A CASE STUDY OF THE PHYSICAL, CHEMICAL AND BIOLOGICAL FACTORS AFFECTING DISSOLVED ORGANIC CARBON IN THE WARREN RESERVOIR, SOUTH AUSTRALIA

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ANOVA	Analysis of Variance
CCA	Canonical Correspondence Analysis
C-S1	Catchment Stream 1
C-S2	Catchment Stream 2
Chl-a	Chlorophyll A
D1	Deep site 1 in the Warren Reservoir
D2	Deep site 2 in the Warren Reservoir
DGGE	Denaturing Gradient Gel Electrophoresis
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
HPSEC	High Pressure Size Exclusion Chromatography
M1	Medium depth site in the Warren Reservoir
PAR	Photosynthetically Active Radiation
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
POC	Particulate Organic Carbon
S1	Shallow site 1 in the Warren Reservoir
SUVA	Specific UV Absorbance
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
UV	Ultraviolet
UV-A	Ultraviolet-A radiation
UV-B	Ultraviolet-B radiation

DECLARATION

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.

Tanja Jankovic-Karasoulos

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ABSTRACT

The dissolved organic carbon (DOC) present in a reservoir comprises allochthonous inputs from the catchment and autochthonous inputs generated within the reservoir itself. The Warren Reservoir, South Australia, is characterised by elevated DOC concentrations and elevated water colour. Inventories and dynamics of DOC concentration and nature in the Warren Reservoir were analysed between April 1997 and August 2001, in order to establish the origins of the reservoir DOC, and determine how the DOC contribution from different sources affects water quality in the Warren Reservoir on a seasonal timescale. During the course of the study, increased DOC levels were accompanied by changes in the nature of the DOC. Between 1997 and 2001, the nature of the DOC pool changed from being dominated by the allochthonous input of coloured organics, to being dominated by the autochthonous input of DOC with lower composition of the coloured material. It is proposed that increased DOC concentrations in the Warren Reservoir and decreased levels of coloured DOC are caused by higher levels of phytoplankton growth during the later part of the study. In addition to annual increases in DOC, seasonal peaks in DOC concentration were observed during summer and winter periods. Summer peaks were found to be a result of increased phytoplankton growth, whereas winter peaks in DOC concentrations were due to increased inflow of water from the catchment into the reservoir. Winter inputs of allochthonous DOC were also found to comprise high levels of coloured organics.

During this study, emphasis was also placed on studying the potential of two natural processes, photochemical degradation (using UV-B radiation) and bacterial decomposition, to remove DOC from the surface waters of the Warren Reservoir. DOC samples were found to be both labile and refractory towards the processes of biotic and/or abiotic degradation and decomposition depending on seasonality, and thus the nature of the DOC material. Exposure of DOC to UV-B radiation resulted in enhanced DOC bioavailability, particularly in the case of allochthonous-dominated winter DOC pool, whereas the summer DOC pool showed enhanced level of direct photo-mineralisation compared to the winter DOC pool. It is proposed that the enhanced DOC bioavailability observed during this study was likely due to the UV-B induced cleavage of higher molecular weight DOC compounds into smaller units. Exposure of reservoir DOC to UV-B radiation was also found to produce changes in the structure of reservoir bacterial communities, as indicated by denaturing gradient gel

electrophoresis (DGGE) analysis of bacterial 16S rDNA sequences, suggesting that certain bacteria may be better able to utilise certain types of DOC compared to others.

As UV-B radiation, which forms a natural component of the solar radiation, is known to be harmful to aquatic organisms including bacteria, further studies were conducted to test the likely effects of UV-B radiation on bacterial communities in Warren Reservoir waters. This was done on the assumption that, although UV-B radiation increased the bioavailability of reservoir DOC, the resulting substrates might not be utilised and degraded by bacterial species in the *in situ* surface waters, as their growth can potentially be inhibited by UV-B radiation. Thus in terms of the cycling of DOC in the Warren Reservoir, it was important to study the response of native bacterial communities to UV-B radiation in order to be able to determine whether lack of bacterial activity, as a result of UV-B inhibition, is likely to cause DOC accumulation in the reservoir in the long term. The combined findings of a laboratory study and a diurnal *in situ* reservoir study suggested that UV-B radiation is unlikely to affect the overall growth of reservoir bacteria whereas it has potential to alter its community structure, most likely through its effects on the DOC cycle.

CHAPTER 1 – GENERAL INTRODUCTION

1.1 DOC AND WATER QUALITY

Dissolved organic carbon (DOC) is a ubiquitous impurity in stored raw water. DOC that survives conventional water treatment processes can be responsible for brown discolouration, odour and unpleasant taste in treated water (Cook et al., 2000; Kilmore et al., 2000). The persistence of biodegradable portions of DOC in treated water promotes bacterial growth in the water distribution system (Ribas et al., 1995; Codony et al., 2003). During chlorine disinfection DOC can be converted into trihalomethane compounds (Siddiqui et al., 1997; Kilmore et al., 2000; Bursill, 2001; Kogut and Voelker, 2001; Jack et al., 2002). These compounds are carcinogenic, and as such represent a health threat to humans and other animals (Bull and Kopfler, 1991; Magnus et al., 1999; Jack et al., 2002). The need to remove DOC has become an important additional requirement to conventional treatment processes that remove or inactivate pathogens through filtration or disinfection (Bursill, 2001).

The problems of DOC are usually addressed at the water treatment stage resulting in high treatment costs (Graveland, 1998; Bursill, 2001). An alternative and complementary approach is to manage the reservoir in a way that reduces the concentration of DOC, or more particularly, reduces those DOC components that are most difficult to remove. This approach requires an improved understanding of how the concentration and chemical nature of DOC is affected by different reservoir processes. Understanding the origin of DOC in reservoirs is also important in terms of optimising reservoir and catchment management strategies to minimise DOC input and thus water treatment costs.

1.1.1 DOC IN RESERVOIR WATER

DOC is a subset of natural organic matter (NOM), defined by Spark (1999) as the sum of organic chemical by-products of living and decaying plant and animal matter. NOM is a heterogenous mixture of compounds covering a wide molecular weight range, from a few hundred to greater than 100000 Da (Pelekani et al., 1999). NOM is usually divided into two

operationally defined fractions – DOC, the fraction that passes through a 0.45 μm filter, and particulate organic carbon (POC), the fraction retained on a 0.45 μm filter (LeCren and Lowe-McConnell, 1977).

The majority of organic carbon in most aquatic ecosystems exists in the form of DOC (Kaplan et al., 1980; Wetzel, 1983; Hobbie, 1992; Mann and Wetzel, 1995; Hoss et al., 2001). Globally, the concentration of DOC varies from less than 1 to greater than 40 mg/L in freshwaters (Pace and Cole, 2002), but are generally within the range of 1-20 mg/L (Moss, 1988). Lakes with low DOC concentrations usually have high water clarity while high-DOC lakes have brown water because of the high concentrations of light-absorbing humic and fulvic acids (Pace and Cole, 2002).

The DOC fraction is itself heterogeneous. In particular, different DOC components can be labile or refractory towards various processes of biotic and abiotic degradation and decomposition. Kirchman et al. (1993) distinguishes three primary classes of bulk DOC based on its rate of degradation: (1) labile DOC with turnover times of hours to days, (2) semi-labile DOC that turns over on seasonal to annual time scales, and (3) non-labile DOC that cycles on time scales of hundreds to thousands of years. Fisher et al. (1998) report that up to 35% of DOC is labile on time scales of days to weeks in both freshwater and marine ecosystems. The refractory nature of DOC is often attributed to the presence of humic substances (Drakare et al., 2002). Humic substances (humic acids, fulvic acids and humin) are coloured and acidic, and mostly refractory to bacterial degradation (Blomqvist et al., 2001; Hoss et al., 2001; Klug, 2002), with less than 15% considered to be directly available to biota (Blomqvist et al., 2001). Humic substances are generally classified on the basis of alkali/acid extraction as shown in Figure 1.1 (Malcolm, 1991). They are believed to be complex polymers of short-chain acids, polycyclic aromatics, and some carbohydrates and amino acids (Moore et al., 2003; Rosenstock and Simon, 2003). Relative proportions of amino acids and carbohydrates associated with dissolved humic substances have only been determined in polar marine environments and one lotic system, indicating that amino acids constitute <3% and carbohydrates around 17% of the humic fraction of DOC (Rosenstock and Simon, 2003). The non-humic fraction of DOC includes carbohydrates, proteins, peptides, amino acids, fats and other low molecular weight biomolecules, which are generally labile and easily degraded by bacteria (Strom et al., 1997; Fisher et al., 1998; Mannino and Harvey, 2000).

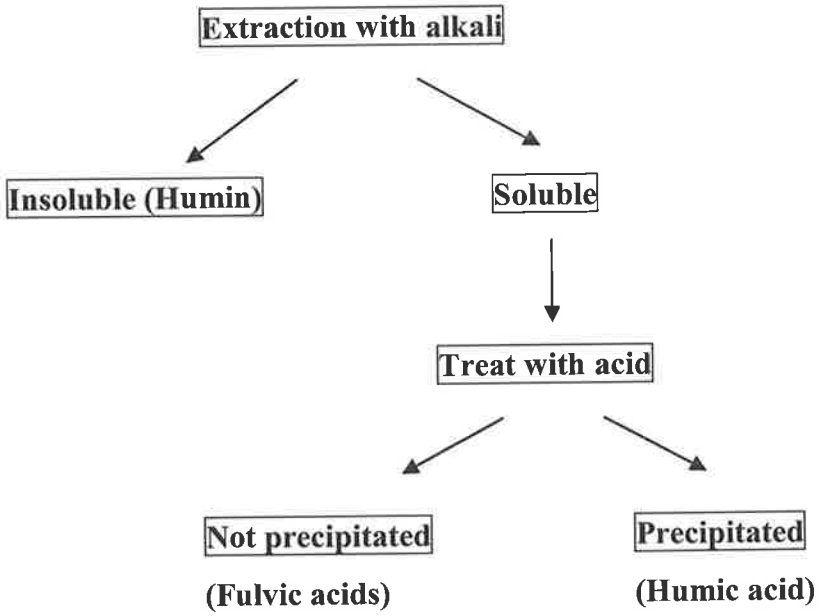


Figure 1. 1: Summary of fractionation of humic material (modified from Malcolm 1991).

1.1.2 SOURCES OF DOC AND ITS FATE INSIDE RESERVOIRS

DOC in water is ultimately derived from terrestrial and aquatic primary producers (V-Balogh and Voros, 1997; Fisher et al., 1998). Labile DOC originates primarily from autochthonous (internal) primary production while the refractory DOC fraction generally originates from allochthonous (external) sources (Wetzel, 1983; Lindell et al., 1995). Once within the reservoir, DOC can regulate ecosystem structure by affecting the chemical and physical characteristics of lakes (Mann and Wetzel, 1995; Vinebrooke and Leavitt, 1998). For example, DOC can bind nutrients, making them unavailable to aquatic communities (Klug, 2002). It can reduce underwater spectral irradiance thus limiting the photosynthetically active radiation (PAR) required by primary producers (V-Balogh and Voros, 1997; Carpenter et al., 1998). It can also act as a source of labile substrate for heterotrophic bacteria and subsequently higher trophic levels (Hobbie, 1992; Hart et al., 2000). The nature and thus origin of DOC is what ultimately determines its fate within reservoirs.

1.1.2.1 Autochthonous DOC

Autochthonous DOC comes from aquatic organisms. Phytoplankton organisms are capable of photosynthesis and are responsible for the significant portion of primary production (synthesis of autochthonous organic matter) in lakes. As such autochthonous DOC can be released from living phytoplankton cells or from lysis or degradation of recently dead planktonic organisms (Hessen, 1992). When algal cells grow and die, some portion of their photosynthetic material is released as dissolved matter. This release may be due to autolysis, lysis by intra- or extra-cellular algal parasites, mechanical breakage by zooplankton, or active excretion of metabolites (Cole et al., 1982; Strom et al., 1997; Middelboe and Lyck, 2002). Figure 1.2 shows a general view of sources and cycling of organic material in pelagic ecosystems derived from Engel and Macko (1993).

The release of DOC by phytoplankton has long been recognised as an important source of high quality carbon (in terms of bioavailability) to bacteria (Baines and Pace, 1991). In clear-water systems, phytoplankton account for most of the carbon production that later, via different processes including exudation, autolysis and grazing, becomes available for bacterial growth (Blomqvist et al., 2001). In such systems, bacterial growth (secondary production) correlates well with rates of phytoplankton primary production and thus autochthonous DOC input (Cole et al., 1982; Hessen 1992; Coveney and Wetzel, 1995; DiSiervi et al., 1995; V-Balogh and Voros, 1997; Findlay et al., 1998). In general, 40-60% of autochthonous primary production is cycled through bacteria (Mannino and Harvey, 2000). Thus, the input from primary production is believed to explain much of the variation in bacterial production in clear-water ecosystems (V-Balogh and Voros, 1997). This is in contrast to humic lakes, where bacteria have access to an allochthonous carbon source that makes them no longer dependent on carbon generated by primary producers (Jansson et al., 1999). The intensity of the coupling of phytoplankton primary production (and ultimate release of internal DOC into reservoirs) with bacterial growth and productivity is shown to vary across trophic gradients, with strongest correlations made in oligotrophic lakes (Lind et al., 1997).

In terms of chemistry, the DOC produced by aquatic phytoplankton is mostly aliphatic and totally devoid of lignin compounds which are a major constituent of the cell walls of vascular plants (Hedges and Ertel, 1982; Goel et al., 1995). Autochthonous input is thus low

in phenolic and aromatic constituents and comprises material such as amino acids, sugars, urea, amino sugars and polypeptides (Moss, 1988). Furthermore, since algae lack cellulosic supportive structures, they have elemental compositions that typically have C/N ratios in the range of 4 to 10 (Engel and Macko, 1993). Conversely, plants having cellulose, such as those of terrestrial origin (grasses, shrubs and trees), contain proportionally less nitrogen and their C/N ratios range from 20 to 80.

Studies of freshwater lakes suggest turnover times for these labile dissolved organic molecules to be only a few minutes or hours for the simple sugars and carboxylic acids (Moss, 1988). Although most labile organics are recycled within the water column, some of the dissolved organics are adsorbed onto clay and other suspended mineral particles, and are transported to the sediment (Killops and Killops, 1993). The amount of organic material reaching the sediment is dependent on the depth of water (ie: the deeper the water, the longer the residence time and thus the exposure to degradation) as well as the amount of primary production in the photic zone.

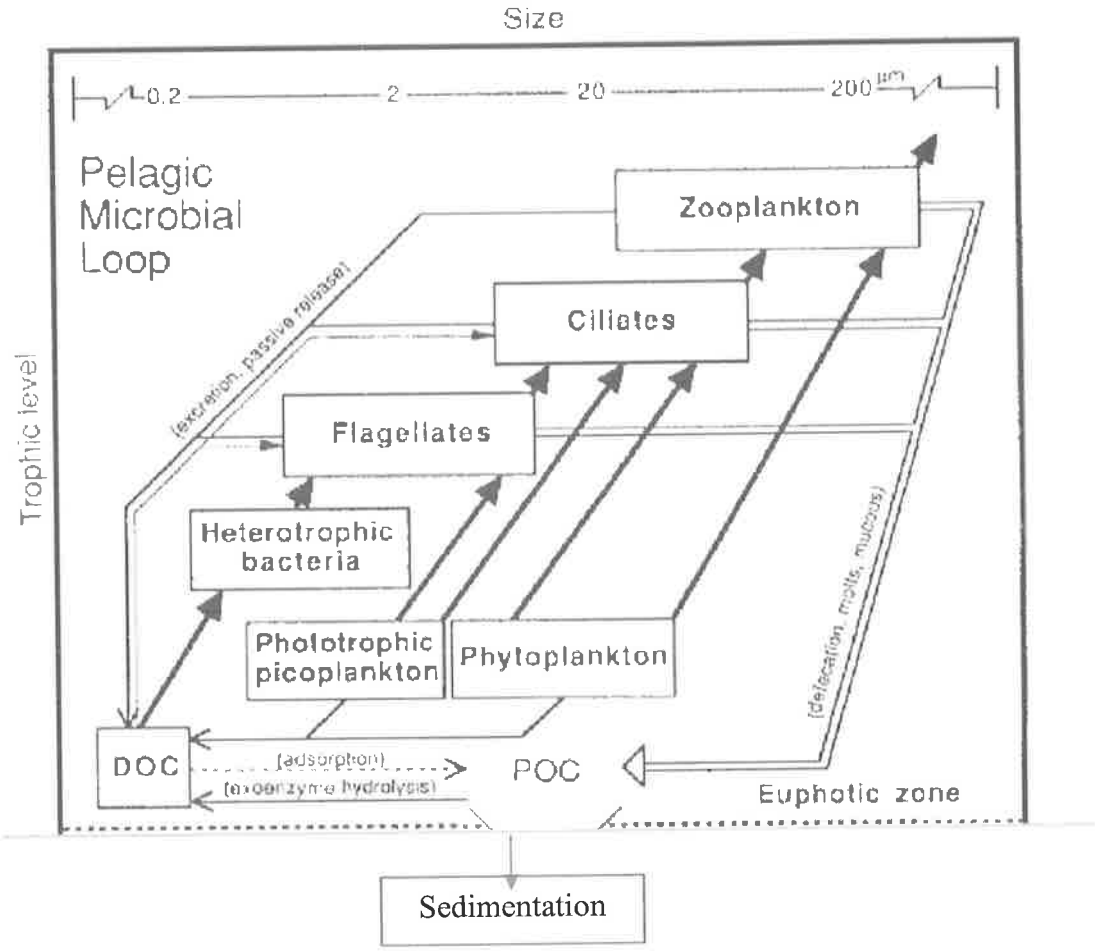


Figure 1. 2: A general description of autochthonous sources and cycling of DOC within surface waters (Engel and Macko, 1993).

1.1.2.2 Allochthonous DOC

Allochthonous sources of DOC include transport of decomposing plant material and soil organic matter transported to reservoirs from catchments (Moore et al., 2003). DOC enters reservoirs through surface runoff, stream inflow and groundwater seepage (Robertson et al., 1999; Spark, 1999; Moore et al., 2003). The nature of the reservoir catchment (physical shape and size, geographic location, rainfall events, vegetative cover etc) impacts on the quantity and nature of the DOC transported to the reservoir. Kaplan et al. (1980) state that, in general, most DOC export from catchments occurs during high-rainfall events, yet low flow conditions prevail about 75% of the year and represent periods of the greatest processing of

DOC. The amount and nature of allochthonous DOC transported from catchments to reservoirs can vary significantly with seasonal changes (Mann and Wetzel, 1995; Spark, 1998). Pace and Cole (2002) show temporal dynamics of DOC to be most likely the result of climatic conditions that affect loading of allochthonous carbon.

In some freshwater lakes, significant loadings of DOC are shown to originate from catchments. For example, in northern European lakes and reservoirs, high DOC concentrations are associated with high allochthonous DOC inputs. Such catchments often comprise a large proportion of peatland (Mattsson and Kortelainen, 1998; Kaplan et al., 1980). Hope et al. (1997) also showed a positive correlation between peat cover and annual export of carbon. Although present in high quantities, DOC of terrestrial origin (comprising mainly humic substances) can be regarded as a qualitatively deficient substrate for bacterial growth, not only because of its recalcitrant nature, but also because of its low content of phosphorus and nitrogen (Hessen, 1992). However, a study of a Swedish humic lake by Jonsson et al. (2001) showed that allochthonous organic carbon can act as a principal substrate for bacterial growth in comparison to autochthonous carbon which contributed as little as 20% of total lake mineralisation. In their study, the importance of autochthonous organic carbon was restricted to the epilimnion and was greatest during a phytoplankton growth maxima in summer (period concurrent with low water inflow). In another study of a humic lake, Hessen (1992) showed allochthonous matter as the main source of carbon for bacteria, accounting for almost 90% of the carbon required to support bacterial growth. Bergstrom and Jansson (2000) also stated that bacterial production may be almost entirely dependent on the utilisation of allochthonous DOC. The authors studied bacterial production in a humic Swedish lake, and showed that bacterial production in the lake varied seasonally. Bacterial production was stimulated by high flow episodes during spring and summer, which brought high amounts of little degraded material into the lake. However, during base flow conditions in autumn (reflecting higher riverine retention times, longer exposures to solar radiation and thus higher bacterial production in the rivers), the allochthonous DOC entering the lake did not stimulate lake bacterial production. This was partly because the input of allochthonous DOC was small relative to the DOC content of lake water and partly because the inflowing DOC during a base flow period was quite degraded before reaching the lake. Thus catchment retention time can play an important role in determining bioavailability and thus the fate of allochthonous DOC in freshwater systems.

The type of vegetation growing in catchments can also influence the quantity, composition and reactivity of DOC in soil solutions leading into reservoirs. Findlay et al. (2001) showed that different land uses in New Zealand catchments (pasture, native forest and pine plantations) affect the quantity and, in particular the nature and bioavailability of DOC moving from terrestrial ecosystems into streams. Vesterdal and Raulund-Rasmussen (1998) showed that coniferous trees generally accumulate more carbon on the forest floor than deciduous trees do. Strobel et al. (2001) showed that different tree species (beech, oak, grand fir and Norway spruce) produce varying quantities of DOC, and litter with different chemical composition and degradability. For example, Norway spruce produced highest DOC concentrations, while grand fir DOC comprised fewer phenolic groups when compared to the remaining three tree species. Baker et al. (2001) also showed that the type of vegetation can affect the nature of DOC. Giardina et al. (2001) compared organic carbon mineralisation from soils under pine and aspen. The authors found that pine soils generated a higher quality organic carbon (in terms of bioavailability) and produced 50% more organic carbon than aspen soils.

On the other hand, some studies focusing on chemical rather than biological properties of DOC, have found minimal differences across a wide range of different catchments. For example, Mattsson and Kortelainen (1998) studied 17 Finish lakes, 6 Finnish streams and 30 Maine (USA) lakes, by fractionating DOC into hydrophobic acids and neutrals, and hydrophilic acids, bases and neutrals. They found that in every sample organic acids dominated the DOC pool (77-85% of total DOC). Estimations of relative proportions of various DOC fractions can be used to indicate the importance or dominance of allochthonous DOC in aquatic systems. For example, changes in the relative abundance of aromatic and aliphatic carbons can be used to indicate changes in the importance of terrestrial sources of DOC relative to total reservoir DOC (Page et al., 2000).

In terms of chemistry, allochthonous DOC comprises a large number of compounds ranging from low-molecular weight aliphatic carboxylic acids to macromolecular fulvic and humic acids. The humic fractions dominate, accounting for more than 90% of allochthonous DOC (Strobel et al., 2001). Thus, allochthonous DOC is the primary source of aquatic humic substances (Mattsson and Kortelainen, 1998). Allochthonous organic matter contains a high lignin component (Goel et al., 1995). Lignin compounds are high molecular weight phenolic polymers that occur in the woody tissues of vascular plants and tend to be concentrated in soil and sedimentary organic matter (Hedges and Ertel, 1982). Decomposition of lignin produces

complex aromatic molecules containing acidic phenol and carboxylic functional groups (Moss, 1988). These degraded substances remain as recalcitrant structures that persist for long periods in catchments as their complexity hinders their decomposition. Thus, although undergoing many chemical transformations en route to the reservoir, allochthonous DOC often arrives as complex and refractory material not easily assimilated by reservoir bacteria (Hobbie 1988; Lindell et al., 1995).

In summary, the prevailing importance of allochthonous and autochthonous sources in freshwater systems appears to be system specific. In eutrophic lakes most organic carbon is likely supplied by primary production of the lake, while in humic lakes the input of allochthonous DOC is considerably higher, and it can replace autochthonous carbon as the dominant energy source (Jonsson et al., 2001).

1.1.3 DOC TRANSFORMATIONS AND REMOVAL FROM RESERVOIRS

Microbial mineralisation and photochemical (in particular UV-B) degradation are two processes reported to have significant effects on DOC in reservoir water (Lindell et al., 1995; Miller and Moran, 1997; Moran and Zepp, 1997; Benner and Biddanda, 1998; Andrews et al., 2000; Bertilsson and Tranvik, 2000; Moran et al., 2000).

1.1.3.1 Impacts of Bacteria on DOC cycling

In aquatic ecosystems, bacteria are the most abundant decomposers and play a key role in the transformation of organic matter and the re-mineralisation of nutrients (Lemee et al., 2002; Muylaert et al., 2002). Bacteria are able to metabolise dissolved organic material originating from both allochthonous (Kaplan et al., 1980; DelGiorgio et al., 1997) and autochthonous sources (Cole et al., 1982; Hobbie, 1992), and repackage this highly dilute organic carbon into concentrated nutrient-rich bacterial biomass that can be assimilated by higher organisms (Jordan and Likens, 1980; Murray and Hodson, 1985; Sherr and Sherr, 1988). Thus, bacteria contribute to carbon cycling in two major ways: (1) by producing new

biomass (bacterial secondary production) and (2) by re-mineralising organic carbon (bacterial respiration) (Lemee et al., 2002).

While the role of bacteria in energy cycling and organic decomposition is well established, technical problems have restricted detailed analysis of interactions between bacteria and DOC in lakes and reservoirs. Difficulties exist in measuring the origin and chemical composition of the DOC, growing the heterotrophic bacteria responsible for the decomposition of DOC and determining the parameters and mechanisms that control this process. Despite these difficulties, it is clear that DOC is the major source of organic carbon and energy in lakes, and that bacteria are the most abundant and important degrader organisms (Hobbie, 1992). As such, they form a fundamental component of the organic carbon cycle in aquatic ecosystems (DelGiorgio et al., 1997). They remineralise the organic material on a large scale, as they are able (under the appropriate conditions) to break down virtually all natural organic compounds and pollutants (Gunnison et al., 1985; Sherr and Sherr, 1988; Lindell et al., 1995). Bacteria are capable of breaking down protein, polysaccharide and starch at a faster rate than cellulose and lignin (Wetzel, 1983; Miller and Moran, 1997; Gunnison et al., 1985). During the mineralisation process, nutrients are returned to the water body, allowing new plant growth and new processes to continue.

Sun et al. (1997) state that only a fraction of the dissolved organic matter present in natural waters supports aquatic bacterial growth, and that this fraction varies from <1% to >75% across aquatic systems. Differences in the amount of the bioavailable organic carbon across aquatic systems can generate broad-scale patterns of bacterial productivity observed in different ecosystems (Findlay et al., 1998). Jordan and Likens (1980) measured the rates of planktonic bacterial production in an oligotrophic lake in the range of 3 to 8 $gC/m^3/yr$. They showed that the annual carbon flux through planktonic bacteria represented 11-31% of the total autochthonous plus allochthonous organic carbon inputs in the lake.

Eiler et al. (2003) examined the impact of carbon source concentration on bacterial growth, biomass and community composition. The authors showed that bacterial biomass increased linearly with the DOC concentration, and the bacterial growth rate in the exponential growth phase exhibited a hyperbolic response to the DOC concentration, suggesting that the maximum growth rate was constrained by the substrate concentration at low DOC concentrations. Composition of microbial communities also showed a gradual change along the quantitative DOC gradient. Rosenstock and Simon (2003) also showed that bacterial consumption of DOC is mainly a function of substrate concentration. These authors

studied bacterial decomposition of humic and non-humic dissolved amino acids and carbohydrates, and those that were extracted in low (<3kDa) and high (>3kDa) molecular weight fractions. They showed that bacterial consumption of dissolved amino acids and dissolved carbohydrates was mainly a function of the initial concentrations of these materials, irrespective of the molecular weight and whether they are bound to humic substances or not.

Although the concentration of bioavailable DOC has been shown to be an important factor in supporting bacterial productivity, the mineralisation of DOC by bacteria also depends on the nature and composition of the organic matter and thus the ratio at which the various sources (autochthonous or allochthonous) contribute to it (Coffin et al., 1993; Muylaert et al., 2002). For example, Findlay et al. (1998) state that benthic bacterial abundance and growth are correlated to inputs of allochthonous organic matter. On the other hand, planktonic bacterial productivity generally correlates well with phytoplankton growth and rates of primary production, where DOC produced by phytoplankton primary production acts as a good source of energy for bacterial growth (Cole et al. 1982; Coveney and Wetzel 1995; DiSiervi et al. 1995; V-Balogh and Voros, 1997). Depending on the trophic state and season, bacteria have been shown to process from 20% to more than 60% of the organic carbon fixed by phytoplankton primary production (Zwisler et al., 2003). In one study, Romani and Sabater (2000) monitored the effects of environmental factors on spatial and temporal variations in heterotrophic activity in three Mediterranean streams. They found algal biomass to be an important factor accounting for the variability in extracellular enzyme activity and heterotrophic activity among streams. The authors state that this relationship is likely due to algae being an important source of labile DOC for heterotrophic bacteria. Crump et al. (2003) studied seasonal changes in bacterial populations in an arctic lake. The authors reported two shifts in bacterial communities that were associated with peaks in bacterial productivity. The first shift was driven by a large influx of labile terrestrial DOC, while the second shift occurred after the development of the phytoplankton community. The authors further showed some bacterial species to be present throughout the year while others were transient and varied according to the source of DOC. Hessen (1992) further showed the importance of allochthonous organic carbon in bacterial production and respiration in a humic lake. The author showed allochthonous DOC to be the main source of carbon for bacteria, accounting for almost 90% of the carbon required to support observed bacterial growth.

Different species of bacteria have different abilities to utilise different fractions of DOC (Lindstrom, 2000). For example, Fandino et al. (2001) analysed the relationship

between bacterial species composition and carbon metabolism during a marine algal bloom. They showed distinct differences between free and attached bacterial communities. Weinbauer and Hofle (1998) studied the distribution and life strategies of two bacterial species in a eutrophic lake, and found differences in their ability to utilise different fractions of DOC. The abundance and growth rate of one species was found to be higher when grown in the presence of unfractionated DOC, whereas the other species thrived on a low molecular weight fraction of the same DOC. Riemann and Winding (2001) studied bacterial community dynamics during an induced algal boom in two freshwater mesocosms by comparing the diversity and succession among free-living and particle-associated bacteria. They found changes in both bacterial populations during the course of the bloom, suggesting that bacterial community structure is dependent on the nature of the organic matter. Van Hannen et al. (1999) studied the development of a bacterial community structure growing on detritus of a green algae and a cyanobacterial species. The authors showed that the origin of detritus could affect the structure of bacterial communities: following 7 days of incubation, a clear divergence occurred in bacterial communities growing on different types of detritus.

Amon et al. (2001) studied bacterial growth and the changes in chemical composition of DOC during a 10-day decomposition experiment with fresh, algal-derived DOC. The initial composition of DOC was characterised by high neutral sugar (14% DOC) and amino acid (7.4% DOC) yields and the dominance of glucose (~75 mol%) and glutamic acid (~25 mol%). During microbial degradation, the neutral sugar and amino acid yields decreased from 14% DOC to <5% DOC and from 7.4% DOC to <3% DOC respectively, and the molecular composition of DOC became more uniform. This indicated that bacteria were capable of altering the chemical composition of marine DOC by selectively removing bioavailable components (eg: neutral sugar carbons and amino acids) and by leaving behind bio-refractory components. Amon and Benner (1996) studied total bacterial community utilisation of high molecular weight (HMW) DOC (>1kDa) and low molecular weight (LMW) DOC (<1kDa) fractions in freshwater and marine systems. They found higher rates (up to 4 fold) of bacterial growth and respiration in HMW than in LMW DOC fractions, in both freshwater and marine systems. This study suggested that the bioavailability of organic matter decreases along a continuum of size (from large to small) and diagenic state (from fresh to old). A study by Sun et al. (1997) further supports this diagenic-reactivity continuum model. These authors studied temporal relationships between the bioavailability of DOC and its bulk chemical composition and found fresh DOC in particulate organic matter leachates to

be more bioavailable than the riverine DOC. They also found that bioavailability of DOC was closely related to the percentage of aliphatic carbon in a sample and that the riverine decreases in bioavailability were mainly attributable to selective degradation of aliphatic carbon.

Thus bacterial mineralisation of DOC among aquatic systems appears to be affected by both organic carbon availability and the nature and composition of the organic material, which is generally determined by the various sources (autochthonous or allochthonous).

1.1.3.2 Impact of UV-B Radiation on DOC Chemistry

Solar radiation in the UV-B range represents less than 1% of total energy reaching the earth's surface (Chatila et al., 2001), yet it can have a profound effect on bacterial communities and DOC cycling (Frederick and Lubin, 1998). UV-B radiation has the potential to alter the chemistry of DOC and thus impact on its subsequent microbial mineralisation in water bodies. UV-B radiation can either increase or decrease DOC bioavailability. It can increase rates of DOC mineralisation by converting larger DOC molecules into dissolved inorganic carbon (DIC) and smaller organic photoproducts that are more susceptible to microbial degradation (Wilson et al., 1970; Lindell et al., 1995; Wetzel, 1995; Miller and Moran, 1997; Benner and Biddanda, 1998; Graneli et al., 1998; Andrews et al., 2000; Bertilsson and Tranvik, 2000; Moran et al., 2000). Seventeen biologically available photoproducts were detailed by Moran and Zepp (1997). These products are shown in Table 1.1. Conversely, the exposure of DOC to solar and/or UV-B radiation can also result in reduced DOC bioavailability, and thus decreased microbial activity (Benner and Biddanda, 1998; Tranvik and Kokalj, 1998; Obernosterer et al., 1999; Waiser and Robarts, 2000).

Miller et al. (2002) estimated the daily production of biologically labile photoproducts in continental shelf surface water to be 3.2μmol C/L/day. The authors also showed that the conversion of DOM to biologically labile photoproducts in these waters was about 13-fold greater than the mineralisation to DIC. This ratio changed with depth to 15:1 at 2m and 16:1 at 4m. The rate of production of biologically labile photoproducts also decreased with increasing water depth.

Table 1. 1: Biologically available compounds formed via photochemical processes from aquatic DOM (Moran and Zepp, 1997).

Compound	MW	Structure	Reference
Low MW Organics			
Acetaldehyde	44	$H_3C\text{-}C(=O)\text{-}H$	Mopper and Stahovec 1986 Mopper et al. 1991 Kieber et al. 1990
Acetate	59	$H_3C\text{-}C(=O)\text{-}O^-$	Wetzel et al. 1995 Dahlen et al. 1995
Acetone	58	$H_3C\text{-}C(=O)\text{-}CH_3$	Mopper and Stahovec 1986
Citrate	189	$^-O\text{-}C(=O)\text{-}CH_2\text{-}C(OH)(C(=O)O^-)\text{-}CH_2\text{-}C(=O)\text{-}O^-$	Wetzel et al. 1995
Formaldehyde	30	$H_2C=O$	Mopper and Stahovec 1986 Mopper et al. 1991 Kieber et al. 1990
Formate	45	$HC(=O)\text{-}O^-$	Wetzel et al. 1995 Dahlen et al. 1996
Glyoxal	58	$O=CH\text{-}CH=O$	Mopper and Stahovec 1986 Mopper et al. 1991
Glyoxalate	73	$O=CH\text{-}C(=O)\text{-}O^-$	Kieber and Mopper 1987 Mopper et al. 1991 Kieber et al. 1990
Levulinate	115	$H_3C\text{-}C(=O)\text{-}CH_2\text{-}CH_2\text{-}C(=O)\text{-}O^-$	Wetzel et al. 1995
Malonate	102	$^-O\text{-}C(=O)\text{-}CH_2\text{-}C(=O)\text{-}O^-$	Dahlen et al. 1996
Methylglyoxal	72	$O=CH\text{-}C(=O)\text{-}CH_3$	Mopper and Stahovec 1986
Oxalate	88	$^-O\text{-}C(=O)\text{-}C(=O)\text{-}O^-$	Dahlen et al. 1996
Propanal	59	$H_3C\text{-}CH_2\text{-}CH=O$	Mopper and Stahovec 1986
Pyruvate	87	$H_3C\text{-}C(=O)\text{-}C(=O)\text{-}O^-$	Kieber and Mopper 1987 Mopper et al. 1991 Kieber et al. 1990 Wetzel et al. 1995
Carbon Gases			
Carbon Monoxide	28	CO	Mopper et al. 1991 Jones 1991 Valentine and Zepp 1993 Schmidt and Conrad 1993 Miller and Zepp 1995
Nutrients			
Ammonium	17	NH_4^+	Bushaw et al. 1996
Phosphate	95	PO_4^{3-}	Francko and Heath 1982

The rate of photochemical mineralisation of DOC depends on highly variable local conditions like solar irradiance, depth, absorption of water and concentration and nature of DOC (Vahatalo et al., 2000). A study by Scully et al. (1997) compared the rates of photochemical mineralisation products in surface waters of clear-water and humic aquatic systems. The authors found that clear-water lakes are likely to experience a greater photochemical response (in percentage terms) to increased UV-B radiation than coloured lakes. They reported 70% increases in mineralisation rates near the surface in the clear lakes, compared with 25% increases in coloured aquatic systems. They further showed that formation of photochemical mineralisation products in humic stained lakes is restricted to the surface (0-0.5m) of such lakes. On the other hand, a study of 38 Swedish lakes by Bertilsson and Tranvik (2000) showed that DOC from oligotrophic humic lakes was more easily photomineralised than DOC from eutrophic lakes with high algal production. A study by Graneli et al. (1998) measured the rates of DOC photo-mineralisation in 4 tropical (Brazilian) and 5 temperate (Swedish) lakes, each region comprising a range of clear water to highly humic systems. The authors found that DOC photo-mineralisation was positively related to the DOC concentration but the DOC from tropical and temperate freshwaters did not seem to differ with respect to sensitivity to photooxidation. The authors further showed that UV-B radiation had a smaller part in photo-oxidation when compared to UV-A radiation and photosynthetically active radiation (PAR), accounting for only 17% of total dissolved inorganic carbon (DIC) production. The rates of DIC production varied from 0.09 to 1.7 mg C/L for the 6 hour incubations.

Lindell et al. (1995) studied the ability of UV-B radiation to increase the availability of dissolved organic matter to bacteria in surface waters of a coloured humic lake. Although the authors did not report losses in DOC concentration as a result of photo-degradation, they found increased bacterial numbers (65%) and cell volumes (360%) with increasing exposure of DOC to UV-B, resulting in an almost sixfold increase in bacterial biomass. The authors propose that the enhanced bioavailability of natural lake DOC to bacteria was most likely due to the cleavage of macromolecules into smaller units. Miller and Moran (1997) studied the interaction between photochemical and biological processes in the degradation of marine DOC rich in humic substances. They reported DOC losses from carbon gas formation (caused by direct photo-mineralisation) and microbial consumption of labile photoproducts to be approximately equal, suggesting that both processes may be important in the degradation of refractory DOC in coastal systems. Similar observations were made by De Lange et al.

(2003), where UV-B irradiation of DOC generated losses in DOC of up to 25% and increases in bacterial abundance of up to 35%. Moran et al. (2000) studied long-term photochemical and biological degradation of estuarine DOC, dominated by terrestrial plant-derived organic matter. They showed that a significant fraction of the terrestrially derived DOC pool was photo-degraded during sunlight exposures. The authors reported losses in DOC concentrations greater than 30%, and showed coloured organic fraction to be most prone do photo-degradation. They further showed positive net effects on biological degradation, with approximately two thirds of the photochemically-derived DOC removed by bacteria. Opsahl and Benner (1998) studied photodegradation of dissolved organic matter, in particular, they examined photochemical reactivity of dissolved lignin in riverine and open-ocean water samples. The authors showed high photochemical reactivity of dissolved lignin in riverine and marine water samples, where approximately 75% of the total dissolved lignin is lost as a result of photooxidation during 28 days of solar exposure. Furthermore, prior to exposure to sunlight, approximately 90% of dissolved lignin in river water was present as high molecular weight (>1000 Dalton) DOM. Following solar exposure, 80% of the remaining dissolved lignin was present as low molecular weight (<1000 Dalton) DOM. The increase in concentration of low molecular weight lignin provided direct evidence for the photo-transformation of macromolecular DOC to smaller molecules.

The exposure of DOC to solar and/or UV-B radiation can also have the opposite effects and result in reduced DOC bioavailability (Benner and Biddanda, 1998; Tranvik, and Kokalj, 1998; Obernosterer et al., 1999; Waiser and Robarts, 2000). Obernosterer et al. (1999) studied the effects of ultraviolet radiation on the bioavailability of DOC to marine bacteria in two different seas. They showed reduced bioavailability of DOM following exposure to solar radiation in waters where the bacterial activity : DOC concentration ratio is high (indicative of labile DOC). The authors reported up to 50% reduction in bacterial activity in the irradiated treatments compared with the dark controls. On the other hand they also reported increases in DOC bioavailability of up to four times following exposure of DOC to solar radiation in waters where the bacterial activity : DOC concentration ratio is low (indicative of more refractory DOC). Benner and Biddanda (1998) studied the effects of photochemical transformations on the bioavailability of marine DOC in surface and deep waters. They found that exposure of surface water DOC to sunlight resulted in a 75% reduction in bacterial production, whereas exposure of deep-water DOC resulted in a 40% enhancement in bacterial production. The authors proposed that photo-mineralisation of

labile DOC and photo-production of refractory DOC most likely contributed to the reduction of bacterial growth in surface waters. Decreased bioavailability of DOC following solar and/or UVB exposure is suggested to be most likely due to the formation of extra bonds generating more complex DOC structures or due to the production of molecules that inhibit bacterial growth.

Thus, in addition to its ability to remove DOC compounds from water, UV-B radiation can also convert labile DOC to refractory forms, decreasing its bioavailability and thus reducing microbial mineralisation processes.

1.1.3.3 Impact of UV-B Radiation On The Biological Potential for DOC Degradation

In addition to its effects on the carbon chemistry, UV-B radiation also has the potential to impact upon the activity and population dynamics of aquatic organisms. UV-B energy is strongly absorbed by DNA and proteins, causing structural changes in these molecules that can interfere with vital cellular processes of growth and reproduction (Karentz and Lutze, 1990; Elasri and Miller, 1999; Buma et al., 2000; Booth et al., 2001; Boelen et al., 2002; Frost and Xenopoulos, 2002). As such, UV-B radiation can be harmful to aquatic organisms such as bacteria, reducing their productivity and altering community structures (Herndl et al., 1993; Lindell et al., 1996; Ferreyra et al., 1997; Booth et al., 2001; Callieri et al., 2001; Chatila et al., 2001; Davidson and Belbin, 2002). As bacteria have a crucial role in aquatic systems as decomposers and mineralisers of organic matter (Jordan and Likens, 1980; Amon and Benner, 1996; Hart et al., 2000; Jonsson et al., 2001; Lemee et al., 2002), there is a potential for an indirect impact of UV-B on DOC cycling. As stated earlier, bacterial species have different abilities to utilise different fractions of DOC (Amon and Benner, 1996; Weinbauer and Hofle, 1998; Lindstrom, 2000; Amon et al., 2001; Fandino et al., 2001; Riemann and Winding, 2001), and in addition to this, not all bacteria seem to be equally susceptible to UV damage (Maranger et al., 2002). Thus, a change in microbial community structure due to UV-B induced population changes, could have an indirect effect on the overall composition of different DOC fractions within a DOC pool. Furthermore, the damage caused by UV-B radiation can result in bacterial matter being recycled into the organic pool as particulate and dissolved organics that can either serve as substrate for further bacterial growth or can accumulate in the surrounding organic carbon pool, altering the DOC structure of the water.

Ogawa et al. (2001) studied the mechanisms of marine DOC formation and found that DOC derived from dead bacterial cells was mostly resistant to microbial degradation, persisting for more than a year in water. In their study, only 10%-15% of the bacterially derived DOC was identified as labile amino acids and sugars.

Karentz and Lutze (1990) monitored the transmission of UV radiation within a water column and evaluated the biological effects of incident and in-water UV radiation by measuring the sensitivity of a DNA repair-deficient strain of *E.coli* to UV radiation at different depths. The authors found that significant amounts of UV-B radiation were transmitted to a depth of 10m, and that biological effects of UV radiation (measured in terms of quantification of cell survival after exposure to radiation at different depths) could be detected as deep as 20 and 30 m. Other authors have also reported inhibiting effects of UV radiation on the survival of aquatic microorganisms. For example, Ferreyra et al. (1997) tested the effects of UV-B radiation on planktonic communities from a saline lake in Canada. They showed marked reductions in cell numbers in the presence of UV-B radiation, suggesting that bacterial populations of this lake were highly sensitive to UV-B radiation. Rae and Vincent (1998) studied the effect of solar UV (UV-B and UV-A) radiation on the natural bacterial communities from a lake and a river in northern Quebec. They found bacterial abundance to be unresponsive to different solar radiation treatments, but the percentage of actively respiring bacteria were significantly inhibited in the presence of UV radiation, decreasing by up to 48% and 59% in lake and river experiments respectively. Herndl et al. (1993) studied the effects of short-term (30 min) solar radiation exposure on thymidine and leucine incorporation into bacteria of the surface layer of the sea by incubating surface water at different UV-B radiation levels. They showed that bacterial activity in the surface layers of the oceans was suppressed by solar radiation. A 50% reduction in thymidine incorporation was observed at UV-B radiation levels of 1.3 W/m^2, whereas a 50% reduction in leucine incorporation was detectable at radiation levels of 0.6 W/m^2. This suggested that, during short-term exposures, bacterial biomass production was more affected than cell division and hence cell production. The authors also showed that in the subsequent absence of UV-B radiation, bacteria are capable of rapid recovery from UV-B induced damage (in terms of cell division and production). Boelen et al. (2002) studied the impacts of natural levels of UV-B radiation on bacteria from the Red Sea. They measured UV-B induced DNA damage, as cyclobutane pyrimidine dimers (CPDs). The authors found that DNA damage accumulated throughout the day. While the numbers of CPDs decreased during darkness,

some residual DNA damage always remained at the end of the night. These results suggested that bacteria were prone to UV-B stress and that dark repair pathways were not sufficient to eliminate damage during or after UV-B exposure, or that part of the plankton community was incapable of repair at all. These authors further showed that a shift in bacterial population structure could lead to a significant change in UV-B sensitivity.

Pausz and Herndl (2002) studied the influence of nutrient availability on the sensitivity of marine bacteria to solar radiation and on their recovery from UV stress. The authors showed that phosphorus availability could influence the sensitivity of bacteria to UV stress and the recovery efficiency from previous UV stress in oligotrophic surface waters. They found that after 4 hours of exposure to the full range of artificial solar radiation, bacterial activity declined by about 46% under P-depleted conditions.

Chatila et al. (2001) studied the effects of UV-B radiation on a natural bacterial community during a 7-day experiment conducted in mesocosms. Although they showed an increase in bacterial abundance of 73% in the presence of UV-B radiation compared to the values measured without UV-B (UV-B free conditions were obtained by implementing filters which exclude UV-B radiation) they also measured a concurrent 40% reduction in thymidine incorporation under UV-B radiation. They suggested that the reduced specific bacterial activity was a direct result of the negative effect of UV-B radiation on bacteria, while an increased bacterial abundance in the presence of UV-B radiation may have been due to decreased grazing pressure and/or increased food supply (labile DOC). Mostajir et al. (1999) showed that increased bacterial abundance (49%) in the presence of UV-B radiation in an estuary was a result of decreased (66%) abundance of predators. They proposed that at a community level, the UV-B radiation is likely to drive the ecosystem toward a microbial food web in preference to an herbivorous food web.

Thus, while Herndl et al. (1993) suggest that the increasing levels of global UV-B radiation might lead to a suppression of the activity of the principal consumers of DOC and ultimately to an increase in potentially labile DOC in the surface waters, it is possible that the net effects of increasing global levels of UV-B radiation on aquatic bacteria may not be entirely negative, but may be balanced by a combination of increased substrate availability and decreased level of bacterial grazers.

1.2 AQUATIC BACTERIAL COMMUNITIES AND FRESHWATER QUALITY

Freshwater lakes and reservoirs provide habitat to a wide diversity of microorganisms. Bacteria are an important component of freshwater microbial communities and represent a major pathway for the flux of organic matter in aquatic ecosystems (Kristiansen et al., 1992; Del Giorgio and Cole, 1998) as discussed in Section 1.1.3.1. In general, they play a key role in the breakdown of organic matter and the remineralization of nutrients, and at the same time they are grazed upon by protozoa and some metazoans thus forming the base of a heterotrophic aquatic food chain (Jordan and Likens, 1980; Kaplan et al., 1980; Cole et al., 1982; Murray and Hodson, 1985; Sherr and Sherr, 1988; Hobbie, 1992; DelGiorgio et al., 1997; Muylaert et al., 2002). Although bacteria are the key components of the trophic web, the species composition of bacteria in aquatic ecosystems is not well defined (Trusova and Gladyshev, 2002). Furthermore, studies attempting to correlate bacterial community composition to biological, chemical and physical parameters in the lakes are limited (eg: Hofle et al., 1999; Lindstrom, 2000; Lindstrom, 2001). On the other hand, enough data is available, for example, to show seasonal as well as spatial variations in lake bacterial communities (Pernthaler et al., 1998; Lindstrom, 2000; Dominik and Hofle, 2002; Lindstrom and Leskinen, 2002; Schauer et al., 2003; Stepanauskas et al., 2003; Zwisler et al., 2003).

Reservoir bacterial communities comprise resident (indigenous) microorganisms and exogenous organisms that enter water through terrestrial runoff and discharge (Lindstrom, 2001; Kistemann et al., 2002). Some of these bacteria are ubiquitous and can proliferate in the most diverse conditions, whereas others remain alive in water for a limited time only. With the aim of identifying typical freshwater bacteria, Zwart et al. (2002) analysed available 16S rRNA gene (rDNA) sequences from plankton of numerous globally distributed lakes and rivers. The authors compared 689 bacterial 16S rDNA sequences from the water column of rivers and lakes in North America, Europe and Asia deposited into the global database. Their analysis indicated that the majority of bacterial sequences were most closely related to other freshwater clones or isolates, whereas relatively few were related to sequences recovered from soils or marine habitats. Thus although bacterial community structures can change with changes in environmental variables, these authors state that freshwater systems generally have a specific planktonic bacterial community distinct from bacteria in neighbouring environments such as soil and sediment and that these planktonic bacteria are distributed in diverse freshwater ecosystems around the world. Although there are similarities in the

compositions of bacterial communities worldwide (Lindstrom and Leskinen, 2002), several investigations of bacterial diversity in lakes and oceans indicate that the species richness of these communities is high but that only a few taxa (generally < 20) dominate (Ferris et al., 1996; Bosshard et al., 2000; Casamayor et al., 2000; Lindstrom, 2000; Pinhassi and Hagstrom, 2000).

1.2.1 ANALYSIS OF AQUATIC BACTERIAL COMMUNITY STRUCTURES

Our knowledge of bacterial community dynamics in the environment is primarily based on studies of pure cultures that have been isolated by selective methods specific to different groups of microorganisms (Rheims et al., 1996; Staley and Gosink, 1999). However, approximately 99% of microorganisms found in nature are unable to be isolated in pure culture media due to their specific growth requirements (Weinbauer and Hofle, 1998; Felske et al., 1999). Furthermore, different growth media can show preferential selectivity for different microorganisms. For example Tabacchioni et al. (2000), tested the influence of two isolation media on the biodiversity of a *B.cepacia* strains recovered from the rhizosphere of *Zea mays*. These authors found that the two different isolation media selected for *B.cepacia* populations with a different degree of genetic diversity. In particular, all strains isolated from one medium were assigned to the *B.cepacia* species, while in the second media only 74% of isolates belonged to the *B.cepacia* species. Despite such biases of culturability, viable plate count or most-probable number techniques have been, and frequently still are, used for quantification of active cells in environmental samples (Amann et al., 1995).

In addition to the lack of culturability and culture-induced biases, most isolated bacteria from natural samples can also not be detected with conventional microscopy, because they are too small, their external structures too simple (Muyzer, 1999; Staley and Gosink, 1999), and because they adhere to soil and sediment particles, therefore remaining invisible (Muyzer et al., 1996). Use of fluorescent dyes (such as acridine orange and DAPI) and epifluorescence microscopy have improved the enumeration of microorganisms such as bacteria in their natural environment, but they yield no information into species diversity. Such fluorescent dyes function by binding to nucleic acids (DNA and RNA), generating fluorescence in such a manner that the fluorescence of the DNA-dye complex is enhanced over that of the unbound dye molecule, enabling visualisation of stained cells and

enumeration of communities of interest (Hobbie et al., 1977; Coleman, 1980). Direct microscopic counts, obtained with the help of fluorescent dyes and microscopy, have been reported to exceed viable-cell counts by several orders of magnitude (Amann et al., 1996), thus it has proven to be a rapid tool for microorganism enumeration without the need for culturing.

Even the analysis of mixed population activities and functioning are not specific enough to enable differentiation between actions of different species. For example, thymidine incorporation assays are commonly used to measure the synthesis of bacterial DNA, bacterial cells and cell biomass carbon of total populations as opposed to individual species (Coveney and Wetzel, 1988). Thus, the limits of traditional techniques in classifying and identifying the structure and functioning of various bacterial communities found in natural ecosystems are apparent.

Recent developments in molecular biological techniques have enabled a study of microbial diversity at the genetic level where microorganisms are grouped according to similarities in their genes, which also reflects their evolutionary relationships (Muyzer, 1999; Zwart et al., 2002). Of particular importance to the study of environmental bacterial communities are the ribosomal RNA genes (rDNA). Bacteria contain three different types of ribosomal RNA genes, 16S, 23S and 5S rDNAs, of which 16S rDNA is the most useful when inferring phylogenetic relationships (MacGregor, 1999). The advantage of rDNA sequences, in particular 16S rDNA, is the generation of an increasingly expanding data set which enables a comparison of newly determined sequences (both from pure culture isolates and those obtained by cloning genes from environmental samples) (Devereux and Willis, 1995). The basis of the rDNA approach lies in the highly conserved DNA regions that span regions of sufficient sequence variability, allowing comparisons of variable regions and thus the establishment of relationships between closely and distantly related groups of organisms (Lane et al., 1985; Devereux and Willis, 1995).

The general principle of molecular microbial ecology is based on isolating nucleic acids directly from their environments containing mixed populations of species, thus eliminating the need for culture induced biases (Staley and Gosink, 1999). There are, however, certain disadvantages associated with this direct approach. For example, in the case of water samples, there is often a need to concentrate bacterial cells prior to extracting the DNA. Vacuum filtration is the most common technique used, concentrating cells on designated pore-size filter membranes (often 0.22-0.45μm) (Pickup et al., 1995). Limitations

are imposed on this method due to clogging of filters and thus low volumes of water that can be processed. Such problems can however be overcome by using alternative methods that filter cells whilst maintaining them in solution (for example, the use of tangential flow filtration, TFF) which enables large (100L) volumes of sample to be processed.

Once bacterial cells are concentrated and DNA extraction is performed, the genomic diversity is most often assessed either by sequence analysis of cloned PCR products of 16S rDNA, or by electrophoretic separation of PCR products via temperature gradient (TGGE) or denaturing gradient gel electrophoresis (DGGE) (Rheims et al., 1996). The former approach, cloning, enables a generation of high copies of the desired gene which can then be sequenced (Hastings, 1999). Such a method is routinely applied when isolating and identifying microorganisms from environmental samples (Fuhrman et al., 1993; Lloyd-Jones and Lau, 1998). For example, Rondon et al. (2000) have used a bacterial artificial chromosome vector to construct libraries of genomic DNA isolated directly from soil. Based on phylogenetic analyses of 16S rDNA sequences, these authors showed a wide diversity of microbial phyla among the uncultured soil microorganisms. In another study of soil bacteria in Dutch grasslands, Felske et al. (1999) also applied the approach of direct DNA extraction, PCR amplification of 16S rDNA and subsequent cloning and sequencing. In addition to studying bacterial species in soil, these authors also compared how well the predominant bacteria, as identified by direct DNA extraction from soil, were recovered by various cultivation approaches (involving 35 different types of media at two different pH values). They found that predominant bacteria in grassland soils remained uncultured when using classical cultivation techniques, possibly due to unknown nutritional requirements, low growth rates of predominant soil bacteria or inhibition by other microorganisms during growth on agar. Cloning of 16S rDNA PCR products has also been applied to water samples. For example, Trusova and Gladyshev (2002) studied the diversity of bacteria of two eutrophic Siberian reservoirs. They showed that a significant number of bacterial 16S rDNA clones were closely related to freshwater bacteria previously found in different aquatic ecosystems, confirming the assumption that some bacterial groups are globally distributed. They also showed that Actinobacteria, together with a β-subclass of Proteobacteria, may be one of the most abundant groups in limnic ecosystems.

Although a technique such as cloning provides better information of the structure of environmental bacterial communities when compared to classical culturing techniques, there are certain disadvantages to using this technique. In particular, this technique is expensive

and labour-intensive. As such, electrophoretic techniques such as TGGE or DGGE are often used as they allow a rapid qualitative analysis of the phylogenetic structure of bacterial communities (Zwisler et al., 2003).

TGGE and DGGE are techniques that provide a profile of the community diversity on the basis of the physical separation of unique nucleic acid sequences, allowing comparisons of bacterial communities from different environments or following the changes in community composition of the same environment over time. These techniques are based on the separation of polymerase chain reaction (PCR) amplified gene fragments, not according to size, but owing to variation in the targeted nucleotide sequence (Edwards, 1999; Hastings, 1999). Thus the DNA fragments analysed are of the same length but different nucleotide content (Lindstrom and Leskinen, 2002). Nucleotide pair dissociation is mediated by increasing temperature or concentrations of chemical denaturants (such as urea and formamide) respectively (Bruggemann et al., 2000). Separation is based on the electrophoretic mobility of a partially melted double stranded DNA molecule in polyacrylamide gels. The double helical structure of DNA will reach its melting point based on its sequence composition as it passes through an increasing temperature or denaturing concentration range (MacGregor, 1999). Once the double stranded structure is lost, the DNA fragment halts migration, appearing as a horizontal band on the stained gel. The number of bands observed in DGGE profiles provides an estimate of species richness, while the relative intensity of each band provides a rough estimate of the relative abundance of each species (Iwamoto et al., 2000). However, as there are many potential errors in the generation of the DGGE data, this method is not used for qualitative purposes but only as a community fingerprint (Lindstrom, 2001). For example, the number of genes coding for 16S rRNA (*rrn*) can vary across different bacterial species (from 1 to 14) (Farrelly et al., 1995). Since information on *rrn* copy numbers is unavailable for all of the uncultured microbial diversity, a quantitative comparison of different bands in a sample cannot be made. Intraspecies heterogeneity can also be observed in a DGGE banding pattern (Dahllof et al., 2000). This is brought on by the presence of multiple copies of the ribosomal genes and the fact that these gene copies have evolved differently. As such, the amplified fragments of 16S rDNA will appear as several bands on a DGGE gel instead of a single band that is representative of that particular species. On the other hand, Felske et al. (1999) demonstrated that different sequences from the same source samples can show the same migration pattern during TGGE, appearing as one band on the TGGE gel. These authors proposed that the assignment of

isolates to matching bands of according environmental fingerprints require additional confirmation, such as sequencing or hybridisation steps. A further potential error in generating a DGGE community fingerprint can be caused by preferential amplification of certain templates over others (Wintzingerode et al., 1997). As such, amplified DNA can only reflect quantitative abundance of species if the amplification efficiencies are the same for all molecules.

Despite the mentioned potential biases in generating the DGGE data, the DGGE method still provides an acceptable view of differences and similarities in the dominating populations of microbial communities (Lindstrom, 2001). One of the strongest points of this technique is the simultaneous analysis of multiple samples that allows monitoring of the complex dynamics that microbial communities may undergo by, for example, diel and seasonal fluctuations or following environmental changes (Ferris and Ward, 1997; Muyzer, 1999; Diez et al., 2001).

Since its initial application to environmental 16S rDNA, DGGE has been used extensively in microbial ecology. For example, Schauer et al. (2003) used DGGE to study the phylogenetic composition of the bacteria in a coastal oligotrophic system during an annual cycle. The authors showed a generally stable taxonomic composition of the bacteria throughout the year, with gradual changes in the dominant bacterial members over the sampling period. The time scale during which a single population appeared in significant amounts in the system ranged between weeks and months. Zwisler et al. (2003) studied seasonal patterns of the bacterial community composition in a large mesotrophic lake. The DGGE analysis of the lake bacterial community showed seasonal and vertical variations of the banding patterns even though several bands occurred throughout the study period and at all depths. Iwamoto et al. (2000) used DGGE to study changes in the overall bacterial diversity during a bio-stimulation treatment experiment of groundwater, where methane was injected as a stimulus for trichlorethylene degrading bacteria. Their DGGE results revealed changes in bacterial community structure upon the treatment. Bacterial community structure continued to change up to 60 days after treatment, after which a relatively stable community structure was formed, different from that of the original community. The authors also report that, based on the DGGE data, there was no reduction in diversity after the bio-stimulation treatment, which often occurs when pollutants are introduced into the system, stimulating growth of those organisms capable of utilising the pollutant. In another bio-stimulation study, Christoffersen et al. (2002) used DGGE analysis to study changes in bacterial community

structure during degradation of microcystins (cyclic pentapeptide toxins of cyanobacteria). The authors showed that indigenous bacterial communities responded quickly to the addition of lysates (microcystins). They reported an increase in the diversity from 14 to 16 bands at the beginning of the experiment, to a maximum of 32 bands after 19 days of incubation. The increase in the number of bands was proposed to reflect an increase in the number of species with a higher relative abundance. In further examples, Macnaughton et al. (1999) applied the DGGE technique to study eubacterial populations of metal-resistant soils. Kilb et al. (1998), studied the community structure of different groundwater habitats using methods of 16S rDNA and DGGE, while Kowalchuk et al. (1997) applied this technique to the study of ammonia-oxidising bacteria in coastal sand dunes.

The use of the DGGE technique to study microbial populations of various environments continues to increase rapidly. Its application has only recently been expanded to integrate studies of community structure and their environments. For example, Lindstrom (2000) had taken the DGGE tool to further lengths, by investigating the relationship between bacterial community composition and physicochemical parameters of five different lakes in Sweden. In that study, biomasses of micro-zooplankton, cryptophytes and chrysophytes, were the three variables found to have strongest correlations to the DGGE patterns, suggesting that these biota had an impact on bacterial community structure. The nutrient content of the lake was also suggested to influence the structure of the bacterial community in these lakes. In a further study of five mesotrophic lakes, Lindstrom (2001) showed that the import of allochthonous bacteria and the interaction with other plankton organisms (eg. predation) in the lakes was the most likely factor to impact the composition of bacterial communities. The approach adopted in these two studies provides the basis for extending the application of the DGGE technique to link the complex environmental fields of biodiversity and biogeography.

1.2.2 THE RELATIONSHIPS BETWEEN PHYSICOCHEMICAL PARAMETERS AND BACTERIAL COMMUNITIES IN AQUATIC ECOSYSTEMS

Due to their short life cycles, bacteria respond quickly to environmental changes, hence their abundance and species composition are likely to indicate the quality of the water mass in which they are found. Bacterial abundance in aquatic systems can vary on the millimetre scale, the variability often occurring in response to changes in certain water

parameters, such as changes in predator abundance or the concentration of organic matter (Long and Azam, 2001). Thus, according to the kind of aquatic habitat, the composition of the bacterial flora can differ widely, depending not only on the water's content of organic and inorganic material used as substrates for growth, but also on the pH, turbidity, temperature, and even the sources from where organisms can enter the water body (DelGiorgio and Cole, 1998). The growth and functioning of bacteria is affected by a great variety of physical and chemical factors that may act with or against one another. Such factors influence not only the size and composition of the bacterial populations, but also the morphology and physiology of the individual bacteria (Morita, 1993). For example, for some bacteria, temperatures or pH values above or below the optimum, or low nutrient concentrations, may lead to considerable changes in metabolism, cell morphology and reproduction (Rivkin and Anderson, 1997; Rae and Vincent, 1998; DelGiorgio and Cole, 1998; Romani and Sabater, 2000; Carlson et al., 2002; Lemee et al., 2002; Montserrat Sala et al., 2002; Simek et al., 2003). On a population scale, changes in community structure can result upon change to environmental conditions.

As bacteria are crucial for DOC mineralisation, information into their relationships with the physical, chemical and biological parameters of reservoir waters are important in elucidating whether any of these parameters affect growth and species composition, and in turn how these relationships reflect on the temporal DOC dynamics of the water body (Cole and Pace, 1995). Temporal changes in various environmental variables have the potential to cause dramatic changes in the activity and composition of the bacterial communities on daily to weekly time scales (Schauer et al., 2003), thus affecting its role in DOC cycling. However, the number of studies addressing temporal changes in bacterial community structures, in relation to the changes in environmental variables, for a whole seasonal cycle in natural environments are limited (Lindstrom, 2000; Muylaert et al., 2002; Schauer et al., 2003). Understanding of diversity and function of aquatic microorganisms with respect to environmental variables is essential in understanding the role of bacteria in the cycling and transformation of organic matter.

For example, temperature is the factor most constant in its effect on individual bacterial species and population communities (Brock, 1997). Living organisms typically encounter temperature changes throughout their life cycle, yet most microorganisms are unable to internally regulate their core body temperature (Michaud, 1991). Temperature exerts a major influence on the biological activity of aquatic organisms, their growth rate, nutritional requirements and to a smaller extent, the enzymatic and chemical composition of

the cells (Wetzel, 1983; Gunnison et al., 1985). Temperature is mainly considered in the context of this study due to its influence on water chemistry. The rate of chemical reactions generally increases with increasing temperatures, which in turn affects biological activity. For example, processes such as decay and mineralisation accelerate with increasing temperature. The breakdown of detrital organic compounds depends in large part on extracellular enzyme activity, which is enhanced under warmer conditions (Gunnison et al., 1985). Lower temperatures however do not halt decomposition process but reduce the rates at which they occur (Erdal et al., 2003). Decomposition and other chemical reactions proceed during winter months as well, even under ice-covered north temperate lakes, but increasing the temperature will accelerate the rate at which these reactions occur, both in pelagic water and in bottom sediments. For example, Kushmaro et al. (1998) studied the *in situ* and laboratory-controlled effects of seawater temperature on the bleaching of the coral *Oculina patagonica* in the Mediterranean Sea. The authors found that coral bleaching by bacteria was closely correlated with temperature. Numbers of bleached colonies rose by about 80% in summer when seawater temperatures were about 28°C and decreased in winter, when seawater temperatures were about 17°C, to values less than 20%. In the controlled conditions, 92% of the corals that were infected with a bacterial agent and grown in the laboratory at 29°C experienced some degree of coral bleaching by 22 days into the experiment, whereas only 45% and 28% of the corals grown in water temperatures of 25°C and 20°C experienced any bleaching. Furthermore, none of the corals in the controlled experiment with water temperature of 16°C experienced any coral bleaching over the 45 days of study. These results demonstrated the temperature dependence of a specific bacterial activity in an aquatic system. On the other hand, Romani and Sabater (2000) showed that water temperature was not an important factor accounting for variability in the heterotrophic activity. These authors studied environmental factors responsible for spatial as well as temporal variations in extracellular enzymatic activities in benthic biofilms in three Mediterranean streams. The authors state that the relationships between temperature and extracellular enzyme activity might be masked if the response of enzyme activity to changing water temperature is delayed.

While the relationships between temperature and bacterial activity have been extensively studied, the information on the effects of temperature on the structure of bacterial communities in their natural environments is limited. Schauer et al. (2003) used DGGE analysis to study seasonal changes in the taxonomic composition of bacteria in a coastal oligotrophic system. These authors found clustering of bacterial populations based on

temporal differences. They proposed that over the year, the main factors affecting bacterial composition are the changes in the supply of the organic matter which in turn is mediated by different algal populations, and the different temperature optima of bacterial populations. Zwisler et al. (2003) also reported clustering of bacterial populations according to the temporal scale. The authors used DGGE analysis to study seasonal patterns in bacterial community composition in a mesotrophic lake, and found highest similarities between populations belonging to the same seasons. Schultz Jr et al. (2003) studied the effects of different environmental variables on the bacterial dynamics in the York River estuary. The authors reported strong relationships between temperature and the studied bacterial properties. For example, both bacterial cell abundance and production showed a seasonal cycle which corresponded to the annual temperature cycles, while concentrations of organic and inorganic substrates were not significantly related to most bacterial properties. The authors proposed that since the organic matter was not limiting, increases in temperature alone could stimulate production and growth rates. Thus, over seasonal time scales, temperature is able to exert strong influence on bacterial processes.

Rae and Vincent (1998) studied the effects of temperature and ultraviolet radiation on microbial food web structures from lakes and rivers in northern Quebec. They incubated microbial populations at two temperatures (10°C and 20°C) and three irradiance conditions (PAR, PAR+UVA and PAR+UVA+UVB). The authors found that the concentration of total bacteria showed no net response to temperature, but the percentage of actively respiring bacteria were up to 57% higher at 20°C when compared to 10°C. This suggested that with climate warming, metabolically active bacteria could become a larger component of the microbial community. Their results also indicated that some populations reacted strongly to temperature, leading to detectable community-level shifts, suggesting that climate change could influence community size structure and composition.

Oxygen is another important water parameter that can influence the activity and community structure of bacteria. Oxygen is produced during photosynthesis and consumed during respiration and decomposition. Dissolved oxygen in water is also derived from inflowing streams and the air. The concentration of oxygen in air is much higher than that in water (21% oxygen in air, and <1% in water), hence the large difference in concentration at the air-water interface causes oxygen molecules in the air to dissolve into the water (Michaud, 1991). Under windy conditions, more oxygen dissolves into the water, as the waves create more surface area, thus more diffusion can occur. Sufficient levels of dissolved oxygen in

water are essential, not only to sustain aquatic life, but for many chemical reactions that are important for lake functioning. For example, dissolved oxygen is an important regulator of decomposition. The lack of oxygen can affect decomposition by limiting the activity of some critical enzymes such as those involved in lignin degradation, which do not function well under anoxic conditions (Wetzel, 1983). More importantly decay in anoxic compared to oxic conditions is not as complete; that is, organic matter is not reduced to the reactants of photosynthesis, being CO_2 and water.

Internal oxygen production, due to photosynthesis, occurs only during the day, while the respiration and decomposition occur during both day and night. During night periods, when photosynthesis cannot counterbalance the loss of oxygen through respiration and decomposition, dissolved oxygen levels can steadily decline, reaching lowest levels just before dawn (Michaud, 1991). As such, oxygen levels can fluctuate on a diurnal scale, potentially limiting the activity of bacteria and altering the community structure. Dominik and Hofle (2002) studied the changes in bacterial community structure at different depths in a German eutrophic lake. They reported changes in bacterial community structure and an overall decreased level of bacterial diversity with increasing depth and decreasing oxygen concentrations and temperature. During spring, oxygen levels were reported to decrease from 20-25mg/L near the surface, to levels below 5mg/L near the sediment, while during winter oxygen levels decreased from 15mg/L at the surface to 2mg/L near the sediment.

Nutrient availability is also important for bacterial activity, as it provides the necessary elements for bacterial growth thus stimulating the rates and dynamics of DOC decomposition. Rivkin and Anderson (1997) studied the effects of inorganic nutrient limitation on oceanic bacterial growth and activity. The authors reported growth rates 5-6 fold higher under phosphate amended conditions than in the controls. They proposed that phosphate limitation of bacterial growth may directly influence the accumulation of DOC in the surface waters and thus have a significant impact on carbon cycling in the sea. They state that if bacterial growth were not constrained by inorganic nutrients, more DOC could be assimilated into bacterial biomass and subsequently transferred to higher trophic levels. Montserrat Sala et al. (2002) have also shown phosphorus to be the main nutrient limiting bacterial growth in the surface waters of the sea, while nitrogen and carbon limitations also occurred at other depths.

Most investigations concerning nutrient influences in the aquatic systems, have dealt with carbon-C, nitrogen-N and phosphorus-P. These three are some of the more abundant elements in the cellular protoplasm and if one of them is available in limited quantities, the

growth of the organism generally becomes proportional to the availability of that nutrient. For example, algal protoplasm has atom proportions of C:N:P as 106:16:1, therefore P is most likely to be limiting (Wetzel, 1983).

Lindstrom (2000) studied bacterial community composition in five Swedish lakes. The author employed canonical correspondence analysis (CCA) to analyse the DGGE patterns of community structure in relation to physical, chemical and biological data from the lakes. The author found no significant correlation between bacterial community structure and inorganic nutrients (eg: P and N). However, inorganic nutrients were suggested to indirectly affect bacterial community composition via their strong correlation with the potential predators of bacteria, which in turn were found to be significantly correlated with bacterial community structure. In another study of the relationships between bacterial community structure and environmental variables, Muylaert et al. (2002) showed that changes in bacterial community structure were, among other parameters, significantly correlated to the concentrations of nitrogen and phosphorus. The authors' findings were based on the information from four shallow lakes that differed in their physical and chemical conditions. The authors state that although nutrient concentration can directly influence bacterial biomass and community composition through effects on growth, the observed relationship between bacteria and nutrients can also be a result of co-variation between nutrient concentration and phytoplankton. Phytoplankton growth often shows strong correlations with bacteria due to its potential to take up a large fraction of dissolved nutrients and the potential to release carbon which in turn can influence bacterial dynamics.

1.3 RESEARCH AIMS

The case study of the Warren Reservoir presented in this thesis was aimed to improve understanding into the short and long-term changes of reservoir DOC under the climatic conditions of South Australian. Interactions between different water parameters and DOC levels and chemical nature were studied in order to determine which parameters drive the DOC changes inside reservoirs. A further aim was to study the effects of UV radiation on altering DOC bioavailability, and to determine whether changes in DOC are likely to cause changes in community structures of reservoir bacteria. This hypothesis was tested on the basis that if certain bacterial species show preference for removing certain DOC fractions, then there may be potential for future treatment technologies to incorporate this approach of biomanipulation during DOC removal strategies. The final aim was to examine the effects of UV-B on reservoir bacteria in order to determine whether the heterotrophic activity of reservoir bacteria is likely to be inhibited by UV-B radiation.

Specific objectives of this case study of the Warren Reservoir were:

1. To measure daily, seasonal and annual variability in DOC concentration and nature.
2. To determine whether and how DOC concentration and nature are affected by biological and physico-chemical reservoir processes.
3. To determine the effects of UV-B radiation on DOC bioavailability.
4. To determine the effects of UV-B radiation on bacterial growth and community composition.

CHAPTER 2 GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter describes the materials and methods employed in completing this study. Methods described in this chapter are all employed throughout the experimental investigations. Methods that are specific to a particular study and which have not been repeatedly used are described under their representative chapters.

2.2 LOCATION OF THE STUDY

The South Para catchment (326km^2 in area) is located 90km North East of Adelaide and comprises three reservoirs (Warren, South Para and Barossa) that supply water to the Gawler, mid-north, northern and north-western regions of South Australia (Figure 2.1). The water originating from the Warren Reservoir flows through the South Para and ends up in Barossa from where it is treated for drinking purposes (Figure 2.2). Thus the Warren Reservoir acts as a connecting body between a river system and other reservoirs (Figure 2.2). The capacity of the Warren Reservoir is 5080 ML (Engineering and Water Supply, 1994), the surface area is 105ha and the area of its catchment is 119km^2. When the Warren Reservoir overflows the water runs into the South Para Reservoir. However, during hot summer months when water is in high demand, the South Para reservoir system is unable to supply sufficient amounts of water. As a result, a branch from the Mannum-Adelaide Pipeline was built to discharge water from the Murray River into the Warren Reservoir (Figure 2.2). A second point of water inflow in the reservoir exists via the Swan Reach-Stockwell Pipeline, connecting to the Warren reservoir, however for the duration of this study, this pipeline was closed.

The Warren Reservoir was selected for this study primarily due to its high levels of DOC (10-20mg/L). Other studies of DOC concentrations and related processes in freshwater systems have also been conducted on lakes with DOC concentrations in a similar range (Lindell et al., 1995; V-Balogh and Voros, 1997; Bergstrom and Jansson, 2000; Bukaveckas and Forbes, 2000; Lindstrom, 2001). The Warren Reservoir was also selected as it was a closed system at the time when the study commenced. The closed system meant that no water

was being pumped in or taken out of the reservoir, other than natural discharge from the catchment and potential overflow from the reservoir into the Para River respectively. In this way the DOC dynamics and the processes which contribute to DOC transformation under the South Australian climate could be analysed with less interference from external factors. However, due to prolonged periods of heat in summer 1998/1999, the water levels in the Barossa and South Para Reservoirs were below the norm thus the Mannum-Adelaide Pipeline was opened in January 1999 and water was continuously pumped into the Warren Reservoir until the end of the sampling period in August 2001. As such, during the course of this study, the Warren Reservoir changed from being a closed system to being an open system.

