 1984, Moore 1989) but has been observed to remain relatively constant during passage through the B-horizon (McDowell and Likens 1988).

The concentration of organic matter in water tends to decrease as it percolates through the soil profile, in temperate forests (McDowell and Wood 1984, McDowell and Likens 1988, Moore 1989, Moore and Jackson 1989), moorlands (Grieve 1990c, b) and grasslands (Hornung *et al.* 1986). This is primarily the result of adsorption of the constituent organic acids as part of the podzolization process (McDowell and Wood 1984, Cronan and Aiken 1985, Thurman 1985, Chittleborough 1992) and the organic matter may be stored for several hundred years. The adsorption is a function of the soil mineralogy, the pH of the percolating solution (Jardine *et al.* 1989) and the availability of adsorption sites which is reduced by the presence of indigenous organic matter (Moore 1988, Fiebig *et al.* 1990, Grieve 1990b). There are also significant interactions with multivalent cations such as aluminium (Palmer *et al.* 2005) and iron. While many catchment properties alter the DOC dynamics, many of these factors are altered or modulated by the catchment hydrology.

Discharge often has a significant influence on the organic carbon concentration in flowing water (Schlesinger and Melack 1981). This influence has been attributed to the flushing and mobilization of old soil water (Pearce *et al.* 1986, Pearce 1990). Regressions between flow rate and organic carbon concentrations suggest that increases in river discharge between 0.1 to 10 Ls^{-1} will result in an increase to between 1.4 and 5.5 times the baseflow organic carbon concentration, although the relationships are sometimes weak (Grieve 1984b, Tipping *et al.* 1988, Grieve 1991, Hope *et al.* 1994). Often a hysteresis is observed with organic carbon concentrations being greater on the rising hydrograph than on the falling hydrograph (Grieve 1984b, Moore and Jackson 1989). The antecedent rainfall and soil moisture conditions are also important to determine the organic carbon concentrations in flowing water (Tate and Meyer 1983) with higher concentrations observed after periods of dry weather (Grieve 1991). The concentration of DOC in the soil solution may also be

seasonally controlled, with summer maxima being attributed to evaporation and time dependant desorption (Grieve 1990a, c). These phenomena may however only be observed within the O/A horizon (Cronan and Aiken 1985) or not at all (Tipping *et al.* 1988).

In Stream Processes

In stream processing will also play a role in the transport and export of organic matter from a catchment. The observation that hydrologic parameters are unable to totally describe the export of organic matter suggests that in-stream retention mechanisms are important (Bilby and Likens 1980). Biological processing of particulate organic debris can be explained via the river continuum concept (RCC, Vannote *et al.* 1980) which considers the river ecosystem as a longitudinal changing physical unit and generalises about the types of particulate organic matter inputs and the functional feeding group relationships that are proposed to be processing it (Minshall *et al.* 1985). The RCC defines these functional feeding groups as collectors, shredders, grazers and predators and postulates that their relative abundance changes depending on the availability of their food sources, which is determined by season, stream order and the nature of the riparian zone. There have various modifications of the RCC since its proposal and they have demonstrated the usefulness of the model to be determined by physical factors, especially current velocity (Wetzel 2001b). The concept of nutrient spiralling was proposed to describe the relative rates of resource processing and longitudinal water movement and describe ecosystem stability (Newbold *et al.* 1981, Minshall *et al.* 1983). The importance of oxidation and respiration of organic material in stream increases with retention time. Removal rates of DOC of 0.1 to 1.1 g m^2 h^{-1} are reported (Hope *et al.* 1994) as the sum of biotic and abiotic mechanisms (such as adsorption, precipitation, oxidation/reduction, complexation, Thurman 1985). Turnover lengths of organic carbon increase down stream from 1 - 10 km in headwaters to 1 - 129 km in the mid-waters to 30 - 250 km in lowland rivers (Newbold *et al.* 1982, Minshall *et al.* 1983). There may also be significant instream production of organic carbon by algae

and macrophytes. For example up to 20 % of the daily DOC export has been attributed to benthic algae in some systems (Kaplan and Bott 1982). This highlights that one of the major variations to the supply of organic carbon is season, through the availability of light for photosynthesis instream, and in the growth and litter production within the terrestrial environment of the watershed.

1.1.2.3. General Characteristics of Stream OM

The flux of organic carbon amongst different catchments is variable, albeit generally in the range of 10 to 100 kg C ha^{-1} yr^{-1} (Hope *et al.* 1994). Despite instream production, the majority of organic carbon found in streams is allochthonous (Fisher and Likens 1972). However stable isotope studies suggest that the organic matter found in streams is less than 40 years old, indicating it is in early diagenesis and is not substantially humified compared to soil organic matter, which is generally 550 to 700 years old (Thurman and Malcolm 1981, Schiff *et al.* 1990).

Models of TOC Export

Models of TOC export may be very simple deterministic equations describing the change in carbon concentration in relationship to a single parameter, usually flow (Pers *et al.* 2001) to very complicated GIS based systems which describe many catchment processes such as groundwater loading, land and stream routing, organic carbon production, sinks and transport (Ouyang 2003). Models of intermediate complexity which are based on a smaller number of catchment parameters and a simpler hydrological model (i.e. without spatial information) are successful at modelling TOC export (De Souza 2004).

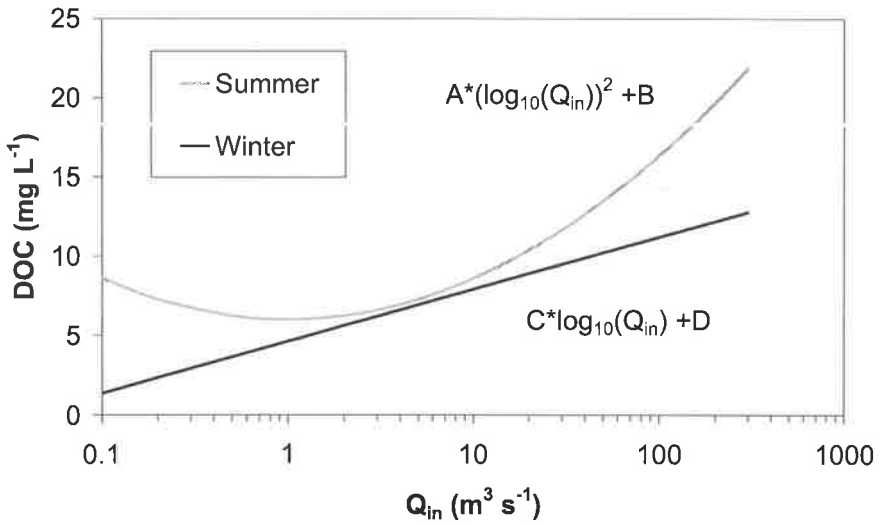


Figure 1.2. DOC concentration in water entering Lake Örträsket (Sweden) from the River Örån (Qin) as modelled by Pers *et al.* (2000). The summer equation was used from June-September and the Winter equation from October to May. Using the Coefficients A = 2.58, B = 6.02, C = 3.28 and D = 4.64 the r^2adj (degrees of freedom adjusted regression coefficient) were 0.78 and 0.67 for the summer and winter models, respectively (N = 32 and 21).

1.1.2.4. Autochthonous

Autochthonous carbon is found as the constituent biopolymers of the aquatic autotrophs from which it is derived. They may be photosynthetic, using light energy to fix CO_2, or chemosynthetic, converting chemical energy of oxidised substrates to fix CO_2 into cell constituents. Autochthonous production may also contribute to both the particulate and dissolved forms of NOM. Particulate inputs are generated by the senescence of tissues of aquatic macrophytes (Godshalk and Wetzel 1978) and the cells and secreted mucilage of planktonic organisms (Cole *et al.* 1984, Baldi *et al.* 1997, Biddanda and Benner 1997). Oligomeric carbohydrates, proteins, lipids, and to a lesser extent, high molecular weight cellulose and lignin, are released during senescence and mortality. Organic carbon, that may be classified as dissolved or colloidal, is excreted in the form of extracellular enzymes and mucilage by many aquatic organisms and may be humified over time (Lara and Thomas 1995). Phytoplankton have also been shown to secrete a range of low molecular weight (LMW) compounds, such as glycolic acid, during photosynthesis (Fogg *et al.* 1965,

Hellebust 1965, Storch and Saunders 1978), and release DOC during cell death and lysis (Lee and Rhee 1997). Autochthonous carbon is generally less humified, more chemically defined and more readily assimilated into the carbon cycle. The fate of the resulting pool of organic carbon is in the various processes of metabolism, photochemistry, sedimentation and export.

1.1.3. Fate of NOM in the Aquatic Environment

The fate of NOM depends upon its chemical character as a substrate for metabolism, its physical characteristics, such as size, and the physical, chemical and biological characteristics of the environment. The prevailing hydrodynamics is a major forcing variable in aquatic systems. Lateral and vertical water movement within the landscape determines the exposure and interaction of the aquatic environment with terrestrial and atmospheric systems. In lotic (relatively quiescent) aquatic systems, such as lakes and reservoirs, vertical water movement can significantly influence ecosystem processes. The vertical movement of water may be restricted by the density structure of the water column. This is determined by thermal flux and physical processes such as wind and artificial mixing and bulk water movement in the form of inflows and outflows (see below). These processes will determine the dilution of influent water, the temperature of the water column and the surface mixed layer. In turn these variables affect the degradation processes by governing the distribution of organic matter, controlling microbial metabolism and determining the exposure to solar radiation.

1.1.3.1. Biodegradation and Assimilation

Microbial heterotrophs change the character of the pool of natural organic matter by breaking down, assimilating and releasing organic compounds. As much of the organic carbon pool is polymeric and too large to be transported across the bacterial cell membrane (Münster 1993), it must first be degraded into its constituent monomers before it can be assimilated. This degradation is achieved by the production of enzymes which are actively

transported across the cell membrane (Chróst 1991). These enzymes may be associated with the cell walls of the organisms (ectoenzymes, Chróst and Siuda 2002) or released into the biofilm matrix or mucilage (Espeland *et al.* 2002) or the aquatic environment (Vetter *et al.* 1998). The production of microbial enzymes is regulated by the organism as part of the dynamic system of feed back regulation of the lytic and uptake mechanisms (Hoppe *et al.* 1988) and can be conceptually represented as two pools connected by valves (Figure 1.3).

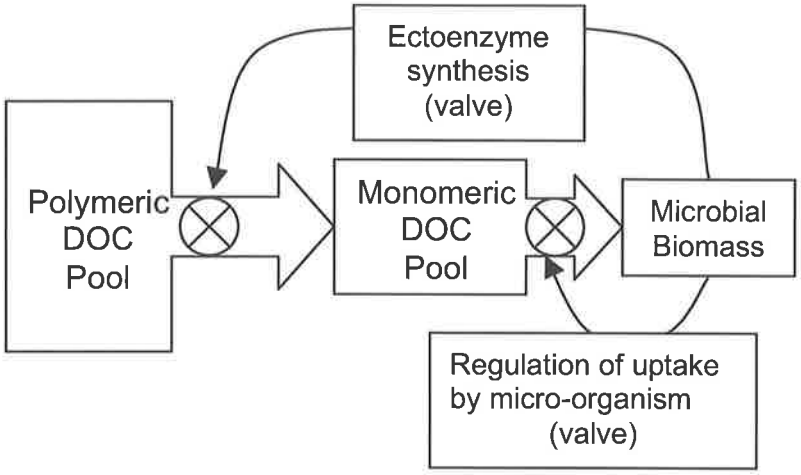


Figure 1.3. Conceptual model of microbial hydrolysis and assimilation of polymeric substrates after Hoppe *et al.* (1988).

The relative magnitudes of uptake and degradation depend upon the trophic state of the water body. Hoppe *et al.* (1998) found that in more eutrophic waters that microbial substrate uptake rate and microbial growth increased more than hydrolytic enzyme activities. This reflects a greater availability of readily assimilable substrates in eutrophic waters and supports the theory that the expression of hydrolytic enzymes is down regulated by feedback inhibition exerted by their assimilable degradation products (Hoppe *et al.* 1998). Similar observations of regulation of chitinase and saccharase activity have been made, however the system may be modulated by mineral nutrient availability (Foreman *et al.* 1998).

The degradation of NOM is also altered by other aspects of the environment (e.g. temperature, pH, ionic strength, redox state) and the composition of the NOM itself. The degradation products of biopolymers and other components of the NOM pool may inhibit

enzyme activity and microbial growth. For example, some aromatic compounds released from NOM can inhibit glucosidase and other enzymes (Freeman *et al.* 2001), and microbial growth may be inhibited by higher plant essential oils (ISO 1996, Boon and Johnstone 1997). Furthermore, moieties that are energetically favourable as a substrate will be consumed preferentially and therefore become depleted from the NOM pool. Interactions between microbial metabolism and the physico-chemical environment provide additional complexity. Low temperatures often reduce the activity of enzymes and slow microbial growth (Vrede 2005) and the prevailing redox environment, which determines the availability of terminal electron acceptors, particularly oxygen, determines the potential for aerobic and anaerobic metabolism and the distribution of microbial taxa over the water column (Nold and Zwart 1998, Glöckner *et al.* 1999).

1.1.3.2. Aerobic and Anaerobic Respiration

Metabolic diversity amongst microbial life forms is extensive, and the range of metabolic substrates and by-products is large. One of the major divisions that can be made within this diversity is aerobic and anaerobic catabolism. The main distinction between these processes is the terminal electron acceptor. In aerobic catabolism, oxygen is the terminal electron acceptor and in anaerobic catabolism, there is a diversity of electron acceptors. These include, the ferric ion (Fe^{3+}), nitrate (NO_3^-), sulphate (SO_4^{2-}) carbonate (CO_3^{2-}) and many organic compounds including humic substances (Capone and Keine 1988, Mattson and Likens 1993, Lovley *et al.* 1996, Coates *et al.* 2002). In an anoxic environment, significant limitations are placed upon metabolic pathways that require the consumption of oxygen or the activity of oxygenases (Bastviken *et al.* 2001).

Anoxic metabolism yields less energy per unit substrate than aerobic metabolism (Bastviken *et al.* 2001). Combined with the observation that anaerobic decomposition suffers from limited degradation of some compounds (Field 2002), it has been proposed that it results in incomplete degradation and therefore preservation of organic matter, for

example in the formation of fossil fuel deposits. While recent work has demonstrated that microbial production is similar in aerobic and anaerobic environments (Cole and Pace 1995), the degree of mineralisation of organic matter is slightly different (da Cunha-Santino and Bianchini 2002). The rate of degradation of labile organic matter has been shown to be greater under aerobic conditions, whereas the converse is true of biologically refractory organic matter (da Cunha-Santino and Bianchini 2002). Bastviken *et al.* (2004) proposed that the degradable DOC could be classified into three fractions. The first was able to be degraded only under oxic conditions, the second was degraded more rapidly under anoxic conditions than oxic conditions, and the third which was degraded at an equal rate under oxic and anoxic conditions. They found that the majority (~70%) of the degradable organic carbon belonged to the fraction that was only degraded under oxic conditions.

1.1.3.3. Sedimentation

A particle that is suspended in a water column, including particulate and colloidal NOM and planktonic organisms, will sediment at a rate determined by its size, density and shape characteristics, the gravitational forces and the turbulent kinetics of the water column (Reynolds 1984). The terminal velocity of a particle in a fluid is described by Stokes Law.

$$w_s = gd^2(\rho_c - \rho_w)(18\eta\phi)^{-1} \quad \text{m s}^{-1} \tag{1.1}$$

Where, w_s is the terminal velocity (m s^{-1}) g is the acceleration due to gravity (9.8081 m s^{-2}), d is the diameter of the particle (m), ρ_c is the density of the particle ρ_w is the density of water, η is the absolute viscosity and ϕ is the coefficient of form resistance. Stokes Law applies to particles of less than 500 μm diameter, and when the suspending fluid flows past the particle in laminar flow (Reynolds 1997b). The downward movement of the particle is hindered by the turbulent velocity of mixing. When the turbulence is sufficient to overcome the settling velocity the particles are considered to be entrained. There is still a

net loss to sedimentation, but it will be very small (Condie and Bormans 1997). Turbulence in the water column is determined by the forces and constraints applied to it, such as wind action on the surface, basin morphometry, deflected internal waves generated by Coriolis' forces and, if present, tidal or other bulk water movements (Reynolds 1997b). Turbulent velocity and the degree of entrainment may therefore vary with depth. Density stratification, due to solar heating and thermal stratification hinders the dissipation of wind generated kinetic turbulent energy through the water column. The buoyancy of the less dense water reduces its probability of being contained in a downward moving turbulent eddy. This results in the formation of and differential entrainment of particles in the epilimnion and the hypolimnion (Figure 1.4).

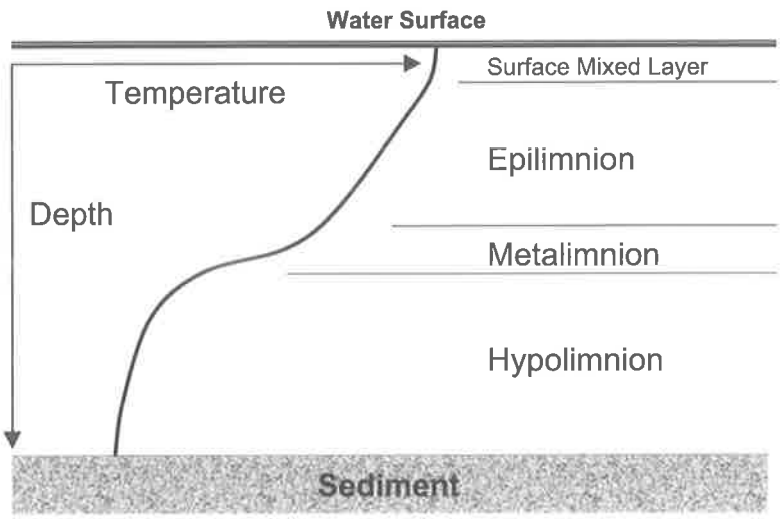


Figure 1.4. Terminology of the water column.

The kinetics of sedimentation of particulate and colloidal carbon may also be affected by the activity of higher trophic levels (Hessen *et al.* 1990, Lawrence *et al.* 1993). The ingestion of particulate carbon by zooplankton provides a significant pathway of detrital carbon cycling and size change (Hessen *et al.* 1990), which will change the distribution of both particulate organic carbon (POC) and heterotrophic bacteria. This has the potential to change the uptake kinetics of dissolved organic carbon by bacteria by changing their small scale distribution in the water column and their subsequent sedimentation in association with faecal pellets (Wassmann 1993, Richardot *et al.* 2001).

1.1.3.4. Photochemical Degradation

The presence of light absorbing chemical bonds in organic compounds, so called chromophores, render them susceptible to photochemical degradation (Zepp and Schlotzhauer 1981). The light absorption capacity is often associated with the presence of delocalised π-electron systems, such as in aromatic rings and conjugated double bonds. Photochemical alteration of organic compounds may be by direct photo-transformation or indirect oxidation by agents generated during the photo-disassociation of other compounds (Schwarzenbach *et al.* 1993). Photo-transformation can cause the fragmentation, rearrangement, isomerisation or dimerisation of molecules, resulting in humification, release of labile compounds, often low molecular weight organic acids, or mineralisation to CO_2, CH_4 and CO (Stevenson 1994, Moran and Zepp 1997, Bano *et al.* 1998). Organic matter must absorb sufficient energy for direct photo-oxidation to occur. It follows that higher energy radiation (shorter wavelength) will be more significant in driving this process. Many authors report that UVB (280 - 340 nm) is responsible for the majority of photo-degradation of DOC (Zepp *et al.* 1981, de Haan 1993, Moran and Zepp 1997). However some authors suggest that UVA (300 - 400 nm) and Photosynthetically Active Radiation (PAR, 400 - 700 nm) also play significant roles (Graneli *et al.* 1998, Vahatalo *et al.* 2000, Wetzel 2001b). The contrasting results indicate that the spectral composition, the character of the organic matter and the inorganic matrix all interact to determine the nature and magnitude of photo-degradation of NOM. For example alkalinity (as $CaCO_3$) can act as a free radical scavenger and reduce the potential for indirect bond oxidation (Hoigne and Bader 1976).

The exposure to radiation within a water body is a function of the hydrodynamics, the intensity and wavelength of incident irradiance and the optical properties of the water. The most relevant optical property of the water column is the spectral extinction at the

photochemically active wavelengths (Morris and Hargreaves 1997). The intensity of light at any depth of the water column is described by the Beer-Lambert Law:

$$I_z = I_0' e^{-z\varepsilon_\lambda} \quad \mu mol\ m^{-2} s^{-1} \tag{1.2}$$

Where, I_z is the light intensity at depth z (μmol m^{-2} s^{-1}), I_o' is the sub-surface light intensity at the wavelength or in the spectral range λ (μmol m^{-2} s^{-1}) ε_λ is the extinction coefficient at the wavelength or in the spectral range λ (m^{-1}), and z is the distance from surface (m).

The extinction coefficient ε_λ, is the result of absorption by multiple components of the water column. It is comprised of the extinction due to water (ε_W), dissolved and suspended material, including autochthonous and allochthonous organics (ε_O), suspended particles (ε_P) and suspended cells (ε_A), which may contain chlorophyll. ε_λ can therefore be described as the sum of these coefficients thus:

$$\varepsilon_\lambda = \varepsilon_W + \varepsilon_O + \varepsilon_P + \varepsilon_A \quad m^{-1} \tag{1.3}$$

The amount of light absorbed by dissolved organic compounds can then be determined by solving for ε_O, and calculating the depth integral:

$$A_O = \int_0 I_0' e^{-z\varepsilon_O} \tag{1.4}$$

In conjunction with measurements of the quantum yield of photochemical products (the number of chemical reactions per photon absorbed) and the photon flux density, it is possible to estimate the amount of photochemical degradation. Some authors have measured the quantum yields of specific products of photo-oxidation of organic matter (Gao and Zepp 1998, Vahatalo *et al.* 2000, Johannessen and Miller 2001). However, due to the variable nature of the chromophoric groups in dissolved organic matter, it is appropriate to use an apparent quantum yield to estimate photo-production. For example, Miller *et al.* (2002) modelled the magnitude of photo-oxidation by application of apparent

quantum yields of formation of biologically labile organic carbon and mineralisation products and concluded it was a significant process in the ocean.

1.1.4. The Carbon Cycle

In its simplest form the carbon cycle can be considered as the pools of inorganic and organic carbon, joined by the fluxes of carbon fixation and respiration. The source and loss processes described above can be incorporated into the carbon cycle, and used to describe and quantify the sizes of various carbon pools, and the rates of flux between them (Wetzel 2001b). Clearly there is complex interaction with higher trophic levels, between benthic and pelagic environments, and between microbial activity and irradiation. Many authors have constructed budgets of organic carbon processing in order to describe these processes (Cole *et al.* 1989, Fitzgerald and Gardner 1993, McConnaughey *et al.* 1994, Kankaala *et al.* 1996, Lyche *et al.* 1996, Wachniew and Rozanski 1997) and consequently there is substantial information on different aquatic systems. The use of stable and radioactive carbon isotopes, have provided valuable information on the relative lability of terrestrial and aquatic derived organic carbon. Figure 1.5 shows the carbon budget calculated using ^{14}C labelled dissolved inorganic carbon (DIC) by Lyche *et al.* (1996). They demonstrated that 70% of bacterial production was fuelled by algal exudates and lysis products. Raymond and Bauer (2001a) found that bacteria preferentially used younger DOC (^{14}C enriched) leaving older less labile terrestrial organic matter to be discharged into the Atlantic Ocean. A review by Raymond and Bauer (2001b) further supports the concept that bacteria preferentially utilize younger DOC.

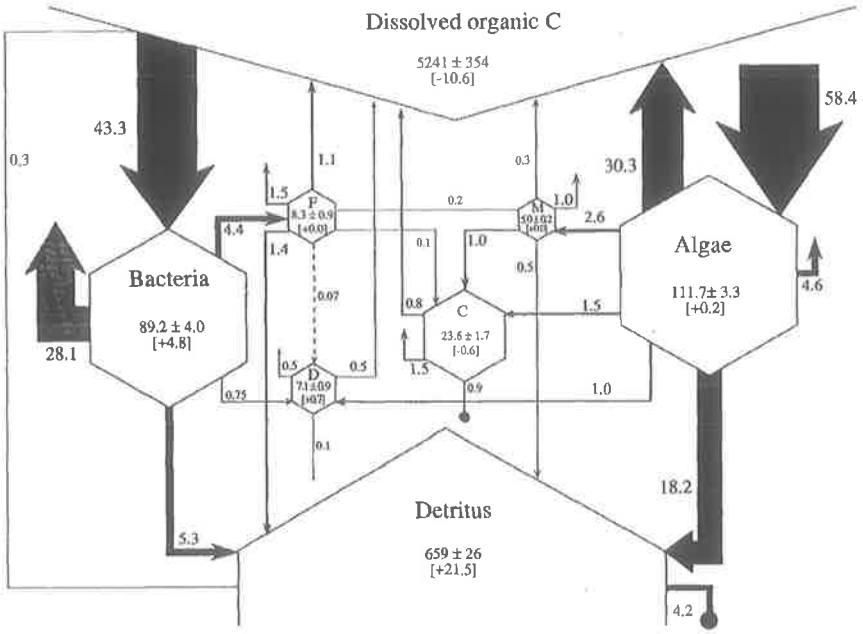


Figure 1.5. Carbon budget from inverse solution based on measured, calculated, and estimated pools and flows. Abbreviations: F-bacterivores (heterotrophic flagellates and nanociliates); M-microzooplankton (rotifers and netciliates); D-*Daphnia* spp.; C-*Cyclops scutijk.* Numbers in boxes are mean and SE of pool sizes in μg C L^{-1}; numbers in brackets are net change of pools from the inverse solution (μg C L^{-1} d^{-1}, where positive and negative numbers mean increase or decrease in pool size); numbers on arrows are carbon flows in μg C L^{-1} d^{-1}. Shaded arrows indicate respiration. Sedimentation- •. Sizes of boxes and arrows are proportional to sizes of compartments and flows. From Lyche *et al.* (1996).

1.1.4.1. Carbon Dynamics in Stratified Systems

While thermal stratification of a water column effectively creates two vertically separated bodies of water, strong vertical stratification of organic carbon is observed less often than other factors (Wetzel 2001b) but has been documented in many systems (AWRC 1979, Bowles *et al.* 1979, Croome 1981, Owen *et al.* 1993, McKnight *et al.* 1997, Kim *et al.* 2000, Choi *et al.* 2002, Fulton *et al.* 2004, Vrede 2005). The general lack of stratification of dissolved organic carbon is attributed to the large proportion of the pool which is resistant to degradation (Wetzel 2001b). However when lakes and reservoirs experience persistent stratification, and oxygen depletion in the hypolimnion impacts on the carbon cycle processes can occur (Osburn *et al.* 2001). Once developed, the epilimnion and hypolimnion have different potential sources of organic carbon. The epilimnion receives

fresh inputs of phototrophic production including the low molecular weight (LMW) acids secreted during photosynthesis and the labile proteins and cell constituents released during cell lysis. Transport of organic carbon from the epilimnion into the hypolimnion is reduced by turbulent mixing. Consequently the most labile fractions of the phototrophic autochthonous organic carbon are assimilated in the epilimnion and are not transported into the hypolimnion (Wetzel 2001a). Autotrophic production in the epilimnion will predominantly be photosynthetic while in the hypolimnion it will be predominantly chemosynthetic. Chemosynthetic bacteria utilise the by-products of anaerobic catabolism to generate energy for cellular activity and are therefore predominantly limited to the hypolimnion and sediment surface (Wetzel 2001a). The distribution of allochthonous inputs is also determined by hydrodynamics. Influent water will predominantly mix with the body of water of most similar water temperature. In temperate systems this is likely to be the hypolimnion or metalimnion. The hypolimnion may also receive inputs from resuspension through bioturbation or the benthic boundary turbulence generated by underflows.

The fate of organic carbon also differs between these compartments. If oxygen is depleted in the hypolimnion then its suitability as habitat for various plankton changes, which in turn alters the way that detrital carbon is processed. The hypolimnetic carbon pool is also protected from the influence of photo-oxidation. The relatively quiescent hypolimnion may also endure greater particulate losses of both organic matter and organisms. This heterogeneity may result in significant differences in the character of organic matter found in these compartments and consequently in the raw water quality of stratified reservoirs that may be used as for potable water production (Bowles *et al.* 1979, Croome 1981).

1.2. The Water Industry

1.2.1. Potable Water Treatment

The removal of NOM from drinking water is an important issue for the drinking water industry. The reasons for this are described below.

1.2.1.1. Coagulant Demand

Natural organic matter can exert a high coagulant demand in the water treatment process and consequently the cost of water treatment may be strongly influenced by the concentration of NOM in the source water. Traditionally, the conventional treatment process results in approximately 50% removal of NOM, while deviation from this value is found depending on water source and NOM character (Crozes *et al.* 1995). Organics are removed by the processes of charge neutralisation of colloids, precipitation as humates or fulvates, and coprecipitation by adsorption to the metal hydroxide (Crozes *et al.* 1995). The cost of coagulant may be significant to the water treatment process, and the NOM that is not removed by the coagulation and filtration stages of treatment passes through to the disinfection stage and the distribution systems and has additional impacts on water quality in these stages.

1.2.1.2. Chlorine Demand, Disinfection By-Products and Bacterial Regrowth

Disinfection of potable water can be achieved using chemical disinfectants such as chlorine and chloramine. Filtered water and the disinfectant are mixed in contact tanks where they react with the constituents of the product water. The reaction of chlorine with various organic carbon species may result in the evolution of disinfection by-products, including trihalomethanes, chloroacetic acids, haloacetonitriles, chloroketones, chloral hydrate and chloropicrin (Kotsakis and Nicholson 2001). Many of these classes of compounds have been determined to have potential toxic and carcinogenetic effects and these compounds are now monitored and regulated to a maximum contaminant level in potable water in the US by the USEPA (Edzwald and Tobiason 1999, Sadiq and Rodriguez 2004, USEPA

2006). In Australia, guideline levels for disinfection by-products are set by the Australian Drinking Water Guidelines (THMs less than 0.25 mg L^{-1}, NH&MRC 2004).

When disinfection of a potable water supply is inadequate, disinfectant residuals may decline to levels that permit unacceptable levels of microbial growth in the network. If this occurs then the residual DOC in the distribution system can act as a microbial substrate. Microbial growth and biofilm development in distribution systems can cause health and aesthetic water quality concerns (Davey and O'Toole 2000, Boe-Hansen *et al.* 2003, Bagh *et al.* 2004) and deterioration of the network through the promotion of corrosion (Lehtola *et al.* 2004).

1.2.2. Management Strategies

The Australian Drinking Water Guidelines gives guidelines for the quality of drinking water in Australia and a framework for management of drinking water quality that promotes the adoption of total quality management (TQM, NH&MRC 1996, 2004). TQM encourages the application of best practices and continuous improvement, real-time process control, the use of multiple barriers to protect public health, the application of preventative as well as reactive management of water quality and the provision of effective leadership within water quality management teams (O'Connor 2002).

In reactive water quality management, the water industry is increasingly using a hazard and critical control point (HACCP) approach to the management of the quality of water supplied to its customers (Havelaar 1994). This is applied in a systems approach, considering the potential points of intervention (critical control points) in the whole water supply system, from the catchment to the customer tap. Strictly, a critical control point is a node in the system where a management decision can result in the real time intervention in the system. True critical control points in water supply systems include:

- the selection of a raw water offtake depth from a reservoir

- the decision to use an algaecide to control an algal bloom
- the decision to artificially destratify a raw water reservoir
- the decision to use an alternative raw water supply for a water treatment plant
- the decision to stop taking raw water from a reservoir for a period of time
- the amount of water treatment chemicals applied during water treatment (coagulants, pH control and disinfectants)

Hence, there is the potential to reactively manage the water supply process at a number of levels. Reactive and preventative management of organic matter can occur in the catchment, the reservoir or the water treatment plant.

1.2.2.1. Catchment Management

As the concentration and character of the organic matter (OM) entering a waterway and ultimately a drinking water reservoir is determined by the conditions present in the catchment, there is potential to manage the production and transport of OM in the catchment. The control of land-use activities in the catchment has the potential to affect many water quality parameters. Catchment management legislation often includes prescription of approved farming activities and development within drinking water catchments. As previously described, different vegetation cover and soil types have different organic carbon export rates. Considering that temperate forests export approximately 3 times as much organic carbon as temperate grasslands (similar to 30 and 9 kg C ha^{-1} yr^{-1}, respectively; Hope *et al.* 1994), grazed catchment may be considered preferable to forested, from the perspective of organic carbon. However, the decision to influence land use practices should consider other aspects of water quality and landscape conservation practice. For instance, the management of catchments for water quality needs to consider sources and transport of pathogens, soil erosion, sediment and nutrient transport.

As soil type and structure has significant influence upon the quantity and character of organic matter leached into waterways (Nelson *et al.* 1993), the application of soil amendments has the ability to reduce export of carbon from soils (Varcoe 2002). It is likely that catchment remedial works, such as riparian planting and creek fencing has the potential to reduce the amount of carbon and nutrients entering streams (Brugger *et al.* 2001, McKergow *et al.* 2003) and therefore drinking water reservoirs. While improvement of riparian and stream bed vegetation and stability is usually associated with lower sediment loads, it may also reduce particulate organic loads to streams (Hope *et al.* 1994). Increasing the retention time of porewater by increasing the physical complexity of the riparian zone may allow the increased assimilation of organic matter (Brugger *et al.* 2001). However, riparian shading has been shown to reduce the bioavailability of NOM passing through the riparian zone (Findlay *et al.* 2001). Further improvement of the complexity and functional diversity of urban and suburban waterways also has the potential to improve water quality in receiving waters by increasing resource interception and processing (Brookes *et al.* 2005a). However, a proportion of the organic carbon found in lakes and reservoirs is produced within the water body (autochthonous) and there is the potential for metabolism and modification of allochthonous organic matter in reservoirs and hence management at this stage is also possible.

1.2.2.2. Reservoir Management

Artificial Destratification

One of the primary tools of reservoir management is the application of artificial destratification which is predominantly applied to control the release of reduced metals (iron and manganese). These metals impart taste and odour and form undesirable particles and scale when re-oxidised in the distribution system. Artificial destratification also has the potential to reduce the growth of problem cyanobacteria or total algal biomass in some conditions (Steinberg and Zimmermann 1988, van der Veer *et al.* 1995, Visser *et al.* 1996,

Simmons 1998, Lindenschmidt 1999). The potential to reduce algal growth is determined by the destratification systems ability to deepen the mixed surface layer of the reservoir and limit the availability of nutrients by reducing the internal load of phosphorus and nitrogen, which may be released under reducing conditions (as phosphate and ammonia).

The main group of phytoplankton that cause water quality issues are the cyanobacteria or blue-green algae. Their increased dominance during summer stratification can be attributed to their ability to regulate their buoyancy, using gas vesicle and carbohydrate ballast content (Walsby 1994) to avoid the sedimentary losses experienced by other phytoplankton (e.g. diatoms and green algae) and successfully regulate their exposure to light (Bormans *et al.* 1999, Brookes *et al.* 1999). The hypothesis that the daily regulation of buoyancy allowed access to both the light and nutrients which become separated in vertically stratified systems (Fogg and Walsby 1971, Ganf and Oliver 1982), however, does not hold in all systems and the physical mechanisms of nutrient supply to the epilimnion can generally explain the observed growth rates (Bormans *et al.* 1999). Therefore, it might be expected that the predominant effect on cyanobacterial populations to be related to changes in the light regime. However, the interaction between light and the nutrient status of the cells also affects their ability to employ gas vesicle regulation (Brookes *et al.* 1999, Brookes and Ganf 2001). Consequently, destratification may indirectly affect cyanobacterial growth by reducing the release of phosphorus from the sediments (Brookes *et al.* 2000a).

The reduction of the internal load is not always achieved by destratification, especially if oxygenation of the sediment water interface is not maintained, or in lakes with very high rates of organic matter export to the hypolimnion (Gachter and Wehrli 1998). The importance of destratification for controlling phytoplankton growth by reducing the internal load is lessened when a significant external load occurs. For example, during a study of artificial mixing to control algal growth in Chaffey Reservoir (Australia),

Sherman *et al.* (2000) concluded that while the internal load and the maximum chlorophyll *a* concentration was reduced, the lack of deepening of the surface mixed layer and the substantial external P load resulted in persistent algal problems.

A conclusion of reviews conducted (e.g. AWRC 1979, Bowles *et al.* 1979, Lewis *et al.* 1991) is that for success in improving water quality, the design of the destratification system must be rigorous. Consequently many design methods for bubble plume aerators have been published (Smith *et al.* 1982, Lemckert *et al.* 1993, Schladow 1993). The design process of many systems subsequently employs a modelling step to ensure a system of appropriate capacity and configuration is installed, however this is often not stated explicitly in the literature (Gomes *et al.* 1996, Antenucci *et al.* 2005b).

Artificial destratification is applied through two main systems, bubble plume aerators and mechanical impellers. Bubble plume aerators use compressed air discharged near the bottom of the reservoir, which then rise to the surface, entraining water and forcing circulation. Mechanical impellers are essentially underwater fans and pump water either from the surface to the bottom of the reservoir or from the bottom to the surface. They may or may not employ a draft tube, which serves to reduce turbulent mixing and slowing of the jet; however they represent a significant structure in the reservoir and can present ongoing maintenance issues. Recently there has been more interest in surface mounted impellers fitted with draft tubes as, unlike bubble plume aerators (Sherman *et al.* 2000), they have been proposed to directly deepen the surface mixed layer. Pumping epilimnetic water containing nuisance phytoplankton out of the euphotic zone will induce light limitation and reduce their growth. This theory has been demonstrated to be valid by modelling approaches (Lewis *et al.* 2002).

Variable Depth Offtake

The use of variable depth offtakes to selectively withdraw water from a particular layer is often used to manage the temperature of water discharged from dams (Stanford *et al.* 1996,

Sutton and Findlay 2003), which in turn has ecological benefit, such as improved fish passage (Khangaonkar *et al.* 2005) and downstream temperature effects (Stanford *et al.* 1996). Selective withdrawal has also been proposed to alter nutrient and phytoplankton dynamics (Straskraba 1996, Barbiero *et al.* 1997). The application of selective withdrawal in vertically stratified drinking water reservoirs may allow system managers to increase water quality or reduce risks by avoiding pathogen rich water or undesirable algal populations by selecting to provide water treatment plants with the water of the best available quality.

Two prerequisites for this mode of management are the infrastructure to selectively withdraw from different depths and information about which vertical layer contains the best water quality. Generally, the latter will depend upon the degree of online monitoring that is performed, the frequency and quality the of water quality monitoring program and the level of understanding of the water quality determining processes that occur in the reservoir in question.

Other Management Practices

Other interventional management options include algaecide dosing and the application of chemicals to clarify the water column. Algaecides are often copper based, either as copper sulphate or a chelated copper compounds. A wide variety of chemicals have been applied as water clarifiers and nutrient binding agents, including alum, PhosLock^{TM} and mixtures of bacterial cultures. Most of these treatments are rarely used in drinking water reservoirs due to the high costs of application to such large volumes or legislative restrictions on the use of these agents in natural environments.

Regardless of the reservoir management practices currently available, the need to treat source water to a potable quality remains. Consequently there is the option to further manage these issues during the treatment process.

1.2.2.3. Treatment Plant Management

Management strategies available in the treatment phase of the water supply system include modifying or altering the treatment system, by altering dose rate, chemical types, or the treatment technology entirely. A great deal of research effort has been committed to the exploration of new treatment technologies for the production of potable water (e.g. MIEX^{TM}, ultra-filtration, nano-filtration etc). However the management of existing conventional treatment plants can result in the improved removal of NOM through alterations in the physical and chemical aspects of the process.

Enhanced Coagulation

In order to attain organic carbon removal rates and disinfection by-product concentrations that comply with the increasingly stringent guidelines (e.g. USEPA 2006 Disinfectant/Disinfection By Products Stage 2 Rule of 80 μg L^{-1} total THMs and 60 μg L^{-1} total haloacetic acids and ADWG 2004 250 μg L^{-1} THM concentration) the application of enhanced coagulation, where coagulant dose rates that exceed that required for colour and turbidity removal alone, are applied under pH controlled conditions, is suggested (LeChevallier *et al.* 2002). Lowering the pH enhances multiple aspects of the coagulation process. The pH during coagulation changes the properties of both the coagulant species and the target organic matter. It causes greater protonation of acidic groups on the organic matter, reducing the total charge of the molecule and the charge repulsion with the metal hydroxide surface. The coagulant is more positively charged at lower pH, which makes adsorption more favourable. At the dose rates used in enhanced coagulation, adsorption has been shown to be the major mechanism of removal (Davis 1982, Jekel 1986, Edwards 1997). Low pH conditions are also likely to increase the rates of colloidal charge neutralisation and coprecipitation (Crozes *et al.* 1995).

Ultimately the decision to alter operational water treatment parameters, such as coagulant dose or coagulation pH, is a trade-off between the increased chemical costs, sludge production and the benefits in water quality.

1.3. Characterisation of Organic Matter

Many of the issues discussed are dependant upon the character, as well as the concentration of natural organic matter. Consequently, much research has been directed towards the methods of characterisation of NOM and many methods have been developed. The methods described are based on physical or chemical properties of NOM, or its interaction with biological entities. A significant review of the methods available for characterisation of aquatic NOM is Leenheer and Croué (2003).

1.3.1. Physical

1.3.1.1. Size Fractionation

One of the simplest ways to characterise organic matter is to separate the NOM components into fractions on the basis of size. The most basic is the functional definition of dissolved organic carbon as that which passes through a 0.45 μm membrane. Size fractionation can be applied at greater and lesser operational magnitudes, from the use of a series of sieves or meshes, or the application of methods such as field flow filtration or ultra-filtration.

Ultra-filtration involves the application of apparent molecular weight cut off (MWCO) membranes in a pressurised chamber. MWCO membranes are manufactured to retain 90% of spherical uncharged molecules greater than the rated cut off mass, and are available from 1 K to 1000 K Dalton MWCO (Pall Gelman Catalogue, 2002). This method is particularly useful for the de-salting and concentration of larger molecular weight organic compounds for the application of other chemical methods that suffer from inorganic interference.

Gel Chromatography and High Performance Size Exclusion Chromatography

Another method to separate molecules on the basis of size is based on their retention time within porous gel media. The gel consists of particles with pores that allow the smaller molecules to diffuse into the intra-particular space and increasing their retention time in the gel. Particles that exceed the size of the pore cannot diffuse into the particle matrix and are passed through the gel without being detained in the column. Ideally, smaller molecules are on average retained in the particle matrix for longer and thus there is a separation on the basis of size. Of course any difference in the functional group interaction between the solid and mobile phase will introduce artefacts. Similarly, the alteration of the chemical environment (pH and ionic strength) of the mobile phase will determine the charge of molecules with ionisable groups and the amount of double later compression, which inturn can influence the solute-gel interactions, size, aggregation and tertiary structure of the analyte molecules.

When applied to NOM the non-size exclusion effects have been shown to be due to electrostatic, salt interaction and specific sorption (Perminova 1999). It is also important to choose standards that have similar electrostatic behaviour to humic substances when determining molecular weight from retention time. For example polystyrene sulphonates are preferable for determining molecular weights than globular proteins, and ideally, charged low molecular weight moieties such as salicylic acid are employed (Zhou *et al.* 2000). The choice of detection wavelength can also affect the calculated molecular weight, as smaller molecules display stronger absorption at shorter wavelengths (O'Loughlin and Chin 2001). Detectors that avoid this issue by directly measuring organic carbon are becoming more widely used (Her *et al.* 2002a, Her *et al.* 2002b, Abbt-Braun *et al.* 2004, Grunheid *et al.* 2005) and in combination with UV detection, can provide more information on the character of the size fractionated organic matter (Allpike *et al.* 2005). The molecular size of organic matter has been shown to effect its utilization by bacteria

(Amon and Benner 1996), its reactivity with chlorine to produce disinfection by products (Gang *et al.* 2003, Weber *et al.* 2005) and determines its photo-reactivity (Thomson *et al.* 2004).

1.3.2. Chemical

1.3.2.1. Functionality

The chemical functional analysis of organic mixtures can be performed by the application of resin fractionation and is particularly relevant to water treatment as this functionality is significant to the coagulation process. The development and application of resin fractionation methods have been described by Leenheer (1981), Lara and Thomas (1994), Lara *et al.* (1997), Croue *et al.* (1999) and van Leeuwen *et al.* (2002). An example of a resin fractionation procedure is as follows. Strongly hydrophobic weak acids and neutrals are first adsorbed to DAX-8 resin at pH 2.0 where their acidic groups are predominantly protonated and charge repulsion is low. Subsequently, moderately hydrophobic organics are adsorbed to DAX-4 resin, again at pH 2.0. The material passing through these resins is then adjusted to pH 8.0 and passed through a column containing an anion exchange resin (e.g. IRA-958) which retains the charged hydrophilic acids, with hydrophilic neutral organics passing through. The DAX-8 and 4 resins are then eluted with NaOH at pH 13.0 and the eluents are passed separately through a strongly acidic cation exchange resin (e.g. Amberlite IR-120). This represents a preparative method that may be used to subsequently characterise the isolated organic matter using other techniques.

If the researcher is interested in the relative sizes of these pools and not in isolating the organic matter, then a simpler scheme can be applied. The 'rapid fractionation' method of Chow *et al.* (2004) estimates the size of each of the fractions isolated by the isolative method, by a series of DOC analyses of the material passing through each of the resins and a subtractive calculation scheme.

1.3.2.2. Jar Test

While the jar test is a routine water treatment research method to evaluate coagulants, dose rates and coagulant aids, it is described here as a method to separate recalcitrant from treatable organic matter. The jar test is a simulation of the coagulation process of water treatment. If standard conditions are applied then it can be used to compare the treat-ability of waters and to separate the removable (at specific pH levels) from the recalcitrant fractions of the NOM. The jar test apparatus consists of multiple stirrers (usually 5 or 6) for separate two litre square acrylic jars (Gator jar). Aliquots of water sample are placed into the jars, coagulant is added and the jar content is 'flash mixed' at high speed (~200 rpm) for a brief period (~1 minute), and then slow mixed for a longer period (~14 minutes). After allowing the flocs to settle for about 15 minutes, the treated quality is determined. Parameters usually include settled and filtered turbidity, UV absorbance at 254nm, DOC, true colour and residual metals.

1.3.3. Microbiological

1.3.3.1. AOC, BDOC and BRP

The Assimilable Organic Carbon (AOC), Biodegradable Dissolved Organic Carbon (BDOC) and Bacterial Regrowth Potential (BRP) assays have all been applied to describe DOC as microbial substrate (van der Kooij *et al.* 1982b, Servais *et al.* 1987, Hambsch *et al.* 1996). They measure various indices of bacterial growth or substrate depletion over a period of time giving an indication of the amount of DOC that is biologically labile. They differ in the type of inoculum and the method of measurement of bacterial substrate use. While superficially they seem to provide similar information, they are conceptually different, and are often complementary (Escobar and Randall 2001). AOC and BRP measure the amount of carbon that is readily converted into biomass, while BDOC measures the amount of carbon that may be mineralised by microbial activity. Escobar and

Randall (2001) argue that measuring either AOC or BDOC alone leaves the researcher open to either over or under estimate the potential for bacterial regrowth.

The AOC assay measures the growth of a model organism over a standard incubation period, determined by heterotrophic plate counts (HPC), intracellular ATP or turbidity increase. *Pseudomonas florescens* Migula (P17) has been demonstrated to be able to grow on a wide range of substrates (van der Kooij *et al.* 1982a), and is used as the industry standard for this assay (Escobar and Randall 2001, Liu *et al.* 2002). The BRP assay is similar but uses an inoculum of the natural microbial assemblage and turbidity to estimate the growth of the microbial population (Withers and Drikas 1998). The BDOC is determined as the difference between the DOC measured initially and the minimum amount found during a period of aerobic incubation. Consequently it relies on the accuracy of DOC measurement and is difficult to apply to low carbon waters and to NOM of low lability. These methods do not specifically address the identity of the biodegradable substrates. This may be achieved using other methods such as enzyme activity or the response of the microbial community to particular substrates.

1.3.3.2. Enzyme Activity

The measurement of a particular enzyme activity is a strong indication that the target substrate of that enzyme is being used for microbial growth; therefore compounds with the chemical structure of the substrate are presumed to be in the environment (Foreman *et al.* 1998). Consequently enzyme activity indicates the structure of carbon available and the patterns of utilisation by microbial communities and may provide one of the best indications of the microbial degradation of NOM that is occurring. Measurement of enzyme activities may be performed in a number of ways. Measurement of the evolution of products or depletion of substrates is possible, but is time consuming and complicated in natural waters containing a mixture of substrates and enzymes. Enzymes may be extracted from the environmental samples before analysis (Ogunseitan 1998), however measurement

of activity in as close to native state as possible provides the most ecologically relevant information on rates of decomposition (Hoppe *et al.* 1988). The methods that are prevalent in modern microbial ecology are the use of synthetic chromophore or fluorophore linked substrates. The use of fluorophoric assays (e.g. methyl-umbeliferyl substrates) has generally superseded the chromophoric assays (e.g. p-nitrophenyl substrates), due to lower limits of detection and shorter incubation times. This allows higher numbers of more accurate assays to be performed. However colorimetric assays are still used today (Yu *et al.* 1997, Igarashi *et al.* 1998, Biely *et al.* 2000, Dunne and Eisses 2001, Sanchez *et al.* 2001).

Glucosidase

β-glucosidase (EC 3.2.1.21) is considered to be the rate limiting enzyme in microbial utilisation of cellulose as a substrate and has received much attention from aquatic ecologists (Chróst 1989, Chróst and Velimirov 1991, Chappell and Goulder 1994b, a, 1995, Freeman *et al.* 1995, Middelboe *et al.* 1995, Becquevort *et al.* 1998, Foreman *et al.* 1998, Shackle *et al.* 2000, Berman *et al.* 2001, Burns and Ryder 2001, Arrieta and Herndl 2002, Siuda and Chróst 2002). As cellulose is the predominant structural polymer of higher plants, its presence in aquatic systems indicates a terrestrial vegetation or aquatic macrophyte input of detrital carbon. Glucosidase activity is not however a specific indicator of the presence of cellulose, as its activity has also been found to be correlated with the degradation of particulate material of algal origin (Caruso *et al.* 2005).

Aminopeptidase

Dissolved proteins are an important substrate for microbial growth in aquatic environments and are rapidly assimilated into bacterioplankton biomass (Halemejko and Chróst 1986, Hoppe *et al.* 1988, Coffin 1989). Their importance as a substrate may be greater for free pelagic (< 0.2 to 3 μm fraction) than particle attached bacterioplankton (Hoppe *et al.* 1998). Proteins are degraded by exo- and endo- peptidases which cleave at the end of the

molecule and within the molecule, respectively (Halemejko and Chróst 1986, Debroas 1998). The nomenclature of aminopeptidases specifies the residue at which cleavage takes place, for example, leucine aminopeptidase (EC 3.4.11.22), is an exopeptidase that cleaves a leucine residue from the end of the polypeptide chain.

N-acetyl-β-D-glucosaminidase

Chitin (poly β-1,4-N-acetyl-D-glucosamine) is a significant constituent of many organisms including arthropods, molluscs, fungi, diatoms and ascarides (Smucker and Kim 1991) and is found in aquatic systems that contain these organisms (Montgomery *et al.* 1990). It is synthesised as a linear homopolymer of β-1-4 linked glucosamine residues and is subsequently modified depending on the organism to yield a variety of sub-forms, including covalent links to peptides and proteins. Its degradation is a two step process, first the activity of chitinase (poly β-1,4-(2-acetamido-2-deoxy)-D-glucoside; EC 3.2.1.14) hydrolyses chitin to tetramers or trimers of β-1,4-N-acetyl-D-glucosamine (GlcNAc). N-acetyl-β-D-glucosaminidase (Chitobiosidase; chitobiose acetamido-deoxyglucohydrolase EC 3.2.1.29) then hydrolyses dimers and trimers of GlcNAc to yield GlcNAc, which is consumed by a wide range of microbes (Riemann and Azam 2002).

Peroxidase

Peroxidases are involved in many biological processes including the polymerisation of organic compounds by oxidative cross linking using hydrogen peroxide or organic peroxides. They have been identified as a potential vector of humification in soils as they are produced in the guttation dew of many grasses (Kerstetter *et al.* 1998). Peroxidases represent the major pathway of consumption of hydrogen peroxide in aquatic systems (Cooper and Zepp 1990), and may therefore reduce the effect of photo-oxidation. Peroxidase has been identified as a major vector of degradation of humic substances (Gramss *et al.* 1999) and lignin (Yokota *et al.* 1990, Paice *et al.* 1995). These lignin and

humin degrading enzymes are produced by basidiomycetes such as *Phanerochaete chrysosporium* along with a suite of other enzymes involved in the process (Broda *et al.* 1996).

Other Enzymes

Other enzyme assays have been applied which reflect the presence and degradation of their substrates. These enzymes include α-1-4 glucosidase, fatty acid esterase, alkaline phosphatase (Foreman *et al.* 1998), cellobiohydrolase, β-xylosidase, phenol oxidase (Sinsabaugh and Findlay 1995) and sulphatase (Freeman *et al.* 1995). While some of these enzymes are not directly involved in the acquisition of carbon substrates, they are relevant to other aspects of microbial growth such as the acquisition of phosphorus (e.g. alkaline phosphatase). They can therefore be used to indicate if the acquisition of other nutrients is limiting the growth of the bacterioplankton.

1.3.3.3. Community Level Physiological Profiling

The community level physiological profiling (CLPP) method is a culture dependant technique that tests the ability of a microbial community to respire and reduce a redox dye in the presence of a range of substrates added to separate wells of a microtitre plate (Garland and Mills 1991, Mills and Garland 2002). The method is similar to the BIOLOG™ phenotype-based bacterial identification system, except that the inoculum consists of the entire microbial community rather than an isolate (Marello and Bochner 1989). The method has been applied extensively to rhizobial microbial communities (Garland 1996, Campbell *et al.* 1997, Larson *et al.* 2002). Some authors have suggested that the method does not necessarily reflect the functional potential of the community, as they found changes in community composition during incubation in BIOLOG plates (Smalla *et al.* 1998). However other authors see fit to draw information on the catabolic competency of microbial communities, on the basis of this technology, in conjunction with other methods (Grover and Chrzanowski 2000).

1.4. Synthesis: The problem with NOM

While much information is available about methods to characterise and classify NOM and the processes that act upon it, significant deficiencies in our understanding of how these processes determine the character of NOM towards the metal coagulants commonly used in conventional water treatment processes. Specific information on the fluctuations in character and concentration of organic matter in response to reservoir management practices does not feature in the published literature. It is more often than not assumed to be a static, unchanging entity, if not explicitly, then implicitly, by simple sampling programs that fail to consider spatial or temporal changes in NOM character and concentration or the impact of hydrology and hydrodynamics. The spatial and temporal change in character and concentration of organic carbon in drinking water reservoirs is the focus of this thesis, particularly as it is affected by hydrodynamic processes, especially riverine influx and thermal stratification. In order to understand the complex array of biological and physical processes that determine the character of natural organic matter in water supplied to the WTP, an understanding of the processes that occur in the system prior to withdrawal must be developed.

Of particular interest from the perspective of lake metabolism is the long term loading of autochthonous and allochthonous organic carbon in absolute and relative terms, which will determine the total organic matter loading to the system. The seasonal differences in allochthonous and autochthonous loads will contribute to variation in organic matter character and concentration. The inter-annual variability in climate has the potential to alter the loading of allochthonous and autochthonous carbon. Short term fluctuations in the concentration and character of OM may occur as a result of inflow events. The onset and persistence of thermal stratification may alter predominantly the autochthonous flux of organic matter and the fate of the NOM pool within the water column, potentially developing a vertical gradient in water quality for potable water production.

1.5. Aim, Objectives and Hypotheses

The aim of the research reported in this thesis was to describe the potential impact of stratification, and therefore destratification, and inflow hydrodynamics on the variation of source water quality of reservoirs of south-eastern Australia, from the perspective of NOM and its treat-ability in conventional treatment processes.

More specific objectives of this study were to:

- Determine the potential of using extracellular enzyme activities in surface waters for assessing changes in the character of NOM through variations in source, seasonal impacts and reservoir management (artificial destratification).
- Determine if the management of reservoirs, through the use of variable level offtakes and artificial destratification has the potential to improve water quality from the perspective of natural organic matter content (concentration and character) and the impact of this on treated drinking water quality.

1.5.1. Hypotheses

The hypotheses addressed in this study are:

1. While the reservoir may act as a barrier to the transport of fresh organics during riverine inputs, large inflow events have the potential to challenge water treatment by increasing the concentration of organic matter at the offtake to the water treatment plant. This hazard may be managed by the water industry by the use of a variable depth offtake system for selection of optimum source water quality to the water treatment plant.
2. The catchment flow regime is important to determine the carbon dynamics and risk to water quality, as it determines the loading of allochthonous organic carbon to the reservoir at short and seasonal timescales.

3. Microbial activity is an important factor in the reduction of the concentration of natural organic matter in drinking water reservoirs.
4. The types and levels of microbial activities reflect the concentration and character of NOM, which in turn, influences the treatment and quality of water for drinking purposes.
5. Vertical gradients of organic carbon concentration and character, associated with summer thermal stratification, will occur in reservoirs that are not artificially destratified. Differences in the treat-ability of the organic matter may be observed as a result.
6. The onset of thermal stratification results in shifts in the phytoplankton and microbial communities that result in changes in the source and processing of organic carbon in the pelagic zone.

1.5.2. Chapter Outline

The thesis is structured into seven chapters, of which, four are experimental chapters addressing the hypotheses above. Chapter 2 gives an outline of the field sites and the methodology employed in this study. Here, an understanding of the trophic states of the water bodies involved in the study and the basic background character and concentration of the organic matter they contain is developed.

Chapter 3 investigates the transport of organic matter to the main field site (Myponga Reservoir) over short timescales, addressing hypothesis 1, above. The influence of the intrusion and dissipation of a large inflow event on the water quality from the perspective of NOM was investigated using sampling and instrumental analysis. The impact on lake metabolism (microbial activity) was investigated using microbial enzyme assays and community level physiological profiling. The potential influence on water treatment plant operation was predicted using empirical models of alum dose and pH correction. The

potential for selective withdrawal to avoid transient changes in the raw water quality is demonstrated. The importance of the organic carbon to flow hysteresis to determine the temporal nature of the threat to water quality is raised.

Chapter 4 presents a flow weighted monitoring program and mass balance model of organic carbon in the Myponga Catchment - Reservoir system, to address hypotheses 2 and 3, above, by demonstrating the inter-annual variability in the load of autochthonous and allochthonous carbon and the change in organic carbon to flow hysteresis over a single winter in Myponga River. The modelling of photo-oxidation and microbial activity shows the relative importance of these processes to the total carbon budget.

Observations of the treat-ability of organic matter in three South-Eastern Australian drinking water reservoirs (Myponga Reservoir, Googong Reservoir and Wartook Reservoir) are presented in Chapter 5, addressing hypothesis 5, above, and demonstrate the importance of site specific factors in determining the persistence of thermal stratification and its impact on organic carbon concentration and character.

Chapter 6 investigates the changes in organic matter character using an *in situ* mesocosm experiment to simulate the onset of thermal stratification in the artificially destratified Myponga Reservoir. This chapter addresses hypotheses 3, 4 and 6 and demonstrates qualitative and quantitative changes in the autotrophic and heterotrophic microbial communities under simulated thermal stratification and the changes in treat-ability of NOM that occur.

Finally, Chapter 7 discusses the observations that were made on the influence of hydrodynamics on the removal of natural organic matter from raw water by conventional treatment, the potential process involved in these and other systems, and outlines conceptual models to assist water quality management using artificial destratification and selective withdrawal.

Chapter 2. Materials and Methods

The general materials used and the majority of methods routinely applied throughout this study are described in this chapter. Details of materials and methods that relate to specific investigations described in following chapters are given in those chapters. These include details of sampling programs, experimental design and specific analyses such as jar testing regimes. In the subsequent chapters, reference is made to specific sections of this materials and methods chapter, where relevant. Some background information is presented about the study sites which is based predominantly on previously published information but does include some information from this study where necessary.

2.1. Field Sites

The field sites for this study were chosen as Natural Organic Matter (NOM) is an important factor for the production of potable water from the raw water they supply. They all have elevated natural organic matter concentrations (see §2.1.5), but differ in other characteristics (see §2.1.1 - §2.1.3). The primary field sites are in three states of Australia, Myponga Reservoir is in South Australia, Wartook Reservoir is in Victoria and Googong Reservoir is in New South Wales (Figure 2.1). Further details of the individual reservoirs are given in the following sections. Three secondary field sites are also described; their bearing on this study was mostly a matter of circumstance, where their management in tandem with Googong Reservoir as the water supply for Canberra (Australian Capital Territory) resulted in Googong Reservoir being severely drawn down. The locations of the Corin, Bendora and Cotter Reservoirs can be seen in Figure 2.5.

Figure 2.1. Location of the primary field sites in Australia.

2.1.1. Myponga Reservoir

Myponga Reservoir (S 35° 21.233' E 138° 25.816') is located 70 km South of Adelaide, South Australia. It has a maximum depth of 42 m and a volume of 26,800 ML at full supply level (FSL). The catchment of Myponga Reservoir is approximately 124 km^2 and contains native vegetation, improved pasture for dairy, beef and hay, and plantations of *Pinus radiata*. A recent survey estimated the major land uses as 62 % livestock and 24 % dairying (Thomas *et al.* 1999). The catchment can be divided into 9 distinct sub-catchments (Figure 2.2B) which have some variability in their slope, soil and vegetation types and land use (Figure 2.3). The reservoir hydrodynamic models DYRESM and ELCOM have both previously been applied and validated for Myponga Reservoir and are able to describe the hydrodynamics of the system (Hipsey *et al.* 2005).