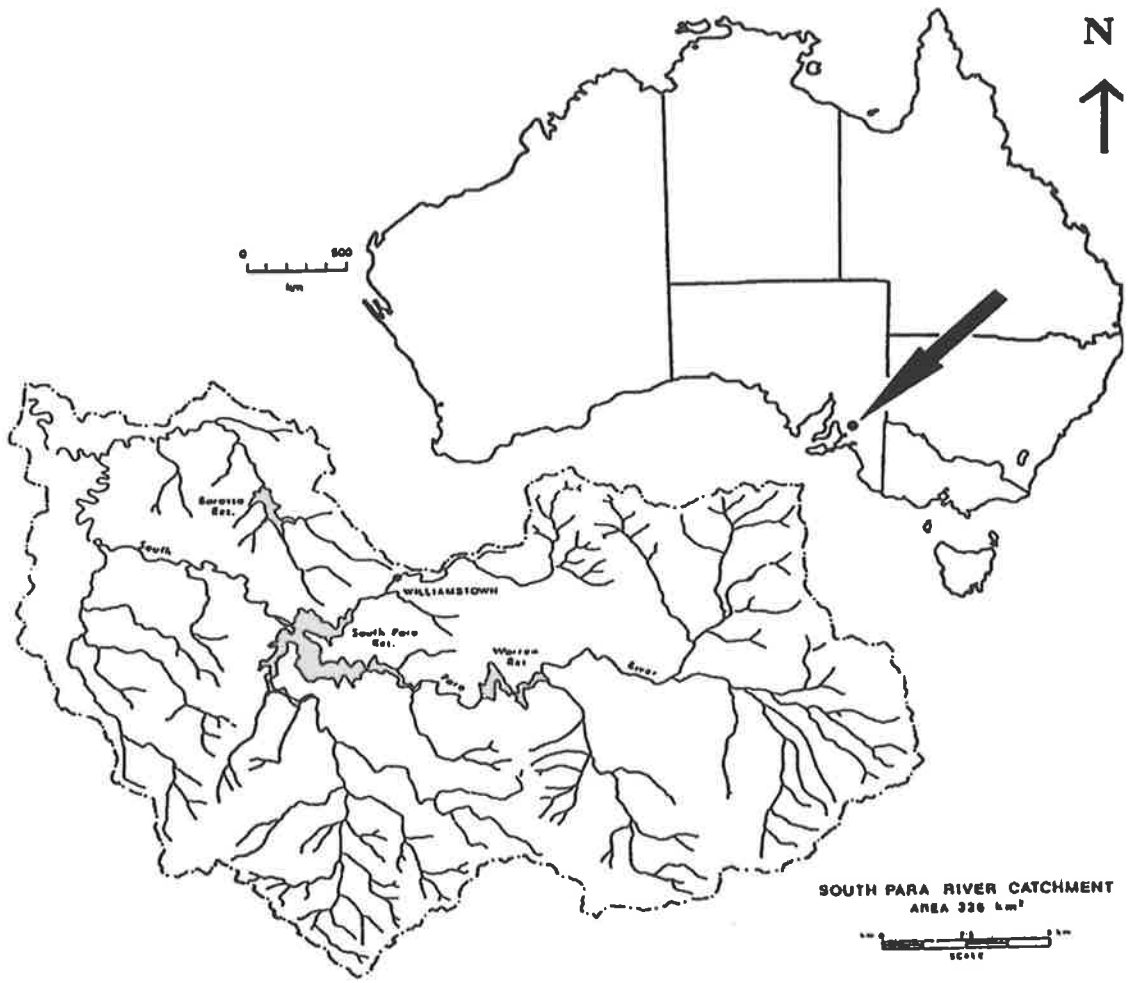


Figure 2. 1: Map of Australia showing the location of the South Para Catchment. The insert indicates the location of the study site, The Warren Reservoir, with respect to the other reservoirs in the catchment.

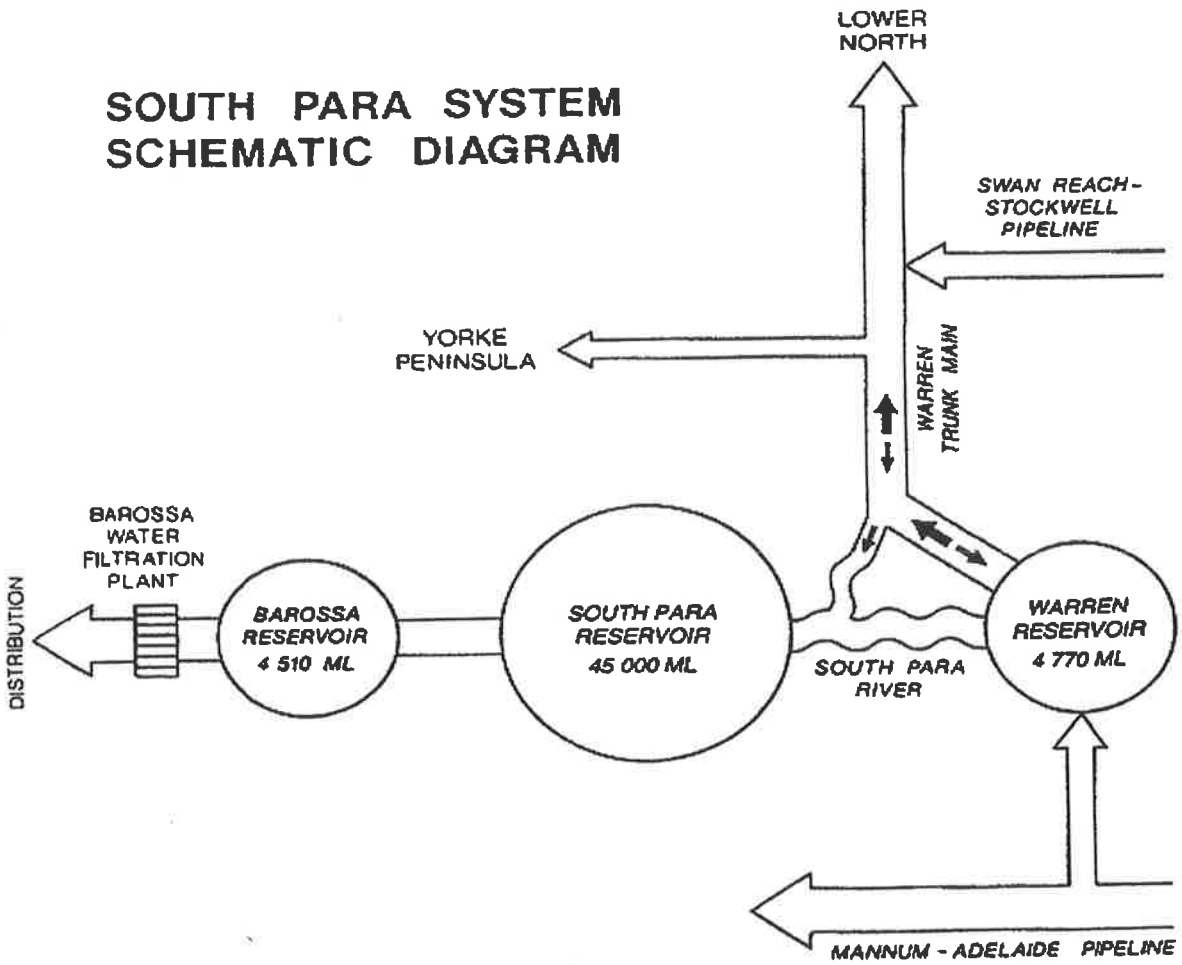


Figure 2. 2: Schematic diagram of the South Parra catchment system located 90km north of Adelaide, South Australia. The diagram shows the position of the Warren reservoir in relation to the entire catchment (SA Water Information Bulletin No.1, November 1993).

2.3 SAMPLING SITES

Four sampling sites within the Warren Reservoir were selected and buoys were used to mark the locations of each site. The four sites represent areas of the reservoir affected by different geophysical conditions. For example, the sites differ in the depth of the water (1m - 15m), in the type of catchment that surround it (pine plantation versus eucalyptus) and by their proximity to the inflow of stream water from the catchment or the point of water outflow.

The four horizontally spaced sites in the reservoir are designated S1, M1, D1 and D2 (Figure 2.3). Site S1 is located at the shallow end of the reservoir where the mean water depth is between 1.5-2m. It represents the furthest end of the reservoir in relation to the dam wall and the point of water outflow. This site is distinguished by its shallow turbulent conditions and is the point at which water enters the reservoir from two catchment streams C-S1 and C-S2 (Figure 3.2). Site M1 is located at the point where the Murray River pipeline enters the reservoir, and has average water depth between 4-5m. D1 is located at the more stable, deeper portion of the reservoir where the average depth is around 9m. It is located half way between M1 and the dam wall and acts as a passing point for water leaving the shallow end of the reservoir on its way to the dam wall. Site D2 is located at the dam wall and represents the deepest point of the Warren Reservoir where the average water depth is around 14m. This site acts as the final point of water flow in the reservoir prior to entering the stream leading to the South Para Reservoir. The waters at this deep end of the reservoir are relatively calm and affected by stratification during warmer months.

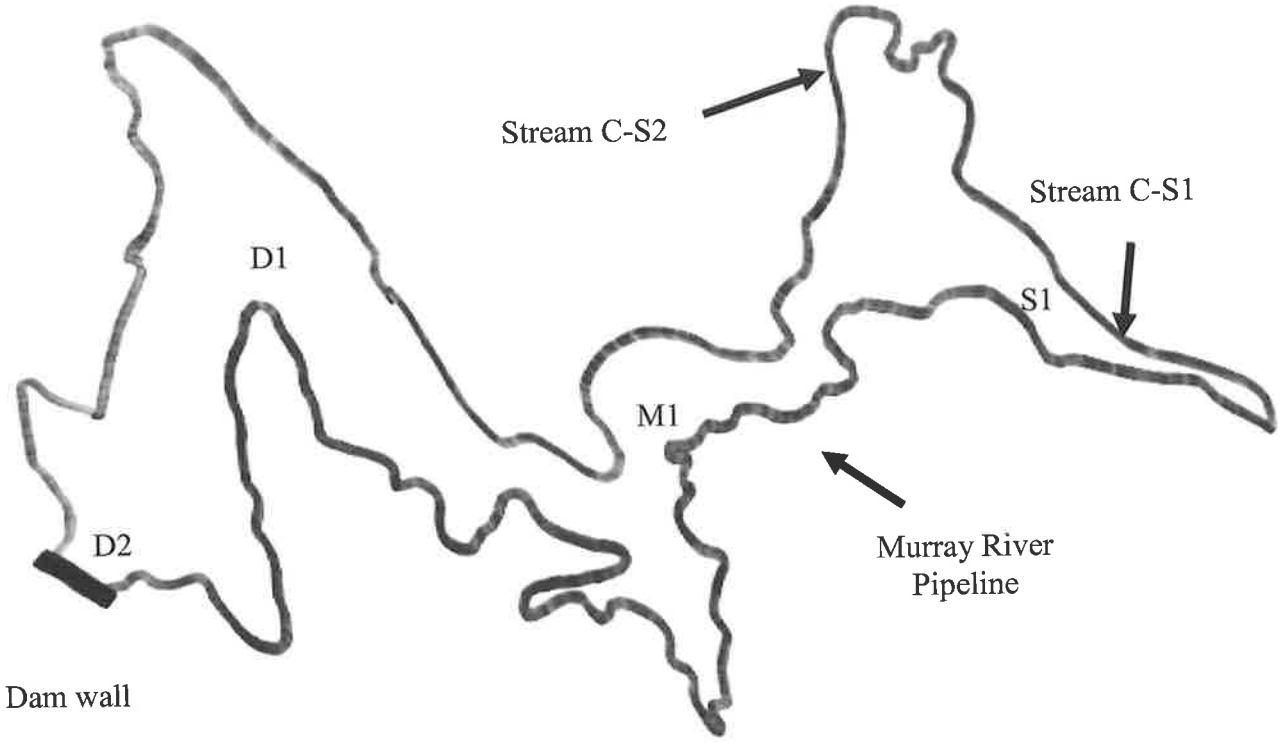


Figure 2. 3: Warren Reservoir, South Australia. Reservoir study sites S1, M1, D1 and D2 are indicated together with the Murray pipeline location and two catchment streams C-S1 and C-S2.

2.4 SAMPLE COLLECTION AND STORAGE

Water samples were collected fortnightly and/or monthly from January 1998 until December 1999, and from December 2000 until August 2001, with an intermittent period between January 2000 and December 2000 during which no data was obtained due to technical difficulties. However, during this period, information on DOC concentration and water turbidity and colour at the deep site D2, were obtained from SA Water Corporation. Water was collected at all times from the epilimnion using the Van-Dorn Sampler. This instrument has been used by other researchers (Marvalin et al., 1989; Aleya and Hartmann, 1995; DiSiervi et al., 1995; Lund and Davies, 2000; Carrias et al., 2002; Iriarte et al., 2003).

It functions by allowing water to penetrate into the bottle at constant speed as it is submerged. Once raised, the inflow-valve shuts, trapping the water from the vertical column. The instrument was submersed to a maximum depth of 1m, collecting 4L of water from the vertical column of the 1m region. Previous studies use water from the depths between 0.5 and 2m (Herndl et al., 1993; Aleya and Hartmann, 1995; Mannino and Harvey, 2000; Blomqvist et al., 2001; Carrias et al., 2002; Jassby et al., 2002). As the depth of the shallow site varied between 1.5-2m, 1m depth was chosen as a consistent sampling depth across the reservoir. Immediately upon collection samples were transported in polyethylene containers to the laboratory and stored at 4°C until analysed or used in experiments.

Upon arrival to the laboratory sub-samples were filtered through 0.45µm nitrocellulose filters (Millipore, Australia) to remove particulates (Miller and Moran, 1997). The filtrates were analysed for nutrients (soluble reactive phosphorus and nitrate) and true colour (measured at 456nm). Total phosphorus analysis was also performed on unfiltered samples.

2.5 PHYSICAL AND CHEMICAL ANALYSIS OF WATER

A Multiparameter Water Quality Monitor (MWQM) 610-DM (Environmental Monitoring Systems, USA) was used to obtain data on various physical and chemical parameters of the water. Analysis was done on site by submerging the MWQM in the water column to a depth of 1m. All data was stored on a computer and later transferred into an Excel type file.

Parameters determined using the MWQM were temperature, turbidity, pH, oxygen concentration, conductivity, salinity and redox potential.

A measure of water clarity, Secchi depth, was also determined at each site. The Secchi meter was used, which comprises a metal black and white disk attached to a rod with a meter length marked. The Secchi meter was submersed in water until the black and white disk was no longer visible. The meter was pulled upward until the disk became visible. The mean between the two depths was recorded. Muylaert et al. (2003) also used Secchi depth as an indicator of water clarity.

2.6 NUTRIENT AND COLOUR ANALYSES

Nutrient content and water colour are commonly used parameters for classification of lakes (Lindstrom, 2000). Colour is often used as an index of the relative humic content of water (Pace and Cole, 2002).

Total phosphorus, soluble reactive phosphorus and nitrate were measured during this study to assess nutrient levels in water samples. All three assays utilized pre-calibrated reagents (provided as powder sachets by Permachem Reagents, USA) and a spectrophotometer for the colorimetric measurements as described in the Standard Methods for the Examination of Water & Wastewater, method 4500-NO_3^- E (18th Ed., APHA, AWWA, WEF, 1992). A DR2000 Direct Reading Spectrophotometer (HACH, USA) was used for colorimetric measurements. Calibrations for the three performed tests were already stored in the DR/2000's ROM eliminating the need for manual conversions from absorbance to concentration.

2.6.1 ANALYSIS FOR TOTAL PHOSPHORUS (TP)

Contents of PhosVer3 phosphate powder sachet (Permachem Reagents) were added to 25mL of raw water sample in a 50mL volumetric flask. 2mL of 5.25N H_2SO_4 was then added to each sample to enable acid digestion, and boiled for 30 minutes on a heating block. Throughout boiling reverse osmosis (RO) water was added to maintain the total volume of sample to no less than 23mL. After cooling, 2mL of NaOH was added to neutralise the pH, and RO water was added to bring the sample volume back to 25mL. For determination of the TP, the DR2000 Spectrophotometer wavelength was set to 890nm and the sample cell was filled with 25mL of the prepared sample. The contents of PhosVer3 phosphate powder sachet were added to the sample cell containing the prepared sample. An 8 minute reaction was initiated during which the sample was mixed for 30 seconds and then incubated for a further 7.5 minutes, during which time the excess phosphate powder was allowed to settle to the bottom of the flask. Following the 8 minute reaction, absorbance at 890nm was determined. The blank was prepared by filling a second cell with 25mL of the appropriate 0.45µm filtrate. Results were expressed as mg/L PO_4^{3-}.

2.6.2 ANALYSIS FOR SOLUBLE REACTIVE PHOSPHORUS (SRP)

This assay measured the concentration of bio-available phosphorus for a given water sample. Each sample (25mL) was filtered through a 0.45μm filter (Millipore, Australia). The method for SRP analysis was identical to that for TP, with the exception of the reaction time, which was 2 minutes. Results were expressed as mg/L PO_4^{3-}.

2.6.3 ANALYSIS FOR NITRATE

To each filtered (0.45μm) sample (25mL) activated carbon was added in excess. Activated carbon binds to the colour-generating organics, thus minimising the effects of background colour formation. After further filtration (0.45μm), the samples were analysed for nitrate, again using the DR2000 Spectrophotometer at 400nm. The content of one Nitra Ver5 Nitrate Reagent powder (Permachem Reagents) was added to the sample cell containing the 25mL of prepared sample. A 6 minute reaction period was initiated during which the sample was mixed for 1 minute and allowed to settle for remaining 5 minutes. During this time cadmium reduction of nitrate takes place, resulting in a colorimetric reaction which is then read as absorbance at 400nm. RO water (25mL) was used as a reference. Results were expressed as mg/L NO_3^- -N (nitrate-nitrogen).

2.6.4 COLOUR

Colour of each filtered sample was determined by measuring the absorbance at 456nm in a 50mm glass cell, as described by Bennet and Drikas (1993) using GBC UV/VIS 918 Spectrophotometer.

2.7 UV-VIS ABSORBANCE MEASUREMENTS

Prior to analysis all samples were filtered through 0.45μm membrane (Millipore). Absorbances were measured between 200nm and 700nm using GBC UV/VIS 918

Spectrophotometer. Deionized water from a Millipore Milli-Q system was used as a reference. UV absorbance was measured in a 1cm quartz cell.

2.8 DISSOLVED ORGANIC CARBON (DOC)

DOC was measured using Sievers 820 Total Organic Carbon Analyser. The method is based on UV/persulphate oxidation of samples at room temperature. This method resulted in formation of CO_2 measured by conductivity. The procedure required the use of a 2% potassium persulphate ($K_2S_2O_8$) solution that was added to the UV reactor. Prior to sample analyses, the analyser was calibrated using 0-25ppm total carbon standards (obtained by dissolving reagent grade potassium hydrogen phthalate, pre dried to a constant weight, in organic free water) and 1-20ppm inorganic carbon standards (obtained by dissolving reagent grade anhydrous Sodium Carbonate, pre dried to a constant weight, in organic free water). Reservoir samples were pre filtered through a 0.45µm membrane (Millipore, Australia). Measured IC values were deducted from TC values to obtain the required DOC concentration of the sample.

2.9 DOC CHARACTERISATION

2.9.1 ABSORBANCE AND RELATED ANALYSES

Organic matter absorbs light over a wide range of wavelengths. For example, the majority of compounds that absorb in the UV region are aromatic groups with various degrees and types of substitutions, including mono-substituted and poly-substituted phenols and various aromatic acids (Korshin et al., 1997). As such, spectrophotometric analyses can be used to indicate the properties of the studied organics.

In this study DOC in water samples were characterised using the Specific UV Absorbance (SUVA) (the ratio of A_{254}/DOC ($m^{-1}mg^{-1}L$)), E_2/E_3 (A_{250}/A_{365}), E_4/E_6 (A_{465}/A_{665}) and specific colour (A_{456}/DOC ratio ($HUmg^{-1}L$)).

As there is a correlation between water colour and its humus content (Hautala et al., 2000), specific colour was used as an indication of the humus component of DOC, and thus as an indication of the allochthonous input of DOC.

Seasonal variations, in both colour and A_{254} of dissolved organics, were reported by Hines and Bursill (1987) in several South Australian reservoirs, values increasing after rainy periods. Thus in addition to specific colour, A_{254} and SUVA were also used as an indication of the allochthonous DOC input into the reservoir.

Absorbance changes with changes in aromaticity, total carbon content and molecular weight (Chen et al., 1977, Hautala et al., 2000), and thus E_2/E_3 ratios (ratio of the absorbance at 250nm and 365nm) were used to monitor the changes in aromaticity and molecular weight. Reitner and Herndl (1997), Lindell et al. (1995) and Graneli et al. (1998) used E_2/E_3 ratios as an estimate of the proportion of smaller versus larger organic molecules in natural waters. E_4/E_6 ratios were used to indicate humification, changes in molecular weight and condensation of aromatic carbon (Hautala et al., 2000; Strobel et al., 2001). Detailed explanations into the use of the E_2/E_3 and E_4/E_6 ratios are provided in Section 4.5.

2.9.2 HIGH PRESSURE SIZE EXCLUSION CHROMATOGRAPHY (HPSEC)

High pressure size exclusion chromatography was used to obtain the molecular weight distributions of DOC. This type of chromatography uses tightly packed columns consisting of small, uniform particles (typically less than 10μm) that are operated at high pressure (>500psi) to provide fast, high-resolution chromatograms (Pelekani et al., 1999).

HPSEC applied in this study was based on the method of Chin et al. (1994). A Waters Alliance System with a built in pump, column heater and a mobile phase degassing system was used. These were interfaced with a computer system and Waters Millennium 32 Windows based software, used for data interpretation. Protein columns (Shodex KW-802.5) were used with a molecular weight range of 0.1K – 50K. The system was calibrated using polystyrene sulphonates (PSS): 35K, 18K, 8K, 4.6K and acetone. Samples were run at a flow rate of 1mL/min with an injection volume of 150μl. The mobile phase was 20mM sodium di-hydrogen phosphate adjusted to pH 6.8 using HCl, with an ionic strength of 0.1M using NaCl. Molecular weight of compounds was estimated for UV absorbing (260nm) compounds only.

2.10 BIOLOGICAL OXYGEN DEMAND (BOD)

A 5-day BOD test was used to determine biological activity in the water samples. This test measures the amount of oxygen (mg/L) that bacteria consume when they oxidise organic matter (Hach et al., 1997). To determine the BOD, the amount of oxygen the bacteria use is calculated by comparing the amount remaining at the end of five days with the amount known to be present at the beginning. The method applied in this study is that of Hach et al. (1997). All tests were carried out at the Australian Water Quality Centre, SA Water Corporation, Bolivar, South Australia, and the results are given as mg/L of consumed oxygen.

2.11 CHLOROPHYLL-A

The determination of chlorophyll in aquatic samples is used as an indicator of phytoplankton biomass (Wetzel, 1983; Aleya and Hartmann, 1995; Coveney and Wetzel, 1995; Moran et al., 2002; Ramaiah and Furuya, 2002). The method used in this study is applicable for the determination of chlorophyll concentration in mixed algal populations as well as single algal cultures. The method is based on the concentration of phytoplankton in water samples by filtration, chlorophyll extraction and spectrophotometric analysis of the extract.

Reservoir water samples were stored at 4°C in the dark prior to analysis, which was conducted within 24 hours of sampling. Herve and Heinonen (1982) reported that whole-water samples stored at 4°C in the dark can be kept up to 1 day without significant degradation of chlorophyll. 400mL of sample was filtered through a Whatman Glass MicroFibre filters (GF/C) to collect phytoplankton. The filter was placed into 10mL of 95% ethanol solution in a tube and vortexed for 1 minute in order to release cells from the filter paper and initialise the lysis of algal cells to release chlorophyll pigments. Ethanol samples were placed in the dark at 4°C for 12 hours to allow chlorophyll extraction to take place. The extracts were then centrifuged at 4300 g for 10 minutes. Absorbance was measured on a DR 2000 Spectrophotometer (Hach, USA) at 750nm, 665nm and 649nm using 95% ethanol as the reference. Chlorophyll concentrations were calculated using equations derived by Wintermans and De Mots (1965) for populations of green algae. The following calculations were applied:

Calculation of extinction coefficients

$E665 = (A_{665} - A_{750})/PL$	$E649 = (A_{649} - A_{750})/PL$

Chlorophyll concentration (μg/L)

Chlorophyll a = $[(13.7*A_{665} - 5.76*A_{649})*$Volume of Ethanol] / Volume of Filtrate

Where PL is the path length of spectrophotometer cell (1cm); A_{665} is the absorbance reading at 665nm; A_{649} is the absorbance reading at 649nm.

2.12 TOTAL BACTERIA CELL NUMBERS

The analysis of bacterial abundance involved techniques such as sample filtration, preservation, staining, slide preparation and microscopy.

2.12.1 COLLECTION AND PRESERVATION OF BACTERIAL SAMPLES

10mL of sample was filtered through a Whatman GF/C filter with a pore size of 1.2μm. This was determined to be the optimum size for removing bacterial predators (such as zooplankton) in the Warren Reservoir samples. The 1.2μm filtrate comprising reservoir bacteria was preserved with a 2% formaldehyde solution (Hobbie et al., 1977). Preservation of bacterial cells was obtained by adding 1mL of filtered formalin solution (37% formaldehyde) to 20mL of sample. Preserved samples were stored at 4°C for up to 3 months.

2.12.2 CELL STAINING FOR EPIFLUORESCENCE MICROSCOPY

Acridine Orange (AO) direct counting method was used to determine the total number of bacteria present in reservoir water samples. It is a standard method used for total cell counts of environmental samples (Bowden, 1977). The AO dye interacts with the nucleic material (DNA and RNA) of bacteria in the following manner. The random coil of the RNA allows so many AO molecules to attach and interact, so that the AO fluoresces as a dimer, producing a red-orange fluorescence in active cells (Hobbie et al. 1977). Conversely, the

inactive bacteria comprise mostly DNA, whose rigid structure allows fewer AO molecules to attach, which do not interact and therefore fluoresce as a monomer, producing a green fluorescence.

0.1mg/mL AO storage solution was prepared using filtered sterile Milli-Q water and stored at 4°C. The dilute stain solution was filtered through 0.2µm filter following preparation and again prior to use. AO was added to make a final stain concentration of 0.005% - 0.01% (5 - 10µg/mL respectively).

0.5mL of 0.1mg/mL AO was added to 10mL of preserved sample. Samples were incubated for 2-3 minutes at room temperature after which they were passed through the filtration apparatus (Millipore, Australia). 25mm black 0.2µm filters (Millipore, Australia) were used to collect stained bacteria during filtration. Black background is used as it is non fluorescent and it improves the contrast between the filter and the fluorescent cells. A damp filter was placed on a glass microscope slide. A drop of non-fluorescent immersion oil was added on top of the filter and a coverslip was placed over the sample to complete slide preparation.

2.12.3 CELL NUMBERS AND BIOMASS

An Epifluorescent microscope (Olympus, USA) was used to provide fluorescence for counting the previously stained bacterial cells. Counting was performed at 1000X magnification, as described by Simek et al. (2003). Prepared slides were scanned for random cell distribution. Vertical and horizontal lines were followed to ensure optimum slide coverage. 30 fields were selected across the filter, each field comprising a 100 square grid contained in an eyepiece. By adopting this method, at least 300 cells were counted and at least 100 cells were measured per filter, which is in agreement with the minimal requirement used by other researchers (Maki and Remsen, 1981; Riemann and Sondergaard, 1984; Lindell et al., 1995; Lind et al., 1997; Benner and Biddanda, 1998; Bergstrom and Jansson, 2000; Brugger et al., 2001; Lemee et al., 2002; Montserrat Sala et al., 2002; Mille-Lindblom and Tranvik, 2003). The total cell number of each sample was calculated by taking into account sample volume, area of each square and total area of the filter. Final results were expressed as cells/mL.

The second eyepiece of the microscope was equipped with a measuring grid enabling measurement of cell size (length and width). Cell volumes were calculated from the formula $V=4/3\pi r^3+(\pi r^2(l-2r))$, which approximates each cell as a cylinder with a hemisphere at either end (r=radius (w/2) and l=length of the cell) (Lindell et al., 1995). Using bacterial concentration data and bio-volume data, population biomass was estimated using the conversion factor of $0.308 pgC \mu m^{-3}$ (Bergstrom and Jansson, 2000).

2.12.4 PREPARATION OF A BACTERIAL INOCULUM

Bacterial inocula used in these laboratory studies were prepared by filtering reservoir water through a 1.2μm Whatman Glass MicroFibre filters, thereby excluding bacterivores. Yields of various filter sizes (0.45 μm, 0.65 μm, 0.8 μm, 1.2 μm and 2 μm) were measured, and 1.2 μm filters were selected, for they provided the highest bacterial numbers whilst removing bacterivores.

2.13 BACTERIAL COMMUNITY ANALYSIS

2.13.1 DNA EXTRACTION

Bacterial community analysis was performed using the molecular techniques of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE). Application of these two techniques on water samples from Warren Reservoir was initially unsuccessful due to the insufficient DNA recovery. Subsequently, samples were concentrated prior to DNA extraction by the Tangential Flow Filtration (TFF) method (Pickup et al., 1995). The TFF unit is designed to concentrate bacteria from water. 20L of raw sample water was passed through the unit, concentrating cells to a final volume of 1L. The unit was cleaned with sodium hypochlorite and sodium hydroxide solutions, followed by rinsing with 10L of RO water in between samples. 400mL of concentrated sample was used for DNA extraction, and the remainder stored at -20°C.

400mL of TFF concentrate was centrifuged at 19000g for 30 minutes. The pellet was resuspended in sterile Milli-Q water to a final volume of 300μl. Ultraclean Soil DNA

Isolation Kit (Mo Bio Laboratories) was used to extract DNA. The procedure is that used by Mo Bio Laboratories for extraction of DNA from soils, with minor alterations applied for water samples. Namely, the initial volume of sample used was 300µl and the final elution step was carried out using sterile Milli-Q water as opposed to Solution S5 of the kit. Final product was stored at –20 °C.

2.13.2 AMPLIFICATION OF 16S RDNA SEQUENCE

PCR is a method that employs oligonucleotide primers and thermostable DNA polymerase to amplify target DNA sequences by temperature-controlled cycles of strand separation, primer annealing and primer extension. Primers can target phylogenetic groups from the strain to the 'universal' level (MacGregor, 1999). For this study, the bacterial population diversity in the reservoir samples was determined through the analysis of 16S ribosomal RNA gene diversity (rDNA) using a set of universal primers binding to conserved regions of the 16S rDNA. Various primer sets were applied, however the best amplification results were generated using the following set of 16S rDNA primers:

Forward Primer

27F-GC	CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCC CGG GGG GAG AGT TTG ATC CTG GCT CAG

Reverse Primer

534R	ATT ACC GCG GCT GCT GG

The two universal primers in combination with each other or with other primers, are commonly used for the amplification of bacterial 16S rDNA (Dahllof et al., 2000; Rheims et al., 1996; Amann et al., 1996; Blackall, 1994; Sperveslage et al., 1996; Suzuki et al., 1997). Problems of inhibition of PCR amplifications, due to interferences of humic substances co-extracted with nucleic acids, were overcome by optimising serial dilutions to provide best yield of amplified DNA. Final dilution of DNA extracts used as template during PCR was 1/10.

Following optimisation of the PCR reaction master mix, final reagent concentrations adopted are shown in Table 2.1.

Table 2. 1: PCR reagent concentrations and volumes.

Reagent	*Initial Concentration*	*Final Concentration*	*Volume (µl)*
Buffer	10 X	1 X	2.5
dNTP	1.25mM	200µM each	4
$MgCl_2$	25mM	1.5mM	1.5
Primer 1	1µM	0.2µM	5
Primer 2	1µM	0.2µM	5
Taq enzyme	5U/µl	0.07U/µl	0.35
DNA			2.5
H_2O			4.15
Final mix			*25*

Specifications of final PCR amplification cycles obtained from the optimisation of PCR conditions are shown in Table 2.2.

Table 2. 2: PCR amplification cycles

Step	Cycle	Temperature (°C)	Time	Number of cycles/step
1	Initial denaturation	94	4′	1
2	Denaturation	94	1′	10
	Primer annealing	65 -1°C/cycle	1′30″	
	Elongation	72	1′30″	
3	Denaturation	94	1′	20
	Primer annealing	55	1′30″	
	Elongation	72	1′30″	
4	Final elongation	72	8′	1
5	End of reaction	4	Hold	1

PCR product confirmation was achieved using 2% TBE AquaPor agarose gels and the pGEM molecular weight markers (Promega). Agarose gels were photographed using a UV

transilluminator (BioRad). Figure 2.4 represents an example of the agarose gel stained for 15 minutes with ethidium bromide, where the 524bp DNA fragment was obtained during the PCR. In the example shown in Figure 2.4, the first lane labelled with 'M' represents the pGEM molecular weight marker whose size is indicated to the left of the column in base pair (bp) units. S1, M1, D1 and D2 are bacterial DNA samples isolated on the 1st September 2000 from the four sites in the Warren Reservoir. Lane 10, marked with 'N' represents a negative PCR control using sterile Milli-Q water as a template, while the last five lanes marked with '*E.coli*' represent positive PCR control where DNA extract from *Escherichia Coli* bacterium was used as a template. Figure 2.4 shows a presence of a single band confirming the correct amplification of the DNA fragment, approximately 524bp in length, for the four environmental samples. PCR amplification of *E.coli* resulted in the formation of one dominant band 524bp in length, and a second less prominent band approximately 1000bp in size. The band present at the top of each *E.coli* lane represents genomic DNA.

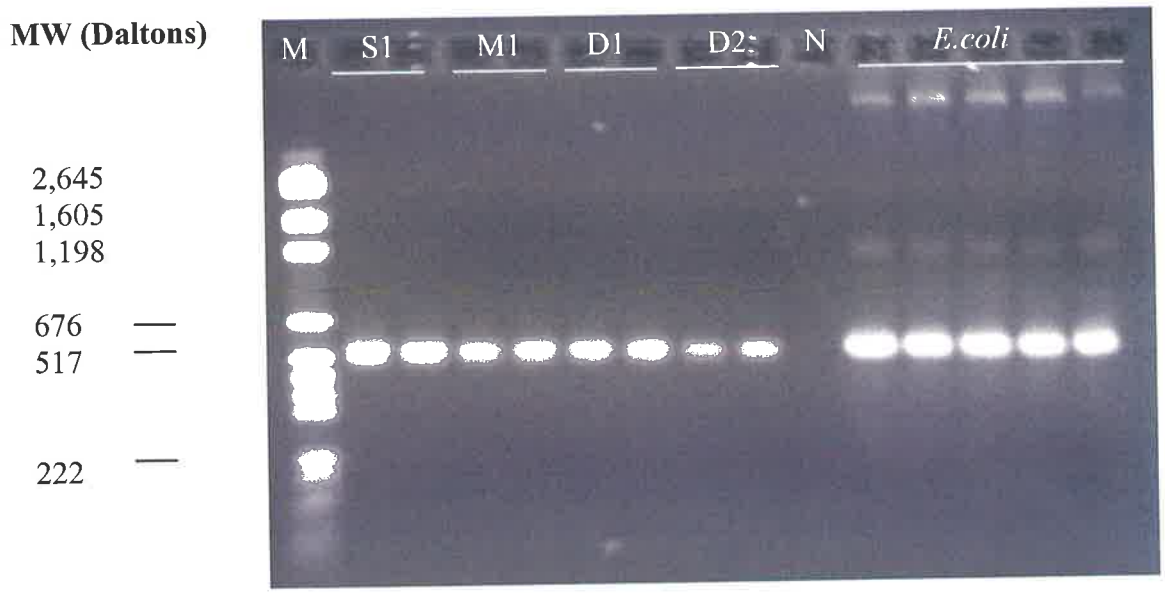


Figure 2. 4: Agarose gel electrophoresis analysis of the 524bp PCR products. DNA extract from a mixed bacterial population within the four reservoir sites S1, M1, D1 and D2 was used as template. Samples were collected in September 2000. Lane 1 contains the pGEM molecular weight marker (M), lanes 2-9 are Warren samples, lane 10 contains the negative control (N) and lanes 11-15 contain E.coli as the positive control during PCR amplification.

2.13.3 DENATURING GRADIENT GEL ELECTROPHORESIS (DGGE)

A perpendicular gradient gel with broad denaturing gradient range (0-80%) was used to determine optimum denaturing conditions for a randomLy selected reservoir sample, as described by Diez et al. (2001). Once a suitable denaturing gradient was determined, parallel DGGE gels were employed, where the range of denaturant was narrowed to allow better separation of fragments. Based on the size of the amplified DNA fragments, an 8% polyacrylamide gel was selected for optimum separation. Final concentrations of the denaturing solutions (based on urea and formamide concentrations) ranged from 46% to 52%.

DGGE gels were obtained with the use of The DCode^{TM} Universal Mutation Detection System (BioRad). Gel casting techniques for both perpendicular and parallel DGGE gels are provided in The DCode^{TM} Universal Mutation Detection System manual.

DGGE gels were prepared using 1 X TAE buffer at a constant temperature of 60°C. Samples were run into the gel at 170V for 5 minutes (to allow DNA to migrate into the gel thus reducing the potential for sample mixing due to circulation pump stirring the samples in the wells), followed by 60V for 23 hours.

Silver Stain Kit (BioRad) was used for DNA staining on the polyacrylamide gels. The procedure provided by the manufacturer was applied with a minor alteration. In the procedure, all three fixatives involved the use of acetic acid, however, for the purpose of this study, the use of acetic acid was omitted, as it mainly applies to proteins and not DNA. The DNA on the gel was fixed by 400mL of 40% methanol solution for 30 minutes. The second fixing solution (10% ethanol, 5% acetic acid) was repeated twice for 15 minutes each time. This was followed by exposure to 200mL of the 10% oxidiser solution for 5 minutes. The gel was thoroughly washed in Milli-Q water until the yellow colour resulting from excess oxidising solution was removed. 200mL of silver reagent was added to the gel for 20 minutes. Excess silver stain was removed by rinsing the gel in Milli-Q water once. Following this, 200mL of the developing solution was applied to the gel. The developing reaction was stopped, once a clear banding pattern was obtained, by adding 400mL of a 5% acetic acid solution.

Stained gel was placed between two moist cellophane sheets on a gel drier (BioRad) and preserved by drying at room temperature for 2 days. Gels were photographed using a transilluminator (BioRad). An example of a silver stained DGGE gel is shown in Figure 2.5.

Based on the banding patterns obtained in the DGGE gels, further analysis into population diversity are possible.

Figure 2. 5: An example of a DGGE gel showing banding patterns of different samples. Lanes 1, 8 and 16 contain the banding patterns of the E.coli marker whereas the other lanes contain Warren Reservoir S1 and D2 samples under different experimental conditions.

2.13.4 CHANGES TO BACTERIAL POPULATIONS

Principal Component analysis (PCA) was used to assess changes in bacterial populations, based on seasonal influences or changes in experimental conditions (Romani and Sabater, 2000; Brugger et al., 2001; Keinanen et al., 2002; Stepanauskas et al., 2003). Canonical Correspondence analysis (CCA) was performed between the bacterial community structure and the physical, chemical and biological variables of the reservoir samples. CCA was designed to elucidate which physical, chemical and biological variables most influenced the variation in bacterial community structure (Lindstrom, 2000; Romani and Sabater, 2000).

Both analyses were based on the information into the band presence/absence on a silver stained gel (Figure 2.5), as well as the differences in peak intensities of each band in the DGGE gel, obtained by using the Diversity Database software (BioRad). An example of the band intensity graph obtained by using Diversity Database software is shown in Figure 2.6.

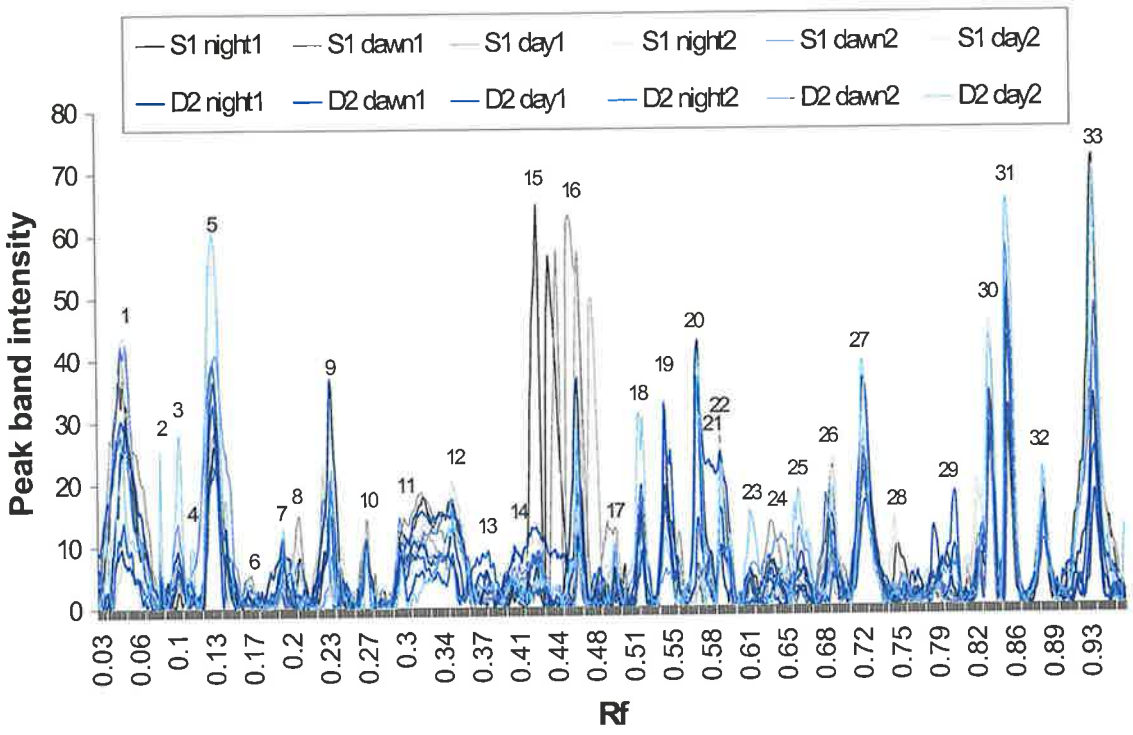
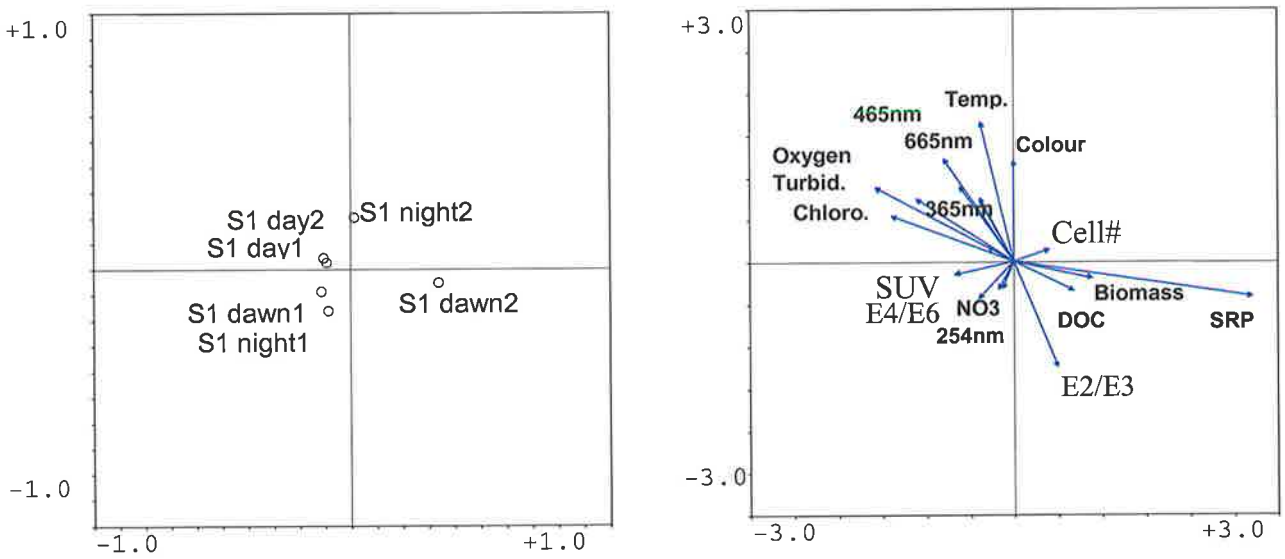


Figure 2. 6: Banding intensities for each sample on silver stained gel are plotted and peak intensities of each band are determined. The Rf value represents the distance the band has travelled in a gel, while the numbers 1-33 represent the number of bands in a sample.

Data of peak band intensities were analysed by PCA and CCA (using CANOCO software), to generate two dimensional PCA and CCA ordination diagrams. The importance of the horizontal and vertical axes on the separation of the data are determined and expressed as percentage (%) explanation of the variance by the X and Y axes (Figure 2.7). In this example, the weight of the X axis on sample separation was higher (51%) than the weight of the Y axis (26%). Thus the differences along the horizontal axis, for example between S1 night1 and S1 dawn2 are stronger than the differences along the vertical axis, for example between S1 night1 and S1 night2.



(A) *(B)*

Figure 2. 7: Principal component analysis (PCA) and Canonical correspondence analysis (CCA) ordination diagrams showing distribution of samples based on differences in bacterial populations during the diel winter study of the shallow site (weighted X axis = 51%; Y axis=26%). Differences between bacterial populations (A) are based on changes in environmental variables (B).

Therefore, the PCA diagrams provide insight into differences between bacterial populations inhabiting different samples without taking the environmental data into account. The CCA quantifies the amount of variation in the community composition explained by a single variable or sets of explanatory variables. The length and direction of the vectors in

CCA ordination diagrams (Figure 2.7 B) indicate the importance of the association of each parameter with bacterial populations. The longer the vector and the closer its position to the axis of higher influence (in this case the X axis), the more important the association is between that parameter and the bacterial population changes. The significance of the relationship between the explanatory variables and community composition was tested using Monte Carlo permutation tests.

CHAPTER 3 - DOC IN THE WARREN RESERVOIR

3.1 INTRODUCTION

This study of the Warren Reservoir aimed to identify the potential sources of DOC in the reservoir and estimate their relative contributions to the total reservoir DOC concentrations. Pine plantation *(P.radiata)* surrounding the shallow regions of the Warren Reservoir had previously been reported to release relatively high DOC concentrations through the catchment run-off (Recknagel et al., 1998). However, as the catchment was dominated by open parklands, with some large concentrations of natural woodland *(E.leucoxylon, E.goniocalyx)*, other sources were also believed to be contributing to significant loading of DOC into the reservoir.

This study also focused on establishing relationships between DOC concentration in the water column and abiotic and biotic parameters of water quality, in the aim of elucidating which water parameters were directly or indirectly associated with DOC cycling in the reservoir. Of particular interest to this chapter was the relationship between DOC and the reservoir bacterial communities.

The two objectives of this study were:

1. To investigate seasonal fluctuations of DOC concentrations in the reservoir and to correlate measured DOC concentrations with possible autochthonous sources (such as phytoplankton growth) or allochthonous sources (such as surface runoff caused by rainfall events or inflow of water from the Murray River).
2. To observe the relationships (unilateral or bilateral) between DOC and certain physical, chemical and biological parameters of the reservoir in order to identify factors associated with DOC cycling.

3.2 MATERIALS AND METHODS

For a detailed description of the reservoir, sampling sites and regime, please refer to Sections 2.2, 2.3 and 2.4 of the General Materials and Methods.

3.3 WATER LEVELS IN THE WARREN RESERVOIR

Data on the water volume of the Warren Reservoir from 1997 to 2001 was obtained from the South Australian Water Corporation, and is presented in Figure 3.1. The mean water volume in reservoir during the study was 4435±24 ML. Significant decreases in water levels were measured between January and August 1998 (Mean ± S.E. 15±2%), and between March and August 2000 (Mean ± S.E. 34±9%). In 1998 water volume dropped from 4756±31ML to the lowest measured levels of 3657±3ML in April 1998. In 2000, water volume decreased from 4856±7 ML to the lowest measured level of 2051±32 ML in June 2000. These decreases were due to water being withdrawn from the Warren Reservoir.

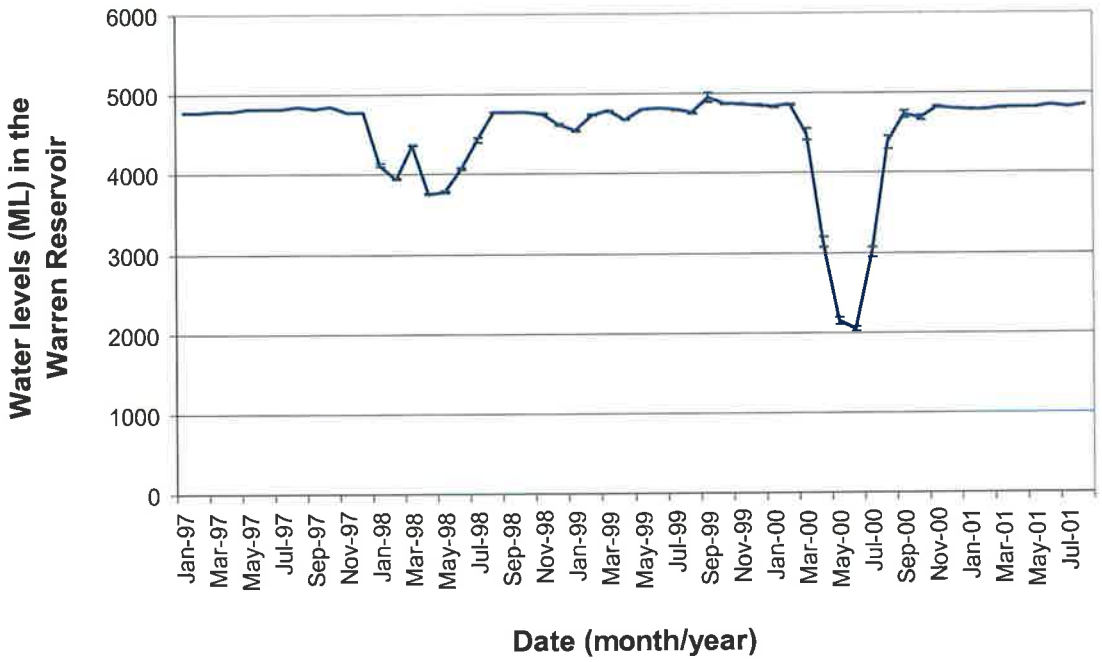


Figure 3. 1: The mean monthly water volume (ML) in the Warren Reservoir from 1997 to the end of the study period in August 2001 (SA Water Corporation). Plotted data is the monthly mean ± S.E. Maximum water volume was 4770ML at which point overflow into the South Para River occurred.

3.3.1 MURRAY RIVER INFLOW TO THE WARREN RESERVOIR

The monthly volumes (ML) of water entering the reservoir from the Murray River pipeline at the M1 site are shown in Figure 3.2. Inflow of water from the Murray River commenced in May 1998 and ended in August 1998, then again in January 1999 and it continued to flow until the end of the study period in August 2001. During the studied period and when the pipeline was operational, the monthly inflow of water from the Murray River pipeline ranged from 144±6 ML to 434±1 ML.

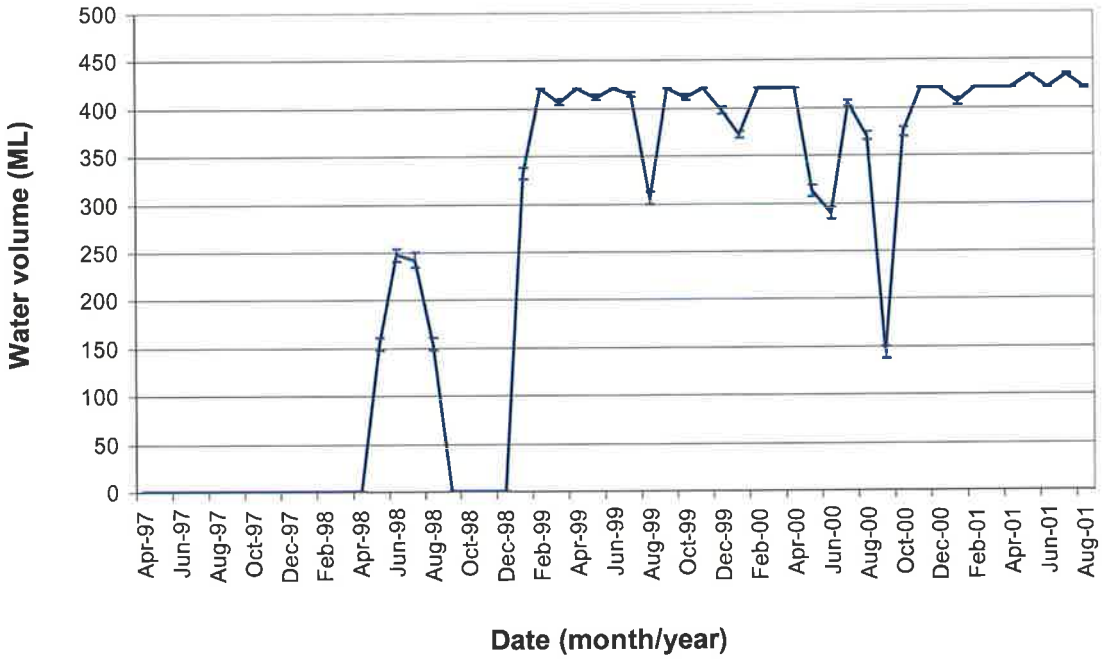


Figure 3. 2: Total monthly volume of water (ML) entering the reservoir from the Murray River pipeline at the M1 site from April 1997 to August 2001 (SA Water Corporation, personal communication). Plotted data is the monthly mean ± S.D.

Using the data presented in Figures 3.1 and 3.2, the quantity of water entering the reservoir through the Murray River pipeline was estimated as the percentage of the mean reservoir volume (Figure 3.3). This was done for the purpose of determining the total monthly contributions of the Murray River flow to the total reservoir volume, particularly in terms of its contribution to the total DOC quantity in the reservoir. The estimated monthly contribution of water from the Murray River to the Warren Reservoir varied from 3% to 6%

during 1998, and remained between 6% and 15% from January 1999 until August 2001, except for September 2000 when it was estimated as 3% (Figure 3.3).

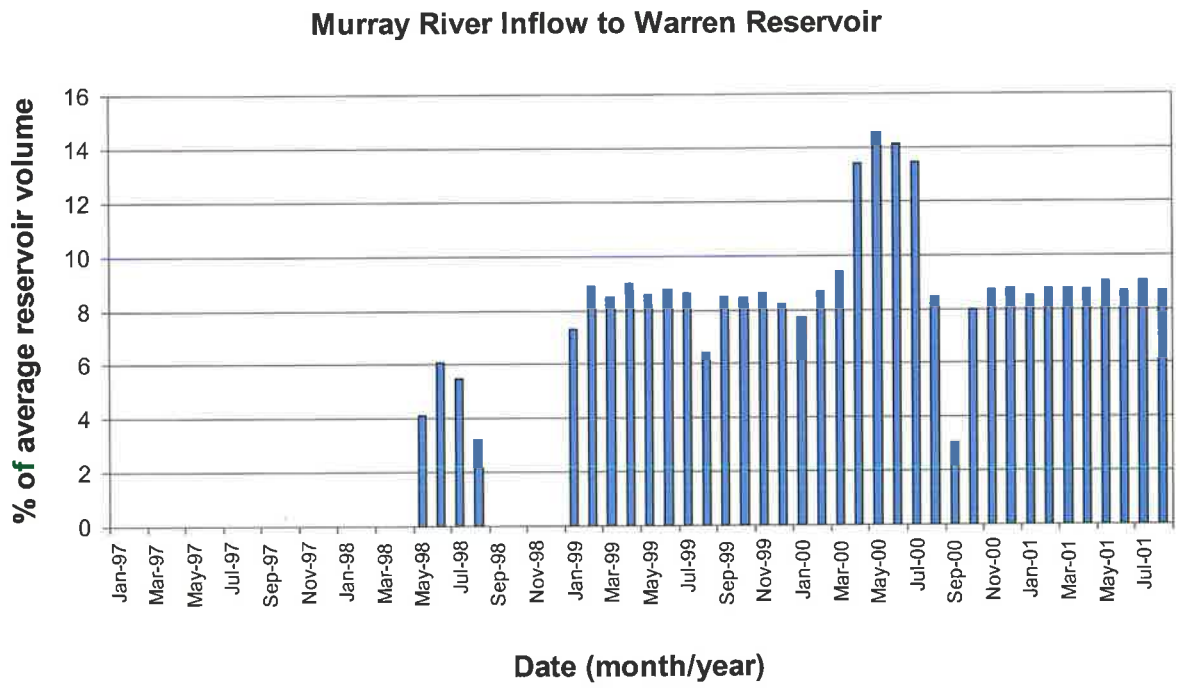


Figure 3. 3: Murray River inflow to the Warren Reservoir expressed as the percentage (%) of the average reservoir volume, from 1997 until the end of the study period in August 2001.

3.3.2 RAINFALL EVENTS

In order to estimate the expected timing for the inflow of water from the two major catchment streams (C-S1 and C-S2), and thus potential allochthonous DOC loading into the reservoir, daily rainfall data was obtained from the Bureau of Meteorology (Adelaide, South Australia), for the duration of the study period between April 1997 and August 2001. The rainfall data is summarised in Figure 3.4. In general, the wet season of each year began around May and lasted until October, although sporadic rain periods were recorded at other times, particularly at the end of summer (March to April). The total measured annual precipitation levels were 554±13mm in 1997, 650±11mm in 1998, 647±11mm in 1999, 713±10mm in 2000 and 831±16mm in 2001.

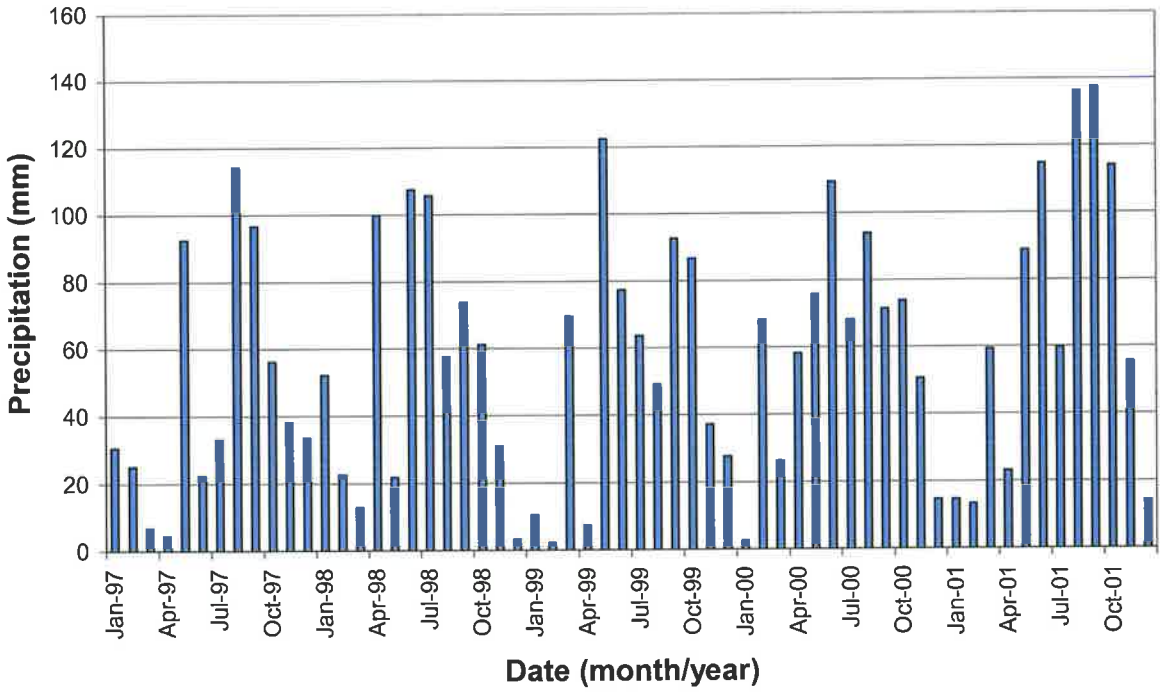
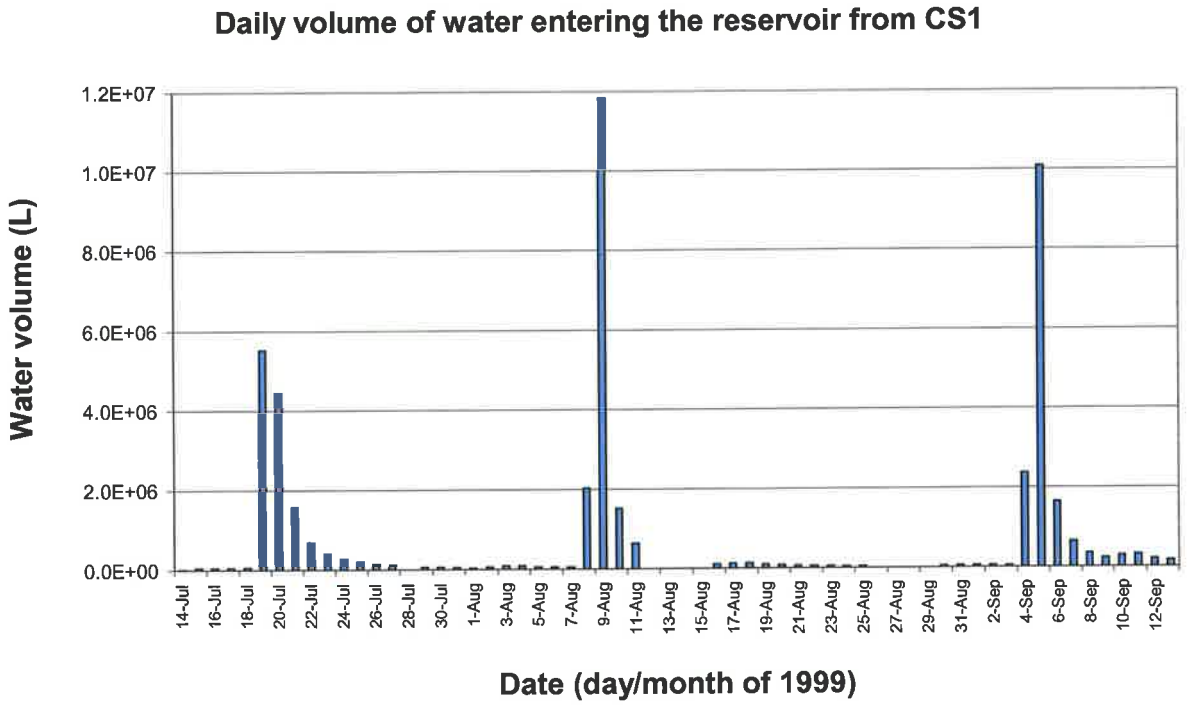


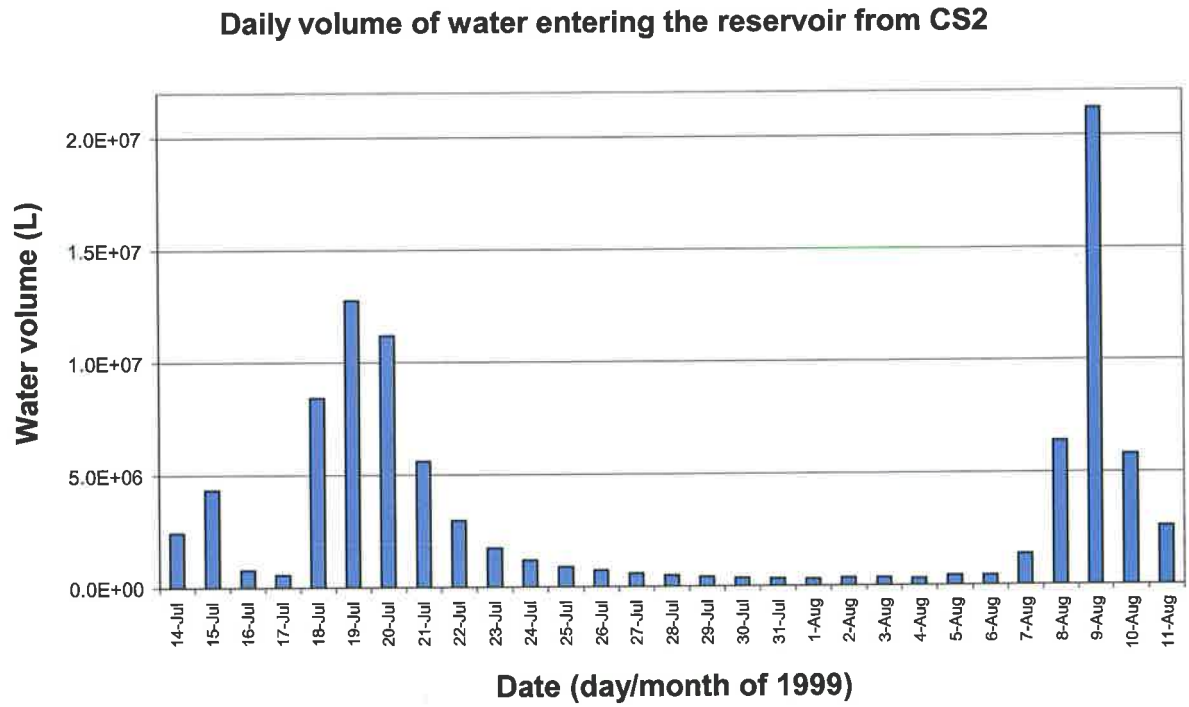
Figure 3. 4: Monthly rainfall data for the Warren Reservoir from April 1997 to August 2001 (Bureau of Meteorology, Adelaide).

3.3.3 CATCHMENT STREAM INFLOW

Rainfall periods between May and October (Figure 3.4) generally produced flow of water in the main catchment streams C-S1 and C-S2 from July to October. In order to estimate the quantity of the DOC delivered to the reservoir via the two streams, the daily stream flow data was measured during winter 1999, on a 24-hour basis (Appendix 1). Using this data, the total daily volume of water entering the reservoir was calculated and presented in Figure 3.5. Water flow data at C-S1 was gathered between 14 July and 12 September 1999, whereas at C-S2 the data was collected between 14 July and 11 August due to the technical problems with the data logger measuring instrument. During the studied periods, the daily water levels entering the reservoir via the two streams ranged from 0.02 ML to 12 ML from C-S1 and 0.3 ML to 22 ML.



A. C-S1

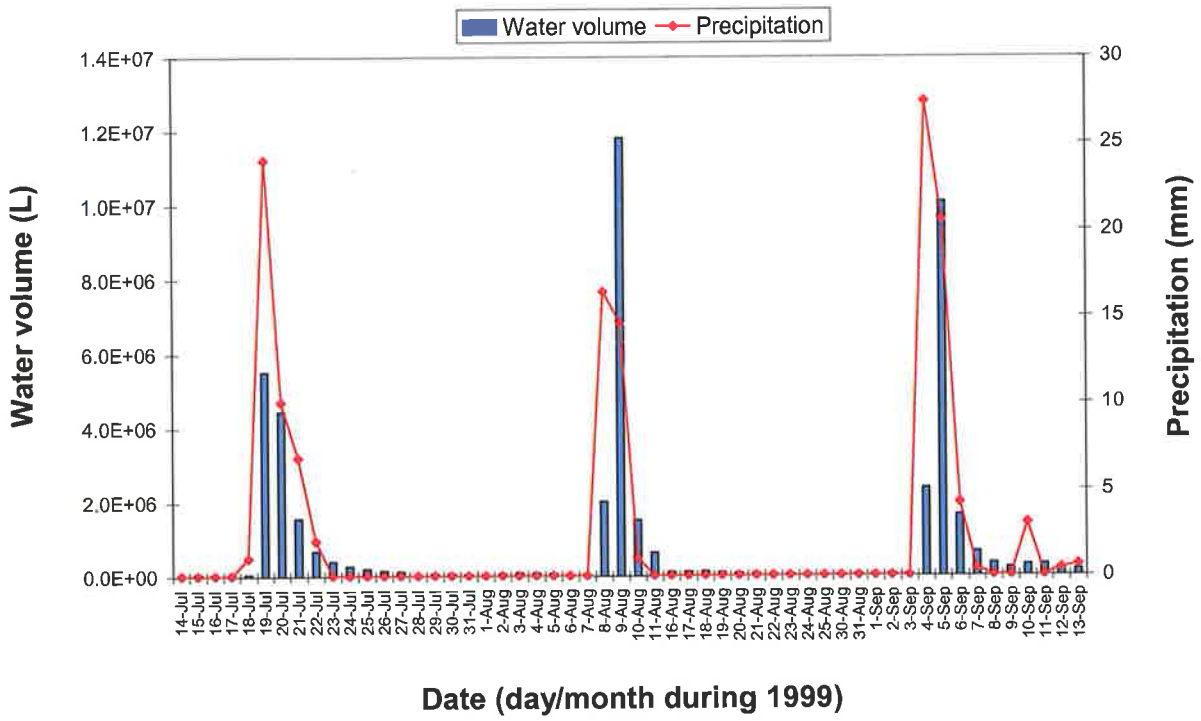


B. C-S2

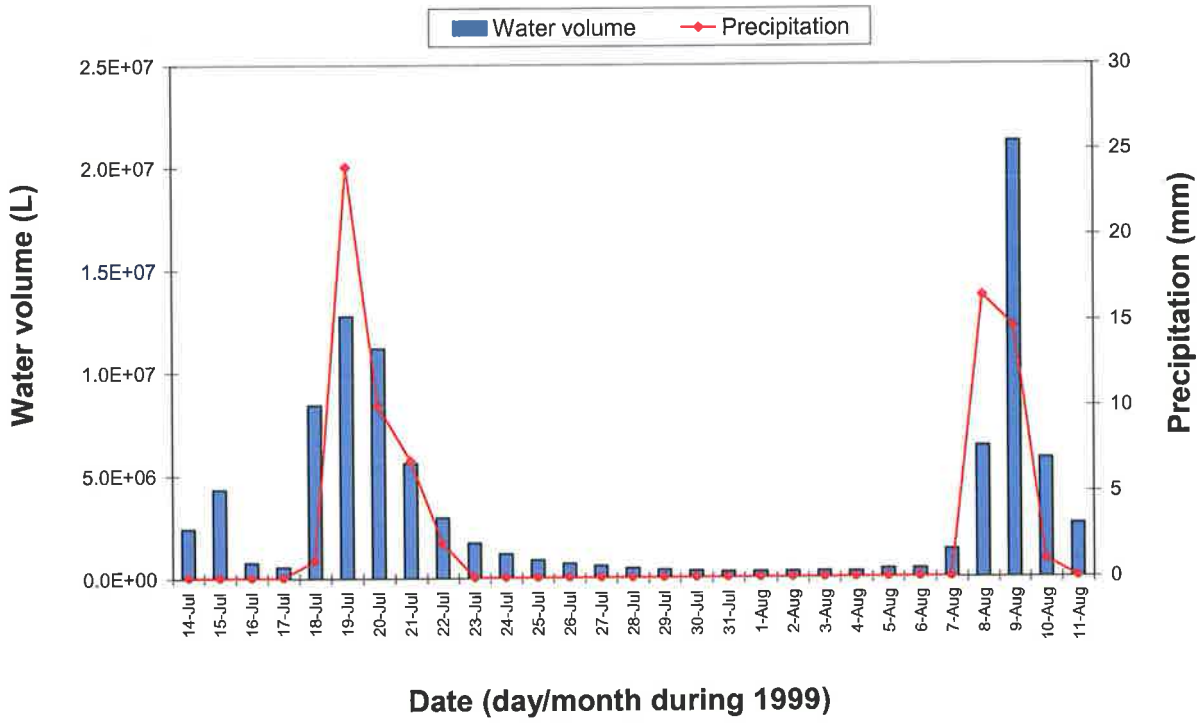
Figure 3. 5: Daily volume of water entering the Warren Reservoir from the two catchment streams (A) C-S1 and (B) C-S2 during the rainy season of 1999.

Based on information presented in Figure 3.5, the total amount of water entering the reservoir via the two streams was 55±2 ML from C-S1 (between 14 July and 13 September) and 95±5 ML from C-S2 (between 14 July and 11 August).

The volume of water inflow from C-S1 and C-S2 streams to the reservoir was correlated to the rainfall data (Figure 3.4) for the area during the same period (Figure 3.6). The data presented in Figure 3.6 shows a strong correlation (R=0.75 and R=0.79) between the level of rainfall and the amount of water entering the reservoir from C-S1 and C-S2 respectively. Based on this strong correlation between the amount of rainfall and the volume of water flow from the catchment streams to the reservoir, estimation was made on the mean volume of stream flow per millimetre of precipitation. This was then used to estimate the volume of water entering the reservoir via the catchment streams for the entire stream flow season of winter 1999.



A. C-S1



B. C-S2

Figure 3. 6: Plot of the rainfall level (mm) and the level of water entering the reservoir from the two catchment streams (A) C-S1 and (B) C-S2. Correlation coefficient for the C-S1 data is R=0.75 and for C-S2 R=0.79.

The estimated mean volume of stream water flow per millimetre of precipitation was calculated as being 1.2±0.4 ML. Based on this, the estimated total water levels entering the reservoir via the two catchment streams C-S1 and C-S2 during the entire wet season of 1999 were 142 ML from C-S1 and 306 ML from C-S2.

The total stream water volume is presented as the percentage of the mean reservoir volume during the entire wet season of 1999 (Table 3.1), in order to determine the potential annual contributions on the stream water DOC to the total DOC quantity in the reservoir.

Table 3. 1: Stream water inflow to the Warren Reservoir expressed as the percentage (%) of the mean reservoir volume during period of stream flow, between July and October 1999.

	Average reservoir volume (ML)	Total volume of stream water entering the reservoir (ML)	% of mean reservoir water
C-S1	4770±5	142	3.0%
C-S2	4776±5	306	6.5%

Thus the annual input of combined (C-S1 and C-S2) stream water during 1999 was estimated to be not more than 10% of the average reservoir volume.

3.4 DISSOLVED ORGANIC CARBON IN THE WARREN RESERVOIR

3.4.1 DOC LEVELS OF THE WARREN RESERVOIR

The DOC concentrations in water samples collected between 1997 and 2001 at the four reservoir sampling sites are detailed in Figure 3.7. Figure 3.7 also shows periods when two reservoir management strategies were applied, namely $CuSO_4$ treatment for controlling algal growth and artificial aeration for preventing stratification at the deep site D2, thus controlling algal growth by creating conditions of light limitation.

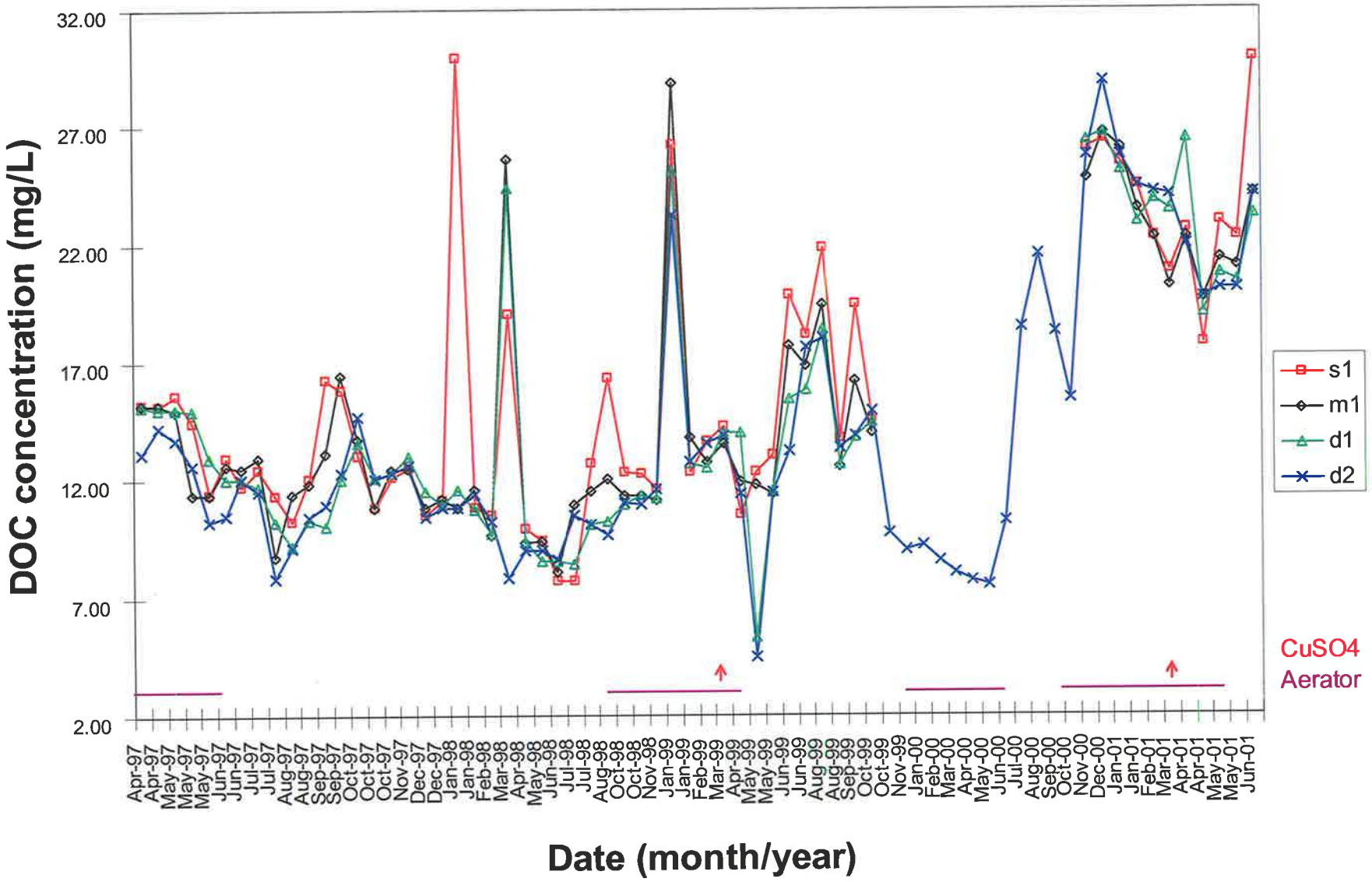


Figure 3. 7: DOC concentrations (mg/L) of water sampled from 1997 to 2001. The figure details the dates of $CuSO_4$ treatment and when an aerator was operating.

Based on data presented in Figures 3.1 and 3.7, the mean monthly quantity of DOC present in the Warren Reservoir was calculated and results shown in Figure 3.8.

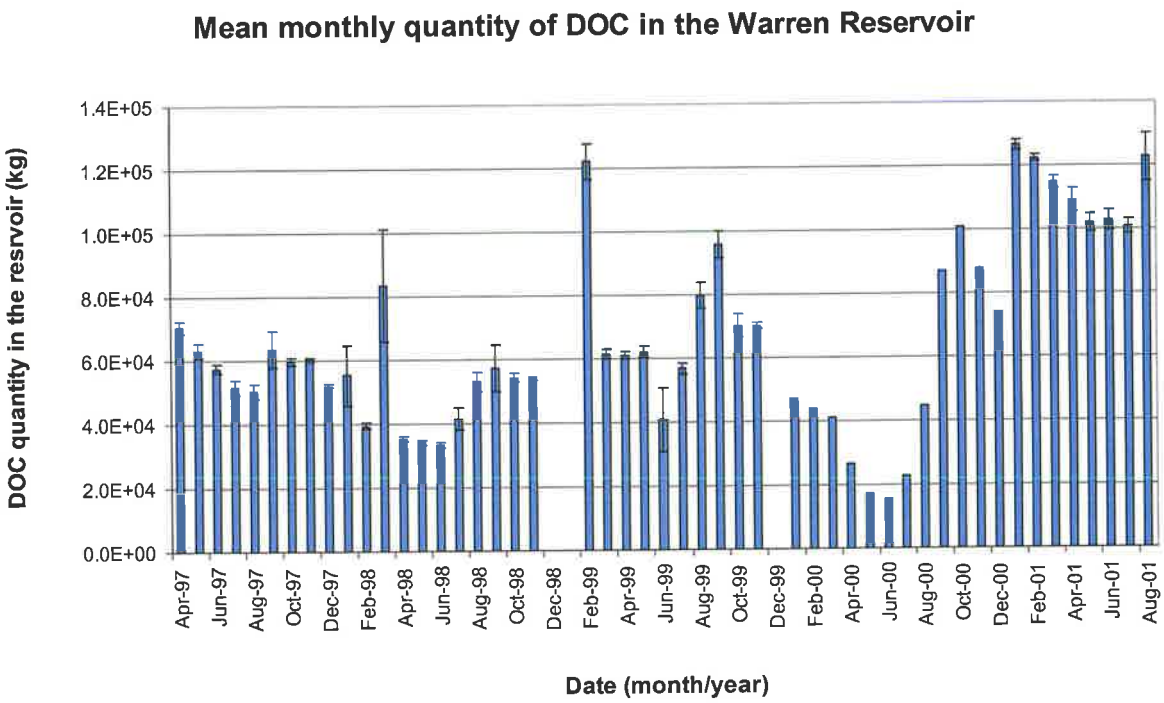


Figure 3. 8: Variations in the monthly load of the DOC (kg) expressed as a mean measure across the reservoir, between April 1997 and August 2001. 2000 data is based on DOC quantity measured at the D2 site only.

On an annual scale, the mean quantity of DOC in the reservoir was estimated as 5.9±0.2 x 10^4 kg in 1997, 4.9±0.4 x 10^4 kg in 1998, 7.2±0.7 x10^4 kg in 1999, 5.1±0.9 x 10^4 kg in 2000 and 14±0.4 x 10^4 kg in 2001. Thus the quantity of reservoir DOC increased over 2 fold from the start of the study period in 1997 to the end of the study in 2001.

An annual increase in the reservoir DOC concentration was observed between 1997 and 2001 (Table 3.2). In 2001, peak summer DOC concentrations were comparable to those measured during the previous years (Figure 3.7). However, the levels of DOC remained at an elevated level through autumn and into winter 2001, whereas in previous years DOC concentration quickly declined following the summer peak. Thus the mean DOC

concentration was significantly higher in 2001 compared to those of the previous years. Mean annual increases in DOC concentrations during the study period are summarised in Table 3.2.

Table 3. 2: Mean annual DOC concentrations (mg/L) at the four sites S1, M1, D1 and D2, from 1997 to 2001. Maximum increases in DOC concentration during the 1997 to 2001 sampling period are expressed as percentage (%). One-way ANOVA was conducted to test the significance of the difference in DOC data between 1998 and 2001.

DOC (mg/L)	S1	M1	D1	D2
1997	12.9±0.5	12.6±0.5	12.3±0.6	11.6±0.4
1998	13.1±1.7	11.7±1.2	11.2±1.2	10.0±0.3
1999	16.1±1.3	15.4±1.3	14.3±1.2	13.9±1.2
2000	n/a	n/a	n/a	12±1.4
2001	23.7±1.0	22.9±0.7	23.5±0.8	23.5±0.9
%	85%*	95%*	110%*	134%*

**Anova LSD P≤0.0001*

In addition to the annual increase in reservoir DOC from 1997 to 2001, a further seasonal trend was found. DOC concentrations in the reservoir showed seasonal variation during summer months, January to March (except in 2000), and during late winter months, August to September (Figure 3.7, Table 3.3). A T-test was used to determine the significance of these DOC variations with respect to levels measured prior to and/or post the DOC peaks. DOC levels measured between January and March were compared to DOC levels measured in the months prior to January and/or post March. Likewise, the DOC levels measured between August and September were compared to DOC levels measured in the months prior to August and/or post September. The T-test results are summarised in Table 3.3.

Table 3. 3: Mean summer and winter variations in the DOC concentrations (mg/L) across the reservoir, from 1997 to 2001. A T-test was conducted at a confidence level $\alpha=0.05$, to test the significance of the seasonal variations in DOC.

	SUMMER		WINTER	
	%	*p*	%	*p*
1997	N/A	N/A	18.3±1.1	*p<0.05*
1998	28.3±4.6	*p<0.05*	21.6±4.8	*p<0.05*
1999	32.1±3.4	*p<0.05*	39.2±1.9	*p<0.05*
2000	8±3.2	*p>0.05*	44±2.3	*p<0.05*
2001	21.5±1.1	*p<0.05*	18.5±4.4	*p<0.05*

In terms of spatial differences in the reservoir DOC concentrations, ANOVA showed no significant differences between sites S1, M1, D1 and D2 in 1997 (p=0.22), 1998 (*p=0.34*), 1999 (*p=0.59*) and 2001 (*p=0.89*).

Furthermore, the application of artificial aeration in the deepest region of the reservoir (where site D2 is located) did not appear to affect surface DOC concentrations at this site when compared with values prior to and post this event. However, levels of DOC were higher prior to $CuSO_4$ treatment (used to control algal blooms) on both occasions, March 1999 (DOC levels reduced by 50±2%) and April 2001(DOC levels reduced by 14±4%).

3.4.2 INFLOW OF DOC FROM MURRAY RIVER PIPELINE TO THE RESERVOIR

Data on the DOC concentrations of the Murray River water between 1997 and 2001 was obtained from the South Australian Water Corporation, and is presented in Figure 3.9. DOC concentrations of the Murray River water were performed on samples collected at the point of uptake of the Murray River to the pipeline. DOC concentrations entering the reservoir via the pipeline varied between 3 mg/L and 11 mg/L. Murray River DOC concentrations entering the reservoir conformed to a seasonal pattern, with DOC concentrations generally increasing at the end of summer and beginning of autumn (February

to March). Mean increases in DOC concentration during this period were 55±10%, reaching peak concentrations around April of each year.

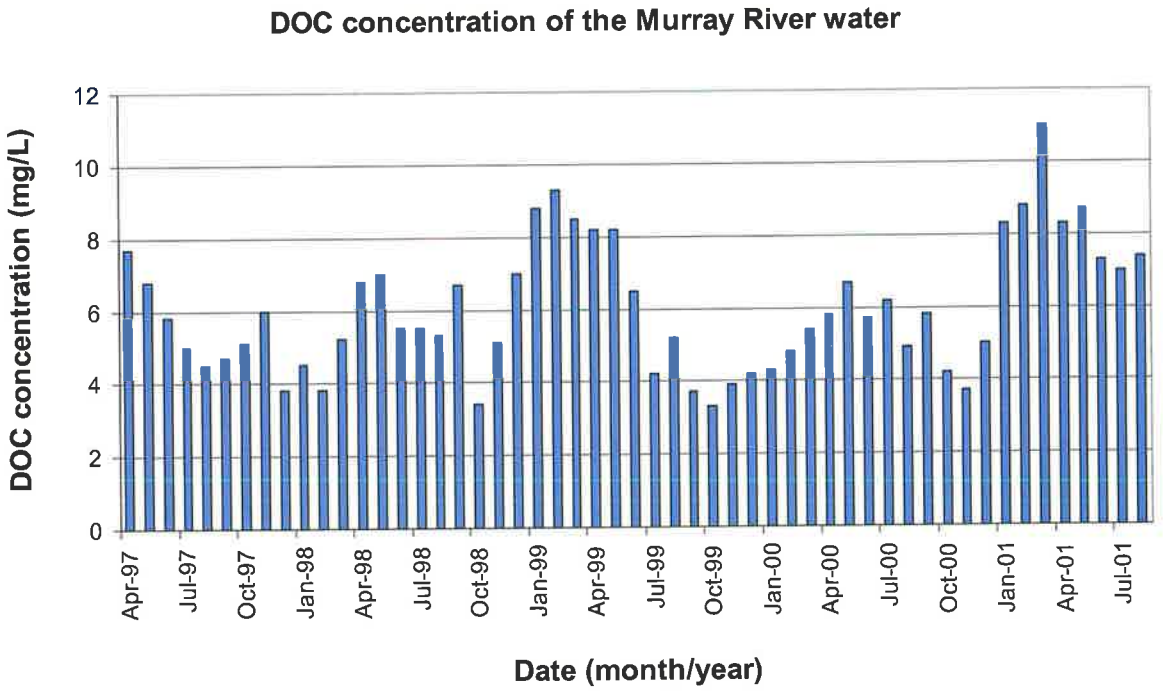


Figure 3. 9: DOC concentration (mg/L) of the Murray River water collected between April 1997 and August 2001 (SA Water Corporation).

Based on the data presented in Figures 3.2 and 3.9, monthly loads of DOC to the reservoir from the Murray River pipeline was calculated and results summarised in Figure 3.10. Monthly DOC load from the Murray River into the reservoir from 1998 to 2001 varied between 816 kg and 4620 kg. There was no DOC contribution from the Murray River to the Warren Reservoir during 1997, as the pipeline was not operating until 1998.

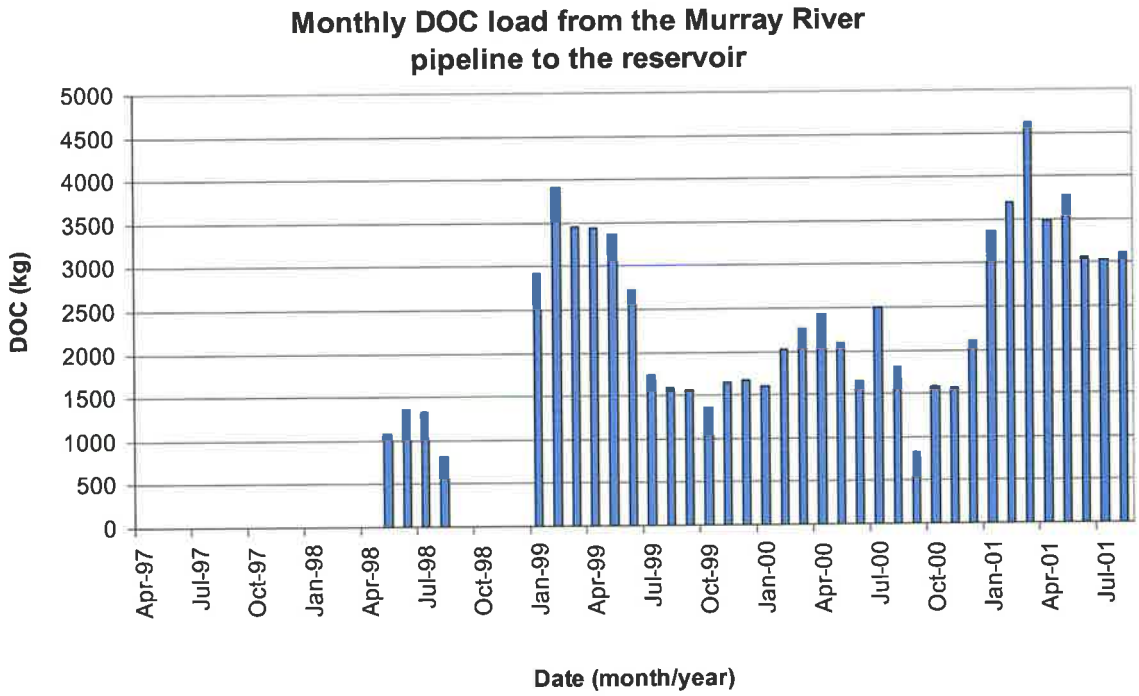


Figure 3. 10: The monthly quantity of DOC (kg) entering the Warren Reservoir via the Murray River pipeline between April 1997 and August 2001.

Based on data presented in Figure 3.10, mean annual DOC quantity entering the reservoir via the Murray River pipeline was calculated and the results are presented in Table 3.4.

Table 3. 4: Annual DOC quantity (kg) of the Murray River water entering the reservoir between 1997 and 2001.

	1997	1998	1999	2000	2001
DOC (kg)	nil	4584	29369	22448	28160

Based on data presented in Figures 3.9 and 3.10, the monthly DOC quantity entering the reservoir through the Murray River pipeline was estimated as the percentage of the mean monthly reservoir DOC quantity (Figure 3.11). This was done for the purpose of determining the total monthly DOC contributions of the Murray River water to the total reservoir DOC

content. Between 1998 and 2001, the estimated monthly contributions of the Murray River DOC to the Warren Reservoir varied between 1% and 12% of the total reservoir DOC quantity (Figure 3.11). The contributions of the Murray River water to the total reservoir DOC quantity showed seasonality (Figure 3.11). Higher contributions were observed between March and June 1999 (mean increase ± S.E. 62±2.2%), March and July 2000 (69%) and between March and May 2001 (22±2.3%), when compared to values prior to and post these months.

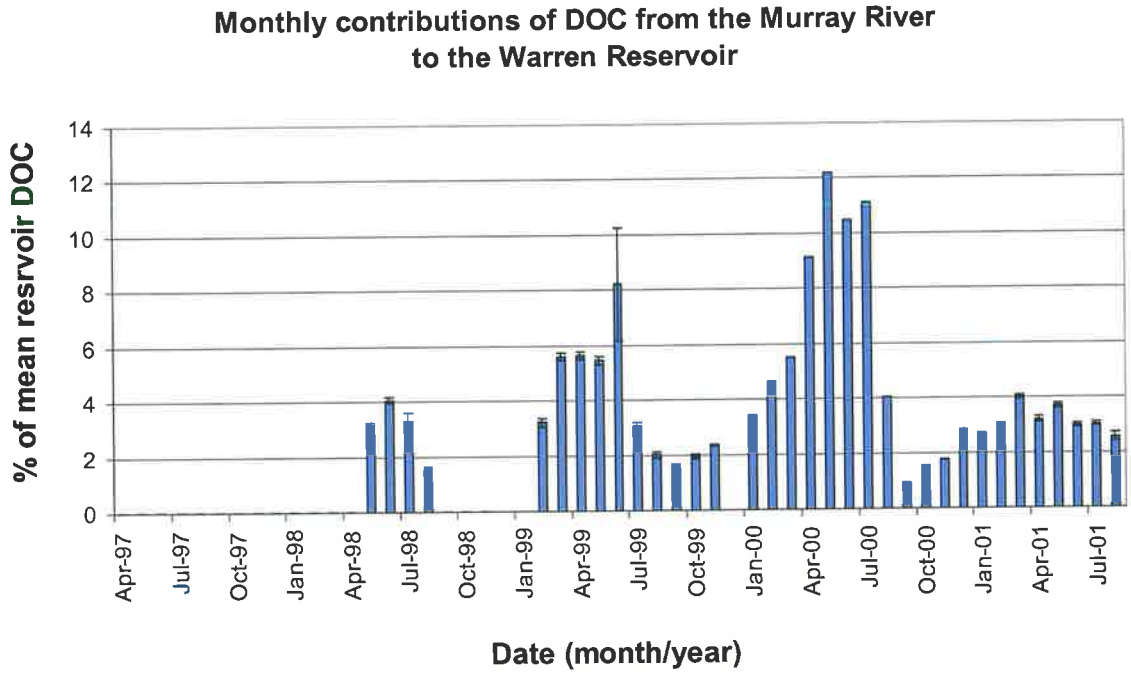


Figure 3. 11: DOC inflow from the Murray River pipeline to the Warren Reservoir expressed as the percentage (%) of the average reservoir DOC quantity, from April 1997 until the end of the study period in August 2001.

Taking into account the mean annual DOC quantities in the Warren Reservoir (Table 3.2) and the total annual DOC quantities entering the reservoir via the Murray River pipeline (Table 3.4), it is estimated that the allochthonous DOC from the Murray River water contributed to 0% of the reservoir DOC during 1997, and approximately 9% during 1998, 41% during 1999, 48% during 2000 and 25% during 2001.

3.4.3 INFLOW OF DOC FROM CATCHMENT STREAMS TO THE RESERVOIR

Inflow of DOC into the reservoir via the two major catchment streams occurred only during the winter months and following the onset of the wet season, as the two major streams, C-S1 and C-S2 only flowed during this period. During the remainder of the year, no flow was measured in either stream, as the streams remained dry.

DOC concentrations of water entering the Warren Reservoir via the two streams C-S1 and C-S2, were measured during winter 1999. This was done to determine whether stream DOC levels were higher than those of the reservoir and if so, whether the influx of higher DOC levels resulted in increased reservoir DOC concentrations observed during winter of every year (Table 3.3, Figures 3.7 and 3.8). Measurements were taken every 3 to 7 days for a period of three months, starting in July and ending in October. Data is summarised in Figure 3.12.

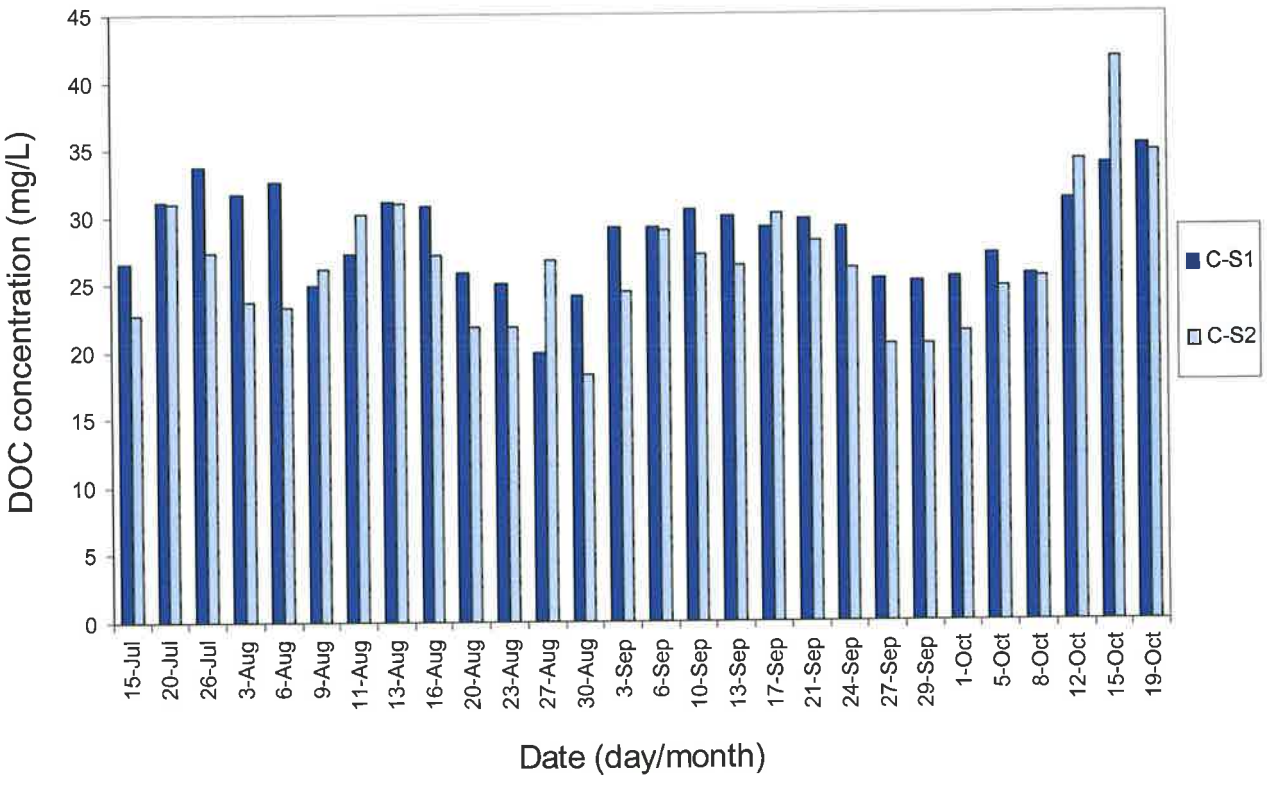


Figure 3. 12: DOC concentrations of water in the two catchment streams, C-S1 and C-S2 discharging into the Warren Reservoir. Measurements were taken during the wet season of 1999.

The concentrations of DOC in the streams ranged from 19.9mg/L to 33.8mg/L in C-S1 and from 18.3mg/L to 41.7mg/L in C-S2. Comparison of the DOC data of the two sites showed that the differences in DOC concentrations between the two streams were not significant (T-test, $p>0.05$).

The concentrations of DOC entering the reservoir near site S1 were compared to DOC concentrations at the S1 site of the reservoir between July and October 1999, using the T-test. Stream DOC concentrations were found to be significantly higher ($p<0.05$) than the average reservoir DOC concentrations in this period at the site S1 (17.6±1.4 mg/L).

The estimated daily loading of allochthonous DOC into the Warren Reservoir during wet season of 1999 was 25±9 kg of carbon and 87±23 kg of carbon from streams C-S1 and C-S2 respectively (Table 3.5). Thus, during winter 1999, an average total of 112 kg of DOC was delivered daily from the catchment into the reservoir at site S1 via the C-S1 and C-S2 streams.

Table 3. 5: Estimated daily DOC loading to the Warren Reservoir from the C-S1 and C-S2 streams, during the wet season of 1999.

Streams	Average DOC concentration (mg/L)	Average daily water volume (L)	Daily DOC loading to the reservoir (kg)
C-S1	28.6 ± 0.7 mg/L	$8.8 \pm 3 \times 10^5$	25±9
C-S2	26.6 ± 1.0 mg/L	$3.3 \pm 0.9 \times 10^6$	87±23

Thus, considering the streams flowed for a period of 3.5 months, between July and October, the estimated total DOC load from C-S1 and C-S2 to the reservoir during the wet season of 1999 was 1.2×10^4 kg.

Taking into account that the mean DOC quantity in the Warren Reservoir during this period (July to October 1999) was $7.6 \pm 0.4 \times 10^4$ kg, it is estimated that the catchment load of DOC via the two streams contributed to approximately 16% of the total reservoir DOC quantity during 1999.

3.5 PHYSICAL, CHEMICAL AND BIOLOGICAL PARAMETERS AFFECTING WATER QUALITY IN THE WARREN RESERVOIR

To obtain information on different parameters of water quality, data for the biological parameters such as phytoplankton biomass, bacterial growth and community composition, and physicochemical parameters such as temperature, pH, salinity, oxygen, turbidity, colour, specific colour, underwater visibility (or light penetration) and nutrients (total phosphorus, soluble reactive phosphorus and nitrate) were collected during 1997, 1998, 1999 and 2001. Only turbidity and colour data are available for the D2 site during 2000, while bacterial abundance and diversity were measured during 2001 only.

3.5.1 PHYTOPLANKTON GROWTH IN THE RESERVOIR

Phytoplankton abundance was analysed by measuring chlorophyll concentrations in water samples. Chlorophyll concentrations in reservoir samples are summarised in Figure 3.13. During 1997 and 1998, concentrations of chlorophyll averaged 0.96±0.3mg/L for S1, 0.64±0.2mg/L for M1, 0.61±0.1mg/L for D1 and 0.56±0.2mg/L for D2. Peaks in chlorophyll concentrations were measured between January and March 1999 at all four sites. These peaks in chlorophyll concentrations were significantly higher than the concentrations measured during the months prior to the peaks (9-fold higher at S1, 34-fold at M1, 36-fold at D1 and 16-fold at D2). High algal biomass (algal bloom), represented by the elevated levels of chlorophyll, was controlled by the application of $CuSO_4$ on the 9th March 1999. From April 1999 chlorophyll levels declined; average concentrations were 3.8±0.9mg/L at S1, 3.7±0.8mg/L at M1, 4.8±0.8mg/L at D1 and 4.1±0.8mg/L at D2.

An increase in chlorophyll concentration was also recorded between 15th January and 1st April 2001 (Figure 3.13). During this period, chlorophyll concentrations were higher in comparison to the concentrations measured prior to this date, by 8-fold at S1, 3-fold at M1, 3-fold at D1 and 2-fold at D2. The algal bloom (reflected by the increased chlorophyll levels) was managed with $CuSO_4$ treatment on the 12th April 2001. By the end of April 2001, chlorophyll concentrations at M1, D1 and D2 were reduced to 10mg/L, 5mg/L and 4mg/L respectively, and remained close to this level for the rest of the study period. Chlorophyll levels at the sites S1 continued to increase even after April to reach 27.6 mg/L.

In summary, a general trend was found where chlorophyll concentrations in the reservoir were higher in summer than in winter months, and they were also higher in 1999 and 2001 when compared to 1997 and 1998.

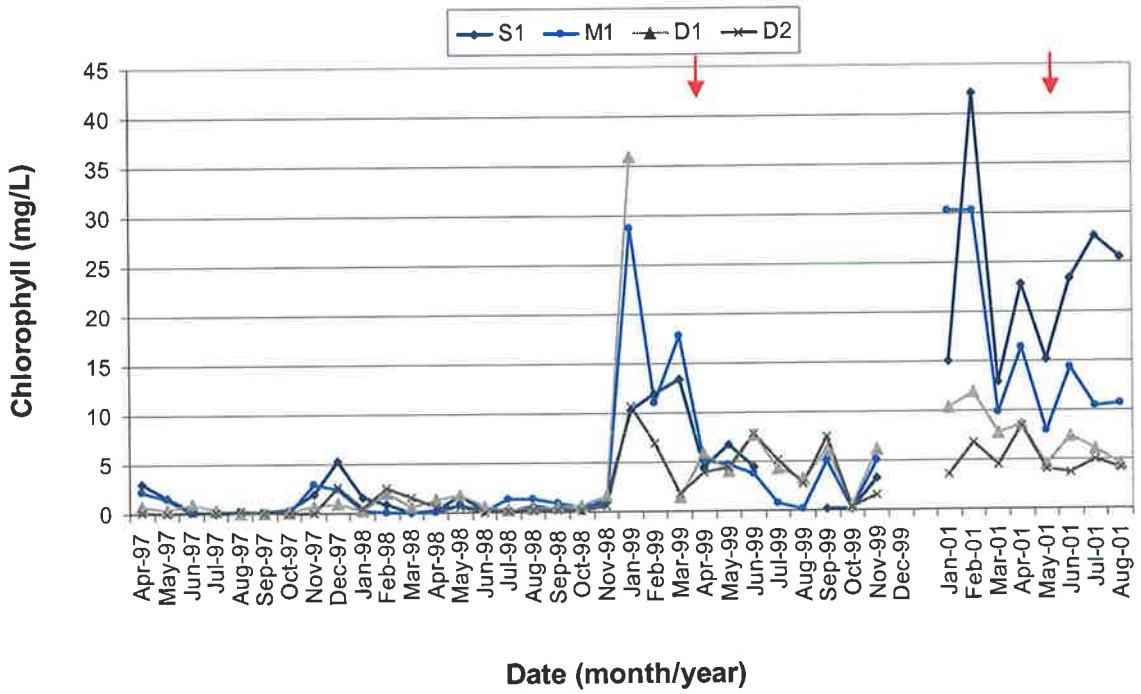


Figure 3. 13: Chlorophyll levels at the four sampling sites in the Warren Reservoir, S1, M1, D1 and D2, measured between April 1997 and August 2001. No chlorophyll data was obtained during 2000. Arrows indicate the application of $CuSO_4$ treatment.

3.5.2 BACTERIAL ABUNDANCE AND BIOMASS IN THE WARREN RESERVOIR

Bacterial numbers in reservoir water samples were determined from December 2000 to August 2001 (Figure 3.14). The highest mean bacterial counts during the study period were observed at site S1 17.4±1.2 $x10^5$ cells/mL (mean ± S.E.), followed by 15.3±1.5 $x10^5$cells/mL at M1, 13.3±1.1 $x10^5$cells/mL at D2 and 12.4±1.7 $x10^5$cells/mL at D1. While bacterial counts at S1 were significantly higher than those at D1 and D2 (one-way ANOVA, $p<0.05$), they were not significantly higher than those at M1 ($\alpha=0.05$, $p=0.27$). No significant differences in bacterial counts were observed between the sites M1, D1 and D2 (one-way ANOVA, $p>0.22$). No seasonal trends and no significant differences were found between

summer and winter bacterial counts at any of the four sampling sites (T-test, $p>0.05$). Variations in bacterial numbers were greatest at the D1 site (numbers in the range of 5 x 10^5 cells/mL to 24 x 10^5 cells/mL) where the lowest mean cell counts were recorded (12.4±1.7 x 10^5 cells/mL). In contrast, variations in cell numbers over the period of nine months was least at the site S1 (numbers in the range of 10 x 10^5 cells/mL and 24 x 10^5 cells/mL), where the highest mean cell counts were measured (17.4.±1.2 x 10^5 cells/mL).

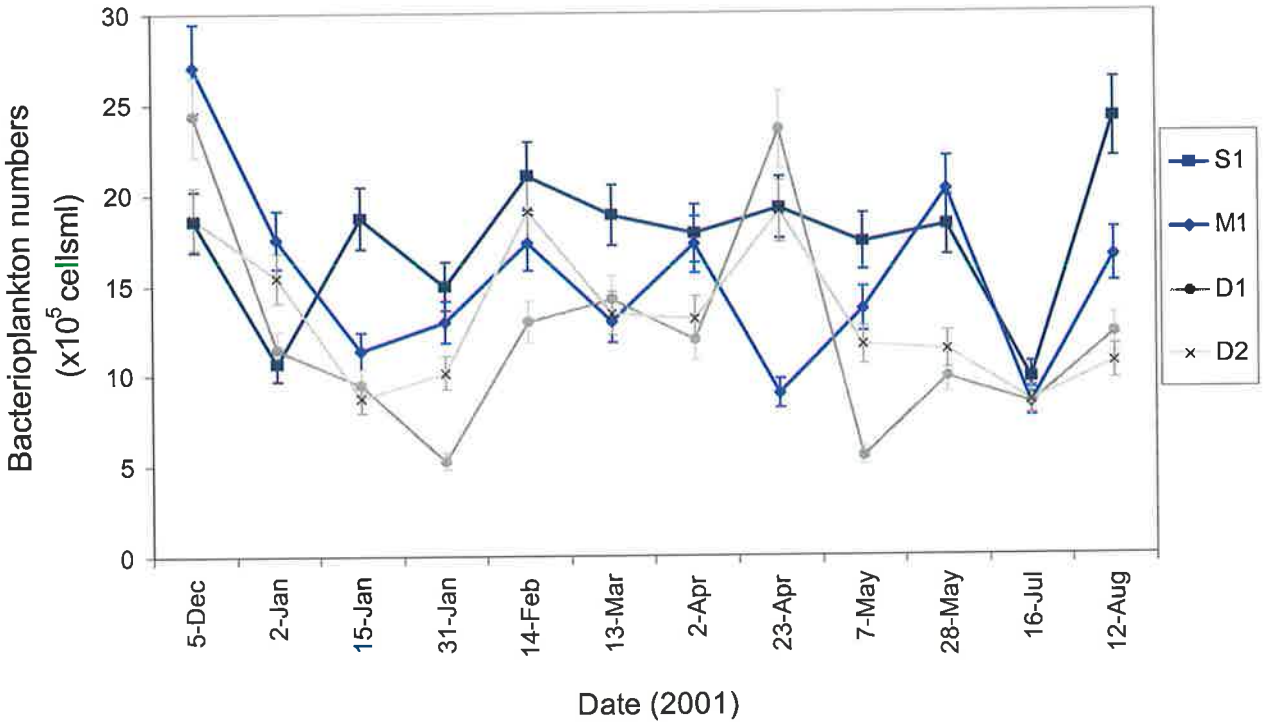


Figure 3. 14: Total bacterial numbers measured from December 2000 until August 2001 expressed as cells/mL, at the four reservoir sampling sites, S1, M1, D1 and D2. Points are the mean of three observations ± SE.

Bacterial biomass in water samples was also determined during the 2000-2001 sampling period, as changes to bacterial biomass can occur independently of changes to bacterial numbers. The mean cell volumes of bacterial consortia were calculated and used to estimate the biomass by applying the conversion factor of 0.308pgCμm^{-3}, as described by Bergstrom and Jansson (2000). The results of biomass calculations are summarised in Figure 3.15. Highest mean population biomass between December 2000 and August 2001 was found

in S1 samples, measuring 51±6 μg C/L, followed by 46±12 μg C/L in M1, 32±12 μg C/L in D1 and 30±7 μg C/L in D2. Significant differences were found in the mean bacterial biomass of sites S1 and D2 (one-way ANOVA, $p<0.05$), while no significant differences in bacterial biomass were observed between any of the other sites. Cell biomass decreased at all four sites from December to January by an average of 78±5%. From January until April 2001 there was a steady increase to approximately double the population biomass. This was followed by a 3 and 2 fold increases in biomass in April at sites D1 and D2 respectively, and a smaller increase of 35% at site S1. From the end of April and early May the population biomass decreased to an average 10±0.7 μg C/L until mid August, when biomass increases between 25% and 85% were measured at the four sites.

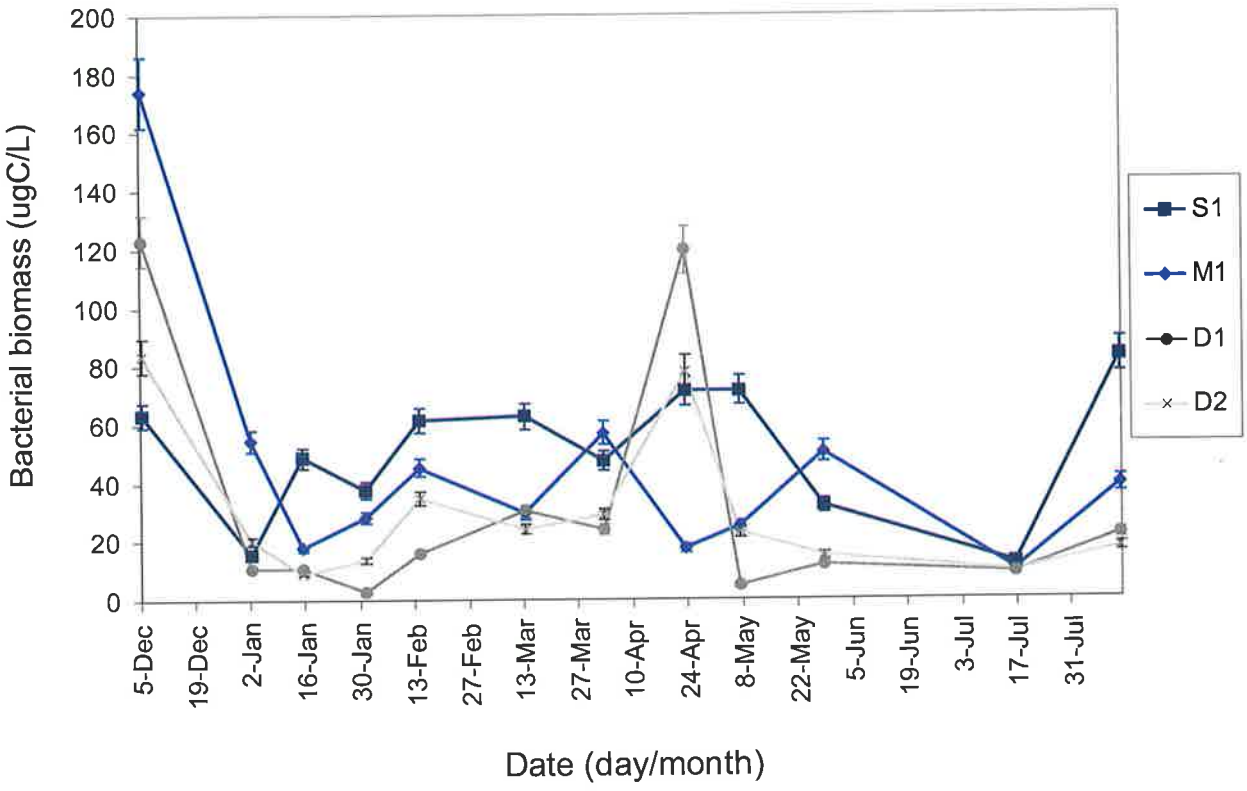


Figure 3. 15: Biomass of bacterial consortia measured at the four reservoir sampling sites, S1, M1, D1 and D2, between December 2000 and August 2001. Points are the mean of three observations ± SE.

3.5.3 BACTERIAL POPULATION DIVERSITY IN THE WARREN RESERVOIR

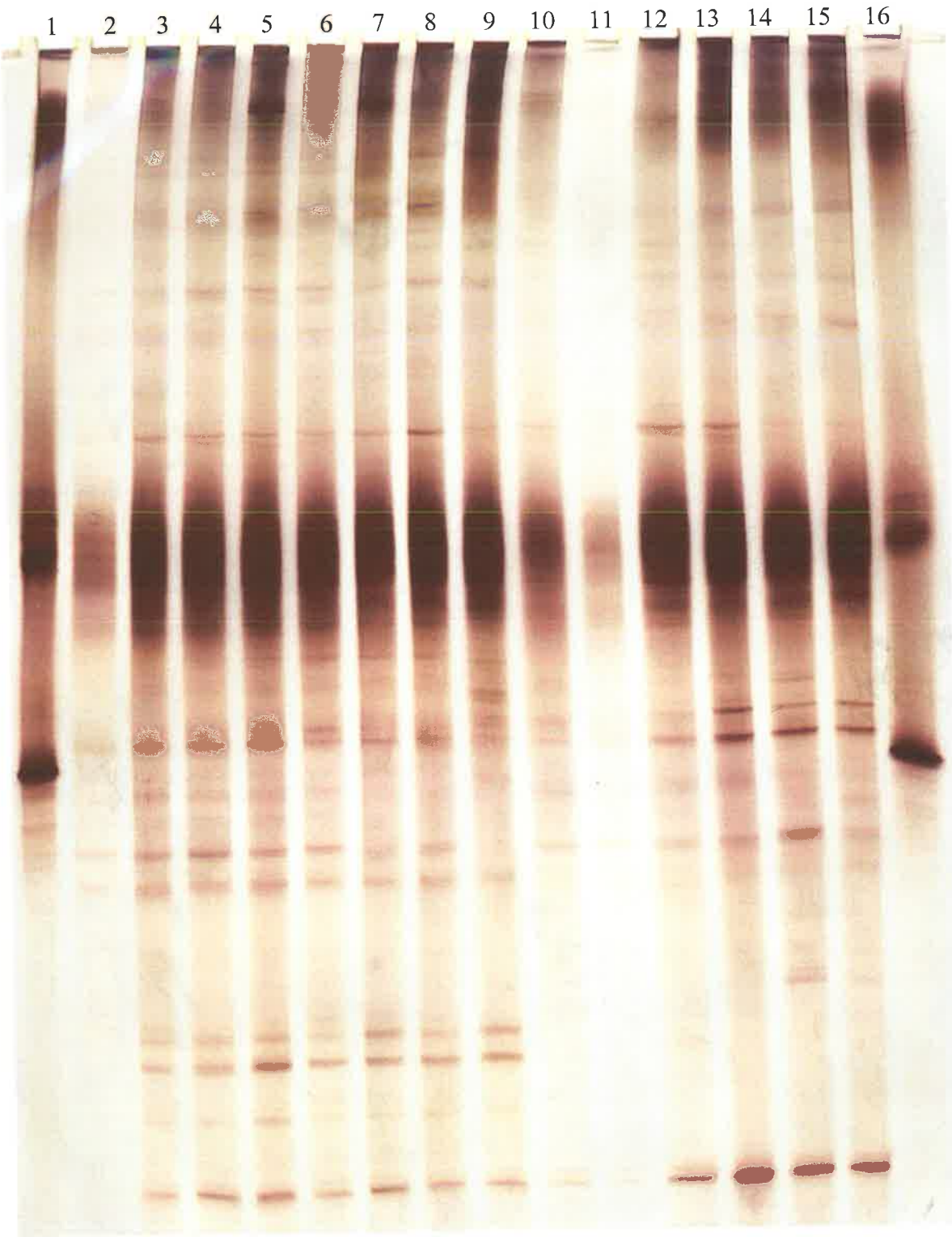
3.5.3.1 Temporal changes in the reservoir population diversity

Bacterial diversity was studied from September 2000 until August 2001 in order to be able to correlate the changes to bacterial community structures to changes in physical, chemical and biological parameters of the Warren Reservoir water. The aim was to determine which water parameters were likely to affect bacterial populations, and in particular whether changes in DOC quality and/or quantity are likely to play a role in altering bacterial community structure.

Amplified DNA from each reservoir sample was separated using Denaturing Gradient Gel Electrophoresis (DGGE) (Figure 3.16). Table 3.6 shows the dates when samples were collected from sites S1, M1, D1 and D2.

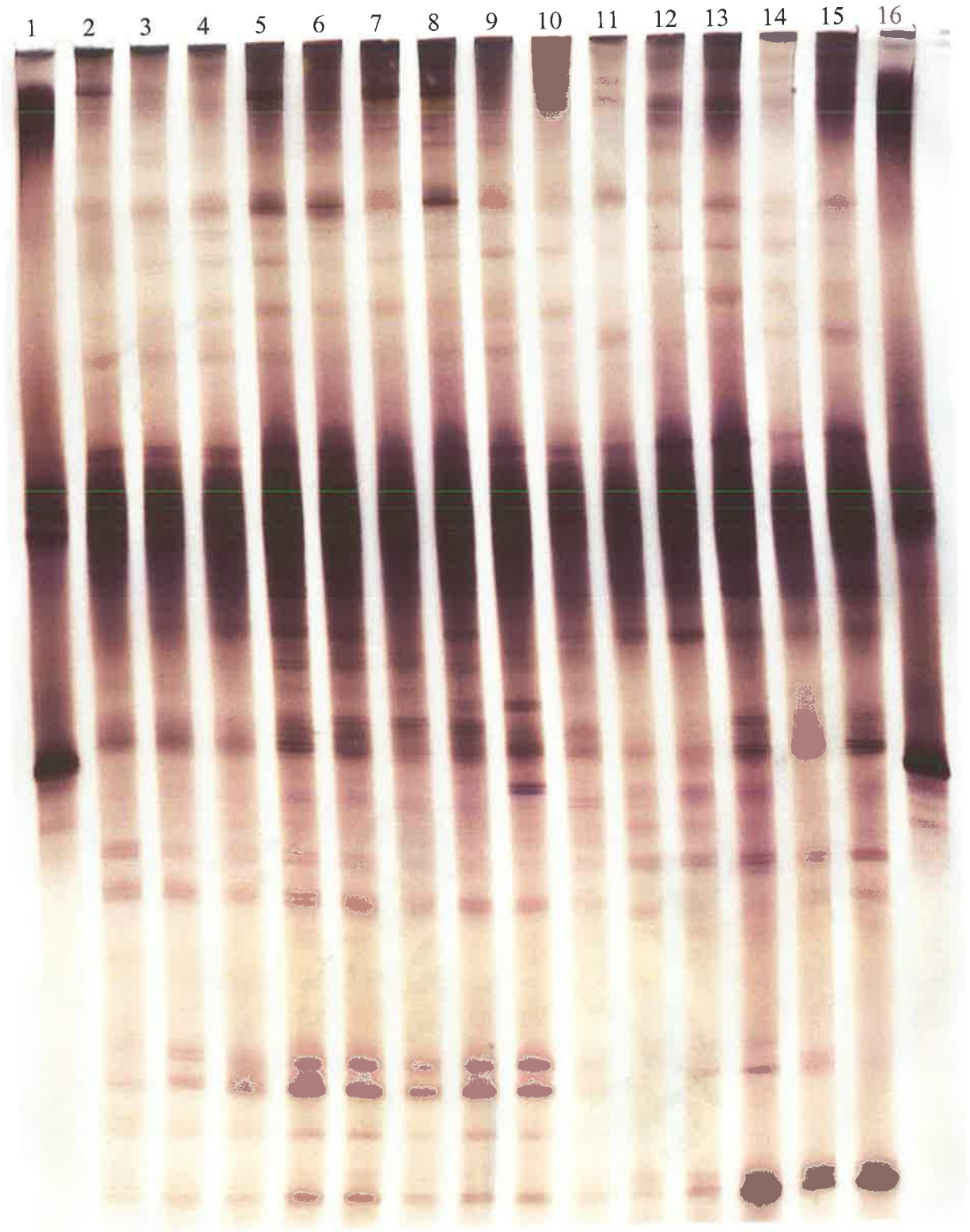
Table 3. 6: Sampling dates for bacterial population analyses by DGGE. Dates indicated for lanes 1-16 are applicable to samples in lanes 1-16 in Figures 3.16 A, B, C and D.

Lane	Date	Lane	Date
1	*E.coli* marker	**9**	02 April 2001
2	24 October 2000	**10**	23 April 2001
3	05 December 2000	**11**	07 May 2001
4	02 January 2001	**12**	28 May 2001
5	15 January 2001	**13**	18 June 2001
6	01 February 2001	**14**	16 July 2001
7	14 February 2001	**15**	12 August 2001
8	13 March 2001	**16**	*E.coli* marker

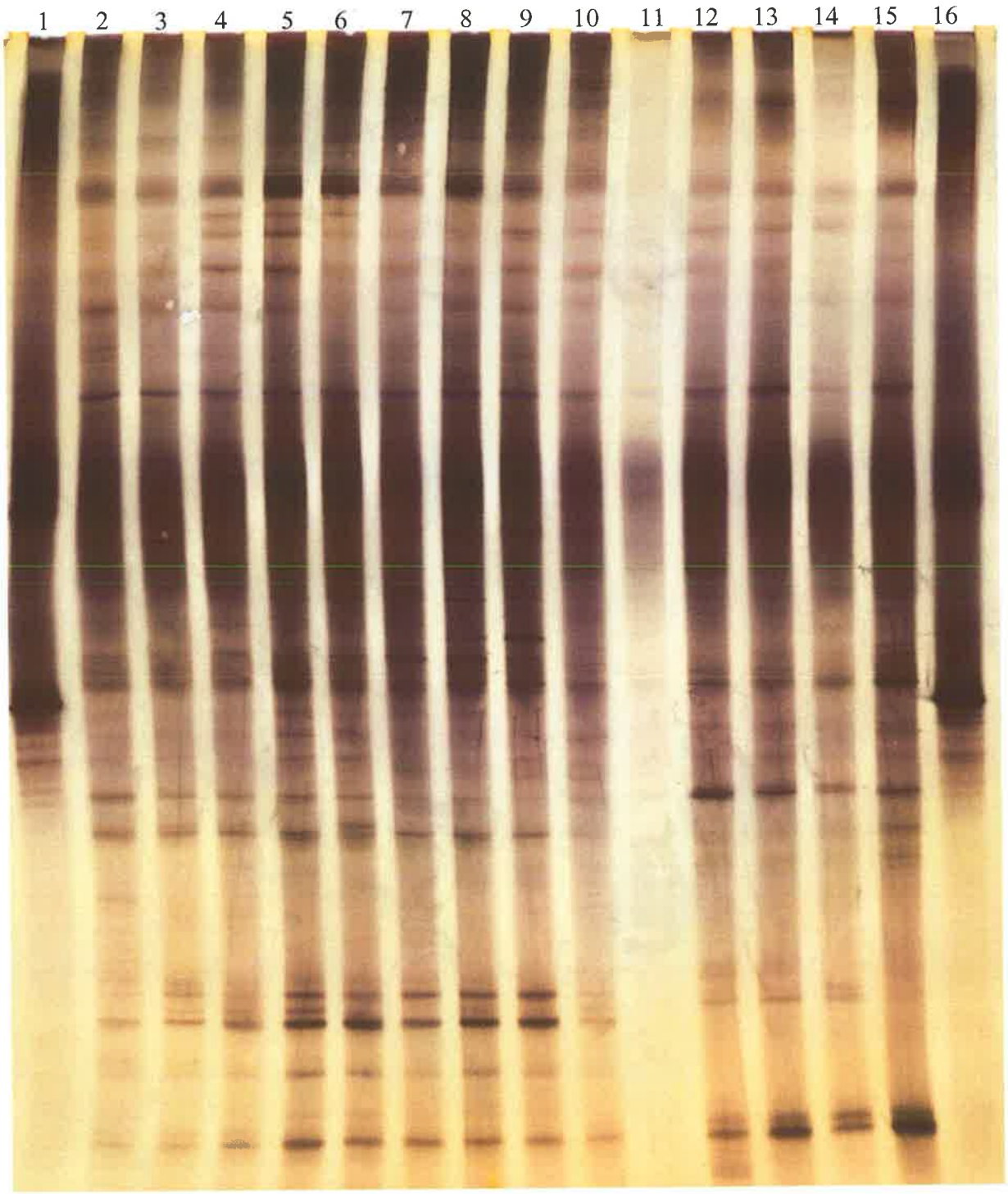


A. Site S1

B. Site M1



C. Site D1



D. Site D2

Figure 3. 16: DGGE analysis of the PCR amplified 530bp 16S rDNA products from mixed bacterial populations collected from October 2000 until August 2001 at the four sites S1, M1, D1 and D2 in the Warren Reservoir. Sampling times for lanes 1-16 of each sample are specified in Table 3.6. The images were processed using Adobe Photoshop to increase the contrast of the gel images.

To enable band analysis of each sample in a gel, complete linkage dice coefficient analysis was performed (using Diversity Database software, BioRad). Phylogenetic trees were constructed based on banding pattern information (Appendix 2) from Figures 3.16 A, B, C & D, enabling comparisons between seasonal samples from each site. The results of this analysis are shown in Figure 3.17. Furthermore, Principal Component Analysis (PCA) was used to substantiate the results from the phylogenetic tree analysis. PCA was based on the band presence as well as the difference in peak intensities of each band in the DGGE gel. Band intensity data was used to generate a two dimensional matrix indicating sample separation along the horizontal and vertical axes. The weight of each axis used to determine the degree of separation between populations is indicated in each figure (Figure 3.18A, B, C & D).

Phylogenetic analysis of samples from shallow site S1 (Figure 3.17A) shows a formation of two distinct groups, one comprising samples from December to April, and the second comprising samples from April to August and an October sample. This finding is supported by the results from the PCA (Figure 3.18A), showing a segregation of two major groups.

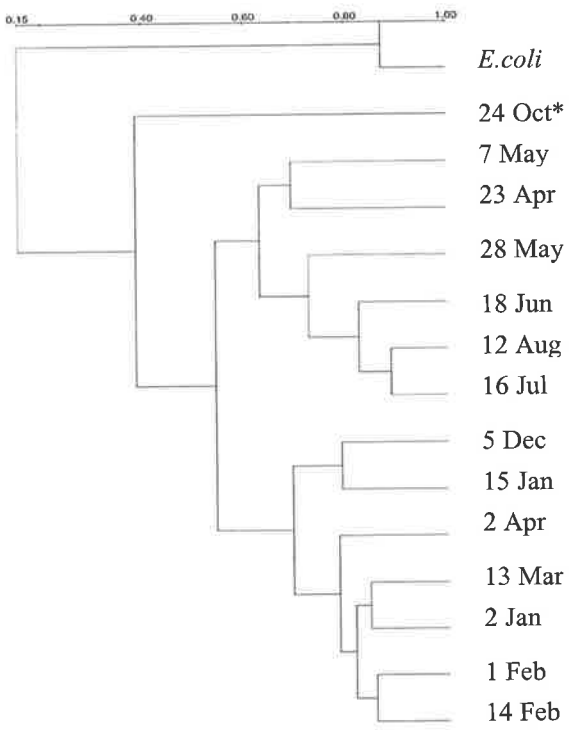
Analysis of the banding pattern of monthly samples from site M1 indicated a formation of four discrete groups. The first comprised two samples from July and August, the second group comprised samples from January to April, the third group had October to January populations, while the fourth group comprised April to June populations (Figure 3.17B). In general, populations from site M1 did not present a clear seasonal segregation, as did the populations from site S1. This lack of temporal segregation of populations can also be seen in the Principal Component Analysis, Figure 3.18B. Although a clear seasonal separation was not found, December to April populations appeared more divergent from April to August populations (Figure 3.18B).

Among D1 populations, two distinct groups were formed, the first comprising samples from late April to August and the second comprising samples from December to early April (Figure 3.17C). The findings from PCA (Figure 3.18C) support the phylogenetic tree analysis, by showing a clear horizontal separation between the two groups December to April and April to August.

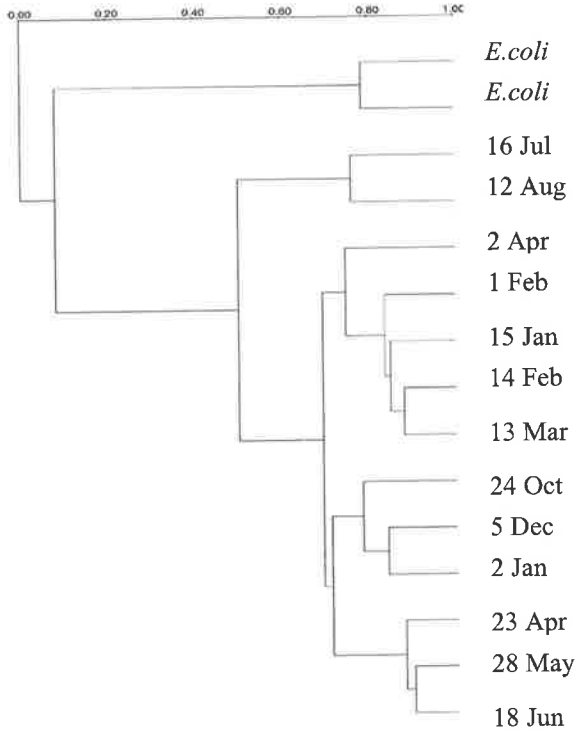
Samples from site D2 formed three separate groups, one comprising samples from May to August, the second comprising samples from October to January, and third with samples from end of January to end of April (Figure 3.17D). Amongst the three groups,

higher similarity was found between December to January and January to April populations, than between December to January and May to August populations. The PCA results showed a separation of December to April populations from April to August populations. Thus, the second and third groups from the phylogenetic tree analysis were grouped as having close similarity.

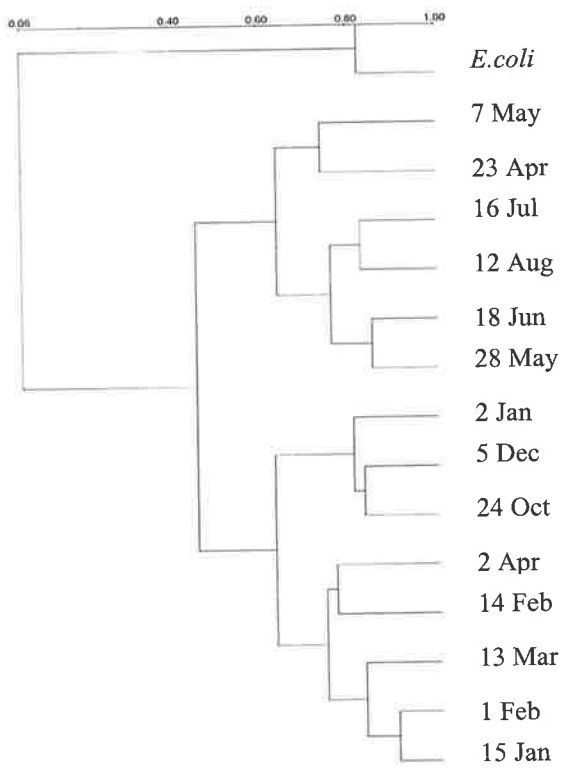
In summary, Figures 3.17 and 3.18 showed varying degrees of seasonal segregation of bacterial populations at the four sites of the Warren Reservoir, with populations at site M1 showing least temporal differences.



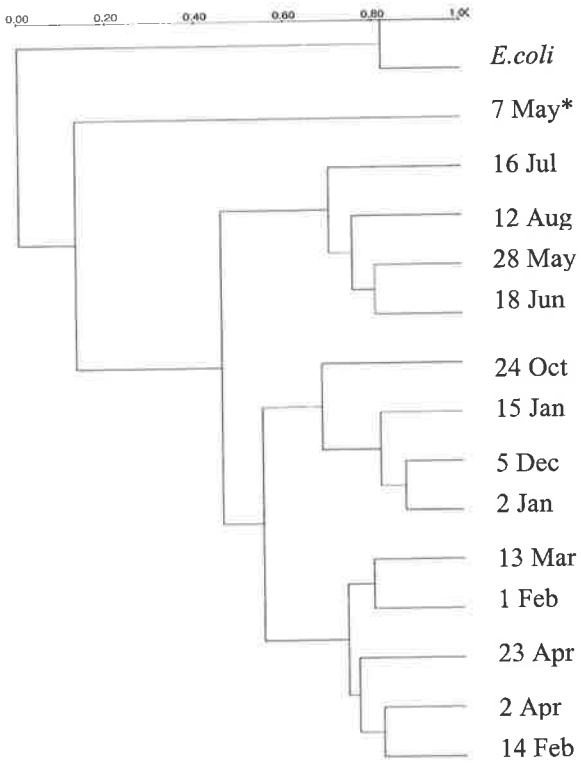
(a) Site S1



(b) Site M1

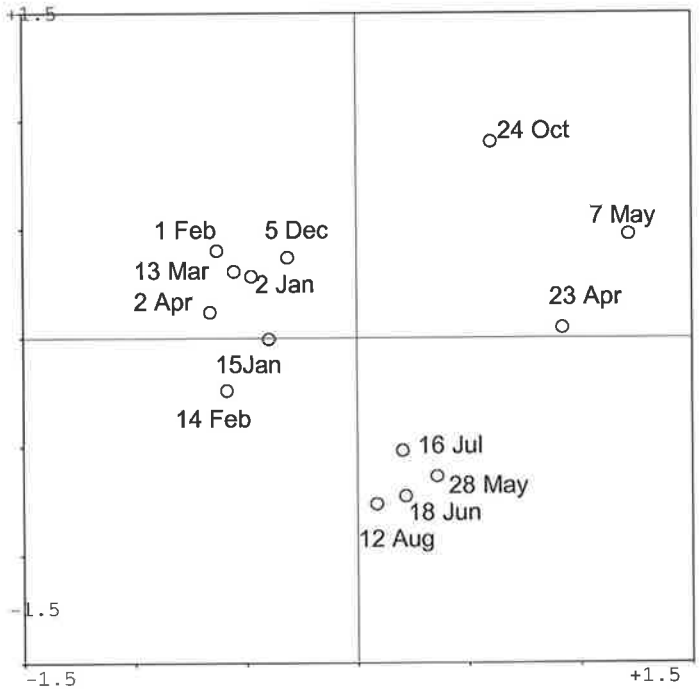


(c) Site D1

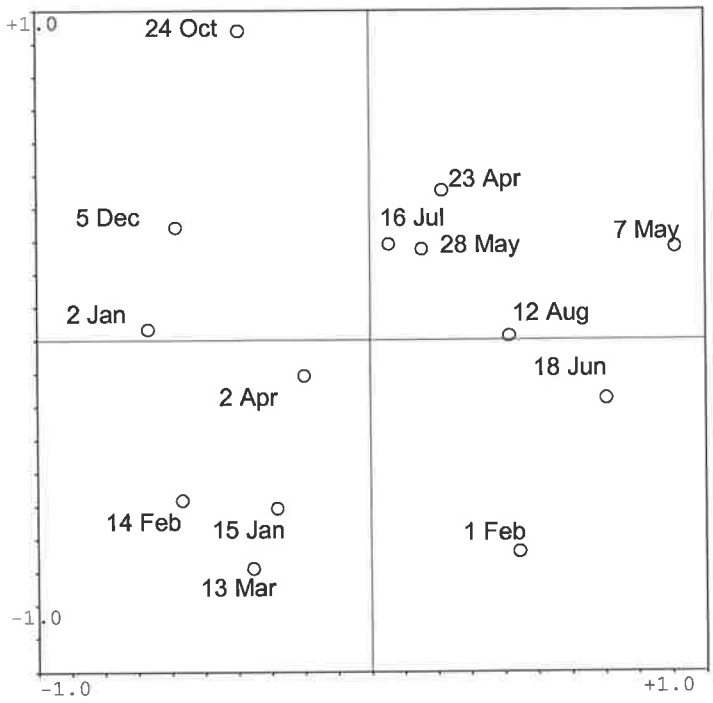


(d) Site D2

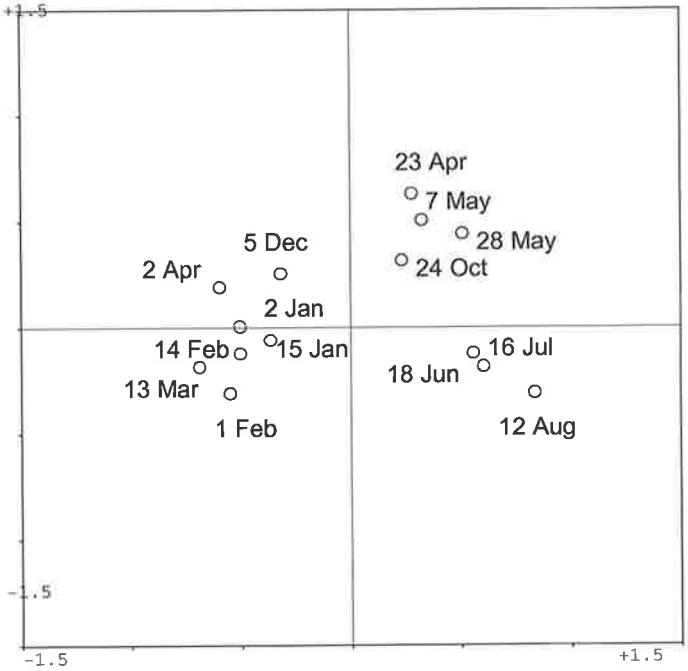
Figure 3. 17: Phylogenetic tree analysis showing similarities amongst bacterial populations collected during different times between October 2000 and August 2001 at each of the four reservoir sites (a) S1, (b) M1, (c) D1, (d) D2. Samples marked with an asteric showed low banding development during DGGE analysis.



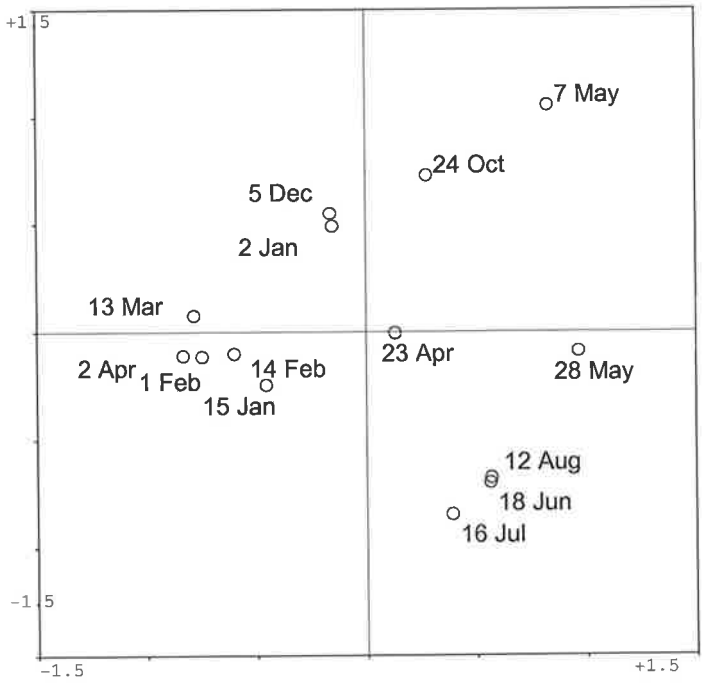
(A) Site S1: X axis=34.6%; Y axis=23.9%



(B) Site M1: X axis=24.3%; Y axis=20.7%



(C) Site D1: X axis=34.3%; Y axis=20.7%



(D) Site D2: X axis=35.4%; Y axis=28.2%

Figure 3. 18: Ordination plot of a principal component analysis showing temporal differences in bacterial populations inhabiting the sites S1, M1, D1 and D2.

Data regarding seasonal segregation of bacterial populations at each site is further summarised in Table 3.7. With reference to the bacterial population found at each site of the Warren Reservoir on 2nd January, percentage similarities were calculated between this population and all other populations found at each site during 2001. The results showed a general trend of declining similarity with time.

Table 3. 7: Temporal changes in bacterial populations during 2001 at sites S1, M1, D1 and D2. Comparisons of bacterial populations with time are expressed as percentage (%) similarity that each population has when compared to the first collected sample on the 2nd of January.

Date	S1	M1	D1	D2
2 January	*100%*	*100%*	*100%*	*100%*
15 January	80.1%	78.3%	77.4%	83.8%
1 Februay	81%	75%	68%	64%
14 February	84.5%	71%	65.9%	71.1%
13 March	84.5%	74.1%	68.5%	66.2%
2 April	78.5%	70.2%	63.3%	70.5%
23 April	61.6%	75.9%	63.3%	68.2%
7 May	<20%	<20%	42%	<20%
28 May	60.4%	71.2%	56.6%	55.6%
18 June	64.2%	74.1%	50.6%	61.9%
16 July	68.4%	58.7%	59.8%	45.9%
12 August	62.1%	63.1%	62.3%	54.1%

3.5.3.2 Spatial changes in the reservoir population diversity

In addition to temporal changes in bacterial populations at each site, spatial differences were also analysed, by comparing populations collected across the four reservoir sites. A phylogenetic tree was constructed (Figure 3.19) comparing populations collected at all four sites during different times of the year. The results of this analysis showed highest

similarities among members of populations at each site (45-50%) irrespective of the time of the year. Comparisons of populations between the four sites showed closest similarities between S1 and D1 populations (47%). Although M1 is a connecting point between sites S1 and D1 in the reservoir, the populations belonging to site M1 showed equally low population similarity to all three sites S1, D1 & D2 (25-33%).

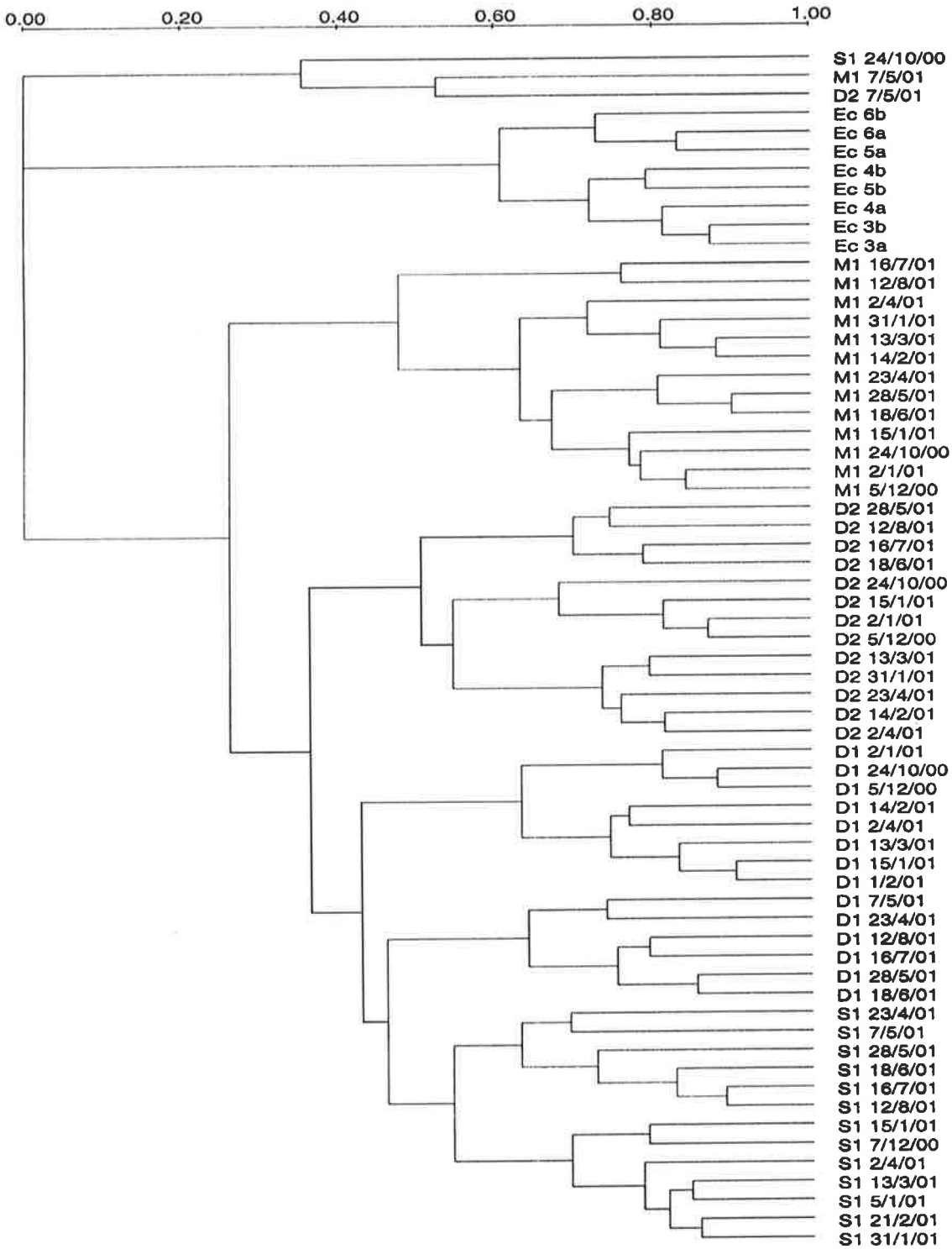


Figure 3. 19: Phylogenetic tree analysis showing bacterial population similarities of samples taken from the four reservoir sites, S1, M1, D1 and D2 and at different times, from October 2000 to August 2001.

3.5.4 THE RESERVOIR WATER COLOUR

Water colour data at the four reservoir sites is summarised in Figure 3.20. There is a general trend where the lowest colour levels (in the range of 19-25 HU in 1998, 1999 and 2000 and 72-86 HU in 2001) occurred during May and June (immediately prior to rainy seasons) (Figure 3.20). During August (a period immediately following the onset of the wet season) of 1998, 1999 and 2000, colour increased across the reservoir by up to 7-fold, 5-fold and 8-fold respectively. In addition to winter colour peaks, a peak was also observed during mid summer (January) 1999.

Highest colour measurements between 1997 and 2001 were obtained at the shallow site S1 (83±6 HU) (Mean ±S.E.), followed by M1 (78±6 HU), D1 (75±6 HU) and D2 (75±7 HU). The spatial differences in colour measurements during the sampling period were not found to be significant (one-way ANOVA, $p>0.05$).

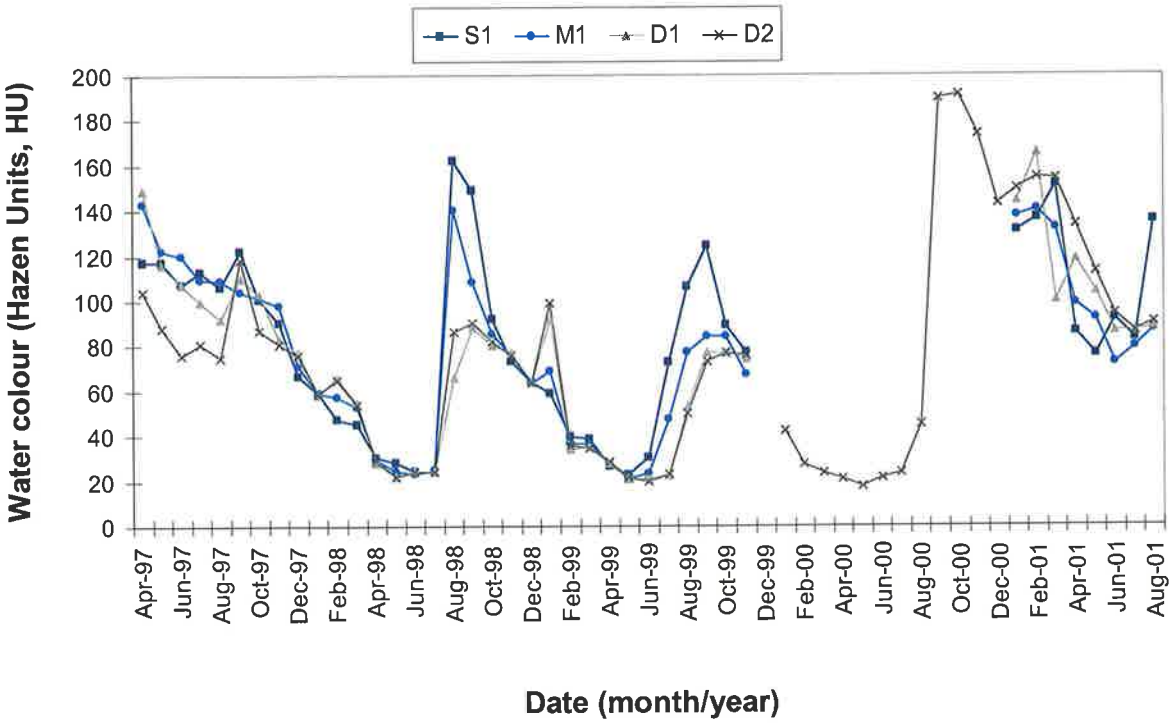


Figure 3. 20: Annual water colour (HU) at sites S1, M1, D1 and D2 in the Warren Reservoir between April 1997 and August 2001.

3.5.5 SPECIFIC COLOUR OF THE RESERVOIR WATER

Analysis of specific colour of water was made by studying the ratios of colour to DOC concentration. This analysis is summarised in Figure 3.21. The analysis was done on the assumption that higher ratios indicated a higher composition of humified and complex structured organic material, such as that originating in catchments.

Specific colour decreased from spring 1997 to summer 1997/1998 (mean decrease across four sampling sites being 67±1%) and increased again during winter 1998 by up to 5-fold. In summer 1998/1999, the specific colour decreased by 80±1%, and then increased by approximately 3-fold during winter of the same year. In summer 2000, a decrease in specific colour of 53% was measured at the site D2, followed by a 4-fold increase in winter 2000. In summer 2000/2001 a mean decrease in specific colour of 42±2% was measured across the reservoir.

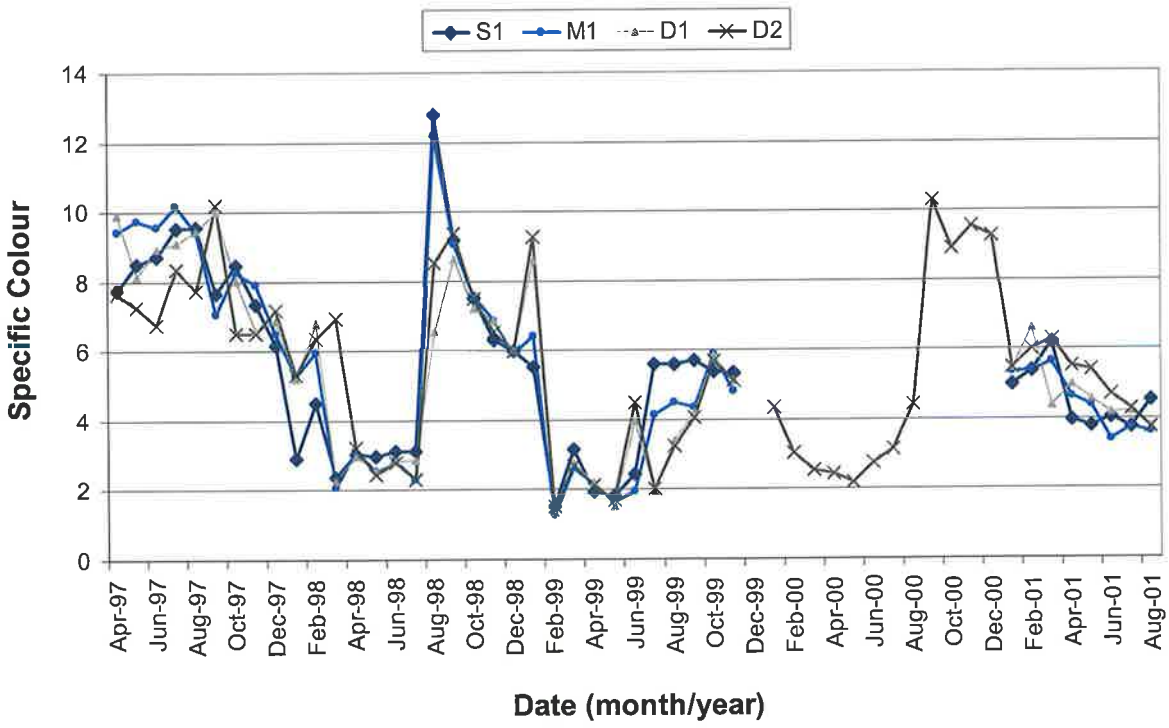


Figure 3. 21: Changes in specific colour (HU $mg^{-1}L$) at the four reservoir sites S1, M1, D1 and D2 from April 1997 to August 2001.