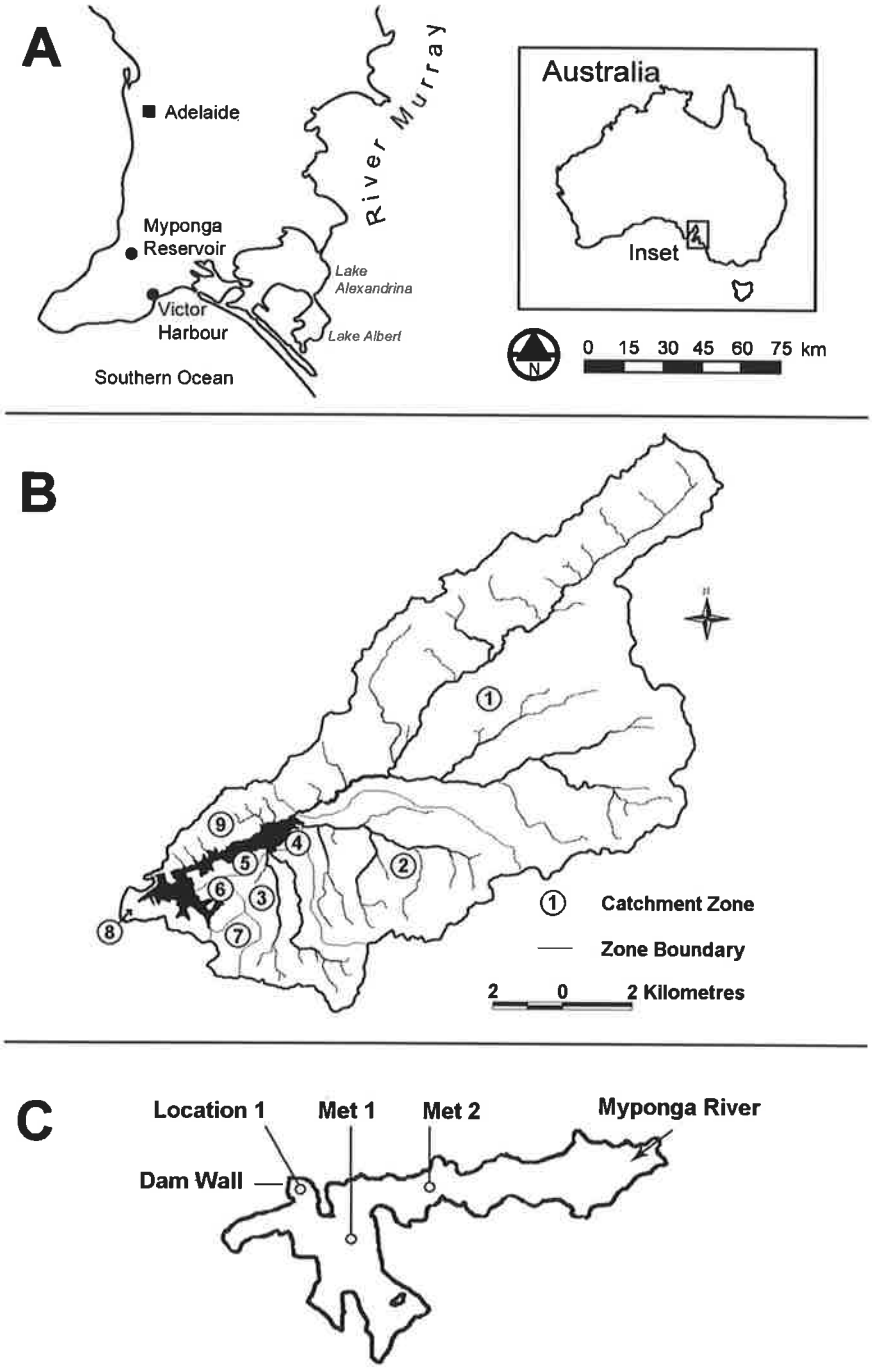


Figure 2.2. A) General location map of Myponga Reservoir and catchment area. B) Map of Myponga Reservoir catchment with sub catchment boundaries and major water courses shown. C) map of Myponga Reservoir, showing dam wall, Myponga River inflow and locations of meteorological station 1 (Met 1), meteorological station 2 (Met 2) and the location 1 sampling location.

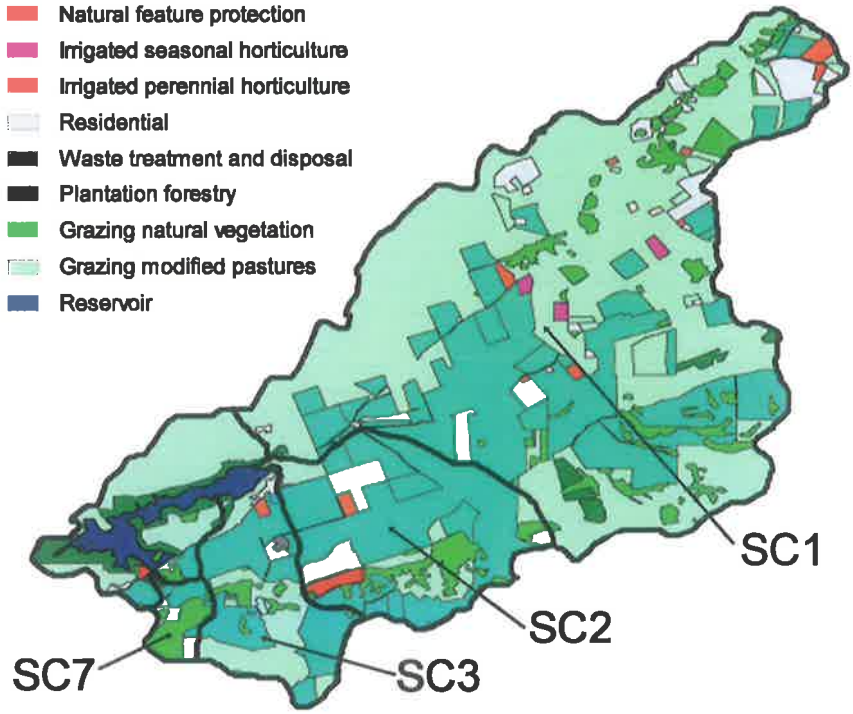


Figure 2.3. Land use map of the Myponga Reservoir catchment modified from Thomas *et al.* (1999). The sub-catchments are marked SC1 to SC7 correspond to those shown in Figure 2.2B.

2.1.2. Wartook Reservoir

Wartook Reservoir (S 37° 05.571' E 142° 26.189') was built between 1887 and 1891 and has a full service level capacity of 29,360 ML (Binney 2000). It is situated in the headwaters of the McKenzie River in the Grampians National Park, Western Victoria. The catchment area of 80 km^2 is almost exclusively native vegetation, and the mean annual flow from the reservoir into McKenzie River is 45,800 ML. The average hydraulic retention time is approximately 0.64 years. Water drawn from the reservoir travels down the McKenzie River in open channels for 50 km before reaching the Mt Zero water treatment plant. The dissolved air flotation and filtration plant can produce up to 30 ML d^{-1} and predominantly supplies the town of Horsham (Grampians-Water).

The data set available for historical data analysis from the Victorian Water Resources Data Warehouse (VWRDW; www.vicwaterdata.net) for the outflow of the reservoir (site reference 415202) has been examined by WATER ECOscience (2003). The reservoir had

good attainment of ANZECC guidelines (ANZECC & ARMCANZ 2000) for physical parameters (temperature, conductivity, pH, dissolved oxygen) but had low attainment of nutrient guidelines, mostly due to intermittently high nitrogen (total and oxidised) and total phosphorus results (WATER ECOscience 2003). The reservoir water is very soft, with alkalinity of 4 - 5 mg L^{-1} as $CaCO_3$. No information on the organic matter properties was available in the VWRDW archive, however the reservoir was known to be highly coloured. Previous studies have investigated the properties of the organic matter in Mt Zero Reservoir, which is supplied from Wartook Reservoir (van Leeuwen *et al.* 2001). Meteorological data was obtained from the Bureau of Meteorology (National Climate Centre, Melbourne, Australia).

2.1.3. Googong Reservoir

Googong Reservoir (S 35° 25.327' E 149° 15.719') is a drinking water reservoir 18 km south east of Canberra which was built on the Queanbeyan River in 1979. It has a capacity of 124.5 GL and a reported catchment area of between 816 and 890.1 km^2 (McIntyre *et al.* 2003, ActewAGL 2005b, Molongolo Catchment Group 2006). Land use estimates suggest the catchment is 34.4 % reserves and 60.4 % has vegetation cover (Molongolo Catchment Group 2006). Some forestry is conducted in the catchment and the remainder of the non-reserve area is predominantly used for pastoral activities (Figure 2.4).

Googong Reservoir has an annual mean chlorophyll *a* concentration of 2.6 µg L^{-1} (ActewAGL monthly monitoring data February 1980 to October 2005; range = 0.1 - 12.7 µg L^{-1}; mean = 2.6 ± 2.1 SD; median = 2.2; N = 454) and the total phosphorus concentration has not exceeded 50 µg L^{-1} since 1992. The routine monitoring has not detected a orthophosphate concentration at the main reservoir sampling site above 6 µg L^{-1} since 2003. The water quality monitoring adjacent to the offtake tower shows that Googong reservoir has medium to high colour (Table 2.1). Between January 2000 and December 2005 the true colour (HU) declined from around 40 Pt-Co units to around 20 Pt-

Co units. The reservoir generally has low turbidity and low to medium conductivity and the hypolimnion can become anoxic. Total depletion of hypolimnetic oxygen does not necessarily occur every summer, however it usually decreases below 4 mg L^{-1} which indicates that iron and manganese have the potential to remain soluble (Wetzel 2001c).

Historically, Googong Reservoir has been used to supply potable water to Canberra only when consumption exceeds the demands of the Cotter River system. The water in the Cotter River system has generally been of better quality than in Googong Reservoir. However the water quality in the Cotter River system was compromised by turbidity and ash input from the catchment following extensive bushfires in 2003. Consequently Googong Reservoir has been relied upon more heavily and has resulted in Googong Reservoir being maintained at a much lower level than normal for the last few years (ActewAGL 2005a). As at June 2005 Googong Reservoir was at just over 30% full (ActewAGL 2005b).

Table 2.1. Raw water quality in samples collected from the offtake of Googong Reservoir from January 1979 to March 2006.

Statistic	*Temperature (°C)*	*True Colour (Pt-Co)*	*Dissolved Oxygen (mg L^{-1})*	*Turbidity (NTU)*	*Conductivity ($\mu S\ cm^{-1}$)*	*pH (units)*
Minimum	6.4	7.5	0.0	0.30	30	6.5
Maximum	25.8	280	13.7	130.00	750	9.0
Mean	13.0	42.4	7.7	4.60	122	7.5
SD	4.6	32.8	2.3	8.74	25.5	0.4
Median	11.3	31	8.1	2.00	120	7.5
N	2886	2734	2839	2778	1194	2636

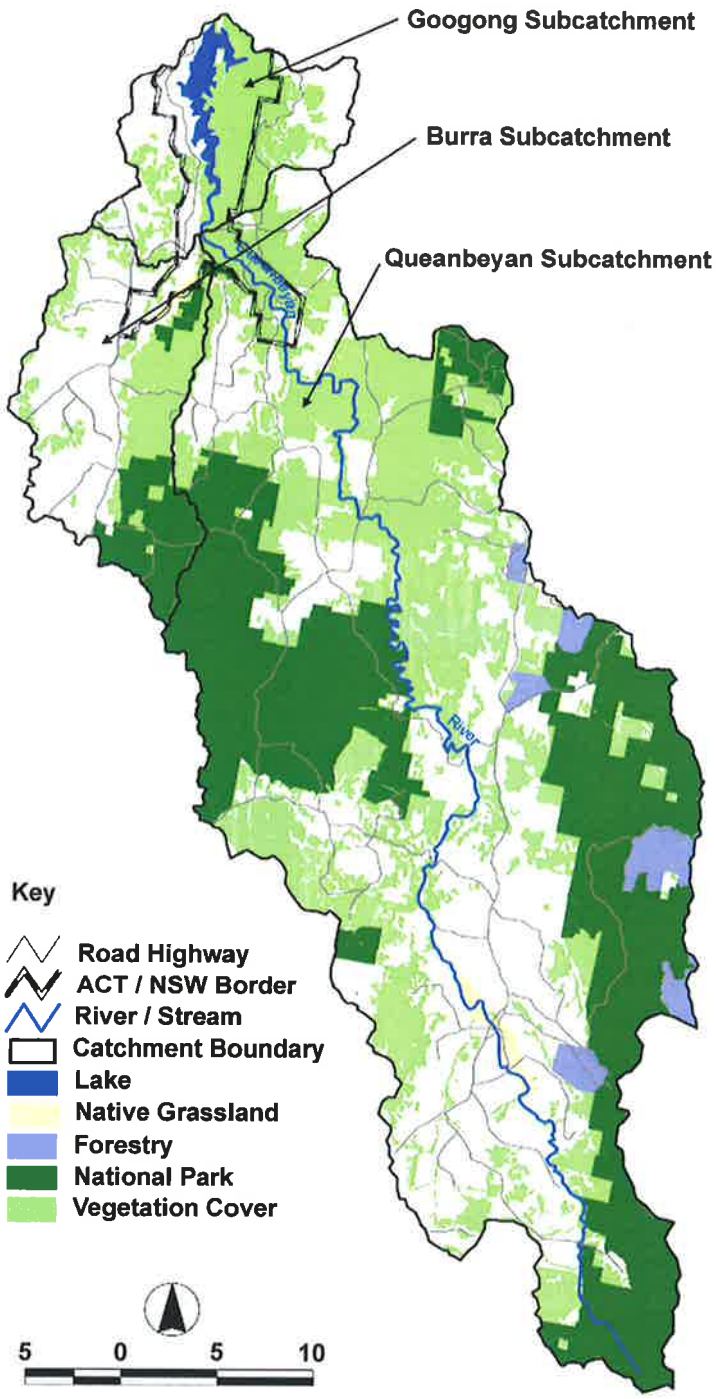


Figure 2.4. Googong Reservoir land use map. Modified from a map prepared for Mongolo Catchment Group by Minchem Pty Ltd. Cadastral, cultural & drainage data supplied by Environment ACT. Attribute data supplied by NSW Department of Infrastructure, Planning & Natural Resources.

2.1.4. Cotter, Corin and Bendoora Reservoirs

The Corin, Bendora and Cotter reservoirs were built on the Cotter River which rises in the south of the ACT, east of the Kosciusko National Park. The majority of the catchment area is within the Namadgi National Park, however one sub-catchment of the Cotter Reservoir

is a government managed pine plantation for forestry (ActewAGL 2005b). Corin Reservoir is contained by an earth and rock fill structure and has a capacity of 75.4 GL. Water flows down the Cotter River to Bendora Reservoir to the North, a double curve concrete arch dam which contains 10.7 GL of water. From Bendora Reservoir a gravity main supplies the service reservoir that in turn supplies the Mt Stromolo WTP. The Cotter Dam is approximately 19 km downstream of Bendora Reservoir, holds 4.7 GL and was the first of the reservoirs on the Cotter River to be built. Since the commissioning of Bendora Reservoir and its gravity main supply to Mt Stromolo WTP in 1961, Cotter Reservoir has rarely been used for potable supply, as water must be pumped to Mt Stromolo, incurring energy and pump maintenance costs in the process.

The water quality in Bendora Reservoir has historically been excellent (e.g. true colour, 15 - 20 HU, low iron and manganese concentrations, Chlorophyll *a* 1 - 3 $\mu g\ L^{-1}$), however due to extensive bushfires in 2003 in the Cotter River catchment, there has been and increase in sediment and pollutant load to the reservoirs (ActewAGL 2005b, Rustomji and Hairsine 2006).

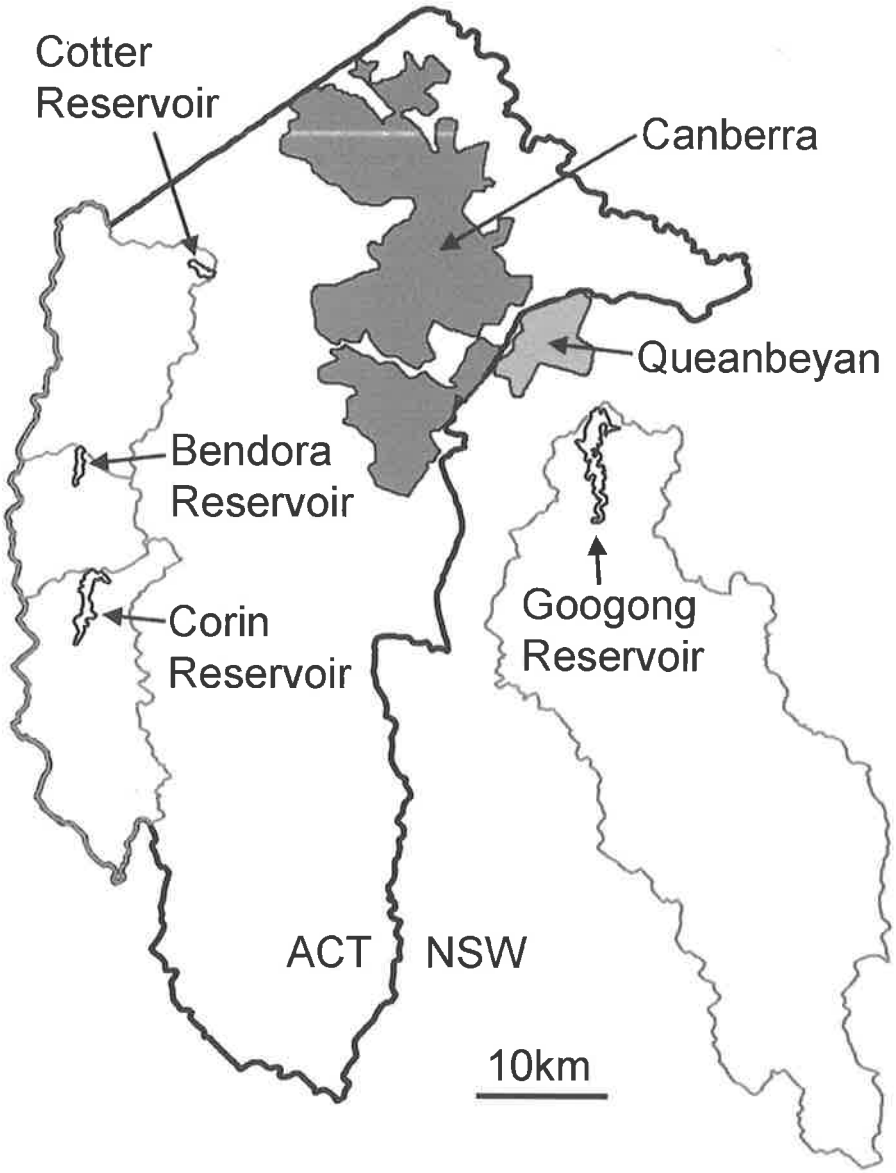


Figure 2.5. Location of Googong, Cotter, Bendora, and Corin Reservoirs.

2.1.5. Comparison of Organic Matter Properties

The typical water quality parameters at the three primary field sites vary apart from the fact that they have generally low turbidity (Table 2.2). Myponga Reservoir is the most highly coloured of field sites and also has higher absolute (UVA) and specific UV absorbance (SUVA). While Wartook Reservoir has a similar dissolved organic carbon (DOC) concentration to Myponga Reservoir, it has considerably lower colour and UV absorbance, suggesting that the dissolved organic matter found there is less aromatic and more aliphatic in character. The aromaticity of the organic matter in Googong Reservoir is probably somewhere in between, as it has a higher SUVA and similar specific colour (SpHU).

Table 2.2. Typical water quality parameters found at the primary field sites, during this study (2003-2005) and from routine monitoring data. DOC, dissolved organic carbon; UVA, ultraviolet absorbance at 254 nm; SUVA, specific ultraviolet absorbance; SpHU, specific colour.

	Myponga Reservoir	*Wartook Reservoir*	*Googong Reservoir*
DOC (mg L^{-1})	10 - 12	9.7	6.4
UVA (cm^{-1})	0.410 - 0.420	0.145	0.130
True Colour (HU)	55 - 65	14	9
Alkalinity (as $CaCO_3$ mg L^{-1})	80	4-5	50
Turbidity (NTU)	<5	<5	<5
SUVA (m^2 g^{-1})	3.0 - 3.4	1.5	2.0
SpHU (HU (mg L)$^{-1}$)	5.4 - 5.5	1.5	1.4

2.2. Field Methods

2.2.1. Sampling - Boating and Navigation

All reservoir sampling was carried out from outboard propelled boats. Field site locations were chosen and either marked with a buoy, or with a GPS waypoint (Garmin eTrex, Kansas, USA), enabling accurate site revisitation. Some locations were previously established through routine monitoring programs of the managing water authority.

2.2.2. Sample Collection

2.2.2.1. Manual Sample Collection

Sample collection was carried out depending upon the types of analyses intended and with logistical considerations such as the water volume required and equipment availability at the field site. The following provides an outline of the predominant sampling methods used for the collection of materials from the reservoirs investigated.

Samples from discrete depths in reservoirs were collected using either a Van Dorn sampler and discharging the sampler into a bucket, or using a length of hose with an impeller or peristaltic pump. All containers were rinsed with the same sample water prior to filling, except where inappropriate, such as when collecting bacteriological samples in sterilised

bottles. Samples collected for determination of heterotrophic bacterial concentrations by Heterotrophic Plate Count at 20°C (HPC) were collected in gamma irradiation sterilised PET bottles without any pre-rinsing and were placed immediately on ice.

Samples for determination of extra-cellular microbial enzyme activities were collected in sterilised bottles when requiring overnight transport from the sampling site to the laboratory, i.e. for samples collected from Wartook (Victoria) and Googong (New South Wales) Reservoirs and from locations in the Australian Capital Territory.

Samples collected for the determination of dissolved organic carbon, colour (true and apparent), ultraviolet absorbance (at 254 nm) and molecular size distribution by high performance size exclusion chromatography were collected in pre-rinsed new PET bottles and capped immediately without an air gap. Samples collected for the determination of microbial enzyme activities, phytoplankton enumeration and chlorophyll *a* and *b* analyses, were collected in similar containers, but with an air gap retained in the bottle. Samples were placed in a temperature insulated container with ice before transportation to the laboratory.

Samples collected for jar testing were stored in 10 to 60 L plastic carboys (LDPE; Silverlock Packaging, Dudley Park, South Australia, Australia). Comparison of UV absorbance, colour and DOC data of sub-samples taken from these large containers with concurrently collected water samples in PET bottles, showed no detectable leaching of organic compounds from the carboys. It was previously established, using high purity water, that there is no detectable leaching of organics, as measured by DOC, from PET bottles. After collection, the 10 to 20L samples were transported immediately to the laboratory under cool conditions and subsequently refrigerated at approximately 4 °C.

2.2.2.2. Automated Sample Collection

Water samples were automatically collected from Myponga River (South Australia) using a refrigerated (4 °C) automatic sample collector (ISCO 3700, ISCO Corporation, Lincoln, Nebraska, USA) at the v-notch gauging weir located approximately 500m upstream of Myponga Reservoir. Sampling events were triggered using the gauge height and temperature data logging instrumentation system (Measurement Engineering Australia, Magill, South Australia, Australia). This instrument allows the user to control the conditions that trigger sampling by specifying a number of parameters, including the gauge height at which sampling begins and the volume between samples. The volume between samples collected was varied depending on the flow conditions of the river at the time and balanced with the capacity of the auto-sampler. Samples were generally collected from the auto-sampler within 48 hours.

2.2.3. Field Data Collection

2.2.3.1. HydroLab Probe

Water temperature, dissolved oxygen, pH, specific conductivity, turbidity, redox potential, chlorophyll fluorescence and depth were measured using a HydroLab 4a sonde (Hach Environmental, Loveland, Colorado, USA). The probe was equilibrated at the surface, the depth sensor was zeroed and the probe set to log at a rate of 1 Hz and lowered slowly by a rope or hand winch. All parameters reported were calibrated according to the manufacturers instructions or checked in the lab prior to the field trip.

2.2.3.2. Temperature Monitoring

Thermistor strings were deployed in Googong Reservoir from 18 November 2003 to 17 May 2004 and in Wartook Reservoir from 4 December 2003 to 30 June 2004. Water temperature was logged every 10 minutes with submersible StowAway TidbiT or Optic Stowaway logging thermistors (Onset Co., Bourne, Massachusetts, USA). The manufacturer report these instruments have an accuracy of ± 0.2 °C, a resolution of ± 0.16

°C and a response time of 5 minutes in water (to 90 %). Their resolution is affected by the 8 bit analogue to digital converter incorporated in their circuitry, limiting them to reporting 256 temperature levels over a 43 °C temperature range (-5 °C to +37 °C). They are suitable for monitoring and describing the thermal stratification at daily timescales but are inappropriate for making calculations of surface mixed layers (e.g. ΔT of 0.05 °C) at any timescale.

2.2.3.3. Meteorological Data

A raft mounted automatic weather station was operated by SA Water on Myponga Reservoir until 8 June 2004, when it was removed for refurbishment or replacement by SA Water Corporation. Prior to this date, data is available for water temperature at 22 depths, wind speed and direction, solar irradiation, air temperature and relative humidity, at ten minute intervals. Rainfall and class A pan evaporation was recorded daily at a site adjacent to Myponga Reservoir and rainfall was also recorded adjacent to the flow monitoring station in Myponga River.

2.3. Laboratory Methods

2.3.1. Sample Filtration

Membrane filtration was routinely employed to determine the concentration of dissolved species (e.g. dissolved organic carbon, ammonia, oxidised nitrogen) in water samples or for analyses where particulate free water samples are required (e.g. UV absorbance at 254 nm and true colour at 456 nm). Samples were filtered either with a vacuum apparatus, a Buchner flask and Milli-Q 47mm filter holder or a 60 mL syringe and a syringe filter. Different filter types were employed for different tasks and are detailed in Table 2.3. The filter or membrane was rinsed with both high purity Milli-Q water and a small volume of sample prior to collection of the sample filtrate to remove the influence of any leachable organic carbon within the filter media.

Table 2.3. Filtration media used in this study. * denotes nominal exclusion by depth media.

Filtration Type	*Pore Size (μm)*	*Material*	*Details*
Dissolved Species	0.45	Cellulose Acetate	Mini-Sart, #16555Q, Sartorius Co.
Filter Sterilisation	0.2	Polyethersulfone	Supor, #4652, Pall-Gelman Co.
Prefiltration or collection of particulates	1.2*	Glass fibre	GF/C, #1822-047, Whatman Co.
Gravity filtration during Jar Testing	11.0*	Regenerated Cellulose	#1 Qualitative, #1001-240, Whatman Co.

2.3.2. Sample Dilution

When a sample was found or was reasonably expected to have an analyte concentration out of range for a given analysis, it was diluted with high purity Milli-Q water using calibrated glassware. A dilution factor of 2 was preferentially chosen. When performing a series of such dilutions, the aliquot of diluent was first measured into the series of containers (e.g. PET bottles or PS vials), followed by the samples, using the same glass volumetric pipette. The pipette was rinsed with a volume of Milli-Q water and the following sample before delivering the sample aliquot. Calculations based on trials of delivering Milli-Q water into clean pre-weighed vials revealed a volumetric pipette delivery error of 0.03 % using both 100 mL (N = 6) and 15 mL pipettes (N = 6).

2.3.3. Extracellular Enzyme Activities

The activities of the enzymes, α-glucosidase (AGL), alkaline phosphatase (AKP), β-glucosidase (BGL), β-xylosidase (BXL), chitinase (CHT), esterase (EST) and leucine aminopeptidase (LAP) were determined in water samples using colorimetric methods, adapted from those described by Vuorinen and Saharinen (1996) and Biely *et al.* (2000). In this study 96 well microplates were used for enzyme reactions and colour measurements. Substrates for these enzymes were 4-nitrophenyl-α-glucopyranoside (N1377), 4-

nitrophenyl-phosphate (N4645), 4-nitrophenyl-β-glucopyranoside (N7006), 4-nitrophenyl-β-xylopyranoside (N2132), 4-nitrophenyl-N-acetyl-β-D-glucopyranoside (N9376), 4-nitrophenyl-butyrate (N9876) and L-leucine-4-nitroanilide (L9125), respectively. All substrates were analytical grade and were obtained from Sigma-Aldrich Corp. (St. Louis, Missouri, USA). Substrate solutions were prepared fresh from powder no longer than a week before being used. All substrate solutions were prepared in Tris-HCl buffer (0.05 M, pH = 7.5 @ 25 °C, Sigma-Aldrich Corp.), except for the esterase substrate which was prepared in high purity sterile Milli-Q water, due to its instability in Tris-HCl buffer. Some published colorimetric assays include an organic solvent such as chloroform (Berman 1970) or toluene (Alef and Nannipieri 1995) to act as an antibiotic to prevent microbial growth and development during the assay reactions. These solvents are incompatible with polystyrene microplates, due to potential effects on the optical characteristics, and thus another antimicrobial agent was required. Actinomycin D (A1410, Sigma-Aldrich Corp.) was chosen as it is an effective broad spectrum DNA dependant RNA synthetase inhibitor, thus preventing the microbial community from responding to the substrate provided (Muschel and Larsen 1969, Dawson *et al.* 1986, Rynolds 1993).

Negative controls were prepared by boiling sub-samples for 10 minutes in a microwave to denature the enzymes present. Any water loss during this process was replaced with high purity sterile Milli-Q water. Positive control solutions were prepared with commercially available purified enzymes (α-glucosidase G0660, alkaline phosphatase P4252, β-glucosidase G4511, β-xylosidase X3501, chitinase C7809 and leucine aminopeptidase L5658, Sigma-Aldrich Corp.) in Tris-HCl buffer (0.05M pH = 7.5 @ 25°C, Sigma-Aldrich Corp.). Standard curve solutions were prepared from stock solutions of 4-Nitroanaline (4-NA, N2128, Sigma-Aldrich Corp.) and 4-Nitrophenol (4-NP, N1048, Sigma-Aldrich Corp.). Standard curves were determined for each individual sample to account for any

optical differences in the samples and quenching or adsorption of the chromophore due to organic matter or particles within the sample.

2.3.3.1. Assay Conditions

Reactions were conducted in sterile 96 well polystyrene microplates (Perkin Elmer SpectraPlate 96 MB or Sarstedt #82.1581). A separate microplate was used for each enzyme activity assay, allowing for 8 samples to be analysed per plate. Plates were arranged as shown in Table 2.4, with three replicate wells for the test, negative control and positive control wells. The microplates were loaded with the solutions (Table 2.5) to the volumes shown in Table 2.6 using a combination of multi-channel pipettes and single and multi-reservoir loading trays to expediate the process. The distribution of reaction wells within each microplate was not randomised as it was deemed an unnecessary impediment to the efficient processing of samples. No significant differences (t-test, $\alpha = 0.05$, JMP, SAS Institute Inc, Cary, North Carolina, USA) were found between any four replicates of the same 4-NP concentration loaded into different columns of the same microplate, in either Tris-Buffer or Myponga Reservoir water. The reactions were initiated by the addition of the enzyme substrate. After initiation, the plate was placed immediately in a MRX-II microplate reader (Dynex Technologies, Chantilly, Virginia, USA) and the optical density (OD) read at 410 nm, with a reference wavelength of 630 nm to account for turbidity and after a 10 s shake cycle. The microplates were wrapped in aluminium foil to protect them from light, and incubated at room temperature (~ 25 °C) on an oscillating shaker at 100 rpm. The plates were read at least 6 times over the following 48 hours. The change in concentration of 4-NP was determined from the standard curve of the initial reading. The rate of 4-NP production was calculated from the change in optical density (OD) with time prior to any effects of substrate limitation or microbial response to the enzyme substrate. These changes are characterised by the curve tending towards a

maximum OD or an increase in the rate of 4-NP production after a lag period, respectively (Figure 2.6).

Table 2.4. Layout of enzyme assay microplate. Each different sample occupies a single row (A - H). NC = negative control, S = standard curve, T = test, PC = positive control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	NC 1	NC 2	NC 3	S1	S2	S3	T1	T2	T3	PC1	PC2	PC3
B												
C												
D												
E												
F												
G												
H												

Table 2.5. Enzyme assay solution contents.

Solution	*Contents*
Tris-HCl Buffer	Tris-HCl buffer pH = 7.5 @ 25°C
Positive Control Solution	AGL, AKP, BGL, BXL, CHT, EST or LAP in Tris-HCl buffer pH = 7.5 @ 25°C
Standard Curve Solution 1	1.0 mM 4-NP (4-NP assays) or 500 µM 4-NA (LAP) in Tris-HCl buffer pH = 7.5 @ 25°C
Standard Curve Solution 2	400 µM 4-NP (4-NP assays) or 200 µM 4-NA (LAP) in Tris-HCl buffer pH = 7.5 @ 25°C
Standard Curve Solution 3	40 µM 4-NP (4-NP assays) or 40 µM 4-NA (LAP) in Tris-HCl buffer pH = 7.5 @ 25°C
Enzyme Substrate	4.0 mM (4-NP-substrates) or 2.0 mM (LAP-substrates) in Tris-HCl buffer pH = 7.5 @ 25°C

Table 2.6. Enzyme assay reaction contents.

Reaction Solution	*Test*	*Negative control*	*Positive control*	*Std Curve 1*	*Std Curve 2*	*Std Curve 3*
Sample (µL)	100	-	100	100	100	100
Tris-HCl Buffer (µL)	50	50	-	-	-	-
Positive Control Solution (µL)	-	-	50	-	-	-
Negative Control Sample (µL)	-	100	-	-	-	-
Standard Curve Solution 1 (µL)	-	-	-	50	-	-
Standard Curve Solution 2 (µL)	-	-	-	-	50	-
Standard Curve Solution 3 (µL)	-	-	-	-	-	50
Enzyme Substrate (µL)	50	50	50	50	50	50
Total Well Volume (µL)	200	200	200	200	200	200

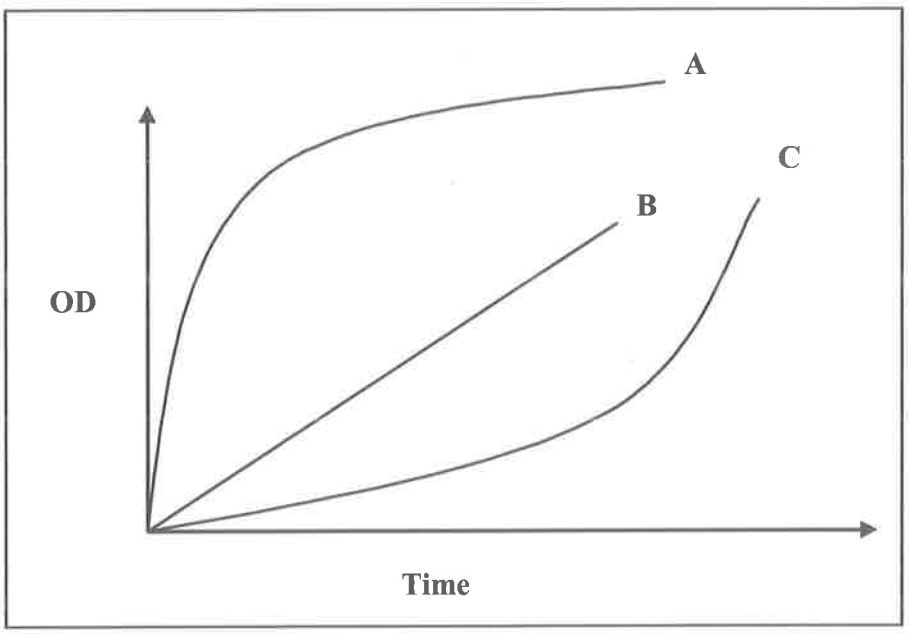


Figure 2.6. Idealised optical density (OD) development curves during enzyme assay incubation. A, rate of production becomes substrate limited. B, linear production with no limitation or microbial response. C, microbial response to substrate causing increase in the rate of OD production.

2.3.4. Community Level Physiological Profiles

The changes in the metabolic competency of the microbial communities was determined using community level physiological profiling (CLPP). BIOLOG Ecoplates™ (BIOLOG Co., Hayward, California, USA) were used according to the product methodology and analysed according to Garland and Mills (1991). BIOLOG Ecoplates™ consist of an array

of 31 simple carbon substrates and one control well (no substrate) repeated 3 times in a 96 well microplate (Table 2.7). All wells also contain tetrazolium violet which is reduced to the coloured formazan when microbial respiration occurs in the well (Mills and Garland 2002). An aliquot of the sample (130 µL) is delivered to each well on the plate and the reduction of the tetrazolium violet is determined by recording the OD of the plate at 550nm after a 10s shake cycle. The resulting data can be interpreted using a number of published methods. The average well colour development (AWCD) was the first method to be published (Garland and Mills 1991). In this method, the individual well optical density is normalised to the maximum, at the point in colour development when the average well colour reaches a certain magnitude (consistent across all plates to be compared). Other methods involve fitting growth curves to the colour development (Lindstrom *et al.* 1998) and those that assess the richness or variability of the microbial response (Harch *et al.* 1997). Subsequent to the initial data analysis various methods have been used to group and discriminate between the bacterial community profiles that are developed (Howard 1999). Much discussion of the relative merits of these methods has been published (Hackett and Griffiths 1997, Garland and Mills 1999, Garland *et al.* 2001). Trials of the application of the fitting of the growth curves as applied in Lindstrom *et al.* (1998) revealed that the data requirements for satisfactory curve fitting was not possible with the demands on the plate reader. Consequently the AWCD analysis method was used.

Table 2.7. Well references and substrates found in BIOLOG Ecoplates™.

BIOLOG Plate Reference	*Substrate*	*Classification (Insam 1997)*
A1, 5, 9	Water	-
B1, 5, 9	Pyruvic Acid Methyl Ester	Carboxylic Acid
C1, 5, 9	Tween 40	Polymer
D1, 5, 9	Tween 80	Polymer
E1, 5, 9	α-Cyclodextrin	Polymer
F1, 5, 9	Glycogen	Polymer
G1, 5, 9	D-Cellobiose	Carbohydrate
H1, 5, 9	α-D-Lactose	Carbohydrate
A2, 6, 10	β-Methyl-D-Glucoside	Carbohydrate
B2, 6, 10	D-Xylose	Carbohydrate
C2, 6, 10	I-Erythritol	Carbohydrate
D2, 6, 10	D-Mannitol	Carbohydrate
E2, 6, 10	N-Acetyl-D-Glucosamine	Carbohydrate
F2, 6, 10	D-Glucosaminic Acid	Carboxylic Acid
G2, 6, 10	Glucose-1-Phosphate	Carbohydrate
H2, 6, 10	D,L-Glycerol Phosphate	Carbohydrate
A3, 7, 11	D-Galactonic acid g-lactone	Carbohydrate
B3, 7, 11	D-Galactonuric Acid	Carboxylic Acid
C3, 7, 11	2-Hydroxy Benzoic Acid	Phenolic Compound
D3, 7, 11	4-Hydroxy Benzoic Acid	Phenolic Compound
E3, 7, 11	γ-Hydroxybutyric Acid	Carboxylic Acid
F3, 7, 11	Itaconic Acid	Carboxylic Acid
G3, 7, 11	α-Ketobutryic Acid	Carboxylic Acid
H3, 7, 11	D-Malic Acid	Carboxylic Acid
A4, 8, 12	L-Arginine	Amino Acid
B4, 8, 12	L-Asparagine	Amino Acid
C4, 8, 12	L-Phenylalanine	Amino Acid
D4, 8, 12	L-Serine	Amino Acid
E4, 8, 12	L-Threonine	Amino Acid
F4, 8, 12	Glycyl-L-Glutamic Acid	Amino Acid
G4, 8, 12	Phenylethylamine	Amine
H4, 8, 12	Putrescine	Amine

2.3.5. Dissolved Organic Carbon

Dissolved organic carbon (DOC) in water samples was determined using a Sievers model 820 equipped with an inorganic carbon removal module and an auto-sampler carousel (Ionics Instruments, Boulder, Colorado, USA), following filtration (see §2.3.1). Each analytical run included high quality Milli-Q water blanks, a 25 ppm organic carbon standard (TC; potassium hydrogen phthalate), a 25 ppm inorganic carbon standard (IC; Na_2CO_3) and a standard that contained both organic and inorganic standard compounds, as above, at 25 ppm each (TC:IC). Additional quality control (QC) was in the form of requirement testing of operator-blind 'high' and 'low' concentration DOC standards (ranging between 10-15 and 1-5 mg L^{-1}, respectively) and of a high purity water sample, prepared and provided by the quality assurance unit of the laboratory (Figure 2.7). Analytical runs were validated if all QC samples were within two standard deviations of the prepared concentrations.

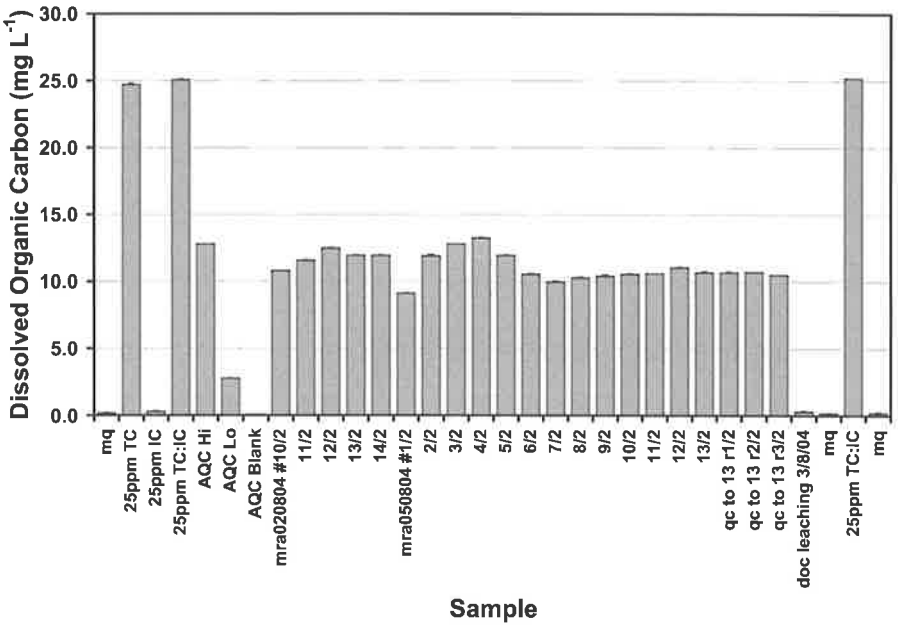


Figure 2.7. Results from a typical DOC analysis run (15 August 2004). Error bars are standard deviations of three determinations within the same sample. Note the analytical quality control samples (AQC; Hi, Lo, Blank), three replicate quality control samples (qc to 13 r#/2) and a DOC leaching during storage sample.

2.3.6. UV Absorbance

The UV absorbance at 254 nm (UVA) of a sample was determined using a Shimadzu UVmini 1240 spectrophotometer. The instrument was zeroed using high purity Milli-Q water and the UV absorbance was measured at 254 nm with a 1 cm path length quartz cuvette. The cuvette was regularly soaked overnight in Deconex laboratory detergent, cleaned thoroughly with a cotton tip and rinsed thoroughly in Milli-Q water. All regular maintenance of the spectrophotometer was conducted as recommended by the manufacturer and regular checks were made against other similar instruments in other laboratories.

2.3.7. True and Apparent Colour

Colour was determined using the method of Bennett and Drikas (1993). True colour was determined using filtered samples; apparent colour was determined using unfiltered samples. Briefly, the absorbance of the sample at 456 nm was determined in a 5 cm glass or quartz cuvette and colour, in Hazen units determined by comparison to a standard curve of platinum-cobalt standards (e.g. 50 and 100 ppm platinum in acidified cobaltous chloride solution).

2.3.8. Jar Tests

Jar tests were conducted using six gator (square-based, 2 L) jars (B^2Ker, or equivalent) on ganged rotary stirrers. When the water had been in cold storage, the water temperature was equilibrated to 20 °C in a water bath before use. Aliquots (2 L) of sample were measured using a volumetric flask and carefully poured into each of the 6 jars. The stirring paddles were lowered into the jars and held approximately 5 cm above the bottom of the jar. The stirrers were turned on at a slow rate (20 revolutions per minute; rpm) and the temperature and pH was monitored using a pH electrode (pH 340i and Sentix 80 electrode; WTW GmbH, Weilheim, Germany) to ensure stabilisation had occurred. Previously determined coagulant doses and pH control reagent doses (acid or alkali) were then placed into 12 x 30

mL polystyrene vials, held in the 'dosing rack' of the rotary stirrer, facilitating concurrent addition of the coagulant and pH control chemicals. The flash mix (200 rpm) of 1 minute was measured immediately after coagulant and pH control solutions were added, followed by 14 minutes of slow mixing at 20 rpm. The flocs were allowed to settle for 15 minutes before filtration was commenced. From the sampling port, the first 50 mL of settled water was discarded and the following 50 mL was collected and set aside to subsequently measure settled water turbidity and pH. A set of six Whatman #1 filter papers (nominal pore size 11 μm, Whatman Co., Brentford, UK) were quarter folded, placed in plastic funnels and rinsed with at least 0.5 L of Milli-Q water. A funnel volume of settled water was used to rinse the pre-rinsed filter paper and the filtrate was used to rinse the sample collection bottle. The settled water was then filtered and collected into sample bottles until the water level in the gator jar dropped to that of the sample removal tap. Approximately 1 L of product water was obtained from each 2 L jar test.

2.3.9. Chlorophyll *a* and *b*

Chlorophyll *a* and *b* were determined spectrophotometrically in ethanol using the method of Wintermans and De Mots (1965). A known volume (generally between 0.5 and 1.0 L) of sample was filtered through a GF/C filter paper (nominal pore size 1.0 μm). The filter paper was placed in 10 mL of cold 95 % ethanol and vigorously mixed for 10 s on a vortex shaker and stored at 4 °C. On the following day the tubes were again vortex mixed for 10 s and the sample filtered through a 0.45 μm membrane syringe filter (Mini-Sart, #16555Q, Sartorius Co. Göttingen, Germany) to remove cellular and filter paper debris and the OD determined at 649, 665 and 750 nm in a Shimadzu UVmini 1240 spectrophotometer. The concentration (μg L^{-1}) of chlorophyll *a* and *b* were then determined by the following equations.

$$E_{665} = \frac{OD_{665} - OD_{750}}{P}$$

$$E_{649} = \frac{OD_{649} - OD_{750}}{P}$$

$$Chlorophyll\ a\,(\mu gL^{-1}) = \frac{13.7E_{665} - 5.76E_{649}}{V_F}(V_E)$$

$$Chlorophyll\ b\,(\mu gL^{-1}) = \frac{-7.6E_{665} + 25.8E_{649}}{V_F}(V_E)$$

Where OD_{649} is the optical density at 649nm, OD_{665} is the optical density at 665nm, OD_{750} is the optical density at 750nm, P is the path length of the cuvette, V_F is the volume of sample filtered and V_E is the volume of ethanol used for extraction.

2.4. Other Chemical and Biological Analyses

A range of chemical and biological analyses were performed through the routine commercial services of the Australian Water Quality Centre (AWQC; a business unit of the South Australian Water Corporation; ISO9001 and National Analytical Testing Authority accredited laboratory). These included nutrient analyses, total phosphorus (TP), filterable reactive phosphorus (FRP), total kjeldahl nitrogen (TKN), nitrite and nitrite (NO_x) and ammonia (NH_3), residual aluminium, trihalomethane formation potential, algal identification and enumeration, heterotrophic plate counts at 20 °C, taste and odour analysis. Generally, the methods applied are the standard methods adopted by the water industry (AWWA 1992) or have been developed at the AWQC and are not presented in detail due to commercial confidentiality. Brief descriptions of these analyses are provided in Table 2.8. The openly published methods are described in more detail in the following sections.

Table 2.8. Brief descriptions of analyses performed at AWQC.

Analysis	*Method*	*Limit of Reporting*	*Average Standard Deviation*
total phosphorus (TP)	Digestion with sulphuric acid and potassium sulphate. Orthophosphate determined by automated flow colorimetry	0.005 mg L^{-1}	0.0063 at 0.188 mg L^{-1} 0.0028 at 0.025 mg L^{-1}
filterable reactive phosphorus (FRP)	As for TP, post 0.45 µm membrane filtration	0.005 mg L^{-1}	0.0023 at 0.020 mg L^{-1} 0.0054 at 0.160 mg L^{-1}
total kjeldahl nitrogen (TKN)	Digestion with sulphuric acid and potassium sulphate. Ammonia-N determined by automated flow colorimetry	0.05 mg L^{-1}	0.0680 at 1.875 mg L^{-1} 0.0294 at 0.25 mg L^{-1}
nitrate and nitrite (NO_x)	Oxides of nitrogen determined by automated flow colorimetry	0.005 mg L^{-1}	0.0021 at 0.040 mg L^{-1} 0.0049 at 0.250 mg L^{-1}
ammonia (NH_3)	Ammonia-N determined by automated flow colorimetry, post 0.45 µm membrane filtration	0.005 mg L^{-1}	0.0033 at 0.075 mg L^{-1} 0.0015 at 0.01 mg L^{-1}
residual aluminium	Standard methods		
trihalomethane formation potential	Buffered water sample (pH 7.4) incubated at 35°C for 4 hours with excess chlorine (~ 20 mg/L). THMs analysed by headspace gas chromatography	1 µg L^{-1}	Unknown
algal identification and enumeration	Preservation with Lugols solution, 10× sedimentation concentration and microscopic examination	1 cell 100 mL^{-1}	10 - 20 %
heterotrophic plate counts at 20 °C	Pour plate onto R2A agar, incubation at 20 °C	1 cfu mL^{-1}	Unknown, 2 significant figures reported
taste and odour	Solid Phase Microextraction - Gas Chromatography / Mass Spectrometry (SPME - GC/MS)	4 ng MIB L^{-1} 2 ng geosmin L^{-1}	Unknown

2.4.1. HPSEC-UV (Method A)

High-performance size exclusion chromatography with ultraviolet absorbance detection was performed by the Water Treatment Unit at the AWQC, using a Shodex KW 802.5 glycol-functionalised silica gel column (5 µm particle size, Pullulan exclusion 60 kDa, Protein exclusion 150 kDa, plate number > 21,000) preceded by a Phenomenex SecurityGuard™ guard column with a GFC-3000 cartridge and a Waters 2690 separations module with vacuum degasser and column thermostat. The separated UV absorbing

organics were detected using a Waters 996 photodiode array detector (PDA) at 260nm. The eluent was 0.1 M sodium phosphate buffer at pH = 6.80. The standards used to determine apparent molecular weight from elution time were 35, 18, 8 and 4.6 kDa polystyrene sulphonates. The column void volume was determined using 2,000 kDa dextran blue. The system was automated using a computer workstation equipped with Millenium^{32} software (Waters Corporation, Milford, Massachusetts, USA).

2.4.2. HPSEC-DOC (Method B)

High-performance size exclusion chromatography with dissolved organic carbon detection was performed at the Curtin University of Technology. The separation system has been described previously (as method A in Allpike *et al.* 2005). Briefly, SEC was performed using Toyopearl HW-50S media (Tosoh Bioscience, Tokyo, Japan; particle size = 30 μm; pore size = 125 Å), packed in a preparative column (length = 250 mm; ∅ = 22 mm) and a Hewlett-Packard 1090 Series II HPLC instrument with filter photometric detection (FPD) at 254 nm. The eluent consisted of phosphate buffer (10 mM, 1.36 g L^{-1} KH_2PO_4, and 10 mM, 3.58 g L^{-1} Na_2HPO_4) at pH = 6.85. The organic carbon detector (OCD) however has novel components. It is based on the detection of UV-persulphate oxidised carbon using a Fourier transform infrared (FTIR) spectroscopy 'lightpipe' detector originally designed for detection of analytes after gas chromatographic separation (Allpike *et al.* 2007).

Apparent molecular weight (AMW) was determined using calibration curves of a series of polystyrene sulphonates (840 – 35300 Da). The calibration curve was validated using a series of other compounds with different functional groups (e.g. trypan blue, 960 Da; cyanocobalamin vitamin B12, 1355 Da; tannic acid, 1701 Da; ribonuclease A, 13700 Da). The calibration curve was linear (r^2 = 0.99) over the AMW range tested.

2.4.3. Particle Size Determination by LISST

Particle size distributions were determined using Laser In-Situ Scattering and Transmissometry (LISST-100, Sequoia Instruments, Bellevue, Washington, USA). The scattering of light in water at small forward angles is primarily determined by diffraction of the light by particles. As transmission makes a very small contribution, the refractive index and composition of the particle is insignificant to the particle size measurement. Therefore, the LISST-100 measures the scattering of a 1 mW red laser beam (670 nm) at 32 angles, mathematically inverted to determine the size distribution and scaled to obtain the volume scattering function (VSF). The size distribution is determined as a concentration of particles ($\mu L\ L^{-1}$) in each of 32 log-spaced size bins. The total volume concentration may be derived as the sum of the particle volume in each bin. As the instrument is able to record data at a rate of 1 Hz most users choose to determine the average volume concentration of multiple readings. The use of multiple readings also allows the calculation of the standard deviation of average total volume concentration, which give an measurement of the variability in the particle size distribution. While this is essentially a field instrument, it may be fitted with a chamber for laboratory use.

Chapter 3. Transport, Metabolism and Fate of Organic Carbon during Flow Episodes

3.1. Introduction

The provision of good quality potable water is dependant on the quality of the source water, the efficiency of the treatment processes and the effective delivery of the water to the customer. The detention of raw water in storage reservoirs provides security to the supply of raw water by acting as a barrier to hazards from the catchment that threaten water quality. Large inflow events, associated with rainfall, have the potential to compromise raw water quality as they can transport a suite of contaminants including protozoan and microbial pathogens (Brookes *et al.* 2004, Hipsey *et al.* 2005), turbidity and inorganic and organic compounds, either natural or anthropogenic (i.e. pesticides, heavy metals and natural organic matter). The increase in concentration of these parameters has the potential to challenge, and possibly overwhelm, the water treatment process and could result in the distribution of aesthetically undesirable or non-potable water (Hawkins *et al.* 2000, Brookes *et al.* 2005b).

The main factors that determine the delivery of inflowing water to the dam wall and the proximal offtake are the densities (determined by temperature and salinity) of the standing and flowing waters, the velocity of the inflow and the distance to the dam wall. If the inflowing water is significantly colder and denser than the water in the reservoir then it will form an underflow, conversely if the inflow is warmer and less dense than the water it will form an overflow. As the inflow mixes with the reservoir water its density may decrease to a point where it forms a subsurface intrusion (Figure 3.1).

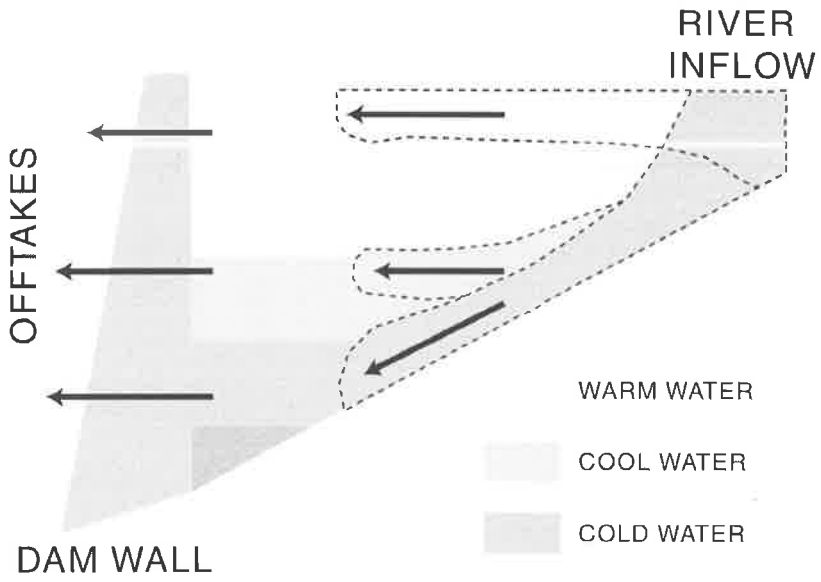


Figure 3.1. Formation of intrusions during inflow events. The density of the water is represented by the shading. The coldest and most dense water is shaded the darkest. Reservoir density stratification has been represented as an arbitrary linear gradient. The depth of formation of the riverine intrusion will correspond to the depth in the reservoir of equivalent density.

Subsequent transport of the riverine intrusion to the dam wall can result in poor quality water being delivered to the water treatment plant. A detailed understanding of the hydrodynamics of inflow events is desirable so that water, not impacted by the intrusion, can be extracted for treatment. While complex 3D models can accurately describe inflow events they are expensive and time consuming to apply. Simpler models that adequately predict the depth of riverine intrusions based on fewer parameters are available and have been proposed as tools to aid management of reservoir offtakes by predicting the timing and magnitude of the threat associated with pathogens (Antenucci *et al.* 2005a).

The quantity and character of organic matter in the raw water is important as it influences the coagulant dose required for its removal. Furthermore, the organic matter remaining after treatment exerts a chlorine demand, contributes to disinfection by-products and can be a substrate for bacterial regrowth in the distribution system (Page *et al.* 2002, Drikas *et al.* 2003). The processes that determine the concentration and character of organic matter in lakes include, riverine transport, enzymatic degradation, microbial assimilation,

sedimentation and photo-degradation (Pers *et al.* 2001). The patterns of enzyme activity present and the patterns of substrate assimilation can provide information on the type of organic matter present in flowing water (Findlay *et al.* 1997). If the transport and distribution of organic matter can be described then the risks to the supply of potable water may be better understood.

It was hypothesised that a large inflow event intruding into a reservoir would result in the increase of dissolved organic carbon concentration proximal to the dam wall and that this threat to water quality would be presented at a similar timescale to that of microbial pathogens. Furthermore, while dilution associated with mixing would occur, an increase in DOC could be expected proximal to the water treatment offtake, sufficient to justify changes in the water treatment plant operations, such as increased coagulant dosing or tighter pH control.

Such an event should result in the influx and distribution of catchment derived microbial activity, followed by changes in the patterns of enzyme activity as the newly mixed autochthonous and allochthonous microbial communities adapt to the substrates that are available. The emerging microbial community function and physiology should reflect the conditions in the reservoir and provide information on the availability, persistence and succession of the different microbial substrates.

From published information on microbial enzymes in aquatic systems, β-glucosidase and leucine aminopeptidase are likely to be prominent in the reservoir (Chróst *et al.* 1989, Vrba *et al.* 1992) and chitinases and xylosidases to be relatively more active in catchment derived samples (Findlay *et al.* 2001). As there is generally a strong association between enzymatic activity and particles (Vrba *et al.* 1992, Hoppe *et al.* 1993, Chróst and Riemann 1994, Debroas 1998, Karrasch *et al.* 2004a) it will most likely result in the sedimentation of a proportion of the particle attached microbial community and its associated activity.

To investigate these issues a sampling program was conducted during a major rainfall driven inflow into Myponga Reservoir that occurred in June-July 2003. This inflow event was studied to determine the transport and metabolism of natural organic contaminants as they travel from the catchment, through the reservoir and towards the water treatment plant offtake. Concentrations of natural organic matter in catchment source streams and reservoir water were determined to assess their flux with inflow and reservoir water mixing. The character of the natural organic matter was studied using basic chemical methods. The influx and attenuation of microbial enzyme activity and physiological competency were measured and used to describe the changes in microbial processes and imply the changes in substrate availability and the composition of the organic matter.

3.2. Materials and Methods

3.2.1. Sampling and Instrumentation

An intensive sampling program was conducted over 6 days from 27 June 2003 (day 1) to 2 July 2003 (day 6), during and after a 60 mm rainfall event which resulted in a large inflow to Myponga Reservoir (~570 ML d^{-1} peak flow from the largest sub-catchment). Samples were collected from 5 creeks draining the 4 larger sub-catchments (1, 2, 3 and 7) immediately upstream of where they enter the reservoir (Figure 3.2). Samples were also collected from 3 locations (Location 1, Met 1 and Met 2) in the reservoir at two depths (surface and 2.5 m from the bottom) at each location (Figure 3.2). Over the 6 day period, each location was sampled between 5 and 8 times. An ISCO 3700 flow-dependant auto-sampler also collected a sample every 50 ML of flow at the v-notch weir in Myponga River. Flow from sub-catchment 2 (SC2) was measured using a Starflow Ultrasonic Doppler Instrument (Model 6526B, Unidata Pty Ltd, O'Connor, Western Australia, Australia) operated in paired stormwater pipes of known internal diameter. This catchment contributed flow to the reservoir proportional to its relative area (16.7% of catchment total, Linden *et al.* 2005) and forms a confluence with Myponga River (SC1) as these waterways

enter the reservoir. During the rain event there was channel restriction in SC2 and there was a back up of water and localised flooding, causing a delay in the flow peak from this catchment (Hipsey *et al.* 2005). In addition to sampling, multi-probe sonde (Seabird; conductivity temperature depth) profiles were conducted at 8 locations and 5 times during the inflow event. Six sets of triplicate sediment traps (see Baker *et al.* 1999 for design details) were deployed on 25 June 2003 and were harvested on 2 Jul 2003. They were deployed at 2.5, 5.0, 10.0, 15.0, 30.0 and 33.0 m depths at the locations shown in Figure 3.2, corresponding to 1.0 m above the reservoir benthos. Particle size discrimination between 2-500 μm was undertaken with vertical profiles conducted at the same time points as the multi-probe sonde (Laser In-Situ Scattering and Transmissometry, LISST-100, Sequoia Instruments, Bellevue, Washington, USA).

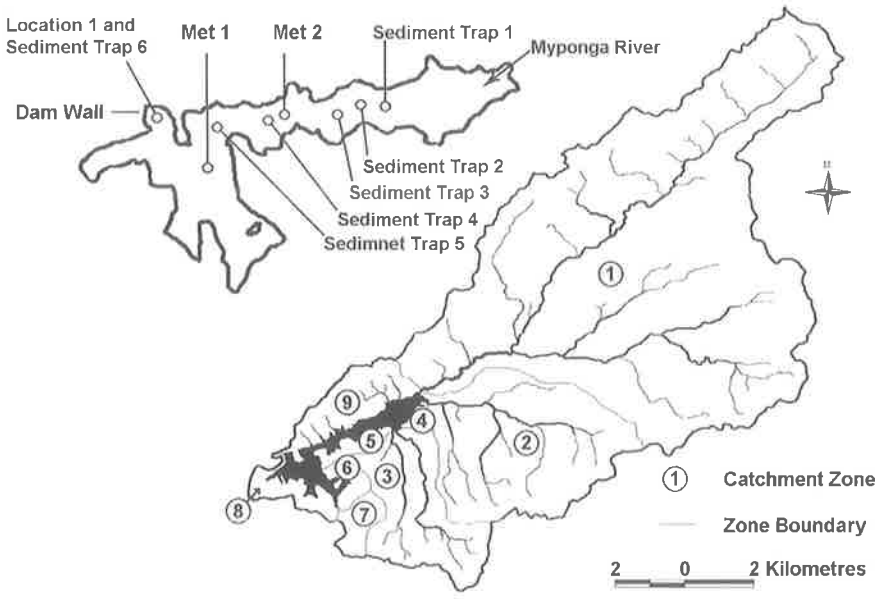


Figure 3.2. Location map showing sampling points and sediment trap locations.

3.2.2. Modelling

The three dimensional hydrodynamic model ELCOM was applied during this experiment to model the fate and transport of carbon. ELCOM is a three dimensional z-coordinate hydrodynamic model, that can simulate the spatial and temporal variation of temperature and salinity. Water movement velocities are resolved using the solution of the Reynolds-averaged Navier-Stokes equations. ELCOM employs a mixed layer turbulence closure

scheme and a mixing-model to directly compute vertical turbulent transport, making it particularly applicable to stratified systems such as lakes and reservoirs. A detailed description of the hydrodynamic model structure and validation can be found in Hipsey *et al.* (2005). Dissolved organic carbon transport was modelled by incorporating DOC into the model as a conservative tracer. The DOC concentration at the beginning of the experiment was set to the average of the unaffected values (see results below) and the monitoring data for Myponga River was used for the inflowing concentration. The model assumes there is no loss of DOC to mineralisation, flocculation or sedimentation and carbon flux from the other sub-catchments was not considered.

3.2.3. Analyses

Each water sample was analysed for DOC, true colour, UV absorbance, turbidity, microbial enzyme activity and microbial community level physiological profiles (see §2.3). Additionally, microbial enzyme activity and microbial community level physiological profiles were determined on selected water samples post filtration, through GF/C or 0.45 μm membranes. Microbial enzyme assays were performed on single samples only, due to the large number of samples analysed. Generally the standard deviation of this method is ± 20%. Water samples collected from Myponga River with the automatic sampler were also analysed for total volume concentration (TVC) of particles using a Laser In-Situ Scattering and Transmissometry instrument fitted with the full path mixing chamber to allow laboratory use (LISST-100, Sequoia Instruments, Bellevue, Washington, USA). Volatile and non-volatile solids were determined from the material collected from the sediment traps, and expressed on an areal basis. Microbial enzyme activity of the sediment trap contents was also assayed.