Variations in annual specific colour (Mean ± S.E.) across the reservoir from 1997 to 2001 are summarised in Table 3.8. Higher DOC concentrations were measured during 1999 and 2001 (Figure 3.7, Table 3.2) when low specific colour values were found (Figure 3.21,

Table 3.8); while lower DOC levels were measured in 1997 and 1998 when higher specific colour values were found. This suggests that during 1997 and 1998, humified organics comprised a higher portion of total DOC when compared to for example 1999 and 2001, when smaller composition of complex humic fractions were possibly dominating the DOC pool in the Warren Reservoir.

Table 3. 8: Mean annual specific colour at the four sites S1, M1, D1 and D2, from 1997 to 2001.

	Average annual specific colour at the four reservoir sites			
Year	**S1**	**M1**	**D1**	**D2**
1997	8.4±0.4	8.8±0.4	8.8±0.3	7.8±0.3
1998	5.8±1.0	6.0±0.9	5.5±0.6	5.7±0.7
1999	3.8±0.5	3.3±0.5	3.1±0.5	3.2±0.5
2000	n/a	n/a	n/a	5.2±0.9
2001	4.5±0.3	4.6±0.3	4.8±0.3	5.3±0.2

One-way ANOVA was used to determine whether the changes to specific colour between 1997 and 2001 were significant. At 95% confidence limit changes in specific colour were found to be significant ($p<0.01$).

3.5.6 STREAM WATER COLOUR AND SPECIFIC COLOUR

Colour measurements of water in both streams, C-S1 and C-S2 were taken between July and October 1999 (Appendix 3), for assessment of any relationship to reservoir water colour and changes to DOC quality of the reservoir. The mean colour for the three-month period was 184±6 HU units for C-S1 and 150±9 HU units for C-S2. These values were approximately 5 to 8 times higher than the reservoir colour values at the time immediately prior to the inflow of stream water from C-S1 and C-S2 (namely 30HU at S1, 19HU at M1, 21HU at D1 and 20HU at D2). At the end of rainy season (October 1999) and the influx of stream water of high colour (150-184HU), there was an increase in reservoir water colour

from 19-30HU units prior to rainy season to 67-124HU (D2 to S1) in October 1999 (Figure 3.20).

The average specific colour values in the reservoir prior to the wet season of 1999 were 2.4±0.3 at S1, 2±0.2 at M1, 2±0.4 at D1, and 2.2±0.4 at D2. The average specific colour values of the stream water measured between July and October 1999 were 6.5±0.2 at C-S1 and 5.5±0.2 at C-S2. Following the onset of the wet season, the specific colour across the reservoir increased by approximately 2-3 fold to 5.5±0.07 at S1, 4.7±0.3 at M1, 4.7±0.5 at D1 and 4.5±0.5 at D2.

3.5.7 RESERVOIR WATER TEMPERATURE

The annual temperatures measured in the top 1m surface waters at each site are summarised in Figure 3.22. Water temperature showed a marked seasonality with lowest water temperature (between 8°C and 11°C) recorded between winter months of June and August, and highest temperature values (between 24°C and 26°C) recorded during summer months of December and January each year.

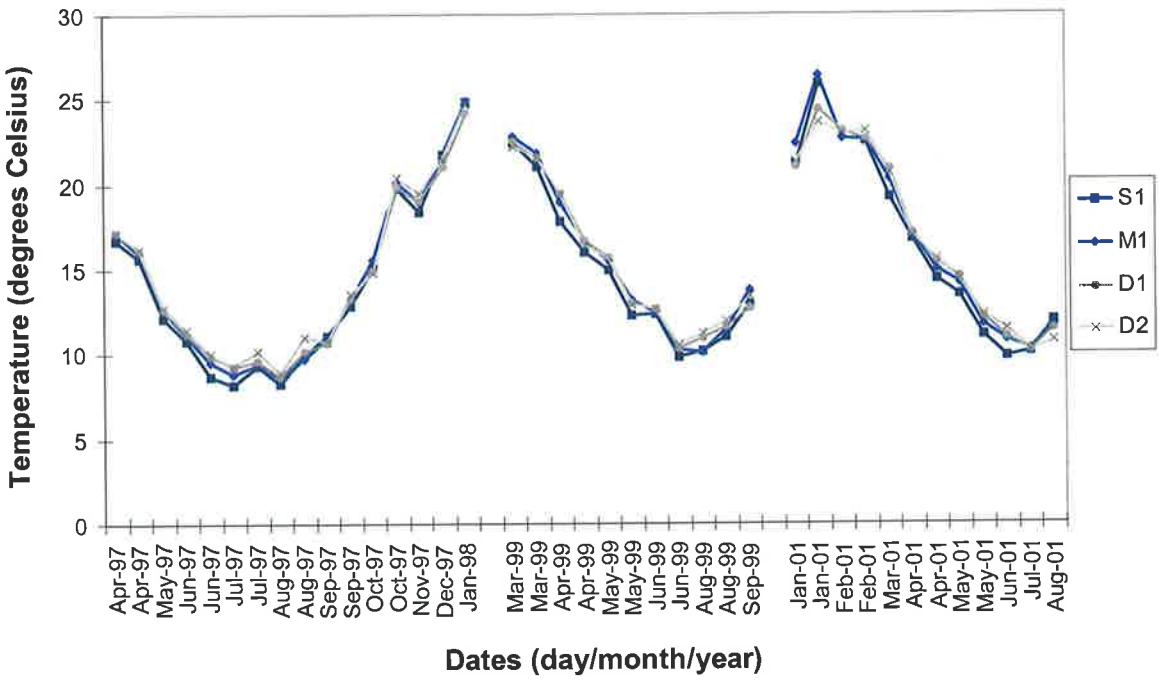


Figure 3. 22: Water temperature (°C) at the four sites S1, M1, D1 and D2 of the Warren Reservoir in 1997, 1999 and 2001.

3.5.8 DISSOLVED OXYGEN CONCENTRATIONS

The annual dissolved oxygen concentrations measured in the top 1m surface waters at each site are summarised in Figure 3.23. Dissolved oxygen concentrations were higher at the deep sites (D1 and/or D2) than at the shallow site (S1) during 1999 (one-way ANOVA, $p<0.05$), however no significant spatial differences in dissolved oxygen concentrations were measured during 1997 or 2001 (Figure 3.23, Table 3.9).

Between January (summer) and August (winter) of 1999 and 2001, increases in dissolved oxygen concentration were measured across the reservoir (40±9% and 114±7%, mean ± S.E., respectively).

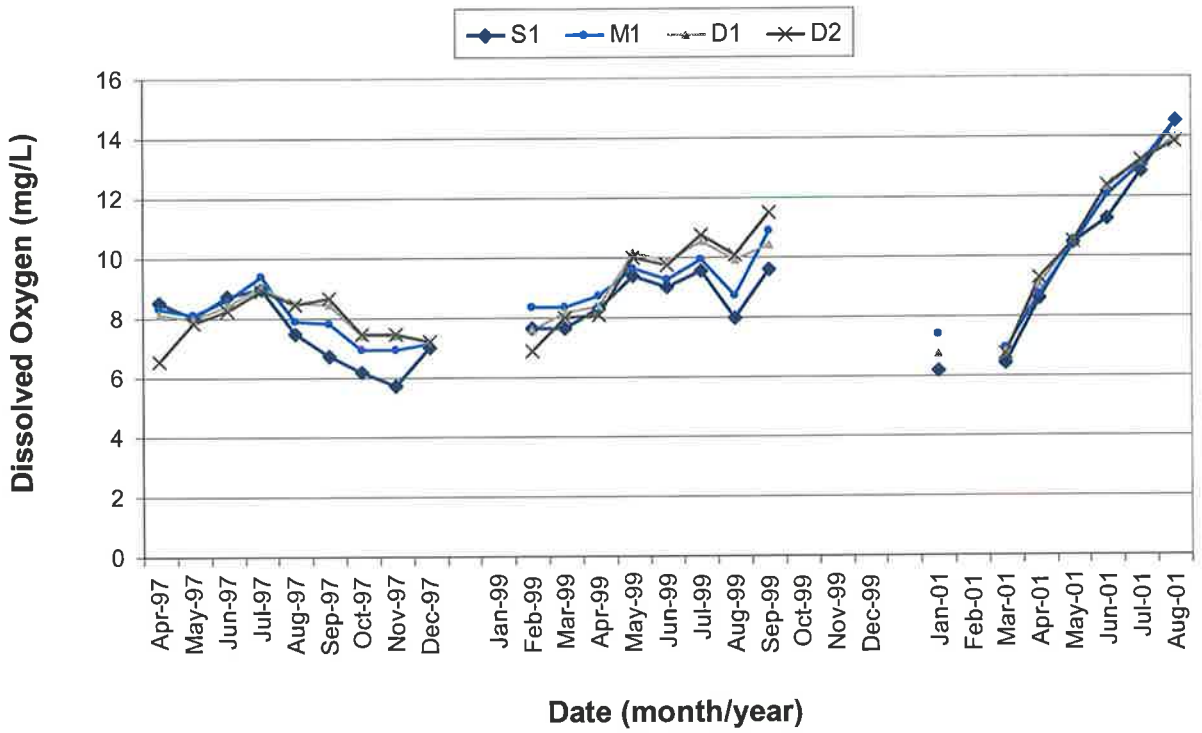


Figure 3. 23: Dissolved oxygen concentrations at the four sites, S1, M1, D1 and D2 of the Warren Reservoir, during 1991, 1999 and 2001.

Temporal differences in dissolved oxygen concentrations across the reservoir were observed between 1997 and 2001, with highest concentrations measured during 2001 (Table 3.9).

Table 3. 9: Mean annual dissolved oxygen concentrations measured during 1997, 1999 and 2001 at sites S1, M1, D1 and D2 in the Warren reservoir.

	Average annual dissolved oxygen concentrations (mg/L)			
	S1	**M1**	**D1**	**D2**
1997	7.5±0.3	7.9±0.2	8.1±0.1	7.9±0.2
1999	8.6±0.2	9.2±0.3	9.4±0.4	9.4±0.4
2001	9.9±0.9	10.2±0.9	10.2±0.9	10.7±0.8

3.5.9 TOTAL PHOSPHORUS (TP)

Total phosphorus (TP) concentrations in the Warren Reservoir are summarised in Figure 3.24. Highest mean TP levels during the study were measured at site S1 (8±0.4 $x10^{-2}$ mg/L), followed by M1 (6±0.5 $x10^{-2}$ mg/L), D1 (5±0.6 $x10^{-2}$ mg/L) and D2 (4.8±0.6 $x10^{-2}$ mg/L). The TP concentrations during 1998 and 1999 were significantly higher in the S1 samples when compared to the samples from the other three sites ($p<0.05$). The differences in TP concentration between M1, D1 and D2 were not significant ($p>0.1$).

Decreases in TP concentrations were measured at sites M1, D1 and D2 from 1997 to 1998 (mean ± S.E., 48±5.3%), and were followed by an increase from 1999 to 2001 (an increase of approximately 3–fold across the reservoir). TP concentrations observed at site S1 did not show the same pattern as the other three sites with little variation between 1997 and 1998 and a less marked increase of 25% from 1999 to 2001. Seasonal patterns in TP distribution were most apparent in deeper waters, sites D2 and D1, where peaks were observed during summer/autumn 1998, summer 1999 and 2001. Seasonal patterns at site M1 were less apparent, however a peak in TP concentration was observed during summer 1999 and 2001. No seasonal pattern in TP concentration was observed for site S1.

Reservoir TP concentrations were compared to those of the catchment, to determine whether stream inflow caused reservoir TP levels to increase. TP levels in two streams C-S1 and C-S2 were measured every 3 to 7 days during the period of three months between 15th July and 19th October 1999 (Appendix 4). Average stream TP levels during this period were

9±0.6 $x10^{-2}$ mg/L in C-S1 and 8±0.9 $x10^{-2}$ mg/L in C-S2. There were no significant differences between TP levels entering the reservoir from C-S1 and C-S2 (p=0.35).

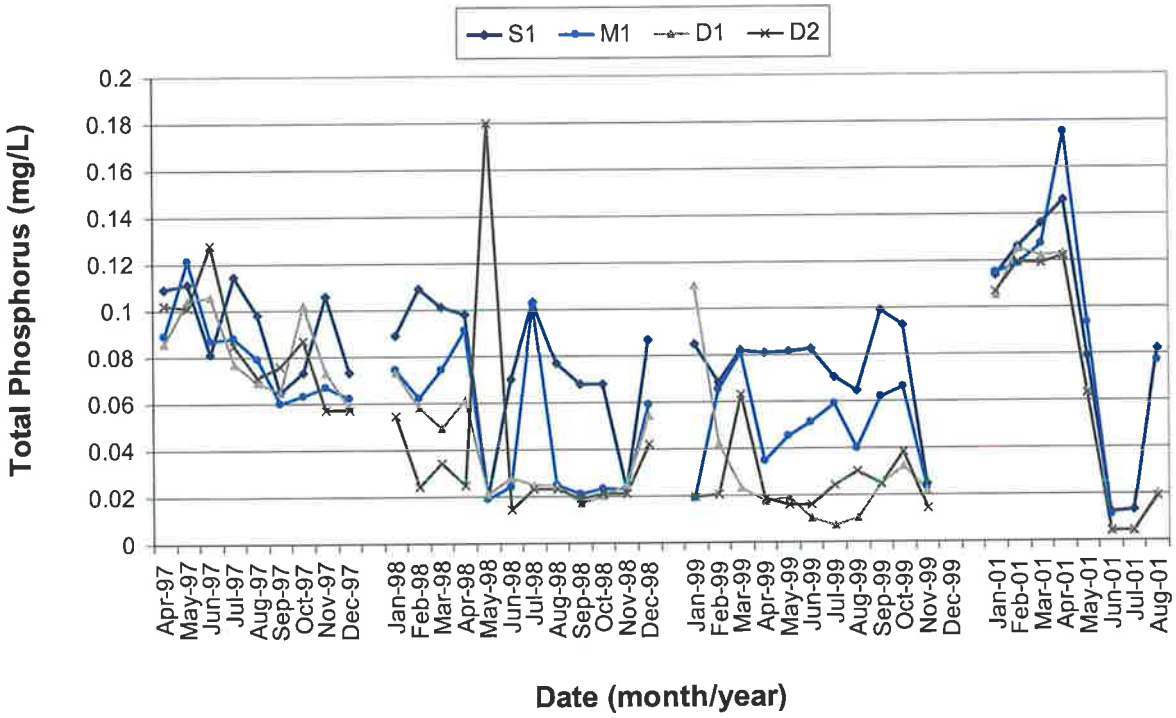


Figure 3. 24: Total Phosphorus (mg/L) determined at the four reservoir sites S1, M1, D1 and D2, during 1997, 1998, 1999 and 2001.

3.5.10 SOLUBLE REACTIVE PHOSPHORUS (SRP)

The concentration of SRP determined in water samples from the four sampling sites is summarised in Figure 3.25.

Water sample SRP concentrations ranged from $3x10^{-3}$mg/L to $1.5x10^{-1}$mg/L. SRP concentrations were generally consistent throughout the study period, with significant peaks observed in February 1998 and 2001 and August 1998. These peaks corresponded to a maximum 20-fold increase across S1, M1, D1 and D2 in February 1998, 10 to 25-fold increases across S1, M1 and D1 in February 2001, and a 3-fold increase at S1 and 10-fold increase at M1 in August 1998.

Mean annual SRP concentrations were higher during 1998 and 2001 (mean ± S.E. 1.8±0.2 x 10^{-2} mg/L and 1.8±0.1 x 10^{-2} mg/L respectively) when compared to 1997 and 1999 (mean ± S.E. 1.1±0.03 x 10^{-2} mg/L and 0.9±0.01 x 10^{-2} mg/L respectively).

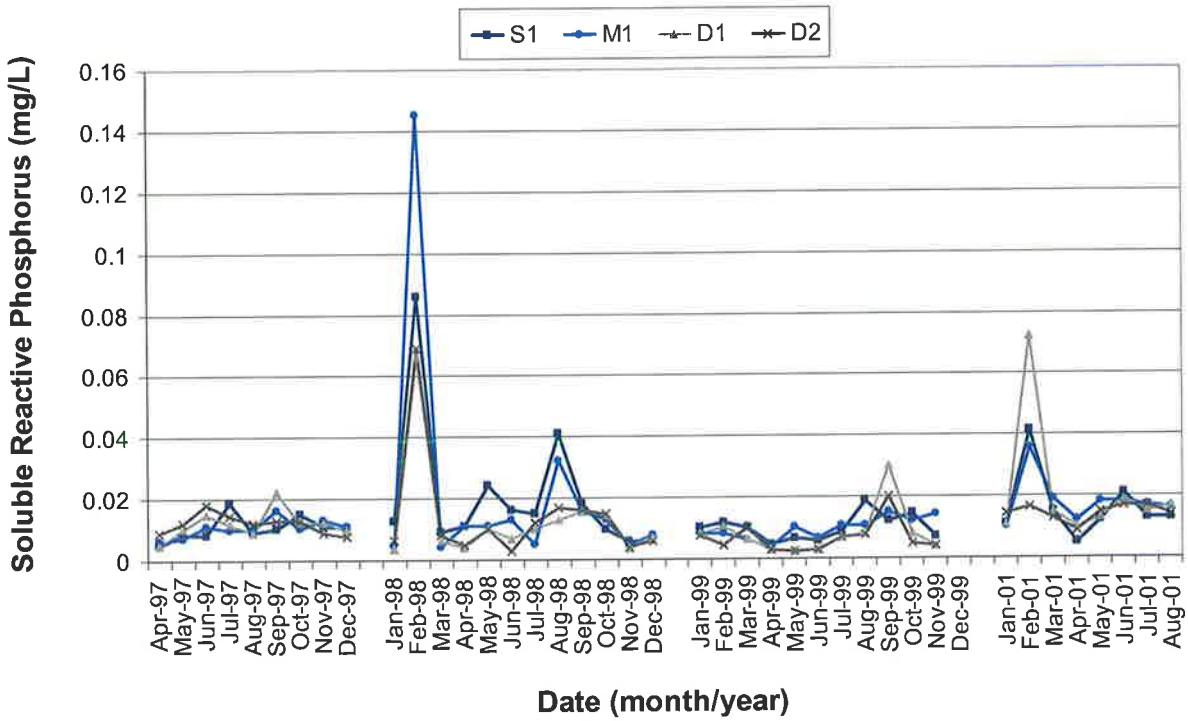


Figure 3. 25: Soluble reactive phosphorus (mg/L) determined at the four sampling sites S1, M1, D1 and D2, during 1997, 1998, 1999 and 2001.

In comparison with stream TP, stream SRP concentrations (Appendix 4) constituted 22% of the total phosphorus levels entering the reservoir via two catchment streams during the three-month study period. Average SRP concentrations in stream water were 2±0.3 x10^{-2} mg/L in C-S1 and 2±0.2 x10^{-2} mg/L in C-S2. The concentrations of SRP in each stream were not significantly different (p=0.21).

3.5.11 NITRATE (NO_3^-)

Nitrate concentrations in reservoir water samples are summarised in Figure 3.26. No significant differences in nitrate concentrations between the four sites were observed throughout the study period (p=0.99), thus changes in nitrate levels occurred irrespective of

the location in the reservoir. Nitrate concentrations did however show seasonality during 1998 and 1999. July to November (winter-spring) nitrate concentrations across the reservoir were 13 to 28-fold higher than the January to May (summer-autumn) concentrations in 1998, and 9 to 19-fold higher in 1999 (Figure 3.26).

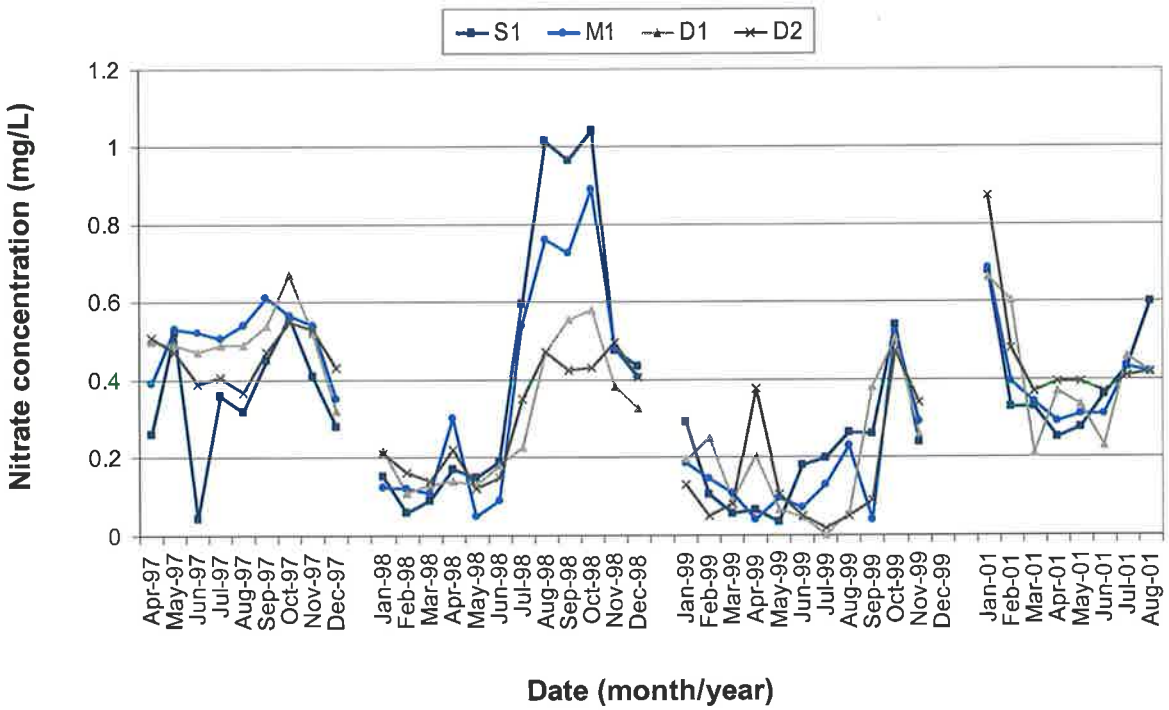


Figure 3. 26: Nitrate concentration (mg/L) determined at the four sampling sites S1, M1, D1 and D2 during 1997, 1998, 1999 and 2001.

The concentrations of nitrate in the two catchment streams were measured during the wet season 1999 (Appendix 4). Mean nitrate concentrations in the stream waters were 0.56±0.07mg/L in C-S1 and 0.49±0.08mg/L in C-S2. A comparison between nitrate levels in two streams and those in the reservoir prior to, during and post winter 1999 suggests that increases in reservoir NO_3^- levels were most likely due to inflow of nutrients from the catchment (mean increase of nitrate concentration across the reservoir being 87±2%).

3.5.12 SALINITY AND pH LEVELS

ANOVA showed no significant differences in water salinity ($p>0.5$) or pH ($p>0.2$) during the study. Furthermore no seasonal or annual patterns were observed in either parameter.

3.5.13 TURBIDITY AND LIGHT PENETRATION

Measures of light attenuation at the four sampling sites, expressed as Secchi depth (m), are summarised in Figure 3.27. Consistent high light attenuation (and thus low underwater visibility) was measured during 1997 and 2001 (depth ranging from 0.39m to 1.4 m, and from 0.2 to 0.6 m respectively) when compared to observations in 1998 and 1999 where Secchi depths of up to 2.8m were recorded.

Mean light attenuation data for the four year study showed lowest visibility at the shallow site S1 (0.53±0.03 m), followed by M1 (0.74±0.04 m), then D2 (0.99±0.07 m) and D1(1±0.07 m). No significant differences in light attenuation were observed between D1 and D2 sites (T-test, $p>0.05$), while significant differences were observed between the sites S1, M1 and D1, D2 (T-test, $p<0.05$).

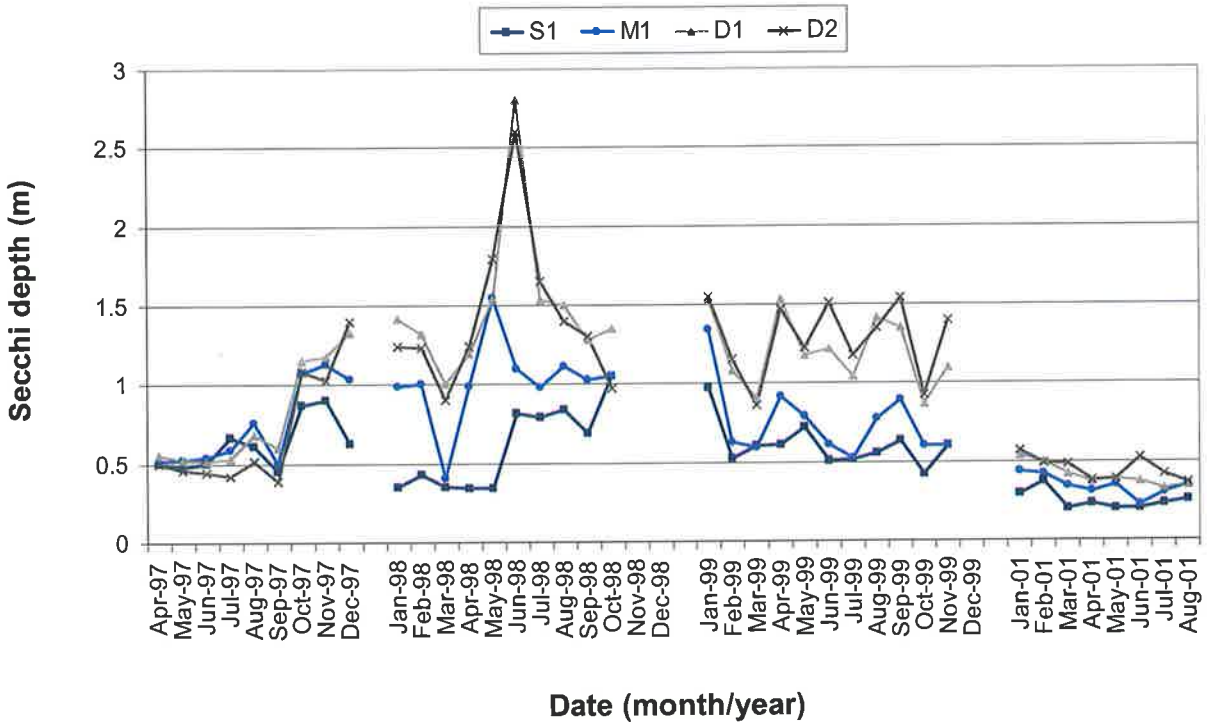


Figure 3. 27: Secchi depth (meters) (representing underwater visibility) at sites S1, M1, D1 and D2.

From 1997 to 2001, water turbidity (Figure 3.28) ranged from 3 NTU in summer to 58 NTU in winter. Turbidity levels between March and June were higher than those measured during the months prior to and/or post this period (46±2% higher in 1998, 82±1.4% in 1999, 54±9% in 2000 and 74±1% in 2001). Except for the first half of 1997, highest levels of turbidity were at the shallow site S1 (mean ± S.E. 35±2 NTU), followed by M1 (30±2 NTU), D1 (24±1 NTU) and D2 (19±2 NTU).

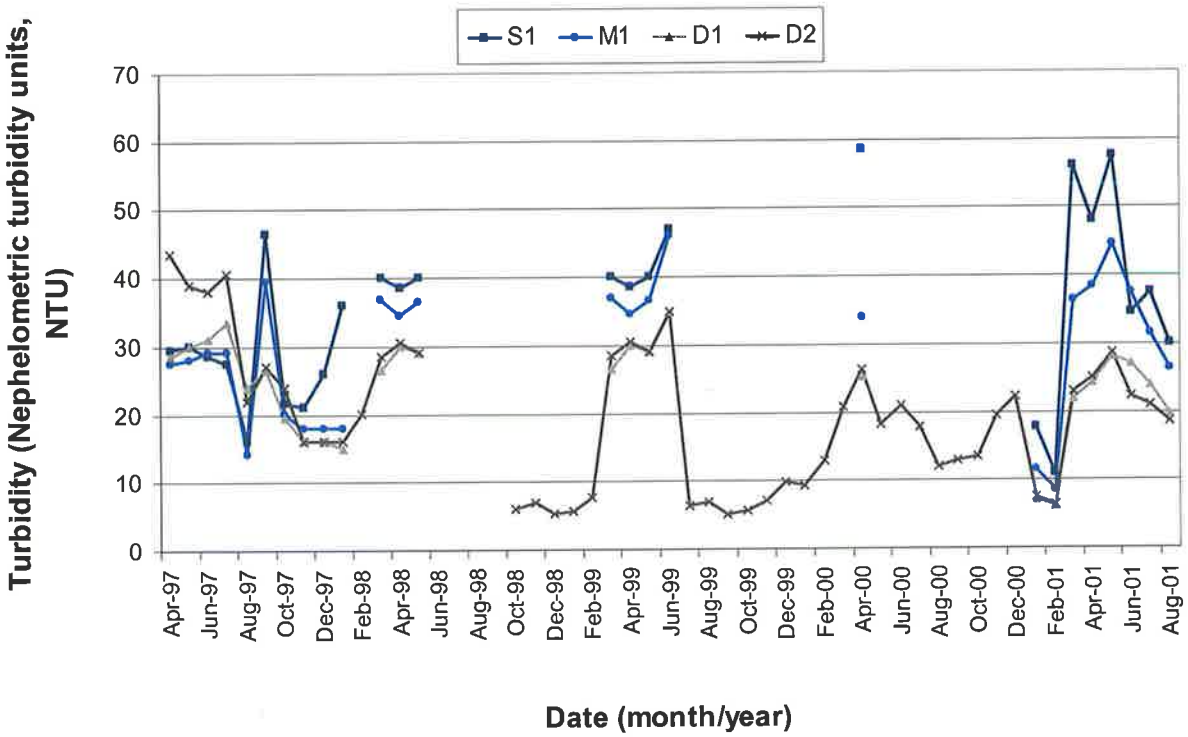


Figure 3. 28: Water turbidity measurements (NTU) at the four sampling sites of the reservoir, S1, M1, D1 and D2, collected between April 1997 and August 2001.

3.6 DISCUSSION

3.6.1 ORIGIN OF DOC IN THE WARREN RESERVOIR

When this project was conceived, the Warren Reservoir was expected to be a closed system for the duration of the study. That is, the only input to the reservoir was to be from the catchment, the reservoir was to remain at full capacity and the only water losses (other than evaporation) were to be over the dam wall. No water was to enter via the Murray pipeline. In such a closed system, the DOC inputs and losses are relatively easy to identify, if not to measure. In actuality, the Warren Reservoir was a closed system for only the first year of this study (1997). Water began to be withdrawn from the reservoir via the pipeline in January 1998, resulting in a 20% decrease in reservoir volume by February 1998 (Figure 3.1). Water was added to the reservoir via the Murray pipeline from April to October 1998, then almost continuously from February 1999 until the end of the study in August 2001. Throughout the duration of the study 12492 ML (or 2.6 reservoir volumes), of Murray water was added to the reservoir. It is estimated that during this time 3563 ML (less than one reservoir volume) of water entered via the two major tributary streams (C-S1 and C-S2). This represents just 29% of the contribution from the Murray pipeline. Thus, the system envisaged at the commencement of the project was very different to the system that was ultimately studied.

The lower Murray River, where this extra water was sourced, is a very different river system to the small catchment streams that flow into the Warren Reservoir, and its DOC load reflects this difference. The lower Murray River is thousands of kilometres removed from the parts of its catchment that yields the bulk of its waters. Most of the Murray waters are sourced from the mountains of the Great Dividing Range in New South Wales and Victoria. There are no major tributaries that enter the Murray River within South Australia. Flow in the Murray River is heavily regulated in South Australia via a series of low weirs. Thus much of the lower Murray River can be viewed as a series of long, shallow reservoirs.

The varying proportions of water sourced from the local catchment and the Murray River throughout this study adds an extra dimension of complexity over and above the usual autochthonous/allochthonous variation in most lakes and reservoirs.

With the exception of 1997, the contribution of the Murray River waters to the annual DOC budgets differed between years. In 1998, 4584 kg of DOC was added to the reservoir via the Murray River inflow, when the Murray River pipeline was open for three months.

This contribution increased to 29369 kg during 1999, 22448 kg during 2000, and 28160 kg during 2001. Since the volume of water and the quantity of the DOC loaded by the Murray River into the reservoir did not change in 2001 with respect to 1999, this implies that other sources were contributing to the higher DOC levels observed during 2001.

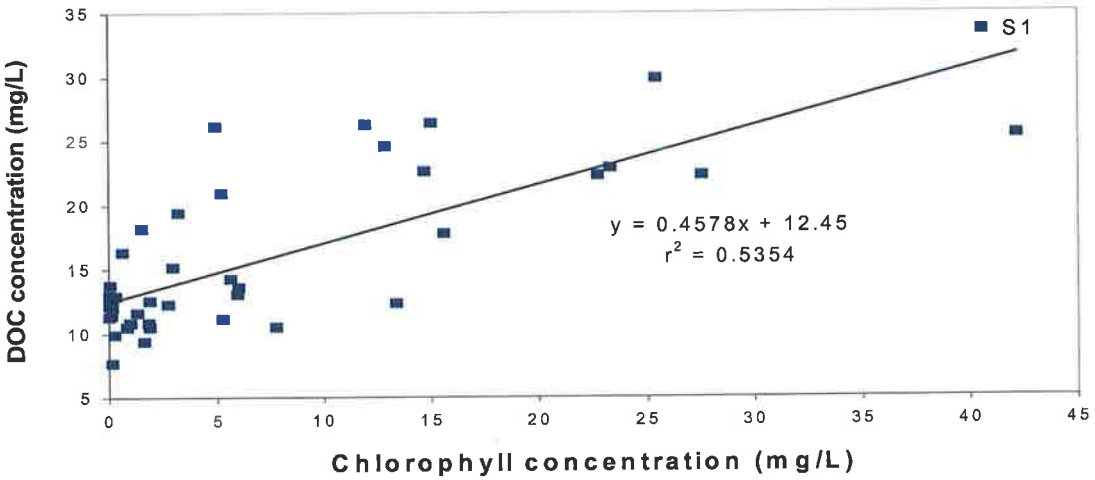
Based on the total level of rainfall during the wet season of each year (554mm in 1997; 650mm in 1998; 647mm in 1999; 713mm in 2000 and 832mm in 2001) and assuming the stream DOC concentrations were similar between the years, the estimated annual contributions of stream water DOC to the total reservoir DOC pool during the course of the study was approximately 10300 kg during 1997, 12100 during 1998, 12000 kg during 1999, 13220 kg during 2000 and 15430 kg during 2001. Thus, on an annual scale, the winter loading of DOC from the catchment streams into the Warren Reservoir was significantly lower than that originating from the Murray River.

Seasonal variations in DOC concentration were observed in the Warren Reservoir from 1997 to 2001 with peaks in DOC concentration in both summer and winter in most years. The contribution of autochthonous and allochthonous DOC can both undergo strong seasonal variation (Hessen, 1992; Jonsson et al., 2001; Pace and Cole, 2002). The production of autochthonous DOC depends strongly on temperature and the level of sunlight irradiance, and hence is seasonal in mid to high latitudes. The contribution of allochthonous DOC depends on rainfall, and hence will be seasonal where rainfall is seasonal. The climate of the Warren Reservoir is Mediterranean, consisting of warm dry summers and cool wet winters. Thus, the summer peak in DOC concentration is most likely due to peak seasonal autochthonous DOC production, and the winter peak most likely reflects higher allochthonous DOC inputs. In order to confirm these sources of seasonal DOC variations, correlation and regression analyses were performed between DOC concentrations and various physical, chemical and biological water parameters.

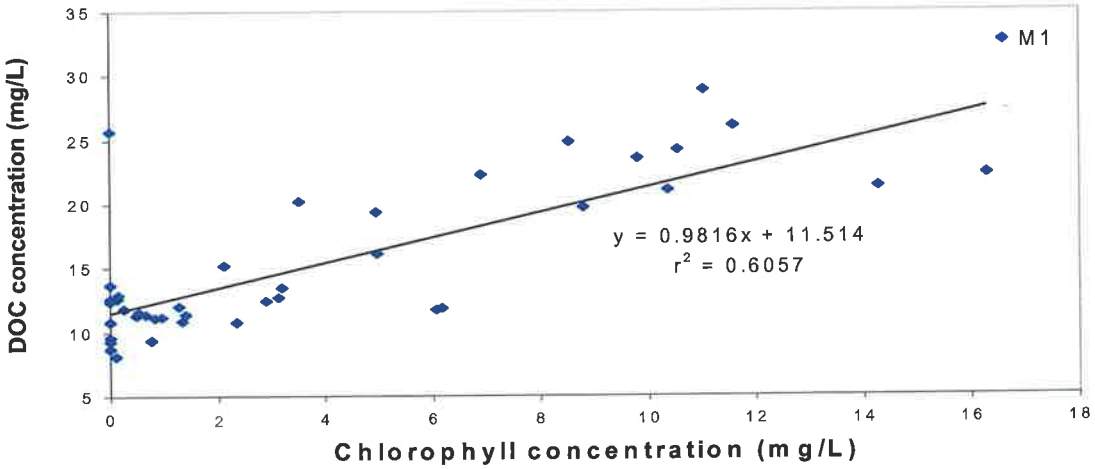
Studying changes in the nature of reservoir DOC can also provide evidence for the contributions of autochthonous and allochthonous DOC in the Warren Reservoir. Generally it is recognised that much of the colour in water comes from humic substances leached from plant and soil organic matter (V-Balogh and Voros, 1997). Decomposition products of aquatic organisms may add to the humic content of water, however terrestrial ecosystems (such as catchments) are generally the primary source of aquatic humus (Mattsson and Kortelainen, 1998). Lakes rich in DOC often have brown water because of the high concentrations of light-absorbing humic and fulvic acids (Pace and Cole, 2002). Based on

numerous correlations between water colour and DOC nature, colour is often used as an index of the relative humic content of water (Waiser and Robarts, 2000; Pace and Cole, 2002). Variations in both DOC concentration and colour reflect the relative contributions of autochthonous and allochthonous DOC (Pace and Cole, 2002). Thus, coupling spectroscopic colour measurement with DOC monitoring can provide information about the nature and source of organic matter (Krasner et al., 1996). In addition to water colour, changes to specific colour (which is the ratio of water colour to DOC concentration) can be used to assess changes to the nature of DOC, on the assumption that higher specific colour indicates a higher composition of more humified and complex structured organic material (Hautala et al., 2000). Increasing DOC concentrations are not necessarily associated with increased water colour (as suggested by Tranvik 1990); it is the nature of DOC that determines the colour of water. For example, certain Canadian lakes contain some of the highest known concentrations of DOC, but are generally less coloured than lakes in humic regions that have a higher input of terrestrial organics (Waiser and Robarts, 2000).

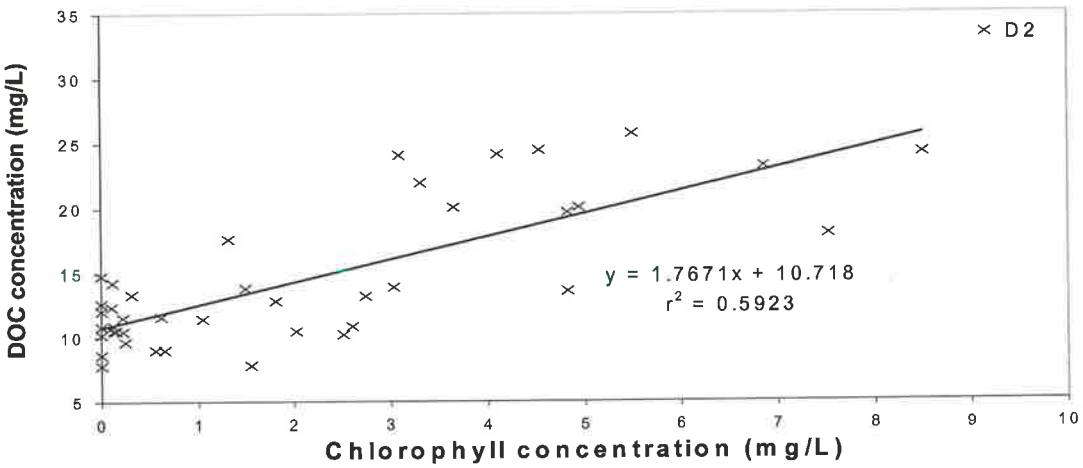
Previous studies showed that phytoplankton is responsible for most primary production (synthesis of autochthonous organic matter) in lakes (Jassby et al., 2002). The organic carbon is released into the water either through excretion or through the lysis of dead cells (Hessen, 1992; DiSiervi, 1995). Thus, depending on the type of water body, the amount of algal biomass may ultimately be associated with DOC levels in the reservoir. Other internal aquatic sources (for example zooplankton) can also contribute to autochthonous DOC inputs (Hessen, 1992; Mattsson and Kortelainen, 1998), but the release of DOC from phytoplankton in most lakes appears to be the most prominent source of autochthonous DOC (Hessen, 1992; V-Balogh and Voros, 1997). To determine whether there was a correlation between phytoplankton and DOC concentration in the Warren Reservoir, the relationship between reservoir DOC and chlorophyll concentrations was studied. The correlation analysis showed a significant positive correlation between the two parameters ($p<0.01$ for each site). Linear regression analysis was performed and the r^2 values are summarised in Figure 3.29.



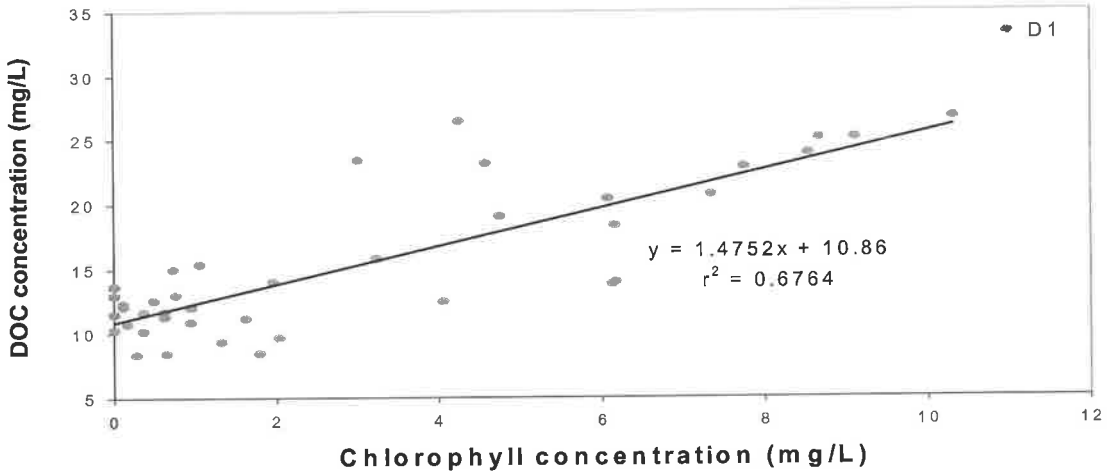
(A) Site S1



(B) Site M1



(C) Site D1



(D) Site D2

Figure 3. 29: Linear regression analysis establishing a relationship between DOC and Chlorophyll concentration at each of the four reservoir sites, S1, M1, D1 and D2. r^2 values are indicated in each figure. Correlation analysis at S1, M1, D1 and D2 was R=0.6, R=0.7, R=0.6 and R=0.5 respectively.

The increases in chlorophyll levels (and thus phytoplankton biomass) were concurrent with increases in DOC concentrations at all four reservoir sites. As DOC increases of autochthonous origin can be temporary, due to autochthonous labile DOC being an important source of high quality carbon to heterotrophic bacteria that can be rapidly removed from the water body (Cole et al., 1982; Baines and Pace, 1991; Jassby et al., 2002), this relationship could explain the short-term increases in DOC observed each year in the Warren Reservoir during the summer months (January-March). The timing of large summer peaks in DOC coincided with occurrences of algal blooms in the reservoir. Based on this information and the correlation analyses, phytoplankton is an important source of Warren Reservoir DOC during warmer months when high temperatures favour its growth.

Although autochthonous DOC is often rapidly removed from water bodies, there is evidence suggesting that portions of this degradable organic carbon can also accumulate in the photic zone (Thingstad et al., 1999). Phytoplankton derived organic compounds decompose within days to weeks but the decomposition rates are shown to decrease with the diagenetic state (that is from fresh to old) (Amon and Benner, 1996; Mannino and Harvey, 2000). This

could explain the measured increases in the average annual DOC concentrations in the Warren Reservoir from 1997 to 2001. Lower phytoplankton levels throughout 1997 and 1998 were corresponded to the low DOC levels. The start of summer 1999 and 2001 were associated with algal blooms, and the chlorophyll concentrations reached progressively higher post-summer levels each year. Water temperatures during autumn and winter months were sufficient to enable phytoplankton growth in the Warren Reservoir (based on the correlation of Warren Reservoir temperatures with those measured in other studies by Coveney and Wetzel, 1995; Rae and Vincent, 1998; Danilov and Ekelund, 2001), which most likely resulted in a continuous input of DOC, causing long-term DOC accumulation in the Warren Reservoir. Increases in algal concentration are generally responsible for eutrophication of waters, which would suggest higher productivity and ultimately higher internal loading of organic matter into the system. Although there is a higher supply of organic material (as was the case in this study), bacterial levels may remain at a constant level due to factors such as competition for mineral nutrients as well as effects of predation (Thingstad et al., 1997). As such, a greater autochthonous input of DOC into the reservoir (when compared to previous years when low phytoplankton levels were found) may result in a continuous accumulation of DOC in the system.

Further evidence that higher DOC levels during 2001 were most likely caused by increased phytoplankton growth can be seen from the regression analysis of the DOC concentration and water colour. As the reservoir water colour is known to be affected by the type of organic material present in water (and ultimately the source of this material) (Pace and Cole, 2002), the colour data was used to elucidate the potential annual source of DOC during the study (ie: autochthonous or allochthonous). A positive correlation between DOC concentrations and water colour was observed (Figure 3.30). The annual increases in total DOC were associated with increased water colour, but the doubling in total DOC concentration from 1997 to 2001 did not correspond with an increase in the water colour. In other words, the specific colour of the DOC was higher in 1997 and 1998, than in 1999 and 2001. This is supported by the mean annual specific colours reported in Table 3.8, and suggests that the DOC pool during 1997 and 1998 contained a higher proportion of coloured allochthonous organic material (steeper slopes in Figure 3.30) when compared to the DOC pool produced during 1999 and 2001.

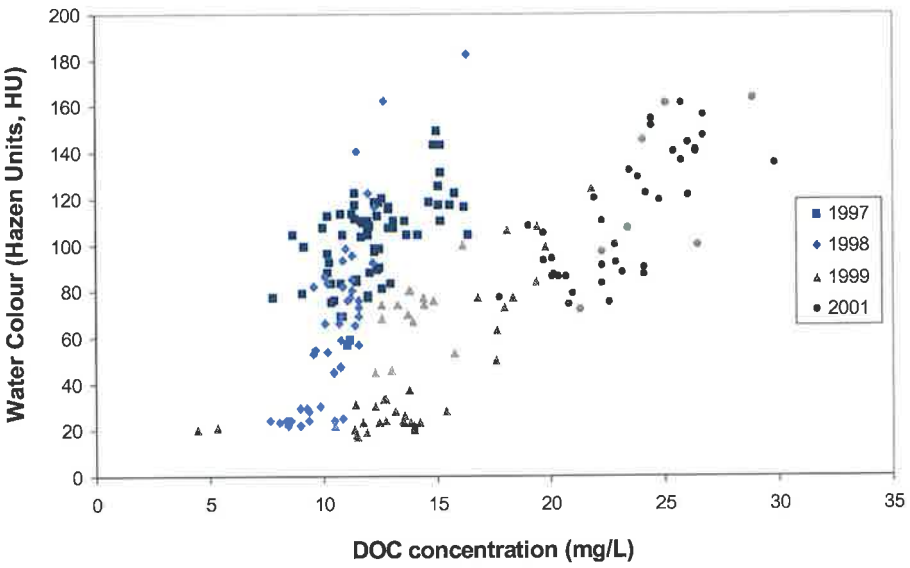


Figure 3. 30: Relationship between water colour and DOC concentration during 1997 (R=0.59, r^2=0.35, y=6.2x+27), 1998 (R=0.84, r^2=0.7, y=20.9x-155), 1999 (R=0.71, r^2=0.51, y=6.7x-43) and 2001 (R=0.73, r^2=0.54, y=7.7x+67).

The decrease in specific colour in 1999 and 2001 corresponds with increasing additions of Murray River water. This may have had the effect of diluting and flushing out humic DOC from allochthonous sources. However, phytoplankton levels were also considerably higher in 1999 and 2001 (Figure 3.13). The increased levels of autochthonous DOC associated with the higher phytoplankton levels would also have the effect of diluting allochthonous DOC (as a proportion of total DOC).

Why higher phytoplankton growth was favoured during 1999 and 2001 when compared to 1997 and 1998 is unknown. Most studies emphasise changes in nutrient status as the main cause of changes in annual primary production, although parameters such as precipitation and water optical properties also play a role (Jassby et al., 2002). Carpenter et al. (1998) state that phytoplankton biomass and production in lakes tends to be increased by phosphorus input and decreased by high levels of coloured DOC. During this study, there were no clear seasonal variations in the studied nutrients (phosphorus and nitrogen). Total phosphorus levels were generally higher during summer 2001 than at other times. This increase in TP was concurrent with the increase in chlorophyll levels although no significant correlation was obtained between the two parameters. Furthermore, no significant

correlations were found between SRP concentrations and phytoplankton ($R=0.3$, $r^2<0.07$), or nitrate concentrations and phytoplankton ($R<0.5$, $r^2<0.3$). However, the optical properties of water (in terms of water colour) did change in the Warren Reservoir. In particular, the specific colour values greater than 6.5 (HU/mgL^{-1}) were always associated with chlorophyll levels that were lower than 3.5mg/L, and vice versa (Figure 3.31). This may suggest that either DOC originating from primary production was of low specific colour value; or that high specific colour levels were having a shading effect on phytoplankton growth in the reservoir (due to inhibition of light required for photosynthesis) (Lind et al., 1997; V-Balogh and Voros, 1997; Bergstrom, 2000). This in turn could explain why low chlorophyll levels were found throughout 1997 and 1998 when specific colour values were mainly high.

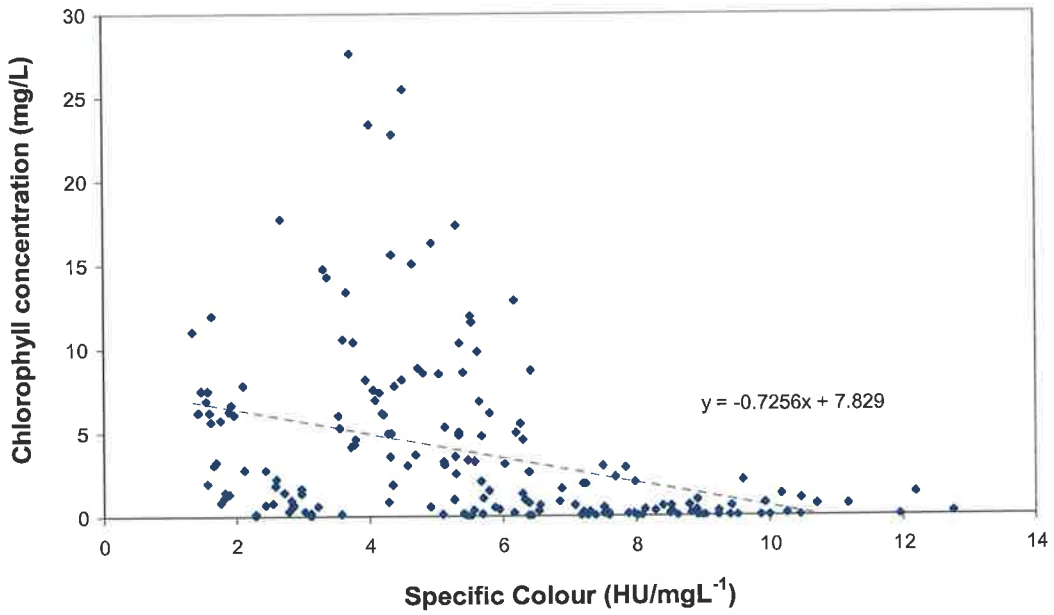


Figure 3. 31: Relationship between chlorophyll concentration and specific colour (Colour/DOC concentration) based on combined data from 1997, 1998, 1999 and 2001 at all four sites of the Warren Reservoir. Results from correlation and regression analyses were $R=-0.4$ and $r^2=0.14$ respectively.

In addition to autochthonous DOC, allochthonous DOC also entered the Warren Reservoir, but only during the rainy months when water flowed in the streams, carrying DOC from the catchment into the reservoir. During 1997, 1998 and 1999 (when entire winter

seasons were studied), increases in reservoir DOC of up to 70% were observed during rainy periods (July to October), however these levels decreased with secession of rain. It is well documented that DOC influx from a catchment into a water body such as a reservoir, is regulated by precipitation and flushing (Vinebrooke and Leavitt, 1998; Findlay et al., 2001). Flushing events are further shown to be seasonal and spatial, depending on the type and location of a water body, often generating variations in not only DOC concentration, but the chemical composition as well (Mann and Wetzel, 1995). Seasonal fluctuations in DOC concentration were also reported for the surface waters of two Australian wetlands (Briggs et al., 1993).

From 1997 until 2000, water colour in the Warren Reservoir was higher in mid to late winter and early spring. Specific colour also increased during the wet seasons of 1997, 1998, 1999 and 2000, suggesting a change in the nature of reservoir DOC toward a higher composition of coloured material and thus humic content. In 2001 winter and spring data is incomplete thus seasonal comparisons could not be made. Based on the DOC quantity and specific colour data, it appears that DOC originating from the catchment exerted only short-term seasonal effects on the overall reservoir DOC levels.

The large increases in the water colour observed over the rainy season indicate incomplete mixing of the water body on the monthly timescale. The colour of the reservoir water at S1 reached values of 130-160 HU (Figure 3.20), which are almost as high as those recorded for the catchment streams (150-200 HU). Even at the deeper sites, colour values increased up to four-fold (from approximately 20 HU to 60-80 HU) during the rainy season. Given that the catchment streams only contribute around 10% of the reservoir volume in a typical wet season, the addition of this highly coloured water (~200 HU) could only result in a doubling of reservoir water colour from typical values of 20 HU before the onset of stream flow, if there was efficient mixing of stream and reservoir waters. The larger increases in colour observed here indicate that much of the water derived from the catchment streams initially remains in the top metre of the water body. The subsequent decrease in water colour following the end of the rainy season can, at least in part, be attributed to mixing of highly coloured surfaces waters with less coloured deeper waters. In other words, the steady decrease in colour observed from September until June each year does not necessarily indicate that the humic substances derived from the catchment are rapidly degraded.

Although the rapid decrease in water colour following the end of the wet season may not signify a rapid degradation of allochthonous DOC, the fact that the reservoir water colour

does appear to return to around 20 HU by the beginning of the wet season (even in 1998, before the addition of substantial quantities of Murray water) indicates that there is significant degradation of allochthonous DOC on the yearly timescale. If there were no degradation of allochthonous DOC, the colour of the reservoir waters would approach that of the catchment streams throughout the year (in the absence of additions of Murray River water). Moran et al. (2000) showed that removal of coloured DOC via photo-degradation was significantly higher when compared to the non coloured DOC pool, which could explain rapid decreases in reservoir colour and DOC following the wet season.

Allochthonous carbon can also be removed by bacterial degradation and utilisation. Although most of the allochthonous organic carbon is regarded as recalcitrant with only 1-10% being readily used and degraded by bacteria (Moran and Hodson, 1990), there are many indications that allochthonous organic carbon serves as a good source of biological carbon cycling in humic lakes. For example, Jonsson et al. (2001) showed allochthonous carbon (supplied either continuously or during high spring flows) to be the principal source for mineralisation in the lake. Waiser and Robarts (2000) showed inflowing higher molecular weight organic material to be 8 times more available to bacteria than low molecular weight material of the lake, which would imply more rapid removal of allochthonous organic material than autochthonous. Bergstrom and Jansson (2000) suggest high bacterial mineralisation of carbon to be mainly due to a large input of 'high' quality allochthonous DOC. As such, events such as the onset of rain during wet seasons, responsible for the flushing organic matter from the catchment into the reservoir, could not only explain increases in Warren Reservoir DOC concentration measured during wet winter months, but possibly its rapid removal from the water upon the end of the wet season and the drying out of two major streams C-S1 and C-S2.

Mann and Wetzel (1995) reported a similar trend in seasonal fluctuations of surface water DOC concentrations, as was observed during the study of the Warren Reservoir. They attributed summer increases in DOC to increased primary productivity of resident macrophytes, periphyton and microbial conversion of POC to DOC.

Comparison of the importance of autochthonous and allochthonous inputs into the Warren Reservoir with those of lakes found in the other regions of the world raise some similarities and differences. In a study of a complex system of rivers and water bodies near San Francisco, Jassby and Cloern (2000) have demonstrated that phytoplankton productivity is an important source of organic matter in all seasons, except winter of above-normal rainfall

years, while it is a dominant source in spring and summer of below-normal rainfall years (Jassby et al., 2002). A study of a Swedish lake implicates allochthonous DOC as the major source of energy for biodegradation even during summer months (Bergstrom and Jansson, 2000). A study by Reitner et al. (1997) showed primary production of a European lake during summer was a significant source of organic input, while the input of humic DOC from allochthonous sources was significant only during a short autumn period but not during winter months as the lake was covered with ice for most of winter. In the case of Warren Reservoir colour-generating compounds (generally known to be tannins, humic and fulvic acids) were mainly dominant during winter inflow of water from the catchment, while DOC of autochthonous origin (not associated with high colour) was dominant for the remainder of the year.

Page (2002) found average specific colour in 13 Australian catchments differing in various biogeochemical parameters to be in the range of 1.2 and 11.6 HU/mg/L DOC. Within the Warren Reservoir alone, fluctuations in specific colour were found to be in a similar range of 1.5-13 HU/mg/L DOC. Analysis of humic acid (Aldrich), fulvic acid (International Humic Substance Society), lignin (Aldrich) and Suwanee River Natural Organic Matter (International Humic Substances Society) standards, (conducted by Leon Linden from South Australian Water Corporation, personal communication), show specific colour values of 58 HU/mg/L DOC, 5 HU/mg/L DOC, 2 HU/mg/L DOC and 7 HU/mg/L DOC respectively; while colour values were 170 HU, 62 HU, 28 HU and 86 HU respectively. Thus, during winter, the specific colour of the Warren Reservoir exceeded that of fulvic acid standards but was below that of humic acid standards. Furthermore, during winter water colour exceeded 180 HU which is similar to the true colour of humic acids. As such, both colour and specific colour during winter were most probably caused by high concentrations of humic acids. As humic acid concentrations in the Warren Reservoir are in all probability less concentrated than those of the standards referenced, its effects on specific water colour of the reservoir would be reduced due to dilution.

Finally, the analysis of the relationships between DOC concentrations and colour during each year showed a positive correlation between the two parameters, suggesting that the increases in total DOC concentrations caused deterioration in water quality in terms of colour. Thus, on a seasonal scale, increases in DOC concentrations resulted in increases in water colour (as suggested by Tranvik 1990). However this relationship failed to hold when

inter-year comparisons were made, suggesting an involvement of additional factors in causing water colour (eg: the nature of DOC).

In summary, it was found that both autochthonous and allochthonous sources were responsible for DOC loading in the Warren Reservoir, but their effects were seasonal. While allochthonous DOC input was dependent on rain, autochthonous input occurred throughout the year, increasing during warmer months. It is proposed that allochthonous DOC is initially present mainly in surface waters during and immediately following the rainy season. Over the following months it is diluted with deeper waters. On a yearly timescale it is assimilated by the aquatic fauna, and removed from surface waters via photo-bleaching (Reitner et al., 1997; Bertilsson and Tranvik, 2000; Moran et al., 2000). On the other hand, the majority of DOC from autochthonous sources was possibly less assimilable by the aquatic fauna and/or less prone to photo-degradation (Benner and Biddanda, 1998) thus accumulating in the system and causing a gradual increase in DOC level during the study. An alternative explanation for the DOC accumulation is that the rate of production of autochthonous material was higher than the rate of its degradation, however this remains speculative, as the microbial activity in the reservoir was not measured.

In addition to identifying the importance of seasonal autochthonous and allochthonous inputs on the DOC quantity in the reservoir, the sources of origin were also found to be important factors influencing the character of DOC in the reservoir. Namely, the analysis of the DOC character (as indicated by the colour and specific colour data) suggests that phytoplankton-derived DOC was different in nature to the allochthonous DOC that comprised colour-generating materials (eg: fulvic acids, tannins and humic matter). While the DOC concentration increased from 1997 to 2001, water colour did not increase proportionately. This implicates the source of DOC as an important factor affecting some of the aesthetic factors of water quality.

The findings of this study also implicate phytoplankton in causing deterioration of water aesthetics. Namely, water colour increased during summer 1999 and 2001 when high phytoplankton levels occurred, while during summer 1998 low colour was parallel to low phytoplankton levels. When this information is combined with the information obtained from specific colour analysis, it appears that summer increases in water colour are not driven by increases of colour-generating DOC compounds, but most likely by other factors such as phytoplankton pigmentation.

3.6.2 ANALYSIS OF THE INTERACTIONS BETWEEN BIOTIC AND ABIOTIC FACTORS IN THE WARREN RESERVOIR

The second objective of this study was to examine the physical, chemical and biological parameters of water quality in an attempt to establish relationships between DOC and physicochemical and biological parameters of the reservoir water (that is, the parameters that are either likely to affect or be affected by the quantity and quality of DOC). Special emphasis was placed on studying changes to bacterial populations over time, in order to determine whether seasonal variations in DOC origin (and thus the nature of reservoir DOC) are associated with alterations to the bacterial community structure.

3.6.2.1 Abiotic factors

The concentration of dissolved oxygen at all four reservoir sites increased from one year to the next. This increase was found to parallel increases in chlorophyll levels during the study period. During periods of growth and photosynthesis phytoplankton release oxygen, so an increase in phytoplankton levels observed during 1999 and 2001 could have resulted in higher levels of oxygen when compared to 1997. An alternative explanation is that the higher oxygen concentrations reflect a lower microbial degradation of DOC. Oxygen consumption can be used to study bacterial utilisation of DOC (Coffin et al., 1993; Amon and Benner, 1996). On an ecosystem scale, increased levels of dissolved oxygen could suggest reduced microbial activity (and/or overall secondary production) in the reservoir as the process of microbial degradation requires oxygen. However this alternative remains speculative, as microbial growth was not analysed for the entire study.

Reservoir temperature was characterised by summer highs and winter lows. High water temperatures in summer can stimulate the growth of aquatic organisms such as phytoplankton (as also suggested by Rae and Vincent, 1998; Schultz Jr et al., 2003) and ultimately increase the input of autochthonous DOC into the reservoir. The lack of correlation between water temperature (thus time of the year) and DOC concentration suggests that both autochthonous and allochthonous sources played a role in DOC loading. Thus temperature can influence the mechanisms of DOC loading into the reservoir but is

unlikely to affect removal pathways of DOC from the reservoir (via the microbial loop), as rapid DOC removal was observed to occur during cold winter months as well.

Among the nutrients that were studied (TP, SRP and NO_3), no seasonal patterns were observed for TP. SRP levels showed little fluctuation for the duration of the study, except when two peaks were observed during summer 1998 and 2001. These peaks may have been a result of the microbial degradation processes, releasing SRP from organically complex molecules as described by Wetzel (1983) and Recknagel et al. (1998). Nitrate levels, however, appeared to undergo some seasonality, with higher levels obtained during late winter months, usually concurrent with the rainy periods. Nutrients can bind to the organic matter in the soils and thus may have been transported to the reservoir along the organics during winter influx in the streams (Bergstrom and Jansson, 2000), which would explain winter increases in nitrate levels of the reservoir. The same authors state that increased input of nutrients (particularly phosphates) stimulates a higher bacterial utilisation of allochthonous DOC, particularly during winter inflow of waters from the catchment. Hessen et al., (1994) further showed that both nitrogen and phosphorus are responsible for stimulating bacterial secondary productivity (and removal of allochthonous DOC) after high flow events. Increases in nitrate levels during winter in the Warren Reservoir were concurrent with winter increases in DOC and its rapid removal from the water. An increase of nutrients (for example nitrate) may stimulate microbial growth, resulting indirectly in a reduction of DOC levels (Rivkin and Anderson, 1997; Romani and Sabater, 2000; Carlson et al., 2002; Olsen et al., 2002). However, when comparisons were made between DOC and all three nutrients, no relationships were found. Nutrient levels can affect both microbial processes as well as phytoplankton growth, thus indirectly affecting the reservoir DOC concentrations. No correlations were found between phytoplankton abundance and nutrient levels in the reservoir, suggesting that nutrients were unlikely to be limiting the growth of phytoplankton.

Water turbidity was found to be significantly higher during winter months than summer. Mixing of the shallow water of the Warren Reservoir was mainly driven by the climatic conditions (most probably wind (Van Duin et al., 2001), whereas artificial aeration was applied periodically at the deep end of the reservoir, causing mixing of the entire vertical column. Highest turbidity was measured at the shallow sites, except for the first part of 1997 where the deeper ends of the water showed higher turbidity. A concurrent event that could explain this was the initial implementation of the aeration system in the deeper end of the reservoir (around site D2) which caused the stirring up of previously undisturbed sediment.

As a result, no significant relationships were found between turbidity and DOC in 1997 (R=0.05, r^2=0.002), while negative relationships between DOC and turbidity were found in 1999 and 2001 (Figure 3.30). Subsequent implementation of the aerator did not appear to increase turbidity at D2.

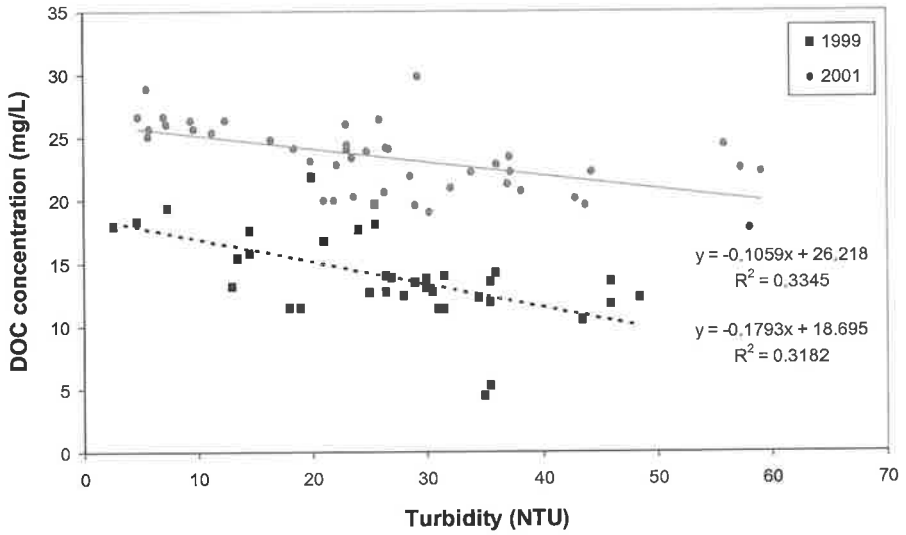


Figure 3. 32: Relationships between DOC concentration and water turbidity across the reservoir during 1999 and 2001. In 1999, R=0.6, r^2=0.32; In 2001, R=0.6, r^2=0.33.

High turbidity can limit the growth of phytoplankton (Lind et al., 1997; Hoyer et al., 2002), and since in 1999 and 2001 phytoplankton was an important source of DOC, this limitation of phytoplankton growth could have caused the observed reduction in DOC concentrations at high levels of turbidity. Implementation of strategies such as artificial aeration to create turbid conditions may prove to be successful in reducing DOC concentrations in the Warren Reservoir, by limiting the growth of phytoplankton.

3.6.2.2 Biotic factors (study of bacterial population dynamics)

Bacterial abundance and community composition of the Warren Reservoir were studied from December 2000 until the end of the study period in August 2001. During this period there was extensive inflow of Murray River water to the reservoir, and as discussed

previously, DOC concentrations were high relative to earlier years of this study, and were most likely dominated by autochthonous DOC. Thus, the findings here relate to the reservoir under these conditions. A different set of conditions, such as observed earlier in the study (lower DOC concentrations and higher allochthonous DOC input), may have a significant impact on the bacterial community in the reservoir.

The purpose of this section of the study was to determine how the size and composition of the bacterial population is affected by the seasonal cycle (in one particular year). Correlation analyses were applied to establish potential relationships between bacteria and other water parameters. Particular emphasis was placed on establishing a relationship between bacterial growth and community structure and seasonal changes in DOC concentration and nature. The purpose was to determine whether DOC seasonality promotes changes in bacterial populations, and if so, whether potential exists for future implementation of bio-manipulation strategies to control aesthetic properties of Warren Reservoir water in the long term.

Bacterial concentrations and biomass fluctuated throughout the study period from December 2000 to August 2001, highest values of both parameters recorded in December 2000. Lind et al. (1997) also found little seasonal variation in bacterial production, but they showed coupling and uncoupling of bacterial biomass to phytoplankton levels (depending on the region of the lake which in turn was affected by different levels of autochthonous and allochthonous input). In the Warren Reservoir, a weak correlation between bacterial numbers and chlorophyll levels (a measurement of algal biomass) was observed ($R=0.4$, $r^2=0.16$). DiSiervi et al. (1995) found strong seasonal variations in bacterial numbers in a reservoir, with highest concentrations during summer and lowest concentrations during winter. These findings were strongly correlated to primary productivity and temperature in that reservoir. In the Warren Reservoir, however, DOC input was not solely dependent on autochthonous production, but a significant portion arises from allochthonous sources, which may explain lack of seasonality in bacterial concentration across the reservoir. An algal bloom occurred in the December/January period of 2001, which may have accounted for high bacterial abundance during December 2000. Following this period, phytoplankton levels remained relatively high (even though water temperature declined), experiencing frequent fluctuations between January and August 2001, and not conforming to a seasonal pattern. Phytoplankton growth has been shown in many studies to be correlated with the growth of bacteria (Cole et al., 1988; Marvalin et al., 1989; DiSiervi et al., 1995; Lind et al., 1997; Zwisler et al., 2003).

Furthermore, allochthonous DOC has also been shown to be correlated with bacterial growth (Bergstrom and Jansson, 2000; Waiser and Robarts, 2000; Jonsson et al., 2001). Both of these sources of DOC were found to be seasonal in the Warren Reservoir, although peak inputs occurred at different times (summer and winter, respectively). The lack of seasonality in bacterial numbers and biomass may reflect a complex relationship with both of these DOC sources, as well as a lack of seasonality of bacteria. Gasol et al. (2002) showed that different water parameters regulated bacterial abundance and activity of the same reservoir. Thus, in addition to changes in DOC source, other parameters such as fluctuations in nutrients and predation can also influence bacterial numbers (Riemann, 1985; Lindstrom, 2000; Gasol et al., 2002; Muylaert et al., 2002).

No correlation was found between bacterial numbers or biomass and DOC concentration during this study ($R<0.1$, $r^2<0.01$). Furthermore no relationships were found between bacterial numbers or biomass with specific colour, which is indicative of the nature of DOC ($R<0.1$, $r^2<0.01$). However, although no obvious relationships were found between bacterial concentration and biomass with any of the physical or chemical water parameters, it does not necessarily imply that those relationships did not exist. Longer and more frequent sampling periods will be required to establish if there are relationships.

On a spatial scale, bacterial concentrations in surface waters of the shallow region of the Warren Reservoir were usually higher than those in the surface waters of the deeper regions, and showed least fluctuation with time when compared to bacterial concentrations in the deeper reservoir regions. An explanation for the differences in the fluctuations of bacterial numbers across the reservoir may lie with its close association with the catchment as well as turbidity. Namely, shallow regions are strongly associated with the catchment and experienced higher turbidity throughout the year than the deeper regions of the reservoir (particularly during winter), resulting in more effective mixing of the entire water column. Continuous stirring of the sediment and water mixing act as continuous supply of substrate for microbial growth (Lind et al., 1997).

In addition to DOC source and turbidity, temperature has been shown to affect bacterial productivity, which in some studies was found to increase with increasing temperatures (Coveney and Wetzel, 1995; Schultz et al., 2003). In other studies, bacterial growth rates and abundance were unrelated to temperature, authors speculating the availability of organic substrates to be the regulating factor in bacterial growth (Coveney and Wetzel, 1995; Rae and Vincent, 1998). The latter may further help explain a lack of

relationship between temperature (thus seasonality) and bacterial concentration and biomass in the Warren Reservoir. Furthermore, temperature was consistent across the reservoir thus unlikely to have played a role in the different bacterial fluctuation patterns observed across the reservoir. As such in the Warren Reservoir, temperature is likely to have an indirect effect on bacterial growth (and thus DOC dynamics) via its correlation to seasonal changes in DOC source, rather than a direct effect on its growth.

Although bacterial abundance and biomass in the Warren Reservoir did not show seasonal variation, temporal as well as spatial changes in bacterial community structures were observed using DGGE analysis of DNA extracted from bacterial assemblages isolated from water samples. The DGGE analysis used in this study was based on both the presence/absence of bands, as well as the intensity of DGGE bands. Due to potential for PCR biases, particularly those that can affect the final ratios of PCR amplicons, this study did not quantify the presence of various species. However, given the high reproducibility of DGGE banding patterns (Diez et al., 2001; Schauer et al., 2003) (which was also tested during this study), changes in band intensities were considered to be most likely due to relative changes in the abundances of the corresponding populations. As such, the analysis of DGGE banding patterns and band intensities was applied for comparative purposes only, to monitor relative spatial and temporal changes in particular populations.

According to the DGGE data, the primary segregation observed in the phylogenetic analysis was based on location in the reservoir. That is, reservoir populations were most similar for samples collected from the same site, regardless of the sampling date. These sites were shown to be different with respect to influence of DOC source, phytoplankton levels, turbidity levels, water colour and underwater visibility, and as such one or more of these factors may have played a role in determining populations of each site. Comparison of all four reservoir sites showed similar bacterial populations in sites S1 and D1, while those in D2, and particularly those of M1 differed strongly from S1 and D1. Samples from site M1 were consistently different from samples from other sites, most likely due to the influence of water inflow from the Murray River pipeline carrying with it external bacterial populations that were found to be different to those in sites S1 and D1. Bergstrom and Jansson (2000) state that high numbers of bacterial cells can be carried into the reservoir together with the allochthonous humic material; these bacterial populations are often different to those of the standing waters of the reservoir. Kirstemann et al. (2002) also show substantial contribution of the total microbial input in reservoirs as a result of rainfall and extreme runoff events;

whereas Gasol et al. (2002) go further to show differences in bacterial communities between different sites of the reservoir that differ geographically (inlet and outlet). As such, it is not unreasonable to expect external bacterial populations to influence reservoir bacterial populations, particularly those in the M1 site of the Warren Reservoir that had continuously been affected by inflow from the Murray River (as compared to other reservoir areas that are not continuously affected by external bacterial populations), except for shallow site during winter (due to inflow from streams C-S1 and C-S2).

In terms of population diversity across the reservoir, there were no major differences between different reservoir sites or sampling times. Namely, throughout the study period banding numbers (representing different taxa) ranged between 24-30 bands, irrespective of reservoir location or sampling time. This suggests that the number of taxa did not vary much, even though the type of taxa and/or the amount of species of a particular taxon may have been different between sites and across seasons (based on the DGGE patterns and band intensity data).

The second type of segregation was based on sampling time, and it was expressed in all four sites (as seen from phylogenetic and principal component analyses). Namely, samples from each site were segregated mainly into two broad groups based on time of sampling; December to April and May to August groups. Samples belonging to M1 site were less defined in their separation pattern, and the population differences that were observed with time at this site were less pronounced than those of the remaining three sites. Seasonal changes in bacterial communities were reported in other studies (Pernthaler et al., 1998; Muylaert et al., 2002; Selje and Simon, 2003; Stepanauskas et al., 2003; Zwisler et al., 2003), while a study by Lindstrom (2000) shows no apparent correlations between community composition and time of the year.

Correlation and canonical correspondence analyses (CCA) were used to study changes in bacterial populations with respect to changes in aquatic variables. CCA has been applied previously to assess relationships between various organisms and their environments (Lindstrom, 2000; Romani and Sabater, 2000; Muylaert et al., 2002). Correlation analyses of changes in bacterial populations with changes in water quality parameters (such as DOC levels or character, temperature, nutrients, turbidity) are summarised in Table 3.10.

Table 3. 10: Summary of the relationships between various water parameters and bacterial population similarity (%). ANOVA was used to test the significance of regression (r^2) and correlation (R) analyses. Significance was tested at a confidence level of 95% and the significant results are highlighted in bold.

	Significance of relationship between water parameters & bacterial population similarity			
Parameter	**S1**	**M1**	**D1**	**D2**
Temperature	**R=0.8, r^2=0.6**	**R=0.6, r^2=0.3**	**R=0.7, r^2=0.5**	**R=0.8, r^2=0.6**
DOC (mg/L)	R=0.3, r^2=0.1	R=0.1, r^2=0.02	**R=0.6, r^2=0.4**	**R=0.5, r^2=0.3**
Chlorophyll	R=0.2, r^2=0.3	**R=0.7, r^2=0.4**	**R=0.6, r^2=0.4**	R=0.3, r^2=0.1
Specific colour	**R=0.7, r^2=0.4**	R=0.3, r^2=0.1	R=0.4, r^2=0.1	R=0.4, r^2=0.2

Temperature changes were most consistent along the reservoir when compared to other water parameters and showed the highest positive correlation to changes in bacterial populations in all the sites studied. Since the reference sample was collected in January (the time associated with higher water temperatures), this correlation suggests that bacterial population differences are more pronounced as the temperatures decrease from January to August. As temperature is directly related to seasonality, it is possible that its association with population changes may have been an autocorrelation rather than a driving force. For example, phytoplankton abundance, turbidity and nature of DOC in the Warren Reservoir were all shown to be seasonal, thus it is possible that this correlation with temperature is not a direct effect on growth of species, but an indirect effect of one or more of these variables (Lindstrom, 2001).

In addition to temperature, phytoplankton abundance, DOC concentration and character were also associated with changes in bacterial populations of the Warren Reservoir. Total DOC concentration and phytoplankton levels were associated with changes in bacterial populations of the deep sites, whereas the nature of DOC was correlated with changes in bacterial populations of the shallow site. For example, changes in the nature and quantity of DOC were observed around May, the same time the bacterial populations changed.

Based on the comparisons of banding patterns (number, position and intensity) between different samples and across seasons, it is proposed that the observed variations in bacterial community dynamics in this study were more related to the difference in the amount of species present at a particular time and location, than to the presence/absence of that species.

The results of the canonical correspondence analysis, summarised in Figure 3.33, were more detailed in the analysis of variables. CCA revealed several significant relationships between explanatory variables and bacterial community composition, the seasonality of which differed according to the region of the reservoir. It is important to note that although significant correlations were obtained between bacterial community structure and certain variables, these relationships were not strong (as indicated by low Eigen values). Temperature, total phosphorus, nitrate, dissolved oxygen and phytoplankton concentrations were generally found to be significantly related to changes in bacterial communities of the deeper waters (M1, D1 and D2). In the shallow part of the reservoir, total phosphorus and dissolved oxygen were still significant in explaining bacterial population changes, but SRP, turbidity and DOC concentration were also found to be important. In terms of how these findings relate to other studies, nutrient concentrations have been shown to influence bacterial biomass as well as community composition through effects on growth (Pinhassi et al., 1999; Keinanen et al., 2002; Muylaert et al., 2002). However, relationships between bacterial community structure and nutrients have also been suggested to be a result of covariation of nutrients with phytoplankton concentrations and/or catchment runoff (Lindstrom, 2000). The phytoplankton can take up large fractions of dissolved nutrients during summer (and may at the same time affect bacterial dynamics via release of carbon in the water), while the catchment runoff can add significant amounts of nutrients during winter months (as was shown to be the case with nitrate in this study), influencing the growth of bacteria (Muylaert et al., 2002). As such, the relationships between nutrients and bacterial community composition observed in the Warren Reservoir was possibly a result of covariation between nutrients and phytoplankton growth as well as catchment runoff. Lindstrom (2000) showed phosphorus concentrations not to be of major importance in determining bacterial community composition of a Swedish lake, and suggests that nutrient content might be indirectly related to the composition of its bacterial community as a consequence of the action of other planktonic organisms.

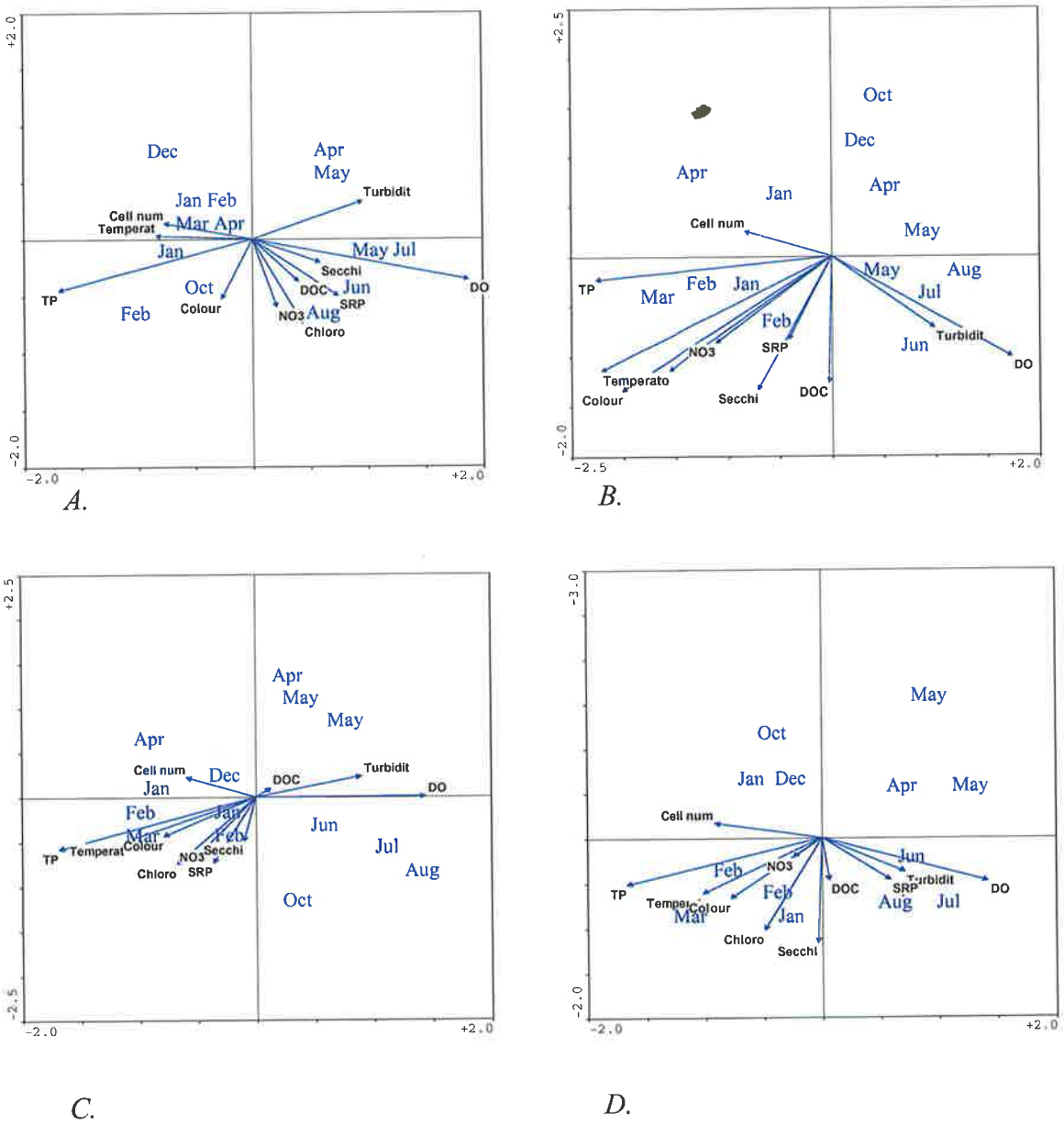


Figure 3. 33: Canonical correspondence analysis (CCA) of bacterial community composition at the S1 site in relation to different water variables. Percentage of variation in bacterial community composition (obtained from Eigen values) caused by the different environmental variables was: (A) 8% Dissolved Oxygen, 7% TP, 4% Turbidity, 3% SRP, 2% DOC concentration; (B)6% Temperature, 6% TP, 6% Colour, 5% Dissolved Oxygen, 3% Nitrate; (C) 9% TP, 7% Dissolved Oxygen, 5% Temperature, 4% Chlorophyll concentration.; (D) 7% TP, 6% Dissolved Oxygen, 5% Temperature, 4% Chlorophyll concentration, 3% Nitrate, 2% DOC concentration. These variations were shown as significant using the Monte Carlo permutation test.

Bacterial community composition was significantly related to phytoplankton biomass in the deep parts of the reservoir, while no such relationship was found in the shallow parts. The shallow site studied is in close contact with the surrounding catchment from which it most probably obtains sufficient supply of DOC, even during summer months, as the shallow waters are continuously stirred, releasing organics from the sediment, as well as from submerged vegetation. Thus, it is unlikely that bacterial populations of shallow waters would be as dependent on phytoplankton for growth as would be the populations in the deeper parts of the reservoir. Lindstrom (1998) showed the bacterial community composition of lakes to be dependent on humic acids. This finding could support the lack of relationship between bacterial populations and phytoplankton in the shallow areas of the Warren Reservoir. However, during a study of a eutrophic Swedish lake (which harbours large quantities of phytoplankton), Lindstrom (2000) showed a correlation between bacterial community composition and phytoplankton growth. Furthermore, comparisons of two different lakes in Belgium (differing in the extent of autochthonous versus allochthonous input of DOC) have shown two different types of relationships between bacterial community composition and phytoplankton growth (Muylaert et al., 2002). In the first relationship bacterial community composition was significantly related to phytoplankton growth because bacteria of those lakes primarily rely on phytoplankton exudates as a carbon source, whereas bacterial community composition of the other lake showed no association with phytoplankton growth, as it relied mainly on other carbon sources (which are considered to be mainly allochthonous (humic acids)). The importance of autochthonous versus allochthonous sources affecting shallow and deep reservoir sites may therefore explain the presence of a relationship between bacterial community composition and phytoplankton growth in some parts of the reservoir but not in others. As such, origin of DOC in the reservoir (which affects the nature of reservoir DOC) is likely to affect the bacterial community composition.

In terms of an association of DOC and bacterial communities, which forms the underlying focus of this study, Weinbauer and Hofle (1998) have shown that different strains of bacteria have different abilities to utilise different size fractions of organic matter. In addition to this conclusion, Zwart et al. (2002) studied numerous freshwater lakes, identifying typical freshwater bacteria. In light of this information and the findings of this study, it would be useful to take this study to the next level and to evaluate not only the chemical structure of DOC but to identify the bacterial species (by sequencing) associated with seasonally different types of DOC. This type of approach (where certain bacterial species may be more strongly

associated with certain types of DOC than other species) may be implemented in the long term to minimise the persistence of certain DOC types in the reservoir.

3.7 CONCLUSION

The concentration and chemical nature of DOC in the Warren Reservoir was found to vary seasonally, and to change on an annual timescale over the period of study. The concentration of DOC exhibited summer and winter peaks. The nature of the DOC during these peaks was substantially different – the summer peak was characterised by low specific colour, characteristic of a primarily autochthonous origin, whilst the winter peak was characterised by high specific colour, characteristic of a primarily allochthonous origin. This is consistent with the Mediterranean climate of the region, in which warm summers promote high levels of algal growth, whilst wet winters result in the addition of highly coloured waters from the catchment.

The annual changes coincided with a change in reservoir management. In particular, from 1999 there were substantial inputs of water from the Murray River via the Mannum-Adelaide pipeline. The input of water from this source greatly exceeded the input of water from the catchment in 1999 and 2001. In these years average DOC concentrations were higher, specific colour was lower, and phytoplankton levels were much higher. These observations are related, since the higher phytoplankton levels are almost certainly responsible for the increase in DOC concentration, and this additional DOC, being autochthonous, had low specific colour. What is not clear, however, is the reason or reasons for the higher phytoplankton levels in 1999 and 2001, or even if they are related to the increased Murray water inputs. Certainly, the increase in Murray water was not directly responsible for the increase in DOC concentration, as analysis of the Murray indicated it had consistently lower concentrations of DOC than the reservoir. Neither was there any indication that the Murray inflows contained high concentrations of nutrients (N and P).

As this study was mainly concerned with overall reservoir characteristics and obtaining an overall picture of DOC levels and sources in the reservoir (as the basis for future work on the reservoir), more detailed study into the character of DOC would be necessary to present definite conclusions into the causes and sources of accumulating DOC in the reservoir, and thus its removal from the water body. Aesthetic properties of the Warren

reservoir water, (namely based on the colour), were found to deteriorate during winter and appeared to be caused mainly by the presence of allochthonous DOC entering the reservoir via the two main streams in the catchment. Therefore, depending on whether the priority of the water industry is to reduce colour and/or DOC levels in the Warren Reservoir, focus should be placed on catchment runoff, as well as phytoplankton abundance and the Murray River contributions respectively.

In terms of natural means of DOC removal from the reservoir, this study implemented the use of DGGE analysis (to study changes in bacterial populations) as a starting point for future studies into interactions of DOC and bacteria (which are the primary consumers in pelagic waters). DGGE analysis indicated that bacterial populations of the Warren Reservoir changed between seasons, and these changes were found to be parallel to different water parameters, including the changes in the nature of DOC. The use of the DGGE technique (extended to band sequencing for species determination) can be applied in correlation with further studies into the DOC chemistry, in order to determine whether certain bacterial species have more affinity toward certain types of DOC molecules. If that is the case, bacterial populations in the Warren Reservoir can potentially be manipulated to enhance DOC removal. This study lays a foundation for future studies by studying the integration of two natural processes of DOC removal, namely photo-degradation and bacterial decomposition, by examining the changes to DOC bioavailability as a result of UV-B radiation, and monitoring how those changes impact on the structure of bacterial communities.

CHAPTER 4 PHOTOOXIDATION OF DOC BY UV-B RADIATION

4.1 INTRODUCTION

The importance of photochemical processes in aquatic systems has gained significant attention due to the effects of stratospheric ozone depletion (Scully et al., 1997; Ziegler and Benner, 2000; Maranger et al., 2002). The increase in anthopogenic gases in the atmosphere is thought to be responsible for the decrease in thickness of the stratospheric ozone layer, which absorbs ultraviolet-B-radiation (280-320nm) (Ferreyra et al., 1997). Mostajir et al. (1999) reported an ozone depletion for the Arctic and north temperate region in the range of 15-20% during winter 1991/1992, 30% during 1996 and as much as 45% in 1997. Stratospheric ozone concentrations over Antarctica during spring commonly fall below 50% of pre-ozone-hole values, leading to a 50-100% increase in UV-B around the summer solstice (Davidson and Belbin, 2002). As a result of ozone depletion, more UV-B radiation reaches the earth's surface today than was the case a decade ago, and these values are predicted to continue to increase (Kaczmarska et al., 2000; Chatila et al., 2001; Davidson and Belbin, 2002). Enhanced UV-B radiation as a result of the thinning ozone layer is, however, not solely restricted to the polar regions; there are strong suggestions that UV-B levels at mid-latitudes are also increasing (Arts et al., 2000).

The increasing levels of UV-B radiation have the potential to impact upon aquatic life as well as DOC cycling in water (Karentz and Lutze, 1990; Ferreyra et al., 1997; Miller and Moran, 1997; Scully et al., 1997; Obernosterer et al., 1999; Bukaveckas and Robbins-Forbes; 2000; Davidson and Belbin, 2002). For example, UV-B radiation can cause cleavage and oxidation of DOC, leading to direct removal of DOC from water (Miller and Moran, 1997; Graneli, 1998), or alternatively, exposure of DOC to UV-B radiation can induce transformations of both the structure and molecular weight, generating low molecular weight DOC products from high molecular weight organics (Miller and Moran, 1997; Bertilsson and Tranvik, 2000; Moran et al., 2000). This in turn has the potential to affect the growth and community composition of bacteria whose role in secondary production can be stimulated or inhibited by changing DOC chemical structure and concentration (Lindell et al., 1995; Amon and Benner, 1996; Benner and Biddanda, 1998; Tranvik and Kokalj, 1998; Obernosterer et al., 1999; Andrews et al., 2000).

In this study of the Warren Reservoir, investigations were conducted to determine how UV-B radiation affects reservoir DOC collected during different seasons. The concentration and nature of the DOC in the Warren Reservoir were found to vary seasonally (Chapter 3). Part of this seasonal variability may be due to seasonal variations in the intensity of the UV-B radiation. The aim of this study was to determine whether the processes of DOC photooxidation cause changes in DOC bioavailability, in terms of affecting bacterial growth and community composition.

The results of laboratory studies into the effects of photooxidation on the chemical composition, concentration and bioavailability of DOC in water from the Warren Reservoir, and the impact of DOC photooxidation on bacterial growth and community composition are reported. Specific objectives of the studies presented in this chapter were to:

1. determine if changes in DOC chemistry and concentration occur in sterile water samples that have been exposed to UV-B radiation, and to make comparisons between the responses of summer and winter DOC pools to UV-B induced chemical changes and bioavailability.
2. study changes in the growth pattern and community composition of mixed bacterial populations following the inoculation of post UV-B treated DOC samples.

4.2 MATERIALS AND METHODS

Samples (3L) collected from the surface waters of the shallow site (S1) and the deep site (D2) during January and August 2001, were filtered though a 0.2μm membrane and decanted into 3L glass jars in duplicate. These sites, located at two ends of the reservoir, were chosen to compensate for any spatial variations in the reservoir DOC at the time of each sampling. Thus quadruplicate samples were incubated under UV-B light (280-320nm, $1\pm0.2 Wm^{-2}$) or in the dark, at 20°C in a shake incubator at 45rev/minute for 5 days (120 hours). This incubation time has previously been shown as sufficient to enable the analysis of changes in DOC and/or its bioavailability (Lindell et al., 1995; Reitner and Herndl, 1997; Benner and Biddanda, 1998; Parkinson et al., 2001). Samples were arranged as shown in Figure 4.1.

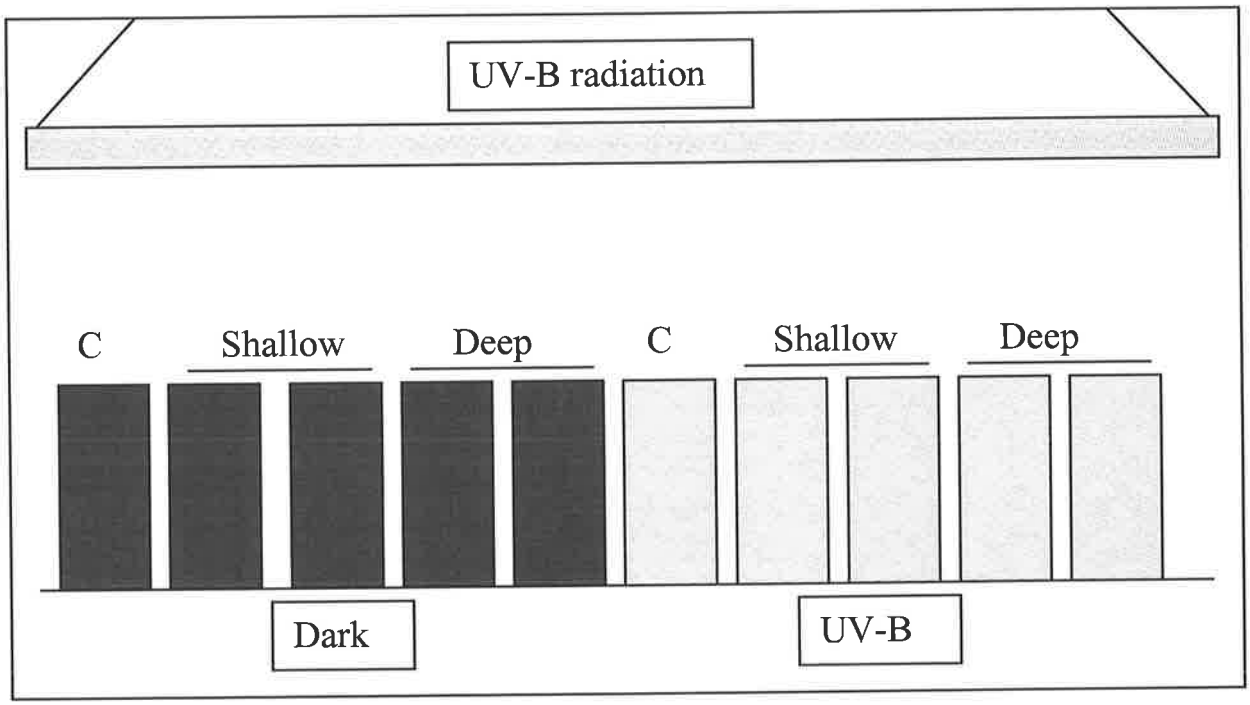


Figure 4. 1: Incubation of water samples, collected from the shallow (S1) and deep (D2) sites of the Warren Reservoir in January and August 2001, in the dark and under UV-B radiation.

Samples were rotated daily under UV-B light to compensate for possible irregularities in the light field. Dark controls were covered with a non-transparent aluminium foil and incubated under the same conditions as the UV-B exposed samples to ensure minimum variability between the controls and the treatments. Milli-Q water was used to monitor for organic and bacterial contamination. Subsamples of Milli-Q water were collected during normal sampling times and tested for DOC concentration and bacterial abundance.

During the 120-hour incubation, subsamples (30mL) were taken from each sample for the analysis of DOC concentration, UV-Vis absorbance (190-700nm) and absorbance ratios (E_2/E_3 absorbance ratios of 250nm/365nm and E_4/E_6 absorbance ratios of 465nm/665nm) at the following time intervals: 0h, 4h, 24h, 48h, 72h, and 120h. At the same time intervals additional subsamples (250mL) were taken from each sample and stored at 4°C.

After irradiation, an inoculum experiment was performed on the 250mL subsamples collected during the UV-B irradiation stage, in order to study the changes in DOC bioavailability as a result of UV-B exposure. During the inoculation part of the experiment,

the 250mL subsamples were placed into 300mL jars and inoculated with 0.5mL of 1.2µm-filtrate of a mixed bacterial population collected from the Warren Reservoir during the same sampling period (Section 2.12.4). Inoculated samples were incubated in the dark at 20°C and 45rev/min for 14 days (336 hours). During the inoculation study, bacterial numbers and population biomass were measured in each sample in order to determine the effects of UV-B irradiation on the ability of DOC to support bacteria growth. Subsamples were analysed following 21h, 48h, 168h and 336h of incubation, for the purpose of being able to determine how different stages of bacterial growth respond to UV-B induced substrate changes. The total inoculation period (336 hours) was also sufficient time to allow bacteria to reach a stationary phase (Lindell et al., 1995). At the end of the bacterial inoculation period (14 days), DGGE analysis was used to examine any changes to bacterial populations as a result of UV-B induced changes to DOC substrates.

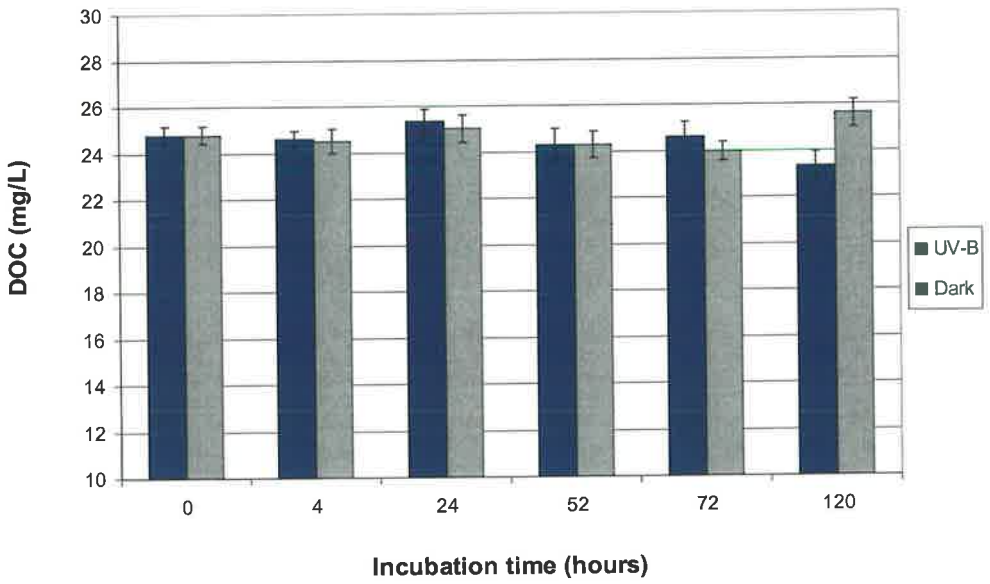
Analyses of standard deviation and standard error were calculated per quadruplicate samples. A T-test (Analysis Tool Pack, Microsoft Office Excel 2000) was used to determine the significance of the variations in measured parameters between UV-B treatments and Dark controls.

4.3 RESULTS

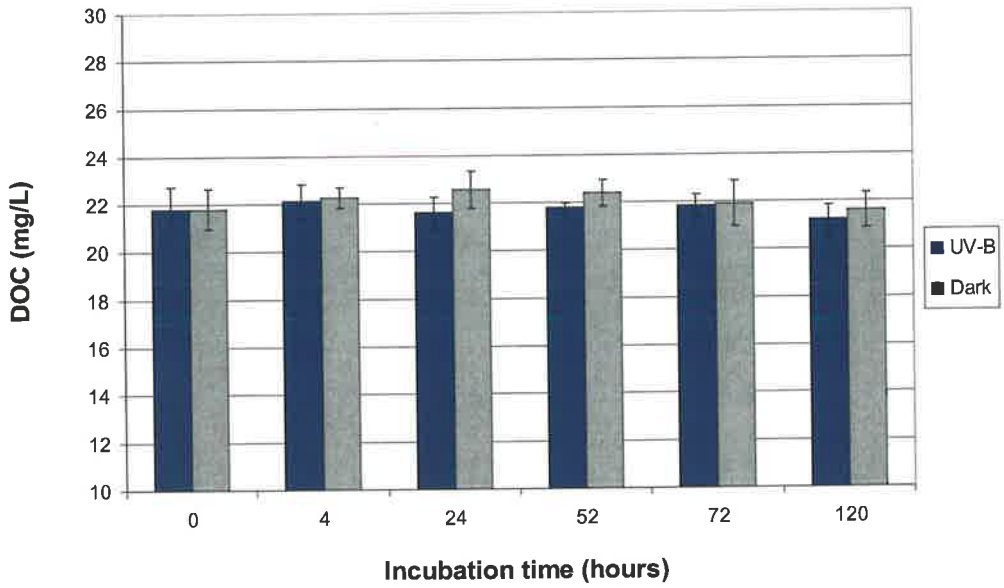
4.3.1 DOC CONCENTRATION

Changes to DOC concentrations, as a result of UV-B irradiation of summer and winter DOC samples, were measured during the 120 hour incubation and the results summarised in Figure 4.2. No significant changes in DOC concentrations of summer samples were measured during 72 hours of incubation, however significant changes in DOC concentration were measured in UV-B exposed samples after 120 hours of irradiation (Figure 4.2A). Mean DOC concentrations in the samples decreased following 120 hours of UV-B exposure by 6±1% from day 1 to day 5. A significant difference in the mean DOC concentration of 2±0.4 mg/L (T-test, $p=0.02$) was measured after 120 hours between UV-B exposed samples and dark controls.

UV-B irradiation of winter reservoir DOC samples resulted in no significant decreases in the DOC concentration (T-test, $p=0.26$) (Figure 4.2B).



(A) Summer

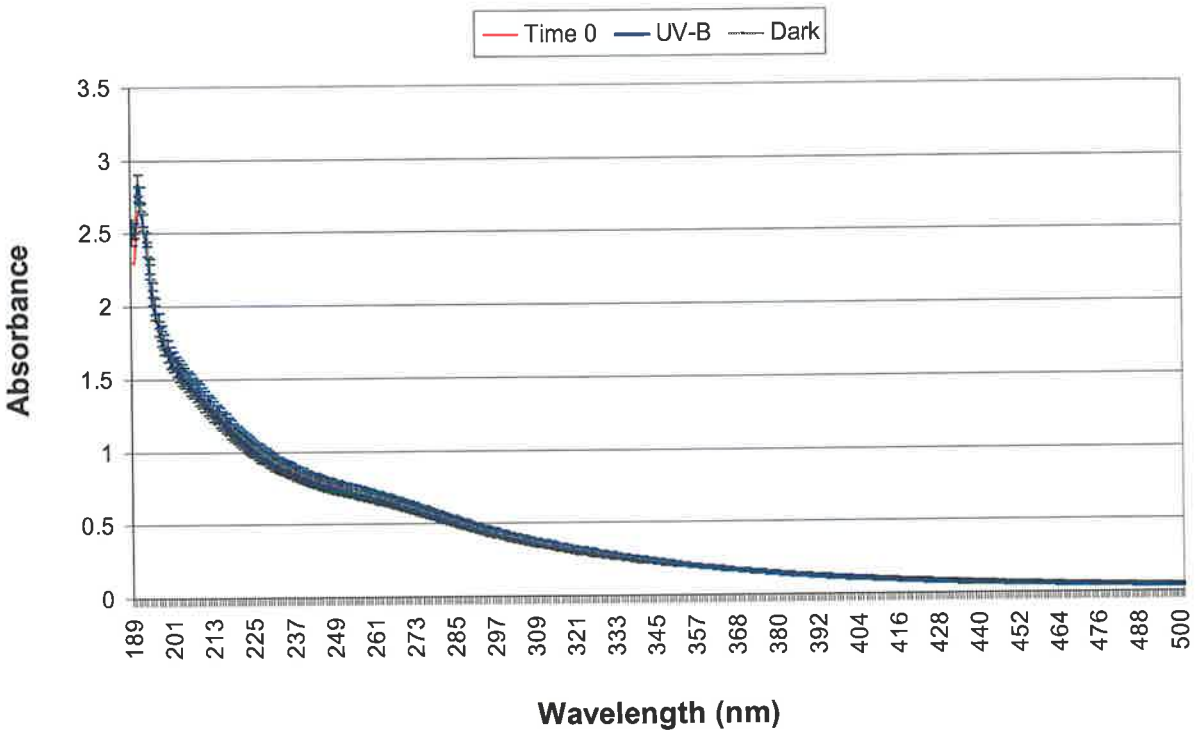


(B) Winter

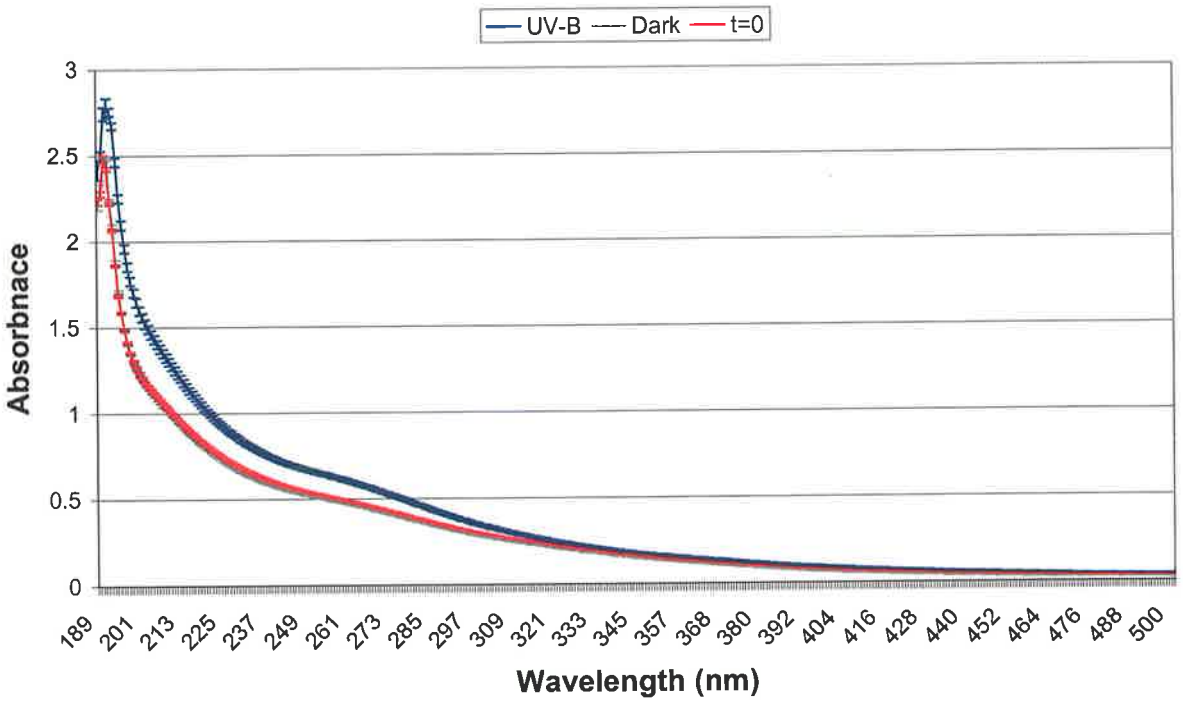
Figure 4. 2: DOC concentration (mg/L) in Warren Reservoir samples collected during (A) summer and (B) winter, and incubated under UV-B radiation and in the dark for a total period of 120 hours (Mean ± S.E.).

4.3.2 EFFECTS OF UV-B IRRADIATION ON THE ABSORBANCE SPECTRA OF DOC

No significant differences were observed in the UV-Vis absorbances after 120 hours of dark incubation during summer or winter (T-test, $p>0.05$). UV-B irradiation of summer samples also showed no significant change in the UV-Vis absorbance even after 120 hours of irradiation (Figure 4.3A). However, 120 hours of UV-B irradiation of winter DOC samples resulted in a significant increases in absorbances (T-test, $p<0.05$) in the wavelength range of 200nm to 360nm when compared to the absorbance at t=0 and the absorbance of the same samples incubated in the dark for 120 hours (Figure 4.3B). The mean increase in absorbance in this wavelength range was 26±0.4%.



(A) Summer



(B) Winter

Figure 4. 3: Absorbance spectra of samples collected during (A) summer and (B) winter from the Warren Reservoir, following 120 hours of incubation under UV-B radiation and in the dark (mean± S.E.).

4.3.3 EFFECTS OF UV-B IRRADIATION ON THE UV ABSORBING PROPERTIES OF DOC

The changes in absorbance at 254nm (A_{254}) caused by UV-B irradiation are shown in Figure 4.4. UV-B irradiation of summer samples caused no significant change to A_{254} during the 120 hours of incubation (T-test, $p>0.05$). During the winter study however, A_{254} increased with increasing irradiation times ($r^2=0.93$). A maximum increase in A_{254} of 28±3% was measured after 120 hours of UV-B irradiation (T-test, $p<0.05$) when compared to the absorbance of the same samples incubated in the dark. Absorbance data at 254nm for UV-B irradiated winter samples was normalised against those of the dark samples to highlight any changes due to UV-B irradiation.

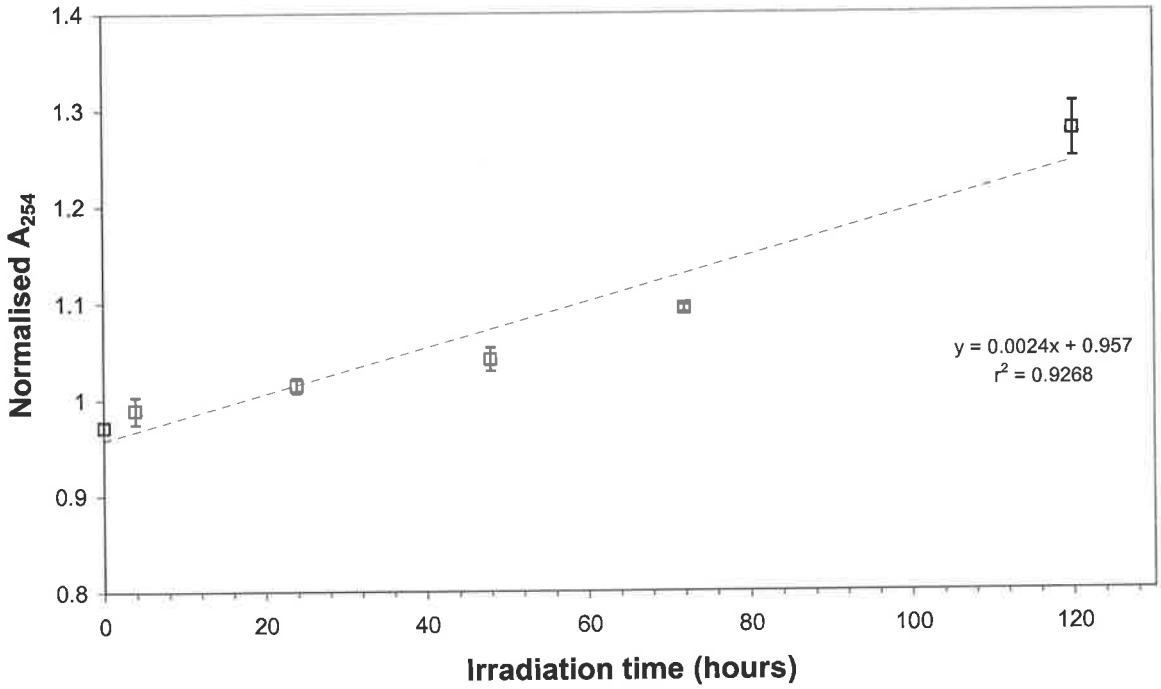


Figure 4. 4: Normalised absorbance at 254nm for winter reservoir samples incubated under UV-B radiation for 0, 24, 48, 72 and 120 hours.

No significant change in SUVA was observed as a result of exposing summer DOC reservoir samples for up to 120 hours of UV-B radiation. During the winter study however, SUVA values increased with increasing irradiation times with significant increases measured after 24 hours of irradiation (T-test, $p<0.05$) (Figure 4.5). SUVA values of the UV-B irradiated samples exceeded those of dark controls by 6±1% after 24 hours, 7±1.5% after 48 hours, 10±3% after 72 hours, and 30±2% after 120 hours.

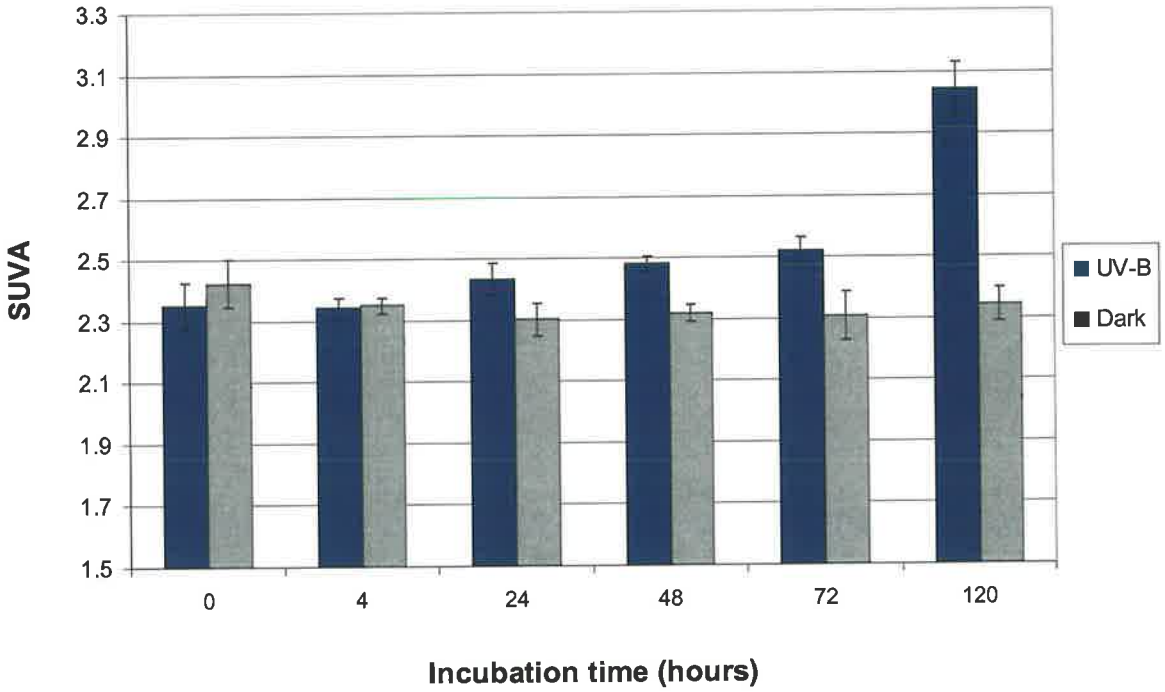


Figure 4. 5: Changes in SUVA in winter samples from the Warren Reservoir incubated under UV-B radiation and in the dark for a total period of 120 hours.

SUVA data for UV-B exposed samples was normalised against those for the dark samples to highlight the effects of UV-B irradiation on winter DOC. These results are summarised in Figure 4.6. Increases in SUVA as a result of increasing UV-B irradiation times are shown, with a regression coefficient of r^2=0.92.

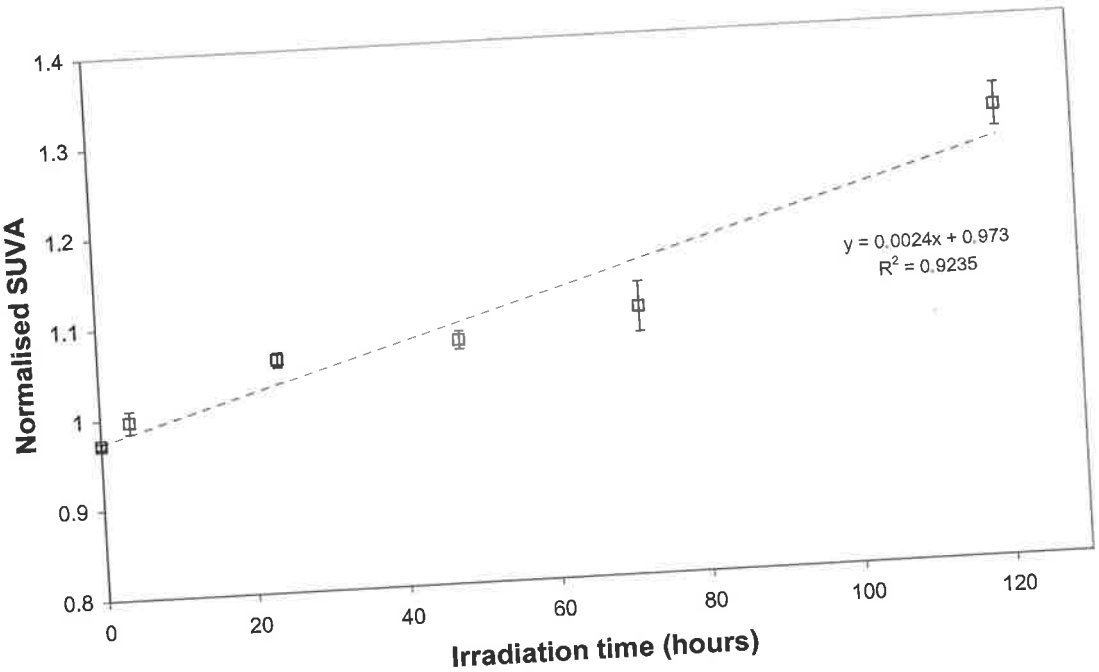


Figure 4. 6: Normalised SUVA (A_{254}/DOC) for UV-B irradiated winter reservoir samples against the values for the same samples incubated in the dark for the same time periods: 0, 4, 24, 48, 72 and 120 hours (Mean ± S.E.).

4.3.4 ABSORBANCE RATIO ANALYSES

E_2/E_3 absorbance ratios (250nm/365nm) of the UV-B irradiated summer and winter samples are summarised in Figure 4.7. Ratios were normalised against the dark controls to highlight changes occurring in summer and winter samples due to UV-B radiation. E_2/E_3 ratios of summer samples, incubated under UV-B radiation for 48 hours, increased by 3±1% when compared to the ratios measured for the same samples that had been incubated in the dark. Conversely, after 120 hours of UV-B irradiation, E_2/E_3 ratios of summer samples decreased by 4±2%.

E_2/E_3 absorbance ratios in winter samples incubated under UV-B radiation increased when compared to the values measured in the same samples incubated in the dark. An increase of 6±2% was measured following 4 hours of irradiation, 6±1% after 24 hours, 10±2% after 48 hours, 9±1% after 72 hours and 6±3% after 120 hours of UV-B irradiation (Figure 4.7).

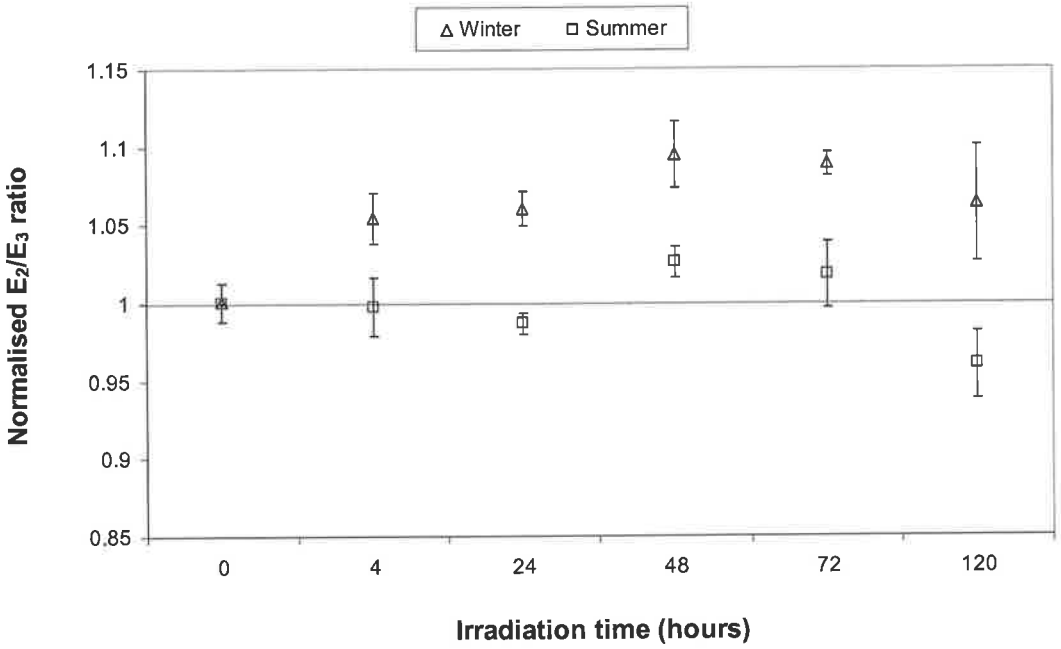


Figure 4. 7: Changes in normalised absorbance ratios E_2/E_3 (250nm and 365nm) for summer and winter samples exposed to UV-B radiation for a total period of 120 hours (Mean ± S.E.).

Normalised E_4/E_6 absorbance ratios (465nm/665nm) are summarised in Figure 4.8. Decreases in E_4/E_6 absorbance ratios were measured in summer samples that had been exposed to UV-B radiation for periods between 4 hours and 120 hours compared to the same samples that had been incubated in the dark for the same periods of time. As a result of UV-B irradiation, a mean decrease in E_4/E_6 ratios after 4 hours was 17±5%, then 30±8% after 24 hours, 25±8% after 48 hours, 26±6% after 72 hours and a maximum decrease of 32±3% was measured following 120 hours of UV-B irradiation.

An increase in the E_4/E_6 ratio was measured in winter samples that had been exposed to UV-B radiation for 4 hours (mean ± S.E. 30±6%). Conversely, winter samples that had been exposed to UV-B radiation for 72 hours and 120 hours, showed a significant decrease in E_4/E_6 ratios (mean ± S.E. 13±4% and 23±8% respectively).

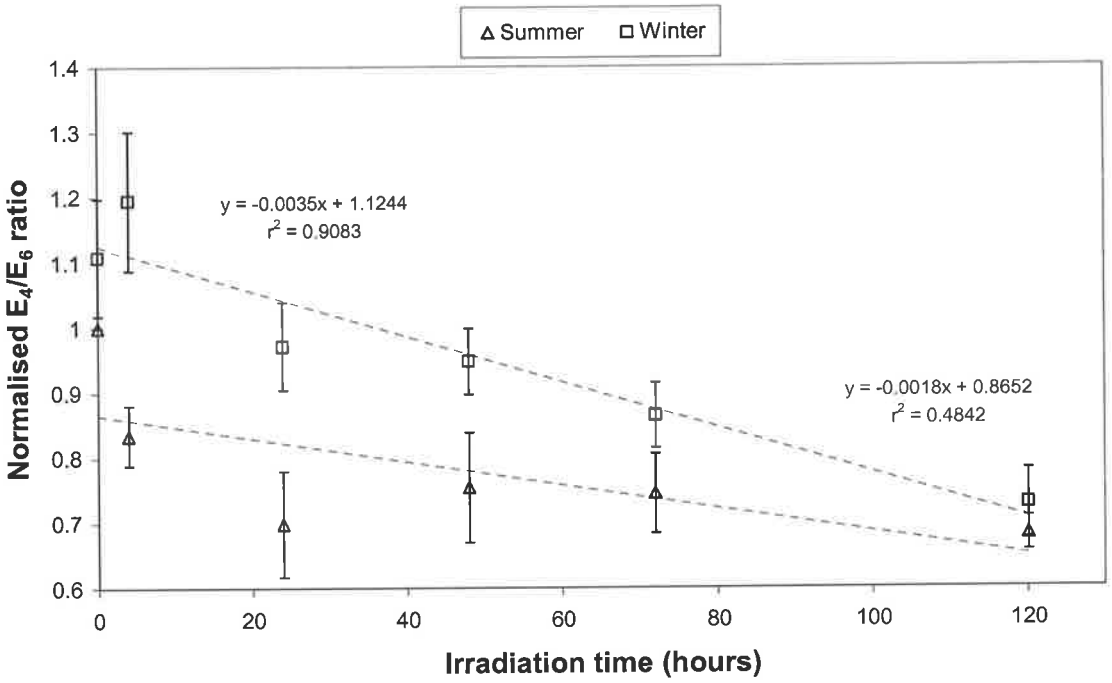


Figure 4. 8: The changes in normalised absorbance ratios E_4/E_6 (465nm and 665nm) of summer and winter reservoir samples during 120 hours of UV-B irradiation (Mean±S.E.).

4.3.5 BIOLOGICAL RESPONSE STUDY

Water samples, which had been exposed for 0, 4, 24, 48, 72 and 120 hours to UV-B radiation and the dark, were inoculated with a reservoir bacterial inoculum. Changes to bacterial growth and community composition, as a result of previous exposure of water samples to UV-B radiation, were measured during the 336-hour inoculation period. The findings of the inoculation study are summarised in Sections 4.3.5.1 and 4.3.5.2.

4.3.5.1 Bacterial numbers and biomass

Bacterial numbers were determined after 21, 48, 168 and 336 hours post inoculation of summer water samples that had previously been incubated under UV-B radiation and in the dark for up to 120 hours. The results are summarised in Figure 4.9.

During the first 21 hours of growth, bacterial numbers ranged from 8.2±0.9 $x10^4$ cells/mL to 1.8±0.3 $x10^6$ cells/mL. Higher bacterial numbers were measured in samples that had previously been exposed to UV-B radiation than in those that had been incubated in the dark. In particular, significantly higher numbers were found in samples that had been exposed to 72 hours and 120 hours of UV-B radiation (increases of up to eleven fold) when compared to the same samples that had been incubated in the dark for the same time periods. However, following 48 hours of inoculation, bacterial numbers in samples that had previously been incubated in the dark had increased to levels that were comparable to those found in the same samples that had been exposed to UV-B radiation (mean ± S.E. 3.5±0.3 $x10^6$ cells/mL). Thus, following 48 hours of inoculation, no significant differences in bacterial numbers were observed between UV-B and dark samples. Further bacterial incubation times resulted in no significant differences in bacterial numbers between samples that had previously been incubated under UV-B radiation and in the dark for 4, 24, 48 or 120 hours. However, bacterial numbers, in samples that had previously been exposed to 72 hours of UV-B radiation, were significantly higher than the numbers found in the same samples that were exposed to dark, for the remainder of the inoculation period (33±14% after 168 hours of incubation and 26±12% after 336 hours of incubation).

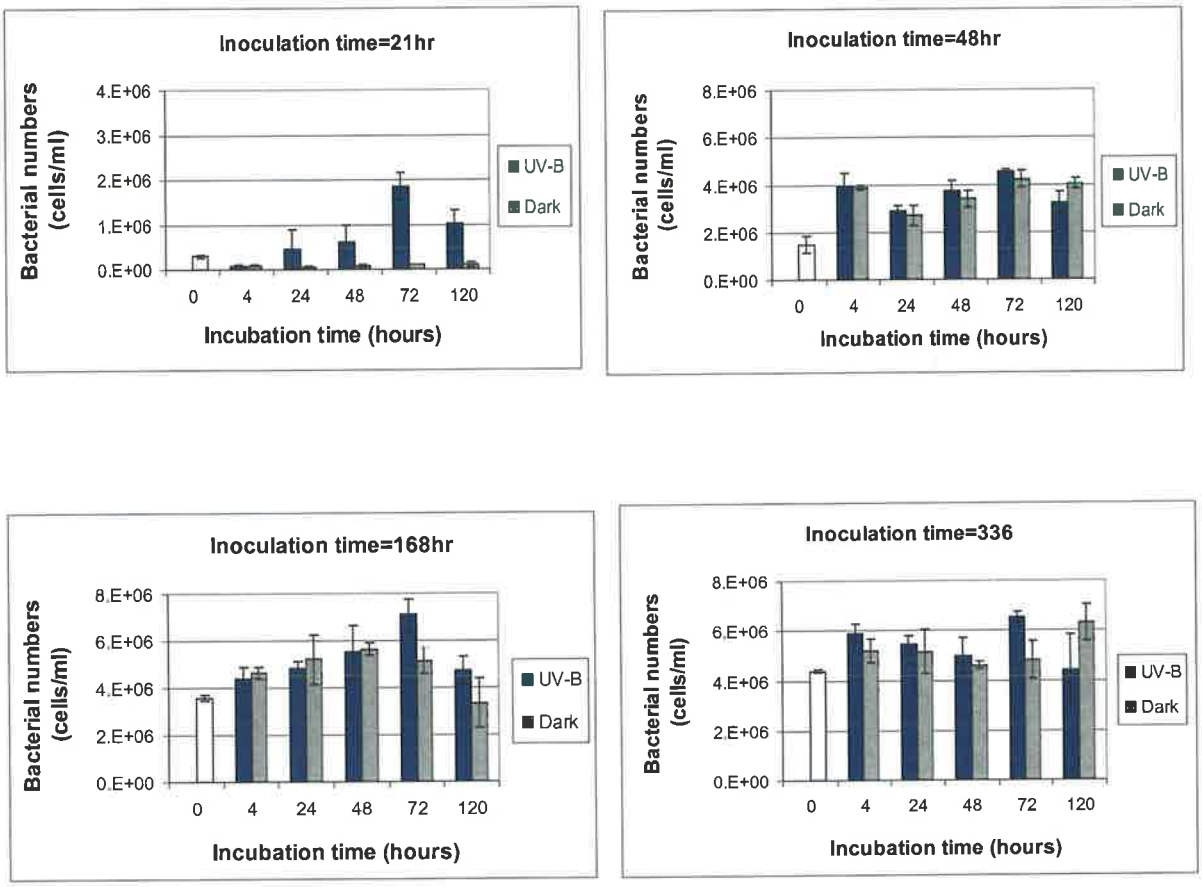


Figure 4. 9: Bacterial numbers (cells/mL) measured following 21, 48, 168 and 336 hours of inoculation of summer reservoir water samples, that had previously been incubated under UV-B radiation and in the dark, for periods of 4, 24, 48, 72 and 120 hours. Plotted data is the mean ± S.E.

Measurements of bacterial numbers, of winter water samples that had previously been incubated under UV-B radiation and in the dark, were obtained after 21, 48, 168 and 336 hours of inoculation. The results are summarised in Figure 4.10.

During the first 21 hours of growth, no significant differences in bacterial numbers were found between samples that had previously been incubated under UV-B radiation and in the dark for period up to 72 hours. However, higher bacterial numbers (33±12%) were found in samples that had previously been exposed to 120 hours of UV-B radiation, when compared to the same samples that had been incubated in the dark.

48 hours after bacterial inoculation, significantly higher bacterial numbers were found in samples that had previously been irradiated with UV-B for 48 hours (mean ± S.E. 35±6%) compared to the same samples that had been in the dark. No significant differences in bacterial numbers after 48 hours of bacterial growth were found in samples previously incubated for other lengths of time. However, following 168 hours of bacterial growth, significantly higher bacterial numbers were found in samples that had previously been exposed to UV-B radiation for periods longer than 48 hours. Bacterial numbers in 48h-UV-B exposed samples were 59±13% higher than those in the dark samples, while numbers in the 72h and 120h exposed samples were 58±6% and 69±3%, respectively, higher than the numbers in the samples previously incubated in the dark for the same periods of time (Figure 4.10).

Following 336 hours of bacterial growth, significantly higher bacterial numbers were found only in the 120h-UV-B exposed samples (54±25%), when compared to the same samples that had previously been incubated in the dark.

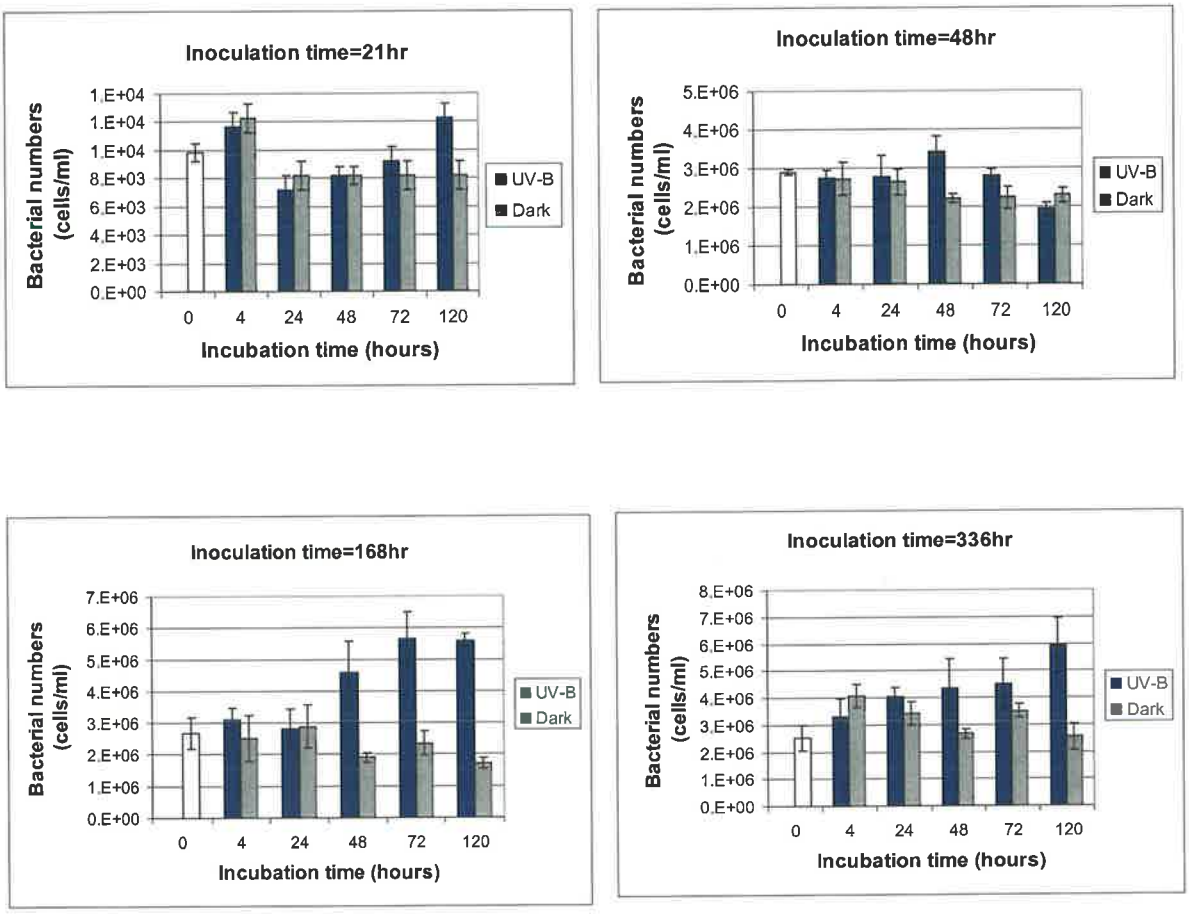


Figure 4. 10: Bacterial numbers (cells/mL) measured following 21, 48, 168 and 336 hours of inoculation of winter reservoir water samples, which had previously been incubated under UV-B radiation and in the dark, for periods of 4, 24, 48, 72 and 120 hours.

Determinations of bacterial biomass, of summer water samples that had previously been incubated under UV-B radiation and in the dark, were obtained after 21, 48, 168 and 336 hours of inoculation. The results are summarised in Figure 4.11.

Twenty-one hours after the inoculation, bacterial biomass in samples that had previously been exposed to 72 hours and 120 hours of UV-B radiation was significantly higher (sixteen fold and fourteen fold respectively) than the biomass measured in the same samples that had been incubated in the dark. No significant differences between the biomass of UV-B and dark samples were observed for other UV-B and dark incubation times.

Following 48 hours of bacterial growth, bacterial biomass in all samples increased from 22±7 µg C/mL (mean ± S.E.) after 21 hours of growth to 155±15 µg C/mL. Bacterial biomass in 24h and 72h incubated UV-B samples was significantly higher (42±3% and

58±8% respectively) than the biomass found in the same samples that were incubated in the dark. No significant differences between the biomass of UV-B and dark samples were observed for other UV-B and dark incubation times. After 168 hours of bacterial growth, significantly higher bacterial biomass (68±13%) was found only in the 72h exposed UV-B samples when compared to the biomass of the same samples that were incubated in the dark. Mean bacterial biomass after 168 hours of growth was found to be 251±19 µg C/mL.

Following 336 hours of bacterial growth mean bacterial biomass was 250±17 µg C/mL. Higher bacterial biomass was measured in samples that had previously been exposed to 4h, 24h, 48h and 72 hours of UV-B radiation, than in the same samples that had been incubated in the dark for the same periods of time (59±9%, 28±14%, 48±16% and 56±16% respectively) (Figure 4.11).

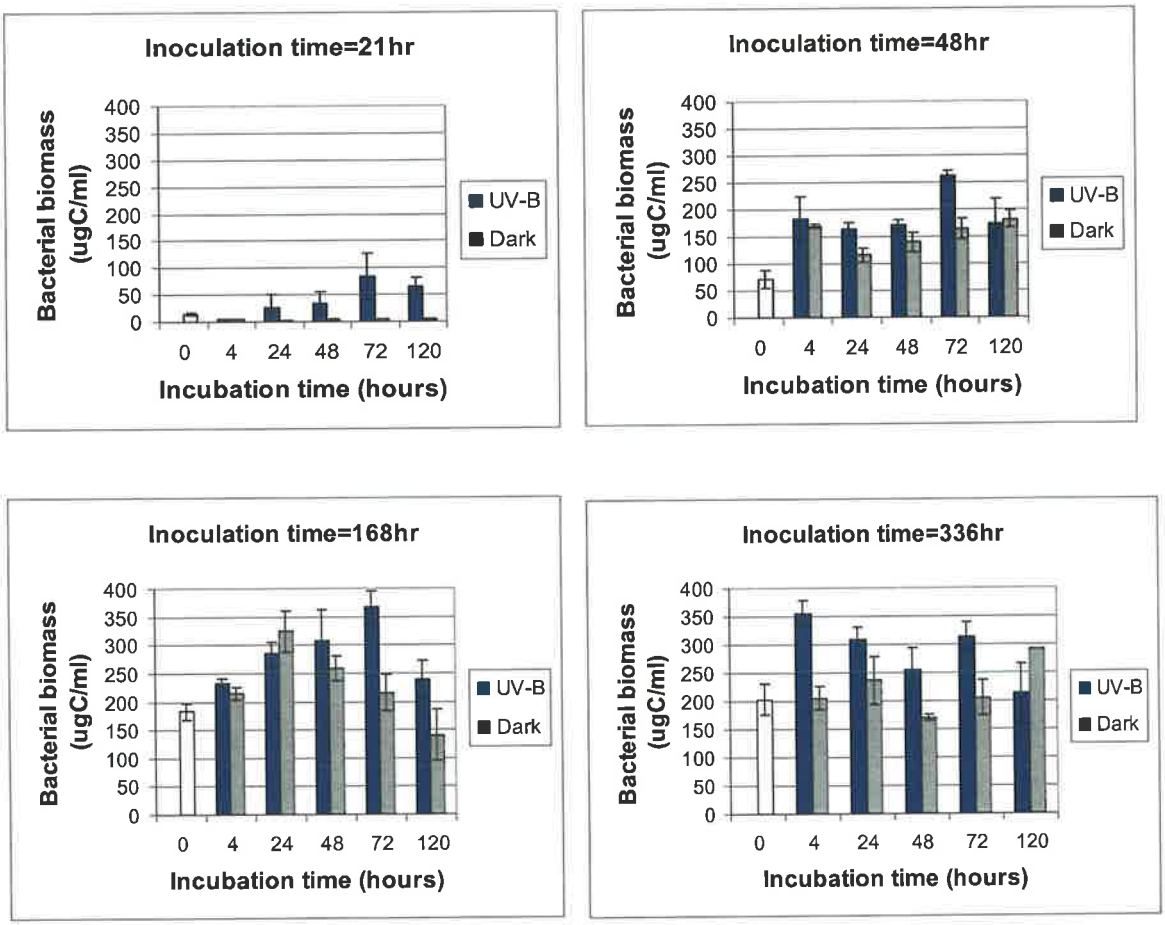


Figure 4. 11: Bacterial biomass (µgC/mL) measured following 21, 48, 168 and 336 hours of inoculation of summer reservoir water samples, which had previously been incubated under UV-B radiation and in the dark, for periods of 4, 24, 48, 72 and 120 hours. Plotted data is the mean ± S.E.

Determinations of bacterial biomass, of winter water samples that had previously been incubated under UV-B radiation and in the dark, were obtained after 21h, 48h, 168h and 336 hours of inoculation. The results are summarised in Figure 4.12.

Twenty-one hours after the inoculation, bacterial biomass in 120h UV-B exposed samples was one fold higher than the biomass measured in the same samples that had been incubated in the dark. No significant differences between the biomass of UV-B and dark samples was observed for other UV-B and dark incubation times.

Following 48 hours of bacterial growth, bacterial biomass in all samples increased from 1±0.08 μg C/mL (mean ± S.E.) after 21 hours of growth to 147±14 μg C/mL. Bacterial biomass in 4h, 48h, 72h and 120h UV-B samples was significantly higher (69±6%, two fold, 96±4% and 26±8% respectively) than the biomass found in the same samples that were incubated in the dark. No significant differences between the biomass of UV-B and dark samples was observed for other UV-B and dark incubation times.

After 168 hours of bacterial growth, significantly higher bacterial biomass was found in the 48h, 72h and 120h exposed UV-B samples (three fold, 2.5 fold and four fold respectively) when compared to the biomass of the same samples that were incubated in the dark. Mean bacterial biomass after 168 hours of bacterial growth was found to be 135±20 μg C/mL.

Following 336 hours of bacterial growth, there was no significant difference in the mean bacterial biomass (146±16 μg C/mL) when compared to the mean biomass measured after 168 hours of growth. Higher bacterial biomass was measured in samples that had previously been exposed to 24, 48 and 120 hours of UV-B radiation, than in the same samples that had been incubated in the dark for the same periods of time (46±17%, two fold and three fold respectively) (Figure 4.12).

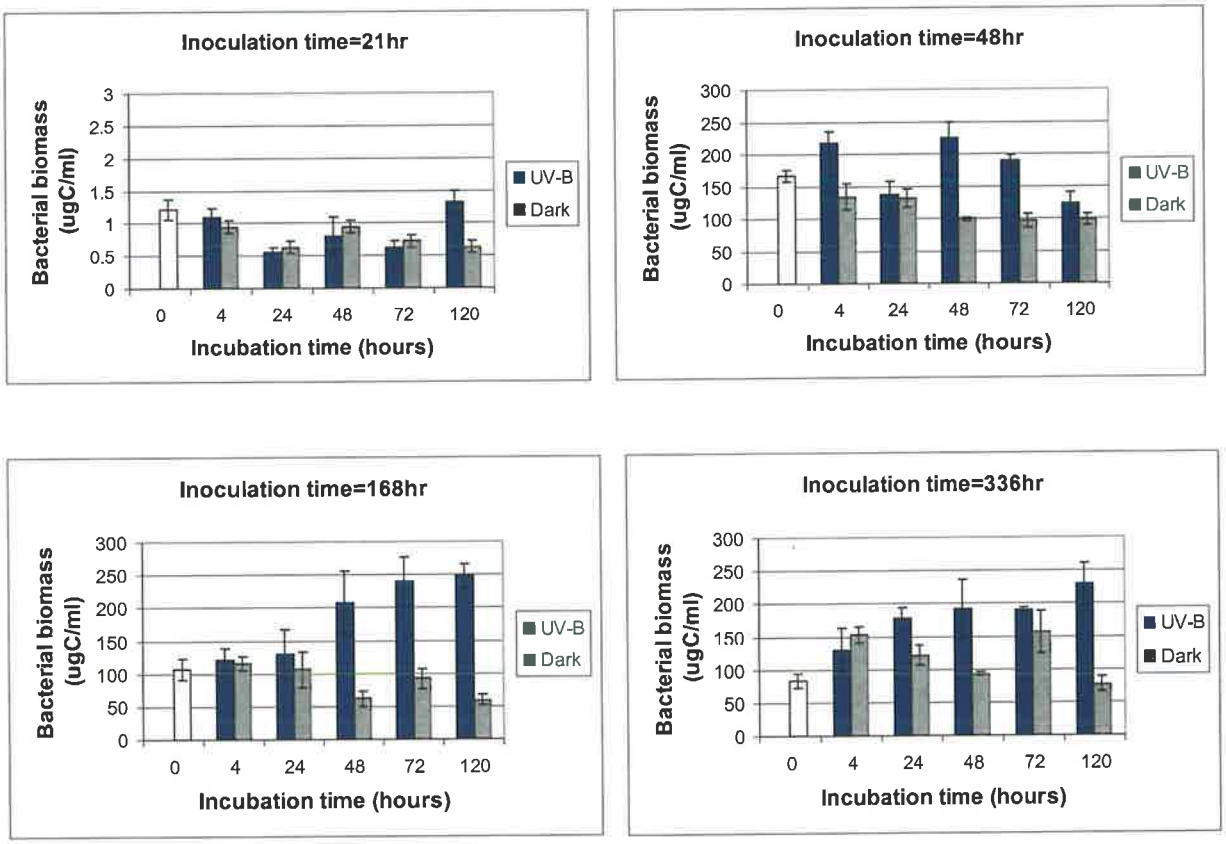


Figure 4. 12: Bacterial biomass (μgC/mL) measured following 21, 48, 168 and 336 hours of inoculation of winter reservoir water samples, which had previously been incubated under UV-B radiation and in the dark, for periods of 4, 24, 48, 72 and 120 hours. Plotted data is the mean ± S.E.

4.3.5.2 Bacterial population diversity

DGGE analysis was employed to determine if the changes in the nature of DOC produced by UV-B radiation are likely to alter bacterial community compositions within the reservoir. This analysis was carried out on water samples collected during both summer and winter. Results of this analysis are shown in Figures 4.13 and 4.15. Sterile water samples irradiated with UV-B and kept in the dark as controls for different time periods (0, 4, 24, 48, 72 and 120 hours) were inoculated with a reservoir bacteria inoculum for a total of 14 days. At the end of this period bacterial community composition in UV-B irradiated samples was

compared to controls to determine whether UV-B irradiation of DOC caused changes to the bacterial species composition and abundance.

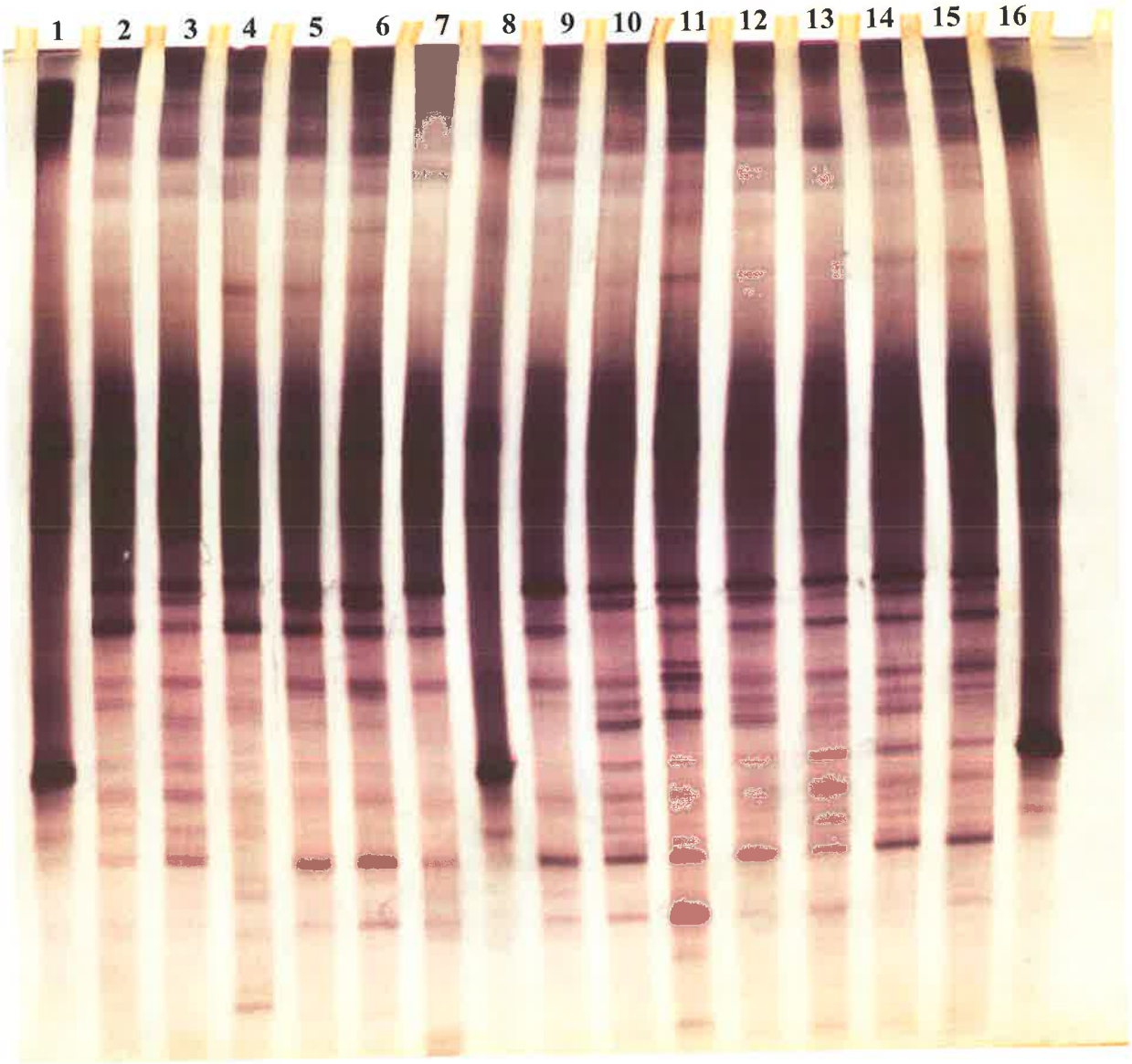


Figure 4. 13: 8% polyacrylamide DGGE gel, showing the separation of520bp PCR products amplified from genomic DNA that was extracted after 336 hours post inoculation of summer reservoir water samples which had previously been exposed to UV-B radiation or incubated in the dark. Lane 1-E.coli marker; 2-0h; 3-4h UV-B; 4-24h UV-B; 5-48h UV-B; 6-72h UV-B; 7-120h UV-B; 8-E.coli; 9-Marker; 10-4h Dark; 11-24h Dark; 12-48h Dark; 13-72h Dark; 14-120h Dark; 15-Marker; 16-E.coli.

Phylogenetic analysis was performed based on the banding patterns of the DGGE gel, based on the band presence/absence as well as information of the relative intensity of the bands (Figure 4.14).

Summer bacterial populations showed segregation between UV-B treated samples and controls. Figure 4.14 shows closest population similarities among samples previously kept in the dark (72%). Bacterial populations in samples previously exposed to UV-B radiation for up to 48 hours showed 64% similarity, whereas longer UV-B irradiation times caused greater differences in bacterial populations when compared to the original population. In particular, samples that had been exposed to UV-B radiation for 72 hours and 120 hours appeared to be most divergent from all other samples (showing 48% similarity to the remaining samples), suggesting significant alteration in bacterial species populating these two samples.