3.3. Results

3.3.1. Hydrodynamics

The largest volume of rain fell between 0800 hrs on 26 June 2003 and 0800 hrs on 27 June 2003 with a smaller volumes falling on the following days (Figure 3.3). The reservoir level rose 0.8 m over four days (Figure 3.3). River flow measured at the Myponga River v-notch weir had a peak flow of 9.54 m^3s^{-1} (Figure 3.5A). This inflow event represents a large (>500 ML day^{-1}), but not unusual, event for Myponga Reservoir; an inflow of this magnitude occurred 16 times between 1991 and 2000 (Linden *et al.* 2005). The water temperature during the inflow event is presented as a series of time points of a longitudinal cross section of the reservoir and shows that the cool, dense underflow reached the dam wall by around 1200 hrs on 28 June 2003 (Transect 3, Figure 3.4). This represents a 36 hour travel time from when the gauge height of Myponga River began to rise at 0000 hrs 27 June 2003. This is a total time of 39 hours from when it started to rain heavily in the Myponga district to when the riverine intrusion reached the dam wall.

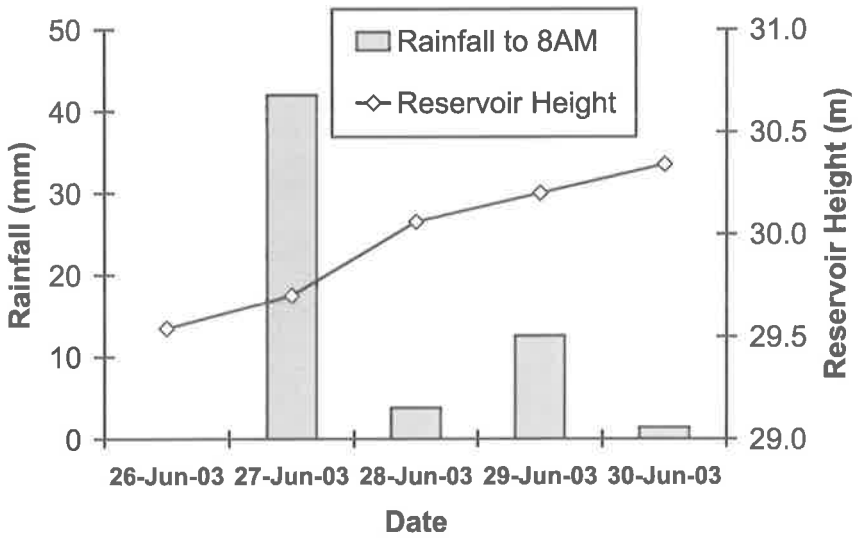


Figure 3.3. Rainfall to 0800 hrs and reservoir gauge height recorded from 26 June to 30 June 2003.

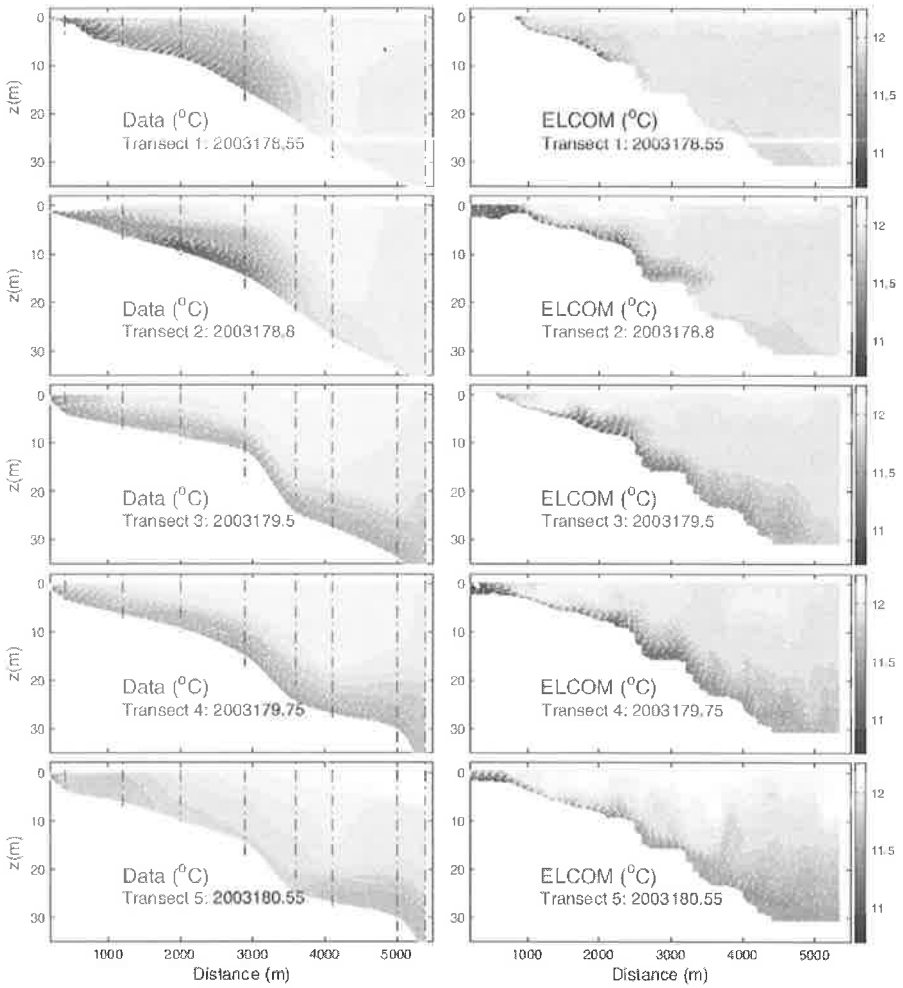


Figure 3.4. Temperature data collected during the inflow event showing riverine intrusion penetrating the reservoir to the dam wall and simulation results from ELCOM, from Hipsey *et al.* (2005). Transect 1: 2003178.55 is 1312 hrs 27 June 2003, Transect 2 is 1912 hrs 27 June 2003, Transect 3 is 1200 hrs 28 June 2003, Transect 4 is 1800 hrs 28 June 2003, Transect 5 is 1312 hrs 29 June 2003. Vertical dot dash lines indicate locations of temperature profiling.

3.3.2. DOC, Conductivity, Turbidity, Colour and UVA

The concentration of DOC in Myponga River decreased slightly during peak flow rates and increased to 28.5 mg L^{-1} after the flow had subsided (Figure 3.5B). Colour and UV absorbance in Myponga River co-varied and reached maxima of 220 HU and 1.1 cm^{-1}, respectively (Figure 3.5C). The base flow conductivity of Myponga River was between 700 and 800 µS cm^{-1} and dropped to ~ 400 µS cm^{-1} at the peak flow before steadily increasing to ~ 550 µS cm^{-1} by the end of sampling (Figure 3.5B). The turbidity in Myponga River increased as the river started to flow and reached a peak of almost 30 NTU. As the flow subsided so did the turbidity, falling to 13 NTU by 2 July 2003 (Figure

3.5A). Initial values of SUVA, SpHU and TVC were approximately 3 L mg^{-1} m^{-1}, 4.6 HU L mg^{-1} and 50 µL L^{-1}, respectively (Figure 3.5D). SUVA increased to approximately 4 L mg^{-1} m^{-1} and SpHU increased to approximately 7 HU L mg^{-1} as the hydrograph peaked and remained at these levels for a number of days. The increases in TVC were more variable, but local maxima of approximately 170, 150 and 200 µL L^{-1} occurred coincident with the rising hydrograph, the peak in turbidity and the small peak in the falling limb of the hydrograph on 30 June 2003, respectively (Figure 3.5D).

The DOC concentration in the smaller sub-catchments followed a similar pattern to SC1, but with slightly higher initial DOC concentration (Figure 3.6A) and similar patterns were observed in the UV absorbance and true colour parameters (Figure 3.6B & C). Based on a simple numerical integration (area under the curve) of flow rate and DOC concentration, this inflow event transported approximately 9.3 metric tonnes of dissolved organic carbon into Myponga Reservoir in the first 24 hours. In the period from 0000 hrs 27 June 2003 to 0000 hrs 2 July 2003 approximately 30.4 tonnes of organic carbon were transported, in the dissolved phase, from sub-catchment 1 into Myponga Reservoir.

The dilution and mixing of this large amount of organic carbon into the reservoir is apparent. At the location closest to the inflow, Met2, the organic matter dynamics were variable (Figure 3.7C). The data suggests that the inflow had already reached this location by the time the first samples were collected, as large differences between surface and bottom samples were already observed (difference in true colour = 18 HU; DOC = 1.6 mg L^{-1}; UVA = 0.057 cm^{-1}). At Met2 the mixing of the inflowing water with the surface water is observed as an increase in the organic matter parameters at the surface (Figure 3.7C). In the deeper main basin of the reservoir (Met1, 35 m depth vs. Met2, 15 m depth) little increase is observed in the surface waters, demonstrating that the mixing of the intrusion with the surface waters has not reached this high in the water column (Figure 3.7A & B). Subsequently, a decrease in the magnitude of all organic matter parameters was observed

in the bottom waters, presumably due to dilution by mixing of the intrusion with surface water. The greatest differences that were observed were true colour, 12 HU; DOC, 0.9 mg L^{-1}; UVA, 0.044 cm^{-1} (Figure 3.7B). The continued dilution of the intrusion is observed at the dam wall, with smaller differences in the peak concentrations and no change in the organic matter parameters at the surface (true colour = 12 HU; DOC = 0.73 mg L^{-1}; UVA = 0.024 cm^{-1}; Figure 3.7A).

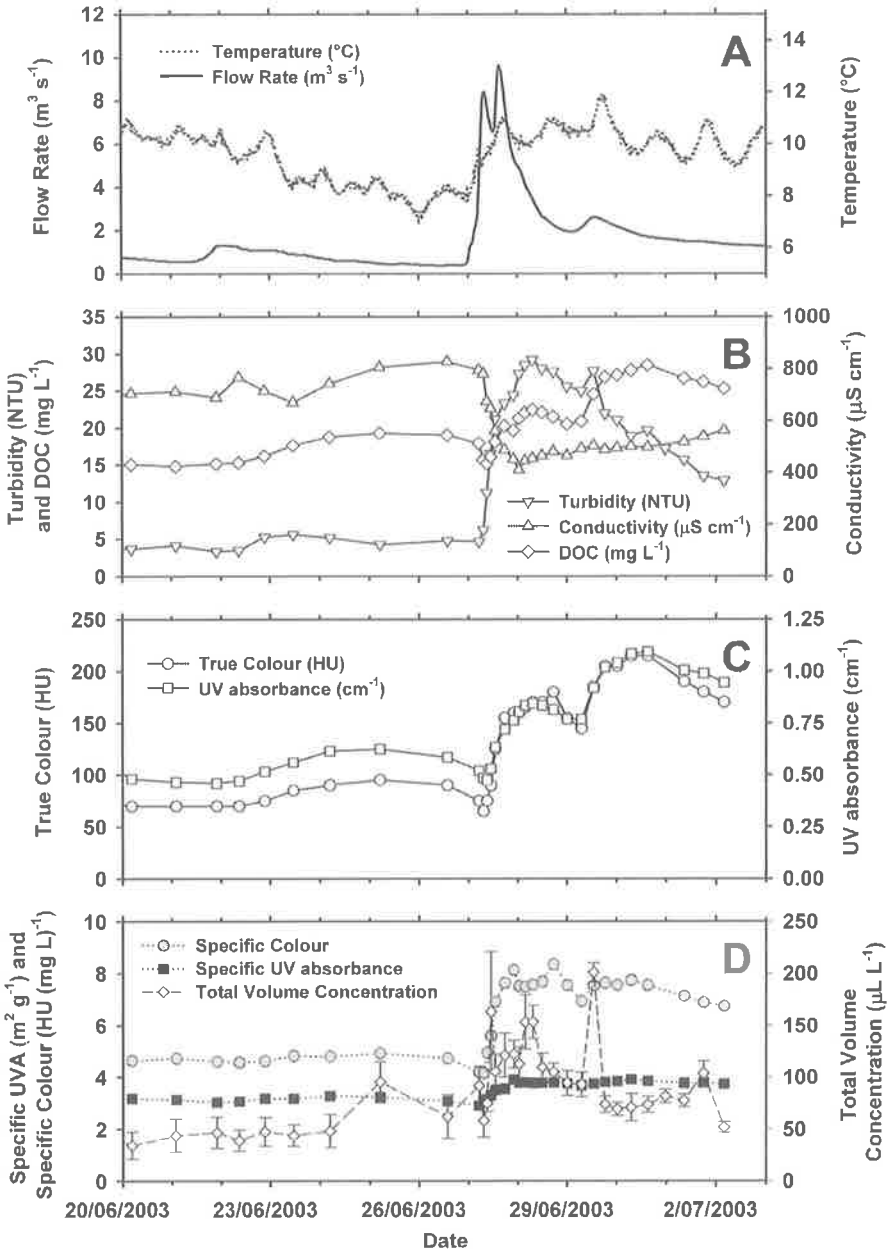


Figure 3.5. Water quality and quantity at the Myponga River v-notch weir monitored using a flow triggered auto-sampler. Samples were collected every 50 ML of flow. A) Flow rate (m^3 s^{-1}) and water temperature (°C). B) Dissolved organic carbon (mg L^{-1}), turbidity (NTU) and conductivity (µS cm^{-1}). C) True colour (HU) and UV absorbance (cm^{-1}). D) Specific UV absorbance and specific colour and average total volume concentration (µL L^{-1} ± SD determined within each single sample).

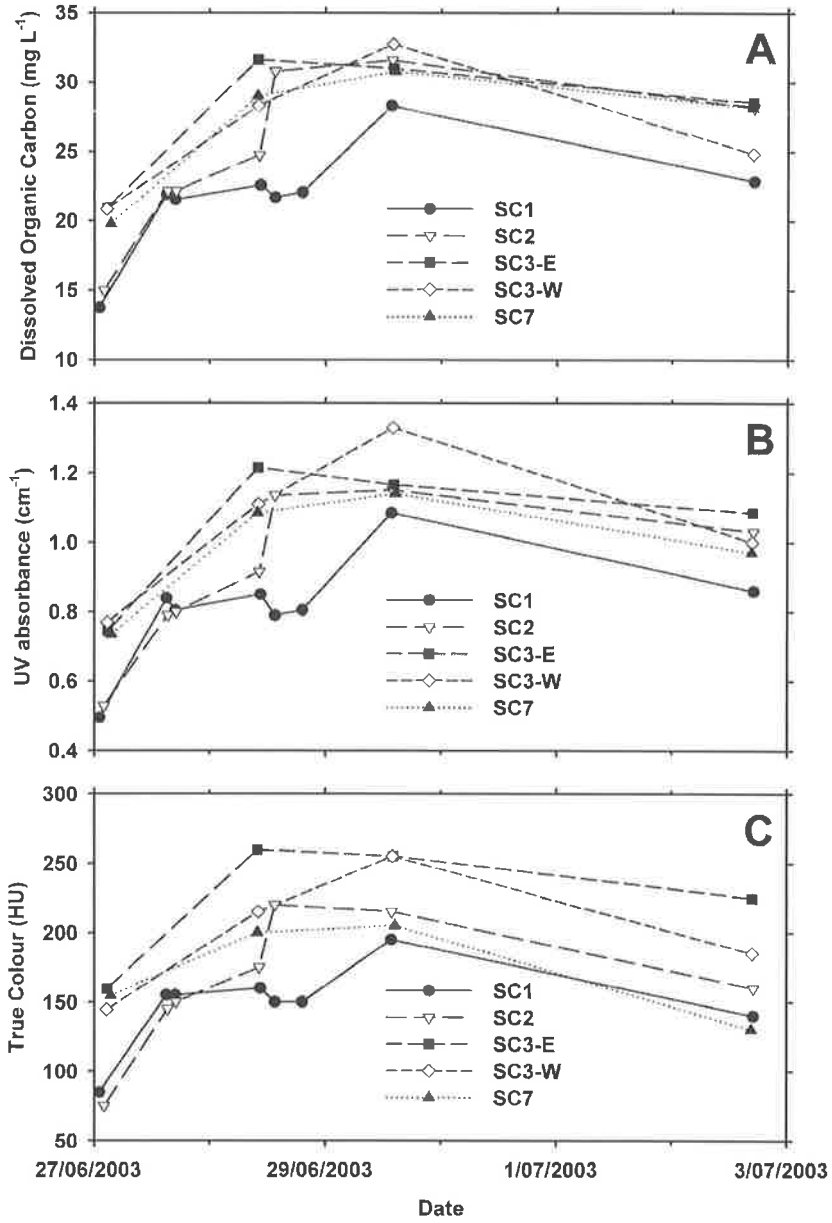


Figure 3.6. Changes in the A) dissolved organic carbon concentration (mg L^{-1}), B) UV absorbance (cm^{-1}) and C) True colour (HU) found in water samples collected from 4 different sub-catchments of the Myponga Reservoir catchment, notated SC1, 2, 3 (East and West) and 7 see Figure 3.2.

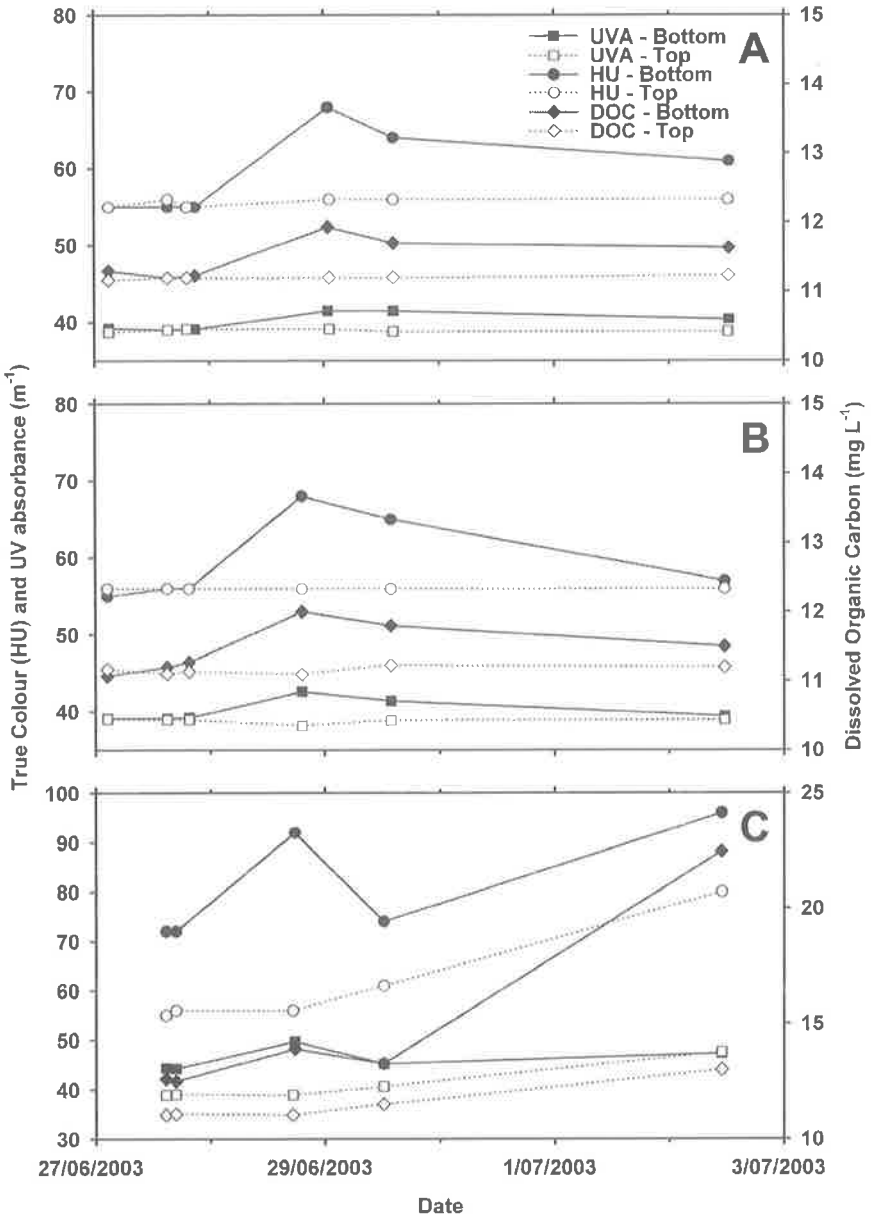


Figure 3.7. Dissolved organic carbon (mg L^{-1}), UV absorbance (note m^{-1}) and true colour (HU) found in water samples collected from A) the dam wall (Location 1), B) meteorological station 1 and C) meteorological station 2, at the reservoir surface (0.5 m depth) and the bottom (2.5 m above the benthos).

3.3.3. Modelling: Temperature and DOC

The simulation of temperature and therefore water mixing and movement was considered to be modelled adequately (Hipsey *et al.* 2005). It is possible that there was greater initial mixing at the point of inflow than captured by the model, as indicated by the vertical temperature gradient proximal to the inflow in transect 1 (Figure 3.4). The simulation of the transport and dissipation of the DOC into the reservoir used DOC data from Myponga

River as the initial input (Figure 3.8A). At Met2, the model initially over estimated the DOC concentration at the bottom but predicted the DOC concentration well at 1130 hrs 2 July 2003 (Figure 3.8B). DOC concentrations in the bottom waters at Met1 were over predicted after the inflow had reached that location (Figure 3.8C). Based on the DOC concentration data, the time of arrival of the intrusion front at the dam wall may have been slightly late, however the DOC concentration was over predicted after the arrival of the intrusion (Figure 3.8D). The discrepancies observed in the prediction of DOC concentration are either the product of error in the mixing dynamics or are artefacts of the DOC dynamics that were not included in the model. These would include, the source of DOC being limited to one sub-catchment, the adsorption of dissolved organics to particles and the flocculation and aggregation of particles and the subsequent settling of these aggregates and the metabolic activity of micro-organisms. It is unlikely that these discrepancies can be satisfactorily attributed to metabolic activity at this timescale and temperatures, and it is more likely that they are the product of chemical and physical processes (sorption, coagulation and settling). It is possible that some of these processes may have occurred in the 500m of waterway between the v-notch weir where the samples were collected and the inflow to the reservoir.

The movement of the riverine intrusion and the increase in DOC concentration along the reservoir thalweg (line connecting lowest points of the valley) can be visualised by longitudinal sections of the reservoir at 24 to 48 hour intervals (Figure 3.9A-L). The initial pre inflow conditions are shown in panel A, and the development of the initial intrusion proceeds through to panel E where it has reached the dam wall (Figure 3.9). In panels E through G a secondary inflow of high DOC water generated by the increase in DOC concentration in Myponga River can be observed, after the passage of the main flow peak (see Figure 3.5). This secondary intrusion of higher DOC water then contributed to the

bottom waters of the reservoir and it was predicted that this body of higher DOC water persisted until 9 July 2003 (Figure 3.9G-L).

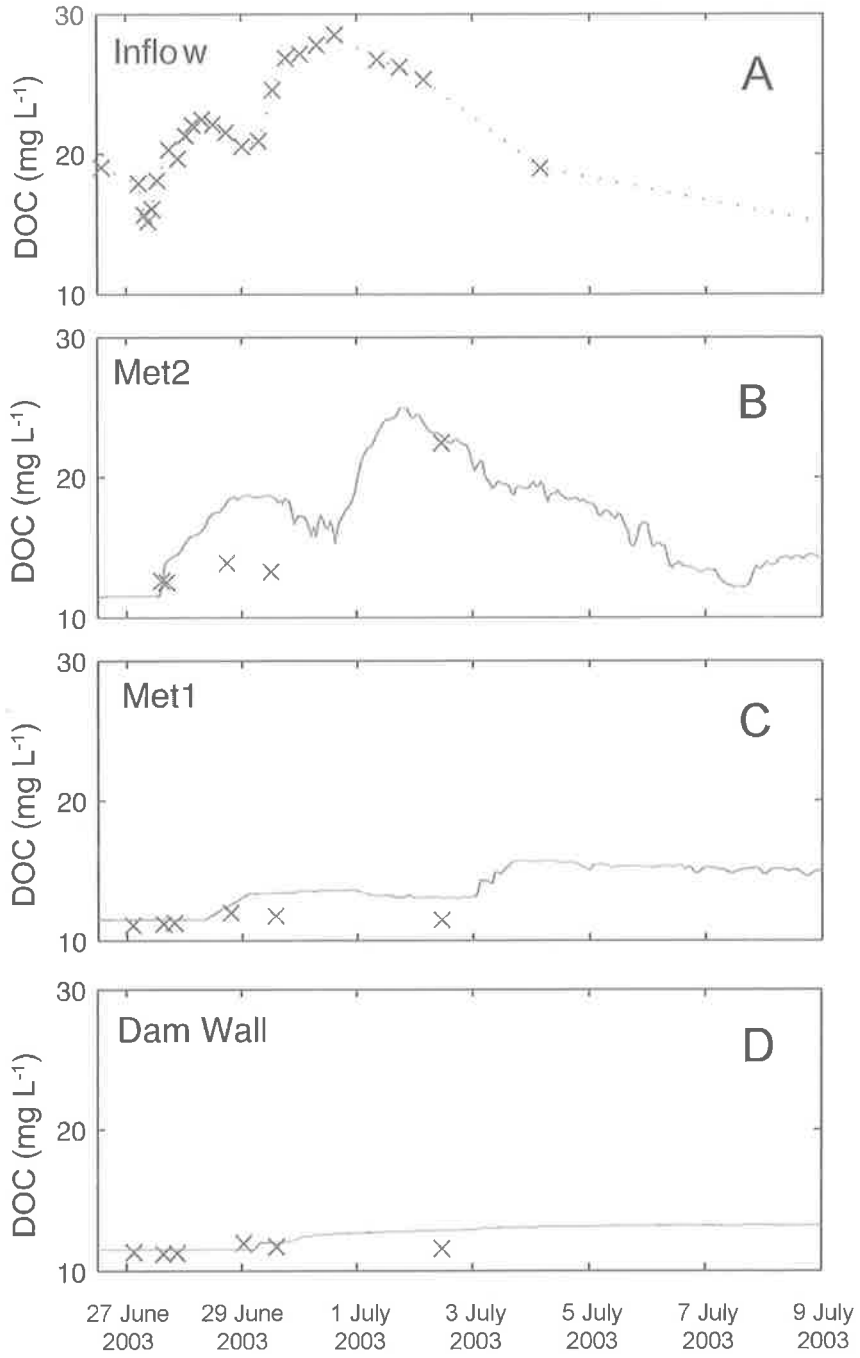
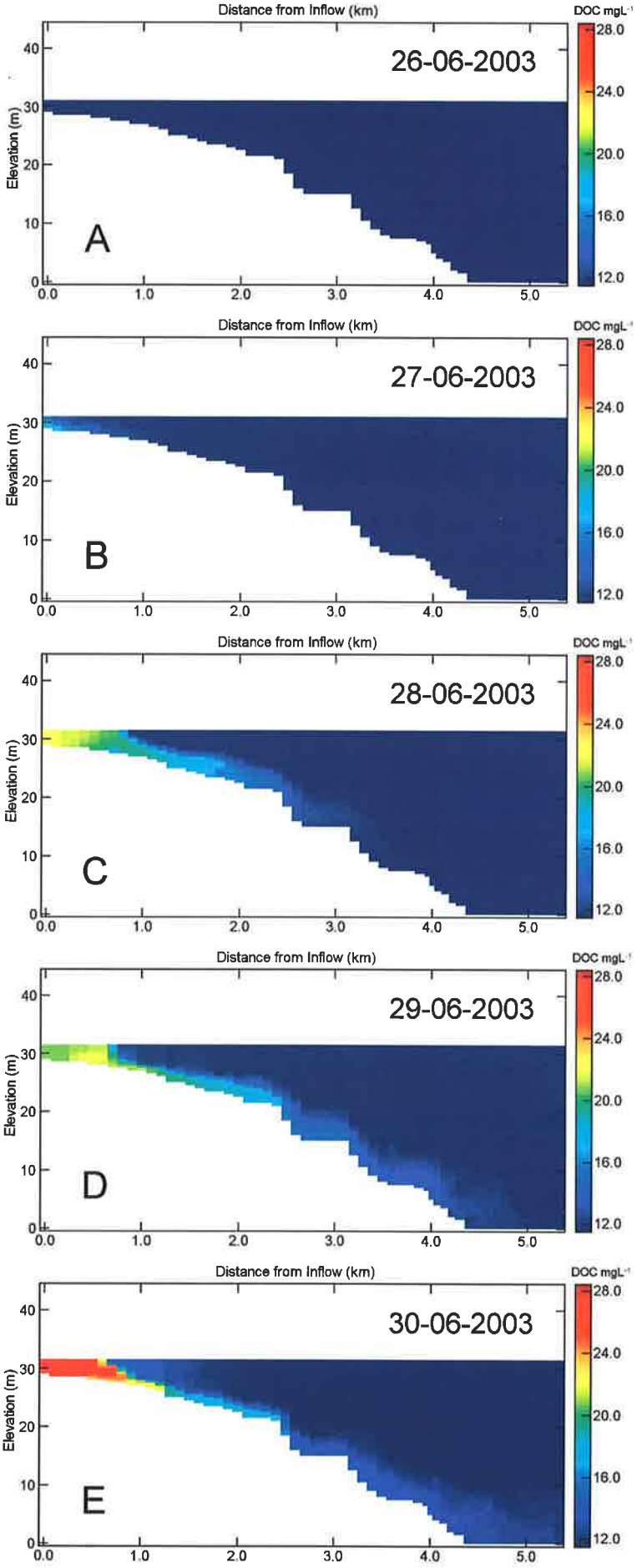
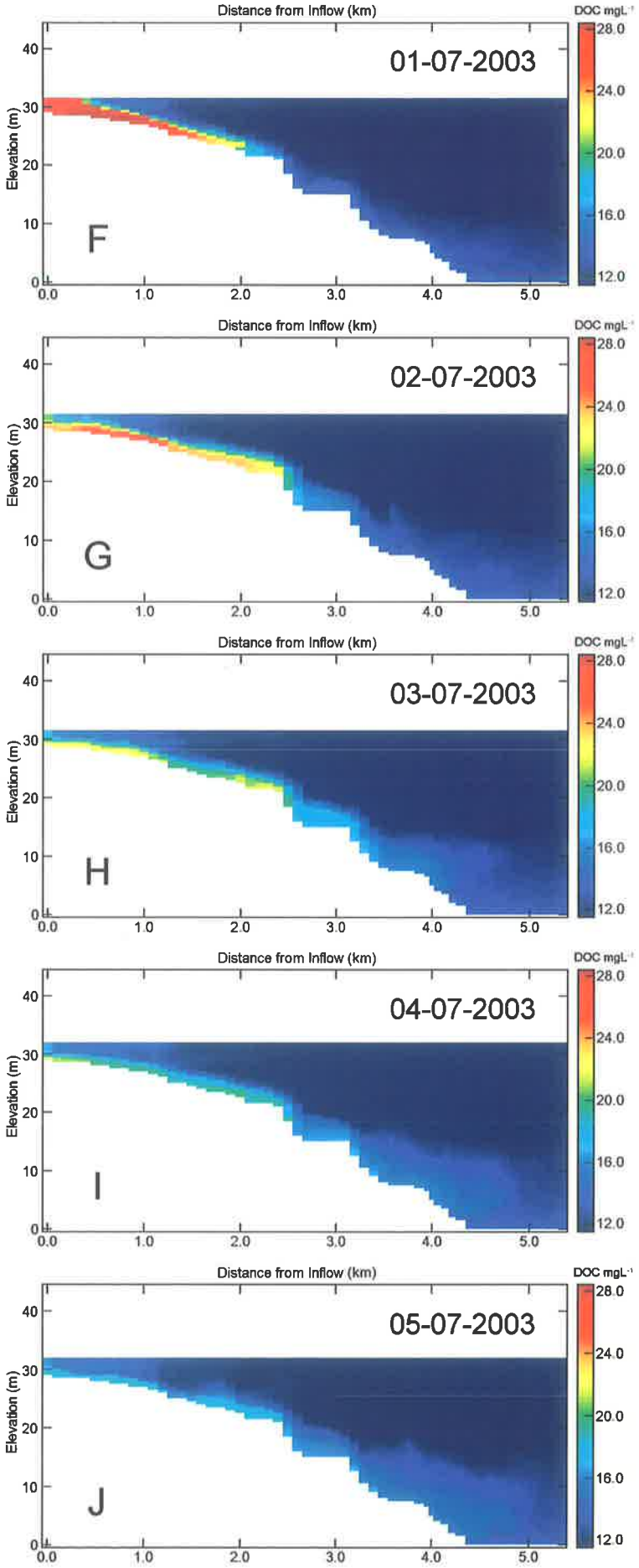


Figure 3.8. Simulation results from ELCOM for dissolved organic carbon dissipation in Myponga Reservoir. A) DOC data (crosses) and interpolation (dotted line) used to force ELCOM predicted DOC concentration in the reservoir at B) Met2, C) Met1 and D) the dam wall. In panels B, C and D, simulation results are shown as a line and measured DOC concentration as crosses.


Distance from Inflow (km)
DOC mgL-1
26-06-2003
A
27-06-2003
B
28-06-2003
C
29-06-2003
D
30-06-2003
E
Elevation (m)
0
10
20
30
40
0.0
1.0
2.0
3.0
4.0
5.0
28.0
24.0
20.0
16.0
12.0


Distance from Inflow (km)
DOC mgL⁻¹
28.0
24.0
20.0
16.0
12.0
Elevation (m)
0
10
20
30
40
0.0
1.0
2.0
3.0
4.0
5.0
01-07-2003
F
02-07-2003
G
03-07-2003
H
04-07-2003
I
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J

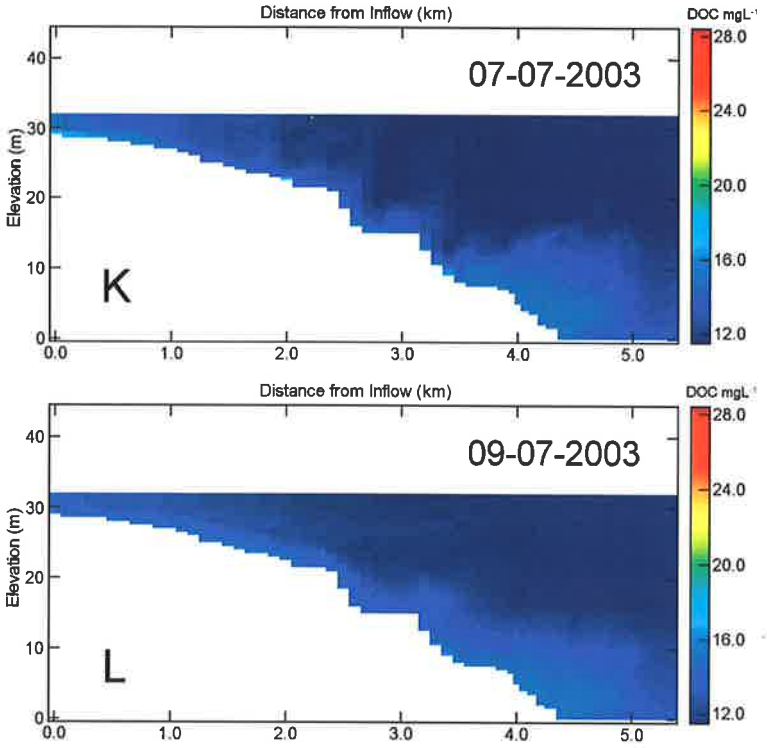

Figure 3.9. ELCOM predicted movement of DOC down the longitudinal axis of Myponga Reservoir. Each longitudinal section is at 0400 hrs on the date give in the top right hand corner of the plot. Met2 and Met1 are at approximately 2.5 and 4.5 km distance from inflow, respectively.

3.3.4. Sediment Traps

The material collected in the sediment traps contained more inorganic than organic material (combustible at 550 °C) at all locations. The rates of deposition of inorganic solids was similar over the longitude of the reservoir, with the only significant difference between sites being between sediment traps 1 and 4 (ANOVA and Tukey-Kramer HSD post test, $\alpha = 0.05$; Figure 3.10). There was no significant differences in the rates of deposition of organic matter between sites. A weak trend in the ratio of inorganic to organic solids was observed with the ratio increasing with distance from the Myponga River inflow (Ratio I:O = 0.48 km + 5.02; $r^2 = 0.27$; $\alpha = 0.038$; N = 16). This may be the result of the resuspension of reservoir sediments of different composition, rather than the differential deposition of organic over inorganic matter.

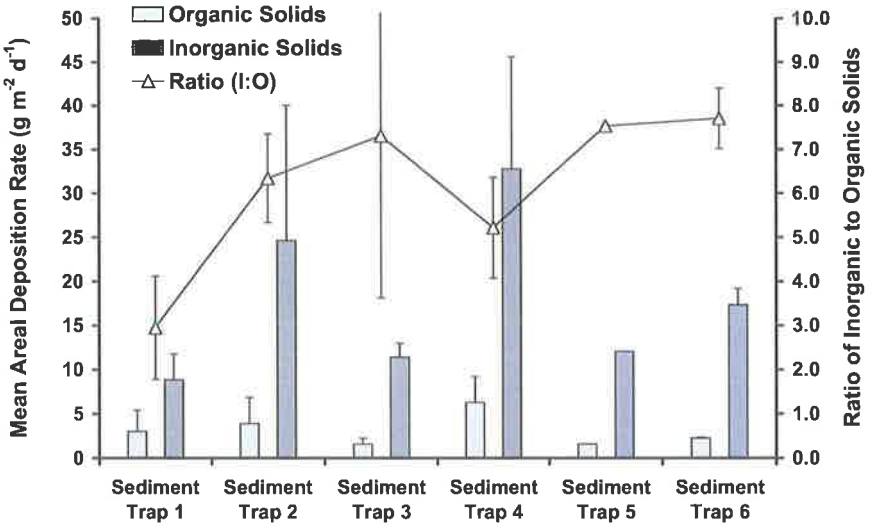

Figure 3.10. Mean sedimentation rates of volatile solids (organic matter) and non-volatile solids (inorganic matter) measured using sediment traps in triplicate at 6 locations in Myponga Reservoir over the period of one week from 25 June 2003 to 2 July 2003 during the inflow event experiment. Error bars indicate one standard deviation of the mean areal rate determined from each set of three traps. Error bars are absent from trap 5 as only two traps were recovered. The absolute differences in the results for duplicate traps for sediment trap 5 were 14 and 9 % of the mean for organic and inorganic solids, respectively. Sediment trap contents were collected on a precombusted GF/C filter paper and volatile solids determined gravimetrically by combustion at 550°C for 1 hour.

3.3.5. Microbial Enzyme Activity

3.3.5.1. Catchment

Alkaline phosphatase (AKP), β-glucosidase (BGL), esterase (EST), and leucine aminopeptidase (LAP) were generally found at an order of magnitude higher activity than α-glucosidase (AGL), β-xylosidase (BXL) and chitinase (CHT) and are presented on different axes in the following figures. In SC1 (Myponga River) the peak activities were generally found in the second sample collected at 1650 hrs 27 June 2003 (day 1). The exceptions are AKP and EST, which had maxima in samples collected on 28 June 2003 (day 2). Microbial activity seemed to follow the pattern of turbidity (see Figure 3.5B) and all enzyme activities assayed were positively correlated with turbidity determined in concurrently collected samples (Spearman rank correlations; Table 3.1). Correlation of microbial activity with conductivity was generally negative, while interestingly, high

correlations with the average and standard deviations of total volume concentration were observed; three of seven enzyme activities were strongly correlated (Prob > |Rho| > 0.05) with the standard deviation of the total volume concentration determined with the LISST profiler. A higher standard deviation of the volume concentration determination suggests that, given that the volume of smaller particle remains similar, that there is larger particles included in the sample, and hence the variation in size is greater. This in turn implies that these larger particles are important sites for the production of microbial enzymes.

The dissolved organic matter character parameters (UV absorbance, true colour and dissolved organic carbon) were not consistently correlated with microbial enzyme activity. This suggests that at the timescale of a flow event that the activity of the microbial population in the flowing water was determined by the properties of the environment of their origin or the particle microenvironment rather than the properties of the water matrix.

Table 3.1. Spearman Rank correlation (Rho) of microbial enzyme activity with water parameters measured in Myponga River (N = 7). Parameters with a Probability > |Rho| of 0.05 are shaded.

	AGL	*AKP*	*BGL*	*BXL*	*CHT*	*EST*	*LAP*	*Sum EA*
Turbidity (NTU)	0.52	0.87	0.23	0.11	0.31	0.65	0.41	0.56
Conductivity ($\mu S\ cm^{-1}$)	-0.72	-0.78	-0.20	-0.32	-0.52	-0.84	-0.67	-0.74
Total Volume Concentration – Average	0.61	0.38	0.74	0.32	0.41	0.40	0.45	0.63
Total Volume Concentration – StDev	0.55	0.07	0.77	0.90	0.85	0.15	0.70	0.59
UVA(cm^{-1})	-0.37	0.04	-0.38	-0.72	-0.59	-0.16	-0.52	-0.34
Colour (HU)	-0.07	0.36	-0.26	-0.63	-0.52	0.24	-0.33	-0.11
DOC (mgL^{-1})	-0.37	0.04	-0.38	-0.72	-0.59	-0.16	-0.52	-0.34

The microbial enzyme activity in SC2 followed a similar trend to SC1, but had an overall higher activity. This is interesting as it had similar concentrations of organic matter throughout the inflow event (Figure 3.6). In SC3 (E and W) the highest activities were found in samples collected on 27 June 2003 (day 1). This may reflect the smaller catchments shorter hydraulic retention times and the earlier onset of flow in these catchments. Generally, microbial enzyme activity decreased between 28 June 2003 (day 2) and 2 July 2003 (day 5) in the smaller sub-catchments. For example, in SC7 LAP

decreased from ~26 to ~5 μmol L^{-1} h^{-1}. This probably reflects wash out of microbial activity from the catchments exceeding the rate of production of activity by the soil microflora.

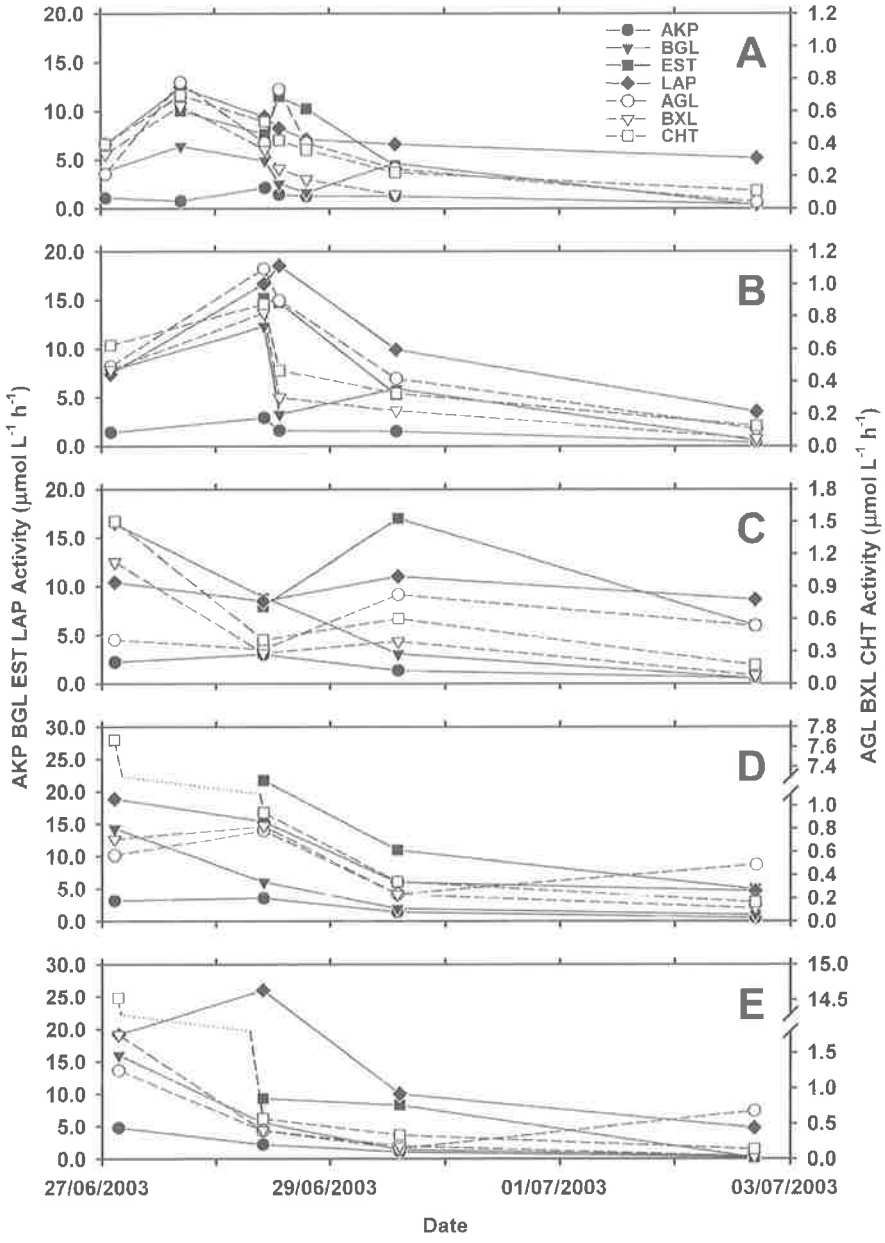

Figure 3.11. Microbial enzyme activity detected in streams of the Myponga Reservoir catchment. A) Myponga River SC1, B) SC2, C) SC3-E, D) SC3-W and E) SC7.

3.3.5.2. Reservoir

Overall, microbial enzyme activity in the reservoir samples was lower than in the catchment samples. There was no detection of AGL activity in the surface water at Met 2 on 28 June 2003 (day 2) and the same sample had a much higher EST activity than on the

previous or following days (Figure 3.12A). AKP activity was low for all of the sampling period. AGL, BXL, CHT and LAP seemed to be increasing between the 29 June 2003 and 2 July 2003, possibly due to the influx of substrates derived from the catchment, while BGL, EST and AKP were unchanged or decreasing (Figure 3.12A). The samples collected from Met 2 at the bottom contained much higher absolute activities of all enzymes tested than at the surface during the inflow event (Figure 3.13A). EST was highly variable with a large peak on 28 June 2003 (day 2) and the maximum on 2 July 2003 (day 6). CHT seemed to peak with the front of the inflow, while LAP and BXL seem to be trending upwards after the inflow event (Figure 3.13A). The activities of BXL, CHT and LAP were higher than or equal to the activities detected in the catchment on this date (Figure 3.11A).

At Met 1, AGL activity was not detected in the surface samples collected the evening of 27 June (day 1) or on 28 June 2003 (day 2). BXL activity was only detected on the 27 June (2 samples) and CHT activity was very low during the entire period. A large EST activity peak was observed on 28 June 2003, while most other activities were approximately stable (Figure 3.12B). In the bottom water samples collected at Met1, the peak activities of CHT and EST were observed on 28 June 2003 (day 2; Figure 3.13B). These are the samples where the peak DOC concentrations occurred (Figure 3.7B). It is interesting to note that the peak activities of LAP and BGL occurred on 29 June 2003 (2.9 and 3.7 $\mu mol\ L^{-1}\ h^{-1}$ respectively; day 3; Figure 3.13B). Subsequently, while LAP activity decreased slightly to 2.6 $\mu mol\ L^{-1}\ h^{-1}$ BGL activity decreased substantially to 0.1 $\mu mol\ L^{-1}\ h^{-1}$.

It is most likely that the high EST and CHT activity found in SC3-W (Figure 3.11D) and the shorter routing distance from the inflow of this sub-catchment to Met1 has produced a temporal displacement in the peaks of the enzyme activities measured. The peaks in EST and CHT were observed at Met 2, however the LAP peak was less obvious. A similar trend of BGL peaking after AGL, CHT and EST was observed at location 1 (Figure 3.13C).

In surface samples collected at location 1, BXL activity was not detected, and AGL activity was only detected on 29 June 2003. The large peak of EST activity that was observed at the other reservoir locations on 28 June 2003 (day 2) was observed early on 29 June 2003 at this location. CHT activity was low and decreased over the period (Figure 3.12C). In samples collected from the bottom, peaks in CHT and EST activity were observed at 0050 hrs on the 29 June 2003 (day 3). The peak in BGL activity followed in the next samples collected at 1430 hrs on 29 June 2003, similar to that found at Met 1. LAP activity also followed a similar trend to Met 1 with a rise over the period to a stable level approaching 2.9 μmol L^{-1} h^{-1} (Figure 3.13C). The stabilisation of LAP activity at an elevated level of around 3 μmol^{-1} L^{-1} h^{-1} is consistent with the continued supply of this enzyme activity at approximately 5, 4, 8, 5 and 5 μmol^{-1} L^{-1} h^{-1} from sub-catchments 1, 2, 3-E, 3-W and 7, respectively.

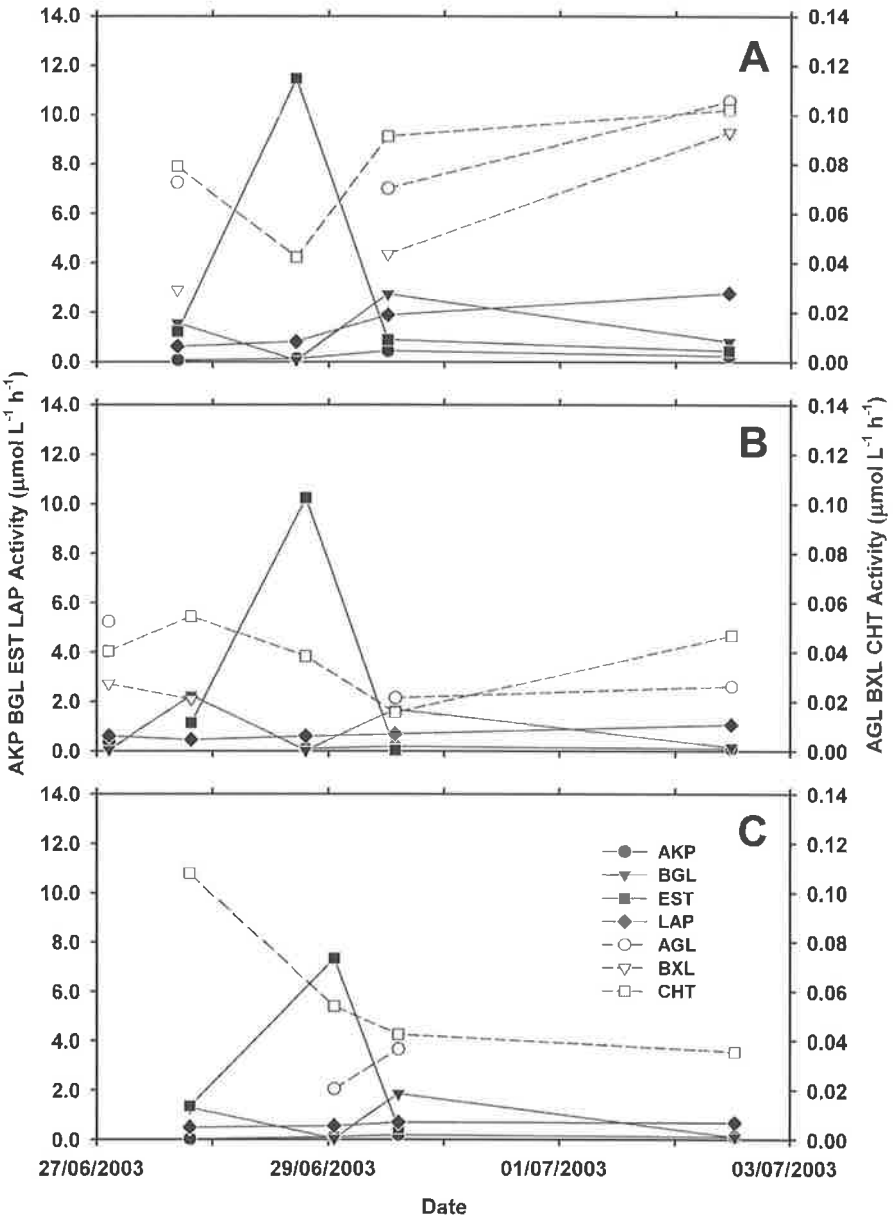

Figure 3.12. Microbial enzyme activity found in samples collected from the surface of Myponga Reservoir at A) meteorological station 2, B) meteorological station 1 and C) location 1. Samples were collected at 0.5 m depth.

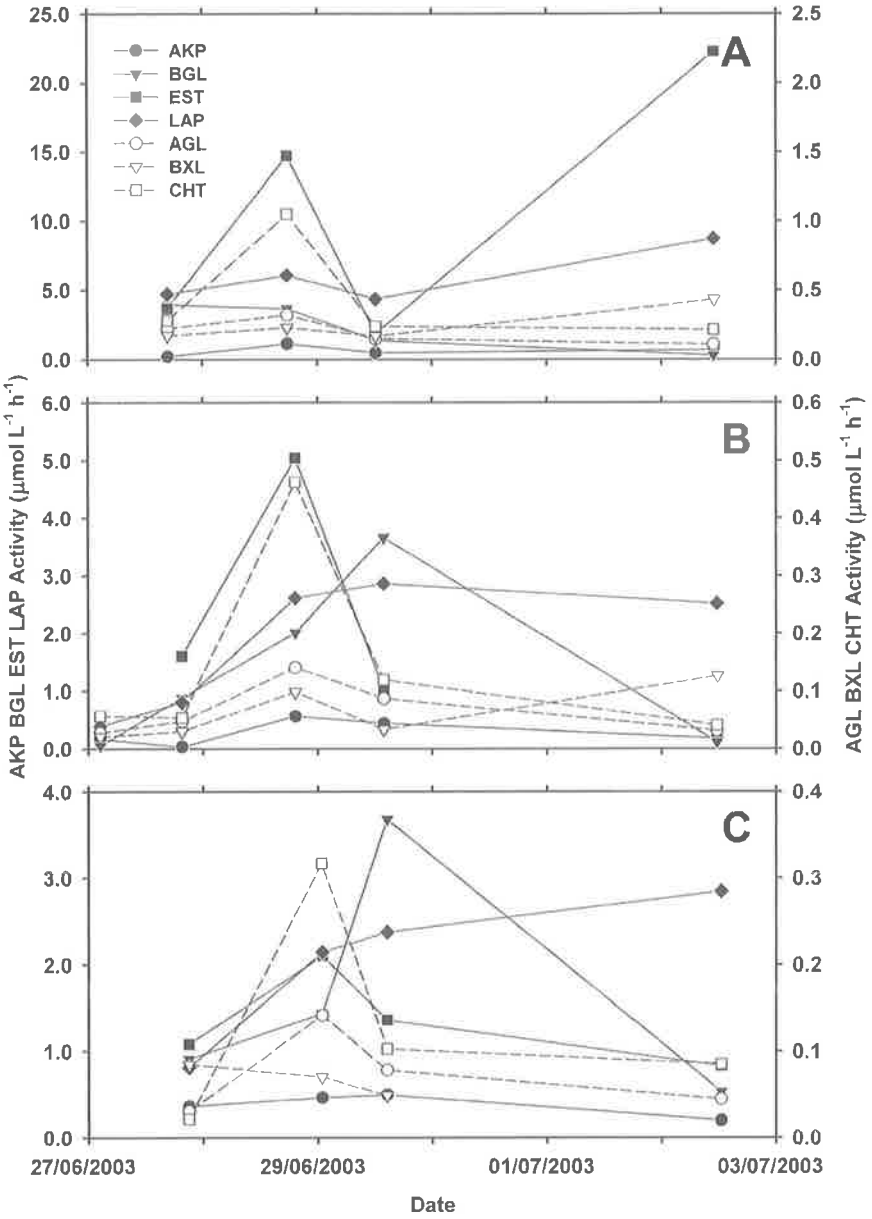

Figure 3.13. Microbial enzyme activity found in samples collected from the bottom of Myponga Reservoir at A) meteorological station 2, B) meteorological station 1 and C) location 1. Samples were collected from 2.5 m above the benthos.

3.3.6. Enzyme Activity – Particle Size Distribution

Examining the distribution of enzyme activity around a 1.0 µm size fractionation (GF/C; nominal), it seems that a greater percentage of the activity is found in the filtrate in samples collected from the reservoir than the catchment. At this size fractionation only BGL stands out as especially distributed in the greater than 1.0 µm fraction (Figure 3.14). In the 0.45 µm filtrate of samples collected from the catchment, AGL, BGL, BXL and

CHT were strongly attenuated and therefore, these enzymes seem to be strongly associated with the retentate (Figure 3.14). Partitioning of AKP to filtrate has been observed by other researchers (Münster 1994). The persistence of LAP activity in the reservoir over the other enzymes may also be the result of reduced losses to settling, due to its association with smaller particles with slower sedimentation rates.

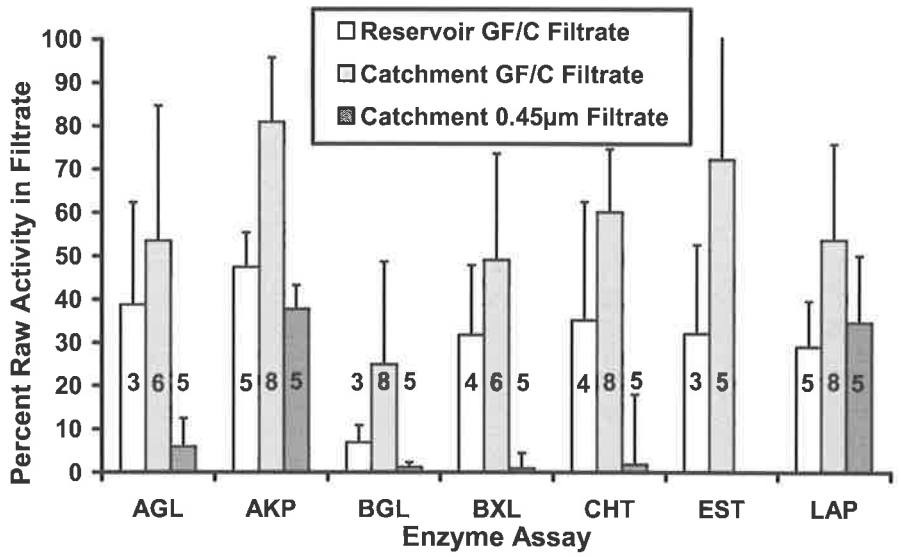

Figure 3.14. Distribution of enzyme activity amongst filtrate of GF/C (~1.0µm) and 0.45 µm membrane filters. Error bars are standard deviation (N given as data labels)

3.3.7. Sediment Trap Enzyme Assays

The sedimentation rate of the seven enzymes determined is based on the assumption that there was little change in the activity within the sediment traps. This is almost certainly an invalid assumption, but is necessary as no information on the change in activity with time was collected. Any response of the settling microbial community is however likely to further reflect the nature of the microbial substrates contained in the sediment trap. AGL, BGL, CHT and EST had strong linear regressions with negative slopes with distance from the main inlet of SC1 (r^2 = 0.93, 0.70, 0.89 and 0.66, respectively) and with the depth of the trap (r^2 = 0.94, 0.85, 0.93 and 0.88, respectively). AKP had strong linear regressions with the rate of both organic and inorganic matter deposition (r^2 = 0.88 and 0.67). BXL and LAP had linear regressions with organic and inorganic matter deposition with r^2 values above 0.5, however the parameter estimates were not significant at $\alpha = 0.05$. The strong

regressions indicate that the enzyme activity found in the sediment traps was determined by both the physical properties of settling in the reservoir and the response of the community after settling. It is also interesting to note that the enzymes with the strongest regressions with reservoir morphology (distance from inflow and trap depth) were those with strongest partitioning to particles in size fractionated material (Figure 3.14). It also seems likely that during growth in the sediment traps that the microbial community has become phosphorus limited and has produced more AKP to access further phosphorus resources, in order to maintain growth.

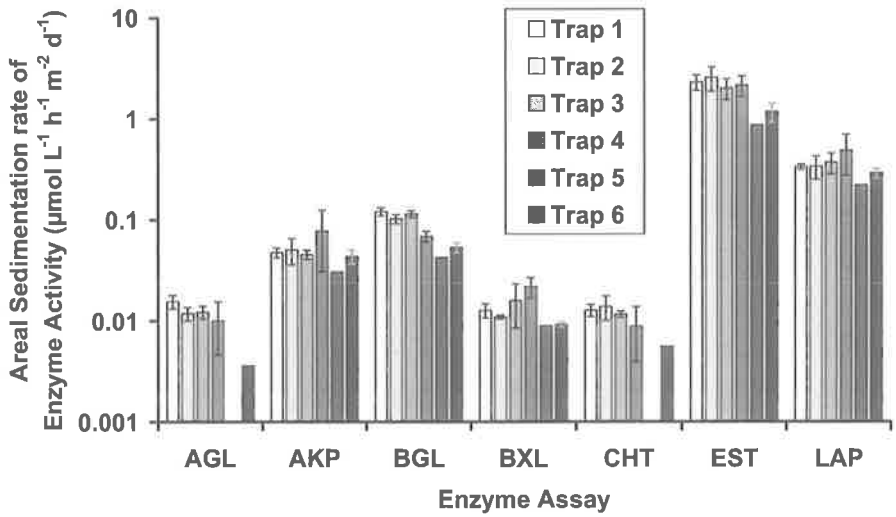

Figure 3.15. Areal sedimentation rate of enzyme activity measured in sediment traps deployed in Myponga Reservoir between 25 June 2003 and 2 July 2003. Calculations are based on the assumption that the activity found in the traps at the end of the period represented the integral of the sedimentation rates over the period.

3.3.8. Community Level Physiological Profiles

3.3.8.1. Substrate use Diversity

Community level physiological profiles (CLPPs) revealed that most substrates were utilised by all the microbial communities present in the samples collected. Most catchment samples were able to utilize all of the substrates present in the BIOLOG Ecoplates (76%), while only 35% of reservoir samples were able to do so. Of these, no microbial community contained in a water sample collected from the surface of the reservoir was able to utilise

all of the substrates presented. The substrates that were not utilised were 2-hydroxy-benzoic acid, α-ketobutyric acid, L-threonine and phenylethylamine, which were utilised in 0, 62, 69, 85% of surface samples respectively. As such, the substrate utilisation diversity (Magurran 1988, Zak *et al.* 1994) was significantly lower in surface waters compared to catchment and reservoir bottom samples (Figure 3.16; Tukey Kramer HSD; $\alpha = 0.05$). While it can be seen that a large number of substrates can be utilised by the microbial communities present, this type of analysis does not compare the qualitative responses of the different communities where significant variation may be found.

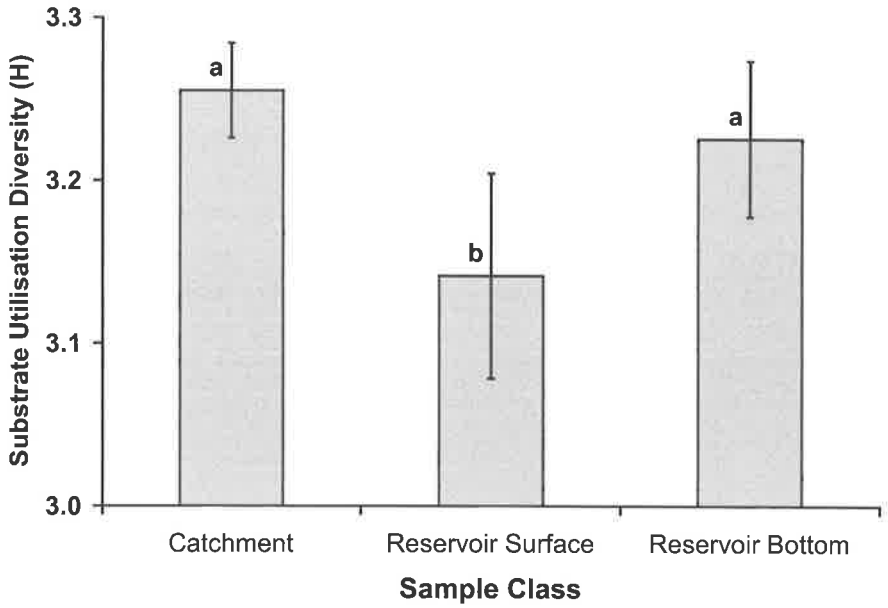

Figure 3.16. Substrate utilisation diversity in samples collected during the inflow event. Labels denote data means which are significantly different using Tukey-Kramer HSD test ($\alpha = 0.05$). Catchment N = 17; Reservoir Surface N = 13; Reservoir Bottom N = 13.

3.3.8.2. Principal Components of Substrate Responses

Principal component analysis of the substrates responses shows the calculated primary and secondary principal components (PCs) account for 46.6% of the variation in the data set (Figure 3.18A). The relationships between the samples and the changes in the metabolic competence of the microbial community is shown more clearly by examining the PC scores of the substrate responses, separated by the days they were collected. On the 27 June 2003 (day 1) there was complete separation of the catchment and reservoir samples,

apart from the single sample collected from Met 2 at the bottom, which had already mixed with catchment water at this time (Figure 3.17B). Here, two regions are defined, labelled C1 and C2, which contain the catchment samples collected between 0100 and 0330 hrs and at approximately 1700 hrs, respectively. These groups can be considered to be pre- or early-flow communities (the gauge height of Myponga River had risen only 0.19 m at the time of sampling) and maximum flow communities (the gauge height at 1700 hrs was 2.91 m, similar to the maximum of 2.98 m). On 28 and 29 June 2003 a tight group of catchment and reservoir bottom samples was observed in a similar position on the PC 1 and PC 2 axes to the C2 group (Figure 3.17C). Furthermore, the filtered catchment samples are closely associated with the C1 group from 27 June (Figure 3.17C). On 2 July 2003 the C2 group still remains but now includes the sample from the surface at Met 2 (Figure 3.17D), which is consistent with the observations of the inflowing water mixing to the surface at this location (Figure 3.7). It is also evident that although the whole samples from Met 2, surface and bottom appear quite similar, the filtered samples have a large separation on these axes, with the surface sample more closely resembling a reservoir community and the bottom sample more closely resembling a catchment community. However it should be noted that at this time the filtered catchment samples had similar substrate responses to what may have previously been considered to be a reservoir surface community.

Further information can be derived from the positive correlation of a substrate with a principal component axis, indicating that samples with high scores on that axis are likely to have had a positive response to that substrate. Plotting the substrates with high correlation values (vectors of modulus greater than 0.5) for the first and second principal components, can be used to visualise the relationship between the samples and their substrate responses. From this it is apparent that catchment samples generally had greater responses to D-malic acid, glycogen, α-D-lactose, α-cyclodextrin, phenylethylamine, L-phenylalanine, 2-hydroxybenzoic acid, D-xylose and 4-hydroxybenzoic acid. Most of the reservoir surface

samples had negative PC 1 scores indicating they were negatively correlated with these substrates. The reservoir surface samples were generally more widely distributed along the PC 2 axis, while the catchment samples predominantly varied along the PC 1 axis.

Mean correlation vectors for each of the substrate classes (as proposed by Insam, 1997) were calculated from the correlation vectors of the most significant substrates (those displayed in Figure 3.18). If these vectors can be interpreted functionally (see discussion to follow) then it implies that the soil derived micro-flora have a greater propensity to metabolise phenolic, amine and polymeric carbon substrates, while the microbial communities present in the reservoir surface waters are more likely to be competent in the metabolism of carbohydrates and carboxylic acids (Figure 3.19).

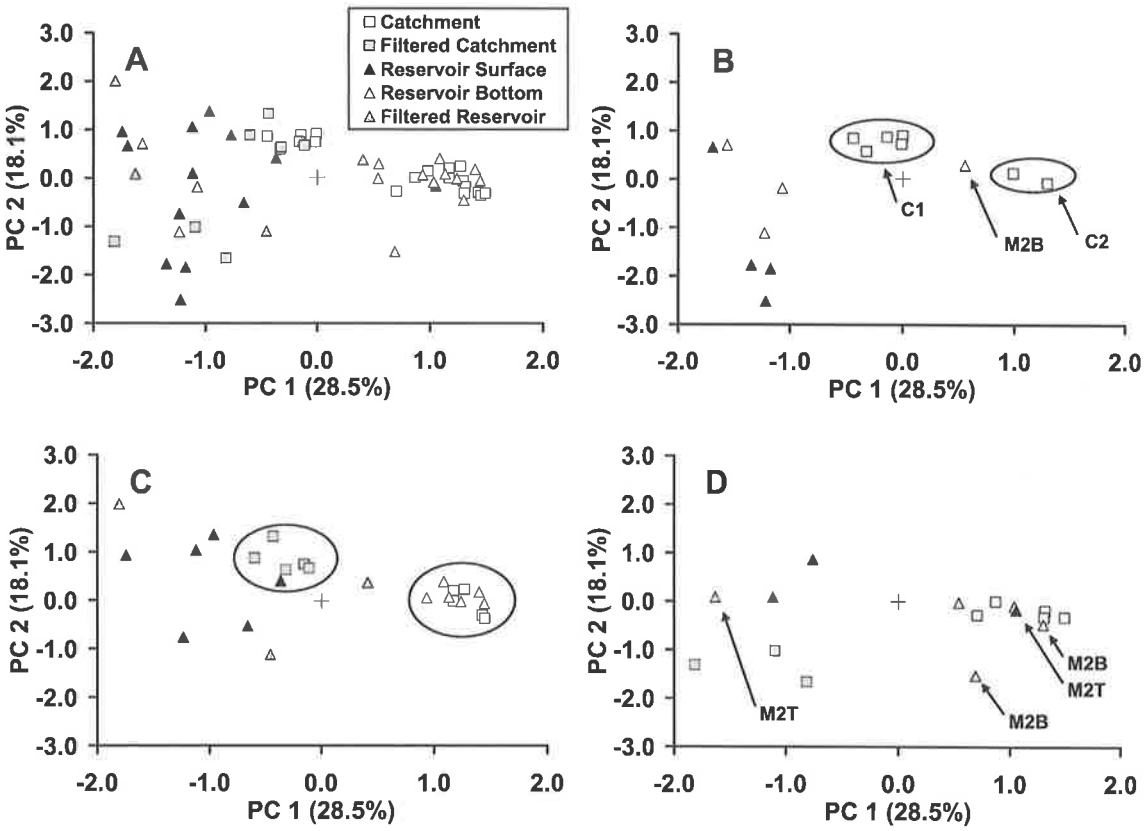

Figure 3.17. Principal component analysis of inflow experiment community level physiological profiles. A) all samples B) samples collected on 27 June 2003 (day 1) C) samples collected on 28 and 29 June 2003 D) samples collected on 2 July 2003 (day 6).

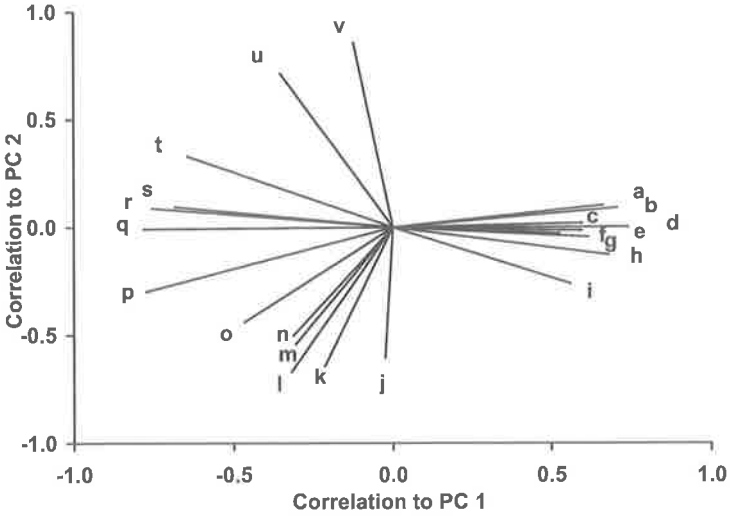

Figure 3.18. Correlation of BIOLOG Ecoplate substrates with the primary and secondary principal components of principal components analysis of community level physiological profiles. The 22 substrates with a correlation vector greater than 0.5 were included. These substrates were a, D-malic acid; b, glycogen; c, α-D-lactose; d, α-cyclodextrin; e, phenylethylamine; f, L-phenylalanine; g, 2-hydroxy-benzoic acid; h, D-xylose; i, 4-hydroxy-benzoic acid; j, L-arginine; k, itaconic acid; l, D-glucosaminic acid; m, Tween 40; n, Tween 80; o, D-galactonic acid γ-lactone; p, D-galactonuric acid; q, L-asparagine; r, D-mannitol; s, D-L glycerol phosphate; t, N-acetyl-D-glucosamine; u, β-methyl-D-glucoside; v, D-cellobiose.

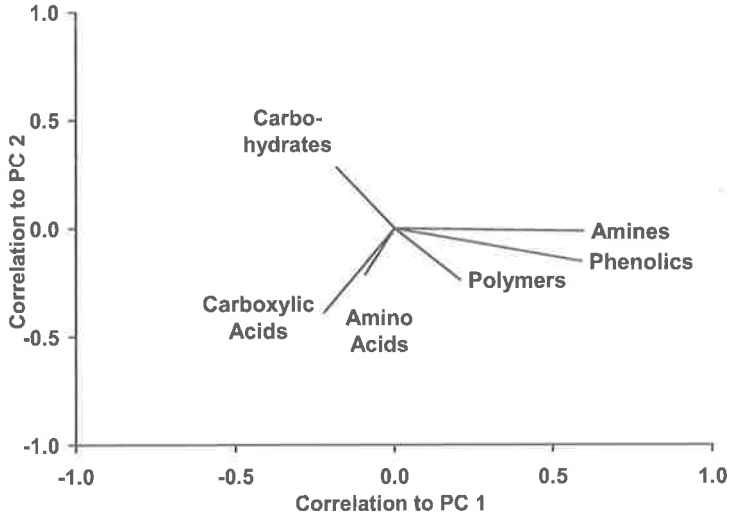

Figure 3.19. Average correlation vectors of BIOLOG substrates shown in Figure 3.18 by classification of Insam (1997).

3.3.9. Interactions between Substrate Degradation and Assimilation

The calculation of the correlations (Spearman rank) between substrate degradation (enzyme activity) and patterns of substrate utilization (CLPPs) should give some information about the regulation of substrate use during the inflow event (Table 3.2).

When all the samples are included in the calculation a number of polymer-monomer pairs or closely related compounds have strong positive or negative correlations, while others have little or no correlation. For example the correlation between the activity of β-xylosidase and the substrate response of the microbial community to xylose was weak (r^2 = 0.19; Table 3.2). Also the correlation between chitinase and the community response to N-acetyl-D-glucosamine was strongly negative (r^2 = -0.38; $\alpha < 0.05$; Table 3.2). LAP activity was strongly correlated to two amino acids (L-threonine, r^2 = 0.38; L-phenylalanine, r^2 = 0.45), while it was strongly negatively correlated to L-asparagine (r^2 = -0.43). Besides these individual relationships, the substrate responses were in general positively correlated with enzyme activity and some substrate responses were negatively correlated with enzyme activity. It may be reasonable to propose that the emerging relationships between the substrate responses and the enzyme activity may be the result of the differences in EA activity or substrate responses found between the reservoir and catchment samples. Consequently, it is appropriate to examine these relationships within these sample classes, to avoid these potential artefacts.

In the recalculated correlation matrix for the reservoir surface samples from location 1 and Met1 (Met2 was excluded due to concerns of catchment influence), only one significant correlation occurred between a polymer-monomer pair, being a negative correlation between BXL and D-xylose (r^2 = -0.73; prob>|Rho| = 0.03). This strong negative correlation was also found in the correlations amongst catchment only samples (r^2 = -0.59; prob>|Rho| = 0.01). The correlation between CHT and N-acetyl-D-glucosamine was positive and significant in the catchment sample correlation (r^2 = 0.50; prob>|Rho| = 0.04). The only other polymer-monomer pair that had a significant correlation was between BGL and D-cellobiose (r^2 = 0.68; prob>|Rho| = 0.003; data not shown). The remainder of the polymer-monomer pairs had weak positive or weak negative correlations, which may be

explained, either by their roles as microbial substrates, or as some artefact of the methods applied.

If the mechanisms by which the negative or positive correlation of a polymer-monomer pair are considered, it may be possible to explain these results further. Positive correlations will be produced by enzyme activity increasing with increasing substrate response. This seems to be the most likely relationship between the activity of an enzyme degrading a polymeric substrate and the response of the community to its monomeric product. The occurrence of a strong negative correlation would occur where as enzyme activity increases, the substrate response of the community decreases. This is more difficult to explain, but may be the result of strong negative feedback on enzyme activity or enzyme synthesis by the monomer product. The absence of significant correlation may result from the lack of sufficient data to detect the relationship or the absence or very low magnitude of the enzyme activity and/or substrate response. Alternatively, the absence of correlation may result when the enzyme is not being produced by the microbial community but its presence in the environment is the result of some other enzymatic process such as phytoplankton lysis (Agusti *et al.* 1998), crustacean moulting (Vrba and Machacek 1994) or protistan grazing (Vrba *et al.* 1996). However in these situations it would seem likely that the corresponding biopolymer monomer was also present in the environment.

These potential scenarios will be considered using the EA-substrate response correlations calculated from separated catchment and reservoir surface data sets, to avoid the influence of the inherent differences in the communities. In the reservoir surface communities, a response to D-xylose was rarely detected in the surface water collected from Met1 and location1 and ranked 22 of the 31 substrate responses (mean AWCD = 0.43 ± 0.29; N = 9) and as such is considered to have a low substrate response. As the activity of this enzyme was only detected in two of the 9 samples being compared, there is insufficient data to consider the negative correlation as meaningful. However in the case of the negative

correlation of BXL and D-xylose in the catchment samples (r^2 = -0.59; N = 17), where activity was detected in all but one sample, BXL activity in the catchment samples seems to have been strongly inhibited by the presence of xylose. This may imply that the utilisation of xylose was occurring at a slower rate than its production and was present in these samples. Indeed, the enzymatic production of substrates beyond the microbial demand is predicted by models of microbial substrate acquisition (Vetter *et al.* 1998).

N-acetyl-D-glucosamine and D-cellobiose were strongly correlated to CHT and BGL activity in the catchment samples, respectively. This implies that these enzymes were not inhibited by their enzyme products, possibly because the products were being rapidly assimilated, or because of the inherent properties of the enzyme. These are likely to be important substrates for these microbial communities.

Table 3.2. Table of Spearman rank correlations between enzyme activities and average well colour development normalised substrate responses in BIOLOG Ecoplates™ in samples collected during inflow experiment. Correlations with a r^2 greater than 0.27 are shaded lightly while those with a correlation less than -0.27 are shaded darker. An $|r^2| > 0.27$ corresponds to significance at $\alpha = 0.05$ at N = 56. Comparisons which may be expected to have relationships due to the relationship between the BIOLOG Ecoplate™ substrate and the enzymes specificity are boxed.

	AGL	*AKP*	*BGL*	*BXL*	*CHT*	*EST*	*LAP*
D-glucosaminic acid	-0.24	-0.48	-0.57	-0.56	-0.48	-0.06	-0.47
Tween 80	-0.36	-0.58	-0.32	-0.46	-0.55	0.00	-0.44
D-mannitol	-0.38	-0.31	-0.23	-0.37	-0.38	-0.24	-0.50
D-galactonic acid γ-lactone	-0.26	-0.45	-0.30	-0.44	-0.45	-0.07	-0.41
D-galactonuric acid	-0.17	-0.36	-0.45	-0.39	-0.43	-0.19	-0.40
D, L-glycerol phosphate	-0.19	-0.41	-0.29	-0.30	-0.43	-0.17	-0.44
N-acetyl-D-glucosamine	-0.31	-0.21	-0.38	-0.30	-0.38	-0.27	-0.34
L-asparagine	-0.18	-0.36	-0.22	-0.26	-0.33	-0.20	-0.43
Itaconic acid	-0.17	-0.49	-0.32	-0.35	-0.31	0.17	-0.36
Tween 40	-0.13	-0.33	-0.19	-0.10	-0.21	-0.21	-0.18
I-erythritol	-0.05	-0.26	-0.02	-0.22	-0.19	0.02	-0.25
Glucose-1-phosphate	-0.26	-0.10	0.10	-0.18	-0.12	-0.19	-0.15
L-arginine	0.07	-0.19	-0.02	-0.05	-0.17	0.10	-0.06
β-methyl-D-glucoside	-0.14	0.10	0.02	0.01	-0.04	-0.21	0.01
γ-Hydroxybutyric Acid	0.00	0.02	-0.01	-0.14	-0.07	0.09	-0.03
D-cellobiose	-0.16	0.16	0.30	0.10	0.09	-0.20	0.05
L-serine	0.26	0.10	-0.22	0.01	0.03	0.06	0.08
Pyruvic acid methyl ester	0.11	0.13	0.18	0.09	0.16	0.07	0.07
α-D-lactose	0.04	0.19	0.28	0.13	0.22	0.07	0.23
α-ketobutyric acid	0.12	0.18	0.31	0.31	0.24	0.10	0.18
Putrescine	0.17	0.17	0.32	0.38	0.34	-0.09	0.23
D-xylose	0.20	0.22	0.15	0.19	0.29	0.31	0.33
Glycyl-L-glutamic acid	0.16	0.08	0.40	0.30	0.40	0.10	0.21
L-threonine	0.28	0.34	0.38	0.31	0.36	-0.03	0.38
4-hydroxybenzoic acid	0.27	0.39	0.28	0.41	0.48	0.07	0.41
Phenylethylamine	0.35	0.37	0.25	0.45	0.41	0.12	0.45
L-phenylalanine	0.31	0.31	0.52	0.52	0.49	0.06	0.45
Glycogen	0.38	0.42	0.46	0.40	0.50	0.06	0.51
D-malic acid	0.38	0.47	0.14	0.37	0.47	0.52	0.47
α-cyclodextrin	0.45	0.58	0.52	0.64	0.64	0.21	0.59
2-hydroxybenzoic acid	0.62	0.55	0.52	0.65	0.65	0.34	0.66

Table 3.3. Spearman rank correlations between polymer-monomer pairs in reservoir surface and catchment samples. In reservoir surface samples, correlations with $|r^2| > 0.67$ corresponds to significance at $\alpha = 0.05$ at N = 9. In catchment samples, correlations with $|r^2| > 0.49$ corresponds to significance at $\alpha = 0.05$ at N = 17. Comparisons with significant positive correlations are shaded lightly, while comparisons with significant negative correlation are shaded darker. Comparisons which may be expected to have relationships due to the relationship between the BIOLOG Ecoplate™ substrate and the enzymes specificity are boxed.

Reservoir Surface Correlations	*AGL*	*AKP*	*BGL*	*BXL*	*CHT*	*EST*	*LAP*
D,L-Glycerol Phosphate	-0.51	-0.37	0.02	-0.09	0.06	0.53	-0.35
N-Acetyl-D-Glucosamine	-0.04	0.37	-0.60	-0.59	-0.40	0.15	0.30
L-Asparagine	-0.45	-0.22	-0.22	-0.46	0.19	0.90	-0.33
Glucose-1-Phosphate	-0.04	0.44	-0.23	0.55	-0.58	-0.02	-0.30
L-Arginine	0.12	-0.03	0.25	0.34	0.12	-0.22	-0.10
γ-Hydroxybutyric Acid	-0.04	0.04	0.65	-0.46	-0.40	-0.34	0.59
D-Cellobiose	0.22	-0.36	0.60	-0.07	0.30	-0.24	0.28
L-Serine	0.38	-0.14	0.12	0.52	0.27	-0.34	0.14
Pyruvic Acid Methyl Ester	0.22	-0.05	0.15	0.07	0.20	0.02	0.03
D-Xylose	-0.59	-0.12	-0.35	-0.73	-0.24	0.22	0.20
L-Threonine	0.31	0.33	0.28	0.73	-0.32	-0.51	-0.15
L-Phenylalanine	0.61	0.22	-0.43	-0.14	0.32	-0.15	0.27
Glycogen	0.42	0.63	-0.08	-0.25	-0.52	0.15	0.45
α-Cyclodextrin	0.40	0.64	-0.17	-0.14	-0.59	-0.46	0.66

Catchment Correlations	*AGL*	*AKP*	*BGL*	*BXL*	*CHT*	*EST*	*LAP*
D,L-Glycerol Phosphate	-0.36	-0.04	0.25	0.15	0.04	-0.60	-0.20
N-Acetyl-D-Glucosamine	0.08	0.37	0.43	0.43	0.50	-0.43	0.29
L-Asparagine	0.00	0.53	0.68	0.68	0.65	-0.44	0.33
Glucose-1-Phosphate	-0.53	-0.47	-0.44	-0.55	-0.63	-0.04	-0.60
L-Arginine	0.03	-0.12	-0.28	-0.26	-0.26	0.59	0.00
γ-Hydroxybutyric Acid	-0.57	-0.48	-0.38	-0.47	-0.53	0.09	-0.68
D-Cellobiose	-0.01	0.62	0.68	0.66	0.67	-0.44	0.41
L-Serine	-0.46	-0.17	0.00	-0.07	-0.08	-0.06	-0.25
Pyruvic Acid Methyl Ester	0.68	0.44	0.37	0.40	0.52	0.19	0.53
D-Xylose	-0.02	-0.46	-0.56	-0.59	-0.61	0.32	-0.48
L-Threonine	0.00	0.07	-0.07	-0.10	-0.08	0.01	0.03
L-Phenylalanine	0.32	0.20	0.38	0.42	0.37	0.05	0.25
Glycogen	0.01	-0.29	-0.30	-0.35	-0.36	-0.23	-0.21
α-Cyclodextrin	0.46	0.29	0.23	0.24	0.31	0.60	0.33

3.4. Discussion

An interpretation of the DOC dynamics in Myponga River is as follows: there was an initial dilution effect due to 'quickflow' in the form of infiltration excess overland flow and precipitation falling within the stream channel, where there was little extraction from the organic layer. Subsequently there is an increase in the DOC concentration as a result of increased infiltration and A/B-throughflow with the extraction of organics from the O-horizon which exceeded the adsorption capacity of the sand to loam A-horizon (Stevens *et al.* 1999, Thomas *et al.* 1999). As the flow rate decreases the residence time of the water in the catchment increases providing a greater contact time for extraction and dissolution of organics; thus the DOC concentration continues to increase. A change in the source of organic matter is also implied by the increase in specific UV absorbance and specific colour at the onset of flow (Figure 3.5D). Previously reported values for organics extracted from the soil and O-horizon from Myponga suggest this is a potential source of this increased specific absorbance organic matter and that it has the potential to produce higher disinfection by-product formation (Page *et al.* 2002).

The prediction that the inflow would produce large changes in the concentration and character of the organic matter proximal to the treatment plant offtake was not realised during this flow event. From the perspective of potable water production, only minor shifts in the organic matter properties occurred proximal to the reservoir offtake (Location 1, Figure 3.7A). The change in predicted optimal dose for NOM removal in the bottom waters at the time of greatest difference in DOC was only 3.5 mg L^{-1} (mEnCo 95.1 c.f. 98.6 mg L^{-1} alum; van Leeuwen *et al.* 2005). These changes alone are unlikely to warrant changes in WTP operation, but in conjunction with the turbidity and pathogen challenge (Brookes *et al.* 2002, Brookes *et al.* 2005b, Hipsey *et al.* 2005) an increase in coagulant demand may be appropriate.

The alum dose predictions above were based on the changes in organic matter parameters (UV absorbance and true colour) and turbidity maintained at 10 NTU (as found on 27 June 2003). If the actual turbidity for the sample from 28 June 2003 is used (20 NTU) an alum dose of 103.9 mg L^{-1} is predicted. This is an increase of 8.8 mg L^{-1} of alum and increases the cost of coagulation and pH control chemicals by 7.2% (pre and post inflow chemical cost estimates were \$29.62 and \$31.77 per ML, respectively, using Alum (52%, $Al_2(SO_4)_3.18H_2O$) \$156 $tonne^{-1}$ and sulphuric acid (H_2SO_4, 98%) \$142.1 $tonne^{-1}$). A simple calculation therefore attributes 40% of the change in the predicted required dose rate to changes in the NOM. Changes in raw water quality and the costs associated with increased coagulant demand can be avoided by adopting a reservoir offtake management strategy that avoids providing the raw water of compromised quality, to the treatment plant and thus control of water treatment processes, providing tangible economic and heath risk benefits.

The lack of correlation between microbial enzyme activities and the dissolved organic matter properties measured (DOC, UVA, HU) found in water flowing from the Myponga Reservoir catchment suggests that the microbial enzyme activity did not reflect the dissolved organic matter characteristics. The correlations with turbidity and the standard deviation of total volume concentration suggests that microbial enzyme activity more closely reflects the composition of particulate matter and the substrates of importance within the soil profile from where they were derived. However, the enzyme activity profile still has the potential to indicate what biopolymers are expected to be found in the inflowing water, given that they are also exported from the soil profile in the particulate or dissolved state. Consequently, the high activities of BGL and LAP found in the inflowing water imply that β-linked glucosides and proteins are an important component of the biochemically defined fractions of the organic matter. Chitin was likely to be a more important constituent of the DOC in waters flowing from SC3-W and SC7, as it was

detected with much higher activities in these catchments compared to the other sub-catchments.

Microbial enzyme activity has been shown to be strongly influenced by land use (Sinsabaugh *et al.* 1994, Chappell and Goulder 1995, Findlay *et al.* 1997) and consequently the higher chitinase activities in SC3-W and SC7 can probably be explained by the greater proportion of native vegetation (albeit grazed) in these sub-catchments (Figure 2.3) as these environments are likely to contain more chitin derived from fungi degrading woody debris (Criquet *et al.* 2000, de Vries and Visser 2001). Other influences on the patterns of enzymatic activity found in these streams can not be discounted however, as a multitude of factors, such as riparian flow path and stream shelter, have also been shown to modulate enzymatic activity (Findlay *et al.* 2001).

Following the flow event most of the enzyme activities in the water samples collected had decreased, presumably due to wash out of the transportable soil microbial community. This also reflects the distribution of activity between the particle associated and free stages; LAP activity, which was distributed more towards the filtrate than the other enzymes, persists late in the event in SC1 and SC3. This may represent continued flushing of the soluble or fine and easily transported particle phase of the soil microbial LAP production.

Strong associations between particles and enzyme activity were observed during this investigation. Interestingly, in the Myponga River samples, the standard deviation of total volume concentration in particular was strongly correlated with enzyme activity; this may indicate that larger particles (which will increase the standard deviation of the average volume concentration if a similar concentration of smaller particles remains present) play an important role in microbial transport. This is supported by the results of the investigation of the distribution of the various enzyme activities by assaying the filtrate, where some enzymes displayed very strong associations with particles greater than 0.45 μm in the catchment samples examined. AGL, BGL, BXL and CHT displayed minimal

activity in the filtrate, whereas approximately 40% of LAP and AKP was found in 0.45 µm filtrate. In the GF/C filtered samples (nominal pore size 1.0 µm) considerably more activity was present in the filtrate, suggesting that a proportion of the activity of AGL, BGL, BXL and CHT was associated with the cell walls of free living or small colonies of microbes. These distributions are consistent with those described in the literature on the size distribution of enzymes in the environment (Hoppe *et al.* 1993, Münster 1994, Vetter *et al.* 1998, Murrell *et al.* 1999, Karrasch *et al.* 2004b, Vrba *et al.* 2004).

Most of the AKP activity was found in the GF/C filtrate, which suggests that this enzyme is predominantly produced by microbes not included in biofilms on particles, or that it is poorly associated with the cell wall or the colonial mucilage, this corresponds well with the literature (Münster 1994). Some contradictory evidence was found in the sediment trap data, where AKP was among the enzymes with strong positive relationships with organic matter sedimentation. This is, however, most likely the result of in-trap production of AKP activity in organic matter rich traps with high phosphorus demands and hydrolysable organic phosphorus present.

Given that glucosides and proteins were important substrates, the observation of metabolic competency for these compounds within the microbial communities of both the catchment and the reservoir, was expected. The main differences in the community level physiological profiles identified between the soil derived catchment communities and the reservoir bacterioplankton was the inability of reservoir communities to utilise 2-hydroxy-benzoic acid, α-ketobutyric acid, L-threonine and phenylethylamine. Reservoir communities showed lower substrate utilisation diversity, suggesting the presence of fewer active metabolic pathways and lower phenotypic and probably genotypic diversity.

While some authors have cautioned against the functional interpretation of substrate responses in BIOLOG plates (Garland and Mills 1991, Garland *et al.* 2001, Mills and Garland 2002), other authors see fit to report functional relationships between substrate

classes and the communities they are studying (Grover and Chrzanowski 2000). If the microbial community is unable to utilise the provided substrate in one well of the BIOLOG plate, it is unlikely that it will be actively using that substrate in the environment. This raises the possibility that reservoir microbial communities are inefficient consumers of some THM precursors. However, while compounds such as hydroxy-benzoic acids are important THM precursors (Norwood *et al.* 1987, Korshin *et al.* 1997), the metabolism of free THM precursors probably plays a minor role in determining the total THMFP, as the majority (~70%) of the THMs are produced from the slow reacting fraction, consisting of functional groups attached to larger humic molecules (Gallard and von Gunten 2002).

In summary, the threat to water treatment by the riverine intrusion, which was large enough to present a significant pathogen threat to the water treatment plant, caused only minor changes in the concentration and character of the dissolved organic matter. Even so, simple management of the reservoir offtake would ensure that even these minor changes are avoided. Large amounts of microbial activity were transported into the reservoir, predominantly associated with particles. The influence of the inflow event on the reservoir microbial community extended past the duration of the sampling program and as such the subsequent adaptation of the microbial community to changes in substrate availability was not observed beyond the influx of the catchment derived microbial consortia.

Chapter 4. Transport of Organic Carbon to Myponga Reservoir from its Catchment

4.1. Introduction

The understanding of the transport of organic carbon from the catchment to the lake or reservoir is important from two main perspectives. First, the influx of allochthonous carbon is one of the two main sources of organic matter in reservoirs (autochthonous production being the other) and therefore plays a significant role in determining the long term concentration of organic matter in a reservoir (Pers *et al.* 2001). Secondly, the flux of organic matter from the catchment has the potential to alter the concentration and character of the organic matter in the reservoir over shorter time scales (Chapter 3, Kim *et al.* 2000, Ouyang 2003). Both the long term concentration of organic matter and the short term variability in natural organic matter (NOM) concentration and character are important from the perspective of potable water production. In the long term, a higher concentration of NOM will generally result in an increase in the cost of water treatment. Short term fluctuations in the NOM concentration or character may result in sub-optimal water treatment. If a water treatment plant (WTP) is unable to adequately respond to sudden changes in source water quality, a reduction in water quality and an increase in the health risk to consumers through increased formation of disinfection by-products may occur. Improvement of the understanding of the delivery of organic carbon from the catchment to the reservoir will enhance our ability to manage these issues and potentially improve the quality of the potable water supplied to the customer tap.

The relationship between flow and organic carbon concentration in flowing water systems within the catchment is determined by catchment properties such as vegetation type, the rate of litter production and the properties of the soil (e.g. development of the profile,

horizon structure, particle size distribution, mineral composition, organic matter content, infiltration rate, hydraulic conductance). Within the context of the catchment properties, the hydrologic characteristics are important, as the rate of water discharge is often observed to have a significant influence on the organic carbon concentration in the flowing water (Schlesinger and Melack 1981). The importance of high flow events in this flow-carbon concentration relationship has been identified (Grieve 1984a), and the need for storm sampling to fully understand the organic carbon dynamics is recognized (Naiman 1982). This influence has been attributed to the flushing of old soil water with higher organic carbon concentrations (Pearce *et al.* 1986, Pearce 1990). Regressions between flow rate and organic carbon concentrations suggest that an increase in discharge of two orders of magnitude can result in increases of between 1.4 and 5.5 times the baseflow organic carbon concentration, although the relationships are sometimes weak (Grieve 1984b, Tipping *et al.* 1988, Grieve 1991, Hope *et al.* 1994). Hysteresis is often observed in the relationship between flow and organic carbon concentration, where the organic carbon concentration is different on the rising and falling hydrographs and is often referred to as a concentration-flow (*C-Q*) hysteresis (Butturini *et al.* 2006). Some authors have reported organic carbon concentration being greater on the rising hydrograph than on the falling hydrograph (Grieve 1984b, Moore and Jackson 1989), while others report variable dynamics in the forms and rotational patterns of hysteric loops within and between catchments with similar characteristics (Butturini *et al.* 2006). The antecedent rainfall and soil moisture conditions are important to determine the organic carbon concentrations in flowing water (Tate and Meyer 1983) with higher concentrations observed after periods of dry weather (Grieve 1991).

Published models of TOC transport vary from simple deterministic equations describing the change in carbon concentration in relationship to a single parameter, usually flow (Pers *et al.* 2001), to complicated GIS based models that describe many catchment processes

such as groundwater loading, land and stream routing, organic carbon production, sinks and transport (Ouyang 2003). Models of intermediate complexity which are based on a smaller number of catchment parameters and a simpler hydrological model (i.e. without spatial information) can be successful at modelling organic carbon export from catchments (De Souza 2004).

Previous investigations of carbon flux from the Myponga Reservoir catchment have not included flow weighted sampling programs (Spark 1998, van Leeuwen *et al.* 2001) or have been limited to small experimental sub-catchments where soil ameliorants have been applied and other elements (nitrogen and phosphorus) have been the primary focus (Stevens *et al.* 1999, Cox *et al.* 2005). Some modelling of total organic carbon (TOC) flux has previously been reported but was based on historical data where high flow episodes are under represented (De Souza 2004).

It should therefore be possible to further describe the relationship between flow and carbon in Myponga River by the application of a flow weighted monitoring program. Some evidence of the relationship that is expected was presented in Chapter 3 (see Figure 3.5). This, however, represents a single event and seasonal variation depending on, for example, the antecedent soil moisture conditions is likely to influence the flow to carbon relationship.

In order to address hypothesis 2 (§1.5.1) and the relative significance of allochthonous and autochthonous organic matter sources, microbial activity and photochemical mineralisation as fate processes and the risk to water quality, some components of these systems must first be considered. What is the relationship between flow and DOC transport in Myponga River? Are there seasonal differences in this relationship, as some authors suggest by applying different empirical models depending on the season (e.g. Pers *et al.* 2001). Can the seasonal DOC concentration be predicted from hydrologic characteristics in Myponga Reservoir? If the challenge to the water treatment plant (WTP) is considered (*sensu*

Chapter 3), then what is more important, peak DOC concentration or the hydrological event size (peak flow)?

These questions were addressed by performing a flow weighted monitoring program during the winter of 2004. The data collected were analysed for carbon concentration to flow relationships and the hystereses examined by applying the numerical analysis of Butturini *et al.* (2006). Further comparison of the significance of allochthonous inputs are examined by constructing a mass balance model to evaluate the quantity of organic matter stored or metabolised by Myponga Reservoir. Photosynthesis, photo-oxidation and microbial metabolism were modelled using published methods, ultimately leading to a discussion of the importance of the source and fate processes that determine the concentration of organic carbon in raw water available for potable water production.

4.2. Materials and Methods

4.2.1. Monitoring Program

Water samples were collected from Myponga River using a refrigerated (4 °C), automated sampler triggered by a data logging system that records gauge height, measured with a pressure transducer, operated at the v-notch weir. The river discharge, in cubic metres per second (m^3 s^{-1}) was determined *in silico* using a gauge height to discharge rating curve. The daily total flow (ML d^{-1}) was calculated *in silico* from the previous 24 hours of instantaneous measurements. The automated sampler was started and the first sample collected when the daily flow exceeded a predetermined flow rate (start sampling flow, SSF; ML d^{-1}). Subsequent sampling events were initiated when a predetermined volume of water had passed over the weir (sampling flow interval, SFI; ML). Sampling was stopped when the gauge height fell to below a predetermined level (sampling stop gauge height, SSGH; m).

Early in the winter, from 9 June 2004, when smaller flow volumes were expected the sampling parameters were 25 ML d^{-1}, 25 ML and 1.5 m for SSF, SFI and SSGH,

respectively. In order to ensure sampling of the entire hydrograph as river flows increased into winter (from 23 July 2004) the SSF was increased to 60 ML d^{-1} and the SFI was increased to 100 ML. From 5 August 2004 to the completion of sampling the SFI was increased to 200 ML. Samples were not collected from 1 July 2004 to 2 July 2004 due to sampler malfunction. Samples were collected from the refrigerated sampler within 48 - 72 hours of the peak flow and transported to the laboratory on ice.

4.2.2. Sample Analysis

Each sample collected was analysed for dissolved organic carbon (DOC), UV absorbance (UVA), true colour (HU) and specific UV absorbance (SUVA) and specific colour (SpHU) calculated as per §2.3. A subset of samples were analysed for conductivity, total organic carbon (TOC), total kjeldahl nitrogen (TKN) and total phosphorus (TP).

4.2.3. Hydrology Budget

A hydraulic budget has previously been developed and validated for Myponga Reservoir based on hydrographic measurements of the catchment and reservoir (Linden *et al.* 2005). Reservoir gauge height, evaporation, rainfall, treatment plant consumption and flow data for Myponga River are used to calculate the water balance. Millimetres of class A pan evaporation was measured daily adjacent to the reservoir, and a pan/lake coefficient of 0.8 was applied to the data. Rainfall was measured at the same location using a manual rain gauge. Reservoir gauge height was measured daily. Data for the consumption of reservoir water by the Myponga water treatment plant (WTP) was provided by United Water International Pty Ltd (Adelaide, South Australia, Australia). The volume of water held in the reservoir was calculated from the recorded gauge height and the bathymetry of the reservoir basin. The volume of water lost to over topping was calculated from records of floodgate opening time, aperture and a flow-rating curve. The water sources that could not be monitored included the groundwater directly entering the reservoir and the overland and

subsurface flow from zones 2-9. An estimate of their contribution was determined by closing the water budget, using the following expression:

$$V_t = V_{t-1} + Fm_t + Fu_t + GWin_t + P_t - E_t - C_t - O_t - GWout_t \quad (4.1)$$

Where, V_t is the volume of the reservoir at time t (ML); Fm_t is the volume of monitored surface flow at time t (ML); Fu_t is the volume of unmonitored flow at time t (ML); $GWin_t$ is the volume of direct groundwater inflow at time t (ML); P_t is the volume of direct precipitation into the reservoir at time t (ML); E_t is the volume of evaporation from the reservoir at time t (ML); C_t is the volume consumed by water treatment plant at time t (ML); O_t is the volume overtopping the reservoir at time t (ML) and $GWout_t$ is the volume of groundwater outflow at time t (ML).

This equation was resolved daily for the time period 1 June 1999 to 31 December 2003. Fu_t, $GWin_t$ and $GWout_t$ were not monitored and are therefore combined as unmeasured flow; the sum of the ungauged surface (catchment zones 2 - 9; Figure 2.2B) and sub-surface flow from the catchment and the groundwater inflow and outflow. E_t was estimated from the measured class A pan evaporation, multiplied by the pan/lake coefficient (0.8), multiplied by the surface area of reservoir at time t. For a period of 8 years, Linden *et al.* (2004), validated this method of calculating the annual hydrological budget and estimated the error in the annual volume estimate to be approximately 3%.

4.2.4. Relationship between Organic Carbon Concentration and Flow

The relationship between flow and organic carbon was first examined using an empirical modelling approach. The available data on particulate and dissolved organic carbon concentration and the flow in Myponga River was divided into calibration and validation data sets using alternate data points to ensure that representative data from the temporally sporadic data set was included in both the validation and calibration datasets (DOC: N_{total} = 180; $N_{calibration}$ = 90; $N_{validation}$ = 90; POC: N_{total} = 70; $N_{calibration}$ = 35; $N_{validation}$ = 35). The

original data set included data from samples collected on a flow weighted basis in 2004 and samples collected at weekly to 3 monthly intervals between 20 October 1998 and 31 October 2000 (CRC WQT project 2.1 unpublished data, DOC only, N = 49). For the flow weighted 2004 samples, the instantaneous flow data (m^3 s^{-1}) was used to calculate the volume of flow in the previous 24, 48, 72 and 96 hours (ML). For the project 2.1 data, where only the daily total flow was available, the 48, 72 and 96 hour flows were calculated as the sum of the corresponding daily flows.

A range of empirical models were fitted to the data including simple linear and natural logarithm models and slightly more complex linear domain and log normal models. The empirical models were chosen based on a visual assessment of the flow-concentration relationship and comparison to model structures presented in the literature and curve fitting software packages (Table Curve 2D, Systat Software Inc., Motulsky and Christopoulos 2004). The equations described represent the range of empirical models that were trialled, including the models used in other studies (e.g. Pers *et al.* 2001), but were found to be unsuitable for Myponga River. Other equations may provide similar modelling capacity. The models were assessed numerically and visually and applied in the mass balance model to estimate the dissolved and particulate carbon loading from the Myponga Reservoir catchment.

The empirical models fitted were as follows:

$$OC_t = aQ_t + c \tag{4.2}$$

$$OC_t = a\ln(Q_t) + c \tag{4.3}$$

$$OC_t = a\exp\left(-\frac{1}{2}\left(\frac{\ln\left(\frac{Q_t}{b}\right)}{c}\right)^2\right) + dQ_t + g \tag{4.4}$$

$$\begin{aligned} OC_t &= aQ_t + c, \forall x : 0 < Q_t < Q_1 \\ &= dQ_t + g, \forall x : Q_1 < Q_t < \infty \end{aligned} \tag{4.5}$$

$$OC_t = a\exp\left(-\frac{1}{2}\left(\frac{\ln\left(\frac{Q_t}{b}\right)}{c}\right)^2\right) + dQ_t + g + hD_{year} \tag{4.6}$$

$$OC_t = a\exp\left(-\frac{1}{2}\left(\frac{\ln\left(\frac{Q_t}{b}\right)}{c}\right)^2\right) + dQ_t + g + hD_{year} + iQ_{33d} \tag{4.7}$$

Where OC_t is the organic carbon concentration at time t; Q_t is the flow at time t; D_{year} is the day of the year; Q_{33d} is the volume of flow in the previous 33 days; a, b, c, d, e, f, g, h and i are fitted parameters for each individual equation. Equation 4.2 is a simple linear model, equation 4.3 is a simple logarithmic model, equation 4.4 is a log normal model combined with a linear model and equation 4.5 is a linear domain model where POC or DOC is described by two linear equations which intersect at Q_1. Equation 4.6 is similar to equation 4.4, except it includes the day of the year multiplied by a fitted parameter. Equation 4.7 is similar to equation 4.6, except it includes the volume of flow recorded in the previous 33 days multiplied by a fitted parameter. The optimum period of 33 days was derived by the sequential fitting of periods from 3 to 40 days flow.

The model parameters were estimated using the Solver Add-in in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, Washington, USA) to maximise the modelling efficiency (EF), which is numerically similar to the widely used coefficient of determination (r^2), calculated as:

$$EF = 1 - \left(\frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}\right) \tag{4.8}$$

Where y_i is the *i*th observed value of y; $\hat{y}_i$ is the predicted *i*th value of y and $\bar{y}$ is the mean of the observed y values (Mayer and Butler 1993).

4.2.5. Hydrologic and Hysteresis Analysis

Nine peak flow events were analysed for concentration-flow (*C-Q*) hysteresis by the methods of Butturini *et al.* (2006). Three hydrological descriptors were estimated from the hydrographic information. The magnitude of the storm event relative to the basal discharge (ΔQ_t) was calculated as:

$$\Delta Q_t = (Q_P - Q_{Bas}) / Q_{Bas} * 100 \quad (4.9)$$

Where Q_P and Q_{Bas} are the storm peak and basal discharges (L s^{-1}), respectively. The slope of the initial phase of the hydrograph recession limb (k d^{-1}) was estimated by fitting a exponential decay curve to the recession curve and determining the initial linear slope of the line. The relative length of the rising limb (*RL*, %) was determined as:

$$RL = R_D / S_D * 100 \quad (4.10)$$

Where R_D and S_D are the length in days of the rising limb of the hydrograph and the entire hydrograph, respectively. The end of the hydrograph was determined as the point where the slope of the fitted recession curve was greater than -0.1 d^{-1}. Δt and ΔQ_{t-1} describe the interval between the present (time = *t*) and the precedent (time = *t-1*) flow events and the magnitude of the precedent flow event, respectively.

Two descriptors of solute behaviour during the flow event were calculated. The relative change in solute concentration and hysteresis trend are described by ΔC (%) as:

$$\Delta C = (C_s - C_b) / C_{\max} * 100 \quad (4.11)$$

Where C_b and C_s are the base flow solute concentration and the storm flow solute concentrations, respectively. C_{max} refers to the maximum observed concentration of the

solute in the data series. The area and rotational pattern of the hysteresis is described by ΔR (%), which is calculated as:

$$\Delta R = R * A_h * 100 \quad (4.12)$$

Where A_h is the area of the hysteresis after standardising the discharges and concentrations to a unity scale. The rotational pattern of the hysteresis is described by R, which takes the values 1, -1 or 0 if the rotation is clockwise, counter clockwise or unclear, respectively. Small hysteresis areas indicate co-occurrence of hydrological inputs from surface and groundwater flow (-20% < ΔR < 20% values Butturini *et al.* 2006).

4.2.6. Modelling of Autochthonous Production

For order of magnitude comparison with the allochthonous inputs to Myponga Reservoir the daily integral of photosynthesis was modelled after Walsby (1997b), with modifications. While the daily integral of photosynthesis is dependant on the depth distribution of phytoplankton through the day (Walsby 1997a), its distribution was assumed to be uniform as detailed information on the distribution of chlorophyll through the water column was not available. The incident photo-synthetically active radiation (PAR; 400 - 700 nm) was calculated from shortwave radiation data from Meteorological station 1 (Middleton EP09 v1.3; λ range 300 - 3000 nm; 10 or 15 minute intervals) and the subsurface PAR was calculated using the surface roughness and solar declination functions at hourly intervals through the day. The PAR at 1 metre intervals to 9 m depth, at the corresponding hour of the day, was calculated using the Beer-Lambert law and the average extinction coefficient (k_d) of PAR measured in Myponga Reservoir between October 2000 to February 2001 (2.02, SD = 0.24, N = 14) using an underwater light sensor (LiCOR-1000).

A photosynthesis-irradiance (PI) curve was determined using a photosynthesis-irradiance (PI) curve calculated using a XE-PAM fluorometer (Heinz Walz GmbH, Effeltrich,

Germany) on 10 January 2000, following the method described in Brookes *et al.* (2000b). Briefly, photosynthetic rate was measured at a range of actinic light intensities using variable chlorophyll *a* fluorescence as:

$$P = I\sigma q_P \left(\frac{\Delta \phi_{\max}}{0.65} \right) \phi_e \eta_{PSII} \tag{4.13}$$

Where P is the rate of photosynthesis (mg O_2 mg^{-1} chl *a* h^{-1}), I is the irradiance intensity (mol m^{-2} s^{-1}), σ is the effective absorption cross section (m^2 mol $quanta^{-1}$), q_P is the photochemical quenching, $\phi_{max}/0.65$ is the fraction of functional PSII reaction centres, ϕ_e is the number of oxygen molecules per photon processed by the reaction centres and η_{PSII} is the number of photosystem II (PSII) reaction centres per mol of chl *a* (1/345). The effective absorption cross-section was measures using the ST-flash assembly using the method of Kolber and Falkowski (1993). The derivation of this formula and further discussion of the calculation of photosynthetic rates by the use of fluorometry can be found in Kolber *et al.* (1998) and Oliver and Whittington (1998).

This PI curve was used to model the rate of photosynthesis throughout the modelling period, using chlorophyll *a* data, interpolated from the routine monitoring program, and the water temperature at depth, monitored by the meteorological stations. The rate of photosynthesis was calculated using temperature adjustment (Q_{10} = 2.0) and integrated at each depth at one hourly time intervals for each day. The rate at each depth was then multiplied by the cross-sectional area of the reservoir at that depth and integrated to calculate the daily gross integral of photosynthesis for the entire reservoir. This value was then divided by the current surface area to calculate areal gross photosynthesis in Myponga Reservoir. It is acknowledged that this is an oversimplification of the processes involved in primary production in lakes and reservoirs and serves only to provide an order of magnitude estimation of the quantity of carbon fixed photosynthetically in Myponga Reservoir.

4.2.7. Estimation of Photo-mineralisation

The photo-oxidative mineralisation of DOC to DIC was demonstrated to have a simple linear relationship between global radiation for Valkea-Kotinen (Sweden; 61°14'N; ~12 mg L^{-1} DOC) by Vahatalo *et al.* (2000), as follows:

$$pm = aI \tag{4.14}$$

Where, *pm* is photochemical mineralisation (mmol C m^{-2} d^{-1}), *a* is a fitted constant (*a* = 0.0647) and *I* is global radiation (300 - 3000 nm; MJ m^{-2} d^{-1}, Vahatalo *et al.* 2000). Comparison of the UV to visible light (300 - 700 nm) absorption properties of the organic matter found in Myponga Reservoir (Sample collected location 4 surface on 3 July 2002; scan with Shimadzu UVmini 1240 spectrophotometer; 1 cm quartz cuvette), revealed higher absolute absorption than that reported by Vahatalo *et al.* (2000). For example the absorption coefficients at 420 nm were 8.2 and 9.6 m^{-1} in Valkea-Kotinen and Myponga Reservoir, respectively. Graneli *et al.* (1996) found that the optical properties of the organic matter did not affect the rate of photo-mineralisation and Graneli *et al.* (1998) found little difference in the photo-oxidative properties of organic matter in temperate and tropical freshwaters. However, two fold differences in the quantum yield of photo-mineralisation between waters are reported (Valentine and Zepp 1993); for example Gao and Zepp (1998) found quantum yields two to four times higher than that reported by Vahatalo *et al.* (2000).

4.2.8. Microbial Production and Respiration

The carbon demand of bacterial production (*BP*) was modelled using the equation fitted to measurements made in nine lakes of various trophic states by Cole and Pace (1995).

$$BP = \frac{35}{\left(1 + e^{2-0.1T}\right)} \tag{4.15}$$

Where *BP* is the bacterial production (mg C m^{-3} d^{-1}) and *T* is the water temperature (°C). Cole and Pace (1995) found this equation described 70 % of the variation in *BP* in the lakes they investigated. The *BP* was estimated with equation 4.15, using the daily average temperature at 1 metre depth intervals over the first 9 metres and integrated over this depth. Bacterial production in the remainder of the reservoir volume was estimated using the temperature at 9 m depth which was very similar to the daily mean temperature at 30 m (regression slope 0.98 and $r^2 = 0.995$).

The carbon demand of bacterial respiration (*BR*) was estimated using the modelled value of *BP* and bacterial growth efficiency (*BGE*). The formula for BGE:

$$BGE = \frac{BP}{BP + BR} \tag{4.16}$$

Was rearranged as follows:

$$BR = \frac{BP(1 - BGE)}{BGE} \tag{4.17}$$

This equation was used to calculate *BR* at daily time steps using published values of BGE from lakes. The reported values of *BGE* range from 0.04 to 0.6 and has a mean and lower and upper quartiles of 0.26, 0.2 and 0.37, respectively (del Giorgio and Cole 1998). The total bacterial carbon demand was estimated as the sum of *BP* and *BP*.

4.2.9. Calculation of carbon budget

The organic carbon budget for Myponga Reservoir was calculated as described in Figure 4.1. Photosynthesis (§4.2.6) and microbial (§4.2.8) metabolism were modelled at hourly time steps and integrated to daily values. Allochthonous loading was modelled at daily time steps using the empirical flow ~ concentration models for dissolved and particulate organic carbon (§4.2.4). Losses of organic carbon to photomineralisation (§4.2.7) and outflow were calculated at daily time steps. The annual values reported were derived from sums of annual values.

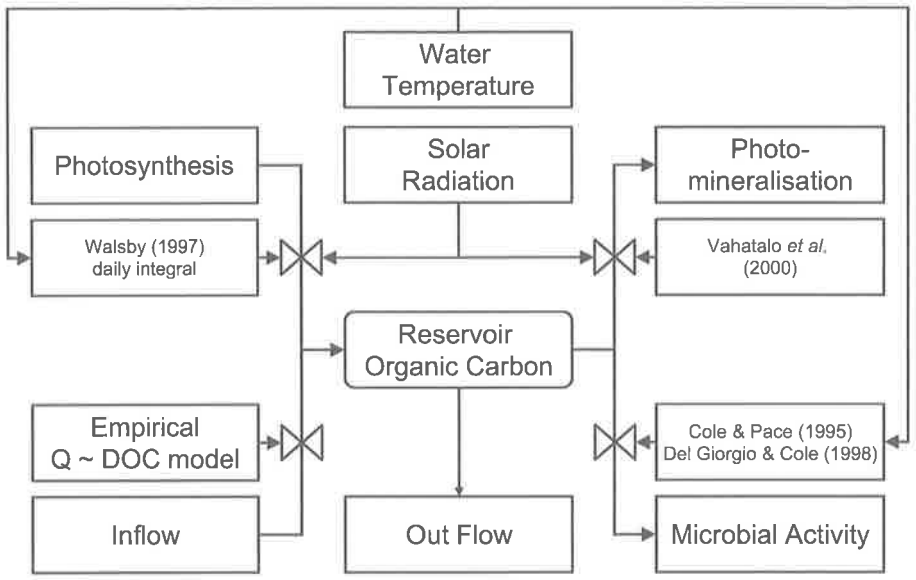

Figure 4.1. Diagram of calculation of organic carbon mass balance for Myponga Reservoir.

4.3. Results

4.3.1. Hydrology in 2004

The total rainfall recorded in 2004 was 758.2 mm which is remarkably close to the reported mean annual rainfall of 760 mm (Thomas *et al.* 1999). The maximum daily rainfall in 2004 was 37.3 mm on 3 August (Figure 4.2B). The flow in Myponga River was less than 2.2 $m^3 s^{-1}$ until 23 and 24 July 2004 when peak flows of 9.8 and 18.2 $m^3 s^{-1}$ were recorded (Figure 4.2A). Peak flow events of 13.0, 18.7, 14.3 and 14.4 $m^3 s^{-1}$ were recorded on 28 July, 3 August, 4 August and 5 August 2004. Following these events, all peak flows were less than 5.0 except for 5.1 on 8 September 2004.

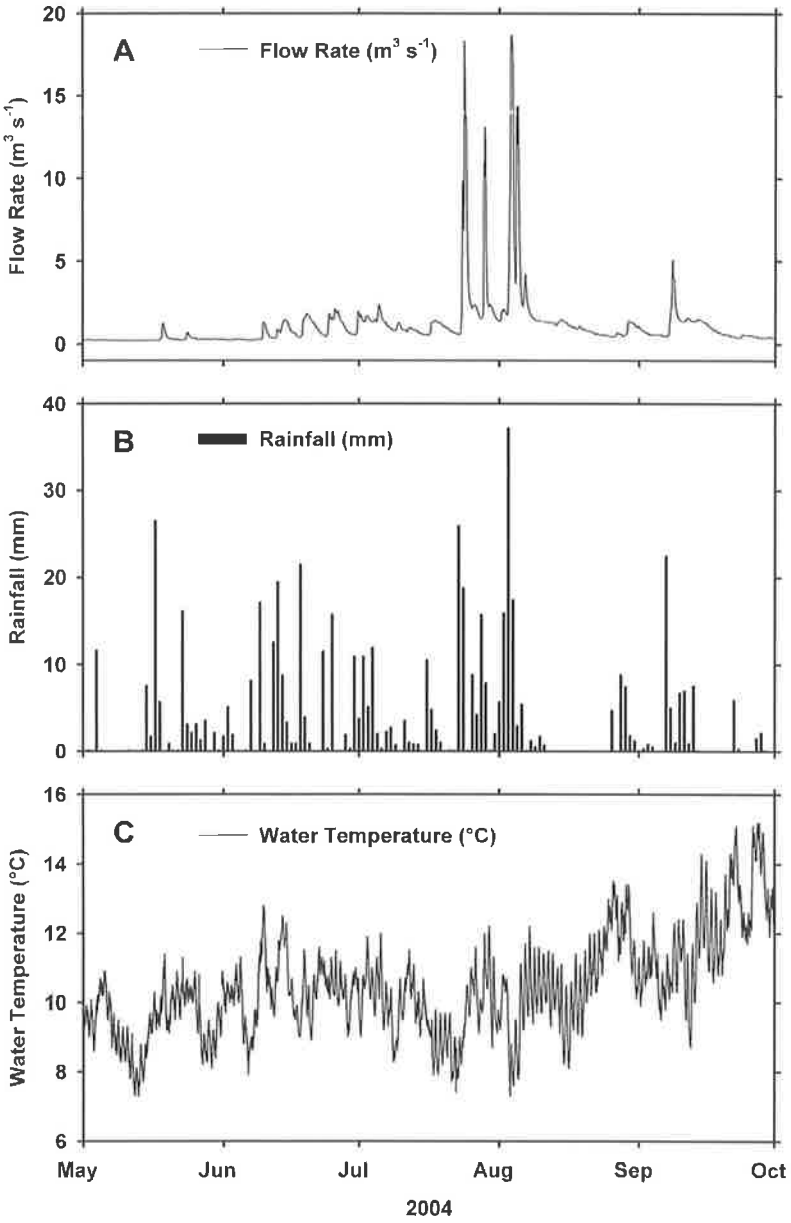

Figure 4.2. A) Flow rate measured in Myponga River, B) rainfall recorded adjacent to the flow monitoring station and C) water temperature measured at the river sampling point.

4.3.2. Organic Carbon Dynamics in 2004

The concentration of POC in Myponga River water was generally much lower than the DOC concentration (Figure 4.3A). Distinct peaks are observed in the DOC concentration early in the season, apparently due to fluctuations in river flow. After the series of large flow events in late July and early August the fluctuations are less pronounced and the DOC concentration remained high for most of August 2004. Dilution of the DOC concentration

during peak flows was observed. The fluctuations in UVA and true colour appear to closely mirror DOC concentration (Figure 4.3B,C). The dilution effect is also observed in the conductivity data (Figure 4.3D). The conductivity also decreased through the winter indicating that the catchment was being flushed of transportable ions beyond their generation by weathering processes.

Some changes in the character of the organic carbon being transported occurred over the winter. Specifically, there were fluctuations in the SUVA of the organic matter early in the season, indicating some differential flushing of organic matter stores or that a differential sorption process was occurring. The SUVA of the organic matter found in Myponga River was very stable following the high flow events at the start of August 2004 (Figure 4.4A). Fluctuations were observed in the specific colour of the organic matter and it was not as stable as SUVA in the late winter (Figure 4.4B). Despite the strong regressions between the concentration of DOC in Myponga River water and the UVA and HU of the sample (UVA = 0.047 × DOC - 0.18, $r^2 = 0.95$, N = 131, $p < 0.0001$; HU = 10.6 × DOC - 62.9, $r^2 = 0.95$ N = 131, $p < 0.0001$), differences were observed in the character of the dissolved organic matter over the season. Linear regressions between the DOC, HU, UVA, SpHU and SUVA of Myponga River samples and the day of year the sample was collected had significant positive slope estimates (Table 4.1), indicating they increased over the winter of 2004.

Table 4.1. Statistics from linear regressions between dissolved organic carbon (DOC), true colour (HU), UV absorbance (UVA), specific colour (SpHU) and specific UV absorbance (SUVA) and the day of the year the sample was collected. N = 131 in all cases.

Parameter	*Regression Slope*	*Regression Intercept*	r^2	*P value of Slope*	*P value of Intercept*
DOC	0.052	9.9	0.17	<0.0001	<0.0001
HU	0.81	-9.9	0.31	<0.0001	0.65
UVA	0.0034	0.10	0.31	<0.0001	0.27
SpHU	0.023	2.8	0.33	<0.0001	<0.0001
SUVA	0.0074	2.3	0.52	<0.0001	<0.0001

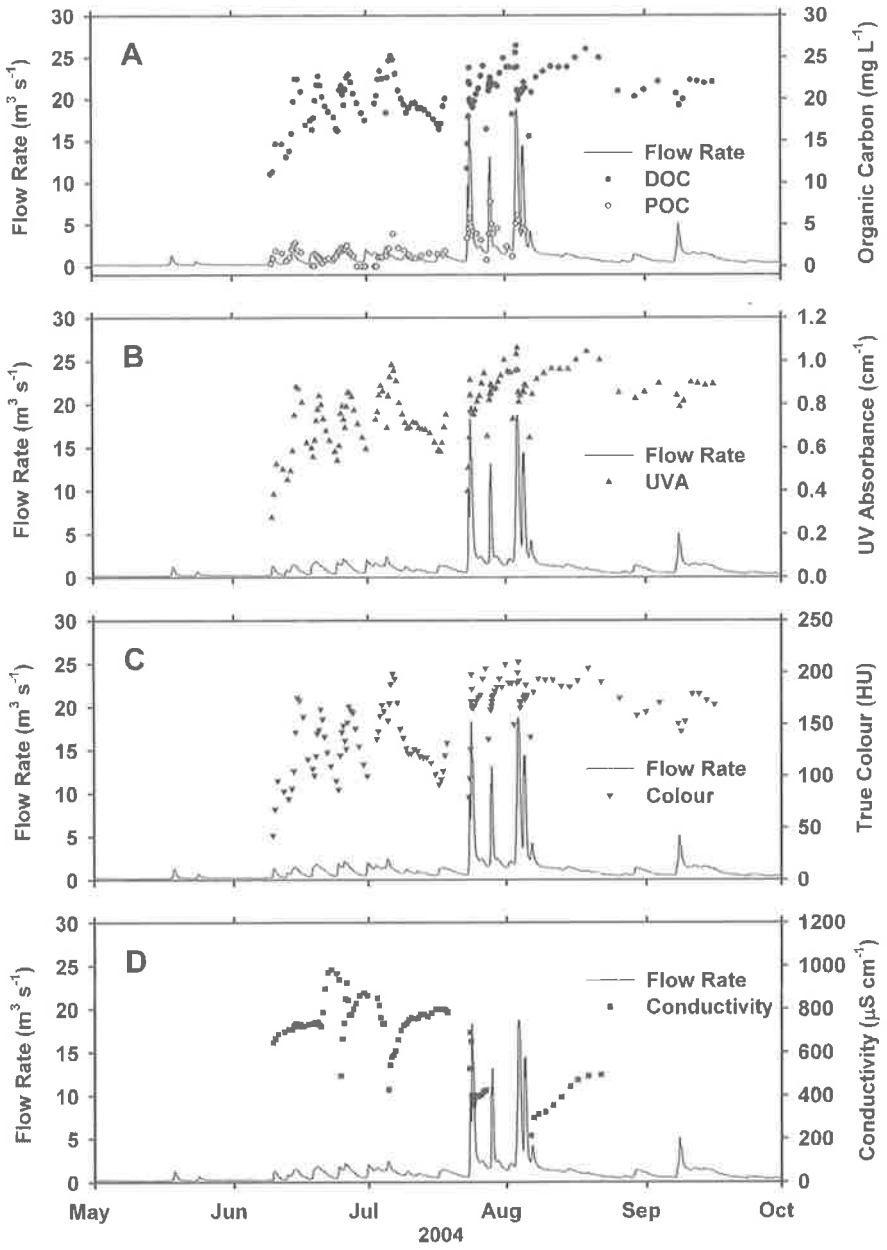

Figure 4.3. A) Dissolved and particulate organic carbon, B) UV absorbance, C) true colour and D) conductivity found in samples collected from Myponga River using an automatic sampler.