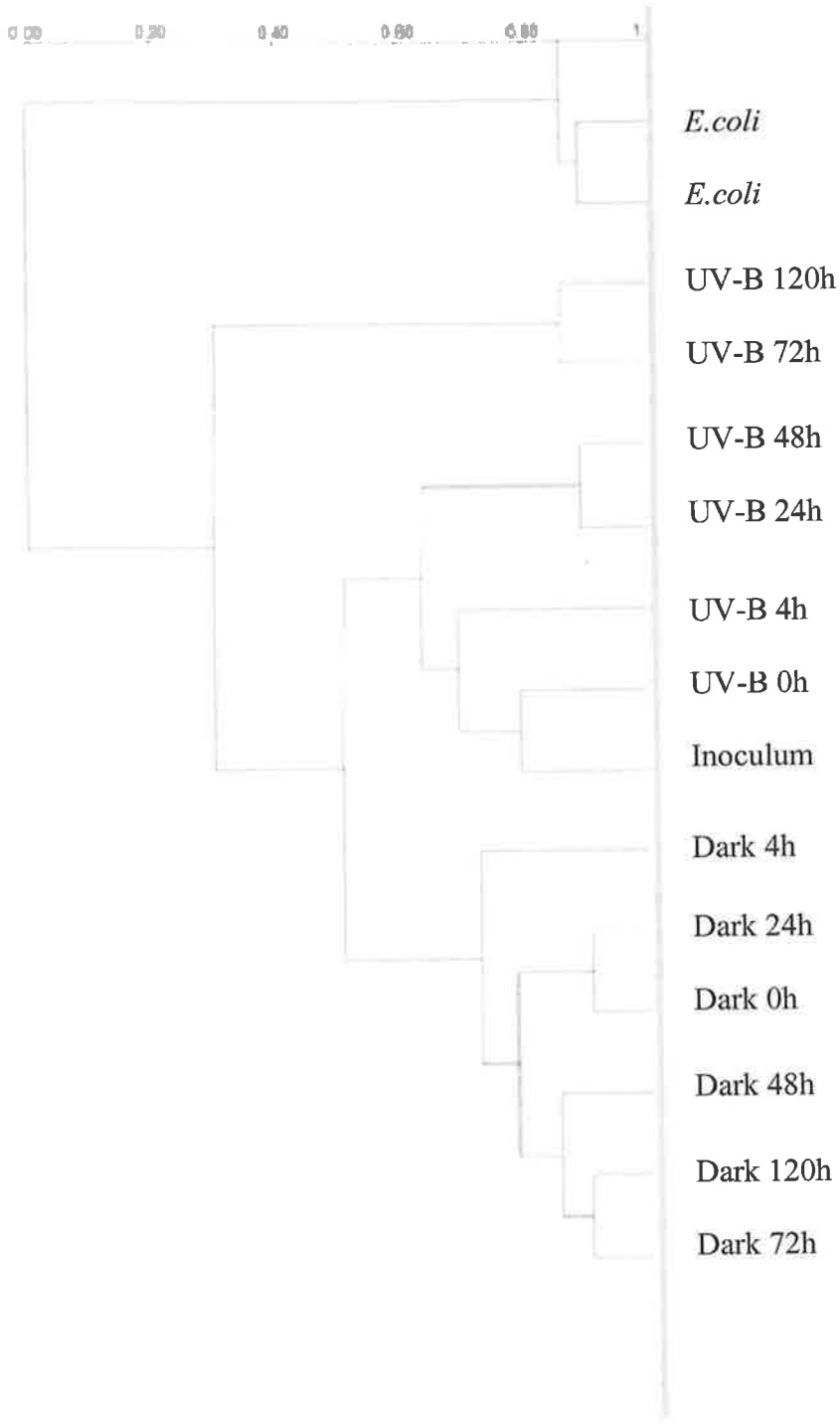


Figure 4. 14: Phylogenetic tree analysis comparing bacterial population changes in summer reservoir samples that had previously been exposed to different periods (0h, 4h, 24h, 48h, 72h and 120 hours) of UV-B radiation and dark.

DGGE analysis was used to determine whether changes in the nature of winter DOC (produced by UV-B radiation) are likely to alter the winter bacteria community structure. Results of this analysis are shown in Figure 4.15.

Figure 4. 15: 8% polyacrylamide DGGE gel, showing the separation of 520bp PCR products amplified from genomic DNA that was extracted after 336 hours post inoculation of summer reservoir water samples which had previously been exposed to UV-B radiation or incubated in the dark. Lane 1-E.coli; 2-Inoculum; 3-0h UV-B; 4-4h UV-B; 5-24h UV-B; 6-48h UV-B; 7-72h UV-B; 8-E.coli; 9-120h UV-B; 10-0h Dark; 11-4h Dark; 12-24h Dark; 13-48h Dark; 14-72h Dark; 15-120h Dark; 16-E.coli.

Phylogenetic analysis, based on the differences in banding patterns and the intensities of relative bands of winter reservoir populations in samples that had previously been incubated under UV-B radiation and in the dark, is shown in Figure 4.16.

Winter bacterial populations in water samples which had been exposed to UV-B for less than 4 hours, showed closer population similarity with the populations of the dark samples (80%) than with populations in other UV-B exposed samples that had been incubated for longer time periods (>24 hours) (55%).

Bacterial populations in water samples, which had been exposed from 24h to 120h of UV-B radiation, shared 74% similarity, with closer similarities found between populations in 24h and 48h exposures (82%) and populations in 72h and 120h exposures (85%). These populations together showed highest divergence from the remaining samples, with only 46% similarity.

Bacterial populations in water samples that had been exposed from 24h to 120h of dark, shared 73% similarity, and showed no pattern of divergence with increasing incubation times, as was the case with the UV-B samples.

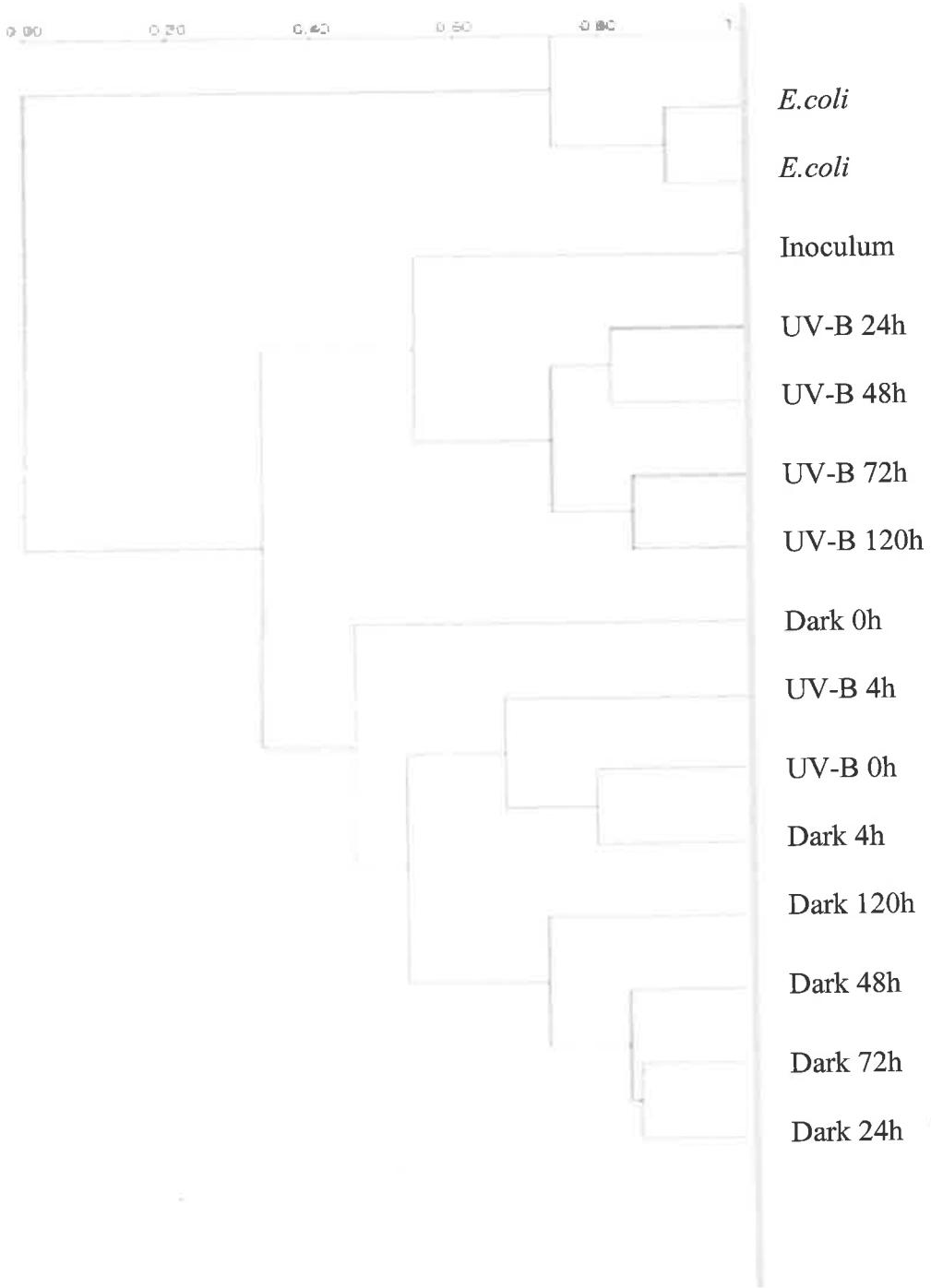


Figure 4. 16: Phylogenetic tree analysis comparing bacterial population changes in winter reservoir samples that had previously been exposed to different periods (0h, 4h, 24h, 48h, 72h and 120 hours) of UV-B radiation and dark.

4.4 TESTING THE EFFECTS OF INCREASED UV-B EXPOSURE TIMES ON DOC NATURE AND BIOAVAILABILITY

A second study was conducted to test the effects of longer UV-B exposure times on DOC quantity and nature. In particular, this study validated the findings of the effects of UV-B radiation on winter reservoir samples (Section 4.4), in terms of the observed changes in absorption properties of DOC.

4.4.1 MATERIALS AND METHODS

The methods for this study are comparable to those shown in Section 4.2 with the following minor changes. Duplicate 15L, 0.2 μm-filtered samples were collected during winter (August) from a 1m vertical column at the shallow reservoir site S1. Filtered water samples were exposed to the following conditions:

1. Continuous exposure to UV-B radiation (280-320nm, 0.1±0.02Wm^{-2})
2. Continuous exposure to dark (controls)

The total duration of the experiment was 21 days (504 hours), which exceeded the 120h incubation times used in Section 4.4. During this period, 50mL aliquots were collected from each sample at 0h, 336h and 504h intervals. A 50mL sub-sample was retained for DOC analyses (such as UV-Vis absorbance, DOC concentration (Section 2.5) and HPSEC used to measure changes in DOC molecular weight distribution).

4.4.2 EFFECTS OF UV-B RADIATION ON THE DOC CONCENTRATION

No changes in total DOC concentrations were detected in either UV-B or dark samples during any of the sampling times. Mean (± S.D.) DOC concentrations of all water sub-samples over the time course of the study were 20.5±0.4 mg/L in UV-B treatments, whereas those of the dark samples were 20.2±0.4 mg/L. These results suggest that 504 hours of exposure of winter DOC samples to UV-B radiation did not cause DOC removal from the reservoir water.

4.4.3 EFFECTS OF UV-B RADIATION ON THE ABSORBANCE SPECTRA OF DOC

The UV-Vis absorbance spectra of DOC samples were measured in the wavelength range of 190 to 700nm. No significant differences in DOC absorbance in water samples incubated in the dark were observed during the incubation period, however significant increases in absorbance between 200 and 290nm were observed in samples incubated for 336 hours ($p=0.004$) and 504 hours ($p<0.001$) of UV-B radiation compared to the absorbance of samples at t=0 (Figure 4.17). This suggests a change in the absorption properties of DOC in water.

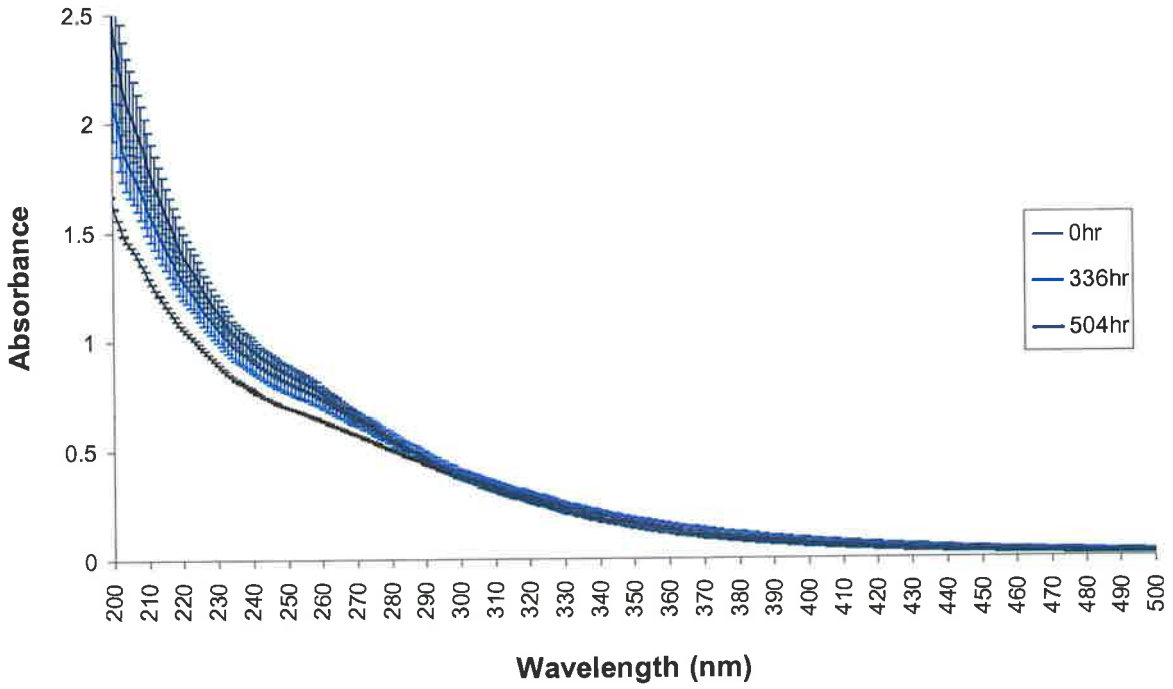


Figure 4. 17: Absorbance scan of water samples incubated under UV-B radiation for 336 hours and 504 hours, and at t=0. Absorbances at each sampling time are shown as the mean ± S.E. (based on duplicate samples only).

The absorbance spectra for the UV-B treated samples were normalised against the controls (Dark) (A/A_0) to further highlight differences between the spectra. Changes in absorbance spectra of 336 hour and 504 hour UV-B-irradiated samples are summarised in Figure 4.18.

Figure 4.18 shows that there was a mean increase in the amount of compounds that absorbed in the wavelength range of 190nm to 400nm after 336 hours of UV-B exposure (mean ± S.E. 13±0.6%). After 504 hours of UV-B exposure, a mean increase in absorbance of 26±1% in the wavelengths between 190nm and 300nm was accompanied by a mean decrease in absorbance of 26±0.8% between 320nm and 500nm.

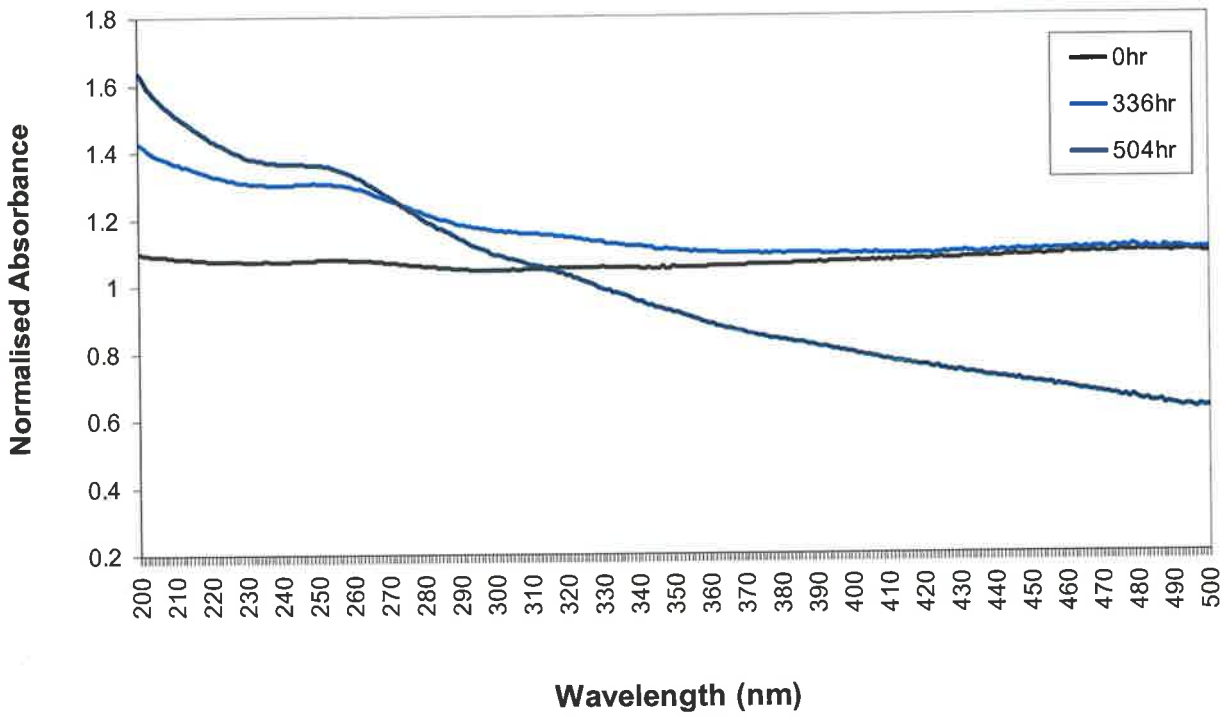


Figure 4. 18: The normalised absorbance spectra of water samples exposed to UV-B radiation for 336 hours and 504 hours, with respect to the same samples incubated in the dark for the same periods of time.

Increases in SUVA of 28±9% and 35±3% were measured after 336 hours and 504 hours of UV-B exposure, respectively, when compared to the same samples that were incubated in the dark (Figure 4.19). After 504 hours of incubation, the SUVA value of dark samples was 2.9±0.06, and that of UV-B samples was 3.9±0.17.

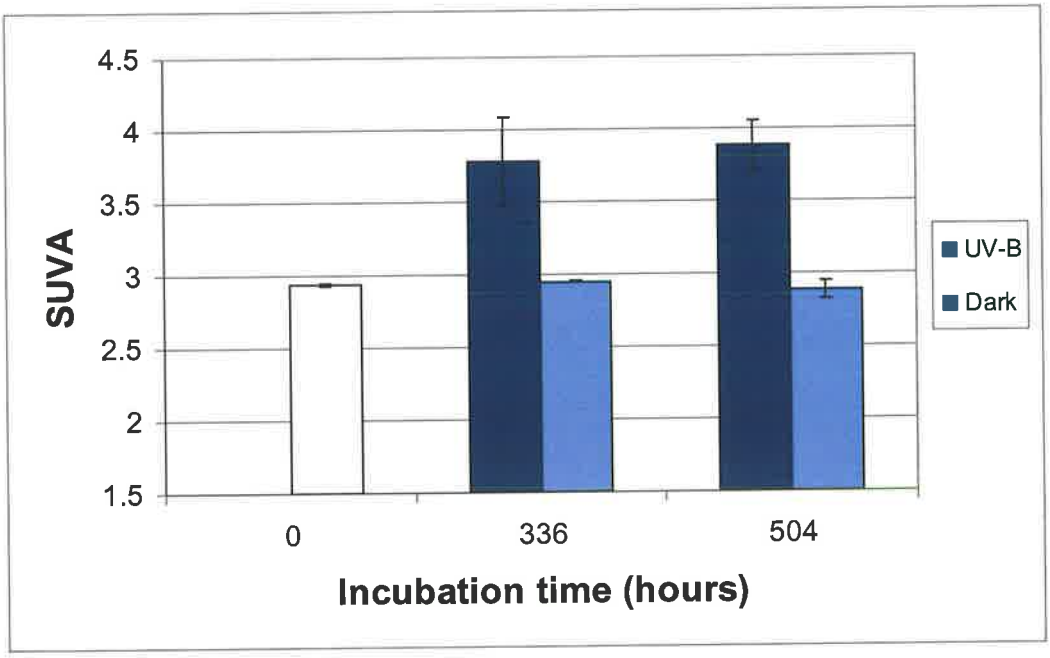


Figure 4. 19: Changes in SUVA in samples exposed to UV-B radiation for 336 hours and 504 hours when compared to the dark controls. Plotted data is the mean ± S.E. based on duplicate samples only.

4.4.4 EFFECTS OF UV-B RADIATION ON THE DOC MOLECULAR WEIGHT DISTRIBUTION

HPSEC analysis was performed on water samples incubated in the presence of UV-B radiation and in the dark, and the results are summarised in Figure 4.20. This molecular weight analysis only reflects the changes to compounds that absorb in the UV range, as the analysis was conducted by reading the absorbance at 260nm. No changes in molecular weight distribution were observed in samples that had been incubated for 336h and 504h in the dark. However, as a result of UV-B irradiation, shifts in molecular weight distributions were observed in the range of 600 Daltons to 1000 Daltons and 3000 Daltons to 10000 Daltons, favouring smaller molecular weight compounds within each range. Thus, this analysis showed changes in molecular weight distribution of UV-absorbing compounds to occur as a result of UV-B irradiation of winter DOC from the reservoir.

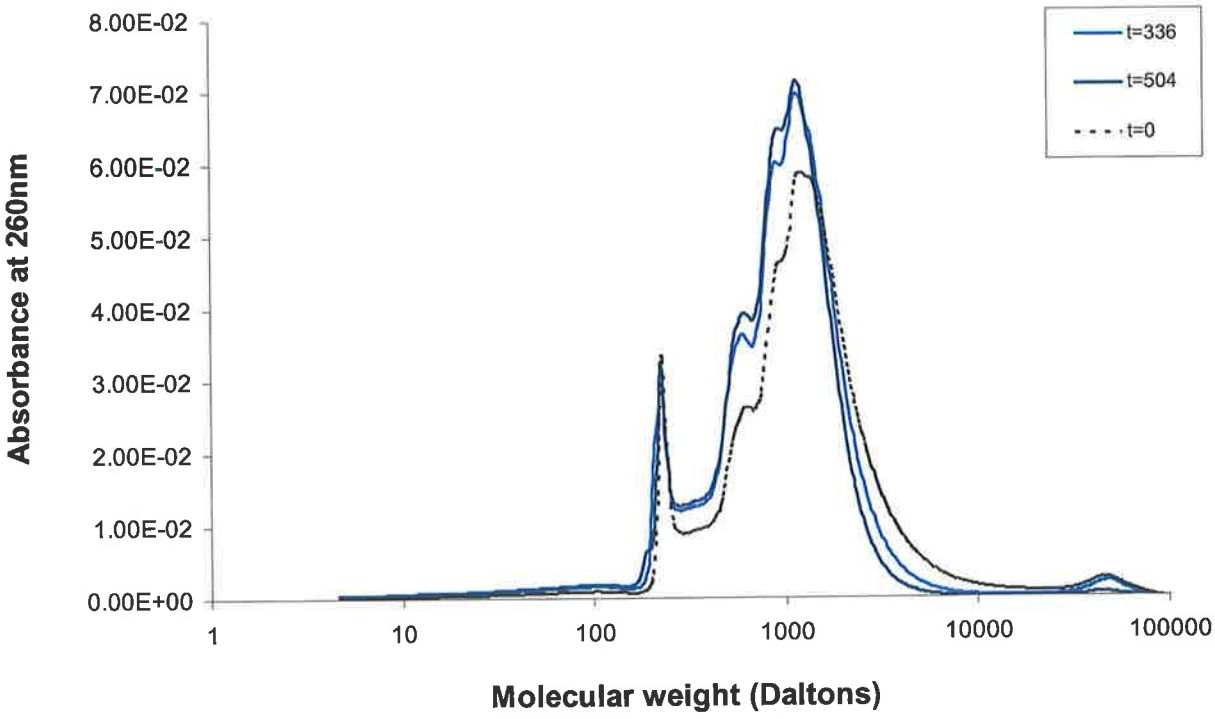


Figure 4. 20: HPSEC analysis showing changes in DOC molecular weight distribution as a result of 336 hours and 504 hours of UV-B irradiation.

4.5 DISCUSSION

This study aimed to determine the effect of UV-B radiation on the quantity and chemical composition of DOC in samples collected from the Warren Reservoir, and whether changes in DOC, resulting from UV-B photooxidation, cause changes in DOC bioavailability, in terms of affecting growth and community composition of indigenous bacteria populations.

With respect to the first objective, the quantitative and qualitative analysis of Warren Reservoir DOC in response to UV-B irradiation, the following observations were made. UV-B radiation caused a reduction in the summer DOC concentrations, suggesting that photo-mineralisation can be responsible for DOC removal from the reservoir during summer. Warren Reservoir DOC is exposed to significantly higher levels of UV-B radiation for longer periods of time during summer (Figure 4.21), potentially making it more photo-labile. As such, further increases to continuous UV-B exposure may have led to small levels of photo-mineralisation that were observed during the summer study. However, no photo-mineralisation of winter DOC from the Warren Reservoir was observed as a result of UV-B irradiation, even during prolonged UV-B irradiation times. The ability of sunlight and UV-B radiation to reduce DOC levels has previously been demonstrated (Obernosterer et al. 1999; Parkinson et al., 2001). However, Tranvik and Kokalj (1998) suggested that the loss of DOC due to photo-mineralisation may too small to detect when measured as a change in total DOC. Similar observations were made by Ziegler and Benner (2000), who failed to detect photo-mineralisation of DOC following 35 hours of sunlight exposure by measuring changes in the DOC concentrations. The loss of DOC from water (via photo-mineralisation) is generally a much slower process in the formation of compounds that, while still part of the DOC pool, no longer absorb light to the same extent as does the parent material (Moran and Zepp 1997; Moran et al., 2000). Based on this, changes in absorbances and related analyses (E_2/E_3, E_4/E_6 ratios and SUVA) were employed to study changes in the nature of DOC in water as a result of UV-B irradiation.

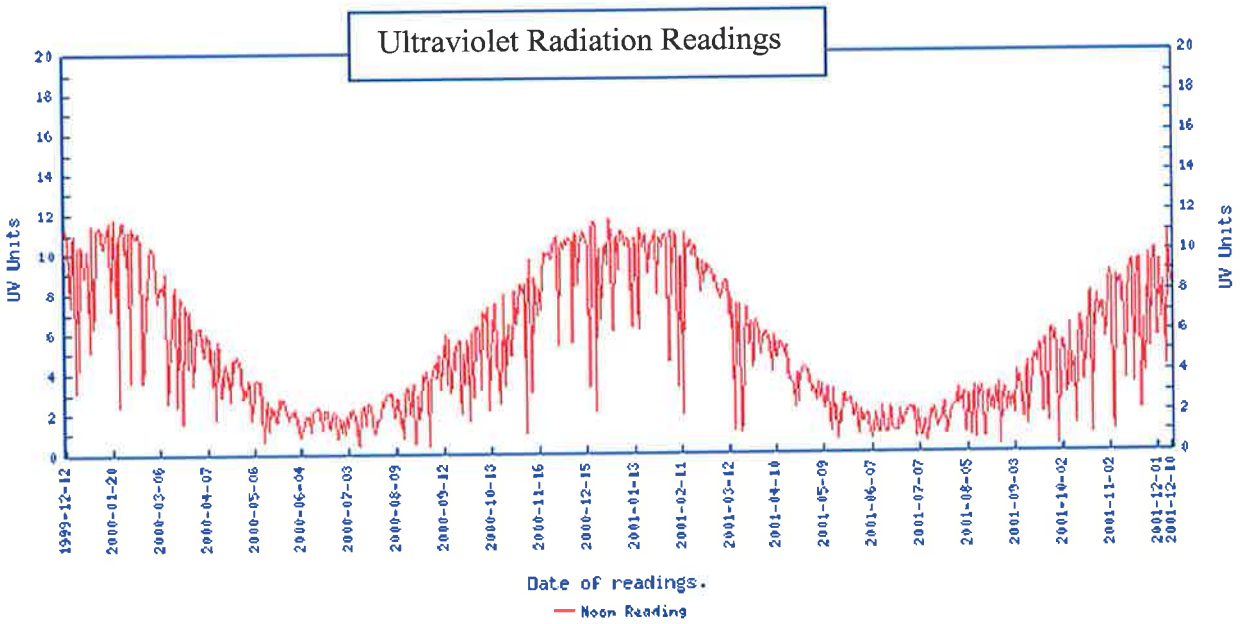


Figure 4. 21: UV Radiation levels (UV units) for the region of the Warren Reservoir between December 1999 and December 2001, establishing seasonal trends in the level of UV radiation reaching the surface waters of the Warren Reservoir. UV radiation in Wm^{-2} is multiplied by 40 to give the UV units presented in this figure (eg: 10 UV units = 0.25 Wm^{-2}) (Bureau of Meteorology, Adelaide).

Changes in the absorptive properties of Warren Reservoir water as a result of UV-B irradiation were observed, indicating that changes in DOC character were occurring. First, increased absorbance in the range of 200 and 290nm was found in UV-B exposed winter samples. The effect increased with increasing irradiation times. Increased absorbance at 254nm suggested increased formation of conjugated double bonds in UV-B exposed water samples collected during winter, as these compounds in particular efficiently absorb short wavelength radiation (Tranvik and Kokalj 1998). Absorbances at the wavelengths used in this study have been used previously for analysing the character of organic matter in water (Goel et al., 1995; Lindell et al., 1995; Reitner et al., 1997; Graneli et al., 1998; Bertilsson and Tranvik, 2000; Hautala et al., 2000). In particular, the absorbance at 254nm is often used as an indication of the presence of conjugated double bonds. Structural changes of DOC were analysed further by studying changes in absorbance ratios. This approach to studying structural complexity of DOC has been explained by Korshin et al. (1997). In particular, the ratio of absorbances at 250nm and 365nm (E_2/E_3) was used to estimate changes to DOC

character in terms of size (Lindell et al. 1995) and in terms of humic fractions (Reitner et al. 1997). That is, short wavelength absorbance has been suggested to represent smaller molecules than long wavelength absorbance. Thus E_2/E_3 ratios were used as an indicator of relative fractions of small to large molecules (Lindell et al., 1995, Strome and Miller, 1978). The E_2/E_3 absorbance ratio increased in UV-B exposed winter samples, suggesting a shift in molecular weight distribution toward formation of smaller molecules. Similar observations were made by Reitner et al., 1997, after exposure of DOC to sunlight radiation for a period of 8 hours and by Lindell et al. (1995), after exposure of DOC to UV-A and UV-B radiation for 100 hours. Although the latter study demonstrated increasing absorbance ratios with increasing UV-B exposure, it also demonstrated an overall reduction in absorbance at all wavelengths studied (250, 365 and 430nm) as a result of UV-B irradiation, in contrast to the findings of the Warren Reservoir study. It is possible that UV-B radiation induced photolysis of winter DOC causing formation of lower molecular weight products (as indicated by the E_2/E_3 absorbance ratio and supported by the HPSEC data) (Miller and Moran, 1997; Bertilsson and Tranvik, 1998), while at the same time it caused some of the DOC molecules to be transformed into more recalcitrant forms (Benner and Biddanda, 1998; Tranvik and Kokalj, 1998; Obernosterer et al., 1999; Ziegler and Benner, 2000) (such as those containing conjugated double bonds as indicated by increasing absorbance at 254nm). Harvey et al. (1983) proposed that in marine systems, dissolved humic matter can be formed by free radical oxidative cross-linking of fatty acids upon exposure of water to solar radiation. This would result in increasing absorbance as a result of sunlight radiation. Tranvik and Kokalj (1998) have further demonstrated that DOC can be transformed by UV radiation into more recalcitrant forms. The production of radicals can result in the formation of side chains and ring products (Mill et al., 1980), which may then bind some of the labile DOC to structures of humic substances. Although these authors did not report how their findings affected UV-Vis absorbance, the reported changes might explain increases in absorbance between 200-300nm in UV-B exposed winter samples found in the Warren Reservoir study. At the same time, a conversion of higher molecular weight products into lower molecular weight products (as suggested by Miller and Moran, 1997; Bertilsson and Tranvik, 1998) can still be occurring which could explain the results of the E_2/E_3 ratio analysis.

The absorbance ratio of 465:665nm (E_4/E_6) has also been used for the characterisation of organic solutes, indicating changes in humification and molecular weight (Chen et al., 1977; Hautala et al., 2000). A decrease in the E_4/E_6 ratio was measured with increasing UV-B

exposure times for the Warren Reservoir DOC study. This suggested a decrease in the amount of humic material in the DOC pool as a result of UV-B irradiation of reservoir samples, particularly in the case of the summer DOC pool. Reitner et al. (1997) studied the effects of UV radiation on various fractions of the DOC pool and concluded that photo-degradation of humic substances was the major source of elevated bacterial growth. The findings of Reitner et al. (1997) are in agreement with the winter results from this study. During summer, the autochthonous DOC pool dominated with respect to the allochthonous pool, and was subject to higher natural UV-B levels than was the case for the allochthonous dominated winter DOC pool (Sections 3.3-3.6). For the summer samples, the higher natural UV-B levels (Figure 4.21) may have already caused considerable structural changes to the DOC. This could explain the decreased response of DOC to UV-B irradiation in the summer experiments (as indicated by the E_2/E_3 data). At the same time, the amount of humic material was observed to decrease during both studies (as indicated by the E_4/E_6 ratios). Dissolved humic material is known to strongly absorb photons, especially of UV radiation, resulting in photolysis and degradation (Reitner et al., 1997; Tranvik and Kokalj, 1998). Reitner et al. (1997) found a higher level of photochemical oxygen consumption in humic fraction of DOC than in non-humic fractions. Thus, it is possible that even during summer (when humic material presents a smaller portion of the total DOC pool in comparison to winter (Sections 3.5.4 and 3.5.5)) reduced E_4/E_6 response may still be observed, suggesting degradation of the humic content of DOC, while not much change is taking place with the bulk of the DOC pool.

The second objective of this section of the study was to determine whether UV-B radiation increases the bioavailability of DOC from the Warren Reservoir and whether changes in the character and molecular size of DOC, brought on by UV-B radiation, cause changes in the bacterial community composition, as a result of certain bacteria species being able to better utilise certain types and/or sizes of DOC with respect to other species (Reitner et al., 1997; Weinbauer and Hofle, 1998).

The biological response study was applied to determine whether DOC bioavailability changes as a result of UV-B irradiation. This approach is reported to be very sensitive in detecting photo-induced changes in DOC bioavailability (Benner and Biddanda, 1998). When solar and UV-B energy are absorbed by DOC, the average molecular weight is often reduced and a variety of photoproducts are formed, some of which include inorganic compounds (such as carbon monoxide, carbon dioxide and other forms of inorganic carbon), while other photoproducts include organic molecules that, although smaller in size than the parent

molecules, remain a part of the DOC pool (Moran and Zepp, 1997). The E_2/E_3 and HPSEC data for the Warren Reservoir study support this. These smaller organic molecules have been shown by many studies to be more biologically active than the parent DOC molecules from which they were formed (Moran and Zepp, 1997; Miller and Moran 1997; Lindell et al., 1995; Parkinson et al., 2001). Conversely, other studies demonstrated contrasting effects of solar radiation on DOC and its bioavailability (Obernosterer et al., 1999).

The general findings of the two studies into the exposure of Warren Reservoir summer and winter DOC samples to UV-B radiation suggested that UV-B irradiated DOC provided better substrates for bacterial growth (as indicated by the bacteria concentration and biomass data). Significant increases in cell numbers and biomass were found in samples previously exposed to UV-B radiation, particularly in the case of winter DOC, suggesting that the UV-B part of the spectrum is potentially important in natural photo-degradation processes. Similar findings were obtained in another Australian study (Parkinson, 2001) that showed increased bioavailability of UV-A and UV-B irradiated DOC samples to be due to the formation of low molecular weight DOC. Other studies also showed increased bioavailability of DOC following photo-degradation (Lindell et al., 1995; Miller and Moran, 1997; Moran et al., 2000).

Exposure of summer DOC to UV-B radiation provided good substrates for bacterial growth, generating higher bacterial numbers during the initial growth phase. However, the bacterial numbers in the controls were found to be comparable to those of UV-B exposed samples after only 48 hours of growth, suggesting the limited production of these substrates by UV-B radiation. On the other hand, exposure of winter DOC to UV-B radiation elicited a slower initial response in bacterial growth, however increasing incubation times resulted in increasing bacterial growth and biomass in samples exposed to UV-B radiation for a minimum of 48 hours. This suggests that exposure of winter DOC to UV-B radiation generated higher levels of labile substrates for bacterial growth when compared to summer DOC. Moran and Zepp (1997) stated that variations in the extent to which the activity of heterotrophic bacteria is enhanced by DOC exposure to sunlight, are likely due to differences in the length and intensity of light exposure. Thus it can be proposed that winter reservoir DOC, which is exposed to less intense solar radiation for shorter periods of time, should be less photo-transformed and thus more prone to further photo-transformations (as indicated by the absorbance analyses and the HPSEC data) when compared to summer reservoir DOC. A study by Waiser and Robarts (2000) showed that in a clear lake, little of the DOC is available

for bacterial growth due to the continuous effects of photochemical transformations, while creek DOC entering the lake did not appear to have been as photochemically changed, and consequently was more available for bacterial growth. Benner and Biddanda (1998) showed that exposure of surface water DOC to sunlight resulted in a 75% reduction in bacterial production, whereas exposure of deep-water DOC resulted in a 40% enhancement in bacterial production. They proposed photo-mineralisation of DOC as well as photo-production of bio-refractory DOC to be the two mechanisms responsible for reduced bioavailability of DOC as a result of UV-B irradiation. As reductions in DOC levels were found during the summer study of the Warren Reservoir but not during winter, it is possible that increased photo-mineralisation was behind the lower responses in bioavailability (in terms of bacterial numbers and biomass) during summer when compared to winter.

As the winter DOC of the Warren Reservoir appears to comprise higher content of humic material when compared to summer DOC (based on the findings of colour and specific colour, Sections 3.5.4 and 3.5.5), these findings indicate that the humic portion of the DOC pool is more prone to UV-B induced transformations, making it more bioavailable. Strome and Miller (1978) showed a UV-B enhancement factor (in terms of bioavailability) of 2.8 for the humic organics of the DOC that were exposed to sunlight. This information further supports the findings of this study, showing winter DOC to be more responsive to UV-B transformation (with respect to bioavailability) than summer DOC. As such, photo-transformation of humic material in the Warren Reservoir is likely to play a significant role in supporting secondary production of the reservoir.

In terms of how UV-B induced DOC transformations affect bacterial community composition, a study of Methe and Zeh (1999) indicated DOC composition to be an important factor in determining the diversity of bacterial communities. As such, it may be assumed that any changes to the DOC structure, caused by UV-B radiation, could also bring about changes in the bacterial community. DGGE analysis showed that UV-B irradiation of both summer and winter DOC resulted in alterations to the bacterial community structures in the Warren Reservoir study. Greater shifts in bacterial populations were generated as a result of exposing winter DOC to UV-B radiation than was the case with the summer DOC (when compared to original community composition and that of the controls). This suggests that UV-B irradiation of humic material, which was higher in the winter DOC pool compared to the summer, caused more pronounced changes in the DOC pool, and these changes were more significant in causing shifts in bacterial community composition. Lindstrom (2000) states the

importance of the correlation between the differences in bacteria community composition and the humic content. However, phytoplankton blooms can also promote changes in the bacterial community structure (Arietta and Herndl, 2002; Christoffersen et al., 2002). Thus, the changes in bacterial community structure observed as a result of exposure of summer reservoir DOC to UV-B radiation can be caused either as a result of changes to humic and/or the autochthonous (algal derived) content. Carlson et al. (2002) stated that the consumption of specific dissolved organic compounds differs among various phylogenetic groups of bacteria. They showed significant changes in bacteria community structure to be correlated with changes in DOC, which resulted from UV-transformation of labile to refractory forms. As such, changes to DOC (irrespective of its original character) are likely to cause changes in bacteria community compositions, as observed during the UV-B irradiation of Warren Reservoir DOC. The exact changes in DOC structure that generate shifts in bacterial communities remain subject for further research.

4.6 CONCLUSION

Reductions in DOC concentration were observed as a result of UV-B irradiation of summer DOC, while structural transformations were observed mainly as a result of exposure of winter DOC to UV-B radiation. Thus, photo-mineralisation of DOC has potential to play a significant role in directly reducing DOC levels in the Warren Reservoir. Furthermore, the UV-B range of the solar spectrum is likely to play a significant role in the cycling of DOC, by causing structural changes in the DOC pool making it more bioavailable. Winter DOC (which has previously been shown to be dominated by allochthonous sources) appeared to be most vulnerable to UV-B transformations, which subsequently caused increased bioavailability. Conversely, the summer DOC pool (which has previously been shown to be affected by the autochthonous primary production) appeared to be less prone to UV-B-induced transformations, resulting in smaller increases in bioavailability. This suggests that depending on the rate of direct photo-mineralisation, the autochthonous DOC may over time enter the recalcitrant DOC pool, particularly considering the relatively high input of allochthonous DOC into the Warren Reservoir, which appears to be more bioavailable in the presence of sunlight and UV-B radiation. As photo-degradation and microbial processes occur simultaneously in the natural environment, UV-B transformation of winter organic material may provide a better substrate for bacterial growth.

Exposure of DOC to UV-B radiation was also observed to cause alterations in the composition of the bacterial community. These results indicate that certain bacteria are probably more capable of utilising certain DOC structures over others. Since bacteria are the primary consumers of organic material in waters, there is potential for future employment of the bio-manipulation strategy, to enhance removal of certain DOC fractions over others.

CHAPTER 5: ROLE OF UV-B RADIATION IN CONTROLLING BACTERIAL GROWTH

5.1 INTRODUCTION

Solar radiation, in particular the UV-B part of the spectrum, has the potential to inhibit the growth and productivity of many aquatic organisms, particularly those in the surface waters, such as bacteria and phytoplankton (Maranger et al., 2002). UV-B radiation is absorbed by DNA and proteins, and as such has the potential to affect physiology, growth and productivity of bacteria species (Karentz and Lutze, 1990; Herndl et al., 1993; Ferreyra et al., 1997; Rae and Vincent, 1998; Elasri and Miller, 1999; Mostajir et al., 1999; Maranger et al., 2002). As a result, some of the side effects that have been shown to occur are a reduced rate of primary and secondary productivity and reduced cell division rates, growth inhibition, DNA damage and a change in phytoplankton community composition (Ferreyra et al., 1997; Mostajir et al., 1999). These effects caused by UV-B radiation are thought to be even more extreme on bacteria than on other aquatic organisms (such as phytoplankton) due to their smaller size, shorter generation time (Mostajir et al., 1999) and lack of protective pigments.

This study focuses on determining whether solar UV-B radiation is likely to inhibit bacterial growth in the Warren Reservoir, to the point that DOC consumption and removal from the reservoir are affected. Although UV-B radiation can increase bioavailability of DOC (as indicated by the findings of the Warren Reservoir study, Section 4.4.5, and studies by Lindell et al., 1995; Miller and Moran, 1997; Moran and Zepp, 1997), the resulting substrate might not be utilised and degraded by bacterial species in the surface waters, as their growth could be inhibited by UV-B radiation. Thus, in terms of the cycling of DOC in the Warren Reservoir, it is important to study the response of native bacterial communities to UV-B radiation in order to be able to determine whether lack of bacterial activity, as a result of UV-B inhibition, is likely to cause DOC accumulation in the long term.

Bacterial species in this study were subjected to two types of UV-B exposure: short-term exposure (simulating natural diurnal cycles experienced in the reservoir) and long-term continuous exposure, in order to determine how bacterial growth and community composition are likely to be affected by UV-B radiation. The specific aims of this study were to:

1. Estimate the UV-B induced changes to bacterial abundance and population biomass of summer and winter populations, and to determine whether UV-B radiation has the potential to induce changes to bacterial community composition.
2. Study the integrated effects of photo-degradation and microbial decomposition on the DOC pool, in order to estimate how UV-B induced bacterial damage is likely to affect the quantity and quality of the DOC pool in the Warren Reservoir.

5.2 MATERIALS AND METHODS

5.2.1. EXPERIMENTAL DESIGN OF THE STUDY PRESENTED IN SECTION 5.3

In January 2000 (during the Australian summer), nine water samples from the top 1m layer of the shallow site (S1) were collected in sterile 2L glass bottles. Samples were filtered through a 1.2μm membrane to obtain a mixed bacterial consortium (Section 2.12.4). Triplicate filtrates (2L) were placed into previously autoclaved glass jars (sterilised at 121°C for 20 minutes) and incubated under the following conditions:

1. Continuous exposure to UV-B radiation (280-320nm)
2. Alternating cycles of 12-hour UV-B exposure followed by a 12-hour dark incubation.
3. Continuous incubation in the dark (control).

The total duration of the experiment was 12 days, during which sampling was carried out at 7 am and 7pm daily, unless stated otherwise. At each sampling time, 20mL aliquots were collected for bacterial abundance analysis performed using Acridine Orange staining and epifluorescence microscopy as described in Section 2.12. Random (blind) counting approaches were applied to eliminate potential of bias during counting. On days 0, 3, 8 and 13, aliquots (150mL) were collected to determine biological oxygen demand (BOD). All samples were incubated in the same shaking incubator (at 85 rpm) equipped with UV-B lamps with the wavelength range of 280-320nm and an average intensity of 0.1±0.02 Wm^{-2}. Control samples held in the dark were covered with aluminium foil. The temperature was held constant at 20°C for the duration of the experiment. Samples were randomLy positioned around the incubator to account for any variability in UV-B energy levels. Prior to each sampling period, sample volumes (in samples where loss of water due to evaporation occurred) were adjusted by adding sterilised high purity, deionised water. Duplicate 2L high

purity Milli-Q water samples were placed under UV-B and dark conditions to check for organic or bacterial contamination.

5.2.2. EXPERIMENTAL DESIGN OF THE STUDY PRESENTED IN SECTION 5.4

During January and August 2001, four 2L samples were collected from the shallow (S1) site of the Warren Reservoir and another four 2L samples were collected from the deep (D2) site of the reservoir, to account for bacterial spatial variability across the reservoir. All samples were filtered through a 1.2μm membrane to obtain a mixed bacteria consortium, placed into sterile glass jars and duplicate samples from each site were placed under:

1. Continuous exposure to UV-B radiation (280-320nm)
2. Continuous exposure to Dark (controls)

The total duration of this experiment was 21 days, during which sampling was carried out daily, unless shown otherwise. At each sampling time 10mL aliquots were collected for bacterial abundance and biomass measurements. These were carried out using Acridine Orange staining and epifluorescence microscopy as described in Section 2.12. Random (blind) counting approach was applied to eliminate a potential of bias during counting. Aliquots (50mL) were collected for measurements of DOC concentration and UV-Vis absorbance. All samples were incubated in a shaking incubator at 85 rpm, equipped with UVB lamps in the wavelength range of 280-320nm and an average intensity of 0.1±0.02 Wcm^{-2}. Dark controls were covered with aluminium foil. The temperature remained constant at 20°C for the duration of the experiment. Samples were positioned randomLy in the incubator to remove any bias of potential irregularity in UV-B energy levels. Evaporation as a result of irradiation was minimal. Any adjustments to the volume were obtained by adding sterilised high purity Milli-Q water. Duplicate 2L high purity Milli-Q water samples were placed under UV-B and Dark conditions as negative controls in monitoring for organic or bacterial contaminations.

At the end of the 21-day incubation period, DGGE analysis was conducted (as described in Section 2.13) on bacteria populations of UV-B irradiated samples and dark controls. This was done to determine whether UV-B irradiation caused changes to summer and winter bacterial community structures. Changes in community structure due to UV-B

irradiation were analysed with respect to dark controls as well as the original community structure prior to incubations.

A pooled estimate of standard deviation method was used to determine the variation of each measurement during this study, as each sample was exposed to UV-B and Dark conditions in duplicates not triplicates. This method combines the estimates of variance of each pair, taking an average of all four sample pairs, with weights equal to their degrees of freedom. This approach gives more weight to the information from the larger sample. The calculation used to determine the standard deviation of a single measurement was:

$$S^2 = 1/8\ [(N_{S1,UVB1} - N_{S1,UVB2})^2 + (N_{D2,UVB1} - N_{D2,UVB2})^2 + (N_{S1,Dark1} - N_{S1,Dark2})^2 + (N_{D2,Dark1} - N_{D2,Dark2})^2]$$

5.3 TESTING THE EFFECTS OF UV-B RADIATION ON BACTERIAL ABUNDANCE AND RESPIRATION

5.3.1 BACTERIAL ABUNDANCE UNDER UV-B, UV-B/DARK AND DARK CONDITIONS

Differences in bacterial counts between the two UV-B treatments in comparison with the controls during the 12 day incubation are summarised in Figure 5.1.

The same bacterial growth pattern was observed for the two treatment types and the controls during the course of incubation. Bacterial concentration increased between days 0 and 4 (by 71±6% in UV-B; 64±6% in UV-B/Dark; 60±5% in controls). Then between day 4 and 7, cell concentration decreased by 55±5% in UV-B, 50±5% in UV-B/Dark and 50±4% in controls. After 7 days of incubations cell concentrations increased again, by 48±4% in UV-B samples, 38±3% in UV-B/Dark samples, and 53±5% in the controls.

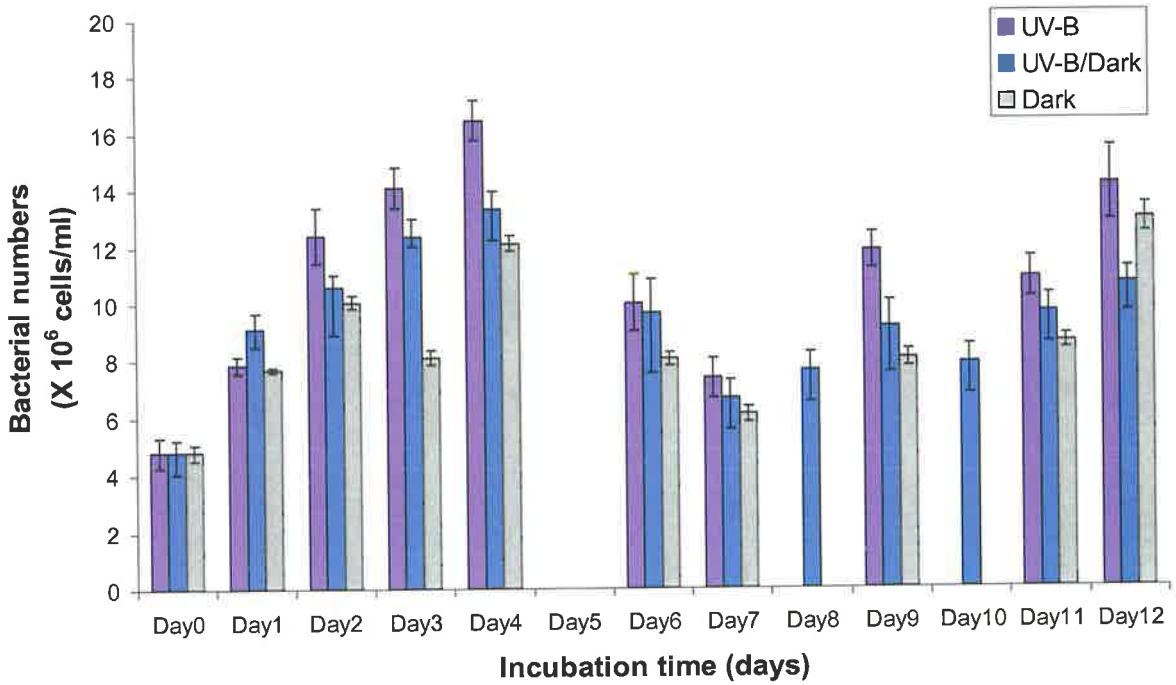


Figure 5. 1: Bacterial numbers in samples incubated under UV-B, UV-B/Dark, and Dark conditions for a period of 12 days. Bacteria numbers for days 5, 8 and 10 were not measured in all incubations. Plotted data is the mean of the triplicate samples ± S.E.

Comparison of populations between samples exposed to continuous UV-B radiation and UV-B/Dark cycles showed that following 2 days of incubation, significant differences in bacterial concentrations were found between the two treatments (T-test $p<0.05$). Furthermore, higher bacterial numbers were always detected in continuous UV-B exposed samples compared to dark controls ($p<0.05$). However, bacterial numbers in UV-B/Dark samples were not significantly higher than those of the controls ($p=0.24$).

5.3.2 BACTERIAL ABUNDANCE DURING A UV-B/DARK EXPOSURE – A SIMULATED DIURNAL STUDY

Bacterial concentration was measured immediately following alternating exposures of cells to UV-B and dark conditions (Figure 5.2). Between days 5 and 8, bacteria concentration was measured only in the morning following the 12-hour exposure to dark. Figure 5.2 shows an increase in bacteria concentration during the first 24 hours. After 24 hours of incubation

there was a trend where lower cell counts were obtained after exposure to 12 hours of UV-B radiation (mean decrease 15±4%), followed by increased cell counts after 12 hours of dark incubation (mean increase 20±3%).

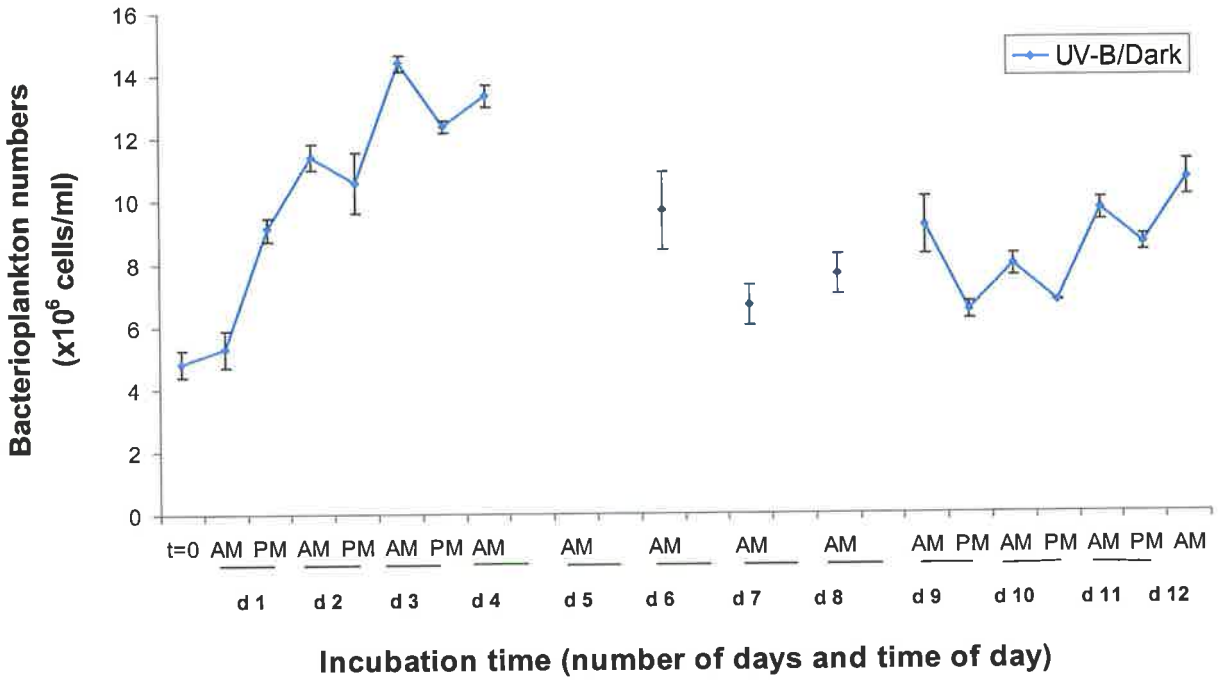


Figure 5. 2: Diurnal patterns of bacterial concentration in samples incubated under alternating UV-B and dark conditions for a total of 12 days. The same sampling times were applied daily, being 7am and 7pm. Plotted data is the mean of the triplicate samples ± S.E.

5.3.3 BIOLOGICAL OXYGEN DEMAND (BOD)

Biological oxygen demand (BOD) was measured in the time 0 sample (S1) and at 3, 8 and 12 days of incubation (under UV-B, UV-B/Dark and dark conditions), in order to determine how bacterial activity was affected by UV-B radiation (Figure 5.3). Following 3 days of incubation, BOD increased by 37±9% in UV-B irradiated samples, but was no different to S1 in UV-B/Dark and Dark samples. At 8 days, BOD decreased in both treatments and in the controls (dark) (when compared to levels measured on day 3), by

24±3% in UV-B samples, 51±4% in UV-B/Dark samples and 47±2% in Dark samples. After 13 days of incubation, BOD increased in all samples (compared to levels measured on day 8) by 52±2% in UV-B samples, 50±11% in UV-B/Dark samples and 50±7% in dark samples.

Significantly higher BOD, irrespective of incubation time, was obtained for samples incubated under continuous UV-B exposure when compared to both UV-B/Dark ($p<0.05$) and dark ($p<0.05$) samples (ANOVA at $\alpha=0.05$). Increases in UV-B/Dark treatments were not significant when compared to dark control ($p=0.5$).

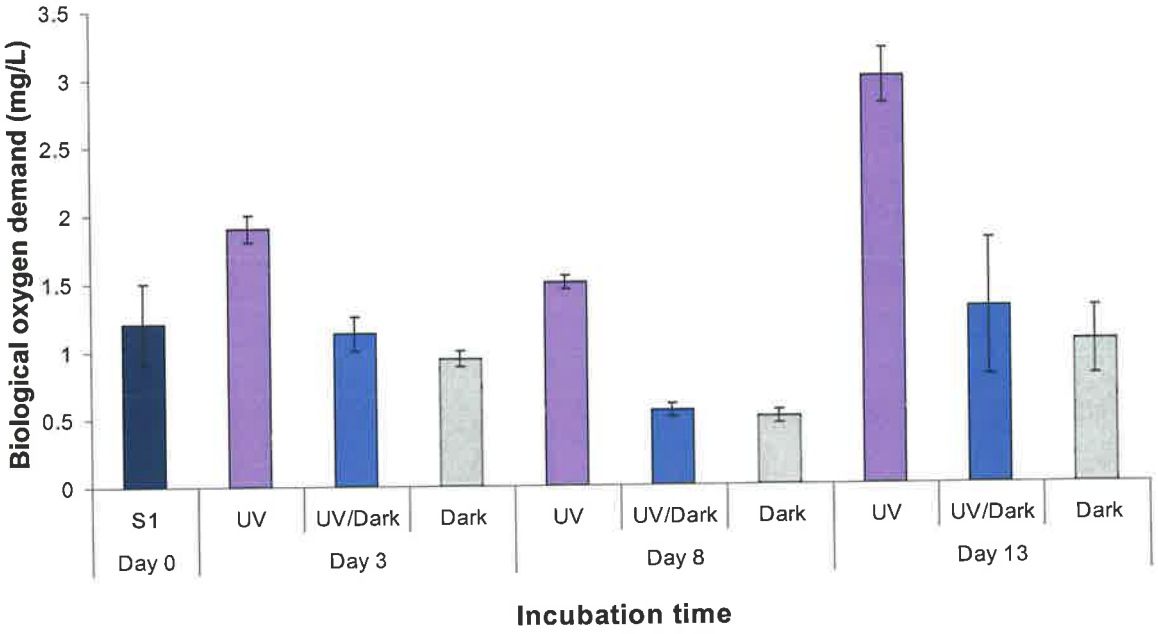


Figure 5. 3: Biological oxygen demand (BOD) data measured at the start of the incubation and following 3, 8 and 13 days of incubation under UV-B, UV-B/Dark and Dark conditions. Plotted data is the mean of the triplicate samples ± S.E.

BOD data of UV-B irradiated samples were normalised against those of the corresponding controls (dark) to highlight the effects of continuous UV-B radiation on the bacterial oxygen demand (Figure 5.4). Two to three fold increases in BOD occurred in the presence of UV-B radiation.

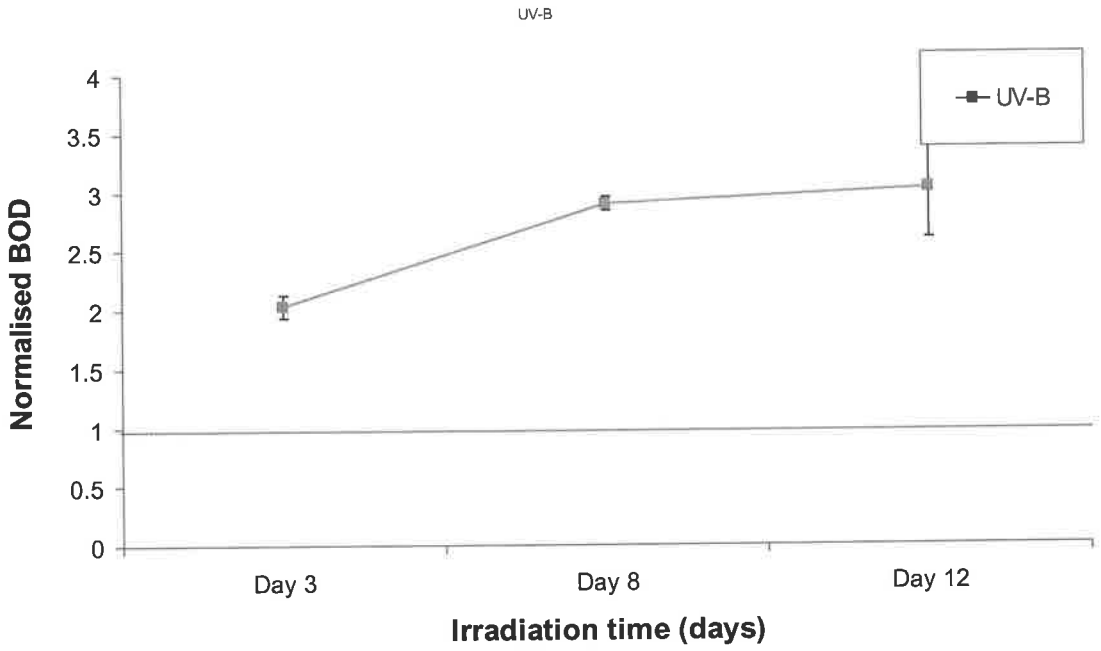


Figure 5. 4: Normalised BOD for UV-B irradiated samples (against that of the dark samples) after 3, 8 and 12 days of irradiation.

BOD values were divided by the bacteria concentration at each sampling time according to the type of incubation, to view changes in community respiration during the course of each incubation. BOD per unit cell increased in UV-B irradiated samples during the course of the incubation by 64%, decreased in dark samples by 52%, and was not significantly different in UV-B/Dark samples (Figure 5.5).

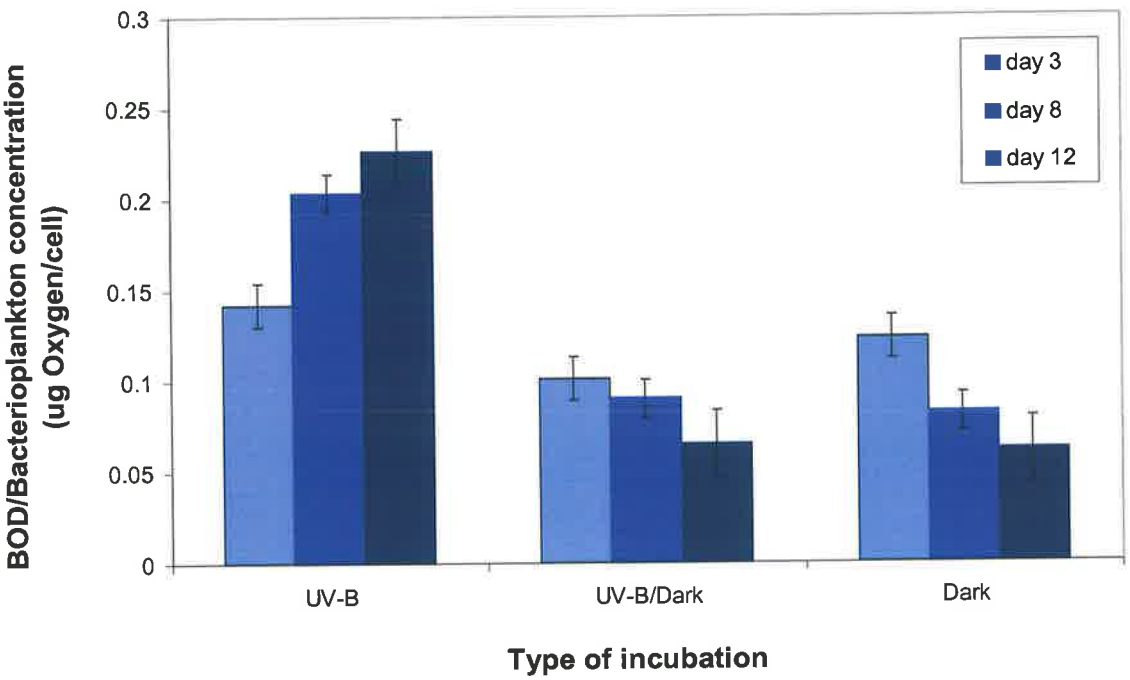
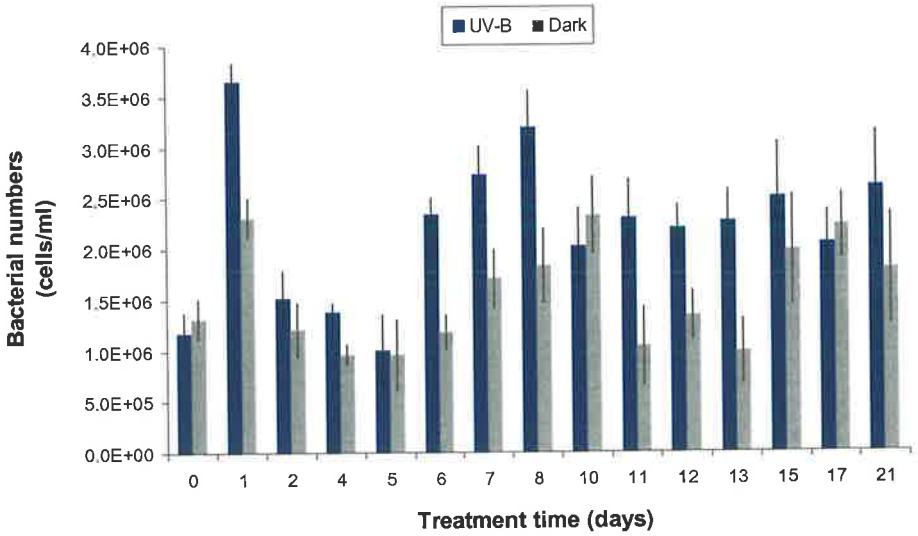


Figure 5. 5: Bacterial community respiration comparison between UV-B and UV-B/Dark treatments and dark controls.

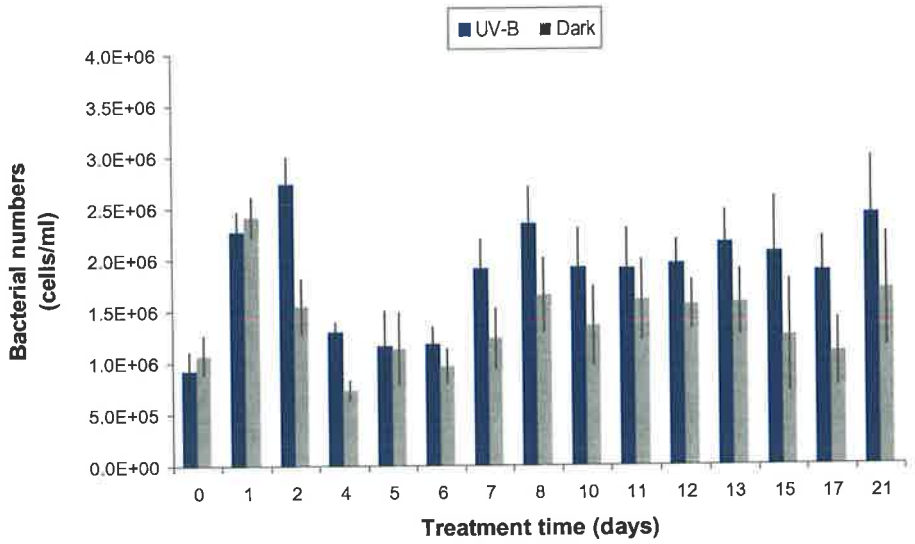
5.4 SEASONAL COMPARISONS OF THE EFFECTS OF UV-B RADIATION ON SUMMER AND WINTER BACTERIAL POPULATIONS

5.4.1 BACTERIAL ABUNDANCE

Summer bacterial numbers during 21 days of incubation under UV-B radiation and in the dark are summarised in Figure 5.6.



(A) Shallow site (S1)



(B) Deep site (D2)

Figure 5. 6: Bacterial concentrations (cells/mL) for summer samples collected from (A) shallow-S1 and (B) deep-D2 reservoir sites, and incubated under UV-B radiation and in the dark for 21 days. Pooled estimators of standard deviation of single measurements are shown.

Cell numbers varied significantly throughout the 21 day sampling period in both UV-B and dark incubations. In the dark S1 samples, cell numbers ranged from 9.6±1.0 $x10^5$cells/mL to 2.3±0.2 $x10^6$ cells/mL, whereas those incubated under UV-B ranged from 1.0±0.4 $x10^6$ to 3.7±0.6 $x10^6$ cells/mL. In the dark D2 samples, cell numbers ranged from

7.3±0.7 x10^5 cells/mL to 24±1 x10^5 cells/mL, whereas those incubated under UV-B ranged from 9±1 x10^5 cells/mL to 2.7±0.3 x10^6 cells/mL. Combined data of bacterial numbers from both sites highlighted the effects of UV-B radiation on the abundance of reservoir bacteria during summer (Figure 5.7). T-test at a 95% confidence level was used to test the significance of the differences between UV-B and dark samples. For the duration of the study, total bacterial numbers from both S1 and D2 sites were higher (44±7%) in the presence of UV-B radiation than in the dark ($p<0.05$).

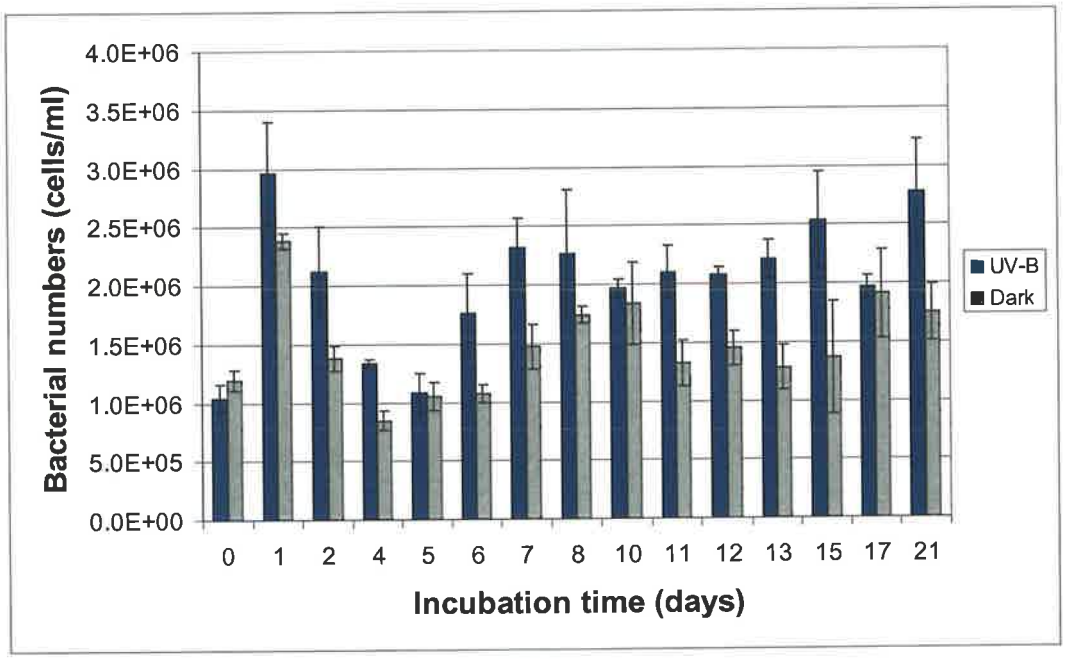


Figure 5. 7: Mean bacterial concentration (cells/mL) for all summer samples incubated under UV-B and Dark conditions for 21 days. Plotted data is the mean ± S.D. for all reservoir samples (S1+D2, thus quadruplicates) under UV-B radiation and in the dark.

For the purpose of comparing the effects of UV-B radiation on bacterial abundance of each site, bacterial numbers of UV-B treated samples were normalised against those of the controls (Figure 5.8). This analysis further supports the data presented in Figures 5.6 and 5.7, indicating enhanced bacterial numbers in the presence of UV-B radiation. Bacterial numbers of shallow S1 and deep D2 samples irradiated with UV-B showed approximately a 1 fold increase when compared to the cell numbers of the corresponding dark samples (Figure 5.8). During incubation, S1 samples had highest cell numbers (compared to the corresponding dark

incubations) after 13 days of irradiation (56±5%), whereas the D2 samples had a highest increase in bacteria numbers in the presence of UV-B after 17 days of irradiation (46±7%) (Figure 5.8).

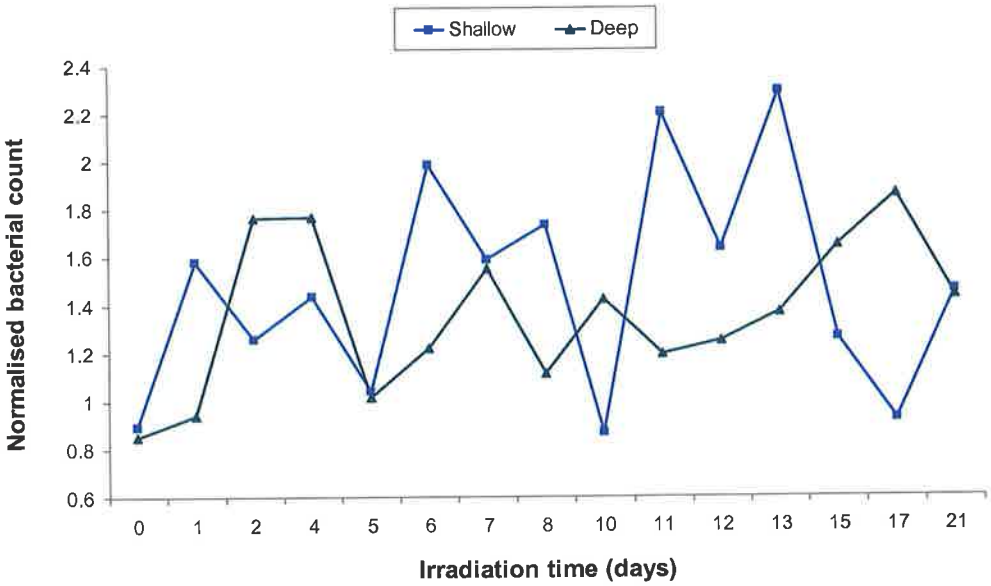
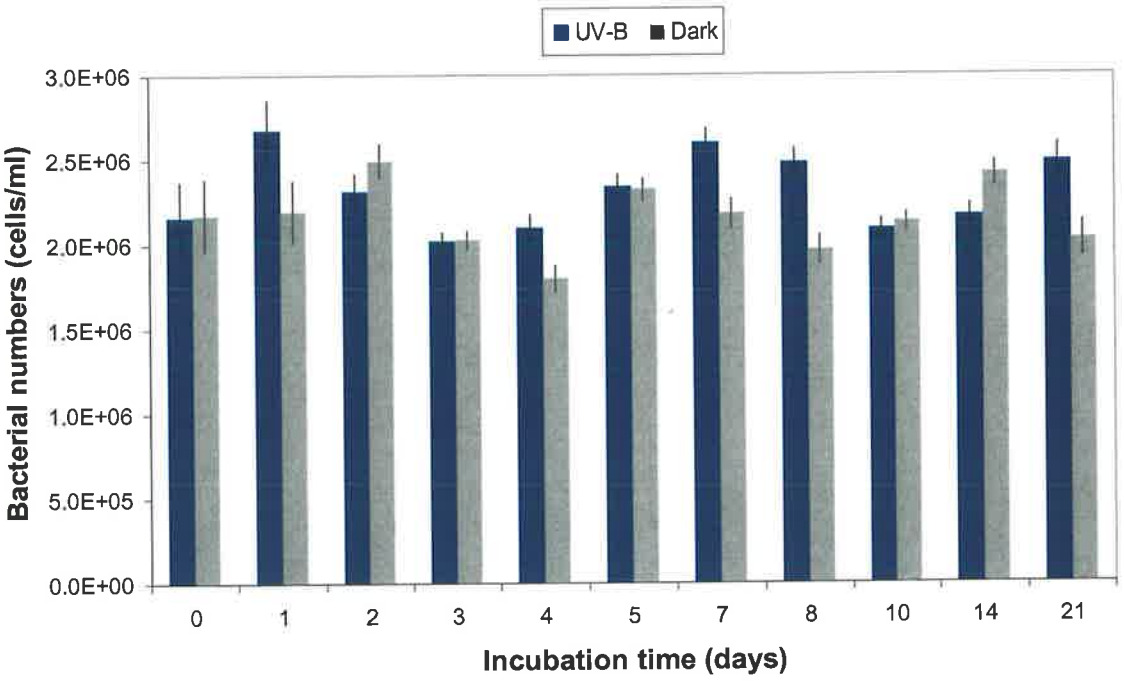
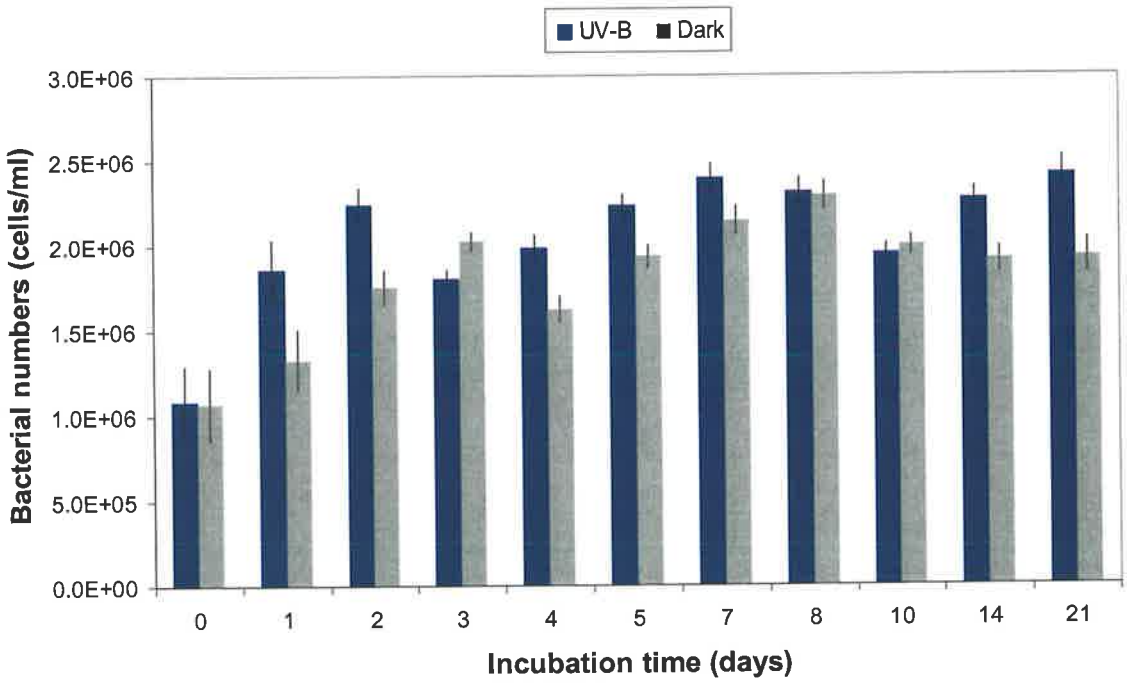


Figure 5. 8: Normalised summer bacterial numbers of S1 and D2 samples treated with UV-B radiation, against bacterial numbers of the same samples that were incubated in the dark for up to 21 days.