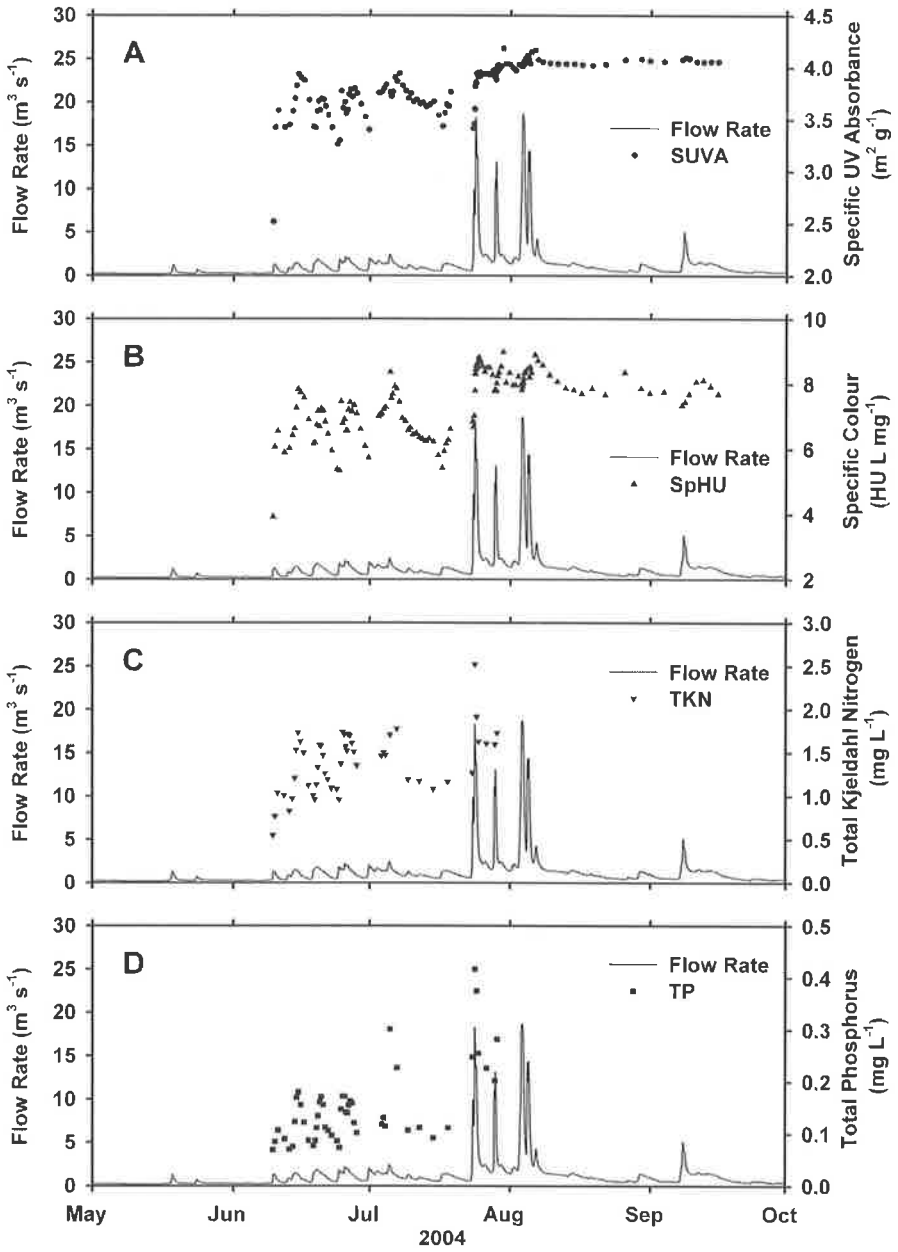

Figure 4.4. A) Specific UV absorbance, B) specific colour, C) total kjeldahl nitrogen and D) total phosphorus found in samples collected from Myponga River using an automatic sampler.

4.3.3. Models of Riverine Transport

4.3.3.1. Dissolved Organic Carbon

The simple empirical models used to predict DOC concentration in Myponga River had variable success (Table 4.2). The linear model fitted the data poorly in the calibration and validation phases and explained up to 22 % of the variation in DOC concentration (Table 4.2). The logarithmic model had better modelling efficiency than the linear regressions with the different flow volume calculations, with 56 to 60 % of the variation explained.

Better performance was found with the log normal distribution model and the linear domain model, with between 61 and 71 % of the variation in DOC concentration being able to be described. The best overall modelling performance, in the models based on flow alone, was achieved by the log-normal model optimised for the 72 hour flow data, with a calibration fit of 72 % and a validation fit of 61 % (Table 4.2). The addition of the linear day of year parameter increased the modelling efficiency of all the log normal-flow only based models, however the model based on 72 hour flow remained the most efficient with a calibration fit of 75 % and a validation fit of 62 %. Further improvement of the modelling efficiency was found when a linear parameter multiplied by the flow over a longer period was also applied. The modelling efficiency increased to a maximum at 33 days when using the 72 hour flow data with a calibration fit of 77 % and a validation fit of 66 %.

The observed versus modelled plots of models A, B and C show that the models appear to have an even distribution of residuals, however all the models tend to under estimate DOC at DOC concentrations above 25 mg L^{-1} (Figure 4.5). Investigating the bias with linear regression between the observed and the modelled values of DOC reveals that the lines of best fit have slopes between 0.71 and 0.77 and intercepts between 4.26 and 5.30 (Table 4.3), with Model C having the parameter values closest to the ideal of a slope of one and an intercept of zero.

The models predicted the DOC concentration well in three years with differing hydrology; 1999 was a very dry year with relatively few but pronounced flow events (Figure 4.6A); 2000 also had below average flow but had flow events with broader peaks than 1999 (Figure 4.6B); 2004 had close to average rainfall and had a number of large flow events (Figure 4.6C). The improved fit of Model C over the other models is most pronounced at the start and end of the wet period and in periods following high flows, for example, in March and April in 1999 and 2000, and August 2004.

Table 4.2. Calibration and validation statistics of six empirical models of DOC concentration in Myponga River based on the volume of flow in the preceding 24, 48, 72 and 96 hours, the day of the year and the total flow in the previous 33 days. The models in bold are presented in Figure 4.4.

Model (24 hour)	*Calibration Fit (EF)*	*Validation Fit (EF)*
Linear	0.129	0.128
Natural Log	0.555	0.530
Linear Domain	0.624	0.575
Log Normal A	0.641	0.579
Log Normal B	0.686	0.618
Log Normal C	0.714	0.662
Model (48 hour)		
Linear	0.157	0.163
Natural Log	0.585	0.557
Linear Domain	0.679	0.617
Log Normal A	0.674	0.591
Log Normal B	0.720	0.629
Log Normal C	0.746	0.667
Model (72 hour)		
Linear	0.180	0.210
Natural Log	0.595	0.576
Linear Domain	0.686	0.606
Log Normal A	**0.715**	**0.613**
Log Normal B	**0.749**	**0.624**
Log Normal C	**0.772**	**0.657**
Model (96 hour)		
Linear	0.224	0.220
Natural Log	0.601	0.576
Linear Domain	0.671	0.611
Log Normal A	0.706	0.610
Log Normal B	0.736	0.620
Log Normal C	0.759	0.655

Table 4.3. Bias in empirical prediction of DOC concentration in Myponga River described by regression statistics between observed and predicted DOC values.

Model	*Slope*	*Intercept*	r^2
Log Normal A	0.71	5.30	0.715
Log Normal B	0.75	4.66	0.749
Log Normal C	0.77	4.26	0.772

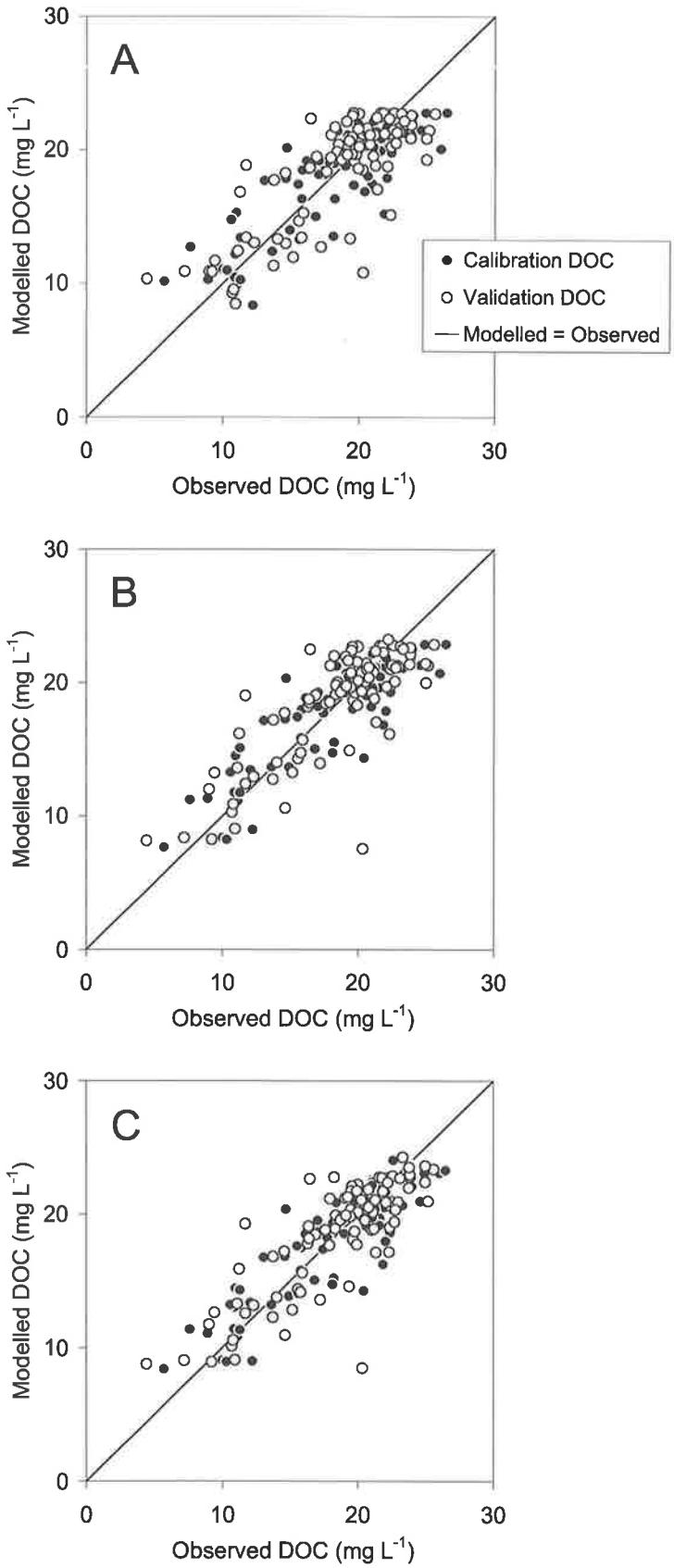

Figure 4.5. Observed vs. predicted plots for models A, B and C. The line in all plots is modelled = observed.

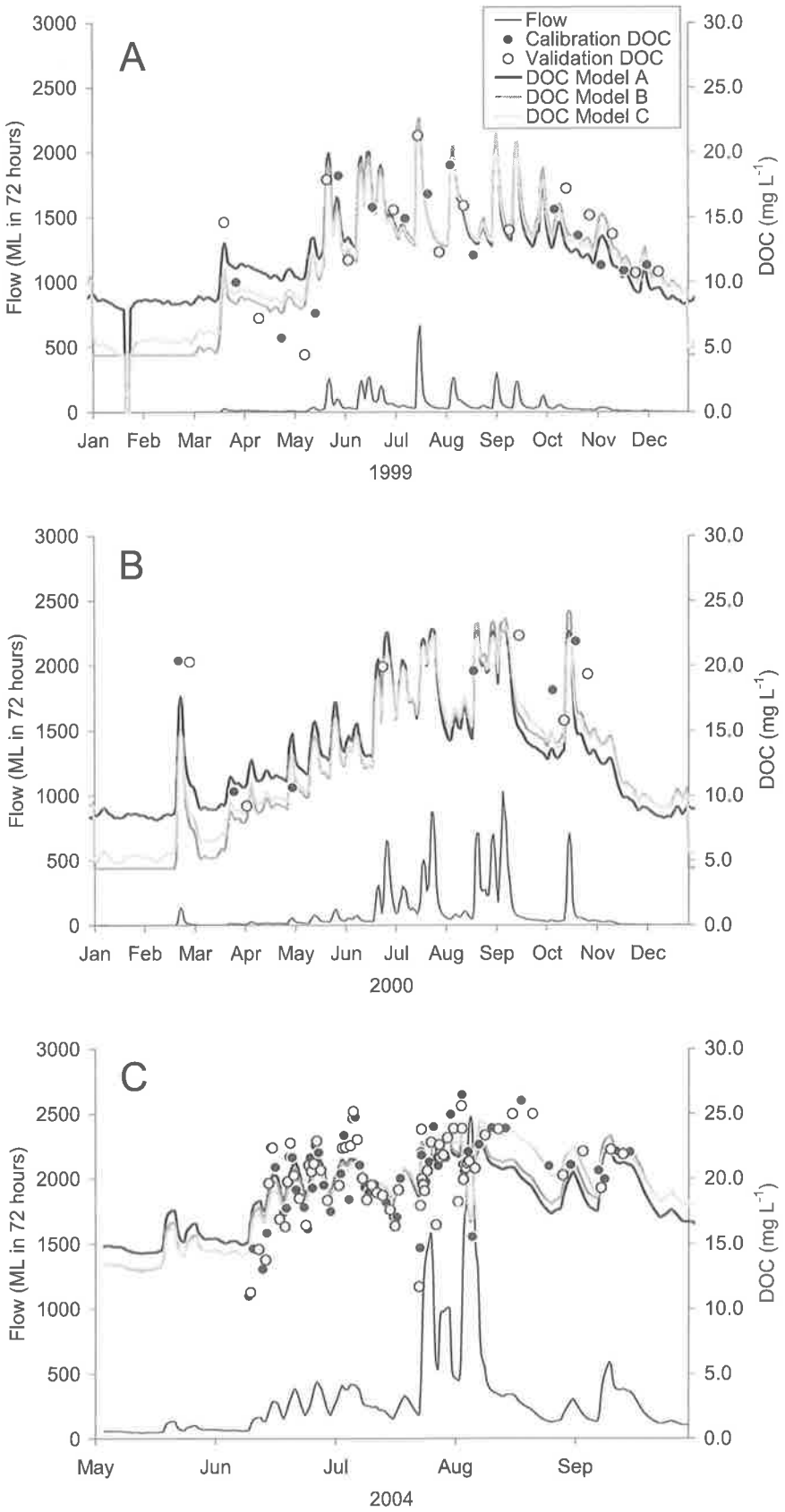

Figure 4.6. DOC concentration in Myponga River simulated with three empirical models (Models A, B and C). The parameters were calibrated against the data represented by filled circles and validated against the data represented by open circles.

4.3.3.2. Particulate Organic Carbon

Particulate organic carbon dynamics were better described by shorter term flow data, compared to DOC. The best empirical model fitted was the linear domain equation (Eq. 4.5) using daily flow data (Table 4.4). The modelling efficiency decreased for each empirical model as the period of summed flow was increased. The linear domain model was chosen for inclusion in the budget model due to its higher modelling efficiency. Unfortunately, no data for POC concentration was available at flows below 25 ML d^{-1} (Figure 4.7), however given that particle suspension should be reduced at lower flows, the form of the of the model is considered to be conceptually sound.

Table 4.4. Calibration and validation statistics of four empirical models of POC concentration in Myponga River based on the volume of flow in the preceding 24, 48, 72 and 96 hours.

Model (24 hour)	*Calibration Fit (EF)*	*Validation Fit (EF)*
Linear	0.520	0.552
Natural Log	0.648	0.616
Linear Domain	0.792	0.720
Log Normal	0.725	0.687
Model (48 hour)		
Linear	0.425	0.489
Natural Log	0.648	0.615
Linear Domain	0.693	0.677
Log Normal	0.453	0.442
Model (72 hour)		
Linear	0.431	0.391
Natural Log	0.501	0.518
Linear Domain	0.612	0.626
Log Normal	0.550	0.582
Model (96 hour)		
Linear	0.353	0.451
Natural Log	0.501	0.518
Linear Domain	0.492	0.575
Log Normal	0.292	0.282

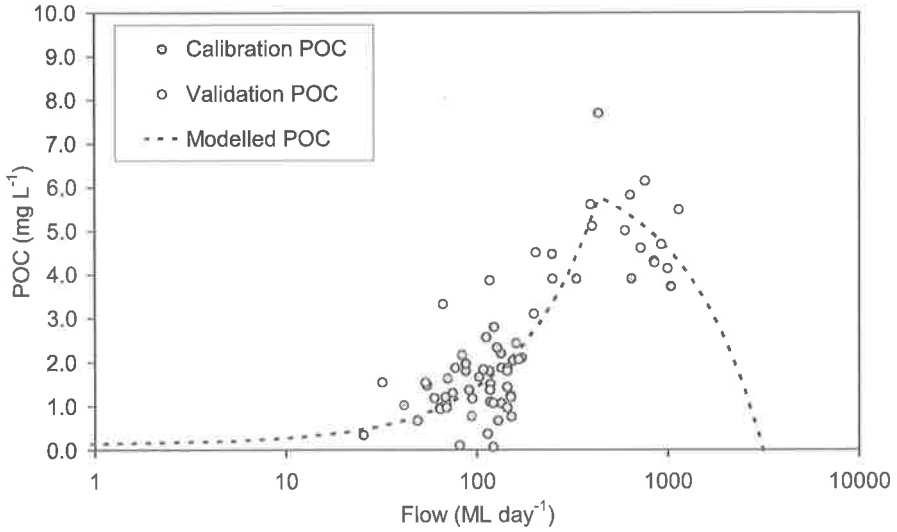

Figure 4.7. Empirical model used to estimate concentration of POC in water flowing in Myponga River.

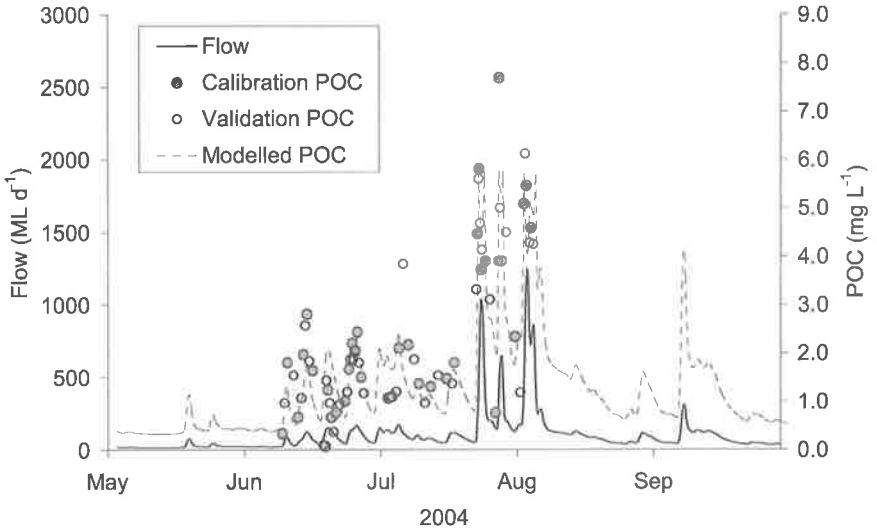

Figure 4.8. POC concentration in Myponga River simulated with a linear domain model (Equation 4.3). The parameters were calibrated against the data represented by filled circles and validated against the data represented by open circles.

4.3.4. Flow~DOC Hysteresis Analysis

The results of the hysteresis analysis imply that there is generally a temporal separation in the surface and ground water contributions to flow occurring in Myponga River as the majority of the ΔR values calculated are not between -20 and 20 % (Table 4.5). A significant positive correlation (Spearman non-parametric) was found between the length of the dry period (Δt) and the magnitude of the flow event (ΔQ_t; Rho = 0.86; prob > |Rho| =

0.0028). Significant negative correlations were found between the magnitude of the previous flow event (ΔQ_{t-1}) and the length of the dry period (Δt; Rho = -0.75; prob > |Rho| = 0.0191) and between the rotation and area descriptor (ΔR) and the initial slope of the recession curve (k; Rho = -0.82; prob > |Rho| = 0.0072). The first two correlations imply that, within this data set, the longer periods between flow events have been followed by relatively larger flow events. The negative correlation between ΔR and k is more interesting as it implies that a broader hysteresis occurred when the decrease in the flow from the peak is slow. A correlation was also found between the day of the year that the event started on and ΔR (Rho = 0.83; prob > |Rho| = 0.0053). This implies that there was a change in the hysteresis rotation over the winter of 2004; the amount of peak flow dilution of DOC concentration and its duration were reduced as winter progressed.

The decrease in the magnitude of the hysteresis has the potential to create a greater challenge to reservoir offtake management later in the winter for two reasons. First, when the peak in DOC concentration trails (temporally) the peak in flow, as in early winter, the time available to respond to the changes in the DOC concentration is slightly longer than the time available to respond to the flow itself (i.e. if responding to a pathogen threat). Therefore if the period between the peak flow and peak DOC concentration is reduced so will be the amount of additional response time. Secondly, the magnitude of the DOC excursions may be greater, as the peak flow and peak DOC concentration are more likely to co-occur, and more DOC rich water will be delivered to the reservoir.

Table 4.5. Results of hysteresis analysis of nine peak flow events in Myponga River in 2004. Parameter descriptions and calculations are given in §4.2.5.

#	ΔQ_t (%)	k (d^{-1})	RL (%)	Δt	ΔQ_{t-1}	ΔC (%)	ΔR (%)
1	320	-0.44	36	4.7	200	35	-71
2	300	-0.43	17	4.6	320	24	-56
3	390	-0.51	24	6.2	300	26	-62
4	82	-0.64	7.9	4.3	320	26	-37
5	2600	-6.6	16	6.3	110	47	-20
6	710	-3.7	17	4.6	2600	32	-29
7	930	-6.4	19	5.9	710	31	35
8	300	-3.6	16	1.4	934	8	-31
9	1000	-1.9	19	9.6	200	11	15

4.3.5. Carbon Budget

The annual allochthonous dissolved organic carbon load to Myponga Reservoir, in the years 2000 to 2003, was estimated to be between 84 and 330 tonnes per annum (mean 240 ± 110 tonnes, N = 4, 1999 was a partial year and not included). For the same period, the annual allochthonous POC load was between 3.5 and 36 tonnes (mean 25 ± 15 tonnes, N = 4). The annual total photosynthetic load of organic carbon to Myponga Reservoir (GPP) was estimated to be between 36 and 110 tonnes (mean 60 ± 34 tonnes, N = 4). The lowest loads of allochthonous carbon occurred in 2002 (Figure 4.9), when the lowest total volume of flow was delivered to Myponga Reservoir from its catchment; there was 4.0 GL of flow in 2002 compared to 9.8 to 13 GL in the other 3 years. Apart from only one year (2000) having significant losses of DOC to over topping, there was very little differences in the year to year estimations of the magnitudes of the other loss processes (Figure 4.10).

Over the entire period (June 1999 to end December 2003) of the budget the total estimated DOC flux from the catchment was 1,100 tonnes while the standing mass of DOC in the reservoir increased by 130 tonnes. Approximately 490 tonnes of DOC was removed with the offtake water and 18 tonnes was lost to overtopping. Assuming on average that 13 % of

pelagic photosynthetic production was lost to the DOC pool (Baines and Pace 1991) then approximately 33 tonnes of autochthonous carbon entered the DOC pool directly in this time. The difference between the standing mass of DOC observed in Myponga Reservoir and the hydraulic balance (balance of inputs and losses to the offtake and overtopping) is shown in Figure 4.11. The loss of organic carbon to photo-mineralisation (PM) was estimated to be 37 tonnes while the losses to bacterial production (BP) and respiration (BR), assuming a BGE of 0.26 (mean for lakes; Cole and Pace 1995), were estimated to be 360 and 1,000 tonnes, respectively. These estimations suggest that the majority of the DOC is likely to be accounted for by microbial processes. It should be noted, however, that the quantity attributed to photomineralisation represents that which is directly oxidised by photochemical energy. A further contribution is made by solar energy towards the clearance of DOC through producing labile products from refractory organic compounds (Lindell *et al.* 1995, Bano *et al.* 1998). The consumption of these labile products, will however be attributed to the microbial budget.

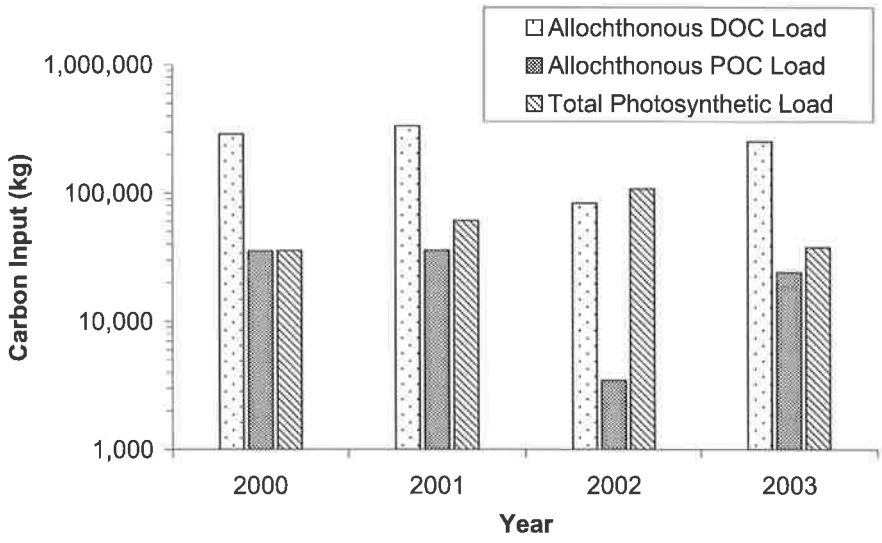

Figure 4.9. Modelled estimates of dissolved and particulate allochthonous organic carbon and total photosynthetic load to Myponga Reservoir.

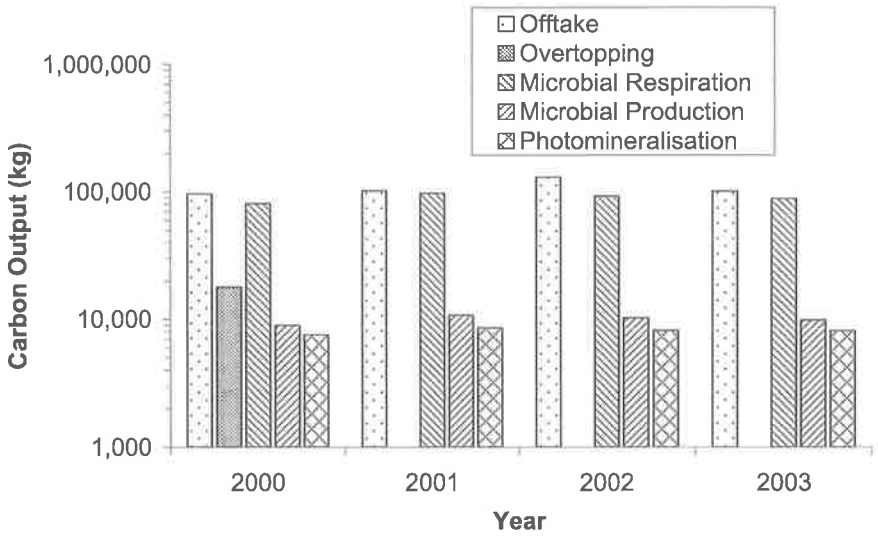

Figure 4.10. Modelled estimates of losses of dissolved organic carbon from Myponga Reservoir.

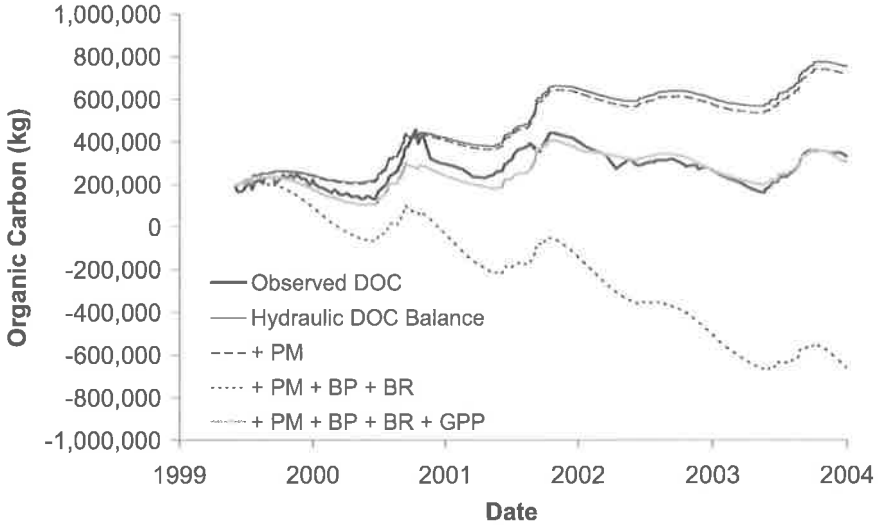

Figure 4.11. Mass balance model of DOC in Myponga Reservoir.

4.4. Discussion

The majority of the variation (up to 71 %) in DOC and POC concentration in samples collected from Myponga River during the winter of 2004 could be described in terms of flow, using simple empirical models. The relationship was nonlinear and the ability to predict DOC concentration was improved in models using flow data as the sum of the previous 72 hours, indicating that the recent hydrology was important to determine the DOC concentration. The seasonal changes, incorporated into the model as a parameterized

day of the year, contributed a slight improvement in the model, from 71 to 75 % of the variability in the data accounted for. Further improvement was achieved by including a fitted parameter multiplied by the flow in the previous 33 days, indicating that longer term flow history also impacts DOC concentration in Myponga River. Other factors that may account for the remainder of the variance are seasonal changes in the concentration of leach-able DOC in the catchment (litter fall and decomposition), seasonal changes in soil water flow paths and other changes in water properties (pH, conductivity, temperature) that determine the equilibrium of DOC between the mobile and adsorbed phases. POC concentration was determined at shorter timescales, with the best relationships found with the daily flow data. Better relationships between instantaneous flow ($m^3 s^{-1}$) and POC were found, but not described here, as the aim was to account for organic carbon at daily timescales.

The range of DOC concentrations found in Myponga River (4.4 - 27 mg L^{-1}) fall into the higher end of the values reported in the literature. Butturini *et al.* (2006) reported ranges of 0.8 to 8 mg L^{-1} for Riera Major, 1.6 to 9.2 mg L^{-1} for Fuirosos and 1.9 to 6.0 mg L^{-1} for Can Vila, three ephemeral streams in North-East Spain. Pers *et al.* (2001) describe ranges of 8 to 40 mg L^{-1} and 5 to 30 mg L^{-1} for Rivers Vårgån and Örån (Sweden), respectively. Kim *et al.* (2000) reported variation in the DOC concentration in River Soyang (South Korea) from 1.0 to 4.5 mg L^{-1}. However, flow is sometimes reported to have no relationship with DOC, for example in the Arizona River (Parks and Baker 1997). In large rivers, such as the Ohio River, phytoplankton density is also reported to be important to determine DOC concentration (Wehr *et al.* 1997).

The annual areal load of DOC from sub-catchment 1 was estimated to be between 7.8 (2002) and 32 kg ha^{-1} yr^{-1} (2001), while the POC load from the same catchment was between 0.3 and 3.4 kg ha^{-1} yr^{-1}. These values are significantly higher than the median values reported for temperate grasslands in north America, New Zealand and Russia

(DOC, mean = 2.9, median = 2.0, range, 1.6 - 5.0; POC, mean = 4.3, median = 5.1, range = 2.1 - 5.6) but are more similar to those reported for temperate forest from the same regions (DOC, mean = 37.9, median = 18.0, range, 3.4 - 417; POC, mean = 6.1, median = 6.6, range = 0.5 - 11.6; Hope *et al.* 1994). This probably reflects the Myponga Rivers catchments history as a forested catchment, over its present state of open grazed grasslands.

The changes in the concentration and character of the dissolved organic matter over the winter of 2004 imply that a greater risk to potable water production would be manifest as the result of large inflow events, late in the winter. The reduction in the hysteresis area implies that there was relatively less dilution of peak DOC concentrations, consequently the short-term loading of DOC to the reservoir would be greater. The higher SUVA values in the flowing water later in winter imply that this organic matter may also carry a greater disinfection by-product formation potential (Page *et al.* 2002). However, the dilution and penetration of these flows through the reservoir will depend on the prevailing temperature structure in the reservoir and the relative temperature of the inflowing water. Using the INFLOW model (Antenucci *et al.* 2005a) to simulate the intrusion of riverine flows (temperature of 10.3 °C) into isothermal water columns of the water temperature found in May, June, July, August and September (17.8, 14, 12, 11.2 and 12.3 °C, respectively), suggest that the dilution of the inflow is reduced as the reservoir temperature decreases.

The accuracy of the estimation of the relative magnitude of allochthonous to autochthonous inputs to the DOC pool depends primarily on the accuracy of the model of photosynthetic production and the fate of this potential or maximum PS. Partitioning of GPP to the DOC pool is reported as varying between 3 to 42 %, with 13 % as the mean (Baines and Pace 1991). The proportion partitioned to the DOC pool depends predominantly on nutritional state and previous light history of the phytoplankton (Baines

and Pace 1991). Zooplankton feeding, phytoplankton senescence and cell lysis can also result in organic carbon entering the DOC pool directly.

Regardless of the proportions of the magnitudes of the allochthonous and autochthonous carbon sources, a significant temporal displacement occurs between the two sources. Allochthonous DOC is determined predominantly by the flow regime and is therefore determined by rainfall in smaller catchments with Mediterranean like climate. Despite the variation in DOC concentration in the river water, the total volume, rather than rate of delivery, is more important to determine the seasonal and inter-annual variation in the delivery of allochthonous DOC to Myponga Reservoir.

The allochthonous inputs to the DOC pool in Myponga Reservoir are most likely an order of magnitude greater than direct autochthonous inputs to the DOC pool. This depends mostly on the proportion lost to the DOC pool during photosynthesis, with the difference being 2.1, 1.4 and 0.9 orders of magnitude at percent losses of 3, 13 and 42 %, respectively. When the total carbon inputs are considered, the allochthonous carbon inputs average 260 tonnes yr^{-1} while the autochthonous inputs average 60 tonnes yr^{-1}.

The hydraulic budget of organic carbon (i.e. neglecting photo-oxidation and microbial utilization) implies that Myponga Reservoir stored, metabolised or otherwise disposed of 92 tonnes of dissolved organic carbon per annum. Had these processes not occurred the resulting DOC concentration as at 31 December 2003 would have been 30.9 mg L^{-1}. Resolution of the fate of this excess 17.3 mg L^{-1} DOC has not been directly addressed in this study, but estimations of its likely partitioning into different pathways was possible using models from the literature. Estimation of the role of direct photo-oxidation was estimated as approximately 9 %, under the assumption that the organic matter in Myponga Reservoir has a similar quantum yield to that reported by Vahatalo *et al.* (2000). If the quantum yield was two to four times higher, similar to that reported in Gao and Zepp (1998), then it would account for between 17 and 35 % of the organic carbon cleared. The

remainder of the organic carbon cleared is easily accounted for by rates of bacterial production and respiration, modelled using reported relationships with water temperature, and therefore most likely accounts for around 70 % of the carbon cleared.

Based on empirical conceptual models of the distribution of the metabolism of organic matter in lakes, it is likely that the majority of the organic carbon in Myponga Reservoir is metabolised in the pelagic. The models proposed by Denheyer and Kalff (1998) predict that, based on mean depth and chlorophyll concentration, that the pelagic in Myponga Reservoir should account for between 85 and 95% of the total lake respiration. The model of Denheyer and Kalff (1998) was based on natural lakes without artificial destratification; given that sedimentary losses will be reduced under destratification, it would seem that an even greater role may be played by the pelagic in the clearance of organic matter in Myponga Reservoir.

Inaccuracies in the Model

The sources of error in the model include the use of a single photosynthesis irradiance curve for estimation of primary production and the use of whole lake averages for parameters known to have vertical and horizontal gradients. For example DOC has longitudinal and vertical gradients during winter inflows (see §3.3.2) and vertical gradients of chlorophyll are generally found in lakes with even small vertical temperature gradients. The littoral and benthic sources of DOC were not considered, despite their frequent importance (Wetzel 2001b), however much of the littoral zones of Myponga Reservoir are sparsely populated by aquatic macrophytes, if at all. The assumption was made that the load of DOC from the remainder of the catchment was similar to that from Myponga River. Some evidence for the similarity in the dissolved organic carbon dynamics for the majority of the sub-catchments was presented in §3.3.2, however very different concentrations and characters have been observed, especially during periods of low flow (Linden, unpublished data).

The sources of error in the modelled estimates of the DOC concentration in Myponga River include the accuracy of the estimation of flow rate and the inherent variation in the determination of dissolved organic carbon concentration in Myponga River (sampling, storage and analysis errors) that the model was parameterised against. As the inherent variation in the DOC concentration determination was not directly investigated experimentally, the model error derived from the measurement of flow, and its implication for the uncertainty of the load estimation from Myponga River will be discussed.

The flow at the weir in Myponga River is determined in the data logger from a gauge height, determined to the nearest centimetre. From this value, instantaneous flowrate (m^3s^{-1}) is determined to 3 significant figures. Propagating the absolute error of the gauge height measurement (± 0.5 cm) results in an estimated uncertainty of instantaneous flowrate varying from 3.2 %, at the minimum predicted DOC concentration, to 0.7 % at the maximum DOC concentration. The percent absolute error in flow rate estimation at the average flow rate for DOC prediction was 0.8 %. Propagating this error estimate through to the DOC concentration estimation model results in an average uncertainty, due to model structure and flow measurement error, of 1.8 % within the validation data set of the log normal model A (equation 4.4). This is probably less than the sum of the sampling and DOC determination error, which is likely to be 5 to 10 %.

Log normal model B (equation 4.6) includes an additional variable of day of the year, which is determined without error. The variable of 33 day flow, included in the log normal model C (equation 4.7), was correlated with 72 hour flow (coeff = 0.69) for the period of 2004. Therefore, only a small amount of additional uncertainty is contributed by this formulation. The uncertainty of the estimation of river flow volume also contributes to the uncertainty of the daily DOC load estimation. The error associated with the estimation of DOC load with model C (equation 4.7), attributed to the estimation of Myponga River flow

rate, was estimated to be between 1.8 and 8.4 %. Given the natural variability in DOC concentration, this level of error is considered to be acceptable.

Conclusions

Myponga Reservoir is a humic mesotrophic lake with a large dissolved organic matter pool, dominated by allochthonous inputs in years of normal flow regime. The allochthonous inputs are likely to be exceeded by algal production in years of low flow, as exemplified by 2002. The main determinant of the ratio of allochthonous to autochthonous carbon inputs is climatic variability in rainfall and runoff. Compared to the source processes, the loss processes are more stable on an interannual basis. The exception being loss to overtopping which is determined the by hydrology and is therefore strongly influenced by climate. Microbial production and respiration most likely accounts for the majority of the cleared dissolved organic carbon, with photo-mineralisation accounting for between 8 and 35%. It is likely that the majority of the DOC standing stock consists of microbially recalcitrant allochthonous DOC and that photo-mineralisation is an important process for the clearance of this DOC. Despite the changes in DOC concentration that occur during high flow episodes, it is predominantly the flow rate of the river and the relative reservoir and intrusion temperature characteristics which determine the dilution and mixing of the intrusion and therefore the potential threat to the WTP offtake.

Chapter 5. Treat-ability of Organic Carbon in Three Australian Reservoirs with Differing Hydrodynamics

5.1. Introduction

The primary aim of work reported in this chapter was to identify the impacts that artificial destratification has on the concentration and character of natural organic matter (NOM) present in reservoir water. In the conventional treatment of water NOM exerts a significant coagulant demand and can act as a precursor for the formation of disinfection by-products following chemical disinfection. One of the main reasons for undertaking artificial destratification is that the different strata of the reservoir have different water qualities. For example, the growth of cyanobacteria in the surface strata and the release of soluble metals into the bottom strata if it becomes deoxygenated (Brookes *et al.* 2000a). If an improvement or a decline in the water quality, from the perspective of NOM, occurs under destratified conditions, then differences in the character of the NOM in the surface or bottom strata will likely be found. These differences may be the result of differential exposure to processes fuelled by photochemical energy, such as photosynthesis and photochemical mineralisation or conformational change, or differential exposure to microbial activity, which is often observed to be vertically stratified (e.g. Cole *et al.* 1993, Amblard *et al.* 1995).

Studies that have described vertical gradients in organic matter character or concentration include (AWRC 1979, Bowles *et al.* 1979, Croome 1981, Kim *et al.* 2000, Osburn *et al.* 2001, Choi *et al.* 2002). These vertical gradients were variously generated by inflow events and persistent thermal stratification. Changes in the optical characteristics (UV-visible absorbance) of organic matter have been observed in the absence of any changes in the concentration of the organic carbon (Osburn *et al.* 2001). A lack of seasonal variation in

the treat-ability of organic matter has been observed, despite the expectations of the authors that significant thermal stratification would produce such variation (Owen *et al.* 1993).

Vertical gradients in factors that result in changes in organic carbon properties are common in aquatic systems. Microbial community composition, cell density and activity varies over depth in lakes (Cole *et al.* 1993, Cole and Pace 1995, Konopka *et al.* 1999) and major differences in carbon metabolic pathways occur between aerobic and anoxic environments (Bastviken *et al.* 2004). The exposure of any part of the water column to solar radiation is determined by its depth and the optical properties of the water. The potential for photochemical modification and autochthonous carbon production is therefore determined by the vertical structure of the water column.

Comparison of the treat-ability of the natural organic matter found in water from different water bodies is often reported in the literature (Chow *et al.* 1999, Drikas *et al.* 2003) and is an important part of demonstrating the utility of a new treatment technology, or the applicability of new ideas or concepts. Seasonal changes in the water quality, or treat-ability in organic matter is also reported (Owen *et al.* 1995, Goslan *et al.* 2002), and is important to understand and manage individual systems at longer timescales. Differences in the quality of the water available for extraction from different reservoir strata have been reported for cyanobacteria (Fastner *et al.* 2001) and pathogens (Hipsey *et al.* 2005). Specific reports on differences in treat-ability of NOM from different strata are limited to those associated with extreme events, such as inflow events following bushfires (CRCWQT 2005). However they do not provide a view of the general changes that can be expected during summer stratification.

Given the diversity of water treatment technologies that are available with various capacities to remove organic matter, the application of a single, well understood, conventional treatment process was selected to provide robust, interpretable data of the

treat-ability of the organic matter in this study. Furthermore, the treatment process was selected so that the results obtained would enable relevant comparisons to current water treatment practises. A jar test procedure including coagulation with alum (aluminium sulphate) and clarification by sedimentation was chosen. As the coagulation pH plays an important role in controlling the coagulation process and removal of DOC (Crozes *et al.* 1995), the majority of jar tests were conducted at a controlled pH. A pH of 5.5 was selected as it has been previously found that this pH results in near maximum removal of DOC with alum (Randtke 1988, Chow *et al.* 1999). Nonetheless, this coagulation pH may not be an operational optimum pH when considering residual aluminium concentration limits and minimising corrosion of WTP infrastructure. Certain jar tests were conducted at a different pH when it was appropriate to provide information about the protonation of acidic groups within the NOM.

The removal of natural organic matter with cationic coagulants (such as alum) occurs by a number of processes. Colloidal NOM can be caused to coagulate and flocculate by charge neutralisation, compression of the charged double layer that surrounds the charged colloid and 'sweep' coagulation, where organics are entrapped during the coagulation of other molecules (Chakraborti *et al.* 2000). Precipitation of aluminium or iron (depending on the coagulant) humate and fulvate complexes occurs as these products have lowered solubility compared to the uncomplexed species, causing the precipitation of a solid. Other organic compounds may adsorb to these metal hydroxide crystals, causing co-precipitation (Jekel 1986). As a wide range of functional groups is found in NOM, the adsorption process is the product of Van Der Waals, hydrogen, hydrophobic and dipole interactions (Crozes *et al.* 1995). The pH plays a dual role in the coagulation process as it affects both the behaviour of the coagulant and the organic matter. At lower pH, a greater proportion of the organic matter is protonated and the coagulant is more positively charged. These characteristics

make adsorption more favourable and the required coagulant dose is reduced. Charge neutralisation is probably also enhanced at low pH (Crozes *et al.* 1995).

In order to predict DOC removal during coagulation, Kastl *et al.* (2004) proposed a model of coagulation of organic matter, further developing the model proposed by Edwards (1997). The model conceptually delineates the DOC pool into three groups, the non-polar group (NPG), the humic acid group (HAG) and the nonsorbable group (NSG). The proportion of the NPG is determined by adsorption alone, while the adsorption of the HAG is determined by pH as well. The NSG is the organic matter that is effectively unable to be removed by the metal coagulant in question. The application of this model, by determining the fitted constants for the DOC absorption by jar testing, allows the prediction of NOM removal for a given water source (Tseng and Edwards 1999, Kastl *et al.* 2004). However it also allows for the prediction of the sizes of the three different groups and therefore quantifies the treat-ability of the NOM from a particular water source, with reference to a particular coagulant. It was therefore considered a potential tool to describe the differences in treat-ability between waters collected from the different strata within our field sites.

The jar testing required for full estimation of the sizes of the HAG and NPG was not always performed (due to time and sample volume demands), however, of greatest significance is the amount or proportion of the organic matter which is unable to be removed; the nonsorbable group. This group can be estimated by the fitting of an exponential decay function to the jar test data of DOC residuals after coagulation and sedimentation with increasing doses at optimal pH. Choosing the optimum alum dose (which may then be altered to extreme and under dosing to provide information on this DOC removal curve) can be achieved using other models of NOM removal. mEnCo can be applied to predict practically suitable coagulant doses and pH control requirements to maximise removal of NOM for a broad range of waters (van Leeuwen *et al.* 2005, Holmes *et al.* 2006).

If differences in treat-ability are found then these need to be placed in the context of the prevailing conditions in the reservoir to determine the possible causes of these differences. Was there persistent thermal stratification that resulted in the production of strata within the water column? If so, what were the differences in the conditions between the sampled strata and how could they have given rise to the observed differences in NOM character. One hypothesis is a difference in the optical characteristics may occur due to the photo-mineralisation of chromophoric organic carbon or vertical differences in autochthonous input. Changes in the redox environment in the hypolimnion, or shifts in the pH during accumulation of respiratory CO_2 may alter the metabolism or the availability of some moieties. To assess these possibilities, it is important to have information on the water column temperature profile, and preferably on the surface layer dynamics prior to collecting the samples.

The field sites used in this study can be considered to represent three broad categories of reservoirs. (1) Myponga Reservoir is moderately deep (> 30 m maximum depth) and is destratified. Some stratification does occur, but mostly at daily timescales. The stratification does not persist and de-oxygenation of any part of the water column is unlikely to occur (Brookes *et al.* 2000a). (2) Googong Reservoir is also moderately deep (> 30 m maximum depth), but is not artificially destratified and would be expected to experience persistent summer stratification and hypolimnetic de-oxygenation. (3) Wartook Reservoir is relatively shallow (< 10 m maximum depth) and while it should stratify during periods of stable weather with high insolation and low wind speeds, it is likely to mix relatively frequently during the summer period.

The main aim of this part of the study was to determine if any differences in the character of NOM occurs in water from different depths of stratified water columns, and furthermore if this character difference causes differences in treat-ability using conventional coagulation-sedimentation water treatment.

5.2. Materials and Methods

Site descriptions can be found in Chapter 2 (§2.1.1, §2.1.2 and §2.1.3). Water temperature was measured at discrete depths using strings of stand alone data loggers in Googong and Wartook reservoirs.

5.2.1. Site visits

Googong Reservoir was sampled for jar testing on two occasions, on 19 November 2003 and 17 May 2004. Wartook Reservoir was visited on three occasions, 6 November 2003, 4 December 2003 and 12 February 2004. Data from Myponga Reservoir is presented from three sampling dates, 13 March 2003, 2 April 2003 and 14 April 2003.

5.2.2. Jar testing

Jar testing was conducted, using procedures detailed in Section §2.3.8, with alum. The coagulation pH was controlled with sulphuric acid and sodium hydroxide solutions. Alum doses were predicted using the mEnCo model using its optimum dose algorithm and its percentage target removal algorithm. An extreme dose was selected by using either 150 or 200% of the model predicted dose (MPD) or the alum dose required to take the pH to 5.5. The conditions applied are presented in Table 5.1. The filtered product water was analysed for UV absorbance (UVA), true colour (HU) and dissolved organic carbon (DOC). The resulting data was fitted to the equation:

$$DOC_{remaining} = a + b\, e^{-D/c} \qquad (5.1)$$

where $DOC_{remaining}$ is the DOC in the product water, D is the alum dose applied (mg L^{-1}) and *a*, *b* and *c* are parameters fitted using least squares minimisation using solver in Microsoft Excel. The fitted parameters describe the shape of an exponential decay curve that describes the behaviour of removal of the organic matter with the coagulant dose and pH applied (Figure 5.1). The fitted parameter *a* is the amount of DOC that theoretically

cannot be removed by alum and $a + b$ is the concentration of DOC in the raw water. The proportion of nonsorbable organic carbon can then be represented as follows:

$$NSG_{proportion} = \frac{a}{a+b} \tag{5.2}$$

To investigate the potential changes in the treat-ability due to the stratification that occurred at each sampling date, the difference in the fitted parameters from samples above and below the thermocline was calculated. This allows the samples collected from the different reservoirs and dates to be statistically analysed as a single data set with a null hypothesis that the mean of the differences is equal to zero. As this data is not normally distributed, the Wilcoxon signed rank non-parametric test was applied, using JMP Software (SAS Institute Inc, Cary, North Carolina, USA).

Table 5.1. Jar test protocol applied on samples collected from Googong, Myponga, and Wartook Reservoirs. Numbers in subscript represent the target pH and the percentages represent the targeted percent removal of DOC dose predicted with mEnCo.

Reservoir	*Date*	*Jar 1*	*Jar 2*	*Jar 3*	*Jar 4*	*Jar 5*	*Jar 6*
Googong	19/11/2003	$50\%_{5.5}$	$80\%_{5.5}$	$MPD_{5.5}$	$2\times MPD_{5.5}$	$MPD_{7.3}$	$2\times MPD_{8.3}$
Googong	17/05/2004	$50\%_{5.5}$	$80\%_{5.5}$	$MPD_{5.5}$	$2\times MPD_{5.5}$	$MPD_{7.3}$	$2\times MPD_{8.3}$
Myponga	13/03/2003	$50\%_{5.5}$	$50\%_{5.5}$	$MPD_{5.5}$	$MPD_{5.5}$	$To5.5_{5.5}$	$To5.5_{5.5}$
Myponga	2/04/2003	$50\%_{5.5}$	$50\%_{5.5}$	$MPD_{5.5}$	$MPD_{5.5}$	$To5.5_{5.5}$	$To5.5_{5.5}$
Myponga	14/04/2003	$50\%_{5.5}$	$50\%_{5.5}$	$MPD_{5.5}$	$MPD_{5.5}$	$To5.5_{5.5}$	$To5.5_{5.5}$
Wartook	6/11/2003	$50\%_{5.5}$	$75\%_{5.5}$	$90\%_{5.5}$	$MPD_{5.5}$	$1.5\times MPD_{5.5}$	$MPD_{6.0}$
Wartook	4/12/2003	$50\%_{5.5}$	$80\%_{5.5}$	$MPD_{5.5}$	$2\times MPD_{5.5}$	$MPD_{7.3}$	$2\times MPD_{8.3}$
Wartook	12/02/2004	$50\%_{5.5}$	$80\%_{5.5}$	$MPD_{5.5}$	$2\times MPD_{5.5}$	$MPD_{7.3}$	$2\times MPD_{8.3}$

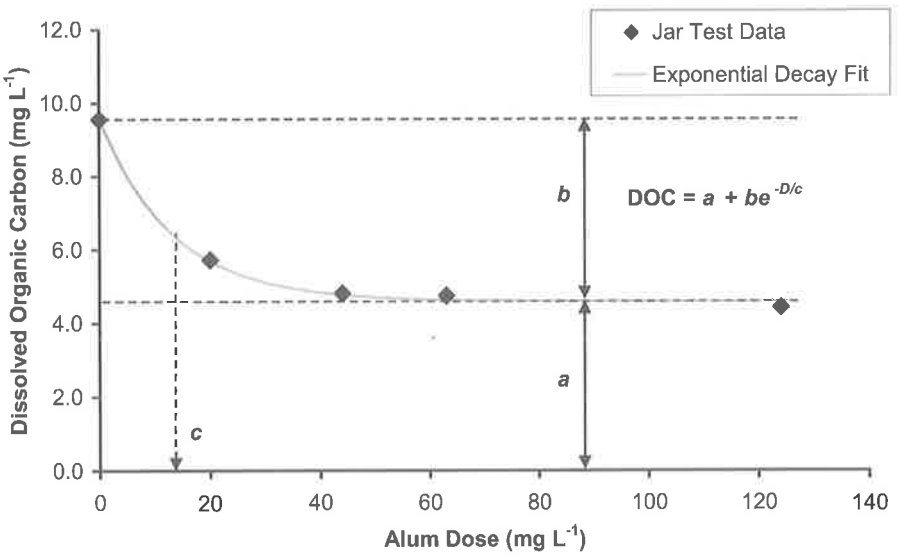

Figure 5.1. Example of exponential decay curve fitted to jar test data.

5.2.3. Sediment cores

5.2.3.1. Experimental Setup

The role of the sediment in determining the DOC character in the overlying water was investigated using sediment cores collected from Wartook Reservoir on 12 February 2004. Shallow sediment cores (67 - 88 mm) were collected in Perspex tubes (internal diameter 57mm) using a gravity-suction corer constructed of heavy galvanised 60mm steel water pipe and plugged beneath the sediment with a rubber stopper. The sediment cores were transported to the laboratory and the original overlying water removed with a hose and peristaltic pump. Three sediment cores and three control tubes (without sediment) were filled with oxygen saturated water collected from the same site from 7.5 m depth and were fitted with oxygen probes (TPS WP82Y meters and YSI 5739 probes) and magnetic fleas (Figure 5.2). All joints in the constructed sediment core chambers were sealed with multiple layers of laboratory film (Parafilm M®). The control cores contained 780 mL of water while the sediment chambers held an average of 580 mL of water and 200 mL of sediment. Stirring was facilitated by arranging the 6 cores around a central magnetic stirrer, ensuring all the magnetic fleas were spinning at an equal rate. The temperature was maintained at between 20 and 27 °C and the cores were incubated for a total of 19 days, in the dark, to prevent algal growth. Dissolved oxygen was logged as the average

concentration at 10 minute intervals. Only two tubes of each treatment were able to be monitored for the entire period, two tubes were only monitored for 5 days due to power supply problems. Each tube was sampled from the centre of the chamber at the end of the experiment, using a tube and peristaltic pump and the water analysed for dissolved organic carbon (DOC), total organic carbon (TOC), filterable reactive phosphorus (FRP), total phosphorus (TP), total kjeldahl nitrogen (TKN), oxidised nitrogen (NO_x) and ammonia (NH_4) (see Chapter 2 for analytical methods).

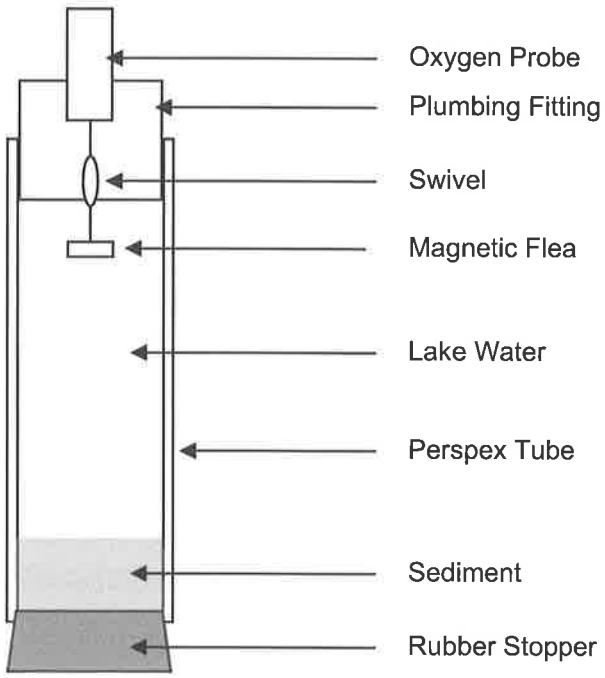

Figure 5.2. Experimental set up for oxygen depletion incubations of sediment cores.

5.2.3.2. Analysis of Oxygen Decay Curves

The oxygen in the chambers was consumed by three potential pathways, microbial activity in the sediment, microbial activity in the water and by the oxygen electrode itself. As the oxygen consumption by the electrode is very low (< 0.01 mg L^{-1} over the entire incubation period or O_2 consumption of 1.1×10^{-5} mg h^{-1}; TPS product information) only the pelagic metabolism and sediment uptake was considered significant. Absorption of dissolved oxygen by sediments is a diffusion limited processes (Tengberg *et al.* 2004) and is therefore be best described by a nonlinear functions such as exponential decay. The

depletion of oxygen in the pelagic is also expected to be a nonlinear function (Ciaccio 1992, Burns *et al.* 1996). A simple exponential model was fitted to daily oxygen concentrations by maximising the modelling efficiency (EF, see §4.2.4) using Solver in Microsoft Excel.

$$DO_t = DO_0 e^{-kt} \tag{5.3}$$

Where DO_t is the dissolved oxygen concentration at time = t (days), DO_0 is the dissolved oxygen concentration at time = 0, and k is a fitted constant. The initial rates of oxygen demand were calculated from the fitted data (slope of curve over first day) and the volumetric sediment oxygen demand was calculated by subtracting the control rate from the sediment column rate. Areal sediment and pelagic oxygen demand were then calculated by multiplying by the effective height of the volume depleted (total volume of water divided by surface area).

5.3. Results

5.3.1. Jar testing

5.3.1.1. Raw Water Parameters

The raw water characteristics were similar within reservoirs but varied considerably between reservoirs (Table 5.2). Myponga Reservoir was the most highly coloured (50 - 65 HU, mean = 58 ± 6 HU), followed by Wartook Reservoir (14 - 44 HU, mean = 31 ± 11 HU) while Googong Reservoir had the lowest true colour (8 - 9, mean = 9 ± 1). The means are significantly different (Tukey Kramer HSD, α = 0.05). DOC and UV absorbance followed a similar trend in the different reservoirs with Myponga having the highest values (DOC, 11.8 - 13.1 mg L^{-1}, mean = 12.5 ± 0.5 mg L^{-1}; UVA, 0.400 - 0.420 cm^{-1}, mean = 0.410 ± 0.008 cm^{-1}), followed by Wartook Reservoir (DOC, 9.2 - 9.8 mg L^{-1}, mean = 9.6 ± 0.2 mg L^{-1}; UVA, 0.145 - 0.254 cm^{-1}, mean = 0.210 ± 0.042 cm^{-1}) and Googong Reservoir

(DOC, 6.4 - 6.9 mg L^{-1}, mean = 6.7 ± 0.2 mg L^{-1}; UVA, 0.123 - 0.130 cm^{-1}, mean = 0.126 ± 0.003 cm^{-1}; all means are significantly different, Tukey Kramer HSD, $\alpha = 0.05$).

Myponga Reservoir had the highest specific UV absorbance and specific colour (SUVA, 3.1 - 3.4, mean = 3.3 ± 0.1; SpHU, 3.8 - 5.2, mean = 4.6 ± 0.5), while the SUVA in Wartook Reservoir (SUVA, 1.5 - 2.6, mean = 2.2 ± 0.4) was not significantly different from Googong Reservoir (SUVA, 1.8 - 2.0, mean = 1.9 ± 0.1; Tukey Kramer HSD, α = 0.05) The specific colour in Wartook (SpHU, 1.4 - 4.5, mean = 3.2 ± 1.1) was significantly higher than in Googong Reservoir (SpHU, 1.2 - 1.4, mean = 1.3 ± 0.1, Tukey Kramer HSD, $\alpha = 0.05$).

Table 5.2. Water quality parameters used to derive model predicted dose for jar testing. These are post-storage values and may differ slightly from those reported elsewhere in the chapter where smaller samples were analysed immeadiately upon collection.

Reservoir	*Date*	*Sample Depth (m)*	*True Colour (HU)*	*UV Absorbance (cm^{-1})*	*DOC ($mg\ L^{-1}$)*	*Turbidity (NTU)*	*Alum to pH = 5.5 ($mg\ L^{-1}$)*
Googong	19/11/2003	0.5	8	0.127	6.6	1.0	120.0
		10	8	0.130	6.5	1.0	119.0
		20	9	0.130	6.4	1.0	116.0
	17/05/2004	0.5	9	0.124	6.9	2.3	132.3
		10	9	0.123	6.9	2.2	132.3
		20	9	0.124	6.9	2.2	131.1
Myponga	13/03/2003	0.5	55	0.400	11.8	3.4	156.0
		19	55	0.410	12.2	3.4	159.0
	2/04/2003	0.5	65	0.415	12.6	3.3	156.0
		13	65	0.420	12.8	3.3	161.0
	14/04/2003	0.5	55	0.415	12.3	3.4	153.0
		13	50	0.400	13.1	3.1	154.0
Wartook	6/11/2003	0.5	42	0.253	9.6	1.0	4.0
		7.5	44	0.254	9.8	1.0	4.0
	4/12/2003	0.5	28	0.217	9.6	1.3	4.2
		7.5	29	0.210	9.7	1.3	4.2
	12/02/2004	0.5	14	0.145	9.7	0.4	7.4
		7.5	29	0.182	9.2	0.8	10.8

5.3.1.2. Assessment of Treat-ability by Jar Testing

Exponential decay curves fitted to the data all had EF above 0.97. Organic matter character varied between reservoirs with the proportion of nonsorbable organic matter being significantly different with relative magnitudes being Wartook > Googong > Myponga (Figure 5.3). When the differences between the fitted parameters above and below the thermocline were considered, with all the reservoirs grouped, the results were not significantly different from zero (Wilcoxon signed rank test, $p > 0.05$). Nor were there any significant differences when the above and below the thermocline differences were considered within the individual reservoirs (Wilcoxon signed rank test, $p > 0.05$). This suggests that there was no consistent trend in the treat-ability between epilimnetic and hypolimnetic samples collected, even within the individual reservoirs.

The number of sampling occasions was small and it may be useful to examine more closely the occasion when the most difference was observed, in samples collected from Wartook Reservoir on 12 February 2004. When the samples were examined using HPSEC-UV (Method A) there were obvious differences in the raw water spectra; the surface samples had lower peak absorbance in the high molecular weight region (~60 kDa) and had a higher UV absorbance through the medium to low molecular weight range (Figure 5.4). The high molecular weight UV absorbing material was totally removed in the both samples at all coagulant concentrations applied. Similar relative differences were observed in the treated waters over the medium to low molecular weight ranges. This suggests that there has been a change in the treat-ability as the 7.5 m sample was jar tested with slightly higher alum doses (0.5 m MPD = 46; 7.5 m MPD = 56). There was a small increase in removal with an additional 20 mg L^{-1} of alum applied to the surface waters. Coagulant treatment was conducted only on the basis of MPD and no comparison was conducted using the same dose for each sample. While there was only a 1 % difference in the proportions of the nonsorbable group of compounds (NSG) between these samples (Table

5.3), there were differences of 3 and 4 % in the non-polar (NPG) and humic acid groups (HAG), respectively. Although these are small differences, they indicate that some modification or change in character of the organic matter may have occurred.

Table 5.3. Estimation of distribution of organic matter into the nonsorbable (NSG), nonpolar (NPG) and humic acid (HAG) groups in Googong and Wartook Reservoirs and over depth.

Reservoir	*Date*	*Depth*	*NSG (%)*	*NPG (%)*	*HAG (%)*
Googong	19/11/2003	0.5 m	43	19	39
		10.0 m	43	19	38
		20.0 m	46	11	44
	17/05/2004	0.5 m	42	22	36
		10.0 m	44	22	35
		20.0 m	42	23	35
Wartook	4/12/2003	0.5 m	48	37	15
		7.5 m	48	36	16
	12/02/2004	0.5 m	46	44	10
		7.5 m	45	41	14

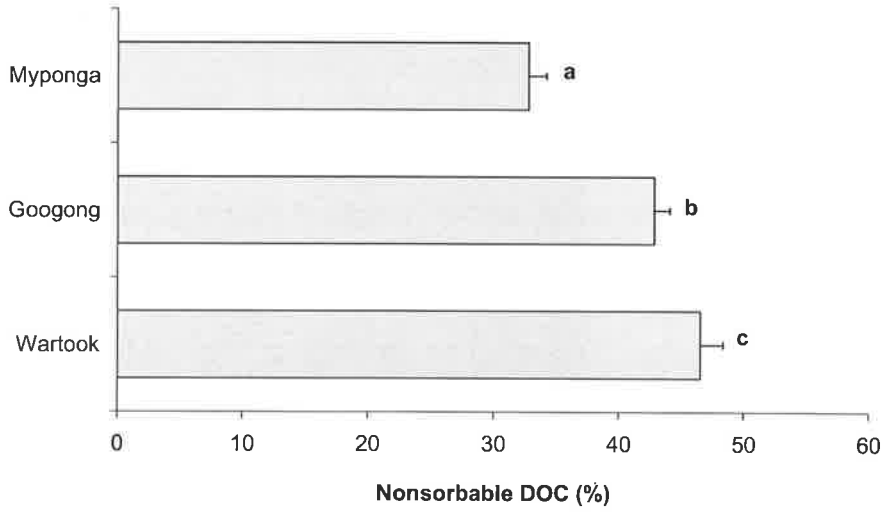

Figure 5.3. Mean (± SD) of the percentage of the DOC which was not coagulated with alum from three different reservoirs (N = 6).

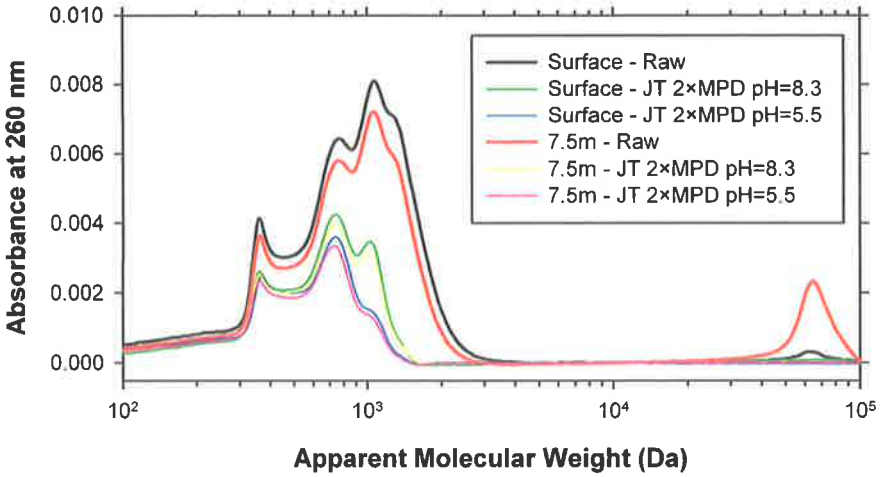

Figure 5.4. Comparison of raw, extreme dose at pH = 5.5 and extreme dose at pH = 8.3 for samples collected from the surface and 7.5 m at Wartook Reservoir.

5.3.2. Placing Treat-ability in Context of Reservoir Conditions

5.3.2.1. Googong Reservoir

The surface mixed layer (SML) of Googong Reservoir on 19 November 2003 was approximately 7 m deep (Figure 5.5). There was abundant dissolved oxygen in the water column and a chlorophyll concentration peaked at 14 μg L^{-1} at between 8 and 10 metres depth, below the SML. Due to the absence of an increase in temperature at ~12 m depth (which would be caused by mixing with the warm surface layer), the length of separation of the epilimnion and hypolimnion was most likely since at least 7 November 2003. On 17 May 2004 there was a small amount of surface heating in the top 1 metre of the reservoir, however the reservoir had recently mixed to greater than 20 m depth, demonstrated by the lack of a temperature gradient from 1 to 21 m (Figure 5.5). Dissolved oxygen was significantly depleted in the hypolimnion, with less than 1 mg L^{-1} remaining at 30 m. The chlorophyll concentration ranged from 3 to 7 μg L^{-1} in the epilimnion and was similar to 3.5 μg L^{-1} in the hypolimnion. The thermistor data reveals that the epilimnion deepened past 15 m around 11 May 2004 and to 20 m around the day of sampling. The sampling equipment only allowed sampling to 20 m and consequently the true hypolimnion was not sampled.

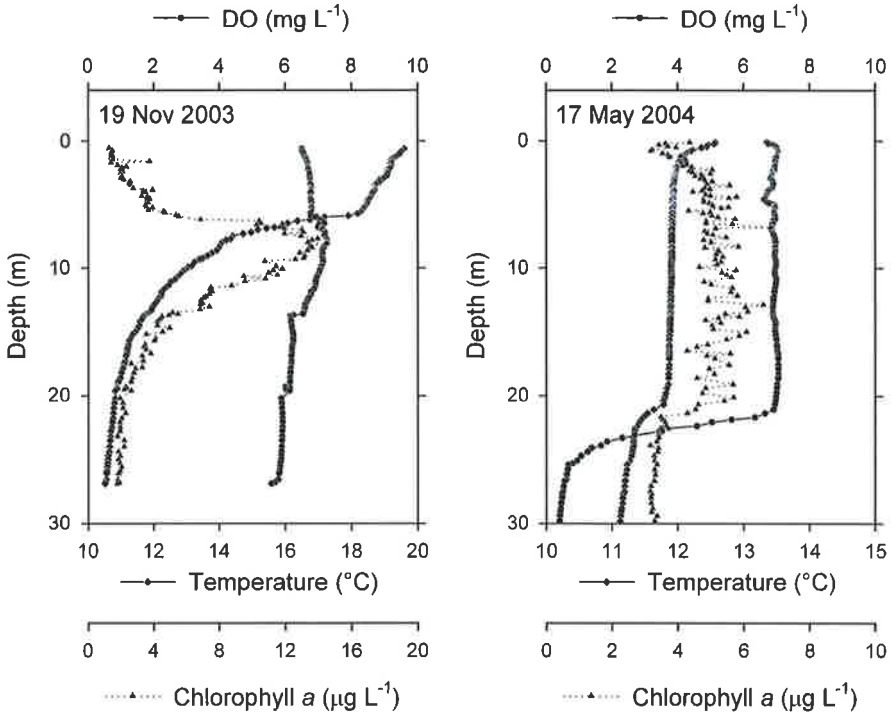

Figure 5.5. Multi-parameter sonde profiles of temperature dissolved oxygen and chlorophyll over depth on 19 November 2003 and 17 May 2004.

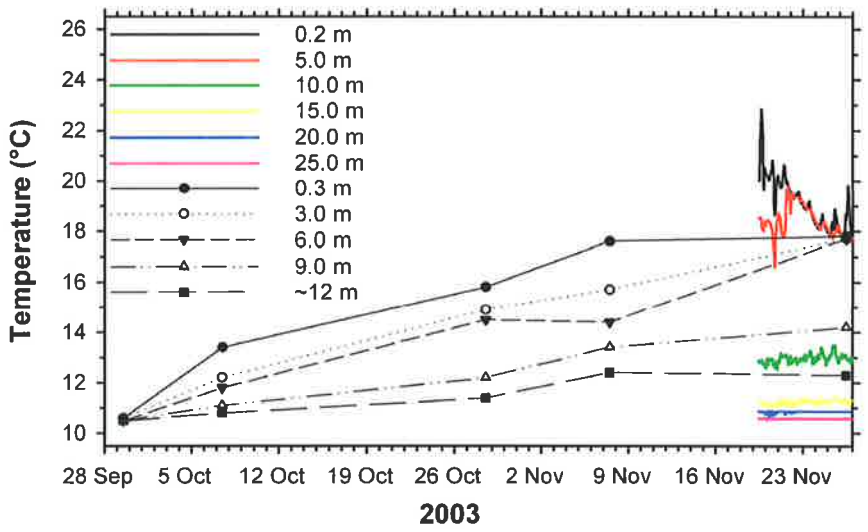

Figure 5.6. Temperature monitoring data for Googong Reservoir, 28 September 2003 to 27 November 2003. Lines without symbols represent monitoring with temperature probes while lines with symbols represent routine monitoring data collected adjacent to the offtake tower, approximately 70 m from the thermistor chain. The data labelled ~12 m was at the deepest depth sampled, between 10.5 and 11.5 m.

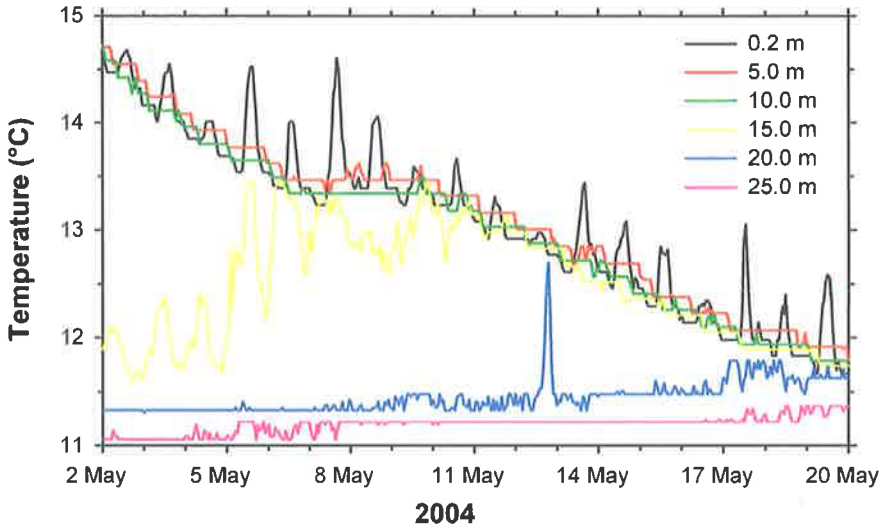

Figure 5.7. Temperature monitoring data for Googong Reservoir 2 May 2004 to 20 May 2004.

5.3.2.2. Wartook Reservoir

Wartook Reservoir had a temperature gradient between 13 and 16 °C and an oxygen saturated water column on 6 November 2003 (Figure 5.8). The chlorophyll concentration was between 5 and 9 µg L^{-1} with slightly higher concentrations found at depth. It is unknown what the precedent hydrodynamics were, as the thermistor chain was installed on this visit and routine monitoring data was not available. On 4 December 2003, the temperature had increased throughout the water column, to between 16.5 and 21 °C, indicating that there had been complete mixing during this time (Figure 5.8). The thermistor chain data suggest that complete mixing had most recently occurred on 24 November 2003 (Figure 5.9). Prior to the sampling on 12 February 2004, the most recent complete mixing occurred on 4 February 2004 (Figure 5.10). The water column had mixed to approximately 4.5 m by the time of sampling on 12 February 2004; at this time there was a gradient in the chlorophyll concentration from about 4 µg L^{-1} at the surface to about 7 µg L^{-1} at the bottom. Dissolved oxygen was between 7.8 and 8.5 mg L^{-1} over the whole water column (Figure 5.8).

Large differences in the apparent colour (surface = 31 HU, 7.5 m = 88 HU) and apparent UV absorbance (surface = 0.183 cm^{-1}, 7.5 m = 0.276 cm^{-1}) were observed, despite the low absolute turbidity of the samples (surface = 0.42 NTU, 7.5 m = 0.78 NTU) and the insignificant differences in the replicate 0.45 μm filtered samples (Figure 5.8). These differences in apparent optical properties were investigated by filtration with a range of membranes with different pore sizes (Figure 5.12) and HPSEC (Figure 5.4). It was observed that while the concentration of organic carbon passing through a 0.4 μm polycarbonate membrane in the 7.5 m sample was almost 1 mg L^{-1} lower than in the surface samples, the concentration that passed through polycarbonate membranes above 2.0 μm was very similar (Figure 5.12A). Despite containing no additional organic matter, the apparent colour and UV absorbance of the 7.5 m samples increased significantly as the pore size was increased (Figure 5.12B,C), while these parameters increased only slightly in the surface samples.

While the initial reaction is to attribute the differences in apparent optical properties to light scattering, the ratio of turbidity (as NTU) to colour (as HU) in both raw samples was less than 0.02 (0.5 m = 0.014; 7.5 m = 0.001); proposed by Bennett and Drikas (1993) as the ratio where light scatter becomes significant and sample filtration is required. It may therefore be concluded that this difference is the result of the optical properties of the organic matter. Further evidence that this is the result of a difference in the organic matter properties is found in the HPSEC chromatograms of the two samples. The HPSEC sample pre-processing involves filtration through a 0.2 μm nylon membrane which is much smaller than the filtrations previously described (Figure 5.12). However a conspicuous difference between the chromatograms is the higher peak absorbance at approximately 60 kDa apparent molecular weight (Figure 5.4). This peak is likely to represent the organic matter that was too large to enter the pores of the gel in the separation column and has eluted without any retardation. Further differences in UV absorbance are found in the

separated organic matter, between 320 and 2000 Da, where the 7.5 m sample has lower absorbance than the surface sample.

The DOC (0.45 μm filtered) measured in samples collected at 0.5 m intervals on 13 February 2004 did not show significant stratification over depth (Figure 5.8). However there appeared to be an increase in true colour and UV absorbance adjacent to the sediments and a similar pattern of increased apparent optical properties was observed (data not shown).

It is unlikely that the differences in the character of organic matter found between the surface and 7.5 m in Wartook Reservoir on 12 February 2004 is from an underflow generated by the rainfall between 29 January and 12 February. The total rain fall in that period was only 9.7 mm with 5 mm falling on 29 January 2004. This amount of rainfall equates to approximately 0.78 ML over the entire catchment and would be unlikely to generate much flow. In the same period the potential evaporation was 72.6 mm approximately 7.5 times greater than the amount of rainfall (Figure 5.11).

A possible explanation for the difference in the organic matter is the polymerisation of low and medium molecular weight organic matter as a result of microbial activity in the water column. This can result as part of the humification process where, for example, the oxidative polymerisation of phenolic groups may occur (Dec *et al.* 2003) and algal biomass can be transformed into humic-like substances (Lara and Thomas 1995). A potential fuel for this activity is indicated by the chlorophyll concentration, which had a maximum at 6.5 m. As this is less than the euphotic depth (1% subsurface irradiance, 5.1 m, vertical extinction coefficient = 0.91 ln units m^{-1}) it most likely indicates that there has been a flux of non-buoyant phytoplankton during the preceding quiescent period. Further evidence of the preceding autochthonous production can be seen in the dissolved oxygen profile. The surface mixed layer (~1 m deep) displays a gradient of DO from the column at the top of the metalimnion demonstrating an out gassing of dissolved oxygen. The super saturation

(~108%) of DO in the metalimnion is evidence of significant recent photosynthesis. Below 4.5 m the DO concentration decreases where recent photosynthetic oxygen production has decreased with light limitation (Figure 5.8). Another explanation may be that the acidification of the hypolimnion by accumulation of excess carbon dioxide, resulting from microbial activity, has caused a shift in the conformation of the humic substances by altering the ionization of carboxylic or phenolic functional groups. Differences in the pH and the alkalinity of these samples was observed, however given the small sample size, no relationships were developed.

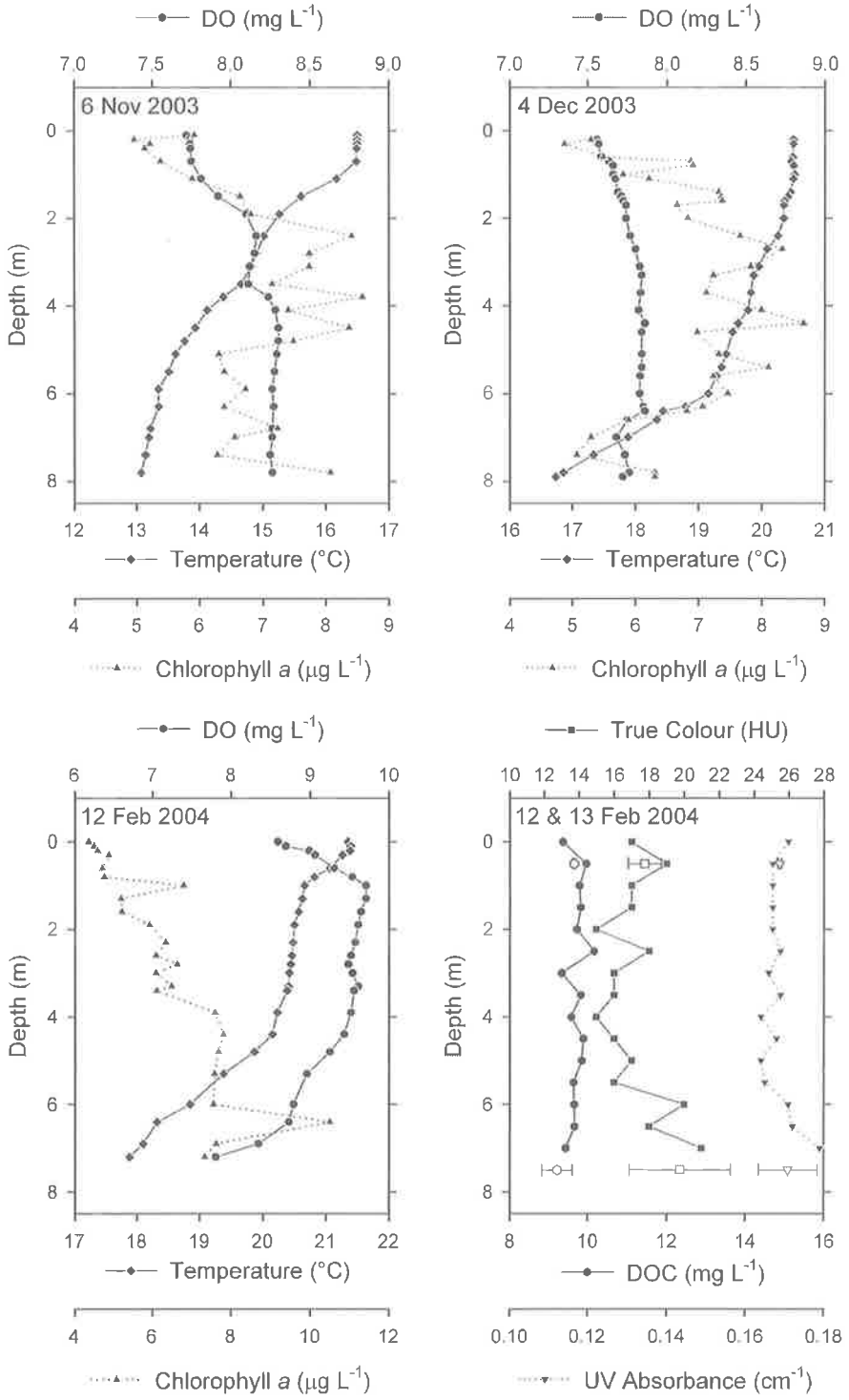

Figure 5.8. HydroLab profiles of the water column on sampling occasions of Wartook Reservoir. The DOC, UVA and HU found at discrete depths on 12 February 2004 (open symbols ± SD, N = 4) and at 0.5 m intervals on 13 February 2004 (filled symbols).

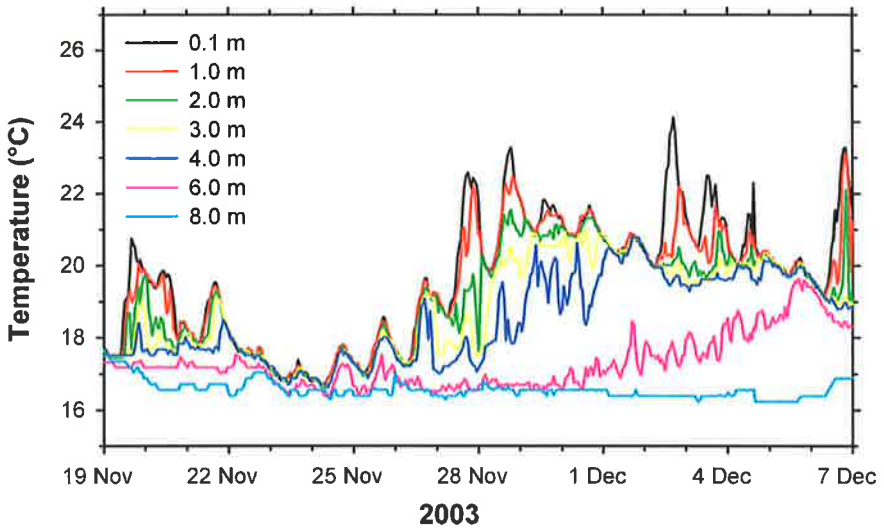

Figure 5.9. Temperature monitoring data for Wartook Reservoir 22 November 2003 to 7 December 2003.

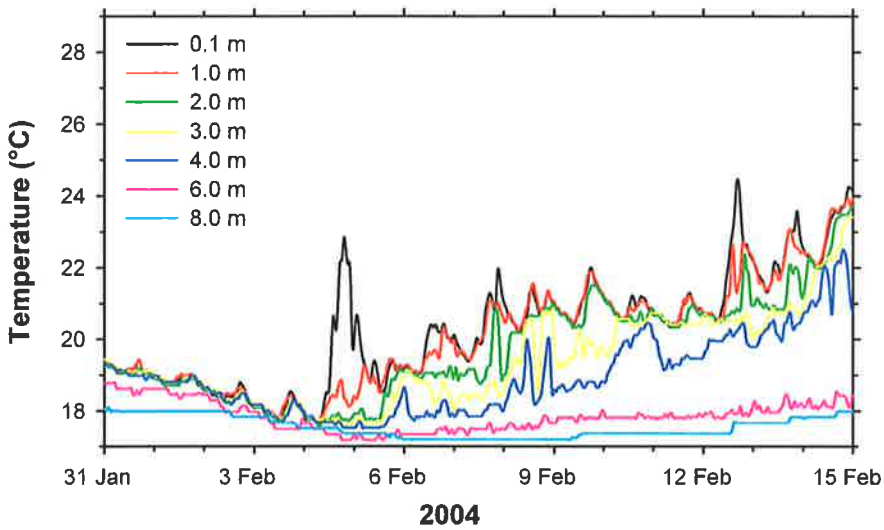

Figure 5.10. Temperature monitoring data for Wartook Reservoir 31 January 2004 to 15 February 2003.

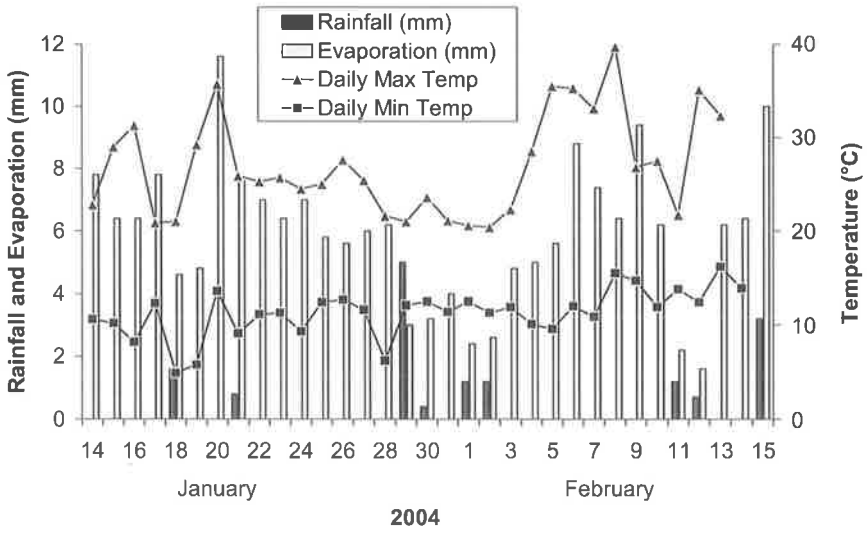

Figure 5.11. Meteorological data preceding sampling of Wartook Reservoir on 12 and 13 February 2004. Rainfall was measured at Wartook Reservoir (BOM station #79046), evaporation was measured at Rocklands Reservoir (BOM station #79052) and daily minimum and maximum temperatures were measured at Stawell aerodrome (BOM station #79105).

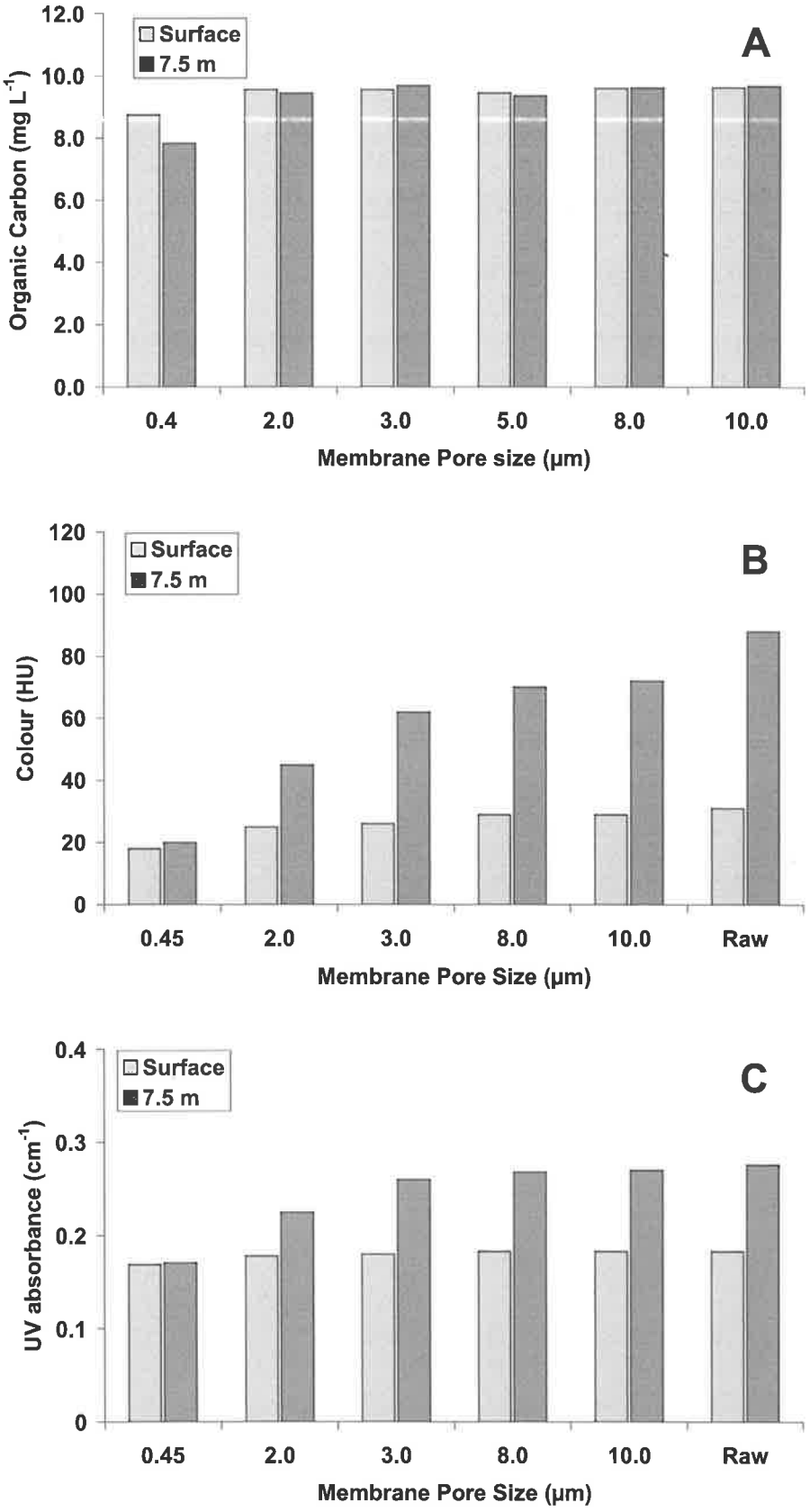

Figure 5.12. Size fractionation of organic mater properties in Wartook Reservoir by membrane filtration.

5.3.2.3. Wartook Sediment Cores

The dissolved oxygen was totally depleted in the chambers containing sediment after 17 days, while approximately 2 mg L^{-1} of DO remained in the control chambers at the end of 19 days (Figure 5.13). The exponential models fitted to the data had EF values greater than 92 % (Figure 5.13 and Table 5.4). The calculated rates of sediment oxygen demand were 0.22 ± 0.02 mg O_2 m^{-2} d^{-1} (± SD; N = 3) and the pelagic demand within the chambers was 0.19 ± 0.06 mg O_2 m^{-2} d^{-1} (± SD; N = 3). Assuming a depth of 7.5 m, the corresponding areal demand for the entire water column at the site where the cores were collected would be 4.5 ± 1.5 mg O_2 m^{-2} d^{-1}. This large ratio of pelagic to benthic oxygen demand is reported in many lakes (Burns *et al.* 1996) and is consistent with conceptual models of lake metabolism (Denheyer and Kalff 1998). The available data indicates that there was a net uptake of phosphorus by the sediments over the incubation. Total phosphorus in the sediment containing columns decreased, on average by 9.3 µg L^{-1} and filterable reactive phosphorus was reduced to below detectable limits in both the sediment and control chambers (Table 5.5). Nitrogen appears to have been released from the sediments, with TKN and NH_3 concentrations increasing in excess of the available oxidised nitrogen. Much of the oxidised nitrogen and ammonia in the control chambers was incorporated into the TKN pool. The high concentration of NO_x in sediment column C (Table 5.5) is probably the result of re-oxidation of accumulated ammonia during sample collection and storage before analysis. The increase in DOC concentration of, on average, 0.9 mg L^{-1} is most likely the result of leaching of organic carbon from the chamber construction material as the sediment cores were incubated in the dark at all times, precluding photosynthetic production. This appears to have been incorporated into the particulate biomass as TOC increased by an average 1.8 mg L^{-1} and the POC increased by an average 0.9 mg L^{-1} (Table 5.5). An alternative explanation would be complexation, coagulation or aggregation of

dissolved organic matter to a size greater than 0.45 μm in processes similar to the formation of 'lake snow' (Grossart and Simon 1998, Simon *et al.* 2002).

Table 5.4. Characteristics of fitted parameters and modelling efficiency of exponential decay curves fitted to data in Figure 5.13.

Parameter	*Sediment A*	*Sediment B*	*Sediment C*	*Control A*	*Control B*	*Control C*
Decay Constant	-0.24	-0.22	-0.23	-0.06	-0.07	-0.12
(EF)	0.98	0.99	0.95	0.93	0.95	0.92

Table 5.5. Nutrient and organic matter concentrations in initial and post incubation samples from chamber incubations. All values in mg L^{-1}.

Parameter	*Initial*	*Sediment A*	*Sediment B*	*Sediment C*	*Control A*	*Control B*	*Control C*
TP	0.023	0.011	0.019	0.011	0.018	0.021	0.026
FRP	0.007	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
TKN	0.59	1.12	2.75	0.99	0.81	0.80	0.74
NH3	0.068	0.824	2.77	0.751	0.016	0.007	0.0017
NO_X	0.032	<0.005	<0.005	0.067	<0.005	<0.005	<0.005
TOC	9.7	8.5	9.5	8.5	11.6	11.3	11.6
DOC	9.4	7.0	8.8	7.1	10.4	10.2	10.4
POC	0.3	1.6	0.7	1.4	1.2	1.1	1.2

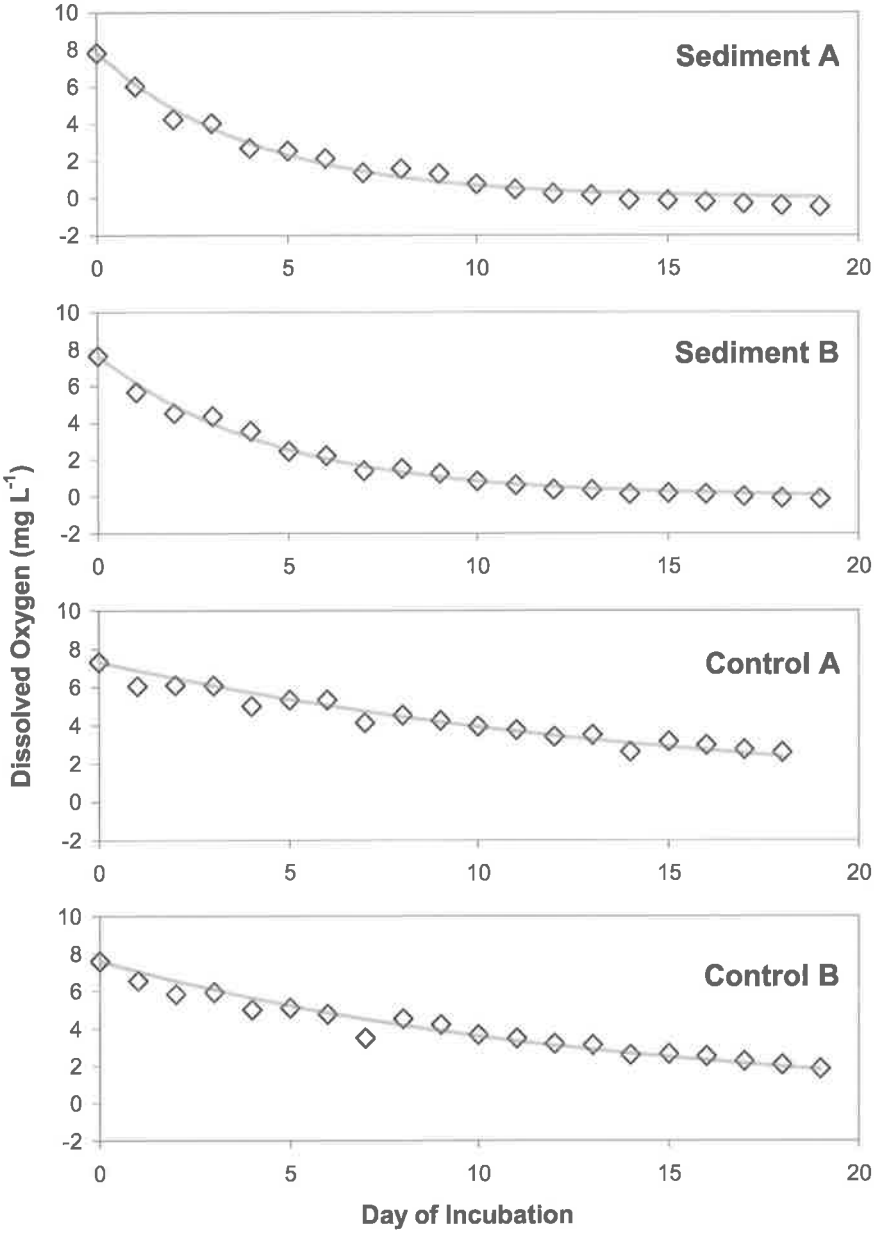

Figure 5.13. Loss of dissolved oxygen in sediment core and control incubations. Fitted line is exponential decay (Equation 5.3).

5.3.2.4. Myponga Reservoir

The temperature monitoring data gives a good indication that Myponga Reservoir was experiencing some surface heating during the time of sampling (Figure 5.14). The cooling detected at 15 m and shown in the temperature traces is evidence of basin scale circulation of cooler water, transported by a convective flow generated by differential heating. The cooling observed results from water flowing into the side arm from the main basin of the reservoir.

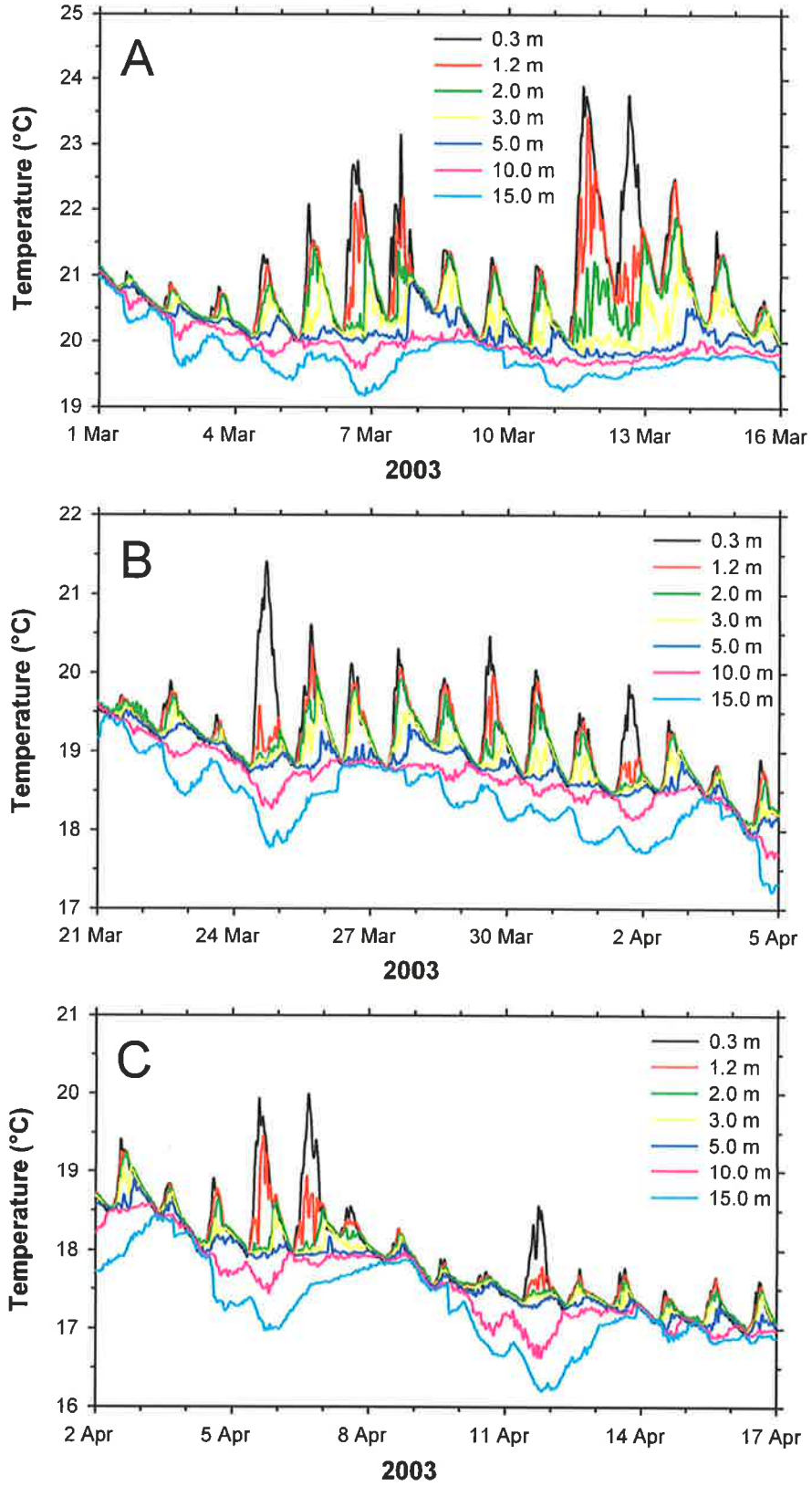

Figure 5.14. Water temperature in Myponga Reservoir monitored at met station 2.

5.4. Discussion

From the data collected in this study, no clear evidence was obtained to indicate that stratification affects the character of NOM and hence its treat-ability with the use of

inorganic coagulants under simulated conventional treatment conditions. Consequently any benefit from destratification on treat-ability was not evident. The impacts of the character of the OM on treat-ability with alum were attributable to water source type rather than water column depth. This probably reflects that the characteristics of the natural organic matter were determined by local catchment and reservoir factors at longer timescales than the periods of stratification that were sampled. It may be that practically small but statistically significant differences may be detected if a larger number of persistently stratified lakes are sampled during summer stratification. Further information may be derived by experimental manipulation, for example using *in situ* mesocosms, to closely examine any changes that might occur in a single system (see Chapter 6).

The most notable differences in the character of organic matter were observed in Wartook Reservoir on 12 February 2004. The differences observed were most likely the result of microbial activity in the water column. Whether it was a result of a direct influence on the NOM, through redox or enzymatic processes, or indirectly through effects upon the inorganic matrix (pH and alkalinity) is unclear. An alternative explanation, that the differences were the result of organic and inorganic complexation processes involving multivalent ions such as iron, manganese or calcium that were released through redox processes from the sediments, is not tenable as the oxygen concentration found at the time of sampling was above the concentration where significant amounts on redox exchange occur (~50% saturation Wetzel 2001c). The lack of accumulation of organic matter above the arbitrary 'dissolved' 0.45 μm boundary (POC see Table 5.5) during the sediment core incubations, compared to the control columns, suggests that the differences in organic character observed was the result of a pelagic, rather than a sediment dependant process.

The timing of sampling of Googong Reservoir did not coincide with prolonged stable stratification, but with the mixing of the surface layer by wind action and cooling driven convection to the depth of maximum sampling depth. Googong Reservoir had the potential

to provide more extreme results, but these conditions were not able to be sampled. The shallow nature of Wartook Reservoir means that thermal stratification rarely persists longer than a few weeks. The period required to deplete the dissolved oxygen in the sediment core incubations was approximately 2 weeks in the absence of the addition of labile autochthonous organic matter. It is therefore implied that oxygen depleted conditions in the hypolimnion of Wartook Reservoir would require a supply of labile organic matter to fuel microbial activity and oxygen consumption.

5.5. Conclusions

From the data of this study, no clear evidence was obtained that demonstrates that artificial destratification affects the character of NOM and hence its removal by conventional treatment processes. The behaviour of the organic matter in the raw water collected and jar tested using enhanced coagulation conditions was significantly different between reservoirs, with each reservoir having different proportions of nonsorbable organic matter. This is consistent with the published literature where it is often assumed that the character of the organic matter towards coagulants does not change at shorter timescales (Edwards 1997, Kastl *et al.* 2004).

Chapter 6. Investigating the Effects of Destratification on NOM using *in situ* Mesocosms

6.1. Introduction

Artificial destratification is used by the water industry to manage the water quality of raw water storages for drinking water production. The changes to the vertical structure of the water column have the potential to alter a suite of pelagic and sediment-water interface processes that determine the quality of water found in a drinking water reservoir. The main aims of artificial destratification are to prevent the release of soluble metals (iron and manganese) and nutrients (predominantly phosphorus) from the sediment under reducing conditions and reduce the growth of taste and odour producing cyanobacteria. Thus, the influence of destratification on the reservoir processes involved in release of ions from the sediment and the growth of cyanobacteria have been extensively studied (Bowles *et al.* 1979, Schladow and Fisher 1995, Visser *et al.* 1996, Lindenschmidt and Chorus 1997, Ismail *et al.* 2002, Lewis *et al.* 2002, Heo and Kim 2004). However, the effect of artificial destratification on the reservoir processes that influence the character and concentration of natural organic matter (NOM) is not well understood.

The nature of organic matter in aquatic systems has been the attention of research for many years from the perspective of water treatment (Frimmel *et al.* 2001), microbial ecology (Findlay *et al.* 2003), protection of aquatic systems from ultraviolet radiation (Zepp *et al.* 1998), food webs (Bunn *et al.* 2003) and ecological stoichiometry (Hessen *et al.* 1990). While the extensive literature on NOM and dissolved organic carbon (DOC) in aquatic systems has provided an understanding of the potential processes and their interactions that may occur during destratification, they remain untested in the context of the management of drinking water reservoirs for potable supply.

Natural organic matter is a significant contaminant in many drinking water reservoirs and prior to 1974 was considered an aesthetic problem, as it imparts an undesirable yellow-brown colour to water if it is not removed. When the potentially carcinogenic nature of many disinfection by products (DBPs) was discovered (Rook 1974), NOM also became a health concern, as it is the main source of the precursor compounds for the production of DBPs (Hubel and Edzwald 1987). NOM also exerts a considerable coagulant demand, which in turn contributes to the cost of water treatment. Consequently any means to reduce the concentration of NOM prior to water treatment is desirable to the water industry. Whether or not artificial destratification has a beneficial or detrimental effect on the concentration and character of NOM could influence the decision to undertake artificial destratification.

Investigating the influence of destratification on reservoir processes is often limited to observational studies (e.g. Chapter 5, Hamilton *et al.* 2001, Antenucci *et al.* 2005b) and comparisons between the hydrodynamic and ecological behaviour of lakes and reservoirs prior to and during destratification (AWRC 1979, Daldorph 1998). The difficulties of scale, associated with making experimental manipulations of whole lakes and the interpretation of inter-comparisons between separate lakes, has led limnologists to the application of other experimental systems. The experimental systems available include *in situ* mesocosms, microcosms or *in vitro* laboratory experiments, which variously allow different experimental conditions to be applied.

In situ microcosms were deemed inappropriate in this study because they would provide insufficient volume for the jar testing assessment of NOM removal. *In vitro* laboratory incubation of large samples with sufficient replication to maintain statistical confidence would require prohibitive space and infrastructure. Furthermore they would be unlikely to satisfactorily simulate the suite of biological interactions that would occur *in situ*. Consequently, in order to experimentally investigate the effect of destratification on the

removal of NOM by conventional treatment, a mesocosm experiment was chosen. Mesocosm experimental systems regularly applied in aquatic ecology have been used to study, phytoplankton succession (Reynolds *et al.* 1982, Reynolds *et al.* 1983), sediment resuspension (Chróst and Riemann 1994), primary production (Gervais *et al.* 1997), flux of organic nitrogen (Søndergaard *et al.* 2000) and acidification and photo-reactivity (Anesio and Graneli 2003).

An experimental design of four replicate benthic and four surface mesocosms was chosen and the experiment was performed in a destratified reservoir, thus, 'stratified' conditions were simulated using the mesocosms and the changes in microbial ecology and the character of natural organic matter were studied. The reservoir was sampled from four adjacent locations in order to provide comparison to the existing destratified conditions. It was assumed that the quiescence induced by enclosure would approximate the physical conditions at the surface found during stratification. Also of importance is the elimination of atmospheric and sediment exchange processes in the benthic and surface mesocosms, respectively.

Certain changes in the physico-chemical conditions found in the mesocosms will be expected if the experimental setup is successful in simulating a water column experiencing thermal stratification. In the benthic mesocosms, the concentration of dissolved oxygen should decrease, while the surface mesocosms remain saturated. Changes in the phytoplankton community were expected, as the quiescent conditions should increase sedimentation losses to phytoplankton species that can not regulate their buoyancy. In the surface mesocosms the conditions should favour the cyanobacteria that can regulate their buoyancy (Visser *et al.* 1997, Brookes *et al.* 1999). It is well documented that a significant proportion of microbial production in the epilimnion is fuelled by recent algal production. Substrates are derived from exudates and matter released from dead and dying algal cells (Vadstein *et al.* 1989, Sell and Overbeck 1992, Lyche *et al.* 1996). Therefore, changes in

primary production and phytoplankton production will in turn alter the availability and type of autochthonous carbon available to the microbial community.

It was hypothesised that there would be a shift in the patterns of enzyme activity and metabolic competency in response to these changes of energy flow. A decrease in the availability of algal substrates in the hypolimnion was expected and the microbial community in the benthic mesocosms might consume less labile components of the NOM when the supply of labile organic substrates from algae were reduced. Two important labile substrates in lakes are dissolved carbohydrates and amino acids (Rosenstock and Simon 1993, Hanisch *et al.* 1996) and consequently, it was expected that changes would occur in enzyme activity and metabolic competency, depending on the polymeric substrate resources that were available.

Other changes in DOC metabolism may occur in the epilimnion. The increase in light dose experienced by the surface waters may result in increased photo-degradation and bleaching of chromophoric organic matter. Photo-degradation of NOM has been reported to both produce microbial substrates (Wetzel *et al.* 1995, Bertilsson and Tranvik 1998) and to reduce the nutritional quality of detritus (Anesio *et al.* 1999). Recent work has shown that the hydrophilic and autochthonous components of NOM are most likely to produce labile substrates under photo-degradation (Anesio *et al.* 2005).

While much attention has been made to the effects of reservoir conditions on microbial activity and photo-degradation, little attention has been paid to the effects of these processes on the removal of natural organic matter by conventional treatment processes. In this mesocosm experiment, it was anticipated that any changes in the properties of the raw water for potable water production would be attributable to the emergent conditions in the experimental hypolimnion and epilimnion of Myponga Reservoir. Furthermore, this experiment should elucidate the potential beneficial or detrimental nature of artificial

destratification on the removal of natural organic matter by conventional treatment processes.

6.2. Materials and Methods

6.2.1. Mesocosm Design and Construction

The experiment was conducted using mesocosms constructed of 200 μm polyethylene tubing with one end held open by a 0.6 m by 0.6 m square of 40 mm PVC pipe (Figure 6.1 J and K). The other end was either tied closed (Figure 6.1M; surface mesocosms) or constricted tightly around a sampling pipe (Figure 6.1H; benthic mesocosms). The square ring of 40 mm PVC was either held down to the sediment by a square of 90 mm PVC pipe filled with sand and scrap iron (Figure 6.1I; benthic mesocosms) or supported on a buoyant empty square of similar PVC pipe (Figure 6.1L; surface mesocosms). The mesocosms held approximately 200 L of water.

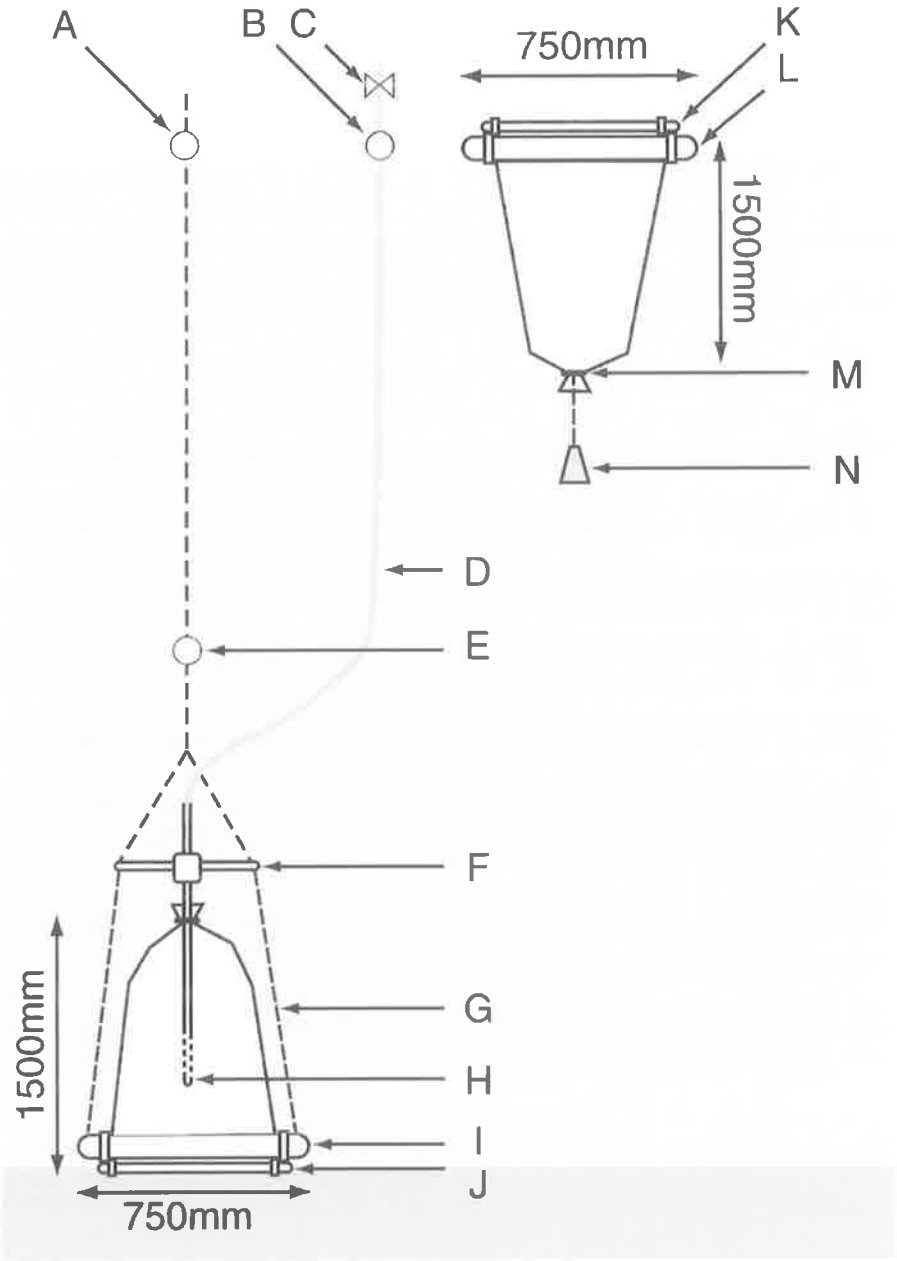

Figure 6.1. Mesocosm design and construction. A, marker buoy; B, sampling hose marker buoy; C, sampling hose valve; D, sampling hose; E, submerged counter buoyancy support buoy; F, sampling pipe support and line spreading framework; G, bottom ring haul line; H, perforated sampling pipe; I, weighted ring (90mm Ø) ; J and K, bag holding ring (40mm Ø); L, buoyant ring (90mm Ø), M, tie off; N, counter weight.

6.2.2. Deployment and Instrumentation

The mesocosms were deployed in a sheltered bay in Myponga Reservoir in water between 4 and 6 metres deep (Figure 6.2A). The benthic mesocosms were deployed and first sampled on 2 March 2005 while the surface mesocosms were deployed and first sampled

on 3 March 2005. A thermistor string was deployed, consisting of independent submersible temperature probe and data loggers (Tid-Bit Onset Corp, Bourne, Massachusetts, USA) at 0.1, 0.5, 1.0, 1.5, 2.5, 3.5, 4.5 and 5.5 m depth, logging temperature every minute (Figure 6.2B, b). The use of this high recording frequency resulted in the total consumption of memory resources by 24 March 2005. However, additional temperature monitoring was conducted within mesocosm 4 (benthic) and mesocosm 6 (surface), using independent submersible temperature probe and data loggers (Optic Stowaways, Onset Corp, Bourne, Massachusetts, USA), logging temperature at 10 minute intervals.

The attenuation of photosynthetically active radiation (PAR, 400-700nm wavelength) was determined with a LI-COR underwater light sensor on 2 March 2005. The attenuation coefficient (k_d) was 1.8 m^{-1} (ln units) and all benthic mesocosms were below the euphotic depth calculated to be 2.6 m (depth of 1% sub-surface irradiance). Ekman grab samples collected in the surrounding area showed the sediment was sandy with a relatively shallow layer of coarse organic matter, containing a significant amount of leaves and woody debris.

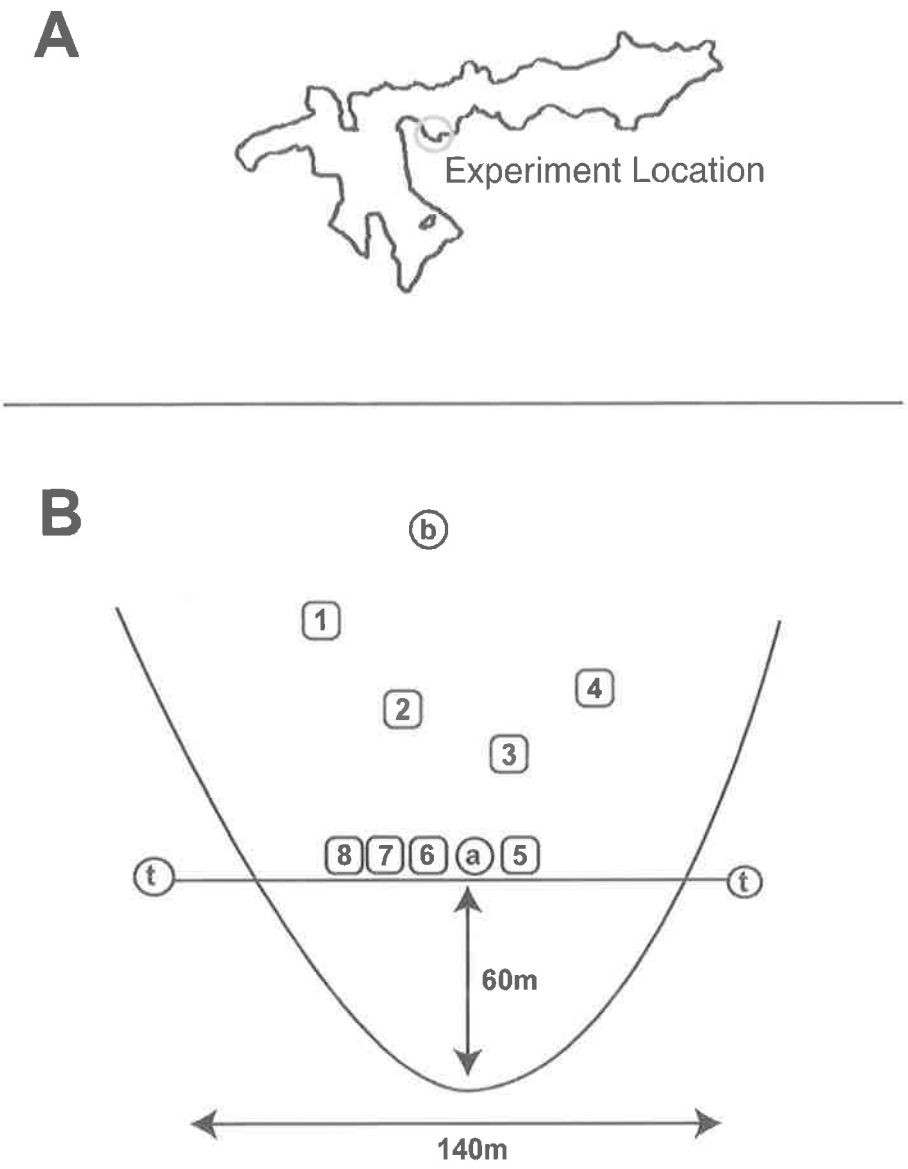

Figure 6.2. A) Location in Myponga Reservoir that the mesocosm experiment was conducted. B) Layout of mesocosms in the sheltered bay. Shaded squares represent benthic mesocosms, open squares represent surface mesocosms. Numbering refers to mesocosm number as described throughout the text. Open circles labelled 'a' and 'b' represent the inshore and offshore sampling sites and the temperature chain was also located at location 'b'. The surface mesocosms were attached to a line fixed to two trees (open circles labelled 't').

6.2.3. Sampling

Samples were collected from the benthic mesocosms using a peristaltic pump (ISCO Corporation Lincoln, Nebraska, USA). Temperature, dissolved oxygen, turbidity and pH were monitored using a HydroLab 4a Sonde (Hach Environmental, Loveland, Colorado, USA) fitted with a flow cell. The instrument reported data in real time to a laptop computer

at 1 Hz, where the data were logged and observed. Samples were collected after at least three sample hose volumes had been pumped and the physicochemical readings had stabilised. Samples from the surface mesocosm were collected using a similar method with a hose submerged to 0.5 m in the centre of the mesocosm. Samples were also collected from the reservoir at surface and at depth from two locations adjacent to the site, again collected by pumping with the peristaltic pump. Profiles of physicochemical parameters were also collected by lowering the HydroLab slowly through the water column while recording the data at 1 Hz.

6.2.4. Sample Processing and Analysis

Sub-samples were analysed for dissolved organic carbon (DOC), ultraviolet absorbance at 254 nm (UVA) and true colour (HU), and the specific ultraviolet absorbance (SUVA) and specific colour (SpHU) were calculated. Sub-samples were analysed for nutrients (ammonia, NH_3; nitrate + nitrite, NO_x; total kjeldahl nitrogen, TKN; filterable reactive phosphorus, FRP; total phosphorus, TP), algal density and heterotrophic plate count at 20 °C. Equal volume composite samples were analysed for the taste and odour compounds MIB and geosmin by gas chromatography (see §2.4). Microbial enzyme activity was determined using chromophoric substrates as described in §2.3.3 Initial and final samples (20 L) were collected for jar testing with aluminium sulphate (alum see §6.2.4.1). The jar test product water was analysed for DOC, UVA, true colour (HU), trihalomethane formation potential, size distribution of NOM by high performance size exclusion chromatography (HPSEC) with UV detection (Method A Chow *et al.* 1999). Representative samples were also analysed by HPSEC with UV/DOC detection (Method B; §2.4.2). Spectra for Method B were baseline corrected with PeakFit 4.12 (SYSTAT, Point Richmond, California, USA) using an initial-final linear algorithm on the full elution time spectra. Apparent number average molecular weights and weight average molecular weight were calculated after Chin *et al.* (1994). Four composite sub-samples were analysed

for bacterial regrowth potential (Withers and Drikas 1998). The concentration of very hydrophobic acids, slightly hydrophobic acids, hydrophilic acids and hydrophilic neutrals in further composite sub-samples were estimated using a rapid method incorporating DAX-8, XAD-4 and IR-958 resins (Chow *et al.* 2004).