Winter bacterial numbers during 21 days of incubation under UV-B radiation and in the dark are summarised in Figure 5.9. Significant variations in cell numbers were also observed during the winter study, in both UV-B and dark incubations. In the dark S1 samples, cell numbers ranged from 1.8±0.1 x10^6 cells/mL to 2.5±0.1 x10^6 cells/mL, while those incubated under UV-B ranged from 2.0±0.1 x10^6 to 2.7±0.2 x10^6 cells/mL. Bacterial numbers in shallow (S1) samples were found to be significantly higher (8±3%) in the presence of UV-B radiation than those incubated in the dark (T-test, $p<0.05$). In the dark D2 samples, cell numbers ranged from 1.1±0.1 x10^6 cells/mL to 2.3±0.1 x10^6 cells/mL, while those incubated under UV-B ranged from 1.1±1 x10^6 cells/mL to 2.4±0.1 x10^6 cells/mL. Bacterial numbers in deep (D2) samples were also found to be significantly higher (14±4%) in the presence of UV-B radiation than those incubated in the dark (T-test, $p<0.05$).



(A) S1 site



(B) D2 site

Figure 5. 9: Bacterial concentrations (cells/mL) for winter samples collected from (A) shallow-S1 and (B) deep-D2 reservoir sites, and incubated under UV-B radiation and in the dark for 21 days. Pooled estimators of standard deviation of single measurements are shown.

For the purpose of comparing the effects of UV-B radiation on bacterial abundance of each site, bacterial numbers of UV-B treated samples were normalised against those of the controls (Figure 5.10). This analysis showed enhanced bacterial numbers in the presence of UV-B radiation. Bacterial numbers of shallow S1 and deep D2 samples irradiated with UV-B increased by less than 50% compared to the cell numbers of the corresponding dark samples. During the 21-day incubation, S1 samples had highest cell numbers (compared to the corresponding dark incubations) after 8 days of irradiation (26±3%), while the D2 samples had a highest increase in bacteria numbers in the presence of UV-B following 1 day of irradiation (40±4%) (Figure 5.10).

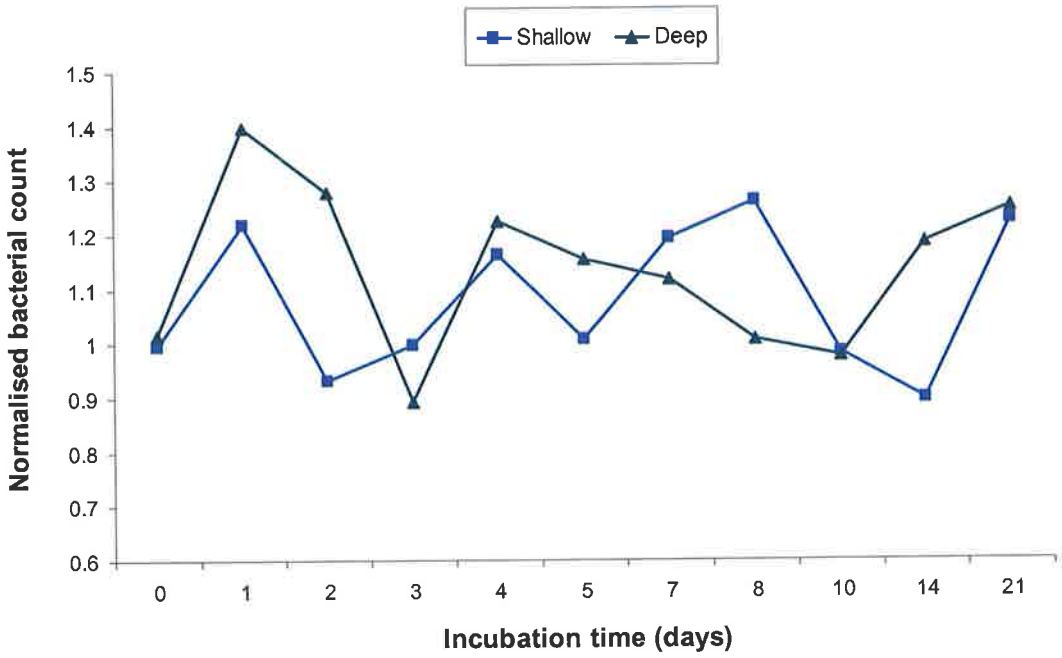
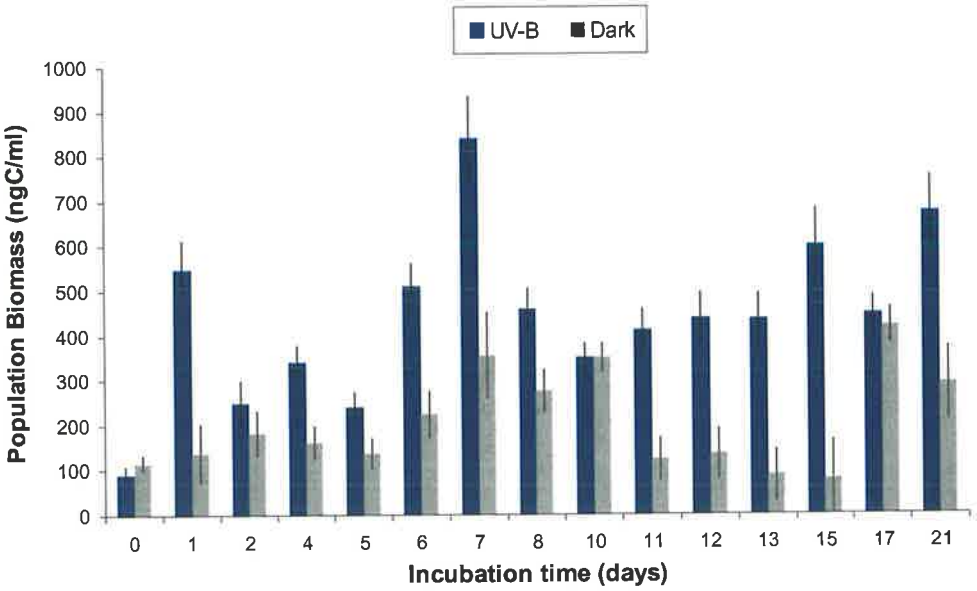


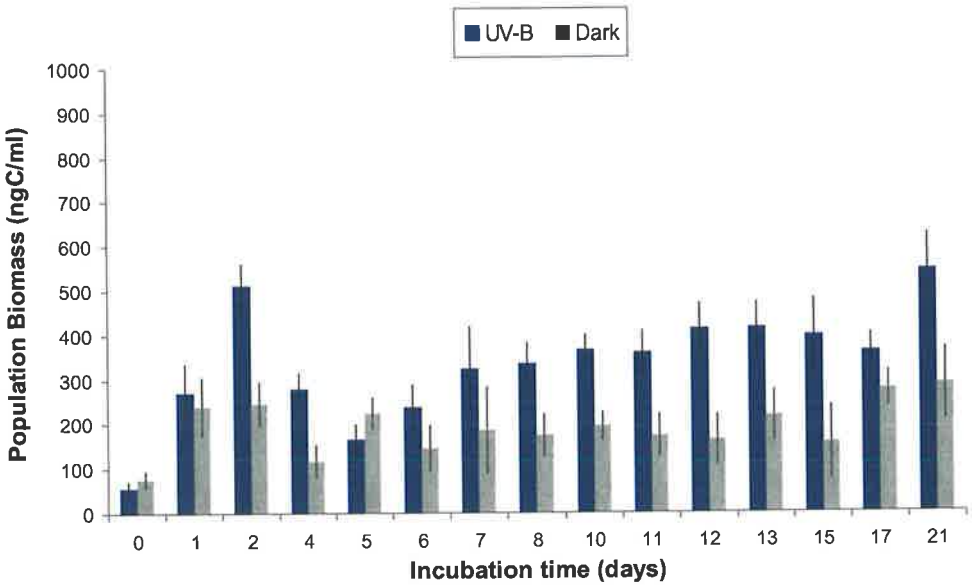
Figure 5. 10: Normalised bacterial numbers of winter S1 and D2 samples treated with UV-B radiation, against bacterial numbers of the same samples that were incubated in the dark for up to 21 days

5.4.2 BACTERIAL BIOMASS

Based on summer bacterial numbers (Figure 5.6) and bio-volume data (not shown), population biomass was estimated using the conversion factor of 0.308 $pgC\mu m^{-3}$ (Bergstrom and Jansson, 2000). Bacterial population biomass is summarised in Figure 5.11.



(A) S1 site



(B) D2 site

Figure 5. 11: Bacterial population biomass (ngC/mL) for summer reservoir samples collected from the (A) shallow-S1 and (B) deep-D2 sites of the reservoir and incubated under UV-B radiation and in the dark for up to 21 days. Pooled estimators of standard deviation of single measurements are shown.

The biomass of both S1 and D2 populations incubated under UV-B radiation was significantly higher compared to that of the corresponding dark incubations (T-test, $p<0.05$).

Significant variations in bacterial biomass were observed during the summer study in both UV-B and dark incubations. Bacterial biomass in dark S1 samples ranged from 87±16ngC/mL to 420±72ngC/mL, while UV-B irradiated S1 samples had biomass in the range of 90±16ngC/mL to 838±78ngC/mL. Bacterial biomass in the dark D2 samples ranged from 76±34ngC/mL to 288±63ngC/mL, whereas UV-B irradiated D2 samples had biomass in the range of 55±22ngC/mL to 546±31ngC/mL.

Combined biomass data of the two sites indicated the overall effects of UV-B on bacterial biomass irrespective of the sample origin (Figure 5.12). Bacterial biomass was higher in UV-B irradiated samples than in dark samples (T-test, $p<0.05$). The data presented in Figures 5.7 suggest a concurrent increase in bacteria numbers and cellular biomass (Figure 5.12), indicating enhanced growth in the presence of UV-B radiation.

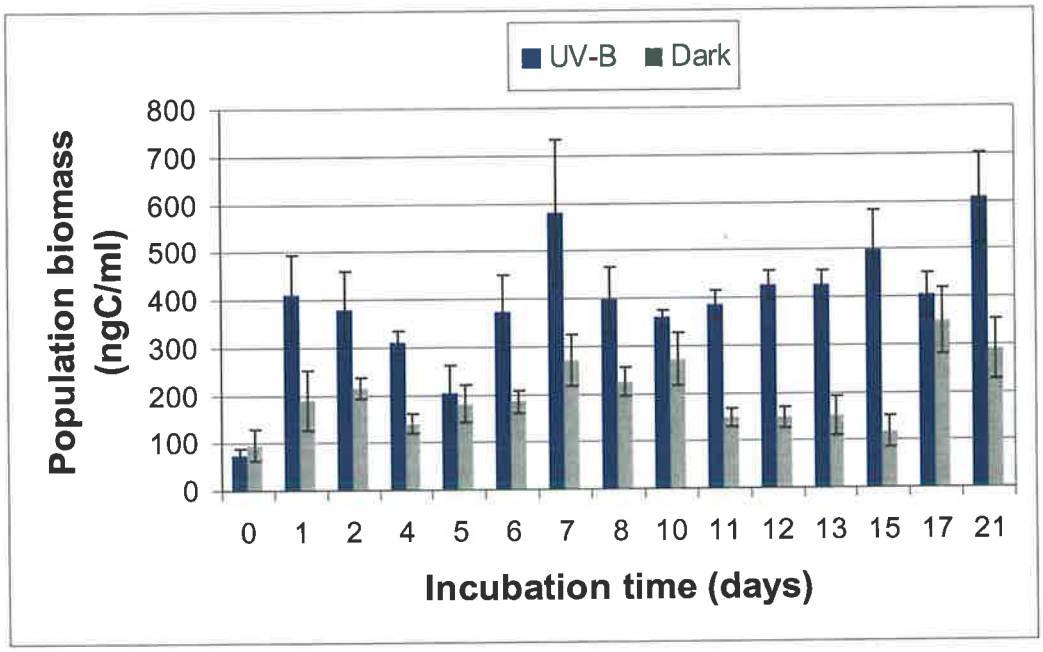


Figure 5. 12: Mean bacterial population biomass (ngC/mL) for all summer samples incubated under UV-B and Dark conditions for up to 21 days. Plotted data is the mean ± S.D. for all reservoir samples (S1+D2, thus quadruplicates) under UV-B radiation and in the dark.

Bacterial biomass data of UV-B treated samples were normalised against those of the corresponding controls to highlight changes due to UV-B radiation (Figure 5.13). This

analysis shows enhanced bacterial biomass due to UV-B radiation. UV-B radiation appears to have more effect in enhancing bacteria biomass of S1 than D2 samples. Increases in biomass of up to 8 times were measured for the S1 samples, while there was an approximate doubling in the D2 samples.

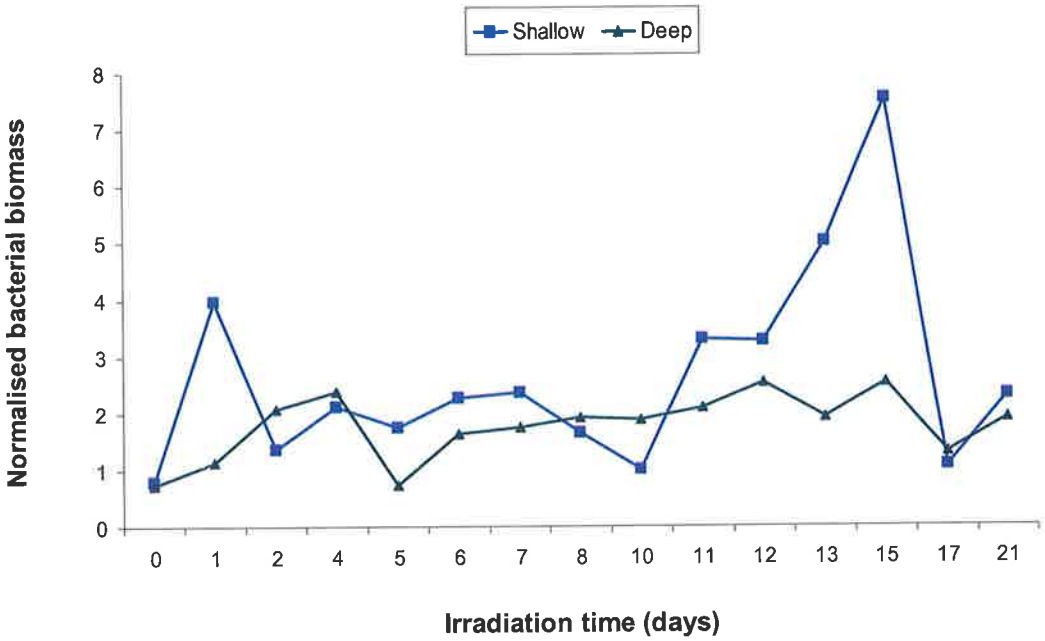
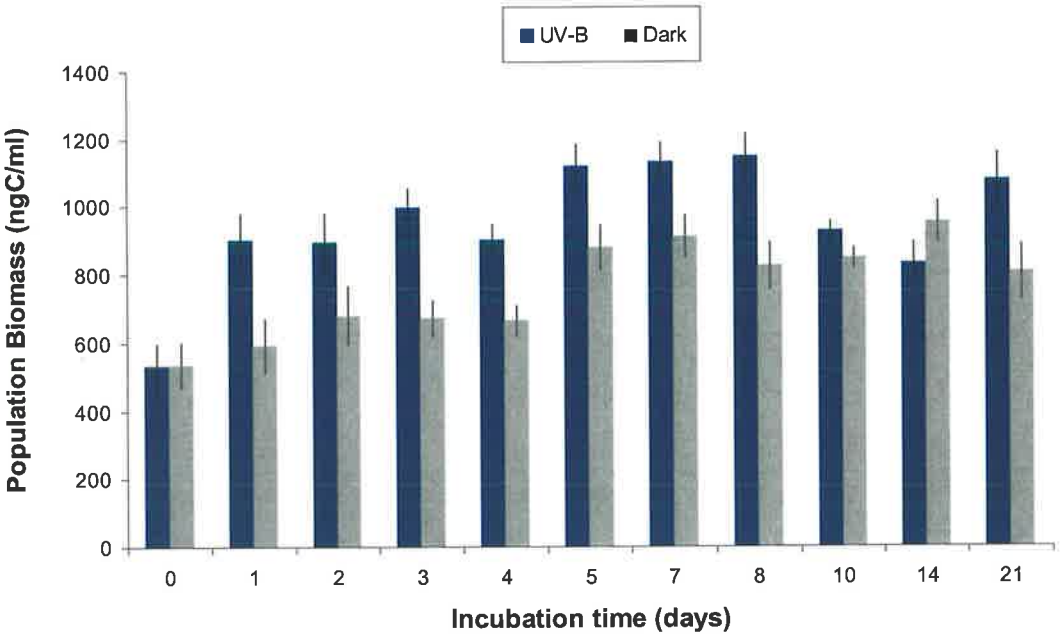


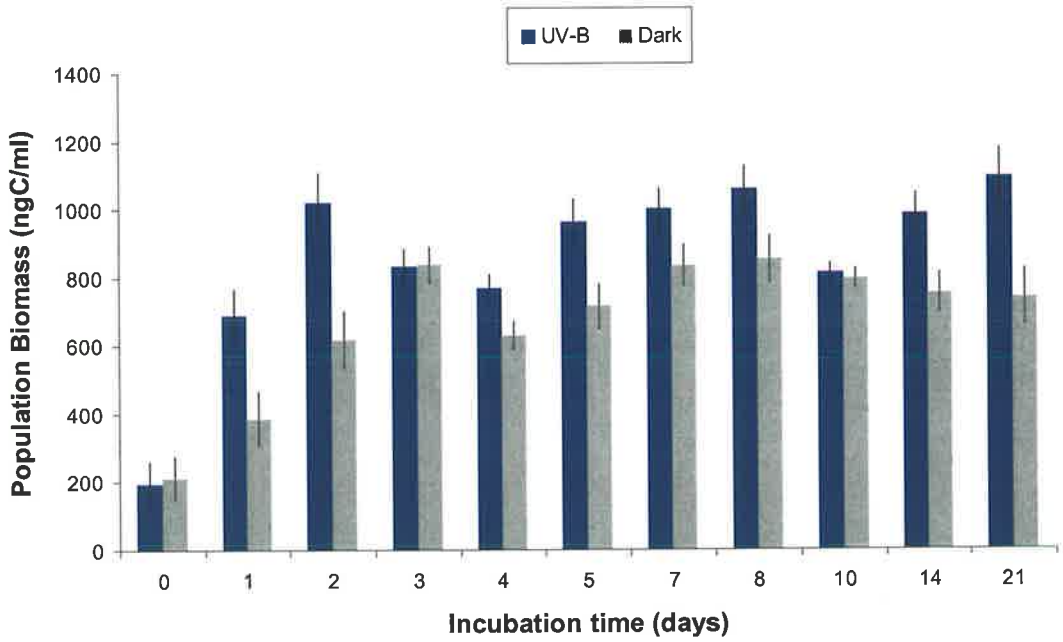
Figure 5. 13: Normalised bacterial biomass of summer shallow-S1 and deep-D2 samples treated with UV-B radiation, against bacterial numbers of the same samples that were incubated in the dark for up to 21 days

Based on winter bacterial concentration data (Figure 5.9) and bio-volume data (not shown), population biomass was estimated using the conversion factor of 0.308 pgCμm^{-3} (Bergstrom and Jansson, 2000). Results are summarised in Figure 5.14.

The biomass of both shallow (S1) and deep (D2) populations incubated under UV-B radiation was significantly higher compared to that of the corresponding dark incubations (T-test, $p<0.05$). Significant variations in bacterial biomass were found throughout the study period, in both UV-B and dark incubations. Bacterial biomass in dark S1 samples ranged from 535±56 ngC/mL to 954±60 ngC/mL, whereas UV-B irradiated S1 samples had biomass in the range of 532±65ngC/mL to 1147±70 ngC/mL. Bacterial biomass in the dark D2 samples ranged from 210±65ngC/mL to 852±70ngC/mL, whereas UV-B irradiated D2 samples had biomass in the range of 193±65ngC/mL to 1093±81ngC/mL.



(A) S1 site



(B) D2 site

Figure 5. 14: Bacterial population biomass (ngC/mL) for winter reservoir samples collected from the (A) shallow-S1 and (B) deep-D2 sites of the reservoir and incubated under UV-B radiation and in the dark for up to 21 days. Pooled estimators of standard deviation of single measurements are shown.

Combined biomass data of the two sites indicated the overall effects of UV-B on bacterial biomass irrespective of the sample origin (Figure 5.15). Bacterial biomass was higher (30±5%) in UV-B irradiated samples than in dark samples (T-test, $p<0.05$). The data presented in Figures 5.9 suggests a concurrent increase in bacterial numbers and cellular biomass (Figure 5.14), indicating enhanced growth in the presence of UV-B radiation.

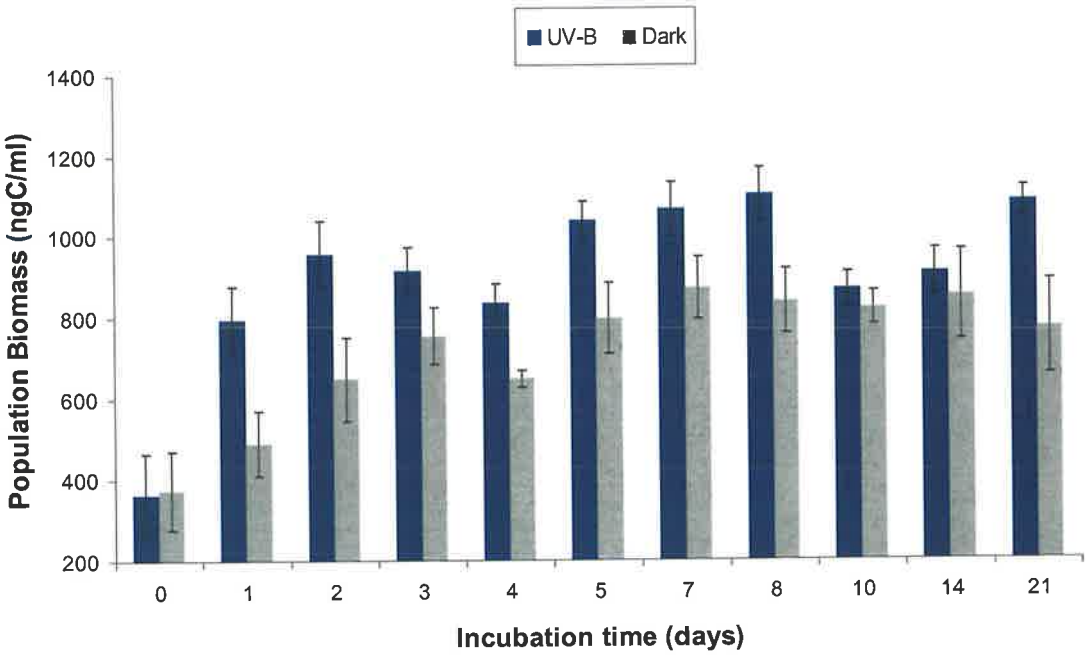


Figure 5. 15: Mean bacterial population biomass (ngC/mL) for all winter samples incubated under UV-B and Dark conditions for up to 21 days. Plotted data is the mean ± S.D. for all reservoir samples (S1+D2, thus quadruplicates) under UV-B radiation and in the dark.

5.4.3 BACTERIAL POPULATION DIVERSITY

To determine whether UV-B radiation had an effect on the community composition of summer bacterial populations, DGGE analysis was employed (Figure 5.16).

Figure 5. 16: 8% polyacrylamide DGGE gel, showing the separation of 520bp PCR products amplified from genomic DNA that was extracted after 21 days of incubating summer reservoir water samples, collected from shallow-S1 and deep-D2 reservoir sites, under UV-B radiation and in the dark. Lane 1-E.coli marker; 2-S1 (Day0); 3-S1 (UVB); 4-S1 (UVB); 5-S1 (Dark); 6-S1 (Dark); 7-E.coli; 8-D2 (Day 0); 9-D2(UVB); 10-D2 (UVB); 11-D2 (Dark); 12-D2 (Dark); 13-E.coli marker.

Based on the differences of banding patterns between different samples in the DGGE gel (Figure 5.16), a phylogenetic analysis was performed to determine whether UV-B

radiation induced changes in population diversity. With the exception of the divergence of *E.coli* markers, the phylogenetic tree in Figure 5.17 shows the presence of two main divisions. The first division groups samples that have been exposed to UV-B radiation irrespective of their reservoir origin (S1 or D2 sites). The second division groups together populations of samples that were held in the dark, with a close similarity link to the original populations found prior to incubations.

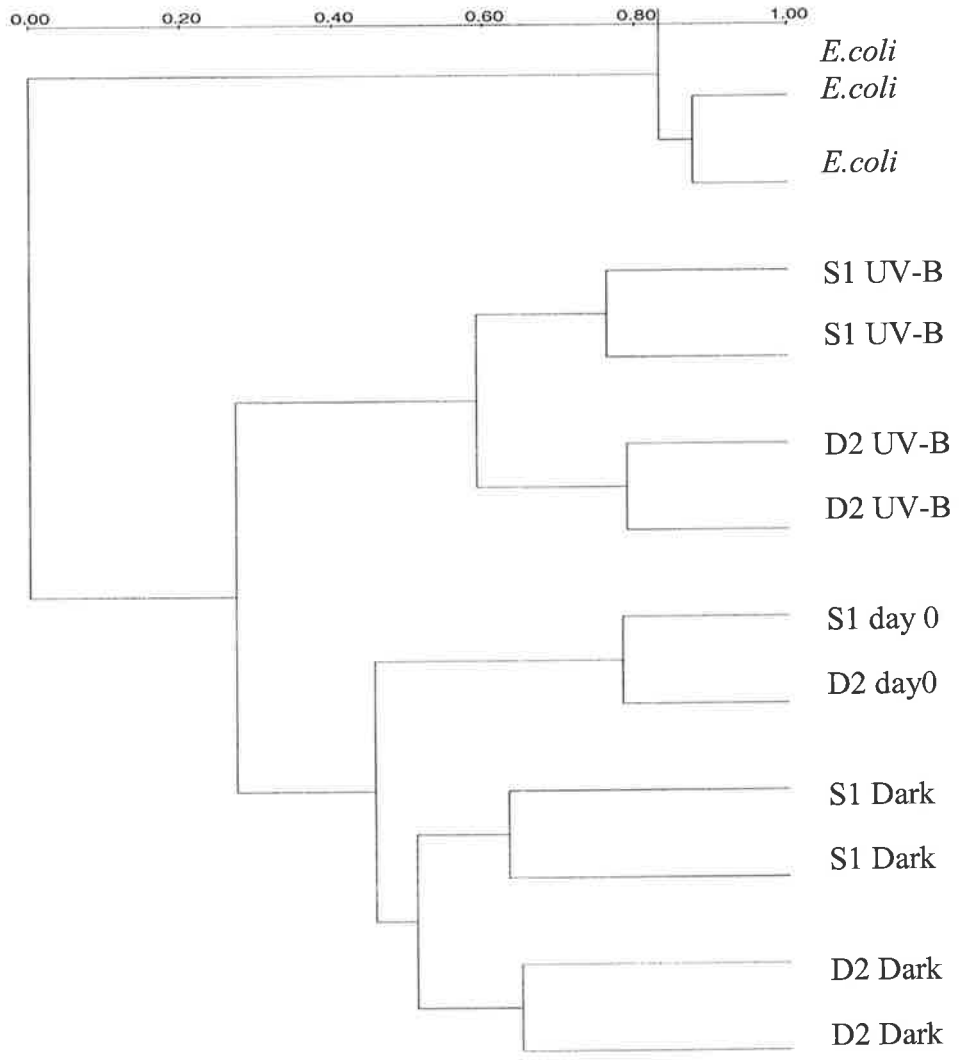


Figure 5. 17: Phylogenetic tree, based on Ward's dice coefficient analysis, indicating varying degrees of relatedness between bacterial populations belonging to shallow-S1 and deep-D2 sites of the reservoir prior to and post 21 days of UV-B and Dark incubations.

Prior to incubations, bacteria consortia belonging to S1 and D2 sites had 80% similarity. After 21 days of dark incubation there was a decrease in population similarity between samples belonging to S1 and D2 sites (50% similarity) but also between samples belonging to the same site (62% similarity). Conversely, after 21 days of UV-B incubation, closer similarity was measured between populations belonging to S1 and D2 sites (60% similarity) as well as between samples belonging to the same site (78% similarity among S1 samples and 80% similarity among D2 samples) than was observed for dark incubations. However, a complete analysis of all the samples contained in the phylogenetic tree shows the UV-B irradiated samples to be most divergent from not only original samples but also from those that were incubated in dark, measuring 45% similarity to these populations.

Principal Component Analysis (PCA) was performed to measure the separation of populations based on type of incubation. The PCA was based on the information of band presence as well as the difference in peak intensity of each band the DGGE gel (Section 2.13.4). Data on band intensity (not shown) was used to generate a two dimensional matrix indicating sample separation along the horizontal and vertical axes (Figure 5.18). The influences of horizontal and vertical axes on sample separation were similar (X-axis=30%, Y-axis=26%). The PCA correlates with the results of the phylogenetic tree analysis (Figure 5.17), showing separation of samples based on they type of incubation (UV-B and Dark). Both UV-B and Dark samples were grouped together irrespective of the origin in the reservoir (S1 and D2).

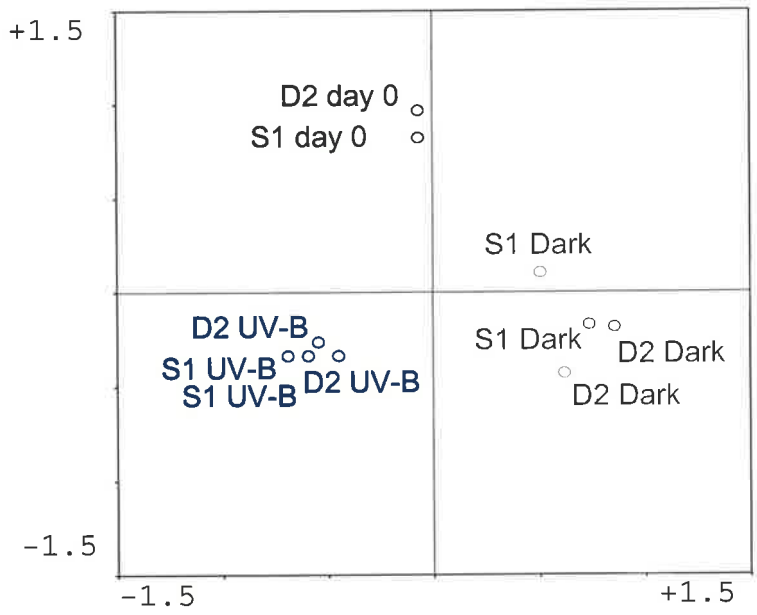


Figure 5. 18: Principal Component Analysis showing differences in shallow and deep reservoir bacteria populations based on treatment type (UV-B and Dark). Influence on separation by horizontal axis is 30% and vertical axis is 26%.

To determine whether UV-B radiation had an effect in altering community composition of winter bacteria populations, DGGE analysis was employed (Figure 5.19).

Figure 5. 19: 8% polyacrylamide DGGE gel, showing the separation of 520bp PCR products amplified from genomic DNA that was extracted after 21 days of incubating winter reservoir water samples, collected from shallow-S1 and deep-D2 reservoir sites, under UV-B radiation and in the dark. Lane 1-E.coli marker; 2-S1 (Day 0); 3-S1 (UVB); 4-S1 (UVB); 5-S1 (Dark); 6-S1 (Dark); 7-E.coli; 8-D2 (Day 0); 9-D2(UVB); 10-D2 (UVB); 11-D2 (Dark); 12-D2 (Dark); 13-E.coli marker.

Based on the banding pattern differences observed between different samples in the DGGE gel (Figure 5.19), a phylogenetic analysis was performed to determine whether UV-B radiation had an effect on altering community composition of winter bacteria populations, and whether the response of winter populations differed to that of summer ones (Figure 5.20).

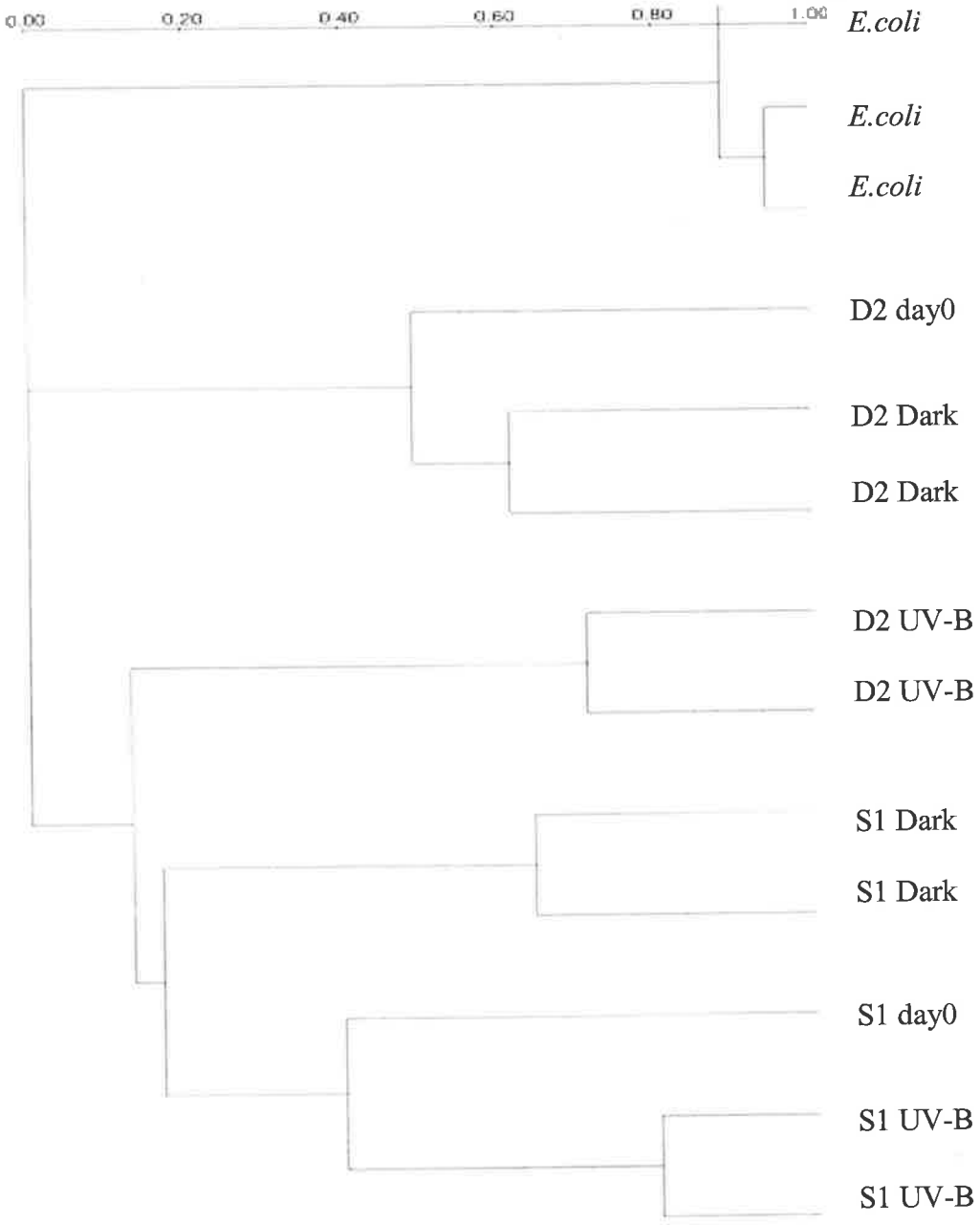


Figure 5. 20: Phylogenetic tree, based on Ward's dice coefficient analysis, indicating varying degrees of relatedness between bacteria populations belonging to shallow and deep sites of the reservoir prior to and post 21 days of UV-B and Dark incubations.

E.coli markers, although showing 90% and higher similarity among each other, are completely divergent from all other samples. Based on the analysis presented in Figure 5.20, increased differences were found between S1 and D2 bacterial populations based on type of incubation (UV-B and Dark), particularly in the D2 samples where following 21 days of UV-B irradiation, bacterial populations diverged from those found in the original samples, showing closer relatedness to S1 samples than any of the D2 samples (pre-treatment or Dark incubated populations, with 10% similarity). In contrast to D2 samples, S1 samples incubated for 21 days in the presence of UV-B radiation showed higher similarity to the original samples than the same samples incubated in the dark, namely 78% and 38% similarity respectively. This suggests that D2 winter bacterial populations were more susceptible to UV-B induced population changes than the S1 winter populations.

Results of Principal Component Analysis (PCA), used to compare bacterial populations based on the type of incubation, are summarised in Figure 5.21. PCA supports the findings of the phylogenetic tree analysis, showing clear separation between D2 samples incubated in dark and in the presence of UV-B radiation. Furthermore, PCA identifies that there was a higher similarity between UV-B irradiated D2 populations and S1 populations, than between the UV-B irradiated D2 populations and the same populations incubated in Dark. Following 21 days of incubation, S1 samples showed separation based on the type of incubation (UV-B and Dark), however the populations were still more closely related than those of the D2 samples. Furthermore, both S1 and D2 populations diverged from those found originally in the samples at the start of incubations. The PCA suggests divergence of populations following 21 days of exposure to UV-B, in agreement with the phylogenetic analysis.

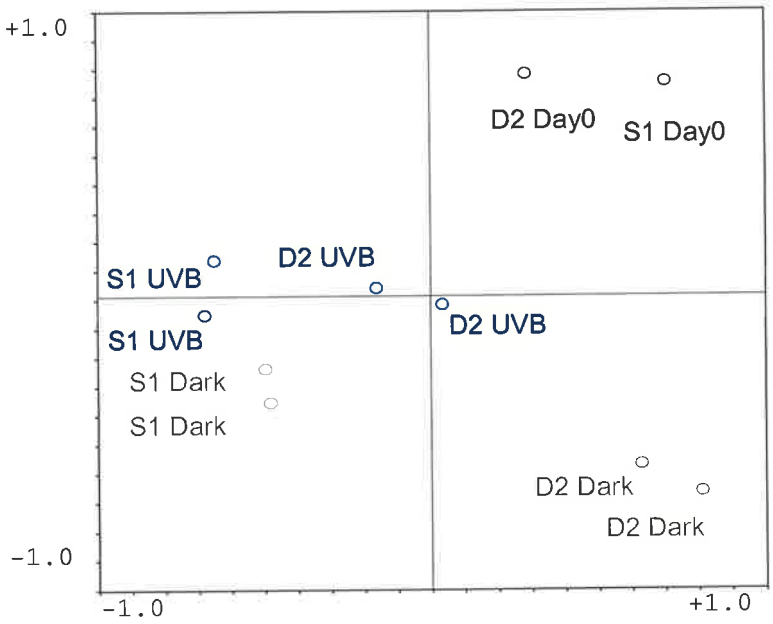


Figure 5. 21: PCA of the differences between winter reservoir bacteria populations exposed to UV-B and Dark conditions. Influence on sample separation by horizontal X-axis=30%, and by vertical Y-axis=22%.

5.4.4 EFFECTS OF UV-B IRRADIATION OF BACTERIAL POPULATIONS ON THE DOC POOL

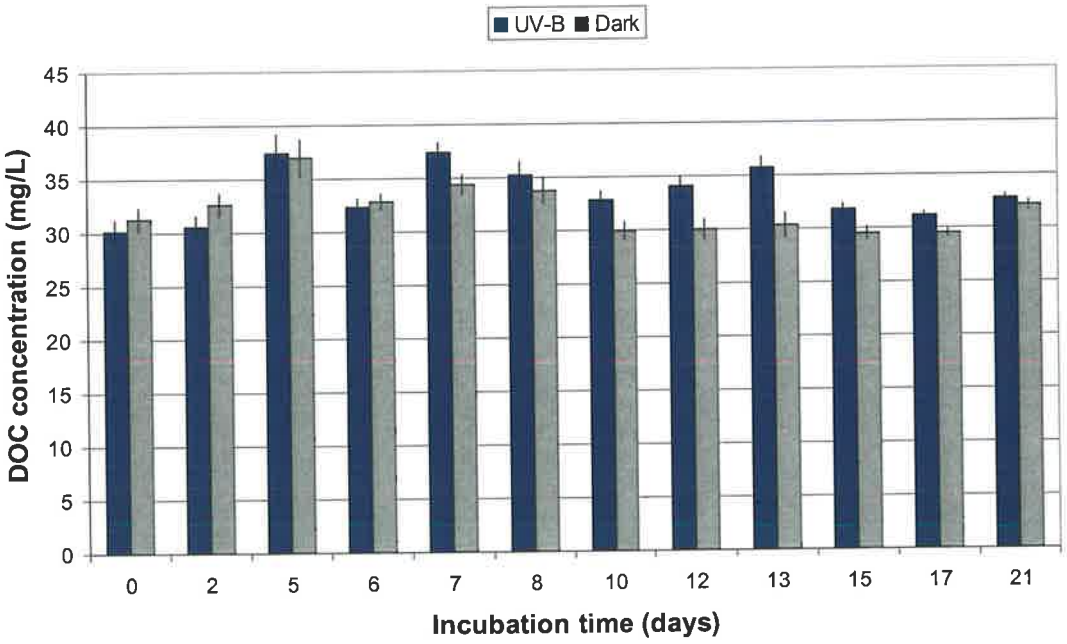
5.4.4.1 Changes in DOC concentrations

DOC concentration during the 21-day incubation of summer bacteria are summarised in Figure 5.22. A T-test at 95% confidence limit was used to determine if the differences in DOC concentrations found between UV-B and dark incubations were significant.

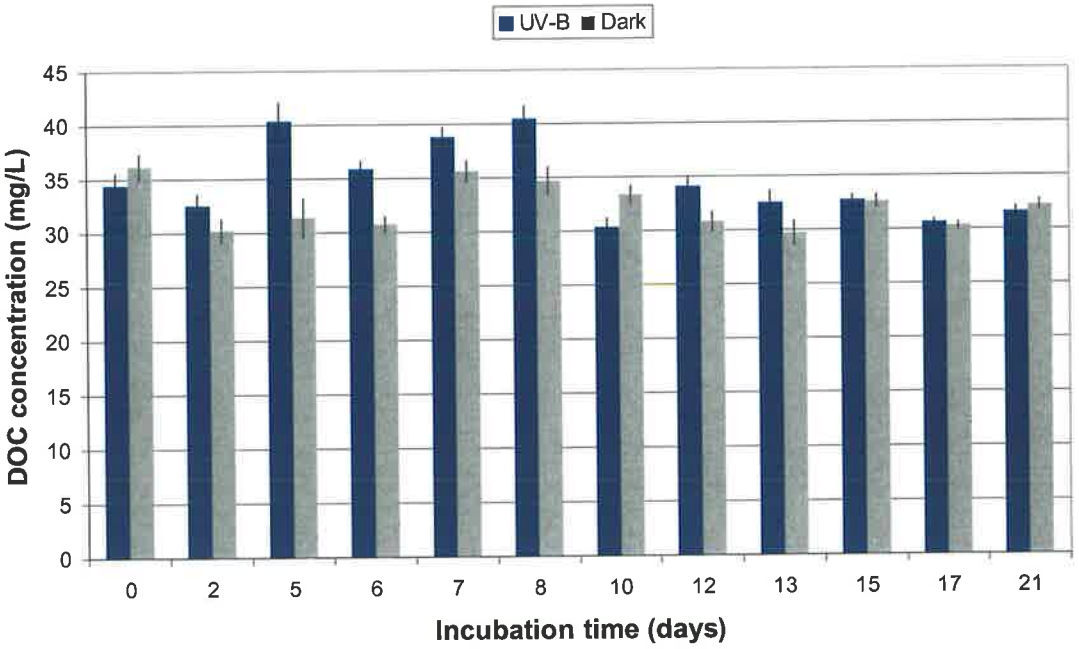
Variations in DOC concentrations were observed in S1 and D2 samples from both UV-B exposed and dark incubations throughout the 21 day study period. DOC concentrations in the shallow-S1 samples ranged between 29±1mg/L and 38±1mg/L. During the first 6 days of incubations, the differences in DOC concentrations between UV-B and Dark samples were not significant ($p>0.05$). After 6 days of incubation (and with the exception of days 8 and

21), shallow S1 samples exposed to UV-B radiation had higher DOC concentrations than the same samples incubated in the dark ($p<0.05$). During this period, DOC levels in the presence of UV-B radiation were higher by 5±0.6% to 18±3%.

DOC levels in the deep-D2 samples ranged between 29±1mg/L and 40±2mg/L. Following two days of incubation and up to day 8, and between days 12 and 13, DOC levels in UV-B irradiated samples were higher than those in Dark samples ($p<0.05$) by 10±2% to 22±3%. On the remaining days there were no significant differences between DOC levels of UV-B and Dark samples.



(A) S1 site



(B) D2 site

Figure 5. 22: DOC concentration measured in (A) shallow-S1 and (B) deep-D2 summer reservoir samples during 21 days of bacterial incubation under UV-B and dark conditions. Pooled estimators of standard deviation of single measurements are shown.

DOC concentrations of all UV-B irradiated samples were normalised against the corresponding controls to highlight the changes to the DOC levels due to UV-B radiation (Figure 5.23). This analysis shows periodically elevated levels of DOC in the presence of UV-B radiation. The data shown in Figure 5.23 further suggests that UV-B radiation caused higher DOC levels in D2 samples than in S1 during the initial period of incubation.

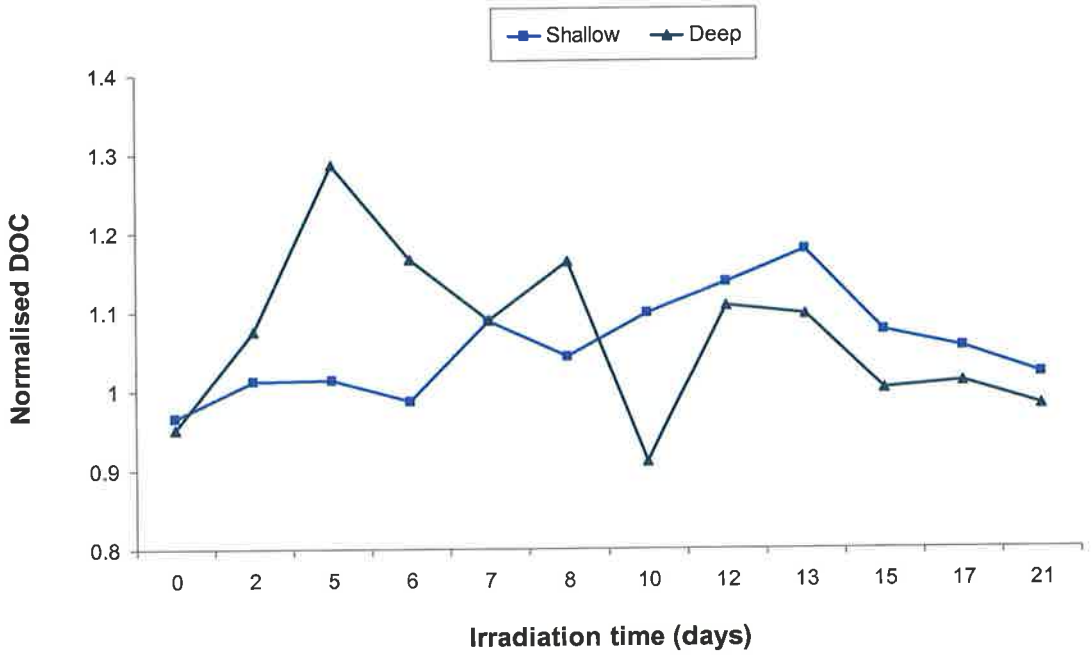


Figure 5. 23: Normalised DOC concentrations, of shallow-S1 and deep-D2 samples incubated under UV-B radiation for up to 21 days, with respect to the DOC concentrations of the dark samples.

Data from Figures 5.22 and 5.23 show periodically higher DOC levels in bacterial samples incubated under UV-B radiation than under dark conditions, suggesting higher levels of internal DOC loading under UV-B radiation.

Whilst regression analysis of DOC levels and bacterial numbers (Figure 5.24) does not show a clear significant relationship, a trend in UV-B incubations, where decreased levels of bacteria resulted in increased levels of DOC, was observed. In the dark incubations the relationship did not appear to hold.

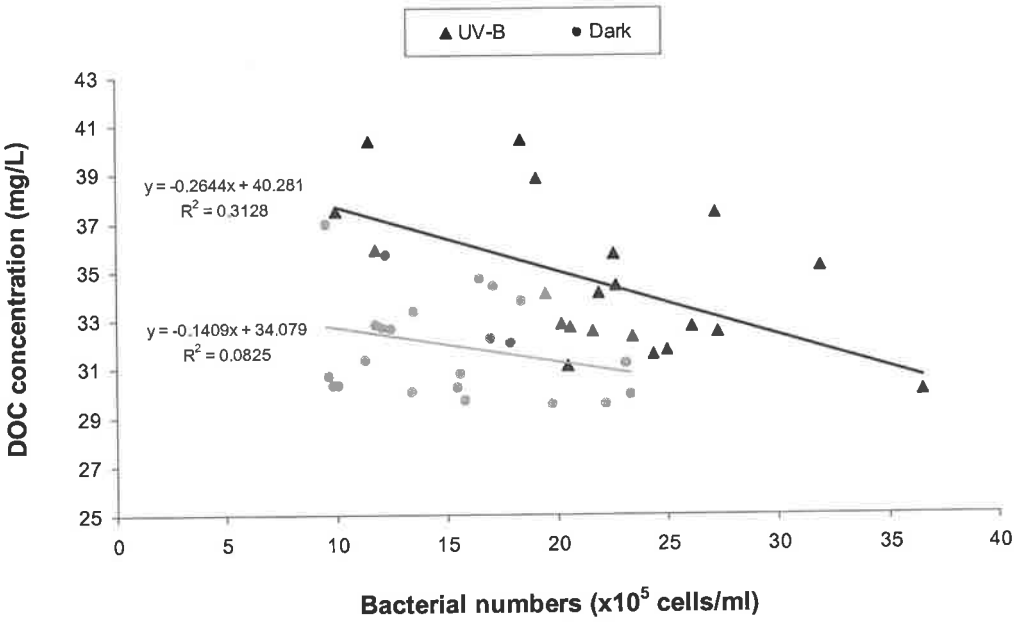


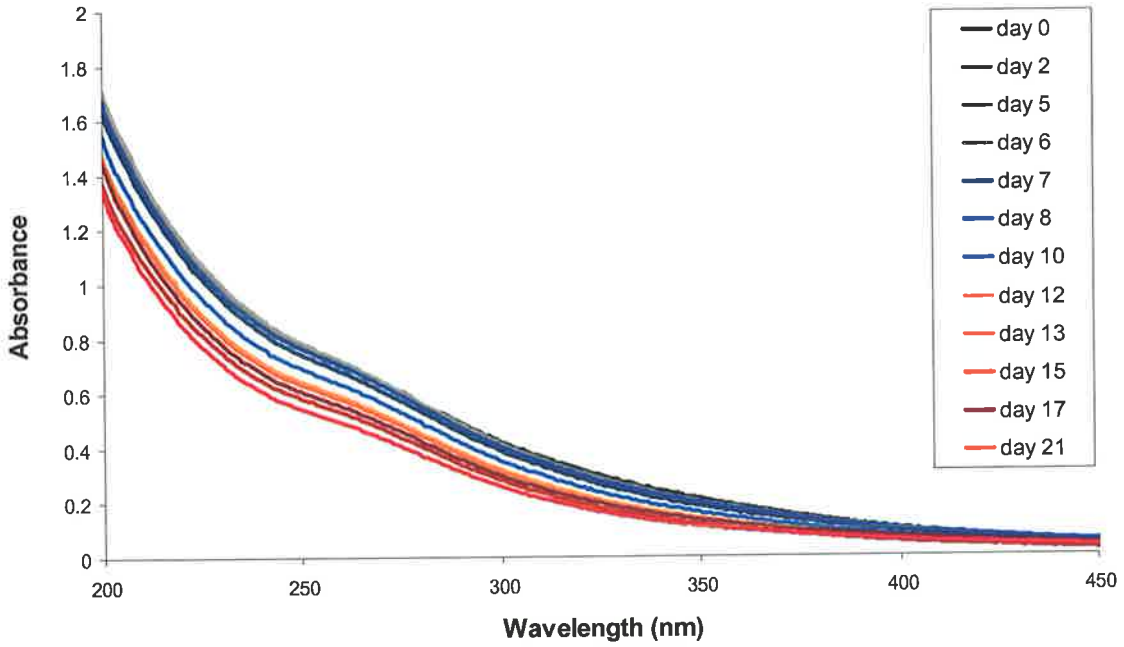
Figure 5. 24: Linear fits of the relationship between bacteria numbers and DOC concentration of all incubations. Correlation coefficient for UV-B was R=0.6 and dark R=0.3.

During the incubation of winter bacterial populations, no significant differences were found in the daily DOC levels between UV-B and dark incubations, in either S1 or D2 samples (data not shown) (T-test, $p>0.05$). Averaged DOC concentrations from S1 and D2 samples gave further indication that UV-B had no significant effect on the internal DOC loading from winter bacterial populations. Furthermore, no relationship was found between winter bacterial numbers and DOC concentration when correlation and regression analyses were performed ($R=0.4$, $r^2=0.1$).

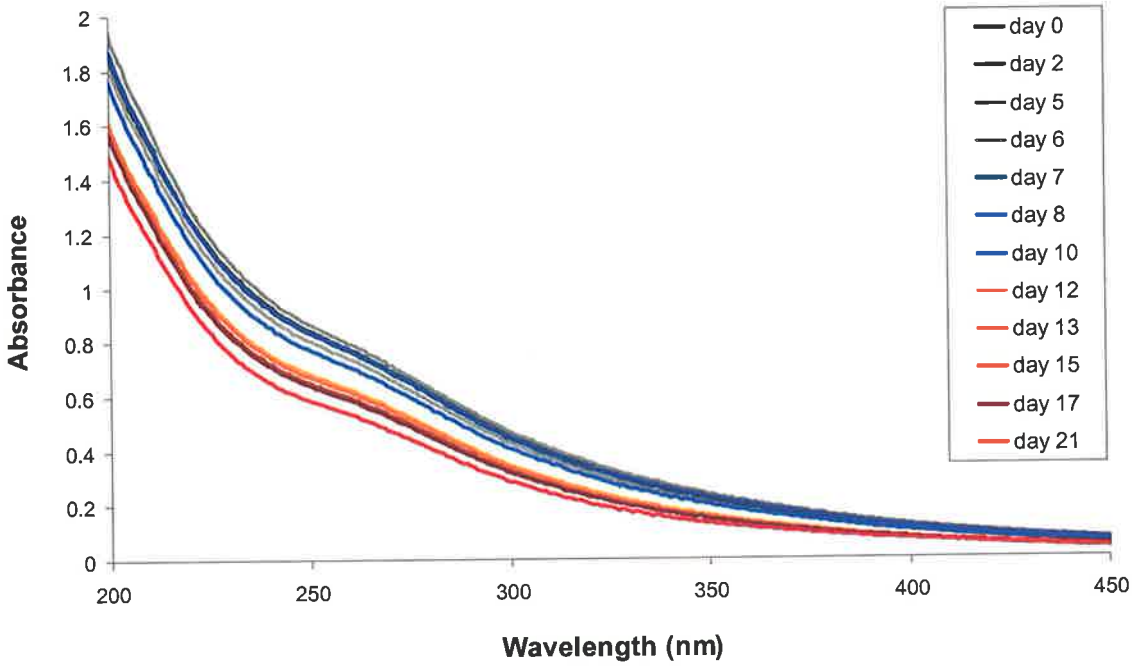
5.4.4.2 Changes in the absorbance spectra of DOC

UV-Vis absorbances were measured during the 21-day incubation period and results are summarised in Figure 5.25. Exposure of summer bacterial populations from S1 and D2 reservoir sites to a longer period of UV-B irradiation resulted in decreasing absorbances in the range of 200 to 450nm. Compared to the absorbances at T_0, the highest decreases were found

after 21 days of incubation, measuring 37±1% ($p<0.05$) in the shallow-S1 samples and 35±1% ($p<0.05$) in the deep-D2 samples. Conversely, S1 and D2 samples that were incubated in the dark showed no obvious difference in absorbance data (data not shown).



(A) S1 site

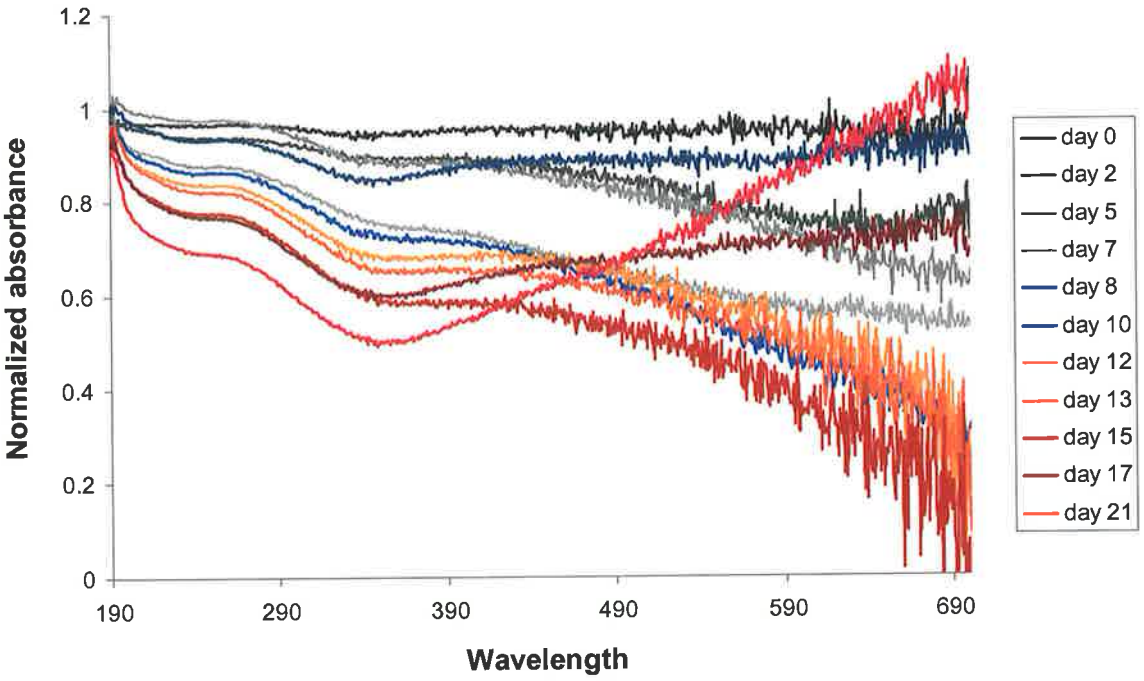


(B) D2 sample

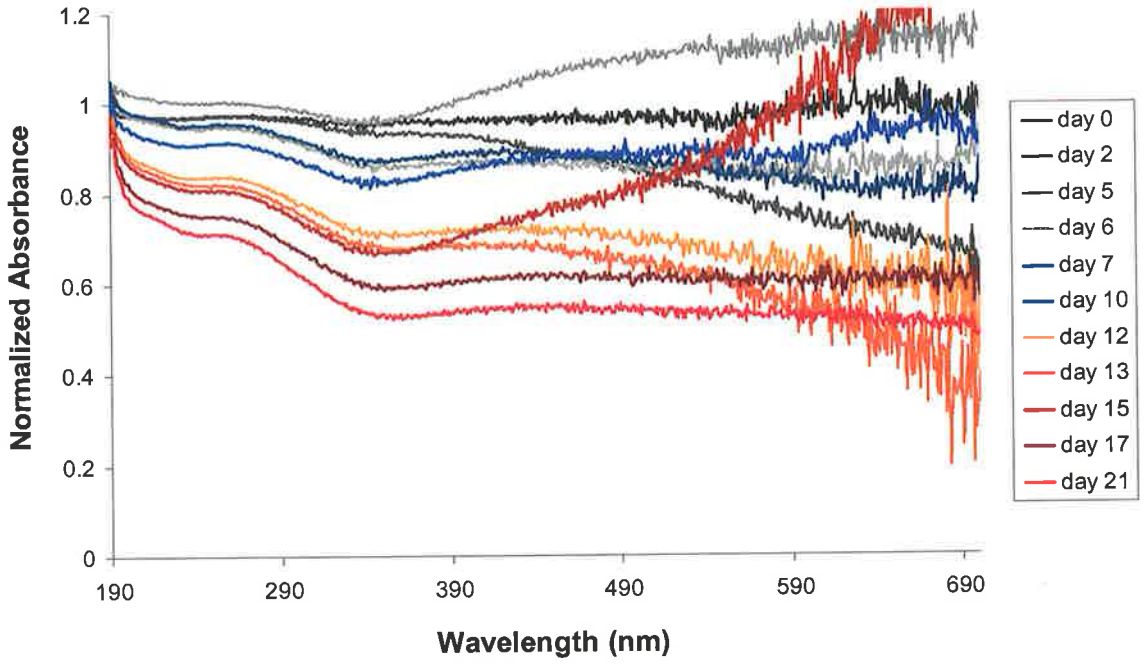
Figure 5. 25: Changes in absorbing properties of (A) shallow-S1 and (B) deep-D2 summer reservoir water samples following 21 days of incubating bacteria under UV-B radiation.

Absorbance spectra of all UV-B treated samples were normalised against the controls (dark) to highlight any changes in the spectra due to increasing irradiation times (Figure 5.26). Incubation times prior to 17 days showed a continuous loss of absorbance in the wavelength region of 200-700nm in the S1 samples. Increased irradiation times (17 and 21 days) resulted in a preferential loss of absorbance in the wavelength region of 200-490nm. The loss of compounds that absorb in the wavelength range of 200-490nm was accompanied by an increase in the amount of compounds that absorb in the range of 490-700nm.

D2 samples incubated for periods less than 13 days showed a continuous loss in absorbance in the wavelength region of 200-700nm. While there was no change in absorbance for 17 and 21 day irradiation times beyond 360nm, increase in absorbance between 360-700nm was recorded following 15 days of UV-B irradiation.



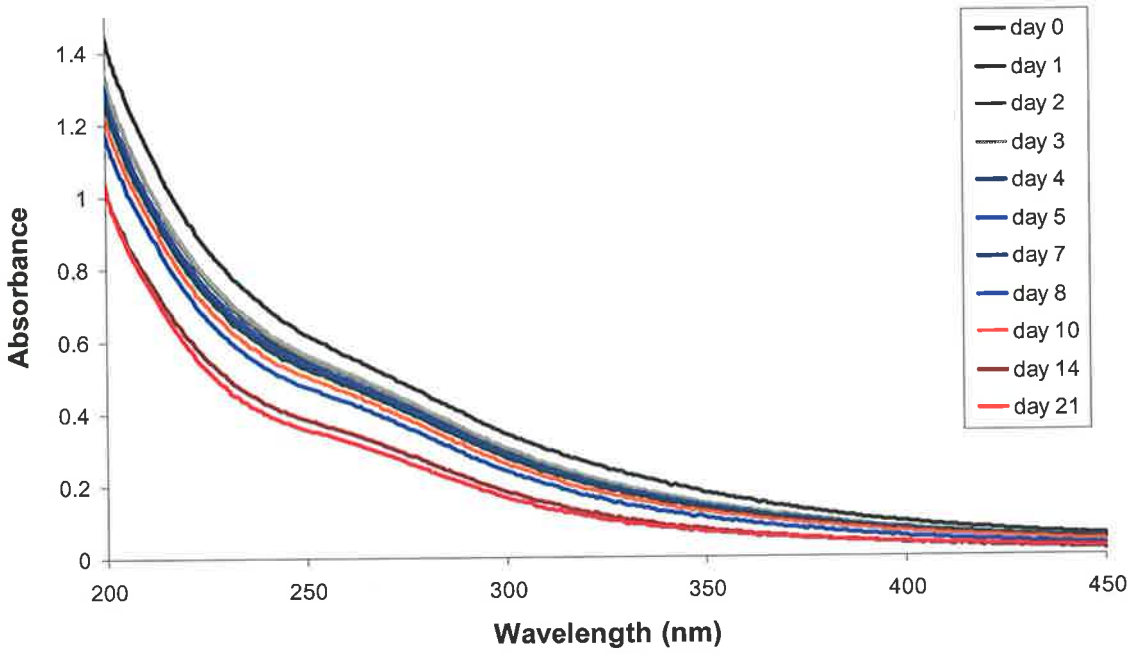
(A) Shallow samples



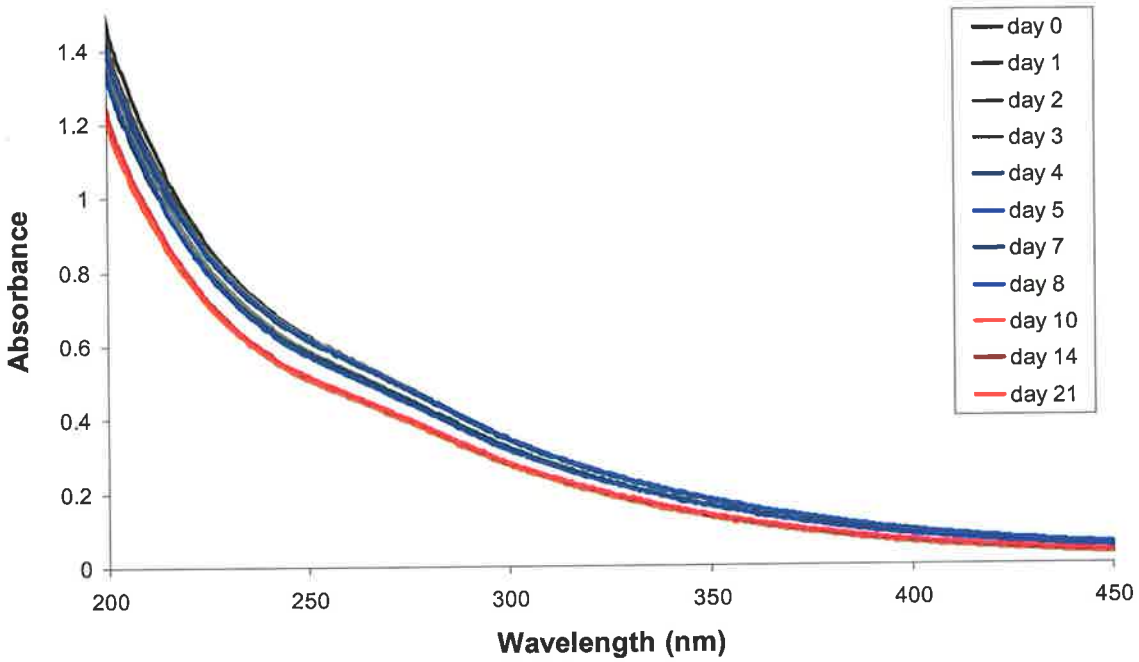
(B) Deep samples

Figure 5. 26: Normalised absorbance spectra ($A_{UV\text{-}B}/A_{Dark}$) for samples irradiated with UV-B for different time periods during the 21 day incubation.

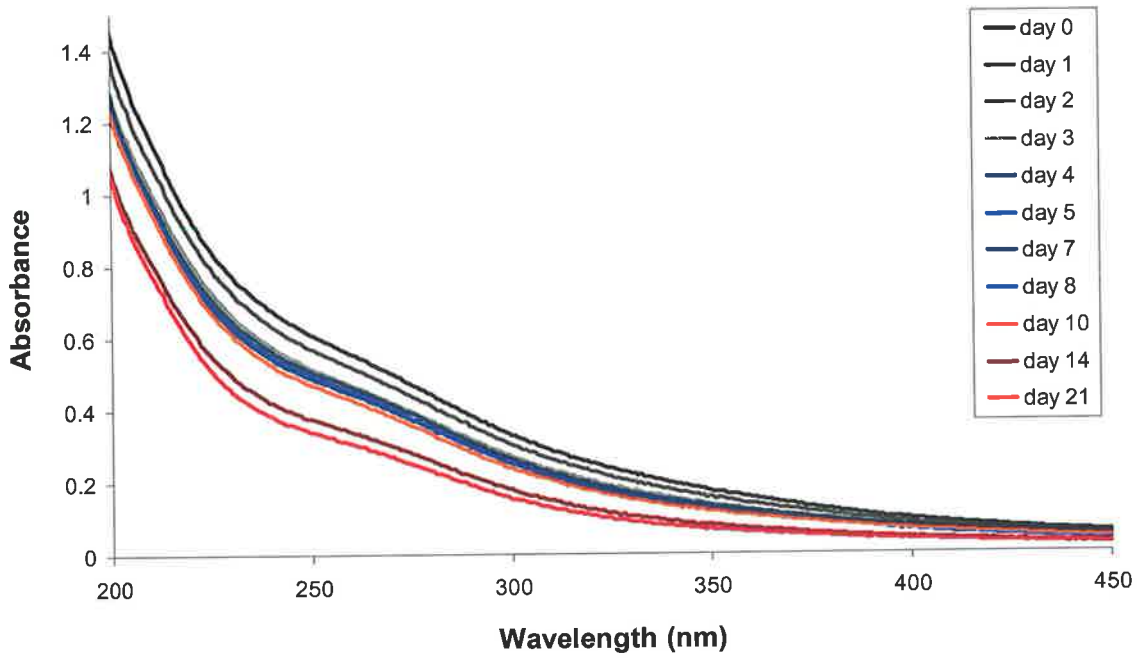
During the 21 day incubation period of winter reservoir samples, decreasing absorbances, particularly in the range of 200-450nm, were found with increasing incubation times in all samples (incubated under both UV-B radiation and in the dark), however higher decreases in absorbance were found under UV-B irradiation when compared to the dark samples (Figure 5.27). After 21 days of incubation and when highest decreases in absorbance were measured, decreases in absorbance in UV-B exposed S1 samples in the 200-400nm range were between 10±2% to 62±3% (mean decrease of 51±1%), whereas in the dark S1 samples, decreases were measured between 4±1% and 28±2% (mean decrease of 22±0.4%). In UV-B exposed D2 samples, absorbances decreased between 9±0.7% and 63±6% (mean decrease of 52±1%) after 21 days of incubation, whereas in the dark D2 samples, decreases were measured between 4±0.2% and 34±0.6% (mean decrease of 26±0.4%). After 21 days of incubation ANOVA showed that changes in absorbance following both UV-B and dark incubations were significant in all samples when compared to the absorbance at T_0 ($p<0.05$).



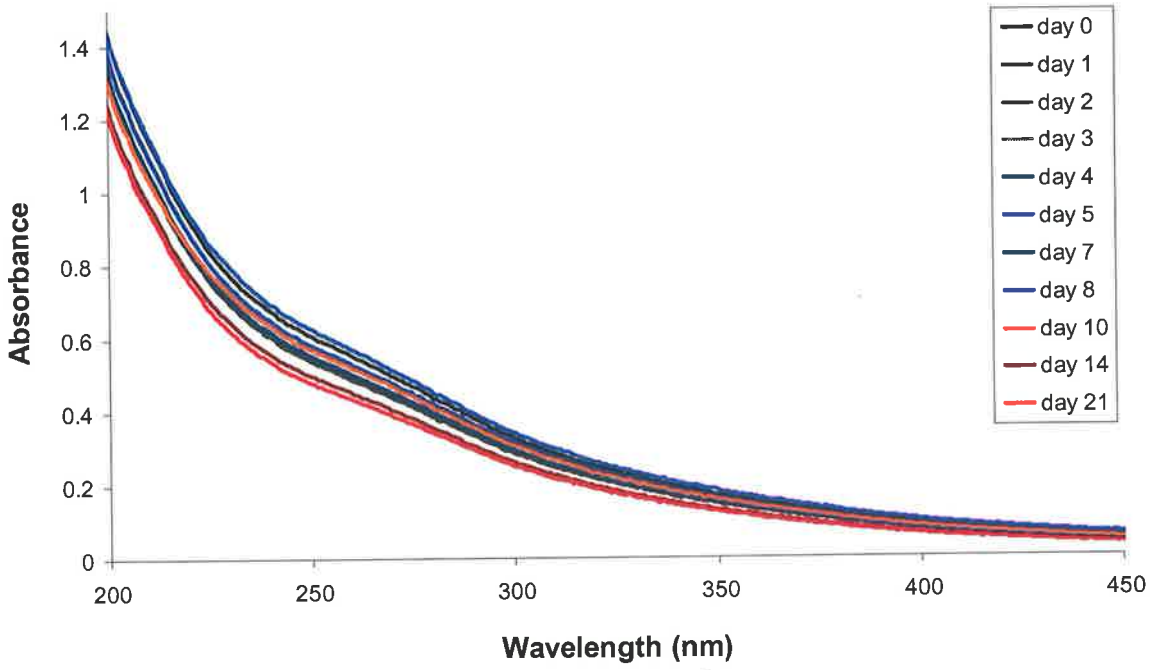
(A) S1 samples under UV-B radiation



(B) S1 sample under dark conditions



(C) D2 sample under UV-B radiation



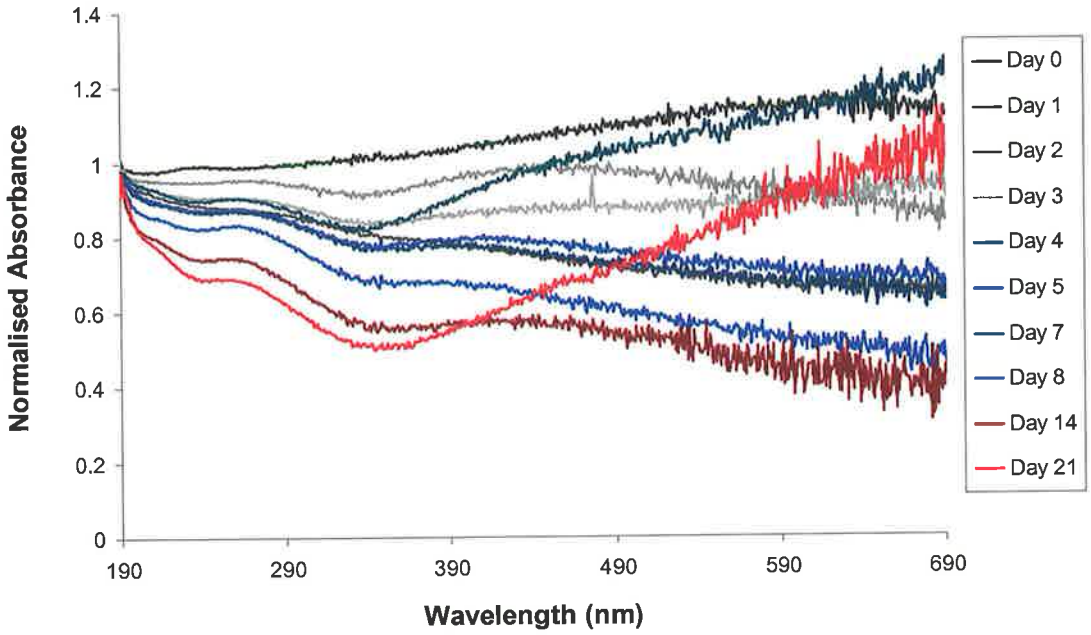
(D) D2 sample under Dark conditions

Figure 5. 27: Changes in absorbing properties of shallow-S1 and deep-D2 winter reservoir water samples following 21 days of incubating bacteria under UV-B radiation and in the dark.