6.2.4.1. Jar Testing Protocols

One sample was processed per day after collection with a rotating schedule of sample type to integrate any effects of storage (i.e. day 1, benthic; day 2, surface; day 3, reservoir; day 4, benthic; etc). The sample to be jar tested (stored at 4°C) was equilibrated to room temperature in a water bath before use. The alum dose for maximizing NOM removal was predicted using the mEnCo model (van Leeuwen *et al.* 2005) after determining the UV absorbance, true colour, turbidity and performing a titration to pH = 5.5 with alum on the day of jar testing. The model predicted dose (MPD) and a dose of 200% of the MPD were each applied in 3 replicate jars at pH = 5.5 according to the protocols described in §2.3.8. Jar testing was carried out in a consistent manner between each sample collected. The same alum solution, acid (0.1M HCl) and base (0.1 NaOH) were used for all the titrations and jar tests performed.

6.3. Results

6.3.1. Simulation of Stratified Conditions

6.3.1.1. Temperature and Physicochemical Data

The temperature and physico-chemical data recorded in the mesocosms suggest that the experiment developed conditions similar to the onset of thermal stratification. The temperature monitoring indicated that the reservoir did not experience permanent stratification during the experiment (Figure 6.3 and Figure 6.4). Some surface heating was observed but this was dissipated through the water column overnight. Maximum daily water temperatures found in the surface mesocosms was often between 25 and 30 °C (Figure 6.4). The average temperatures found in the surface and benthic mesocosms over

the period were similar, yet were more variable at the surface (Figure 6.4; benthic, 19.7 ± 0.5; surface, 19.8 ± 2.5 °C; mean ± SD, N = 5767, 1200 hrs 3 March 2005 to 1200 hrs 12 April 2005). At the time of sampling, between 1200 and 1500 hrs on all occasions, there was between 1 and 2 °C difference in the mean temperature of the sampled water and the differences amongst the replicate mesocosms was small (Figure 6.5A, coefficient of variation $cv < 5.7$ % [standard deviation as a percentage of the mean]). On all sampling occasions following deployment, dissolved oxygen concentration and saturation was lower in the benthic mesocosms and had low variability between replicate mesocosms (Figure 6.5B and C, highest *cv* was 13.0%). The pH in the benthic mesocosms decreased from 8.2 to 7.0 over the course of the experiment, while in the surface mesocosms pH increased slightly to 8.6 units by 12 April 2005 (Figure 6.5D).

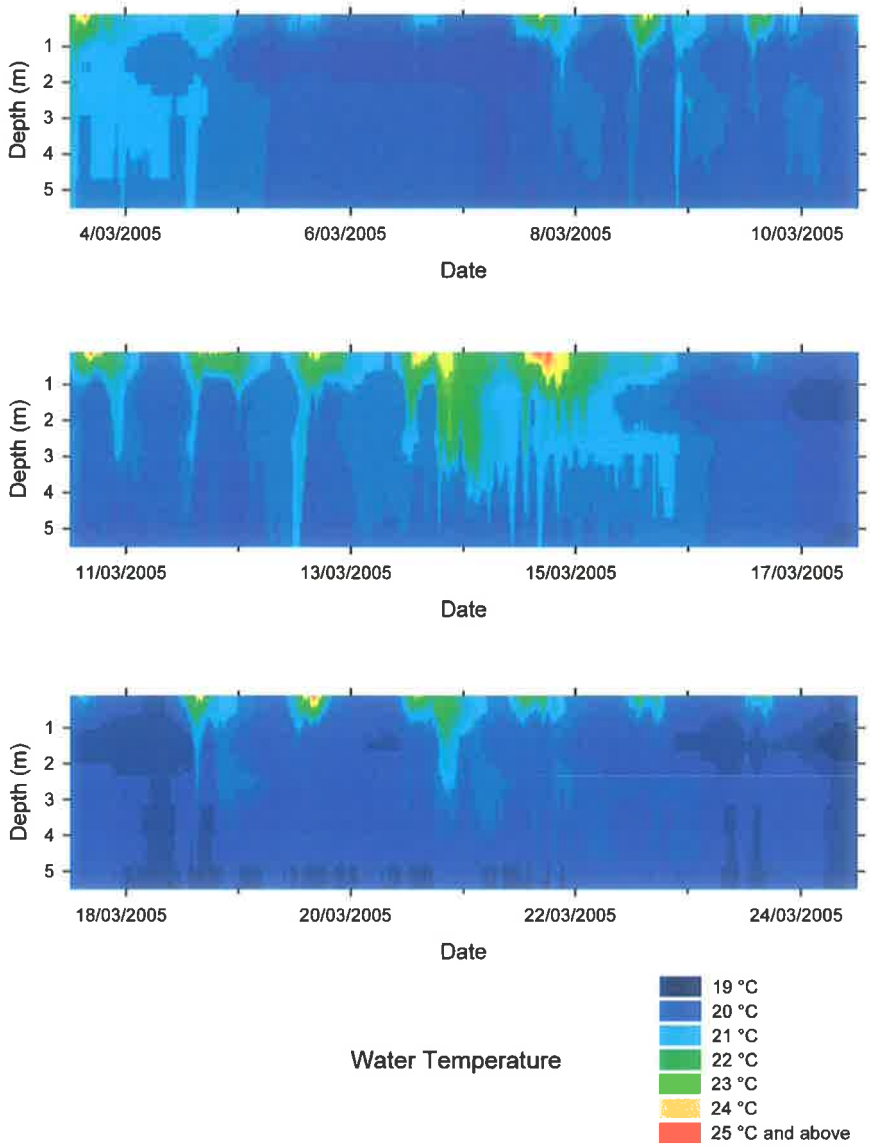

Figure 6.3. Water temperature recorded during the mesocosm experiment using a chain of thermistors suspended at 0.1, 0.5, 1.0, 1.5, 2.5, 3.5, 4.5 and 5.5 metres depth.

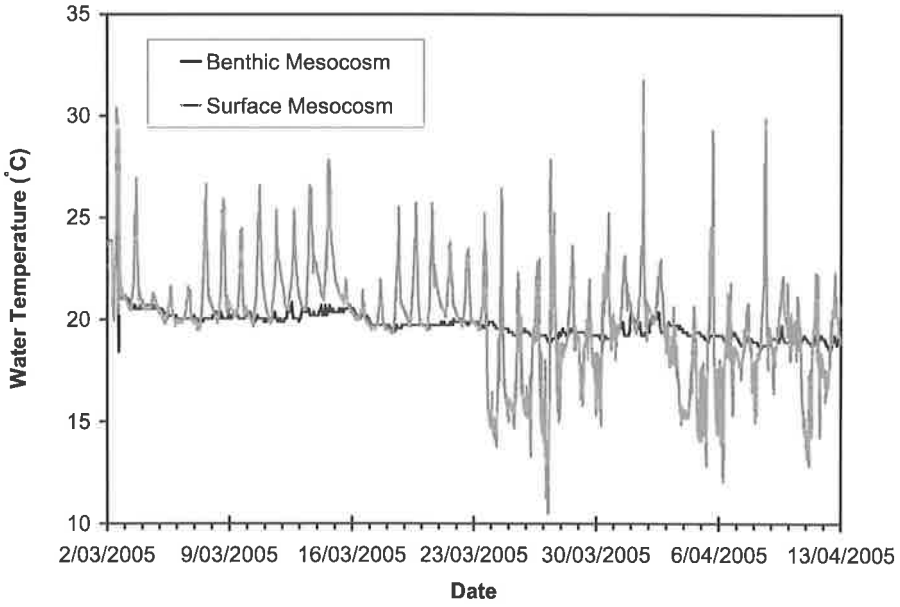

Figure 6.4. Temperature measured within the benthic and surface mesocosms deployed in Myponga Reservoir from the 2 March 2005 to 12 April 2005.

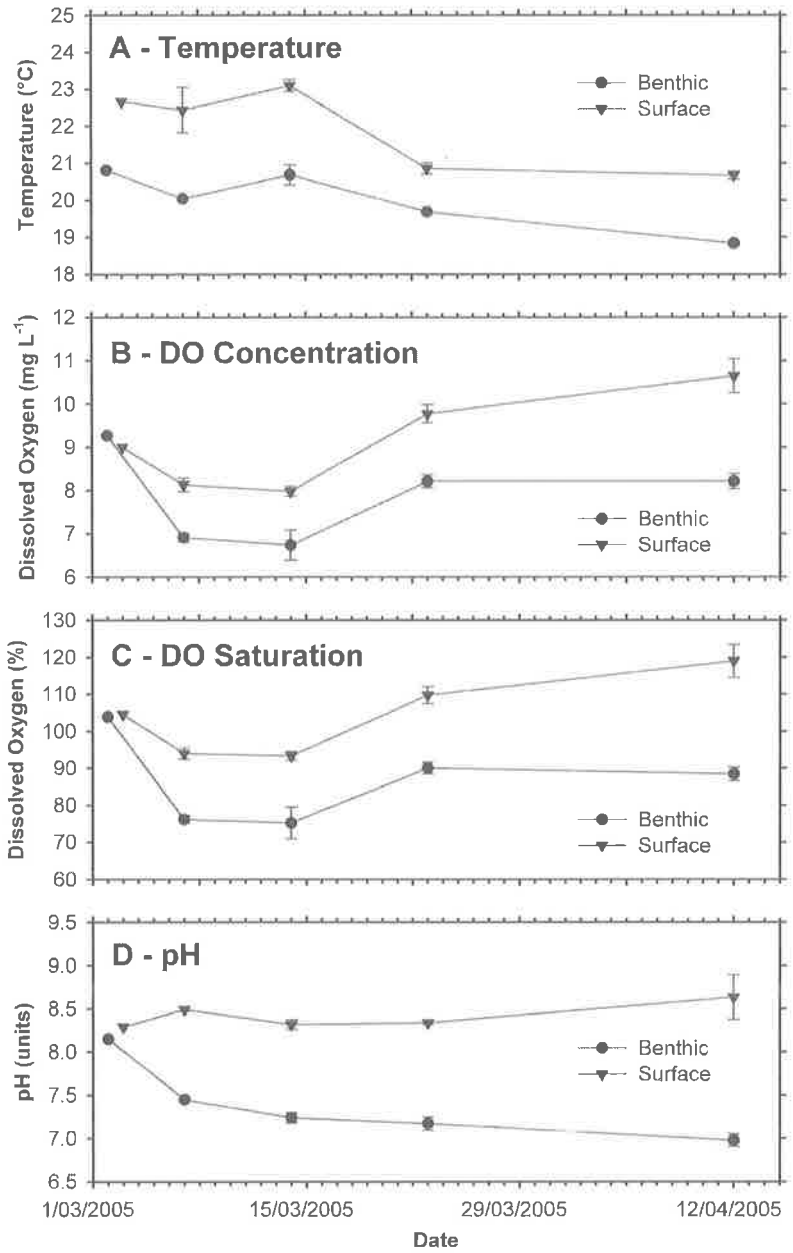

Figure 6.5. Physico-chemical conditions recorded using a multi-parameter sonde and through-flow cell during sampling from benthic (circles) and surface (triangles) mesocosms.

6.3.1.2. Nutrients

Initially there was an accumulation of ammonia in the benthic mesocosms with the maximum concentration occurring on 14 March 2005. This then decreased to a similar level to the surface mesocosms and the reservoir samples for the remainder of the experiment (Figure 6.6A). This indicates that there may have been a layer of deoxygenated water lying over the sediments within the benthic mesocosms, as ammonia is usually

released under anoxic conditions (Jones 2001). The levels of oxidised nitrogen also increased in the benthic mesocosms and remained at higher concentrations until the end of the experiment. The concentration of total kjeldahl nitrogen was significantly greater in the surface mesocosms on 12 April 2005 compared to all days except for 23 March 2005 (Tukey-Kramer HSD $\alpha = 0.05$). This may be explained by the growth of *Anabaena circinalis* in the surface mesocosms, which has the potential to fix atmospheric nitrogen (see §6.3.1.3 below). In the surface mesocosms, the mean FRP concentration decreased to less than the limit of detection (LOD) by 14 March 2005, and remained at or near to the LOD for the remained of the experiment. The mean FRP concentration was significantly higher in the benthic mesocosms on the 14 March 2005 and 12 April 2005 compared to the initial samples. The mean FRP in reservoir samples was significantly lower on 14 and 23 March 2005 and 12 April 2005 compared to 2, 3 and 7 March 2005. Total phosphorus concentrations showed less change than FRP; there were no significant differences in the mean concentrations in the benthic mesocosms by date. There were some significant differences amongst dates in the surface mesocosms and the reservoir, but the differences in the means were very small (< 0.019 mg L^{-1} in all cases). The accumulation of nitrogen and phosphorus in the benthic mesocosms indicate that the microbial flora were unlikely to be nitrogen or phosphorus limited during the experiment. The surface mesocosms may have become phosphorus limited as filterable reactive phosphorus concentrations fell to below the LOD, however this does not necessarily confirm phosphorus limitation.

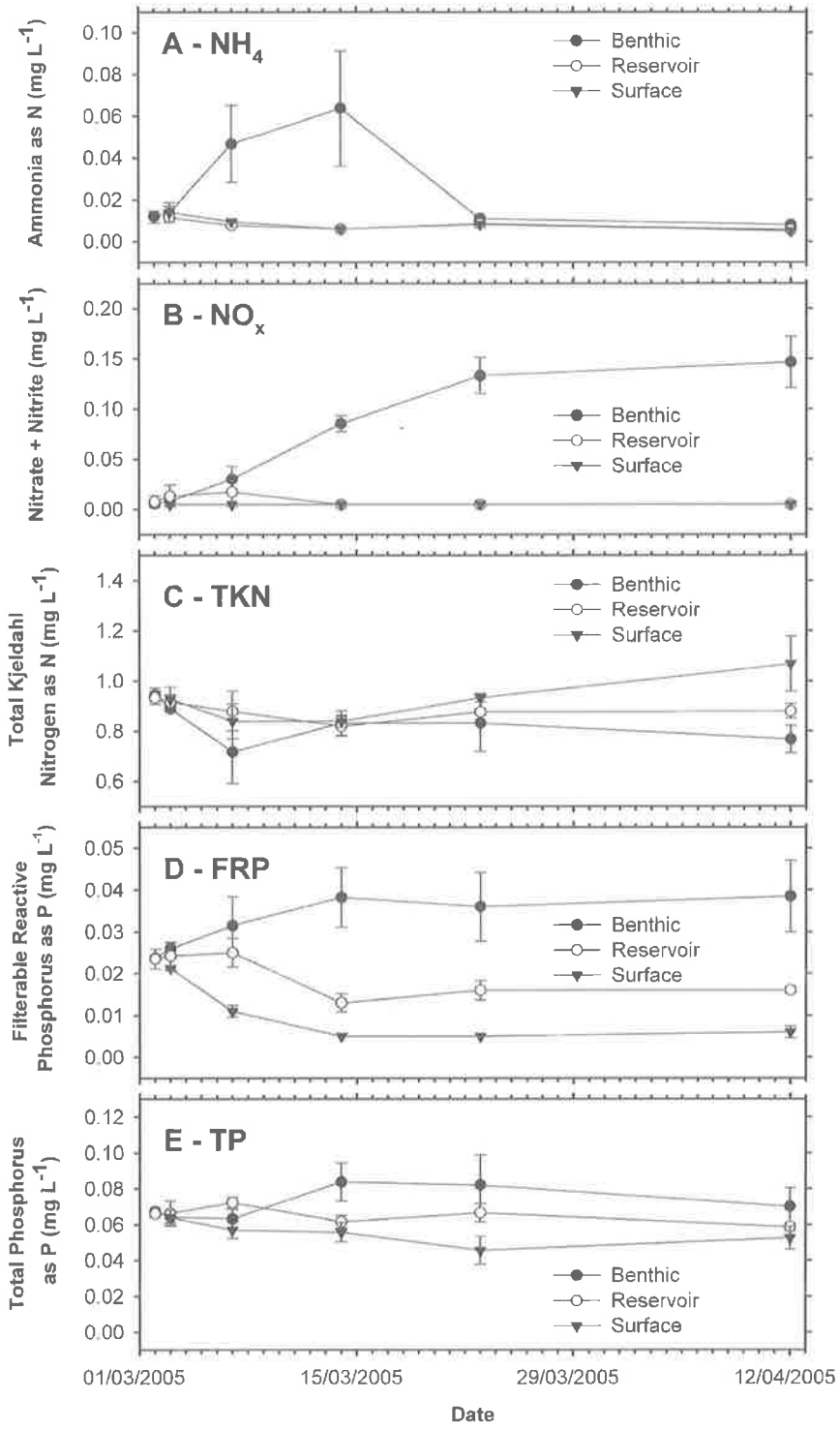

Figure 6.6. Mean nutrient concentrations (± SD, N = 4) in samples collected from benthic (filled circles) and surface (filled triangles) mesocosms and Myponga Reservoir (open circles).

6.3.1.3. Phytoplankton Dynamics

The patterns of algal abundance and succession in the experimental enclosures were consistent with those expected under stratification. *Anabaena circinalis* abundance was

low in the reservoir (1 - 10 cells mL^{-1}) and was only detected in the benthic mesocosms on 3 March 2005 (Figure 6.7A). In the surface mesocosms, however, *A. circinalis* grew at 0.37 d^{-1} (ln units) between 3 March and 23 March 2005 (ln(*A. circinalis*) = -0.07 + 0.37 × day; r^2adj = 0.95; N = 13; $p < 0.0001$). If the entire experimental period is considered then the estimate of growth rate is 0.19 d^{-1} (ln(*A. circinalis*) = 1.5 + 0.19 × day; r^2adj = 0.72; N = 17; $p < 0.0001$). *Ankistrodesmus*, *Nitzschia* and *Scenedesmus* abundance declined in the benthic mesocosms with estimated logarithmic decay constants of -0.06, -0.14 and -0.05 d^{-1}, respectively (ln units, r^2adj = 0.56, 0.77 and 0.74 respectively; N = 24; $p < 0.0001$; Figure 6.7B,C and D). These loss rates correspond closely to the estimate of loss rate of chlorophyll *a* in the benthic mesocosms of 0.05 d^{-1} (ln(Chl *a*) = 2.3 – 0.05 × day; r^2adj = 0.84; $p < 0.0001$). The chlorophytes persisted in the surface mesocosms, indicating there was sufficient turbulent mixing to maintain the population in suspension, as would be expected in a shallow surface mixed layer. It could therefore be assumed that the loss in chlorophyll *a* between 3 March and 23 March 2005 (ln(Chl *a*) = 2.2 – 0.04 × day; r^2adj = 0.47; $p = 0.002$), was the result of reduction in the per cell quota of chlorophyll, which is expected at higher light doses (Reynolds 1997a). Chlorophyll *a* concentrations recovered to approximately the initial concentrations by the end of the experiment.

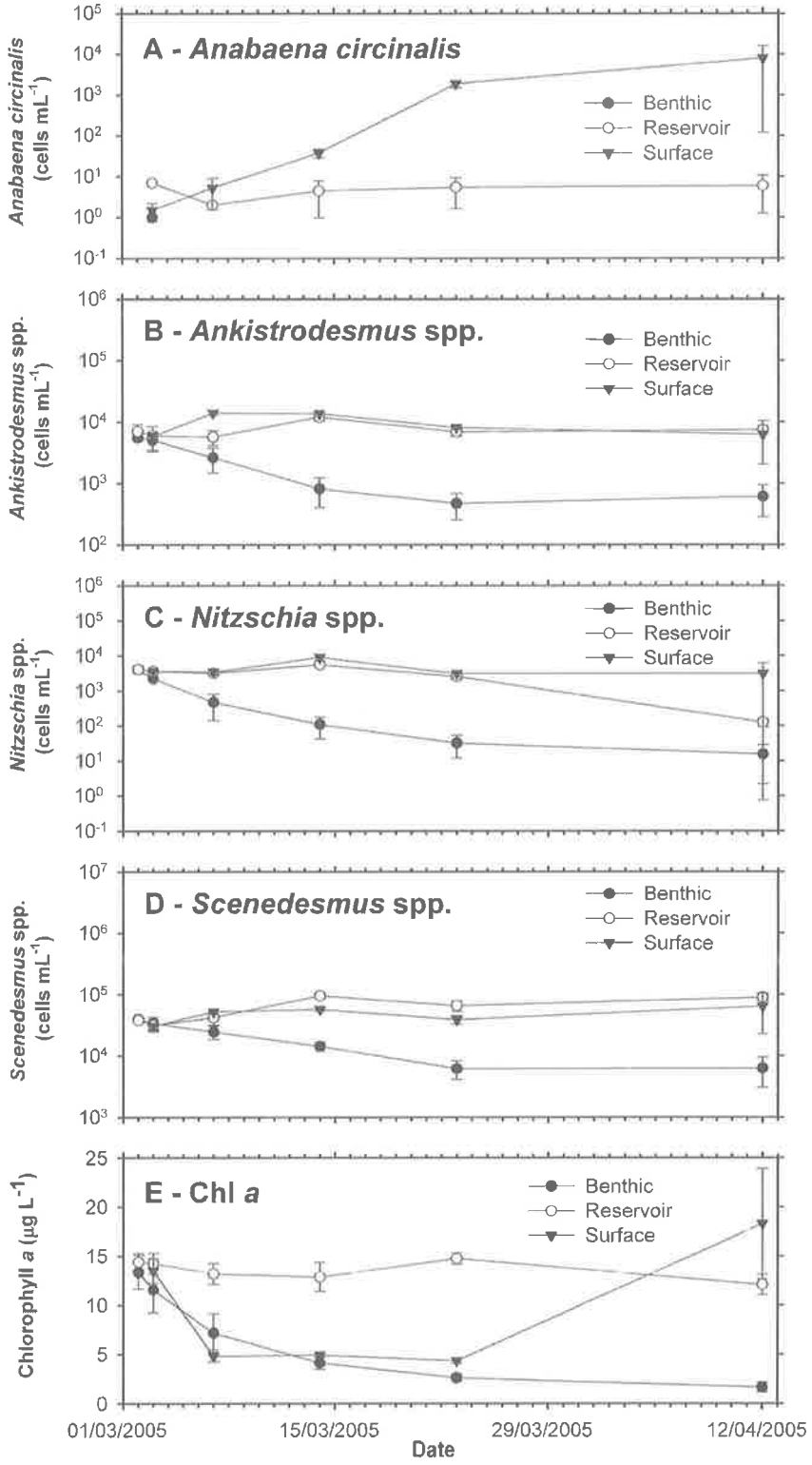

Figure 6.7. Algal numbers and chlorophyll *a* in samples collected from benthic (filled circles) and surface (filled triangles) mesocosms and Myponga Reservoir (open circles). *Anabaena circinalis* was only detected in the benthic mesocosms in the initial sample.

6.3.2. Emergent Microbial Communities

6.3.2.1. Enzyme Assays

There were no regressions between time and the activity of α-glucosidase (AGL), β-xylosidase (BXL), chitinase (CHT) or esterase (EST) with statistically significant slope estimates, and while some fluctuations in activity were found, no clear trends in these enzymes occurred (Figure 6.8). The means and standard errors of the mean activities for each enzyme over the entire experimental are also presented (Table 6.1). There was a large peak in the EST activity in both the surface and benthic mesocosms following set up (Figure 6.9B) which may be the result of lysis of phytoplankton (e.g. Agusti *et al.* 1998). The means and standard errors, calculated after excluding the EST data from 3 March 2005, are also presented (Table 6.1). BGL activity increased in the benthic mesocosms to a maximum on 23 March 2005 (Figure 6.9A), and the regression between time and BGL activity had a statistically significant slope estimate (Table 6.2). The regression between time and AKP activity had a significant slope estimate, however the slope was very small (Table 6.2) the increase in AKP in some of the surface mesocosms on the last sampling date suggests that phosphorus limitation had begun to occur. The regressions between time and LAP activity suggest that it was decreasing in the benthic mesocosms and increasing in the surface mesocosms (Table 6.2 and Figure 6.9C). These results suggest that the benthic mesocosm community began to use more carbohydrates and less amino acids/proteins while the surface mesocosm communities began to rely more upon amino acid or proteinaceous substrates.

Table 6.1. Means and standard errors of mean enzyme activities over the experimental period. * indicates the mean is significantly greater than means of the other treatments (Tukey-Kramer HSD $\alpha = 0.05$). [1] indicates the mean was calculated after exclusion of data from 3/03/2005.

Enzyme	*Benthic*	*Surface*	*Reservoir*
AGL	0.033 ± 0.007	0.031 ± 0.01	0.041 ± 0.005
AKP	0.14 ± 0.016	0.15 ± 0.024	0.14 ± 0.014
BGL	0.11 ± 0.031*	0.046 ± 0.012	0.037 ± 0.004
BXL	0.048 ± 0.011	0.045 ± 0.014	0.027 ± 0.003
CHT	0.038 ± 0.007	0.047 ± 0.010	0.043 ± 0.003
EST	3.45 ± 1.82 1.67 ± 0.471	3.00 ± 1.26 1.76 ± 0.21	1.39 ± 0.26
LAP	0.26 ± 0.054	0.53 ± 0.15	0.40 ± 0.078

Table 6.2. Statistics from linear regressions between time and enzyme activity.

Enzyme	*Treatment*	*Slope estimate (units d^{-1})*	*Standard error of parameter estimate*	r^2	*Significance of slope estimate (α)*
AKP	Reservoir	-0.002	0.0004	0.41	0.0007
BGL	Benthic	0.004	0.0009	0.43	0.0005
LAP	Benthic	-0.007	0.0012	0.59	<0.0001
LAP	Surface	0.019	0.0039	0.56	0.0001

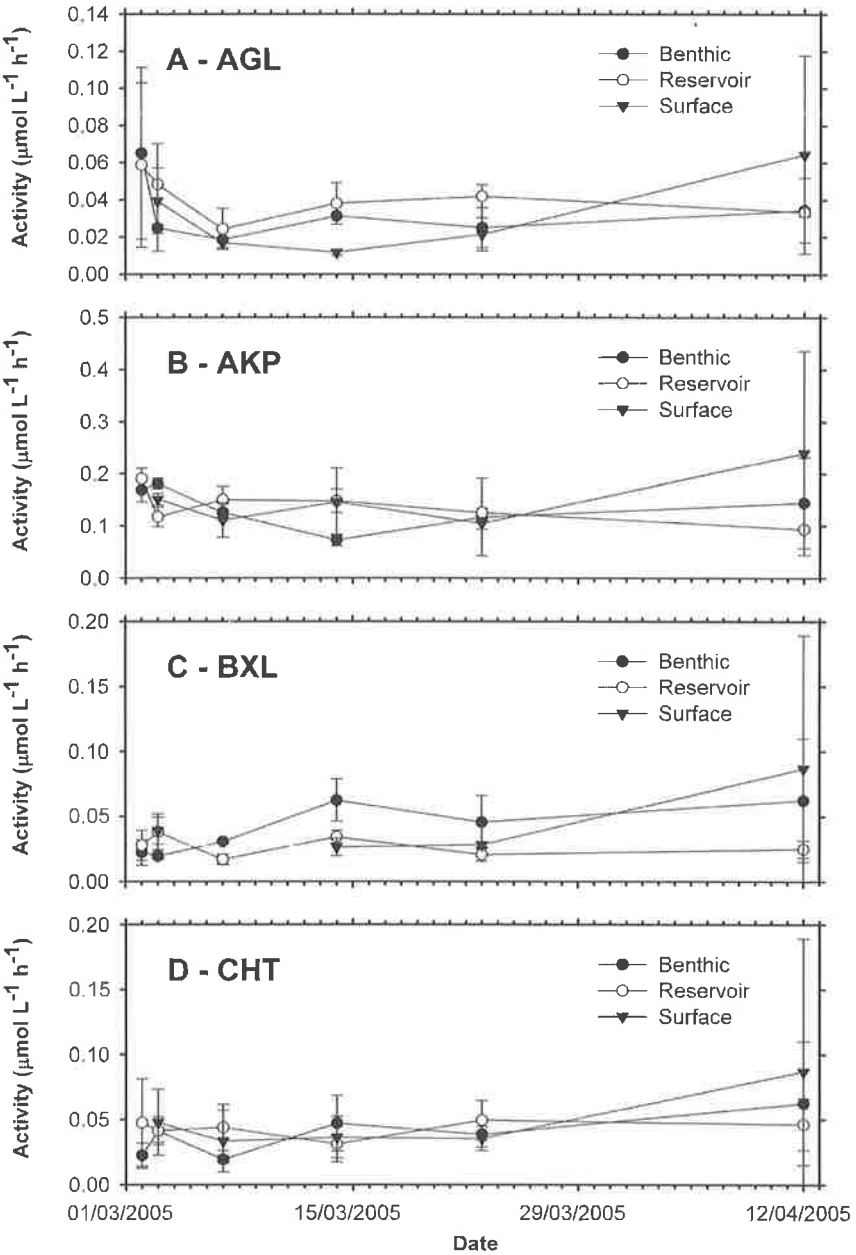

Figure 6.8. Mean activities (± SD; N = 4) of A) α-glucosidase (AGL), B) alkaline phosphatase (AKP), C) β-xylosidase (BXL) and D) chitinase (CHT) in samples collected from benthic (filled circles) and surface (filled triangles) mesocosms and Myponga Reservoir (open circles).

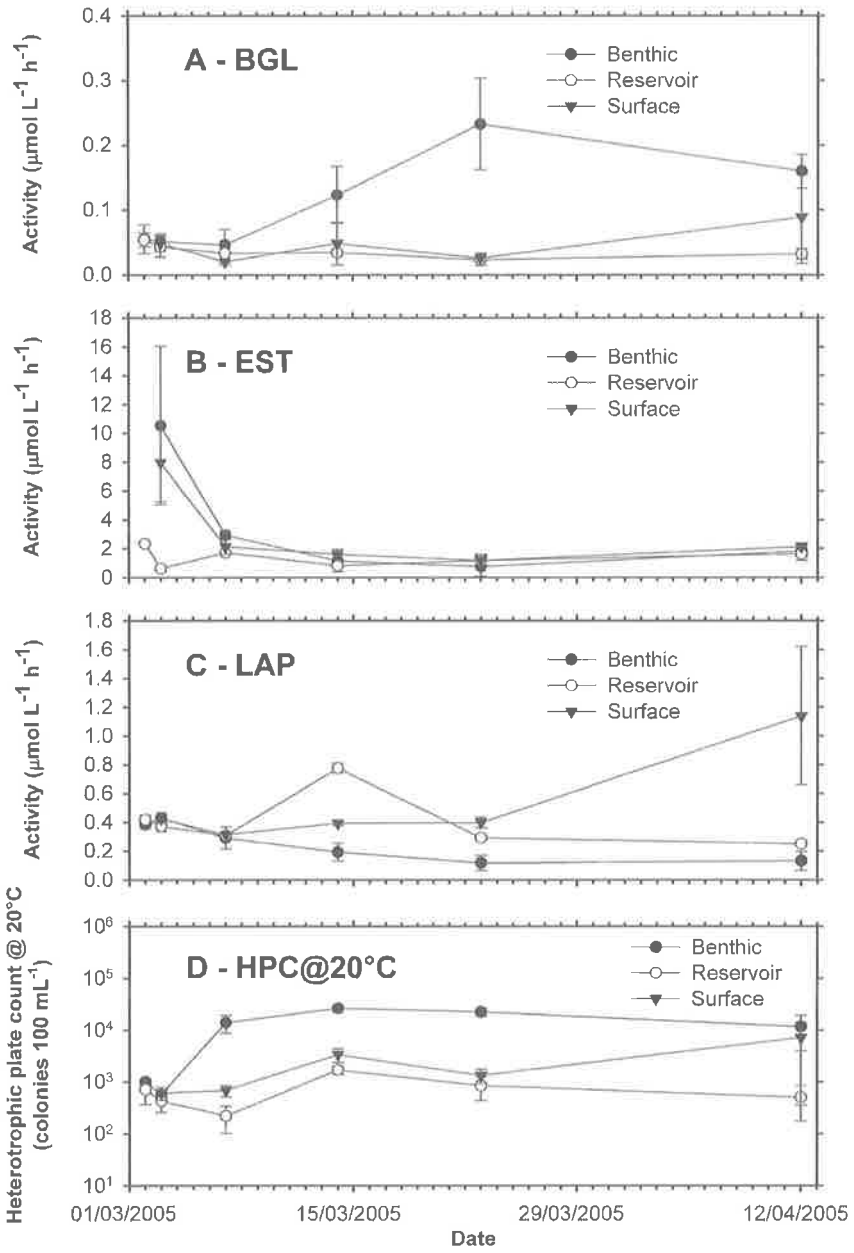

Figure 6.9. Mean activities (± SD; N = 4) of A) β-glucosidase (BGL), B) esterase (EST) and C) leucine aminopeptidase (LAP), and D) mean heterotrophic plate counts (± SD; N = 4; HPC@20°C) in samples collected from benthic (filled circles) and surface (filled triangles) mesocosms and Myponga Reservoir (open circles).

6.3.2.2. Heterotrophic Plate Count

The number of bacteria detected by heterotrophic plate counts at 20°C increased rapidly in the benthic mesocosms following enclosure (note log scale, Figure 6.9D) and remained at an order of magnitude higher abundance compared to the reservoir for the length of the experiment. This may be the result of the reduction in growth inhibition from exposure to solar ultra-violet radiation or be the product of movement of bacteria from the sediment, a

change in the density or activity of grazers (e.g. heterotrophic nanoflagellates or zooplankton, Jardillier *et al.* 2004) or the changes in mineral nutrition (nitrogen and phosphorus, Coveney and Wetzel 1992, Hessen *et al.* 1994). It must be acknowledged that enumerating environmental bacteria via plate counting is strongly criticised as the majority of the microbial community are not amenable to growth under the plate conditions (Konopka *et al.* 1998) and many members of the community are not enumerated (Kirchman *et al.* 2001).

6.3.2.3. Community Level Physiological Profiles

The substrate utilisation diversity (H) was 2.85 ± 0.06, 2.91 ± 0.12 and 3.04 ± 0.10 in the benthic mesocosms, surface mesocosms and the reservoir samples, respectively (mean ± SD, N = 4). The substrate utilisation diversity (H) was significantly different between the experimental treatments (ANOVA; $\alpha = 0.05$) with the mean of the benthic mesocosms being significantly lower than in the reservoir (Tukey-Kramer HSD; $\alpha = 0.05$). This may indicate that the benthic mesocosm reservoir community has lost some phenotypic diversity due to reduction in access to autochthonous substrates or sedimentation of particle associated microbial consortia.

Ordination of average well colour development (AWCD) normalised substrate responses shows there was greater variation in substrate responses amongst reservoir samples compared to benthic or surface mesocosm samples, corroborating the substrate utilisation diversity calculations. Both the mesocosm treatments formed tight groupings, each with one replicate falling further from the group mean (M3 of the benthic mesocosms and M7 of the surface mesocosms; Figure 6.10). 18 of the 31 substrates had correlation vectors with the first and second principal component axes greater than 0.5 (Figure 6.10). Nine substrates had significant differences between treatments (Student's t-test $\alpha = 0.05$). Both benthic and surface mesocosm treatment communities showed a greater propensity to metabolise 4-hydroxy benzoic acid, L-serine, D-malic acid and D-galacturonic acid,

compared to the reservoir community. 4-hydroxy benzoic acid and malic acid have been proposed as potential low molecular weight subunits of humic acids (Hayes *et al.* 1989, Perminova *et al.* 1998). The reservoir community responded more than the mesocosm communities to L-threonine, L-arginine, glycogen and D,L-glycerol phosphate. The surface communities had a greater response to D-cellobiose than the benthic mesocosm and reservoir communities, showing that while the surface community had shifted towards greater amino acid use over carbohydrates (as indicated by the enzyme activities §6.3.2.1), they retained the ability to rapidly respond to carbohydrate substrates.

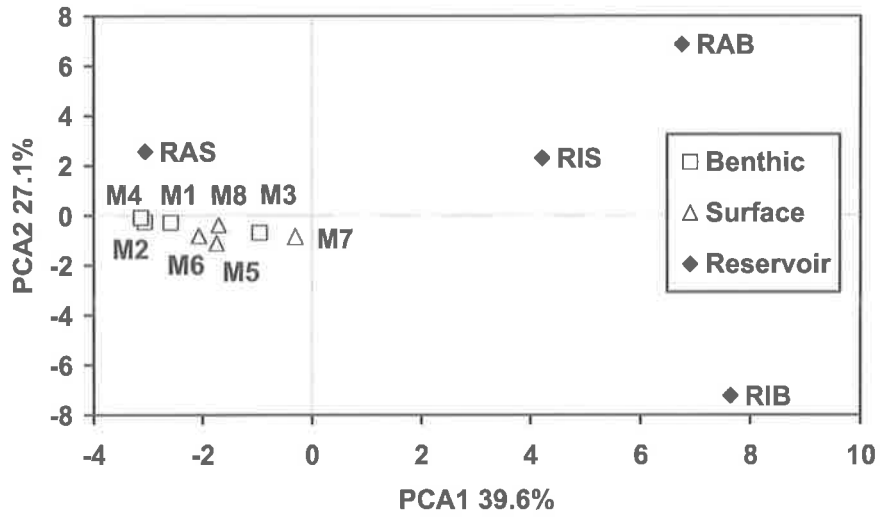

Figure 6.10. First and second principal components of the principal component analysis of the AWCD normalised responses of the microbial communities from four reservoir samples, four replicate surface mesocosms and four replicate benthic mesocosms. RIS, reservoir inshore surface; RIB, reservoir inshore bottom; RAS, reservoir away surface; RAB, reservoir away bottom; M1-4 refer to benthic mesocosms; M5-8 refer to surface mesocosms.

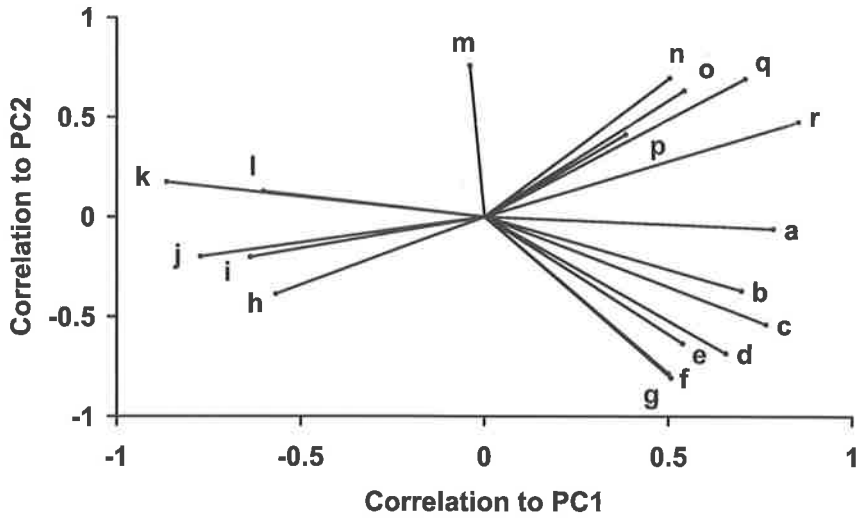

Figure 6.11. a, α-D-lactose; b, D,L-glycerol phosphate; c, α-ketobutyric acid; d, glucose-1-phosphate; e, D-glucosaminic acid; f, Tween 40; g, I-erythritol; h, D-malic acid; i, L-serine; j, 4-hydroxy benzoic acid; k, L-asparagine; l, putrescine; m, Tween 80; n, D-xylose; o, β-methyl-D-glucoside; p, L-arginine; q, glycogen; r, L-threonine.

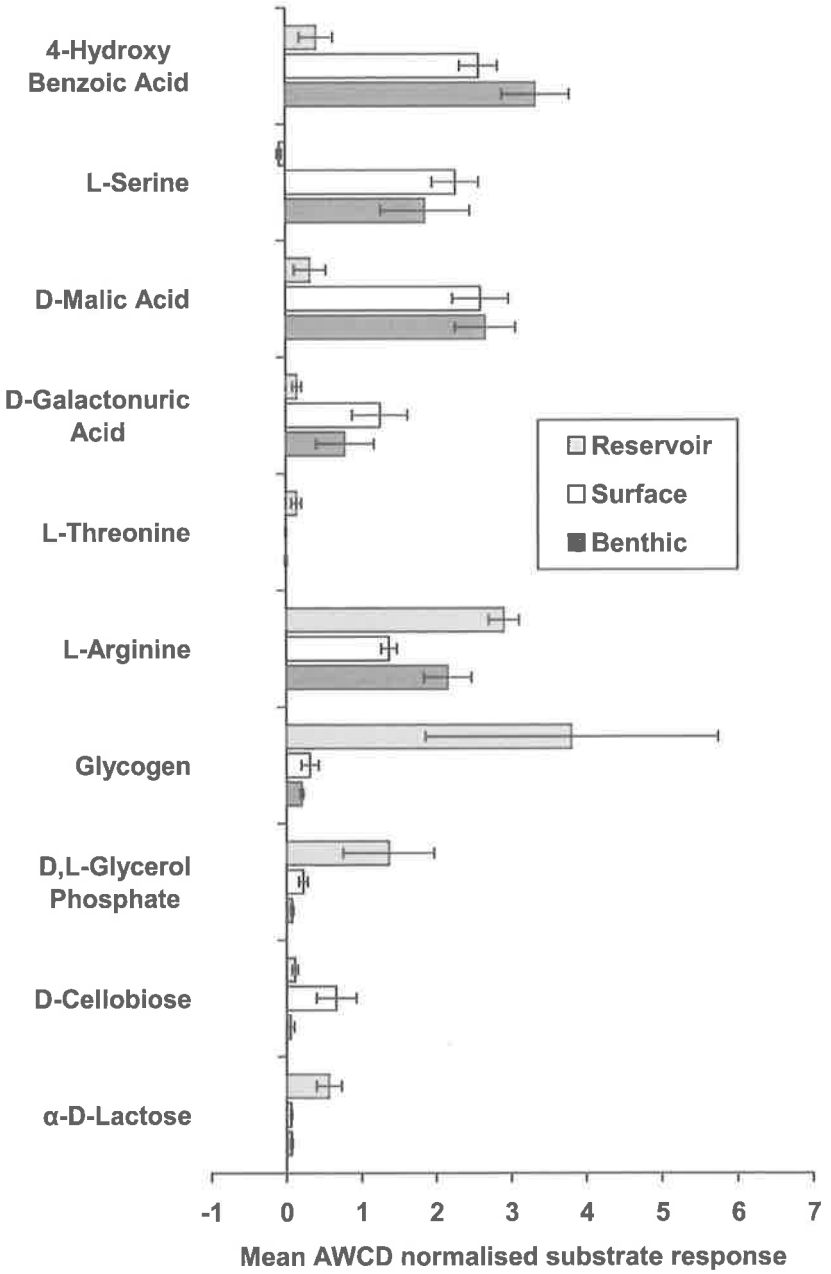

Figure 6.12. Mean (± SE; N = 4) of mean AWCD normalised substrate response in samples collected from surface and benthic mesocosms and Myponga Reservoir. These substrates all had significantly different AWCD substrate responses (Students t-test; α = 0.05).

6.3.3. Changes in Organic Matter and Character

6.3.3.1. Organic Matter

Benthic mesocosm 4 had a very different DOC dynamic to the other benthic mesocosms; the DOC concentration in benthic mesocosm 4 increased to approximately 16 mg L^{-1} by 23 March 2005, 3.5 mg L^{-1} greater than the mean of the other benthic mesocosms, and consequently was excluded from further analysis (data not shown). It is possible that this

mesocosm included the burrow of a yabbie (*Cherax destructor*) or a fish was enclosed in the mesocosm causing perturbation of the sediments and releasing DOC along with interstitial water. Alternatively, microbial uptake may have been disrupted, but this does not appear to have occurred, as nitrogen and phosphorus were not limiting (Figure 6.6), nor was microbial growth inhibited (Figure 6.9).

All linear regressions between time and the organic matter parameters DOC, UVA, colour (HU), SUVA and SpHU in the different experimental treatments (benthic mesocosms, surface mesocosms, reservoir), except for SUVA in the benthic mesocosm had fits with significant slope estimates ($\alpha < 0.05$, Table 6.3). All the linear slope estimates were negative (concentration decreasing with time) in the reservoir and the benthic mesocosms. The regression between DOC concentration and time in the surface mesocosms had a positive slope. The regression slopes suggest that DOC, UVA and colour decreased at greater rates in the benthic mesocosms than at the surface, while SUVA and SpHU decreased faster at the surface. From 14 March 2005 until the conclusion of the experiment, the mean DOC concentration in the surface mesocosms was significantly higher than that in the reservoir and benthic mesocosm samples (Figure 6.13A, Tukey-Kramer, HSD $\alpha = 0.05$). The final mean DOC concentration in the surface mesocosm was significantly greater than the initial mean concentration (initial mean = 12.8 mg L^{-1}, N = 4; final mean = 13.5 mg L^{-1}, N = 4; Tukey-Kramer HSD $\alpha = 0.05$).

Table 6.3. Statistics from linear regressions between time and DOC, UVA, HU, SUVA and SpHU.

Parameter (units)	*Treatment*	*Slope estimate (units d^{-1})*	*Standard error of parameter estimate*	r^2	*Significance of slope estimate (α)*
DOC ($mg\ L^{-1}$)	Benthic	-0.028	0.011	0.22	0.020
	Surface	0.017	0.005	0.41	0.0023
	Reservoir	-0.009	0.001	0.62	<0.0001
UVA (cm^{-1})	Benthic	-0.0016	1.6×10^{-4}	0.81	<0.0001
	Surface	-2.1×10^{-4}	7.0×10^{-5}	0.30	0.012
	Reservoir	-6.3×10^{-4}	3.0×10^{-5}	0.96	<0.0001
True Colour (HU)	Benthic	-0.43	0.027	0.91	<0.0001
	Surface	-0.17	0.044	0.52	0.0003
	Reservoir	-0.17	0.013	0.92	<0.0001
SUVA	Benthic	-0.0038	0.0022	0.12	0.094
	Surface	-0.0065	0.0010	0.68	<0.001
	Reservoir	-0.0023	0.00050	0.49	0.0001
SpHU	Benthic	-0.020	0.0045	0.47	0.0002
	Surface	-0.022	0.0048	0.54	0.0002
	Reservoir	-0.012	0.0012	0.81	<0.0001

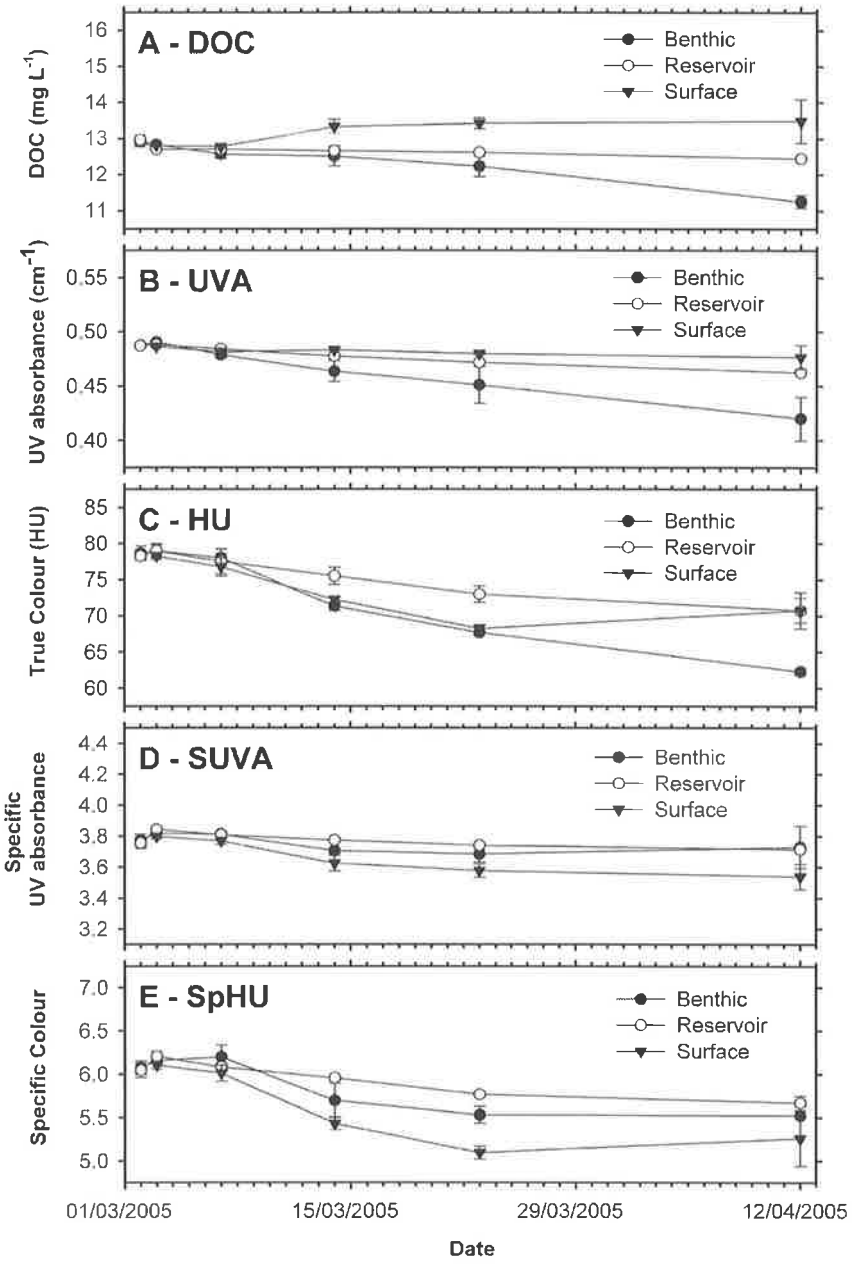

Figure 6.13. Mean (± SD; N = 4) values of A) dissolved organic carbon (DOC), B) UV absorbance (UVA), C true colour (HU), D) specific UV absorbance (SUVA) and E) specific colour (SpHU) in samples collected from benthic (filled circles) and surface (filled triangles) mesocosms and Myponga Reservoir (open circles).

6.3.3.2. Jar Testing

The model predicted doses (MPDs) for the different treatments, except for initial compared to surface, were significantly different (Table 6.4, Tukey-Kramer HSD, $\alpha = 0.05$). However there were no significant regressions between the alum dose and the product water quality parameters (data not shown). Therefore, comparisons are made between the product water quality of the different experimental treatments, neglecting the differences in

alum dose. At the MPD, significantly higher values of DOC and UVA were found in product water from the surface mesocosms (Table 6.4, Tukey-Kramer HSD $\alpha = 0.05$). No significant differences in colour were found between any of the experimental treatments. The mean SUVA found in MPD treated product water from the surface mesocosms was significantly lower than the means of the benthic mesocosms and the reservoir samples, but not the initial samples.

The application of another 94 to 99 mg L^{-1} of alum removed only another 0.4 to 0.5 mg L^{-1} of DOC (Table 6.5). There was also no significant regressions between the 200%MPD alum doses and product water parameters and will be compared, neglecting the differences in alum dose, as per the MPD treatments. A significantly higher mean concentration of DOC was found in the product water from the surface water mesocosms compared to the means of the other treatments (Table 6.5). The mean UVA of the product water from the 200%MPD jar tests of the surface mesocosm samples was significantly greater than the mean of the reservoir.

Table 6.4. Model predicted dose jar testing results. Three initial samples were collected and one from each of the four mesocosms and four from the reservoir. Three replicate jars were conducted from each sample collected. The mean (± SD) of all replicates is presented. Means ± SD followed by the same superscript label ($^{a\text{-}l}$) are not significantly different, as determined by Tukey-Kramer HSD ($\alpha = 0.05$).

Level	*Initial*		*Reservoir*		*Benthic*		*Surface*	
Number of replicates	9		12		12		12	
Alum Dose (mg L^{-1})	99.0	± 0.1 ^{a}	97.4	± 0.1 ^{b}	93.8	± 1.4 ^{c}	98.3	± 0.6 $^{a, d}$
UVA remaining (cm^{-1})	0.093	± 0.001 ^{e}	0.092	± 0.003 ^{e}	0.091	± 0.004 ^{e}	0.098	± 0.006 ^{f}
DOC remaining (mg L^{-1})	4.8	± 0.1 ^{g}	4.6	± 0.1 $^{g, h}$	4.6	± 0.2 ^{h}	5.1	± 0.4 ^{i}
Colour remaining (HU)	5.9	± 0.3 ^{j}	5.5	± 1.3 ^{j}	5.5	± 1.1 ^{j}	4.9	± 0.7 ^{j}
SUVA	1.95	± 0.02 $^{k, l}$	2.01	± 0.04 ^{m}	1.99	± 0.07 $^{k, m}$	1.93	± 0.05 ^{l}

Table 6.5. 200% model predicted dose jar testing results. Three initial samples were collected and one from each of the four mesocosms and four from the reservoir. Three replicate jars were conducted from each sample collected. The mean (± SD) of all replicates is presented. Means ± SD followed by the same superscript label ($^{a-l}$) are not significantly different, as determined by Tukey-Kramer HSD ($\alpha = 0.05$).

Level	*Initial*		*Reservoir*		*Benthic*		*Surface*	
Number of replicates	9		12		12		12	
Alum Dose (mg L^{-1})	197.9	± 0.2 ^{a}	194.7	± 0.2 ^{b}	187.7	± 3.1 ^{c}	196.5	± 1.2 ^{a,d}
UVA remaining (cm^{-1})	0.082	± 0.002 ^{e,f}	0.079	± 0.001 ^{e}	0.081	± 0.004 ^{e,f}	0.085	± 0.004 ^{f}
DOC remaining (mg L^{-1})	4.4	± 0.1 ^{g}	4.2	± 0.02 ^{h}	4.2	± 0.1 ^{g,h}	4.6	± 0.3 ^{i}
Colour remaining (HU)	4.8	± 0.4 ^{j}	4.3	± 0.7 ^{j}	4.4	± 1.0 ^{j}	4.1	± 0.3 ^{j}
SUVA	1.87	± 0.03 ^{k,l}	1.92	± 0.02 ^{k,m}	1.93	± 0.07 ^{m}	1.84	± 0.04 ^{l}

6.3.3.3. THMFPs

No significant differences in the mean THMFP of the 200% MPD were detected (ANOVA; $\alpha = 0.05$) and no significant regressions were found between the organic matter parameters (DOC, UVA, colour (HU), SUVA, SpHU) and the formation potential of any of the different halogenated compounds. This is most likely due to the small range of the OM parameters in the product water and the THMFP concentrations.

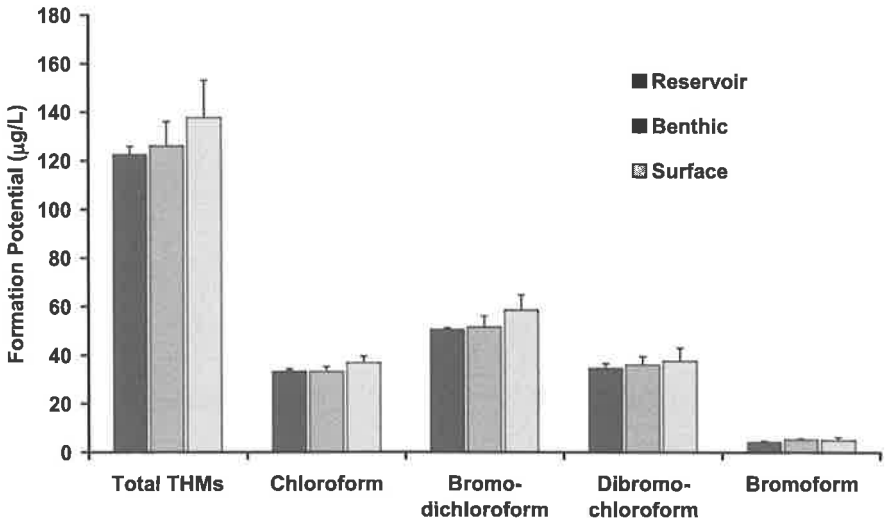

Figure 6.14. Trihalomethane formation potential of 200% MPD product water.

6.3.3.4. Resin Fractionation

Resin fractionation was conducted on 200%MPD product water in order to determine if there had been any changes in the functional character of the non-removable organic

matter under mesocosm conditions. The resulting changes in concentration of the DOC fractions were of the minimum reportable increment and were less than or equal to the uncertainty of the method (0.2 mg L^{-1} for VHA, SHA and CHA; 0.1 mg L^{-1} for NEU; Chow *et al.* 2004). There was an extended period of storage of these samples (refrigerated at 4 °C stored in PET bottles) due to a DOC analyser malfunction. At the time of analysis only 3.0 - 3.4 mg L^{-1} of DOC remained in the samples compared to 4.2 - 4.6 mg L^{-1} directly after jar testing. Alternatively there was a problem with the DOC analyser which was not identified during analysis, however given the stringent QC that is undertaken, this is unlikely. Consequently, while the samples show some small differences, caution should be used when interpreting these results.

The reservoir samples had identical fractional composition to the initial samples, while the surface mesocosm composite samples were reported to have 0.1 mg L^{-1} more of each, VHA and CHA. The benthic mesocosm composite samples were reported to have 0.1 mg L^{-1} less of each VHA and SHA and 0.1 mg L^{-1} more of CHA (Figure 6.15). If it is accepted that the reported values represent the proportions of these fractions of the DOC, then the results imply that the VHA fraction, which is generally assumed to represent the biologically refractory humic substances, was reduced in concentration in the benthic mesocosms or was rendered more amenable to coagulation. This may indicate that the microbial community began to utilise less favourable substrates, or that the physicochemical conditions were more amenable to its degradation or slightly altered its coagulation properties (decreased pH, for example). Also reduced in concentration in the treated water from the benthic mesocosms were the SHA and NEU fractions, of which, the NEU fraction is particularly recalcitrant to removal with alum.

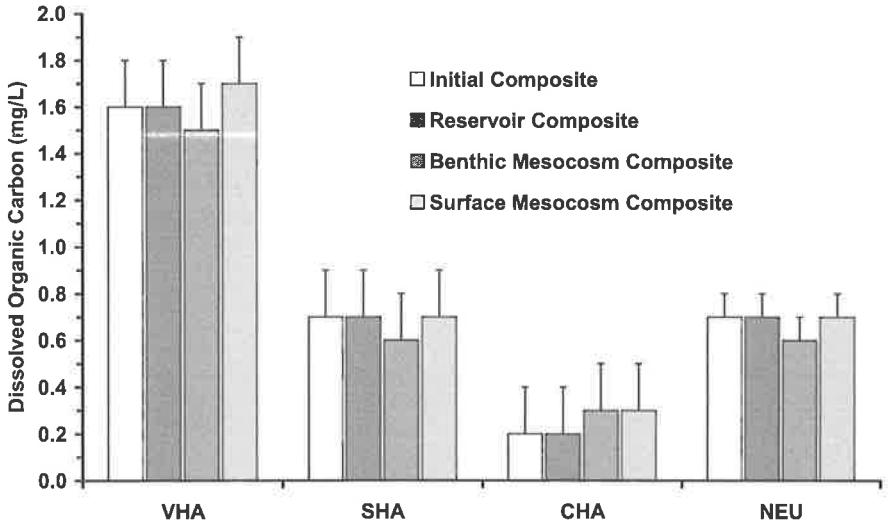

Figure 6.15. Concentration of different classes of organic carbon in the 200%MPD product water, as determined by resin fractionation. Very hydrophobic acids (VHA), slightly hydrophobic acids (SHA), hydrophilic charged (CHA) and hydrophilic neutral (NEU). Error bars indicate the experimental uncertainty reported by Chow *et al.* (2004).

6.3.3.5. Bacterial Regrowth Potential

Bacterial regrowth potential was determined to evaluate any influence on the distribution system stability of the product water, using an inoculum derived from a composite of all the samples analysed, ensuring a similar and high metabolic diversity. The values reported are the mean of two duplicate analyses of composite samples. The reservoir composite had a BRP of 142 μg L^{-1} (acetate equivalents) while the surface and benthic (-M4) mesocosm composites were very similar with 94 and 92 μg L^{-1}, respectively. Benthic mesocosm four (M4) which had been analysed separately as it had an accumulation of DOC during the first 2 weeks of the incubation, had a BRP of 344 μg L^{-1}, which is much higher than all the other composite samples. It would appear that a significant amount of labile carbon passed through the treatment process in this sample.

6.3.3.6. High Performance Size Exclusion Chromatography

HPSEC-UV (Method A)

No significant differences in the number average (M_n) or weight average (M_w) molecular weights were found between the mesocosm treatments in either the raw or 200%MPD

treated jar test product water. The polydispersity (M_n/M_w) was statistically significantly higher in the reservoir than the surface mesocosms (Tukey-Kramer HSD, $\alpha = 0.05$) though the difference between the means was only 0.009 (~0.7% of the mean) and is most likely not meaningful. No significant differences in polydispersity were found in the 200%MPD treated UV absorbing organics.

The spectra of the reservoir replicate samples and the 200%MPD treated product water were practically indiscernible (Figure 6.16), providing good confidence in the repeatability of the method. The shape of the spectra is similar between the treatments with peaks at approximately, 370, 820, 1400, 1500 and 61000 Da and only small differences in the relative magnitudes of the peaks (Table 6.7). The 61 kDa peak represents material that was unable to permeate into the stationary phase of the media and the size estimate is outside the calibrated range of standards. Consequently the apparent molecular weight of this peak should be considered as arbitrary and its size will only be used as a term of reference. Comparison of the raw water spectra to the 200%MPD treated product water suggests total removal of UV absorbing NOM above 1100 Da, significant removal from 700 to 1100 Da and minor removal from 250 to 700 Da (Figure 6.16). The variation between the raw water spectra from the replicate benthic and surface mesocosms was greater than the reservoir (c.f. Figure 6.17 and Figure 6.18 to Figure 6.16). Most of the variation occurred above 1000 Da, with the benthic mesocosms having lower absorbance peaks than the reservoir samples and the surface mesocosms having equal or higher absorbance peaks than the reservoir samples. It was observed that there was a reversal in the order of peak absorbance between the peaks at approximately 1400 Da (range 1365 - 1401) and approximately 61 kDa (range 60.6 to 62.1 kDa) and a strong linear regression with significant parameter estimates was found between the maximum absorbance of these peaks (Figure 6.19; 61 kDa absorbance = -0.29 × 1.4 kDa absorbance + 0.01; r^2adj = 0.68; N = 12; $\alpha < 0.0001$). The nature of the distribution of the data does suggest that there is more than one process

occurring as the surface mesocosm data forms a very consistent series contiguous with the reservoir samples (61 kDa absorbance = -0.50 × 1.4 kDa absorbance + 0.02; r^2adj = 0.99; N = 8; $\alpha < 0.0001$), while the benthic mesocosm samples are more variable (Figure 6.19).

It appears that a significant proportion of this apparent shift of organic matter from the 1.4 kDa to the HMW size range was the result of aggregation driven by changes in the pH of the water column. This is demonstrated by the regression between the pH of the sample at collection and the ratio of the 1400 Da to 61 kDa absorbance across all three treatments (Figure 6.20; ratio peak absorbance = 5.9 × pH - 32.4; r^2adj = 0.34; N = 12; $\alpha < 0.05$). When the relationship within each treatment (reservoir, benthic, surface) are examined, only the benthic samples have significant regression parameter estimates (ratio peak absorbance = 43.5 × pH - 293; r^2adj = 1; N = 4; $\alpha < 0.0001$). One of the surface mesocosms (M7) sits well out of an apparent linear trend in the other three mesocosms (Figure 6.20). Another process that could be proposed to explain this result is the oxidative polymerisation of the NOM (Piccolo 2001, Piccolo *et al.* 2005).

Table 6.6. Mean number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity (M_n/M_w) of NOM as determined by HPSEC-UV (Method A). N = 4 in all cases. Determined for NOM between 100 and 7000 Da apparent molecular weight.

Sample	*Treatment*	*Mean Mn*	*SD*	*Mean Mw*	*SD*	*Mean M_n/M_w*	*SD*
Reservoir	Raw	1073	1.2	1417	1.3	1.32	0.0003
Surface Mesocosm	Raw	1066	5.4	1397	14.1	1.31	0.007
Benthic Mesocosm	Raw	1054	17	1386	22.8	1.31	0.002
Reservoir	200%MPD	457	1.0	632	2.0	1.38	0.002
Surface Mesocosm	200%MPD	460	7.1	631	3.8	1.37	0.014
Benthic Mesocosm	200%MPD	456	6.2	630	1.6	1.38	0.019

Table 6.7. Mean apparent molecular weight peaks and relative magnitudes (to peak absorbance) of the raw water as determined by HPSEC-UV (Method A).

Benthic		*Surface*		*Reservoir*	
AMW Peak (Da)	*Relative Magnitude*	*AMW Peak (Da)*	*Relative Magnitude*	*AMW Peak (Da)*	*Relative Magnitude*
371	0.27	372	0.28	370	0.28
815	0.44	818	0.46	822	0.46
1381	0.94	1388	1.00	1392	1.00
1526	1.00	1508	0.95	1501	1.00
61216	0.10	61778	0.05	61472	0.09

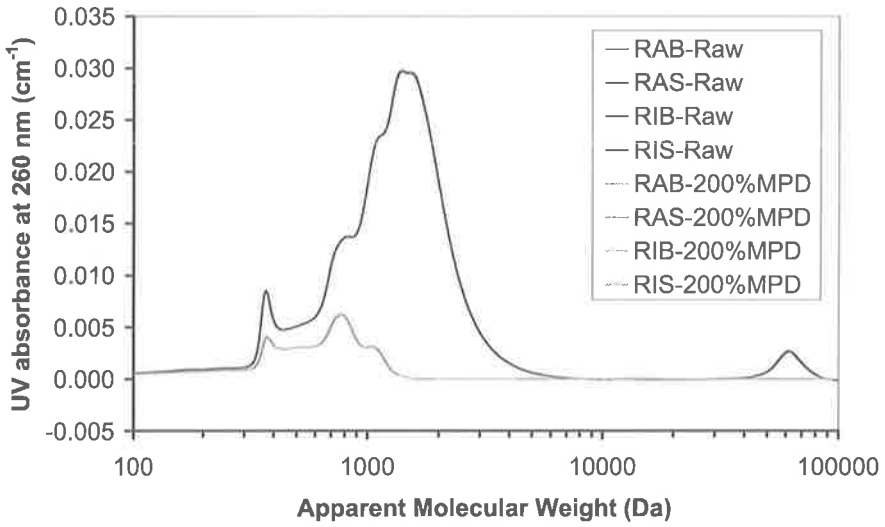

Figure 6.16. HPSEC-UV spectra (Method A) of the Raw and 200%MPD treated replicate reservoir samples.

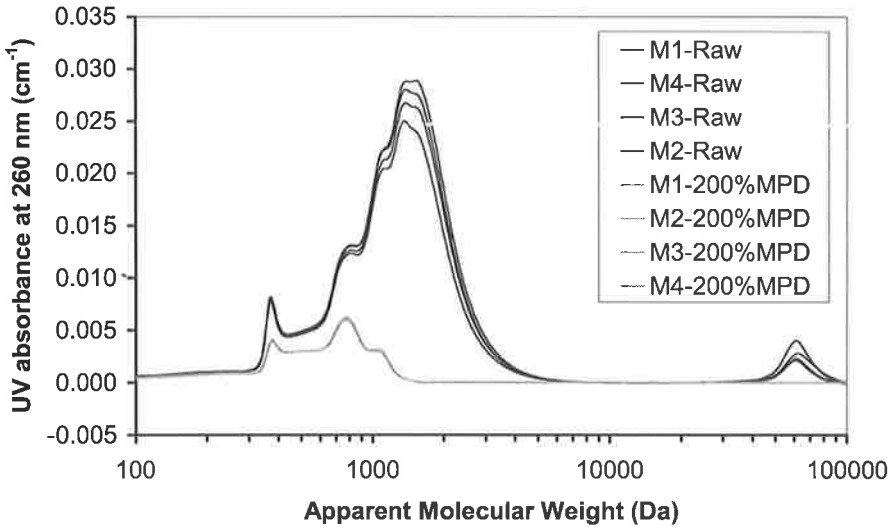

Figure 6.17. HPSEC-UV spectra (Method A) of the raw and 200%MPD treated replicate benthic mesocosm samples. Legend labels for the raw samples are in the order from highest to lowest absorbance maxima.

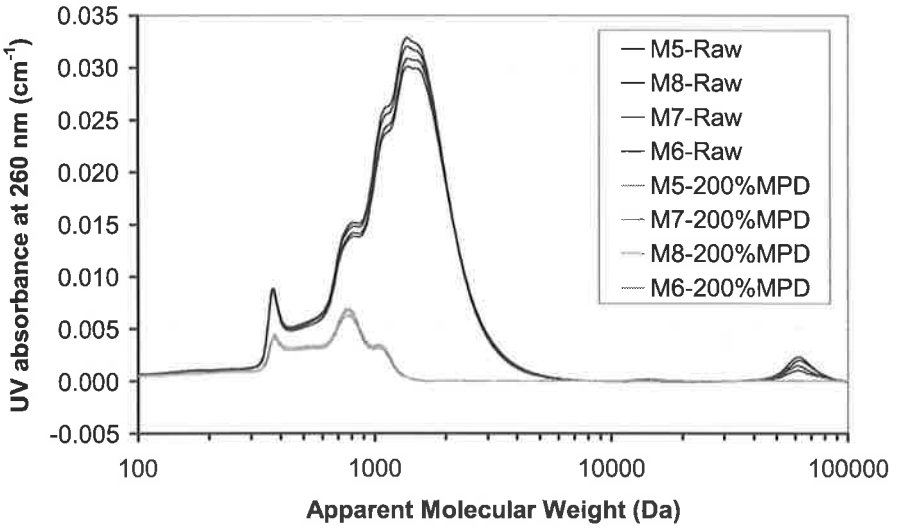

Figure 6.18. HPSEC-UV spectra (Method A) of the raw and 200%MPD treated replicate surface mesocosm samples. Legend labels for the raw samples are in the order from highest to lowest absorbance maxima.

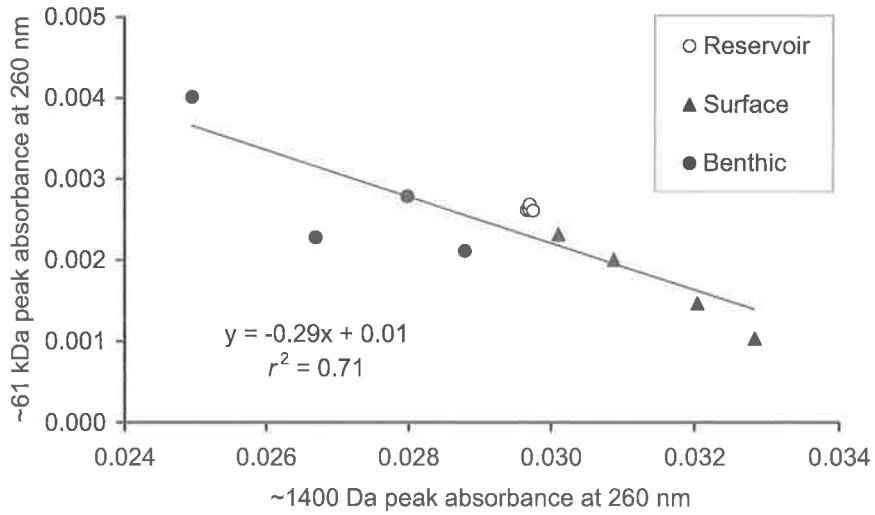

Figure 6.19. Linear regression between the peak absorbance at approximately 1400 Da and the peak absorbance at approximately 61 kDa in samples collected from Myponga Reservoir and surface and benthic mesocosms determined by HPSEC-UV (Method A).

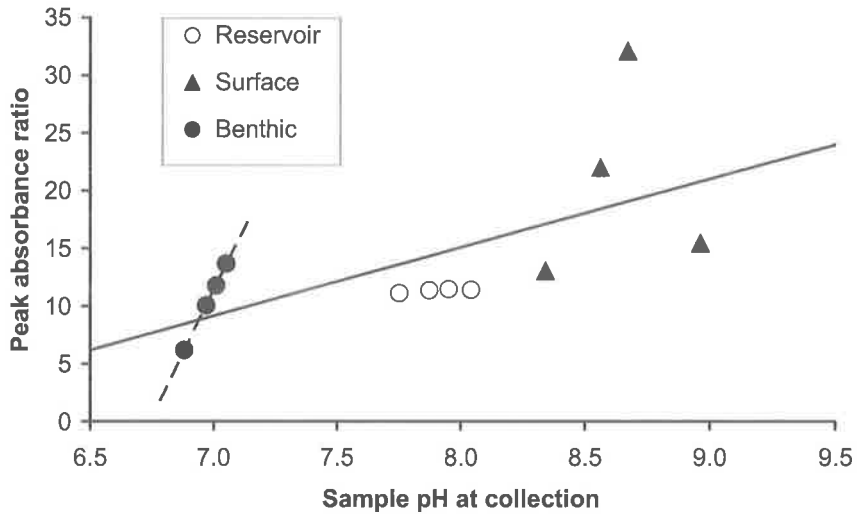

Figure 6.20. Ratio of the peak absorbance at approximately 1400 Da and 61 kDa in samples collected from Myponga Reservoir and surface and benthic mesocosms determined by HPSEC-UV (Method A) compared to the pH of the sample at the time of collection. The solid line is the all data regression (ratio of peak absorbance = 5.9 × pH - 32.4; r^2adj = 0.34; N = 12; $\alpha < 0.05$) and the dashed line is the benthic data regression (ratio of peak absorbance = 43.5 × pH - 293; r^2adj = 1; N = 4; $\alpha < 0.0001$).

HPSEC-DOC (Method B)

The separations performed using two different systems (see §2.4.1 and §2.4.2 for details) show different separation characteristics, as expected when different separation columns and conditions are used (Perminova 1999, Peuravuori and Pihlaja 2004, Allpike *et al.* 2005). Method A identified 5 peaks while method B resolved 7, however, while there are

shifts in the apparent molecular weight peaks and their relative magnitudes, the spectra share qualitative similarities. Method B identified low molecular weight peaks at 450 and 760 Da which are similar molecular weights to Method A. It seems that more separation was achieved in the medium molecular weight range in Method B as this is where the main differences occur between the separation methods. Method B identified peaks at 1500, 2500 and 4100 Da. The high molecular weight material formed two peaks in Method B of 18 and 23 kDa apparent molecular weight but again it is likely that this represents the organic material that has not permeated the media and has eluted at the void volume (dextran blue, 200 kDa, V_e = 24.24 mL).

In the jar test product water spectra, the ~4000 Da peak is almost totally removed (Figure 6.22), suggesting that the partially removed shoulder at 1100 Da in Method A corresponds to the 2500 peak in Method B. This difference in apparent molecular weight is most likely the product of the difference in ionic strength (I) and pH of the eluent used in the two methods (Method A, I = 160 mM, pH = 6.8; Method B, I = 120 mM, pH = 6.85). The higher ionic strength of Method A will increase the double layer compression of the molecules and result in enhanced permeation of the pores of the stationary phase. This will increase the elution volume and thus decrease the molecular weight estimate (Peuravuori and Pihlaja 1997). While the ionic strength of the mobile phase is generally considered the most important factor, the differences in column solid phase may have also played a role, for example in hydrophobic interactions.

However, in both methods the removal of the UV absorbing organics appears similar, i.e. there is total removal above a certain apparent molecular weight and progressively less removal with decreasing molecular weight. The reversal in the magnitude of the absorbance peaks between the medium and the high molecular weight UV absorbing organics is also observed in the Method B spectra, however as only three samples were analysed, no attempt to develop a regression was made.

There are obvious differences in the spectra with DOC detection. The trend of reversal of peak magnitude between the medium and high molecular weight peaks is not apparent, implying that these differences are either the product of changes in the specific absorbance of the compounds in these peaks or the addition or subtraction of organic matter to these peaks. The HMW peak between 10 and 30 kDa has a higher peak DOC response in the surface mesocosm raw sample. There also appears to be a corresponding peak in the treated water spectra, which, as there is no corresponding increase in UV absorbance in the UV spectra, indicates that this is UV transparent material and is probably composed of carbohydrates or other aliphatic compounds. As there is apparently no equivalent peak in the reservoir or benthic sample, it can be assumed that this is the result of accumulation of high molecular weight organic matter in the surface mesocosms. This is consistent with the conclusions made by Her *et al.* (2002) and Leenheer and Croué (2003) that high molecular weight organic matter predominantly consists of polysaccharides and proteins, the residues of microbial cell walls. Further evidence for this being carbohydrate in nature is the increases in BGL activity found in the surface mesocosms and the microbial consortia's metabolic response to cellobiose in the CLPP analysis. It appears, therefore, that there is a number of processes occurring that are altering the size distribution of the organic matter in this experiment.

The number average molecular weight, weight average molecular weight and the polydispersity estimates based on the Method B separations were all higher than the Method A estimates (c.f. Table 6.6 to Table 6.8). Again, this is likely the result of the lower ionic strength of the eluent in Method B causing greater apparent size of the molecules. The polydispersity of the UV absorbing NOM in the raw waters (Method A) was greater than that calculated for the treated water (Table 6.6), however the opposite trend was observed when these parameters were determined on the basis of the separation made by Method B, both with DOC (Table 6.8) and UV response (Table 6.9).

The concentration of DOC in each peak was calculated on the basis of the integrated peak area of the elution volume against DOC detector response spectra (Table 6.10). The original DOC of the sample was divided by the relative area under the elution volume to DOC response curve to give the relative concentration of DOC in each fraction. These calculations assume no loss of DOC on the column and reveal that in the surface mesocosms that the increased DOC concentration is spread across the entire spectra (0.1 - 0.3 mg L^{-1} difference from reservoir; Table 6.10). In the treated water however, the main differences in concentration occur in the 3.0 - 13.7 kDa size range (0.16 mg L^{-1} difference; Table 6.10).

Table 6.8. Average number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity (M_n/M_w) of NOM as determined by HPSEC with DOC detection (Method B). Determined for NOM between 100 and 7000 Da apparent molecular weight.

Sample	*Treatment*	*Mn*	*Mw*	M_n/M_w
Reservoir	Raw	2970	1930	1.54
Surface Mesocosm	Raw	2880	1830	1.57
Benthic Mesocosm	Raw	3070	2000	1.53
Reservoir	200%MPD	1380	1080	1.28
Surface Mesocosm	200%MPD	1380	1020	1.36
Benthic Mesocosm	200%MPD	1400	1040	1.35

Table 6.9. Average number average molecular weight (M_n), weight average molecular weight (M_w) and polydispersity (M_n/M_w) of NOM as determined by HPSEC with UV detection (Method B). Determined for NOM between 100 and 7000 Da apparent molecular weight.

Sample	*Treatment*	*Mn*	*Mw*	M_n/M_w
Reservoir	Raw	4020	2380	1.69
Surface Mesocosm	Raw	3390	2310	1.47
Benthic Mesocosm	Raw	4780	2440	1.95
Reservoir	200%MPD	1350	1040	1.30
Surface Mesocosm	200%MPD	1330	1010	1.32
Benthic Mesocosm	200%MPD	1280	874	1.46

Table 6.10. Calculation of concentration of DOC in size fractions (mg L^{-1}) on the basis of HPSEC-DOC (Method B) chromatograms. The DOC of the sample was divided by the relative area of the size fractions, determined from the elution volume against DOC detector response spectra.

Peak	*1*	*2*	*3*	*4*	*5*	*6*
Approximate Size Range	*13.7 -31.3 kDa*	*3.0 - 13.7 kDa*	*2.0 - 3.0 kDa*	*1.0 - 2.0 kDa*	*500 - 1000 Da*	*300 - 500 Da*
Reservoir - Raw	0.38	5.6	2.6	2.3	1.2	0.40
Benthic - Raw	0.46	4.7	2.3	2.2	1.1	0.42
Surface - Raw	0.55	5.8	2.9	2.6	1.4	0.50
Reservoir - 200%MPD	0.01	0.09	0.63	1.9	1.1	0.45
Benthic - 200%MPD	0.02	0.09	0.63	1.8	1.0	0.56
Surface - 200%MPD	0.07	0.25	0.66	2.0	1.1	0.54

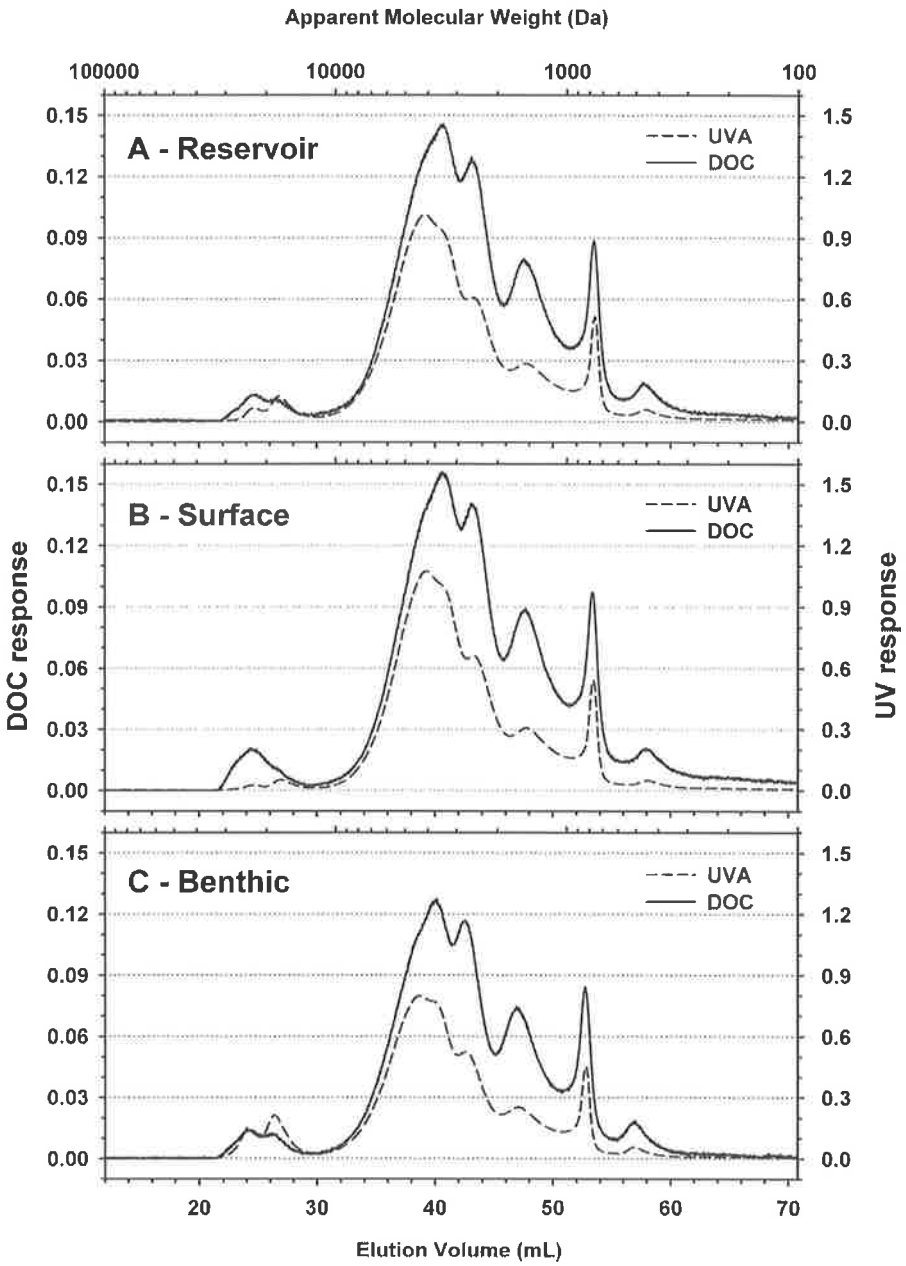

Figure 6.21. HPSEC spectra generated using Method B of raw waters before jar testing. A) reservoir sample (RAS), B) surface mesocosm (M8) and C) benthic mesocosm (M2). Solid line is the arbitrary DOC response and the dashed line is the arbitrary UV response at 254 nm.

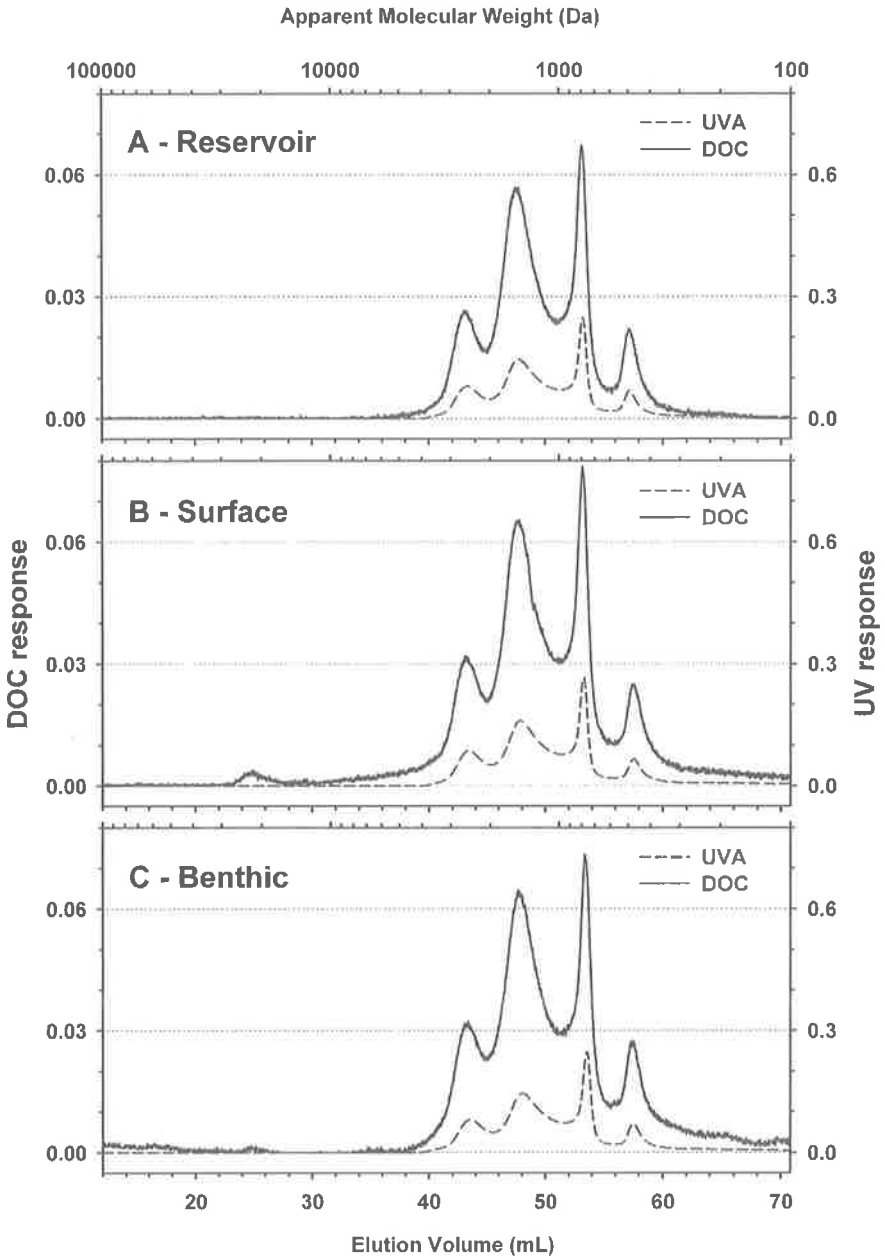

Figure 6.22. HPSEC spectra generated using Method B of 200%MPD treated product water from jar testing. A) reservoir sample, B) surface mesocosm and C) benthic mesocosm. Solid line is the arbitrary DOC response and the dashed line is the arbitrary UV response at 254 nm.

6.3.3.7. ATR-FTIR of Flocculated Material

The FTIR spectra of the flocculated material from the 200%MPD treatments had only subtle differences. Absolute differences were observed in the raw spectra (Figure 6.23) due to the differences in sample composition, for example the ratio of organic carbon to alum used in the jar testing procedure, and the presentation of the sample to the ATR sampling

apparatus. As flocculated material was analysed, a large peak at 550 cm^{-1} dominates the spectra. The form of the spectra in the fingerprint region (E) does not provide any indication of any change in functionality of the flocculated organic matter.

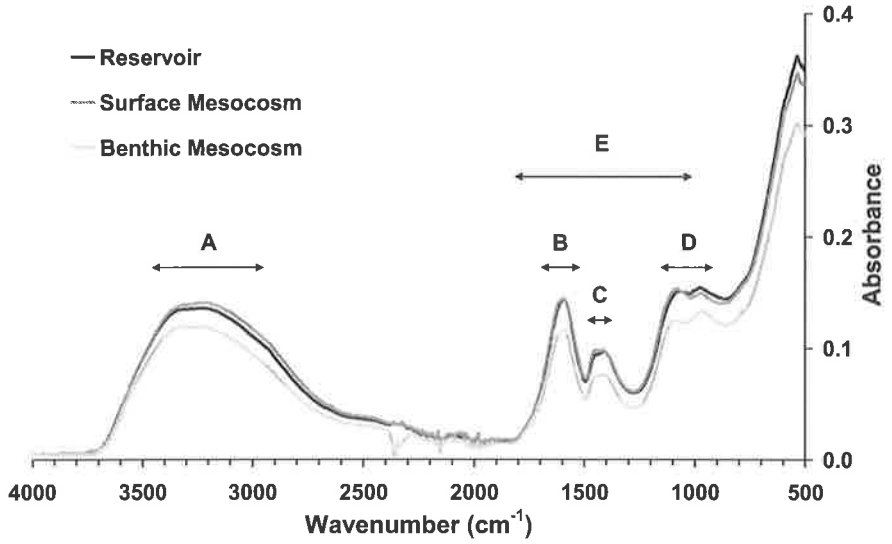

Figure 6.23. Raw ATR-FTIR spectra of flocculated material from jar testing of mesocosm experiment water. The labelled regions are A, OH group stretch of structural and adsorbed water, B, asymmetric (protonated) carboxylic acid (COOH), C-C ring and amide (I and II) stretch, C, symmetric carboxylic acid (COO^-) and CH_2 stretch, D, C-O-C ring stretch of carbohydrates, E, the fingerprint region.

6.4. Discussion

The changes in microbial metabolism, organic matter character and the treat-ability of the NOM found in this study were subtle. The changes in some of the water treatment parameters were statistically significant; however their significance from the perspective of water treatment is probably minor. Nevertheless, this work highlights some of the potential interactions between micro-organisms, natural organic matter and stratified water columns. Sediment adsorption plays an important role in the removal of organic compounds, as observed in the decrease in DOC in the benthic mesocosms, however this did not result in improved product water. The data indicates that it is medium to high molecular weight, and most likely hydrophobic moieties that are lost to the sediment. These compounds are easily removed by conventional water treatment processes and have little impact on the product

water quality. It did however, result in reduced dose rates to achieve the same water quality (c.f. benthic MPD to reservoir MPD and product water quality; Table 6.4).

An estimate based on the cost of water treatment chemicals suggests that water from the benthic mesocosm would be 3.7% cheaper to treat than the reservoir water. Similar calculations suggest the surface mesocosm water would be 2.4% dearer (reservoir, benthic and surface chemical cost estimates were \$30.19, \$29.07 and \$30.92 per ML, respectively, using Alum (52%, $Al_2(SO_4)_3.18H_2O$) \$156 tonne^{-1} and sulphuric acid (H_2SO_4, 98%) \$142.1 tonne^{-1}). If a comparison was made directly between the surface and benthic mesocosm, the benthic mesocosms would represent a 6.4% saving in water treatment chemical costs, before final pH correction is considered.

The changes in the concentrations of organic matter in the mesocosm treatment occurred mostly in the medium to high molecular weight range, according to the HPSEC data. It seems that some UV transparent, high molecular weight material that was not amenable to removal by conventional processes was produced in the surface mesocosms. The apparent rise in DOC in the surface mesocosms is most likely the result of phytoplankton production (carbon fixation through photosynthesis) and some evaporative concentration. The DOC flux may have occurred via excretion, 'leaky' cells, cell lysis and/or zooplankton 'sloppy' feeding, all well documented carbon flux pathways in aquatic systems (Hessen *et al.* 1990). An alternative explanation is that the microbial activity in the surface mesocosms was inhibited by an increase in exposure to solar UV irradiation (Zepp *et al.* 1998). This is however unlikely as there was no decrease in the number of culturable bacteria by heterotrophic plate count or microbial enzyme activity.

Evidence for the conformational change or oxidative polymerisation of the UV absorbing organic matter was found using HPSEC (Figure 6.16, Figure 6.17 and Figure 6.18). Regressions attributing the proportion of this shift which is described by the pH differences in the samples suggests pH is the predominant factor in the benthic mesocosms (Figure

6.20). In the surface mesocosms, the corresponding decrease in UV absorbing HMW organic matter was observed using HPSEC Method B, however the DOC detector identifies an accumulation of UV transparent HMW organic matter. It is tempting to attribute this peak to phytoplankton cellular debris, due to the significant algal growth that occurred in the surface mesocosms, however no direct evidence for this suggestion is available. The possibility of oxidative polymerisation of phenolic groups within the organic matter, fuelled by microbial activity on algal exudates, seems unlikely as the HMW DOC in question has low UV absorptivity.

The physico-chemical conditions that developed during this experiment were mild compared to the depletion of dissolved oxygen and changes in pH that can develop during persistent stratification. Much greater differences may be expected under extreme conditions, however they will not necessarily be similar to what was found in this experiment, as large changes in the metabolic activity will occur under anoxic and anaerobic conditions. The changes in microbial enzyme activity that were found were also subtle. However the observed changes were consistent with current understanding of the regulation of microbial substrate degradation and acquisition. It appears that BGL activity was up-regulated in the benthic mesocosms, as a result of the change in substrate availability that occurred when the recently produced phytoplankton substrates were depleted. It is likely that the terrestrially derived coarse organic matter found on the sediment surface became an important substrate. The benthic mesocosms developed a much larger (culturable) microbial population than the reservoir or the surface mesocosms. While the individual data are not presented, the surface mesocosm with the greatest heterotrophic plate count also had higher enzyme activities, suggesting this abundance was supported by the degradation of polymeric substrates.

Persistent thermal stratification can lead to many water quality problems in drinking water reservoirs. While the results of this study indicate that small benefits in alum usage might

be gained by harvesting water from the hypolimnion during the onset of stratification, it is likely that the redox conditions would rapidly compromise the raw water quality through the solubilisation of iron and manganese. Given this and the other water quality problems that destratification controls or reduces, this water harvesting strategy could not be recommended. It is more likely that the more homogenous water column provided by destratification will provide a more consistent raw water quality from the perspective of natural organic matter. The effects of stratification on biogeochemical processes other than those that affect natural organic matter are likely to be relevant from the perspective of the water industry. The acidification of the hypolimnion may however be an important factor in the long term sedimentation dynamics of organic matter in humic lakes and reservoirs.

Chapter 7. Discussion and Conclusions

This thesis highlights the importance of hydrodynamics in determining the quantity and character of natural organic matter (NOM) in drinking water reservoirs. Hydrology was a major determinant of the concentration of dissolved and particulate organic carbon (DOC and POC) in Myponga River. The intrusion and mixing hydrodynamics determined the characteristics of DOC transport to the reservoir dam wall. Changes in the vertical structure of simulated water columns and the associated changes in physicochemical conditions resulted in changes in organic matter molecular weight distribution. The major findings of the investigation are discussed in three sections, (1) inflows, (2) microbial activity and (3) treat-ability, and a conceptual model of the major effects of hydrodynamics on NOM is presented. These concepts are further developed by presenting a management framework considering NOM in the context of other water quality hazards.

7.1. Inflows

The hypothesis that large inflow events challenge water treatment by increasing the concentration of organic matter at the offtake (Hypothesis 1, §1.5.1) is supported by the field experiment that tracked a large inflow event at Myponga Reservoir (Chapter 3). The potential for short term fluctuations in the concentration, and to a lesser extent, the character of organic matter present in reservoir strata available to the offtake, was demonstrated, but were not severe. However, they do support the hypothesis and highlight that this hazard to raw water quality should be considered by reservoir managers. The empirical modelling of optimum dose predicted an increase in treatment costs of 7.2 %, of which 40 % is attributable to changes in the NOM properties. The potential for the temporal displacement in the threat from NOM from the threat presented by pathogens and turbidity was also observed, the result of the fluctuation of organic carbon concentration with flow in Myponga River.

The threats to water quality presented by pathogens and NOM have some distinct differences in their characteristics. The concentration of pathogens may change by orders of magnitude over short timescales (Brookes *et al.* 2005b). The concentration changes in NOM that were observed were relatively small, however NOM presents a constant challenge to water treatment. This observation gives managers the opportunity to modify the management of the reservoir offtake, accounting for this temporal disparity between the pathogen and the organic carbon threats. The combination of offtake management and optimisation of water treatment conditions for both particle and colour removal will ensure multiple barriers are presented to water quality hazards, such as protozoa, enteric bacteria, disinfection by-products and bacterial regrowth substrates.

It was hypothesised that the catchment flow regime determines the loading of allochthonous organic carbon and therefore plays a major role in determining the carbon dynamics and therefore the water quality (Hypothesis 2, §1.5.1). This hypothesis is supported by the strong relationships found between dissolved and particulate organic carbon (DOC and POC) concentration and the hydrological parameters, using simple empirical models (Chapter 4). Changes in the concentration and character of the inflowing NOM were also observed at the seasonal timescale (Table 4.1).

Current predictions of climate in the coming century suggest that increases in storm intensity and prolonged periods of drought will occur in temperate systems (Huntington 2006). This will almost certainly have a profound effect on catchment hydrology and result in fewer, yet larger inflow events to drinking water reservoirs. Furthermore, catchment models predict an amplification of the effect of changes in precipitation on the patterns of run-off (Chiew *et al.* 1995, Chiew and McMahon 2002). The likely result is prolonged periods of decreased catchment water yield, punctuated by larger flow events. Water resource management must therefore cope with reduced catchment yield and the potential deterioration in water quality caused by intruding inflows into drinking water reservoirs.

7.2. Microbial Activity

The hypothesis that microbial activity plays a significant role in determining the concentration and character of NOM in drinking water reservoirs (Hypothesis 3, §1.5.1) is upheld by the volume of literature on the humification processes that occur in the terrestrial, soil and aquatic environments and the evidence presented here (Chapter 3 and Chapter 4). In Chapter 3 it was demonstrated that the inflowing water contained significant amounts of microbial activity, which mixed with the reservoir water and persisted in the reservoir microbial community. The influx of soil derived catchment microbes were an important source of metabolic diversity during and following the inflow event. This is consistent with observations of the influence of catchment microbial communities on phenotypic and genotypic diversity in the microbial communities of other lakes and reservoirs (Chapter 3, Lindström and Leskinen 2002, Lindström and Bergstrom 2005, Kritzberg *et al.* 2006).

The variable distribution of the activity of the different microbial enzymes between particles, the cell surface, and in free suspension, demonstrates the importance of the particle associated microenvironment for the different substrates. Notably those associated with the degradation of higher plant polymers and algal cell wall components tend to be associated with particles (Chapter 3). The surface of particles is a 'hot spot' of the humification process and microbial activity is an important part of this process (Steinberg 2003). The greater partitioning of leucine aminopeptidase (LAP) and alkaline phosphatase (AKP) to the >1.0 μm fraction (Chapter 3) suggests these enzymes are more important to free living microbes or are released from the near cellular space, and may play an altruistic role (Vetter *et al.* 1998).

While hypothesis 3 (§1.5.1) is also supported by the large percentage of organic matter found to be mineralised by microbial processes (Chapter 4), it is likely that a large quantity of microbially recalcitrant organic matter is turned over by photochemical processes, or

requires the involvement of photochemical as well as microbial processes for mineralisation (Rosenstock *et al.* 2005, Amado *et al.* 2006). As photochemical mineralisation accounts for relatively less of the organic matter processed, in terms of mass turnover (Chapter 4), the microbially refractory organic matter persists in aquatic ecosystems.

The hypothesis that microbial activity reflects the concentration and character of NOM (Hypothesis 4, §1.5.1) seems to hold predominantly for particulate organic matter, and therefore has little relevance in determining the water quality for drinking water purposes where flocculation and filtration are employed. Significant correlations between microbial enzyme parameters and the characteristics of dissolved organic matter were not found (Table 3.1). This is probably due to the bulk organic matter parameters measured (DOC, UVA, Colour) being primarily the product of microbially refractory organic matter. If parameters like free and hydrolysable amino acids, or free and hydrolysable carbohydrates had been measured, then correlations with microbial enzyme activity may have been expected to be observed.

7.3. Treat-ability

A field sampling program was conducted to test the hypothesis that vertical gradients of organic carbon concentration and character will occur in thermally stratified reservoirs (Hypothesis 5, §1.5.1). The efforts made to sample Googong Reservoir following a substantial period of thermal stratification were prevented by the deepening of the surface mixed layer beyond the depth of the sampling equipment available. The sampling event conducted in May 2004 was originally scheduled for March 2004, however unavoidable logistical problems delayed the trip. The shallow nature of Wartook Reservoir means that it only stratifies for short periods during stable weather with low wind speeds. This resulted in a vertical gradient of organic matter being observed on only one of three sampling occasions (12 February 2004; Figure 5.4, Figure 5.8 and Figure 5.12). On the basis of these

observations and the differences in the raw water organic matter properties observed in simulated hypolimnia and epilimnia (Chapter 6) it is likely that similar differences would have been observed in Googong Reservoir, had it been sampled at the appropriate time. It seems that the hypothesis that vertical gradients in the treat-ability of organic carbon will require further investigation before a conclusive rejection of the null hypothesis may be made.

It was apparent from the mesocosm experiments that a large amount of the change in the apparent molecular weight distribution of the organic matter could be attributed to the pH of water at the time of sampling (Figure 6.20). Considering the results from Wartook in light of this observation, a similar relationship can be observed (Figure 7.1). This is probably due to changes in the supramolecular conformation of the NOM at the lower pH (sensu Piccolo 2001, Piccolo *et al.* 2005). Despite the observed change in raw water properties, little difference in the treat-ability of the raw water was observed. Most likely as the pH induced differences are not so important during alum coagulation which reduces the pH significantly below the environmental conditions, and would induce similar conformational changes in the NOM.

Any potential benefit of this pH driven shift towards higher apparent molecular weight would be realised over time as the molecular weight of the organic matter may increase to the point where it is of sufficient size to be subject to sedimentation, become part of the lake snow and be subjected to further microbial colonisation and degradation (Grossart *et al.* 1998, Grossart and Simon 1998, Simon *et al.* 2002). It is likely that the formation and sedimentation of organic aggregates was an important loss process in the benthic mesocosms (Chapter 6), although this process was not measured directly. The long term effect of hypolimnetic acidification on the loss of organic carbon from humic lakes and reservoirs may warrant further research.

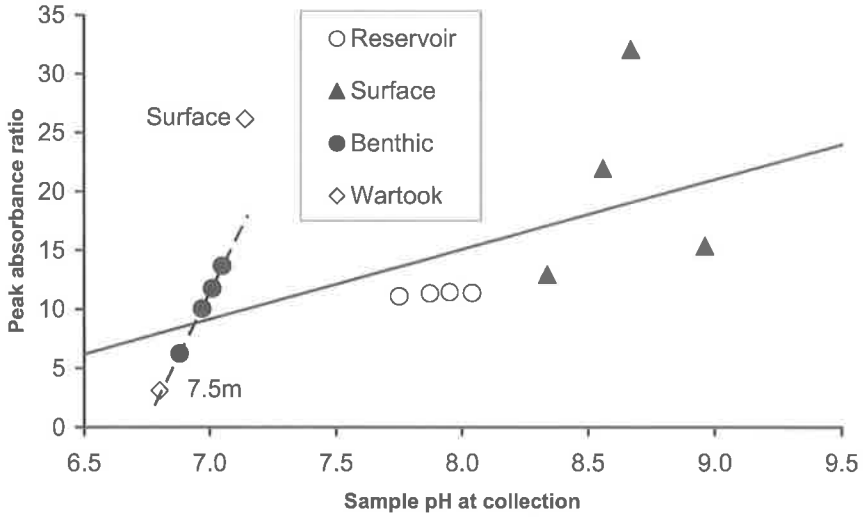

Figure 7.1. Relationship between pH at sample collection and the ratio of high molecular weight organic (peak absorbance above 50 kDa) matter and the peak absorbance of the HPSEC spectra below 50 kDa. Data from Myponga Reservoir Mesocosm experiment (Chapter 6) and sampling of Wartook Reservoir 12 February 2004. See Chapter 6 for regression results. No regression was calculated for the Wartook results.

Changes in the composition of the phytoplankton communities (Figure 6.7) and the abundance and metabolic activity of the microbial communities (no identities were determined, Figure 6.9, Figure 6.10 and Figure 6.11) were observed in the mesocosm experiment, consistent with hypothesis 6 (§1.5.1). Also consistent with this hypothesis, changes in the microbial degradation and assimilation of substrates was observed. The changes implied that β1-4 linked carbohydrates (i.e. cellulose) rapidly became a more important substrate in the benthic mesocosms and that proteins gradually became more important in the surface mesocosms. The loss of physiological competency was observed in both the surface and benthic mesocosms. In the ordination of the substrate responses, the mesocosm samples had primary and secondary principal component scores that were more similar to each other than the reservoir. This may be the result of sedimentation losses of particle attached microbial communities in quiescent mesocosms. This raises the question of the effect of artificial destratification on the dynamics of particulate organic carbon in reservoirs in the long term.

7.4. Conceptual Model and Management Framework

The results of this study can be used to construct a conceptual model for reservoir management that provides insight into the factors that impact on water quality especially NOM. The conceptual model (Figure 7.2) represents the majority of the processes that determine the quantity and character of NOM. The endpoint of the conceptual model is three important aspects of treated water quality that are determined by NOM (bulk treat-ability; disinfection by-product formation potential, DBP-FP and biological stability). The bulk treat-ability characteristics of the NOM is determined predominantly by the character of the refractory dissolved organic matter. The amount of organic carbon unable to be removed during water treatment is determined by the treat-ability characteristics and the water treatment conditions applied. Therefore, the DBP-FP of the product water is strongly influenced by the refractory dissolved NOM. The biological stability characteristics of the raw water is primarily the product of the labile dissolved NOM. Although this is true for raw reservoir water, interactions with oxidants during water treatment and distribution (e.g. chlorine, ozone and permanganate) will cause the production of microbial substrates from previously refractory compounds (Vuorio *et al.* 1998, Escobar and Randall 2001, Hem and Efraimsen 2001).

The complexity of the system is indicated by the numerous feedbacks and interactions between different parts of the system. This makes it difficult to make any broad statements on the effect of artificial destratification on NOM dynamics across reservoirs. However, it is possible to make predictions based on the knowledge of other reservoir characteristics, such as the concentration and internal load of inorganic nutrients and the loads of autochthonous and allochthonous organic carbon. If the internal nutrient load is high, then artificial destratification is likely to be beneficial from the perspective of organic carbon concentration, as reduction of the internal load may reduce the autochthonous organic carbon load to the reservoir. In a reservoir where the external nutrient load alone is

sufficient to sustain the potential autochthonous production, then some reduction in the autochthonous organic carbon load may be achieved by altering the physical characteristics with artificial destratification. This would be achieved if increasing the mixing of the water column would reduce the average photosynthetic light dose of a given parcel of water. If the physical characteristics alone will limit the autochthonous production, then it is most likely that artificial destratification will be beneficial when the reduction in light dose is achieved by deepening the surface mixed layer. Therefore, reservoirs with significant autochthonous carbon loads may benefit from the application of artificial destratification from the perspective of natural organic matter, in addition to the reduction of algal biomass and the likely-hood of encountering a significant cyanobacterial population.

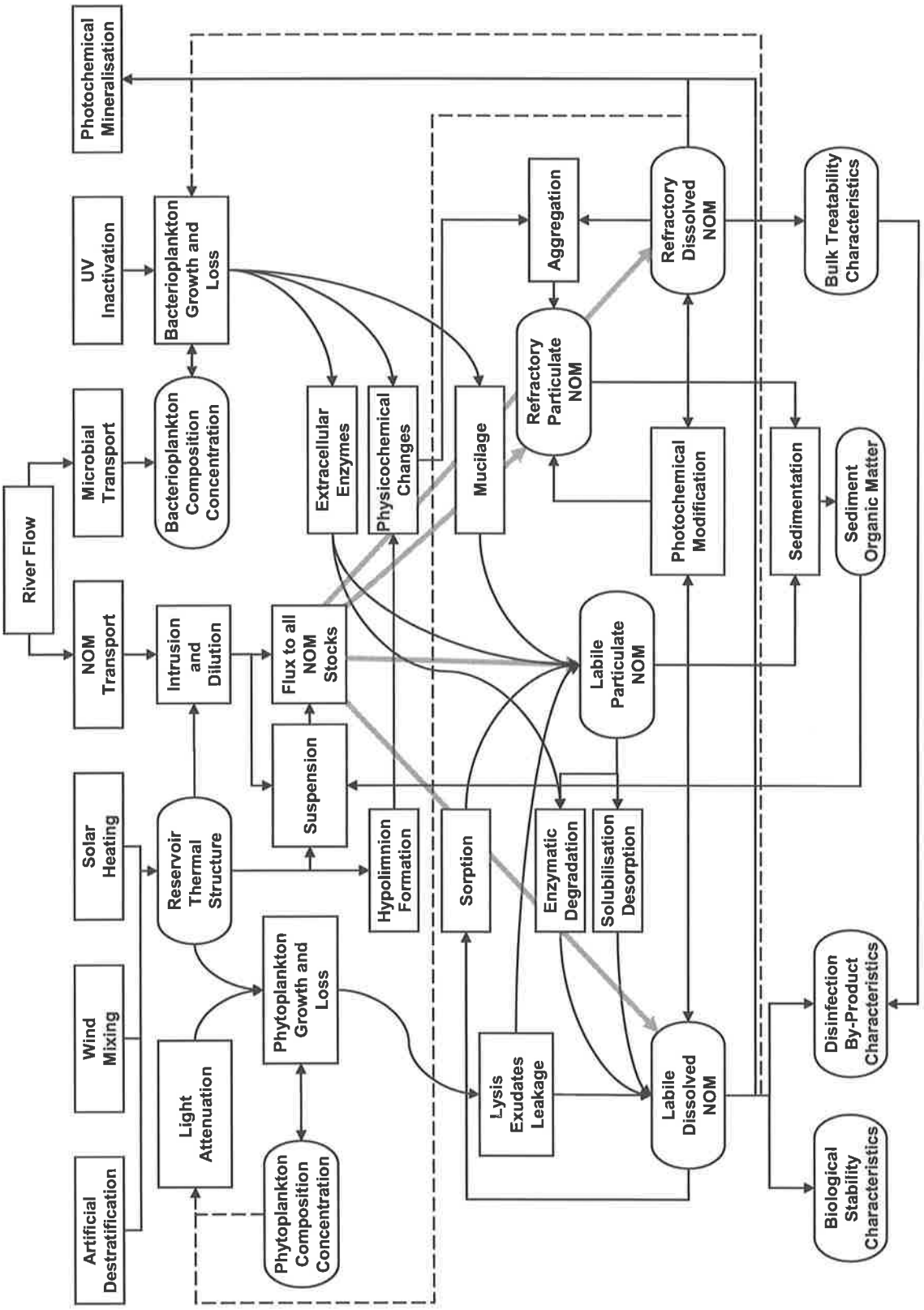

Figure 7.2. Conceptual Model of NOM dynamics. Boxes represent processes. Rounded boxes represent results or stocks. Some arrows are dashed for clarity. Grey arrows represent fluxes originating from more than one source.

It is informative to consider NOM management within a framework that considers other water quality hazards, as it places NOM management in context with whole of reservoir management. Figure 7.3 illustrates a simplified model of NOM dynamics, but with the inclusion of water quality hazards that need to be managed, such as phytoplankton, iron and manganese and pathogens. Further information on the management of these hazards can be found elsewhere (Brookes *et al.* 2002, Reynolds and Maberly 2002, Lewis *et al.* 2004, Hipsey *et al.* 2006). The management framework also presents flows of information through online monitoring that can be used to conduct risk assessment using established methods (Havelaar 1994, Szewzyk *et al.* 2000, McRae *et al.* 2001, Brookes *et al.* 2002). These risk assessments are then used to make decisions on the operation of artificial destratification, selective withdrawal and water treatment plant conditions. Monitoring programs of these four water quality hazards that provide regular yet intermittent data are also used to manage these systems. However, this does not represent true 'critical control point' management, as the frequency of the data is too sparse. These data are, however, fundamental to the process of developing an understanding of individual systems, and validating the use of the modelling framework (Brookes *et al.* 2002).

This management framework highlights the precedence of other water quality hazards over NOM. If suitable conditions for algal growth problems or release of iron and manganese occur then artificial destratification should be deployed to control these hazards despite any small benefit for NOM treat-ability. Further, if the offtake is threatened by an inflow that may transport increased concentrations of pathogens then the operational response should be made at the earliest possible time. However, given the observations of the persistence of the increase in DOC concentration made following the inflow event (Chapter 3), the decision to return to previous offtake operation should consider the NOM dynamics.

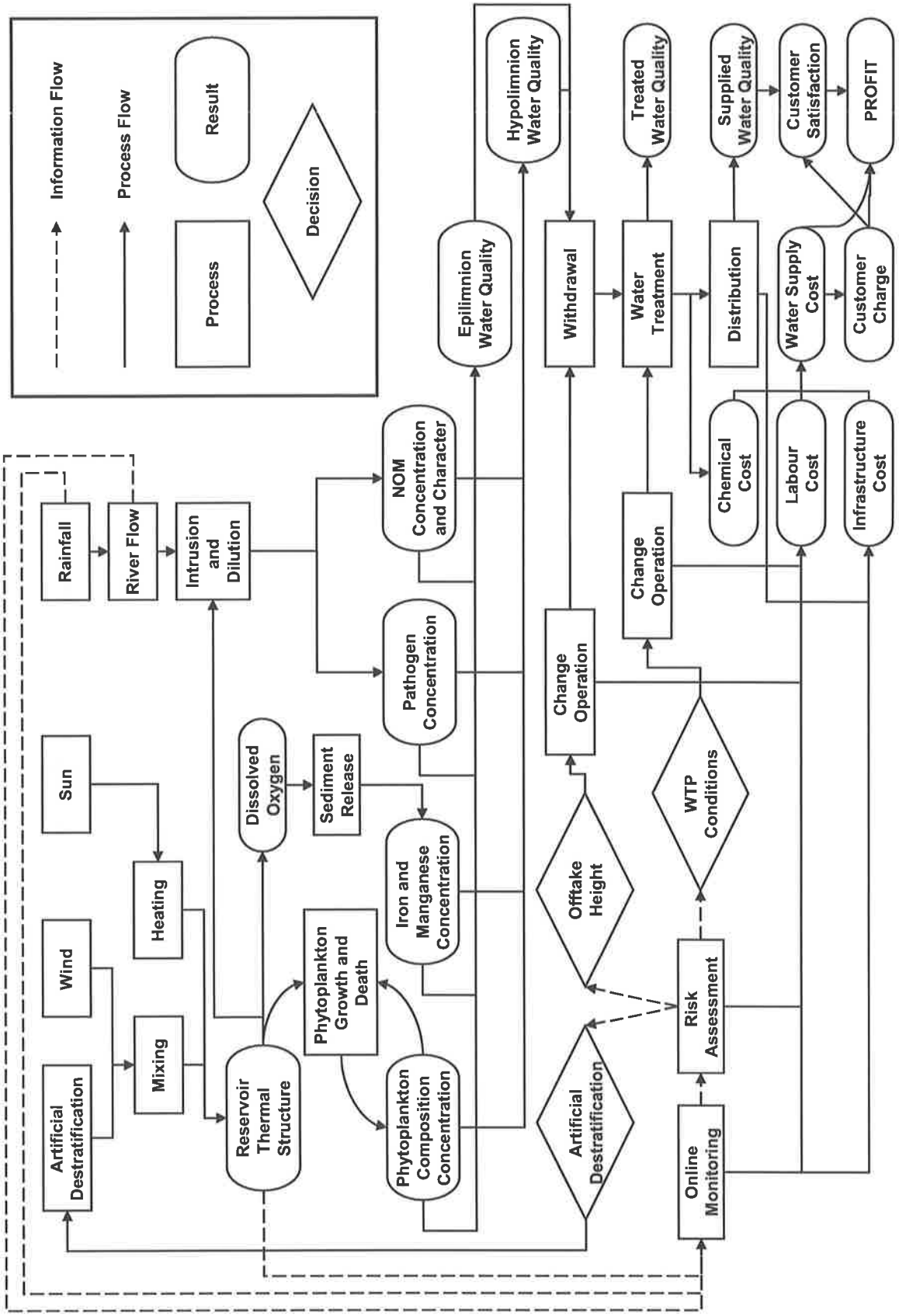

Figure 7.3. A simple reservoir water quality management framework.

A more general conceptual model of the system is presented in Figure 7.4. Here the importance of climate as a driving factor of stratification, inflow and photochemical and microbial modification of the reservoir NOM is shown. Further, the parts of the system where effective management will provide the most benefits are shown. Reduction of the allochthonous organic carbon load and the external nutrient load may be possible using catchment management, while short term increases in reservoir NOM concentration may be avoided using offtake management. Artificial destratification has the most potential to alter the autochthonous load of organic carbon by the reduction of stratification and the limitation of the internal nutrient load. Ultimately, the application of these management methods should result in improved water quality for potable water production.

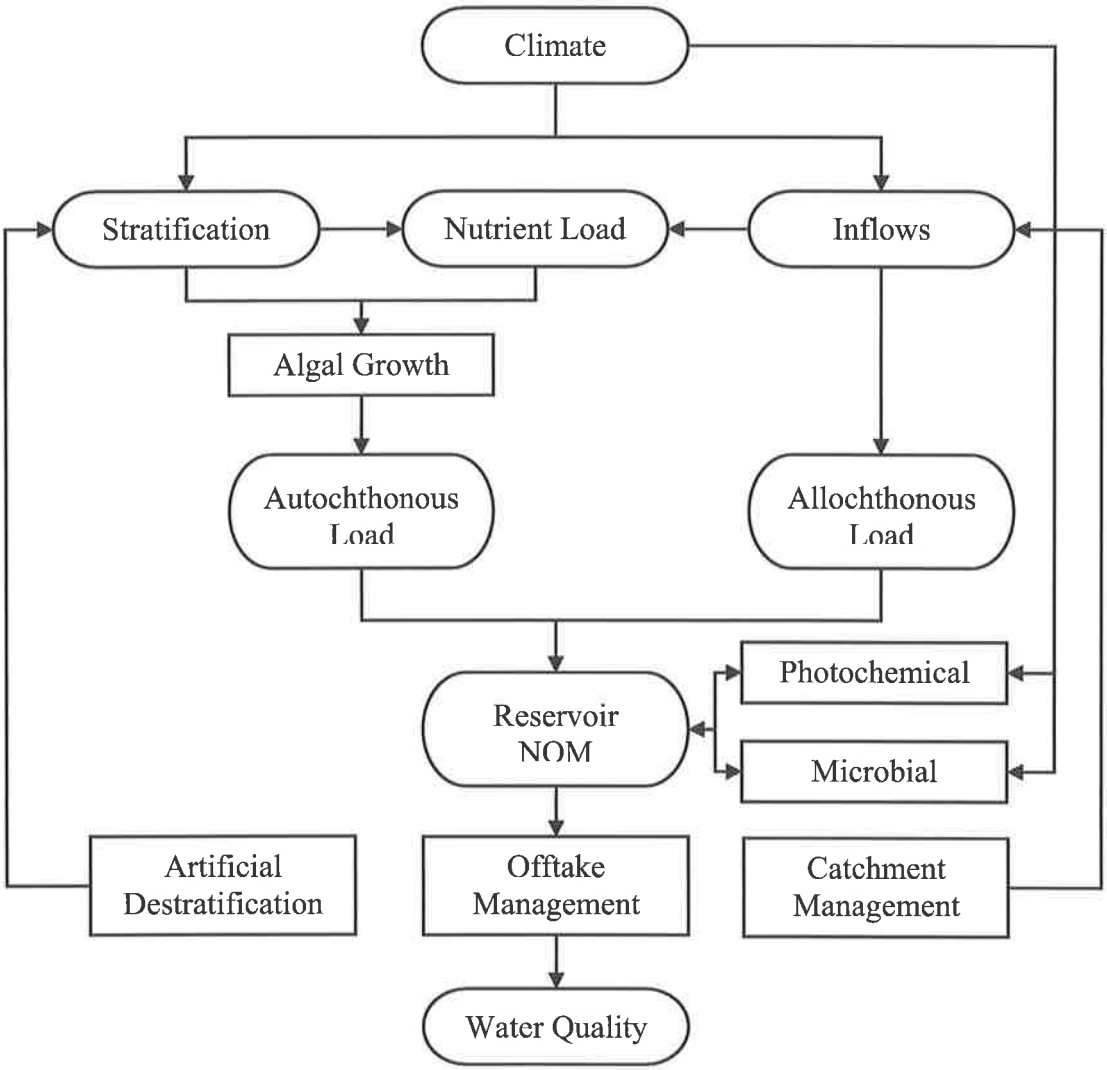

Figure 7.4. General conceptual model of the management of NOM in drinking water reservoirs.

7.5. Knowledge Gaps

This study concentrated on the effects of destratification on the character and concentration of NOM, which in turn impacts on the removal of NOM by conventional treatment processes. The effects observed on the size distribution of the organic matter have the potential to affect other water treatment methods such as membrane filtration and ion-exchange processes. Insights from the mesocosm experiment suggest that there can be dramatic shifts in the labile fraction of organic matter during periods of stratification, which may have implications for the bacterial regrowth potential (BRP) of the product

water (§6.3.3.5). No effect of destratification on the disinfection by-product (DBP) formation potential (FP) of the raw water was found, most likely as the DBP concentration is determined by the microbially refractory portion of the NOM. Systems where DBP-FP is determined by organic matter derived from algal biomass will most likely differ from these results.

7.5.1. Global Change

Climate effects on water quality of surface water systems need consideration to ensure future water quality as well as quantity. The effects of climate change on the cycles that determine organic carbon concentrations include changes in both catchment and reservoir processes. Recent predictions of climate change made for the near future suggest that increases in average temperature of 0.2 °C per decade can be expected in the short term given greenhouse gas emissions continue at current rates (IPCC 2007). Changes in precipitation and run-off from catchments are likely to occur (Chiew and McMahon 2002, Huntington 2006) and affect the management of catchment-reservoir systems through alteration of the catchment flow regime. The predictions of the increased occurrence of extreme weather events, with more intense storms, and therefore even larger runoff events (Chiew and McMahon 2002), will increase the risk of through-flow challenge of reservoir offtakes by pathogen and NOM hazards. The numerical analysis of the increase in risk is beyond the scope of this project, however predictions of potential increases of runoff of up to 15% on the east coast of Australia by 2030 have been made using hydrological models and future climate scenarios (Chiew and McMahon 2002). The delivery of greater volumes of water in fewer events will compound the risk to water quality.

Other long term effects may also occur, for example, soil organic carbon mobilization may increase due to increases in enzymatic activity at increased temperatures (Worrall and Burt 2004). Increases in organic carbon export are also attributed to increasing soil water pH which has occurred under decreased acid deposition (Evans *et al.* 2006); it is widely

accepted that increases in temperature will increase soil organic matter mobilisation (Davidson and Janssens 2006). These predicted and observed increases in soil water organic carbon concentration have the potential to result in further increases in the risk of NOM challenges through increases in the initial inflowing concentration.

These predictions of increased allochthonous organic carbon loading also have implications for the long-term trends of organic carbon concentration in drinking water reservoirs. There are some indications that the potential increases in terrestrial carbon export may be met by increased microbial and photochemical degradation in aquatic systems. A 57 % increase in the percentage of actively respiring bacteria between samples incubated at 20 °C compared to 10 °C has been observed in experimental manipulations (Rae and Vincent 1998). Further complexity is introduced by other global change factors such as ultraviolet radiation (UVR). Global stratospheric ozone depletion also has potential effects on carbon cycling in terrestrial and aquatic ecosystems (Zepp *et al.* 1998). Leavitt *et al.* (2003) conclude that many lakes may experience order of magnitude declines in algal abundance as a result of future climate, NOM and UVR interactions. If this decline in algal abundance is realised it may result in improved water quality, but will also depend on any shifts in the phytoplankton community composition. It would seem that the impact of these global factors on ecological processes and their potential influence on water quality will become more relevant to the water industry as time passes and greenhouse gas emission continues.

7.6. Conclusions

The management of the hazard presented by inflows carrying elevated concentrations of organic matter are worthy of consideration when planning reservoir offtake management strategies. It is unlikely that NOM will become the primary focus of offtake management strategies, as the threat posed by pathogens seems likely to temporally precede the increase in NOM risk. However, as NOM presents an ongoing challenge to water treatment, the

optimisation of the reservoir management strategies that influence its character and concentration deserve ongoing consideration. The monitoring of concentration-flow hysteresis effects for a single winter indicated that the potential risks were greater in the late winter due to the hydrologic and organic character parameters (catchment saturation etc); however the hydrologic characteristics are likely to be more important in determining the risk. Therefore current hydrology monitoring systems such as online rainfall and gauge height sensors should be sufficient to inform management response to large inflow events, given sufficient understanding of the NOM dynamics. Minimising the amount of NOM entering the water treatment plant, and therefore the concentration of disinfection by-product precursors, bacterial regrowth substrates and the coagulant demand, will always be beneficial from the perspective of potable water supply.

A high background concentration of microbially refractory organic matter seems to dominate the effects of stratification, and therefore destratification, on natural organic matter. This is generally the result of large external loads of allochthonous organic matter. The implication for the manager of such a reservoir is that the decision to artificially destratify will have little bearing on the character of the organic matter. However, one could propose an entirely different scenario for a reservoir that has a sufficiently high organic matter concentration which is the result of a large internal load of organic matter, for example in a highly eutrophic lake or reservoir.

If one conceptualises the organic matter pool as consisting of a microbially labile autochthonous fraction and a microbially refractory component, then the relative abundance of these differing sources in the NOM pool will determine the potential for artificial destratification to elicit a change in the overall character of the NOM pool. If the predominant fate of chromophoric, microbially refractory organic matter is photochemical, then little influence over its concentration or character can be achieved through artificial destratification. Apart from small changes in the easily removable high molecular weight

organic carbon, facilitated by pH shifts or microbial activity on phytoplankton cellular debris, the bulk character of the NOM pool is determined by the stable long lived organic matter present in the reservoir, derived predominantly from the catchment with a smaller contribution from microbially refractory autochthonous organic matter. It is therefore proposed that the potential of artificial destratification to alter water quality for potable water production from the perspective of organic matter may be realised in reservoirs with lower ratios of allochthonous to autochthonous organic matter than those investigated in this study.

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