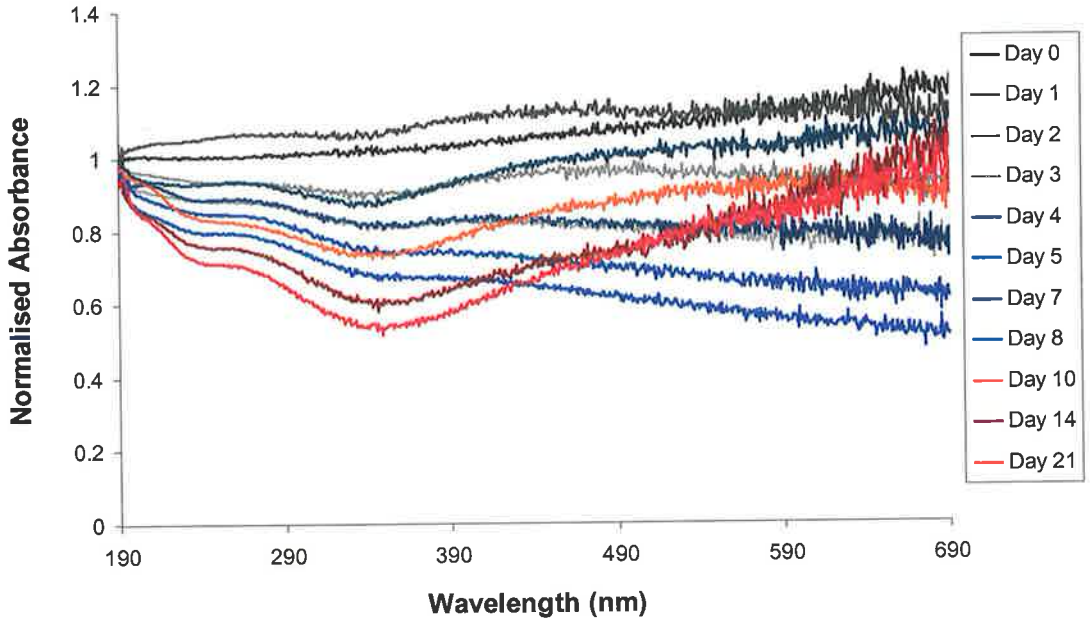
Absorbance spectra of all UV-B treated samples were normalised against the controls (dark samples) ($A_{UV\text{-}B}$ /A_{DARK}) to highlight changes in the spectra due to increasing irradiation times (Figure 5.28).

Decreases in absorbances were observed at all wavelengths with increasing UV-B irradiation times. S1 samples showed a continuous decrease in absorbance at all wavelengths except in samples collected on days 7 and 21, where a preferential loss of absorbance occurred only at the lower wavelength range (200-350nm). The loss of compounds that absorb in the wavelength range of 200-350nm was accompanied with an apparent increase in compounds that absorb in the range of 350-700nm.

Depending on the time of sampling, D2 samples showed an increasing loss of absorbance with increasing wavelength, or a preferential loss of absorbance in the wavelength range of 200-350nm followed by increasing absorbance with increasing wavelength.



(A) Shallow samples



(B) Deep samples

Figure 5. 28: Normalised absorbance spectra ($A_{UV\text{-}B}$ / A_{Dark}) of shallow-S1 and deep-D2 reservoir samples irradiated with UV-B for different time periods during the 21 day incubation.

Absorbances and absorbance ratios used for DOC characterisation during the summer incubations are listed in Table 5.1. 21 days of UV-B irradiation during summer incubations caused reductions in absorbances at various wavelengths between 250 and 465nm, and an increase in E_2/E_3 absorbance ratio, in both shallow and deep samples. At 665nm the decreases in absorbance were no longer observed (Table 5.1).

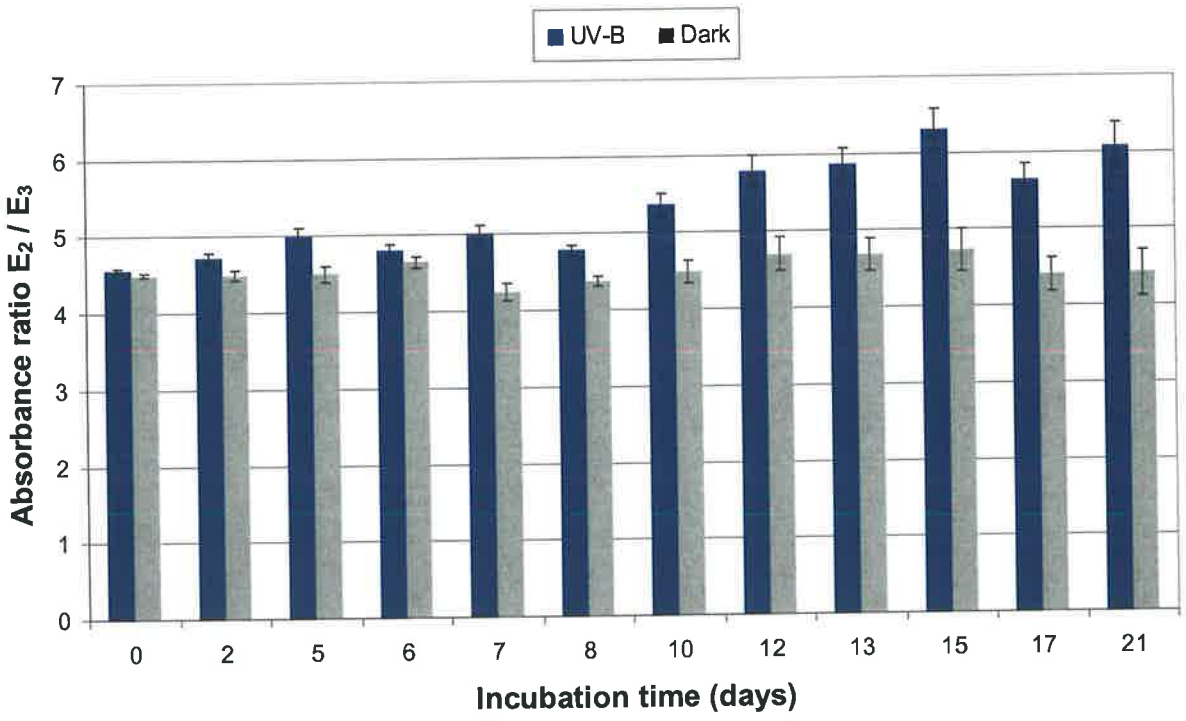
No significant changes were detected in absorbance spectra of summer samples incubated under dark conditions (data not shown).

Table 5. 1: Mean absorbances of shallow-S1 and deep-D2 summer reservoir samples prior to and post 21 days of UV-B treatment. Shown are % increases and decreases in absorbances after 21 days of incubation in comparison to the original samples, with pooled estimators of standard deviation of single measurements. Significant changes are highlighted.

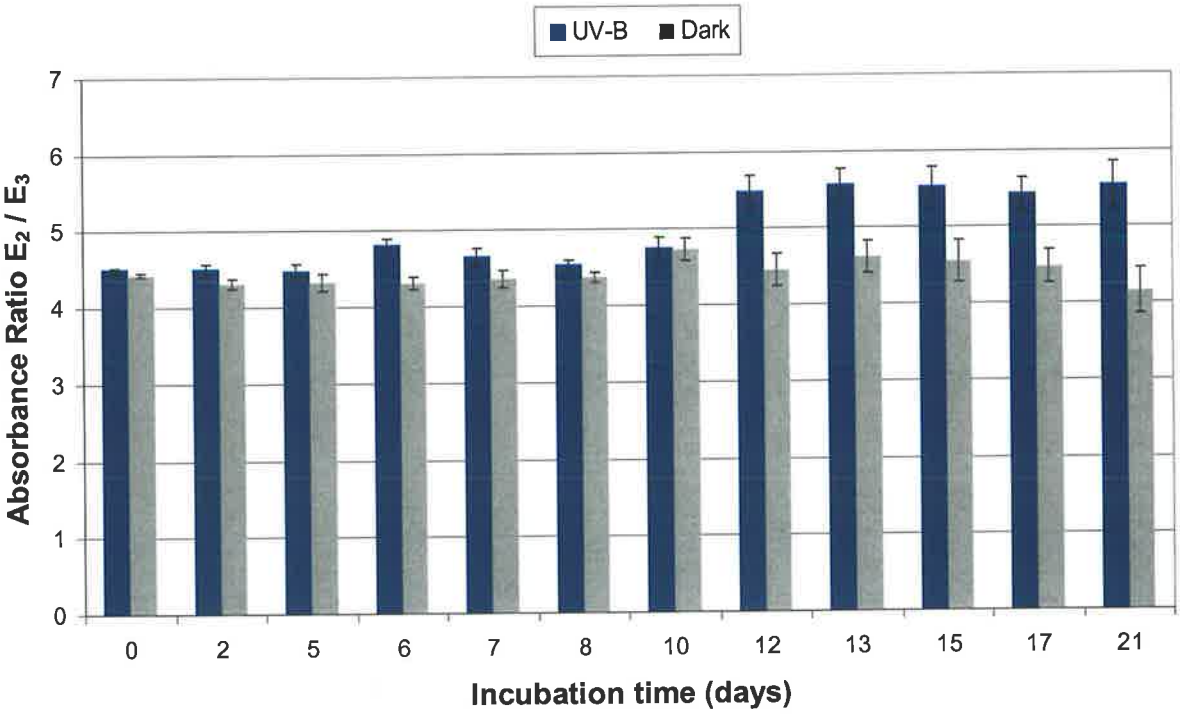
	Shallow-S1			***Deep-D2***		
Wavelength	***0 days***	***21 days***	***%inc/dec***	***0 days***	***21 days***	***%inc/dec***
250nm (E_2)[1,3,4,9]	0.784	0.538	***-31±1***	0.840	0.582	***-30±1***
254nm[5,7]	0.753	0.522	***-31±1***	0.809	0.565	***-30±1***
272nm[1]	0.628	0.422	***-33±2***	0.676	0.463	***-32±2***
280nm[1]	0.560	0.367	***-34±2***	0.605	0.402	***-34±2***
365nm (E_3)[3,8,9]	0.172	0.090	***-48±2***	0.187	0.106	***-43±2***
465nm (E_4)[1,6]	0.045	0.030	***-33±4***	0.051	0.035	***-31±4***
665nm (E_6)[3,6]	0.012	0.012	*0*	0.013	0.013	*0*
E_2 / E_3 [2,3,8,9]	4.6±0.02	6.0±0.2	***+25±1%***	4.5±0.02	5.5±0.2	***+19±1%***

References: (1) Hautala et al. 2000 (2) Reitner et al. 1997 (3) Lindell et al. 1995 (4) V-Balogh and Voros 1997 (5) Andersen et al. 2000 (6) Strobel et al. 2001 (7) Goel et al. 1995 (8) Graneli et al. 1998 (9) Lindstrom 2000

In the presence of UV-B radiation, E_2/E_3 ratios increased by 25±1% (S1) and 19±1% (D2) after 21 days (Table 5.1). A more detailed daily analysis of the changes in E_2/E_3 absorbance ratios during 21 days of incubation is summarised in Figure 5.29. During the 21 days of incubation, E_2/E_3 absorbance ratios of dark samples did not show much variation, with a mean value of 4.5 ±0.1 and 4.4±0.1 for S1 and D2 samples respectively. Conversely, the absorbance ratio for UV-B incubated S1 and D2 samples started to increase after 2 and 6 days of incubation respectively. Highest E_2/E_3 ratios in shallow samples were found after 15 days of UV-B irradiation (6.3±0.3, increase of 28% from day 1), and in deep samples it was measured on day 13 (5.6±0.2, increase of 19% from day1).



(A) Shallow-S1 summer samples



(B) Deep-D2 summer samples

Figure 5. 29: Changes in E_2/E_3 absorbance ratios for summer (A) shallow and (B) deep reservoir samples incubated under UV-B radiation and in the dark for up to 21 days.

E_2/E_3 absorbance ratios of all UV-B treated summer samples were normalised against those of the controls (dark) to highlight changes in the ratios due to increasing irradiation times (Figure 5.30). This analysis shows a trend of increasing absorbance ratio in both S1 and D2 samples with increasing irradiation times.

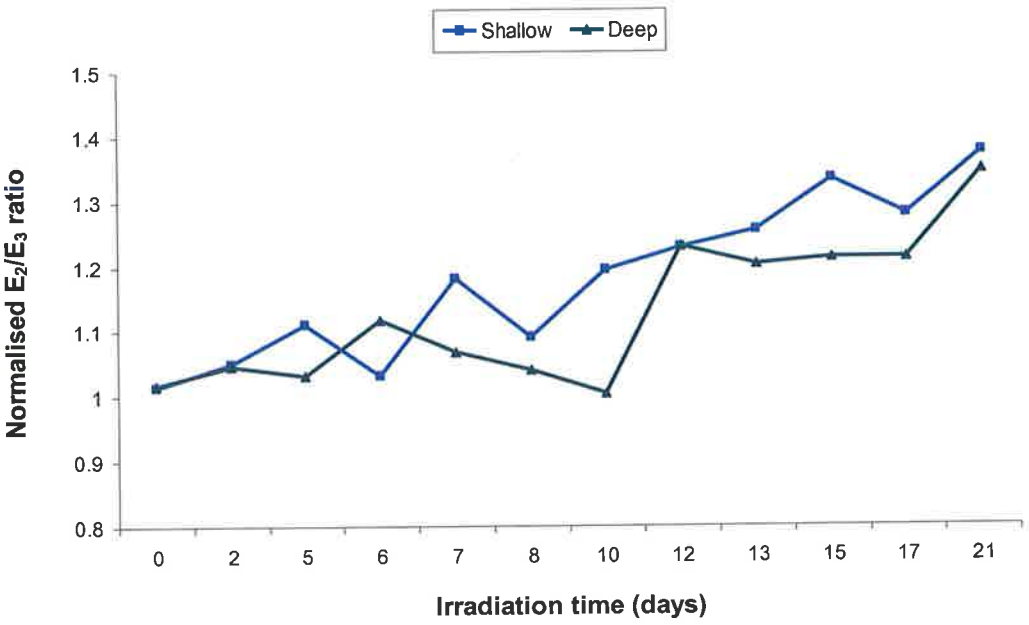


Figure 5. 30: Normalised E_2/E_3 absorbance ratios of shallow-S1 and deep-D2 summer reservoir samples incubated under UV-B radiation for periods up to 21 days, with respect to the E_2/E_3 absorbance ratios of the dark samples ($A_{UV\text{-}B}/A_{Dark}$).

Absorbances and absorbance ratios used for the characterisation of DOC during the winter study are listed in Table 5.2. Significant losses of absorbance were measured after 21 days of UV-B irradiation between of 200 and 700nm (ANOVA at α=0.05, p<=0.05). Dark incubations also showed significant decreases in absorbance for the entire spectrum, although these decreases were lower than those observed for UV-B incubations (Table 5.2 A and B).

Table 5. 2: Mean absorbances of shallow-S1 and deep-D2 summer reservoir samples prior to and post 21 days of incubation under (A) UV-B radiation and (B) Dark. Shown are % increases and decreases in absorbances after 21 days of incubation in comparison to the original samples, with pooled estimators of standard deviation of single measurements. Significant changes, as determined using a t-test are highlighted.

(A) UV-B incubation

	SHALLOW			DEEP		
Wavelength	**0 days**	**21 days**	**%inc/dec**	**0 days**	**21 days**	**%inc/dec**
250nm (E_2)	0.620	0.356	**-43±5**	0.611	0.340	**-44±5**
254nm	0.595	0.343	**-42±5**	0.588	0.327	**-44±5**
272nm	0.495	0.276	**-44±5**	0.489	0.262	**-46±5**
280nm	0.449	0.241	**-46±5**	0.439	0.229	**-48±5**
365nm (E_3)	0.147	0.055	**-62±7**	0.145	0.054	**-63±7**
465nm (E_4)	0.051	0.020	**-60±5**	0.051	0.021	**-60±5**
665nm (E_6)	0.019	0.007	**-62±5**	0.019	0.007	**-60±5**
E_2 / E_3	4.3±0.2	6.5±0.3	**35±8**	4.2±0.1	6.3±0.2	**33±8**

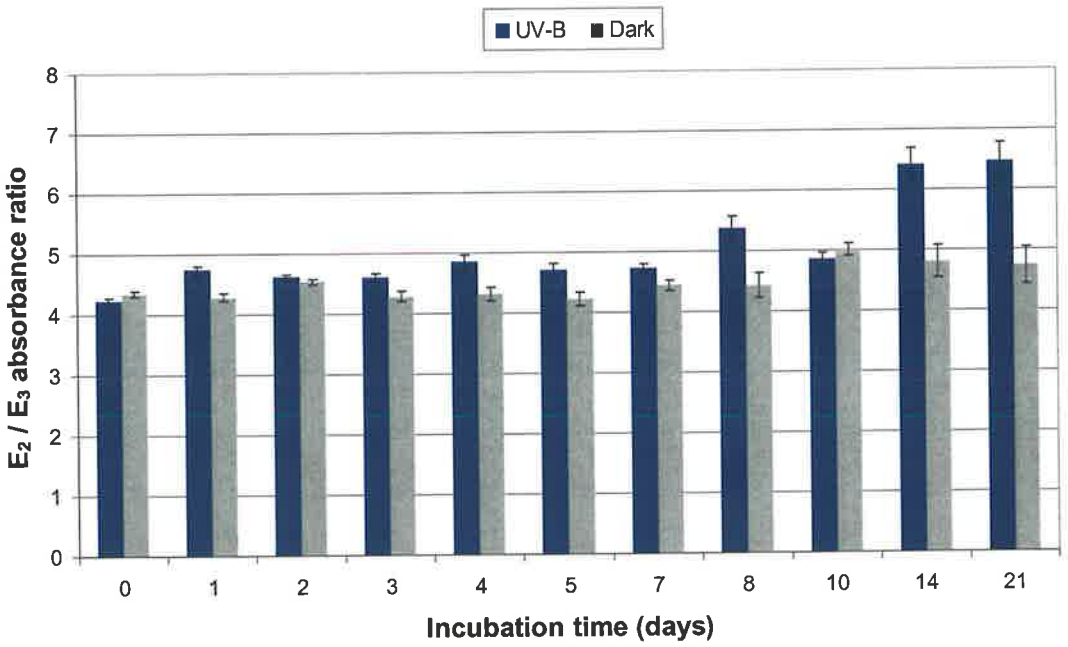
(B) Dark incubation

	SHALLOW			DEEP		
Wavelength	**0 days**	**21 days**	**%inc/dec**	**0 days**	**21 days**	**%inc/dec**
250nm (E_2)	0.627	0.517	**-18±5**	0.608	0.479	**-21±5**
254nm	0.603	0.497	**-18±5**	0.585	0.462	**-21±5**
272nm	0.502	0.412	**-18±5**	0.485	0.380	**-22±5**
280nm	0.452	0.372	**-18±5**	0.435	0.339	**-22±5**
365nm (E_3)	0.145	0.109	**-24±7**	0.142	0.099	**-30±7**
465nm (E_4)	0.047	0.030	**-36±5**	0.048	0.030	**-38±5**
665nm (E_6)	0.016	0.008	**-53±5**	0.016	0.008	**-51±5**
E_2 / E_3	4.3±0.1	4.7±0.1	*8.3±8*	4.3±0.1	4.8±0.1	*11±8*

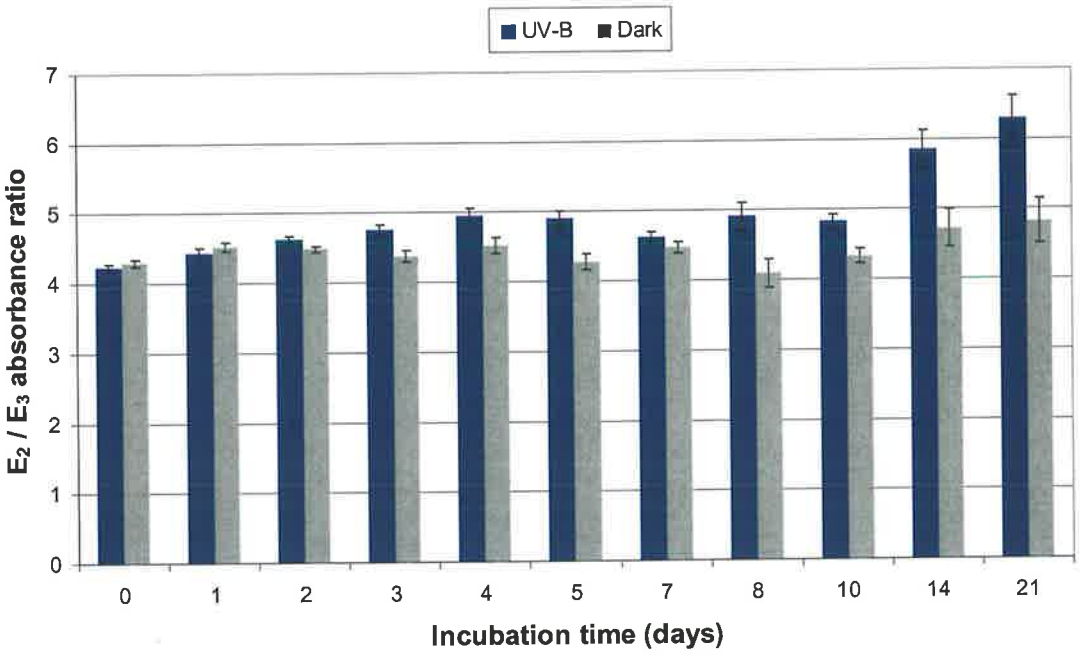
During the 21 day incubation of winter bacterial populations, E_2/E_3 ratios increased in the presence of UV-B radiation by 35% (S1) and 33% (D2), while no significant increase was measured in the dark incubations (Table 5.2). Daily variations in E_2/E_3 ratios between UV-B and Dark incubations are summarised in Figure 5.31.

During the 21 day incubation period, E_2/E_3 absorbance ratios in UV-B exposed S1 samples were generally higher than those in the dark samples (with the exception of days 2 and 10) (mean increase of 18±4%). The highest increase of 35±8% was recorded after 21 days of incubation (Table 5.2).

Following two days of incubation, higher E_2/E_3 absorbance ratios were also found in UV-B irradiated D2 samples when compared to the dark samples (mean increase of 14±3%). The highest increase of 33±8% was found after 21 days of incubation (Table 5.2).



(A) Shallow-S1 site



(B) Deep-D2 site

Figure 5. 31: Changes in E_2/E_3 absorbance ratios for winter (A) shallow and (B) deep reservoir samples incubated under UV-B radiation and in the dark for up to 21 days.

E_2/E_3 absorbance ratios of all UV-B treated winter samples were normalised against those of the controls (dark) to highlight changes in the ratios due to increasing irradiation times (Figure 5.32). This shows a trend of increasing absorbance ratio in both S1 and D2 samples with increasing irradiation times, where data from days 7 and 10 are not included.

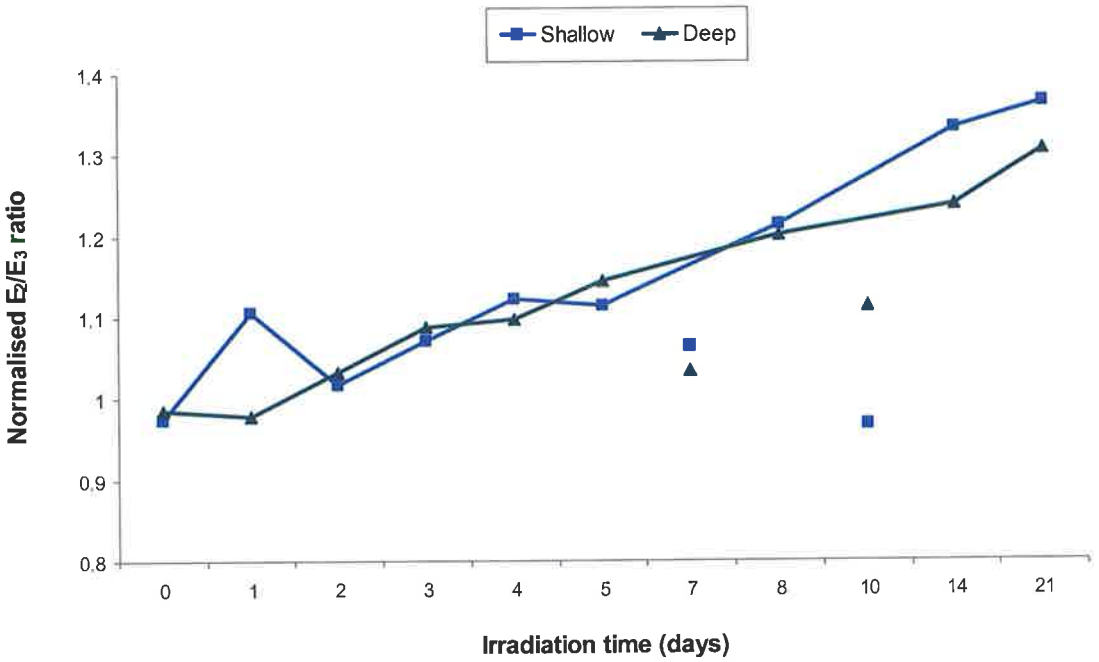


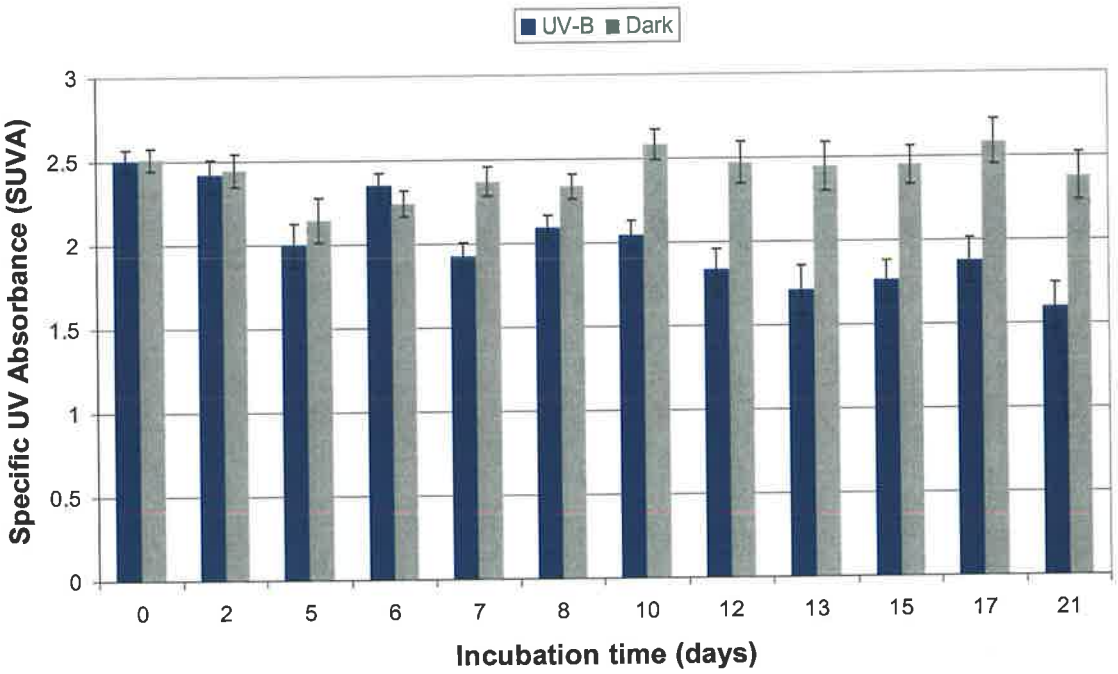
Figure 5. 32: Normalised E_2/E_3 absorbance ratios of shallow-S1 and deep-D2 winter reservoir samples incubated under UV-B radiation for periods up to 21 days, with respect to the E_2/E_3 absorbance ratios of the dark samples ($A_{UV\text{-}B}/A_{Dark}$). Data from days 7 and 10 are not included here for trend evaluation.

5.4.4.3 Changes in Specific UV Absorbance (SUVA)

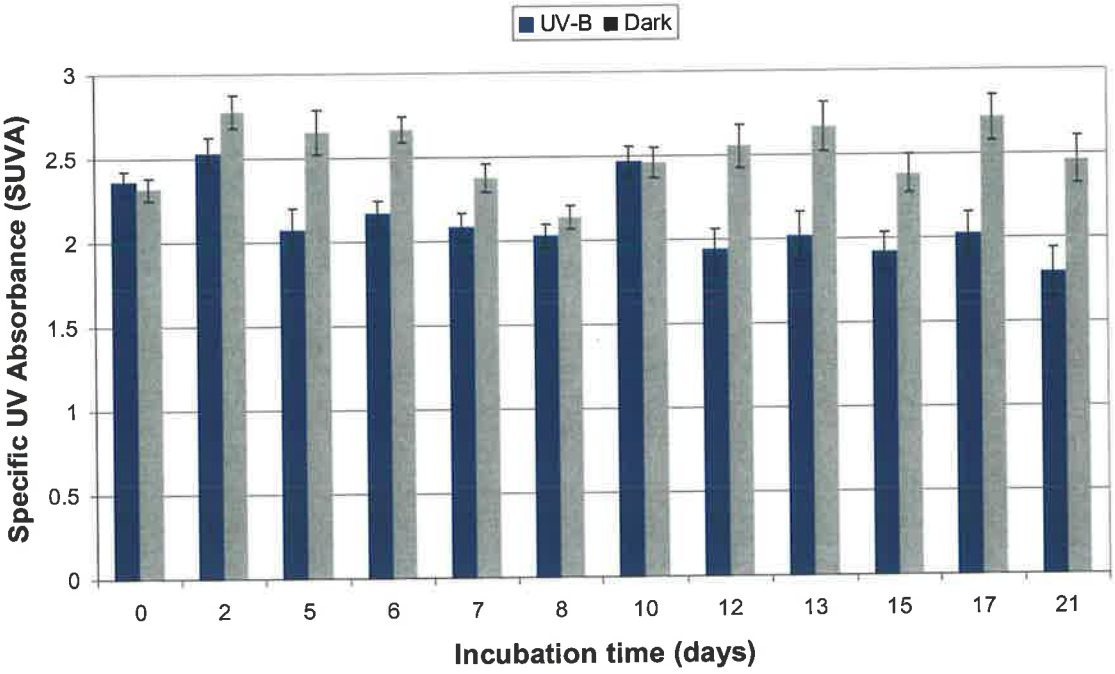
Data of SUVA during summer incubations are summarised in Figure 5.33. Significant variations in SUVA were observed in S1 and D2 samples incubated under both UV-B radiation and in the dark. Following 5 days of UV-B irradiation (with an exception of day 6), significant decreases in SUVA (mean ±S.E. 35±4%) were observed in S1 samples compared to T_0, with a highest decrease of 57% measured after 21 days of irradiation. Furthermore, following 7 days of incubation, significantly lower SUVA values were found in UV-B irradiated samples when compared to dark samples (mean decrease ± S.E, 33±4%). S1 samples incubated in the dark showed no significant change in SUVA during the 21 day incubation.

Following 5 days of UV-B irradiation (and with the exception of day 10) significant decreases in SUVA (mean ±S.E. 18±2%) were observed in D2 samples compared to T_0, with the highest decrease of 32% measured after 21 days of irradiation (Figure 5.33). Furthermore,

following 5 days of incubation (and with the exception of days 8 and 10), significantly lower SUVA values were found in UV-B irradiated samples compared to dark samples (mean decrease ± S.E, 24±4%). D2 samples incubated in the dark also showed significant variations in SUVA values during the 21 day incubation period. Following 2 days of dark incubations, higher SUVA values were observed throughout the 21 day incubation (with the exception of day 8) when compared to T_0 (mean increase of 10±2%).



(A) Shallow-S1 site



(B) Deep-D2 site

*Figure 5. 33: SUVA ($(A_{254}$*100%)/DOC mg/L) of (A) shallow-S1 and (B) deep-D2 summer reservoir samples incubated under UV-B radiation and in the dark, for up to 21 days.*

SUVA values of all UV-B treated summer samples were normalised against those of the controls (dark) to highlight changes in SUVA due to increasing irradiation times (Figure 5.34). Although fluctuations in SUVA occurred in both S1 and D2 samples during the 21 day incubation, compared to T_0, Figure 5.34 shows a general trend of decreased SUVA as a result of UV-B irradiation. Decreased SUVA values caused by UV-B radiation indicate a removal of compounds that absorb light at 254nm, suggesting decreased aromaticity due to UV-B irradiation.

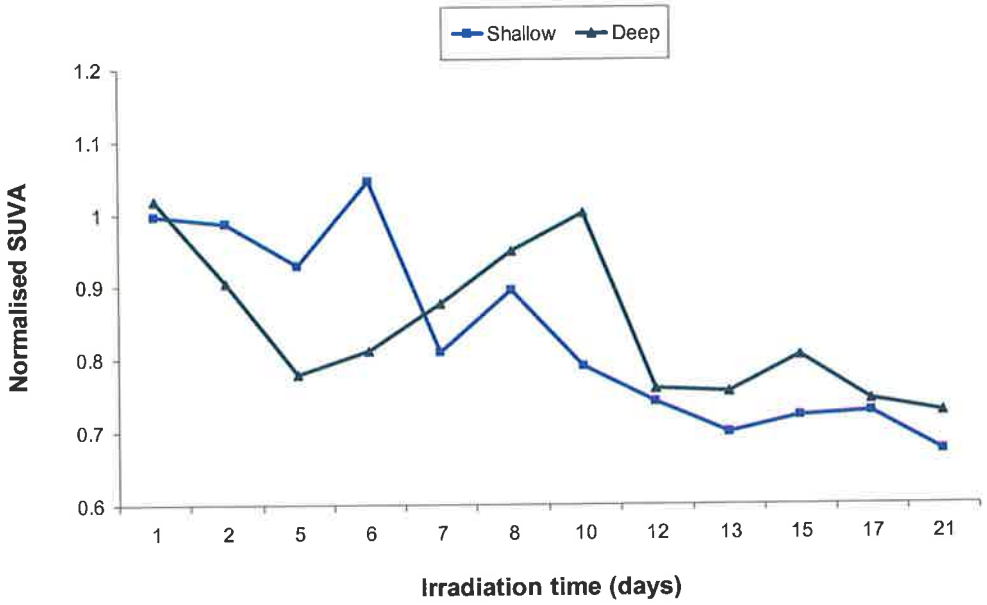
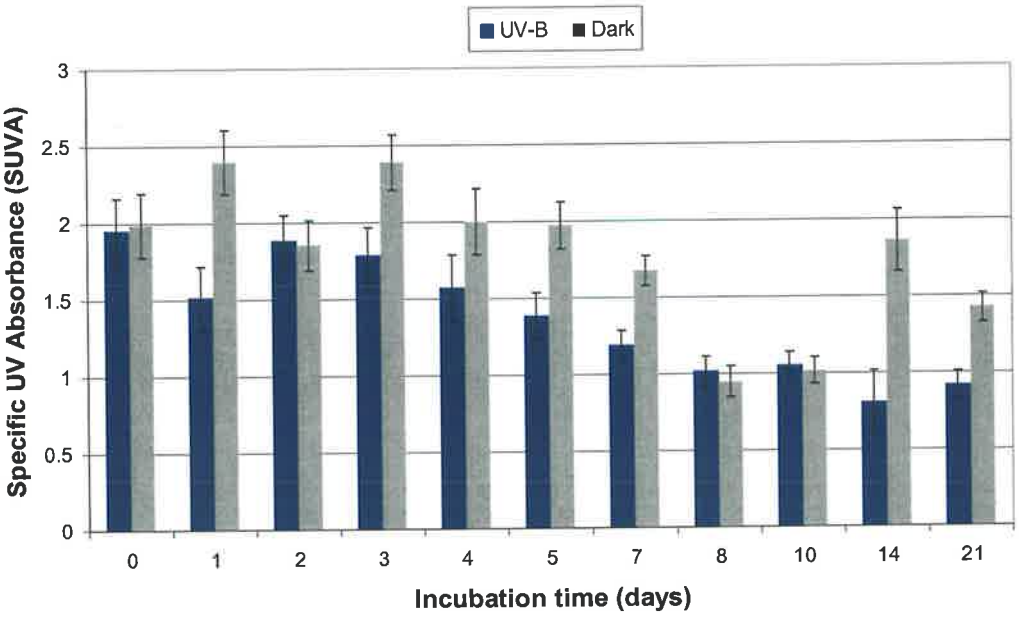


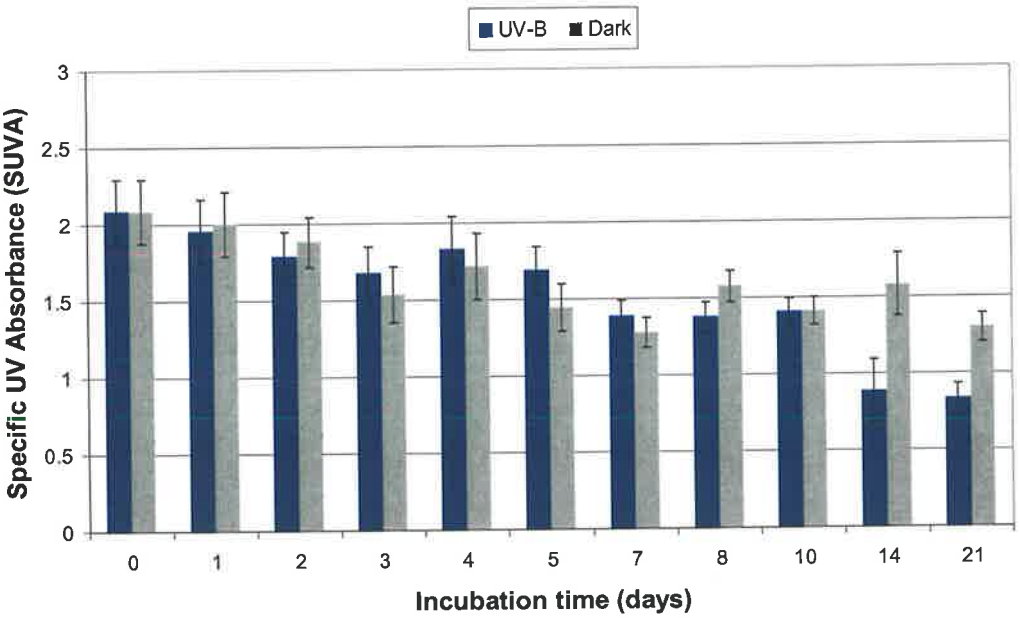
Figure 5. 34: Normalised SUVA of shallow-S1 and deep-D2 summer reservoir samples incubated under UV-B radiation for periods up to 21 days, with respect to SUVA of the dark samples ($SUVA_{UV\text{-}B}/SUVA_{Dark}$).

Significant variations in SUVA were observed in S1 and D2 winter samples incubated under both UV-B and dark conditions (Figure 5.35). Following 5 days of UV-B irradiation, SUVA values of S1 samples decreased with respect to T_0 (mean decrease of 29±4%), with a maximum decrease of 58% observed after 14 days of incubation. Furthermore, following 1 day of irradiation (and with the exception of days 2, 8 and 10), SUVA values were significantly lower in UV-B irradiated samples when compared to those of the dark samples (mean decrease ± S.E. 34±4%). S1 samples incubated in the dark for 8 days and 10 days also showed significant decreases in SUVA when compared to T_0 (mean decrease ± S.E. 50±8%), while no significant differences were measured in these dark incubations at other times.

Following 7 days of UV-B irradiation, SUVA values of D2 samples decreased with respect to T_0 (mean decrease of 34±7%), with a maximum decrease of 63% observed after 21 days of incubation (Figure 5.35). Furthermore, SUVA values were significantly lower in UV-B irradiated samples when compared to those of the dark samples only following 14 days of incubation (mean decrease ± S.E. 48±13%). Following 5 days of dark incubations, D2 samples showed significant decreases in SUVA when compared to T_0 (mean decrease ± S.E. 47±6%).



(A) Shallow-S1 site



(B) Deep-D2 site

Figure 5. 35: SUVA ($m^{-1}mg^{-1}L$) of (A) shallow-S1 and (B) deep-D2 winter reservoir samples incubated under UV-B radiation and in the dark, for up to 21 days.

SUVA of UV-B exposed samples was normalised against those of dark samples, highlighting changes caused by UV-B irradiation (Figure 5.36). Whilst fluctuations in SUVA occurred with respect to T_0, UV-B irradiation of shallow-S1 winter samples generally caused

reductions in SUVA. Conversely, UV-B irradiation of D2 samples appeared to have little impact for the first 7 days after which reductions in SUVA occurred to 21-day incubation.

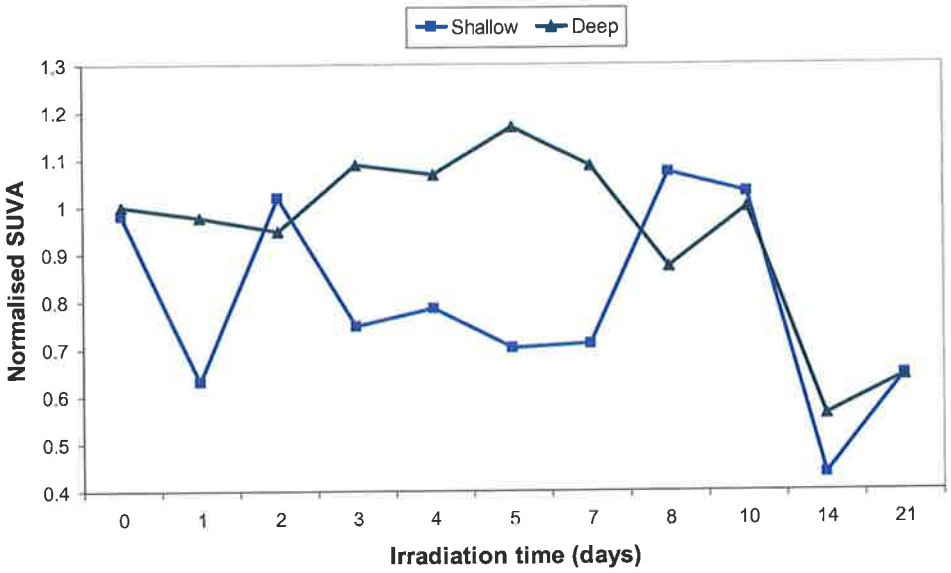
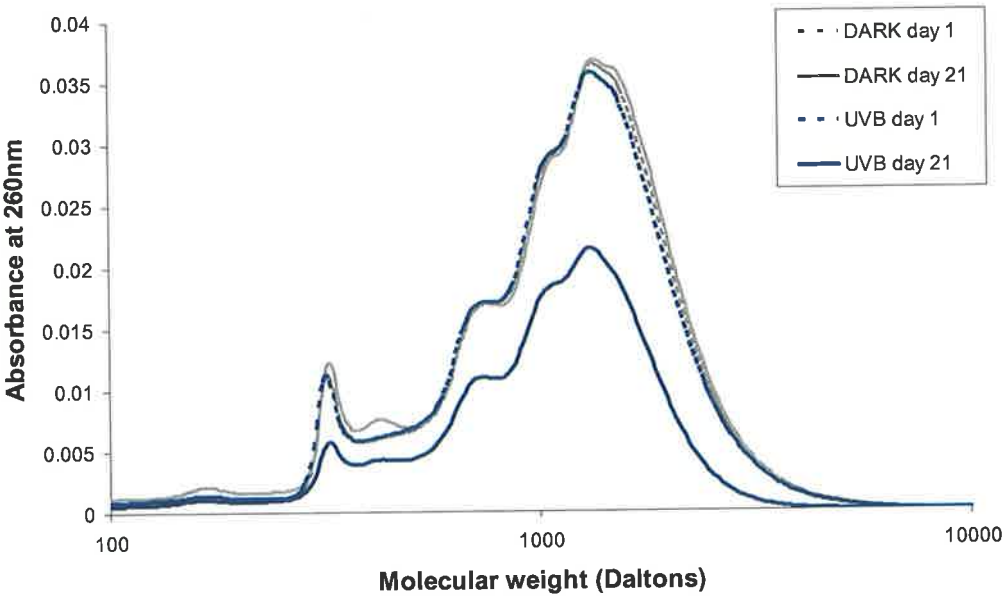


Figure 5. 36: Normalised SUVA of shallow-S1 and deep-D2 winter reservoir samples incubated under UV-B radiation for periods up to 21 days, with respect to SUVA of the dark samples ($SUVA_{UV\text{-}B}/SUVA_{Dark}$).

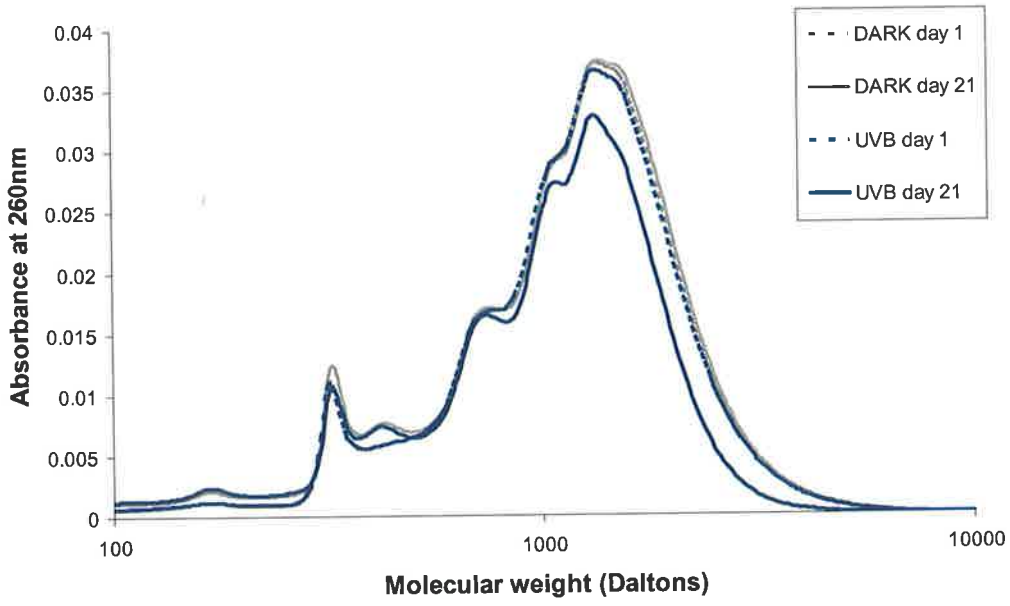
5.4.4.4 Changes in molecular weights of the DOC pool

Changes in DOC molecular size distribution, following UV-B irradiation of reservoir bacterial populations, are summarised in Figure 5.37. No changes in DOC molecular size distribution were observed in samples incubated in the dark. However, after 21 days of UV-B irradiation a significant loss in absorbance of 47±3% and 13±2% in the wavelength range of 300 to 5000 Daltons was observed in S1 and D2 samples. The observed changes in molecular weight distribution were detected with absorbance at 260nm only. As such, the plotted changes to molecular size only reflect the changes to UV-absorbing compounds in the DOC pool. This data supports the absorbance data presented in Section 5.4.4.2, showing significant reductions in absorbance at lower wavelengths (250–400nm) in UV-B irradiated samples, with no changes to dark incubations. Based on this data, UV-B radiation appears to enhance

the removal of UV-absorbing DOC molecules in the molecular weight range of 300 to 5000 Daltons.



(A) S1 sample



(B) Deep sample

Figure 5. 37: The HPSEC chromatograms for (A) shallow-S1 and (B) deep-D2 samples at T_0 and following 21 days of incubation under UV-B radiation and in the dark.

Further molecular weight analysis showed that the average molecular weight of DOC samples decreased with increasing UV-B irradiation times (Figure 5.38), while there was no change in the average molecular weight of the corresponding dark incubations.

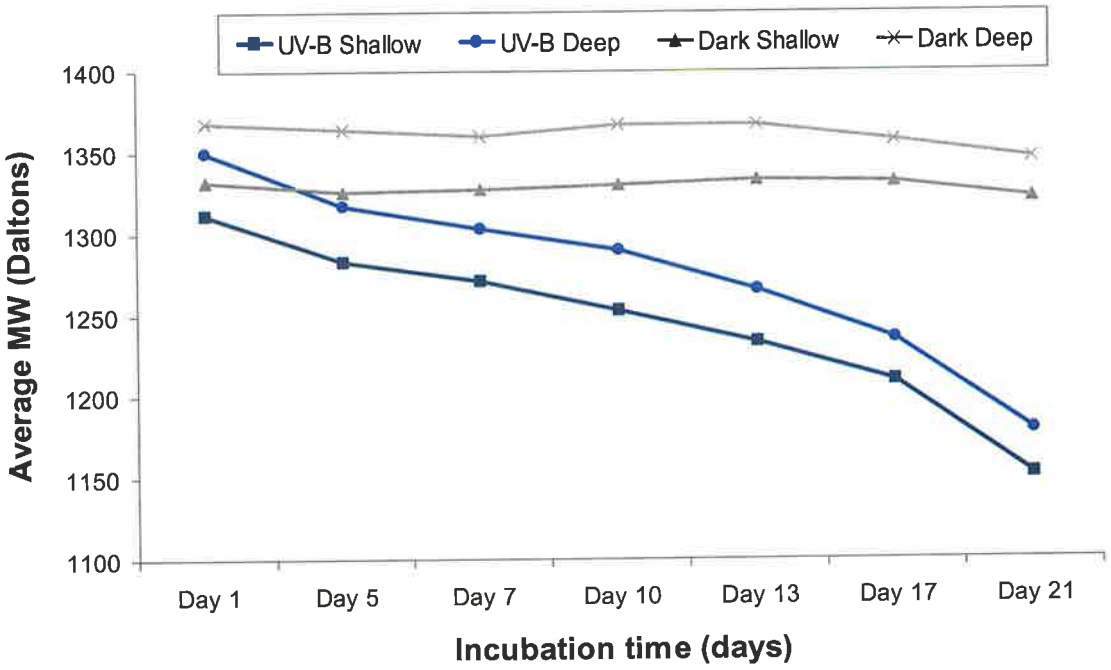


Figure 5. 38: Variations in the average molecular weights (MW) measured by HPSEC for shallow and deep reservoir samples incubated under UV-B radiation and in the dark up to 21 days.

5.5 DISCUSSION

The main aim of this section of the study was to determine if UV-B radiation is likely to inhibit the growth of bacteria in the Warren Reservoir, and how it affects the community structure. Two summer and one winter study showed that UV-B radiation significantly affected the abundance and biomass of bacteria in water collected from the Warren Reservoir, and caused changes to the bacterial community structure.

A direct negative effect of UV-B radiation on bacterial growth was observed during the short-term exposure studies simulating diurnal situations. Bacterial numbers decreased following UV-B exposure periods, suggesting that UV-B radiation reduces bacterial growth, however cell numbers recovered during dark periods to levels higher than those prior to irradiation. Other studies have also shown that UV-B radiation exerts negative effects on bacterial abundance, biomass and productivity (Karentz and Lutze, 1990; Herndl et al., 1993; Ferreyra et al., 1997; Rae and Vincent, 1998; Mostajir et al., 1999). For example, Reitner and Herndl (1997) reported a decline in bacterial activity during UV-B irradiation, and showed that bacteria rapidly recover from previous UV stress in the absence of UV-B radiation, which is in agreement with the findings of this study. Microorganisms have developed several mechanisms to repair DNA lesions that are either induced under solar radiation in the presence of UV-A and PAR (photosynthetically active radiation), or induced in the dark (Maranger et al., 2002; Pausz and Herndl, 2002). DNA repair mechanisms were most likely active during the dark (post-UV exposure) periods, resulting in higher bacterial counts indicative of bacterial recovery. Similar findings were reported by Jeffrey et al. (1996) and Boelen et al. (2002) who studied diel patterns of UV-B radiation induced DNA damage in marine bacteria. These authors reported that DNA damage in bacteria accumulates throughout the day and decreases during the night.

The observed growth recovery during dark periods in this study may also have been aided by a higher internal loading of DOC (shown to occur during the second summer study), generated by an enhanced level of UV-B induced bacterial damage, as well as increased level of DOC bioavailability (which has been shown to occur as a result of exposure of Warren Reservoir DOC to UV-B radiation, Chapter 4) (as suggested by Herndl et al., 1993; Lindell et al., 1995; Ferreyra et al., 1997). Bertilsson and Tranvik (1998) showed that photochemically produced organic molecules such as carboxylic acids can accumulate in sunlight exposed samples, serving as high quality bacterial substrates after mixing into deeper layers, or during

periods of darkness. On the other hand, Reitner et al. (1997) found that direct exposure of bacteria to short term (<4 hours) UV radiation led to reduced bacterial activity even though it was accompanied with an increase in available DOC. These authors suggested that UV-B radiation exerts direct negative effects on bacteria growth (as has been suggested by Ferreyra et al., 1997; Rae and Vincent, 1998; Mostajir et al., 1999) and that the presence of labile substrate alone is not sufficient to enable post-UV recovery, but that sufficient dark periods or periods of low UV-B intensity are required for the recovery of bacterial growth (as suggested by Reitner and Herndl, 1997; Bertilsson and Tranvik, 1998). Whilst these findings are consistent with the changes in bacterial abundance observed during the diurnal study presented in this chapter, they do not explain the findings of the long-term exposure studies where generally higher abundance and biomass were obtained under continuous UV-B irradiation with no UV-B-free periods provided for DNA repair and growth recovery. In fact, very few studies have reported positive effects of UV-B radiation on bacterial populations. For example, a study by Herndl et al. (1993) focused on the short-term effects of UV-B on oceanic bacteria. The authors reported varying responses of bacteria to UV-B radiation, depending on the intensity of UV-B radiation used. A 50% reduction in activity was reported in the presence of UV-B radiation levels of up to $1.3Wm^{-2}$, while increased bacterial activity was reached by using lower UV-B radiation energy ($<0.2Wm^{-2}$). They proposed the lower bacterial activity to be a direct effect of UV-B exposure, whereas the higher bacterial activity was suggested to be an indirect result of UV-B irradiation caused by an increase of organic substances that are readily metabolised. During the study presented in this chapter, UV-B radiation levels of $0.1\pm0.02Wm^{-2}$ were applied, thus the observed increases in bacterial abundance are comparable to those reported by Herndl et al. (1993). Ferreyra et al. (1997) also found similar responses of bacteria to UV-B radiation. Bacterial abundance increased markedly in the presence of low intensity UV-B but decreased in the presence of high UV-B intensity. Their study also reported increased bacterial activity in the presence of UV-B radiation, suggesting the enhanced growth to be an indirect response to UV-B exposure caused by UV-B induced modifications to the DOC pool. Total UV-B intensities used in this study were within the range of those used in other studies.

Mostajir et al. (1999) also examined the effects of long-term (7 days) UV-B exposure on the plankton consortia of an estuary and found bacteria to exhibit two different responses during the week long experiment. Initially (<4 days), bacterial abundance and biomass were lower in the presence of enhanced UV-B radiation, showing direct negative effects of UV-B

exposure. These findings were in support of those obtained during the short-term studies by Herndl et al. (1993) and Ferreyra et al. (1997). However, after 5 days of exposure, cell numbers and biomass increased by 49% and 48%, respectively, in the presence of enhanced UV-B radiation, with higher increases reported under higher UV-B energy levels. These increases in abundance and biomass were primarily associated with decreases in predator numbers and the substrate changes brought about directly by UV-B radiation. This study by Mostajir et al. (1999) distinguishes between direct and indirect effects of UV-B exposure. It shows the negative effects of UV-B radiation on bacterial abundance and biomass to be the result of direct UV-B irradiation, but suggests that these negative effects are overcome by the indirect positive effects that UV-B exerts on the aquatic system, in this case predator elimination and substrate alteration. Although predation did not form a component in this study, changes in the nature as well as the quantity of the DOC pool were observed in the presence of UV-B radiation, and as such could have provided better substrate for bacterial growth, thus supporting increased bacterial abundance observed under continuous UV-B exposures.

In general, when comparing results from this study to those of other studies, it is important to consider the different effects of short and long-term UV-B exposures, and to consider the variations in energy levels used in each study.

For example, short term UV-exposure studies (including the diurnal study described in this chapter) are likely to assess the direct effects of UV-B radiation on bacterial populations, whereas the growth responses observed during the long term studies are likely to be due to both direct and indirect effects of irradiation. In the case of this study, changes to bacterial abundance and biomass were most probably brought on by the changes in the nature and/or quantity of the DOC pool (as observed from absorbance, absorbance ratios and HPSEC analyses). Maranger et al. (2002) state that UV-B can exert direct negative effects on bacteria by causing cellular damage or by producing toxic photo-products, but it can also have positive effects on the growth of aquatic bacterial communities through the photochemical transformations of the DOC pool. Thus differentiation between direct and indirect effects of UV-B on bacterial growth must be taken into account. This can often be hard to achieve, particularly in the case of integration between bacteria and the non-living matter, as small qualitative or quantitative changes to organic matter are mostly undetected.

In terms of variations in energy levels between different studies, total UV-B intensities used in this study were $0.1\pm0.02 Wm^{-2}$, although the energy distribution per wavelength

between 280-320 nm is not known. During their studies, Herndl et al. (1993) measured energy levels between 0.15-1.3Wm^{-2}; Reitner et al. (1997) measured 0.3Wm^{-2} at 305nm and up to 5Wm^{-2} for 320nm; Ferreyra et al. (1997) measured a maximum energy of $4x10^{-4}$ Wm^{-2} at 300nm. In addition to the issue of total energy levels, all studies reported using light in the UV-B range of 280-320nm, but the emission spectra of the lights used is also important. For example, light at 295nm has 1,000 times the sunburning effect on human skin as the light at 320nm (Karentz and Lutze, 1990). Therefore, the emission spectra should be comparable between different studies in order to be able to compare various responses to UV-B radiation. Since no data on the energy per wavelength is available in this or other studies, there is a possibility that in this study most energy was emitted at higher wavelengths as opposed to other studies. If this were the case, the damage on the bacterial populations would not be expected to be as strong as if lower wavelengths were dominating the energy.

Further explanations why higher bacterial numbers were observed in the presence of UV-B radiation may be found in terms of water quality and bacterial community interactions. Kaczmarska et al. (2000) state that certain types of DOC (such as humic material and aromatic fractions), absorb UV radiation and by doing so they offer protection for organisms such as bacteria. DOC from the Warren Reservoir was shown to be photo-reactive (Chapter 4), the absorbance analyses suggesting structural changes in DOC to occur as a result of UV-B irradiation. These structural changes were associated with compounds that absorb light mainly in the 280-400nm range (such as aromatic fractions). As such, the DOC pool may have produced some degree of protection by absorbing UV-B radiation and providing shade to bacteria, allowing them to periodically recover from UV-B induced stress. Another possibility may lie with the bacteria themselves. Bacteria are capable of forming aggregates by adhering to each other, thus growing in communities as opposed to individually (Garcia-Pichel, 1994; Elasri and Miller, 1999). This mode of existence can be brought about under certain conditions, in this case enabling protection by physical shielding against UV-B radiation. Elasri and Miller (1999) showed an increased survival rate of bacteria that have formed aggregates following exposure to UV-B radiation. Either mechanism of shading, mentioned here, may explain why bacterial abundance and biomass persisted under continuous UV-B radiation. The presence of some shading factor and a change in the DOC pool toward bioavailable DOC products of photolysis, may explain why both summer and winter bacterial growths were higher under UV-B radiation than in the dark.

A study of community composition using DGGE analysis showed that at the end of the 21-day incubation period, summer bacterial populations were clearly grouped according to the type of treatment, whereas the divergence of winter bacterial populations was not as strong. During the study of summer populations, both S1 and D2 samples that were kept in the dark had higher similarity among each other and with the original populations than did the S1 and D2 populations incubated under UV-B radiation. Thus UV-B treated summer samples were found to be most divergent from both the original samples and dark samples. This result suggests that summer bacteria populations were more prone to UV-B induced changes in community structure than the winter samples. However, since bacterial abundance of summer samples was enhanced in the presence of UV-B radiation, the results of the DGGE analysis probably suggests that some species were more prone to UV-B induced stress than other species. Maranger et al. (2002) state that not all bacteria are equally susceptible to UV damage. Thus, the induced changes in community composition may have been a result of some species being more sensitive to UV-B radiation than others, or they may have been driven by UV-B-transformations of DOC substrates. UV-B-transformations of the summer DOC pool were shown (Chapter 4) to cause pronounced changes in community composition of inoculated bacteria when compared to communities incubated in samples that were previously held in the dark. Based on this, it is likely that higher bacterial abundance and more pronounced changes to the community composition of summer samples exposed to UV-B radiation during this study were due to changes in the DOC pool providing better growth substrate for certain species than for others. Lindstrom (2000) states, that different strains of bacteria have different abilities to utilise different types of DOC. If UV-B radiation was inducing changes in summer DOC pool at the same time as it was affecting bacterial growth, the possibility remains that the observed changes in community structure may have been in a large part a reflection of the DOC substrate changes.

UV-B induced changes in winter bacterial populations were not as apparent as those observed for the summer samples. UV-B induced changes in the winter DOC pool were previously shown (Chapter 4) to cause pronounced changes in community composition of inoculated bacteria, driving shifts in their population structure. It is possible that substrate changes may have caused some alterations to the winter bacterial populations as well, although these changes were not as pronounced as those of summer populations. A possible reason may be that the winter DOC pool provided a higher degree of shading from UV-B radiation than was the case for the summer DOC pool. Namely, coloured DOC compounds

are known to absorb strongly in the UV part of the spectrum (Belzile and Vincent, 2002), and the winter DOC pool has been shown to comprise significantly higher levels of coloured organics than the summer DOC pool in the Warren Reservoir (Sections 3.5.4 and 3.5.5). As such, it is possible that higher levels of coloured organics during winter provided higher degree of protection for winter bacteria, resulting in less pronounced structural changes at a community level.

The second main aim of this study was to investigate the integrated effects of photo-degradation and microbial decomposition on the DOC pool, in order to estimate how UV-B induced bacterial damage is likely to affect the quantity and quality of the DOC pool in the Warren Reservoir.

During the summer study, increased levels of DOC were measured in the presence of UV-B radiation, as opposed to the winter study where DOC level did not change as a result of UV-B irradiation (in comparison with dark controls). In the summer study, a trend of increasing DOC levels with decreasing bacterial abundance was obtained under dark conditions. This relationship was enhanced in the presence of UV-B irradiation, suggesting that UV-B enhanced bacterial turnover increased the internal loading of DOC. Higher DOC inputs in the presence of UV-B (when compared to dark controls) are most likely a result of enhanced bacterial numbers and thus enhanced turnover rates resulting in more DOC being released into the water at any point in time. Ogawa et al. (2001) showed that the DOC produced by bacterial turnover is relatively resistant to decomposition. Conversely, other studies state that this DOC can serve as substrate for bacteria that are not damaged as a result of UV-B radiation. For example, Middelboe and Lyck (2002) studied the effects of viral lysis on bacteria and state that the DOC released by bacteria during lysis can be an important substrate source stimulating the growth on non-infected bacterial populations. This could explain the relationship between bacterial abundance and DOC concentration in summer UV-B exposures. Increased bacterial abundance was parallel to reduced DOC levels (period of bacteria growth and DOC consumption) and decreased bacterial abundance was parallel to increased DOC concentration (period of bacterial turnover and DOC release). During the winter study, however, there was no difference in the DOC input between the dark and UV-B samples. Considering the differences in the nature of the summer and winter DOC pools discussed previously, it is possible that due to a higher composition of coloured organics a greater protection was provided to winter bacterial populations, resulting in lower turnover

rates of winter bacteria and thus no observable differences in the internal DOC loading as a result of UV-B induced bacterial damage.

In terms of qualitative changes to the DOC pool, the following observations were made. Increasing loss of absorbance was measured with increasing wavelength (between 200-500nm) for some incubation times, while for other incubation times, highest losses in absorbance occurred between 200 and 360nm. Losses in absorbance in this area (particularly between 254 and 280 nm) are often used as an indication of the changes in conjugated, unsaturated double bonds and aromaticity (Andersen et al., 2000; Hautala et al., 2000). During the winter study, losses in absorbance were measured in dark samples as well, although higher losses occurred in the presence of UV-B radiation. Highest losses in absorbance were measured after 21 days of incubation for all samples (UV-B and dark). The loss of absorbance (particularly at 254nm) can be representative of reduced DOC levels. However, considering that the loss in DOC levels was not linear with respect to the length of incubation (instead it fluctuated during the 21-day incubation period), these losses in absorbance were most likely representing increased changes to the nature of DOC over time.

Increased changes in the nature of the DOC pool with incubation time were most likely a result of not only UV-B photo-transformations, but bacteria as well. Depending on the nature of the initial DOC pool, bacteria can alter the DOC pool by consuming certain fractions of DOC while releasing autochthonous DOC during its growth and turnover. Summer DOC mostly originates from autochthonous production, while the winter DOC is highly influenced by allochthonous input. Greater changes should be expected to the winter DOC pool as a result of bacteria growth and turnover (with or without the presence of UV-B radiation). During the winter study, changes in the absorbance were found not only in UV-B irradiated samples but in dark samples as well. Increased incubation times caused loss of absorbance in winter samples under both UV-B and dark conditions, whereas losses in absorbance in summer samples were only found under UV-B exposure. Direct effects of UV-B radiation on the DOC most likely caused the summer changes in absorbance properties (as they could only be found in UV-B irradiated samples), while the winter changes in absorbance were most likely a reflection of UV-B induced transformations in the DOC pool, as well as changes in the dominant fractions of DOC (which in winter samples would have changed from allochthonous to autochthonous).

In terms of how bacterial activity and input of DOC is likely to affect the summer and winter DOC pools, the following observations were made.

In the summer, highest absorbance losses were measured in the wavelength range of 200-365 nm. These losses were most likely associated with aromatic fractions, which absorb highly in this spectral range (Andersen et al., 2000; Hautala et al., 2000). Decreased absorbance at 254nm in particular suggested a decrease in the amount of conjugated double bonds, as these in particular, efficiently absorb short wavelength radiation (Tranvik and Kokalj, 1998). Furthermore, the E_2/E_3 absorbance ratio increased in UV-B exposed summer samples, suggesting a shift in molecular weight distribution toward the formation of smaller molecules (Lindell et al., 1995). In the winter study, highest losses (in both UV-B and dark incubations) occurred between 365-665 nm, whereas losses in the 200-365 nm range were higher under UV-B radiation than in dark samples. The loss of absorbance in the 365-665 nm range was most likely associated with coloured (optically active) fractions of the DOC (which absorb light of higher wavelengths) (Andersen et al., 2000), and was probably the result of direct bacterial removal of these fractions from water (given the changes in the absorbance data of higher wavelengths during the dark incubations). Conversely, losses in the 250-365 nm range were most likely associated with reduced aromatic fractions, most likely due to the effects of UV-B irradiation. Short wavelength absorbance has been suggested to represent smaller molecules than long wavelength absorbance (Lindell et al., 1995), as such preferential losses in absorbance at certain wavelengths can also be indicative of changes to molecular size fractions.

In general, a number of possibilities might explain the observed changes in absorbances and thus the likely changes in the nature of the DOC pool caused by interactions of bacteria growth and turnover and UV-B irradiation.

First is the case of preferential removal of certain DOC fractions over others. DOC fractions that absorb light at higher wavelengths (365-665 nm), were preferentially consumed by winter bacteria populations (as indicated by dark as well as UV-B data), causing a more rapid removal of these fractions from water. These fractions were also removed by UV-B irradiation but to a lesser extent (as indicated by the UV-B absorbance data), possibly via transformation to other forms or through mineralisation. In this case, losses in overall absorbances and/or an increase in the E_2/E_3 absorbance ratio would be expected. Reduced absorbances were found as a result of increasing incubation times, suggesting overall removal of compounds from water. Greater losses of compounds that absorb in the higher wavelengths (365-665 nm) were observed, suggesting a reduction in the average DOC molecular weight (Lindell et al., 1995) due to bacterial activity and/or turnover. Furthermore,

E_2/E_3 absorbance ratios increased with increasing irradiation times, suggesting a conversion of higher molecular weight products to lower molecular weight products (Lindell et al., 1995; Andersen et al. 2000). DOC fractions that absorb in the 250-365 nm range were more prone to UV-B transformation (in comparison to more bioavailable fractions which absorb in the higher wavelength range). A shift from higher to lower average molecular weight was observed (for UV-absorbing fractions), suggesting photochemical transformation of DOC compounds. The changes in molecular weight distribution were detected with absorbance at 260nm only, thus the measured changes in molecular size only reflect the changes to UV-absorbing compounds in the DOC pool. The reduction in absorbances at lower wavelengths (250–365nm) was caused by UV-B radiation (as no changes to molecular weight were detected for dark samples). Therefore, UV-B radiation appears to enhance the removal of UV-absorbing DOC molecules in the molecular weight range of 300 to 5000 Daltons.

The second case is that of bacterial turnover causing changes to the nature of the DOC pool. DOC entering the water due to bacterial turnover consisted typically of compounds that absorb light in the UV-B (short) wavelength range. Short wavelength absorbance has been suggested to represent smaller molecules than long wavelength absorbance (Lindell et al., 1995), which would imply that the products of bacterial turnover are of low molecular weight. For example, the increase of E_2/E_3 ratios with increasing incubation times (thus an increase in the amount of UV-B absorbing compounds with respect to other compounds) was parallel to an increase in the input of lower molecular weight DOC pool, suggesting that bacteria release lower molecular weight DOC products. Furthermore, SUVA values decreased during the course of incubation (in both UV-B and dark), indicating reduced aromaticity and average molecular weight of the DOC pool with increasing irradiation time) (Goel et al., 1995; Strauss and Lamberti, 2002). Thus bacterial turnover appears to cause a decrease in the proportion of aromatic fractions of the DOC pool, by contributing lower molecular weight DOC compounds that are easily utilisable by growing bacteria and susceptible to photo-transformation and/or mineralisation, thus not accumulating in the system. Andersen et al. (2000) showed that increased input of lower molecular weight organics (indicated by HPSEC data and increased E_2/E_4 ratios) into the dissolved organic pool forms a good nutritional source for heterotrophic consumers. However, given that these compounds were continuously entering the system (as a result of bacterial turnover), their removal was not as great as that measured for compounds absorbing light at higher wavelengths (as indicated by the absorbance data).

In summary, changes to the nature of the DOC pool observed during the course of incubation were not only caused by changes to the initial DOC pool (as a result of UV-B transformations or microbial decomposition), but also due to loading of fresh organics originating from bacteria. This organic matter appeared to be of low molecular weight, comprising a high portion of molecules that absorb light in the UV range (as suggested by E_2/E_3, SUVA, HPSEC and absorbance spectrum data). These molecules served as good substrates for supporting further bacterial growth (as indicated by a lack of DOC accumulation in the water). Goel et al. (1995) showed that internally produced DOC originating from both algae and bacteria, comprises material that is mostly aliphatic and low in phenolics and aromatic constituents. These structures act as a better substrate for growth and, as such, may be one of the factors responsible for stimulating higher bacterial numbers.

5.6 CONCLUSION

Positive and negative effects of UV-B radiation on bacterial populations of the Warren Reservoir were observed during this study. Alternating exposures to UV-B and dark conditions showed that UV-B radiation can reduce bacterial numbers, but the dark periods (simulating the diel events of the natural reservoir environment) allow recovery of bacterial numbers. Surviving cells were able to reach higher concentrations probably due to increased bioavailability of substrate due to either UV-B induced transformations of the DOC pool or due to high-quality of internally loaded DOC.

Conversely, increased bacterial abundance in the presence of UV-B radiation was most likely due to the abilities of these organisms to induce some form of behavioural adaptation to UV-B radiation (such as self-shading) or due to the ability of the reservoir DOC pool to absorb light in the UV-range, thus forming a degree of protection for bacteria. Furthermore, bacterial growth appeared to contribute low molecular weight products to the DOC pool, which absorbed light in the UV range. Thus UV-B induced death of some species may be supporting growth of surviving species, not only by acting as available substrate, but by absorbing UV-B light and thus providing a degree of protection against UV-B radiation.

No accumulation of DOC was observed in the water, suggesting that total bacterial population mortality due to UV-B irradiation is unlikely to result in DOC accumulation in the

Warren Reservoir during either summer or winter, as a result of either reduced bacterial consumption of DOC or as a result of bacterial turnover contribution to the DOC pool.

As the UV-B energy levels applied during this study are within the ambient UV-B levels measured for the area of the Warren Reservoir (Figure 4.20), the findings of this study can be used to represent the potential effects of solar UV-B radiation on reservoir bacteria, and ultimately the potential effects of UV-B radiation and bacterial activity on the DOC pool.

CHAPTER 6 DIEL VARIATIONS IN PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES OF WATER FROM THE WARREN RESERVOIR

6.1 INTRODUCTION

During the *in situ* study of the Warren Reservoir (Chapter 3), emphasis was placed on the seasonal association of DOC with various physical, chemical and biological parameters, such as the temperature, nutrient levels, phytoplankton and bacteria. This section of the study examined short-term fluctuations in water parameters during the diel cycles (diurnal and nocturnal periods). Diel DOC concentrations and character were correlated to various physical, chemical and biological parameters. This was undertaken in an attempt to determine if correlations, which may not have been resolved on a seasonal scale, can be drawn on a finer diel scale, between DOC and the parameters which cause its accumulation and removal in the Warren Reservoir.

Kaplan and Bott (1982) associated diel DOC fluctuations with algal growth. They also showed a correlation between DOC, algae and bacterial abundance in the short-term accumulation and removal of DOC. Conversely, DiSiervi et al. (1995) demonstrated fluctuating rates in bacterial activity (with maximum values occurring during the night and early morning), however there was no temporal coincidence between algal primary production (autochthonous DOC) and bacterial secondary production as reported by Kaplan and Bott (1982).

Particular emphasis in this section was placed on determining if bacterial concentrations or community structure in the reservoir conform to a diurnal-nocturnal cycle, as was indicated during the laboratory study in Chapter 5 (where reduced bacterial numbers were observed after exposure to short-term UV-B radiation while sufficient recovery of cell numbers was observed during dark periods). The aim was to determine whether correlations exist between the changes in DOC concentration or nature and changes in bacterial concentration and community structure on a finer diel scale.

Diel patterns of physical, chemical and biological parameters were monitored during both summer and winter to determine whether potential short-term fluctuations in DOC and other parameters are season dependent. Two diel studies were conducted during summer (February) and winter (August) with the following objectives:

1. examine diel water quality patterns, with the main emphasis on the association between DOC concentration or character with bacteria and phytoplankton, in summer and in winter.
2. analyse short-term relationships between various physical, chemical and biological parameters of the reservoir, with the aim of determining which parameters may be responsible for DOC accumulation and/or removal from the Warren Reservoir.
3. determine if the effects of solar radiation on bacterial growth inhibition or stimulation can be measured *in situ*.

6.2 MATERIALS AND METHODS

In situ measurements and water sampling were conducted at the shallow (S1) and deep (D2) sites of the Warren Reservoir during February (summer) and August (winter) 2001. Measurements and sampling were performed at dawn, midday and night for a total period of 48 hours. Physical and chemical properties of water such as oxygen concentration, temperature, pH and water turbidity were measured on site. Water sampling involved collecting 22L of water from 1m below the water surface, followed by transportation to the laboratory for further analyses. All analyses (except DGGE) were carried out within one hour of sampling. Sampled water was allocated in the following manner. 20L of water was concentrated to 1L using Tangential Flow Filtration (TFF). The concentrates were stored at 4°C until DNA extraction and DGGE analysis 48 hours later. From the remaining 2L, 100mL was filtered through a 1.2μm membrane (Whatman GF/C) and samples were fixed for bacterial counting (Section 2.12), while the remaining volume was filtered through a 0.45μm membrane (Millipore) for chemical analyses. The chemical and biological analyses performed were as follows: DOC concentration, total phosphorus, soluble reactive phosphorus, nitrate, colour, absorbance, chlorophyll, bacterial abundance and diversity. Methods for each analysis are detailed in Chapter 2.

6.3 WATER QUALITY PARAMETERS: DIEL FLUCTUATIONS AND TRENDS

6.3.1 DOC CONCENTRATION

Diel DOC changes were analysed using ANOVA to determine if the differences in DOC concentrations between diurnal and nocturnal cycles were significant. Although different DOC concentrations were measured between summer and winter samples, ANOVA at α=0.05 showed no significant difference in the diel DOC concentrations at either S1 or D2 sites during summer or winter (S1 summer p=0.8; S1 winter p=0.4; D2 summer p=0.2; D2 winter p=0.7) (Table 6.1).

Table 6. 1: Diel differences in DOC concentrations during two summer and winter diel cycles, at the shallow (S1) and deep (D2) ends of the reservoir.

	Night 1	Night 2	Dawn 1	Dawn 2	Day 1	Day 2
Summer S1	27.7±1.3	25.1±0.1	26.4±0.6	25.5±0.3	25.5±0.3	25.8±0.1
Summer D2	26.7±0.3	26.7±0.3	27.1±0.1	26.4±0.1	25.1±0.6	26.1±1.0
Winter S1	31.1±0.6	31.0±0.3	31.0±0.1	31.0±0.3	31.0±0.3	30.0±0.1
Winter D2	25.4±0.1	23.2±0.3	23.0±0.1	24.0±0.3	24.0±0.2	24.1±0.1

6.3.2 ABSORBING PROPERTIES OF DOC

ANOVA showed no significant differences in diel absorbances (at 250nm, 254nm, 365nm, 465nm) or the E_2/E_3 absorbance ratios at either site during summer or winter (Table 6.2). Significant diel patterns in absorbance at 665nm were found at the deep site during winter (p=0.02) (Figure 6.1). A_{665} increased from night to dawn by 39% and 26% during the first and second diel cycles respectively. Significant diel differences were also measured for E_4/E_6 absorbance ratio at the deep site of the reservoir during both summer (p=0.05) and winter (p<0.01) (Table 6.2). During the 48-hour summer and winter studies maximum E_4/E_6 absorbance ratios were measured at night (Figure 6.2). The decreases in ratios from night to dawn were between 60% and 80% in summer and 20% and 25% in winter.

Table 6. 2: ANOVA for the diel fluctuations in specified absorbance at the shallow and deep sites during summer and winter. Significant differences in absorbance (at α=0.05) between dawn, day and night are highlighted.

p values	SUMMER		WINTER	
	Shallow	**Deep**	**Shallow**	**Deep**
250nm	0.77	0.98	0.69	0.70
254nm	0.79	0.98	0.70	0.82
365nm	0.83	0.89	0.84	0.16
465nm	0.86	0.62	0.57	0.09
665nm	0.41	0.24	0.23	**0.02**
E_2/E_3	0.88	0.73	0.46	0.07
E_4/E_6	0.16	**0.05**	0.30	**0.01**

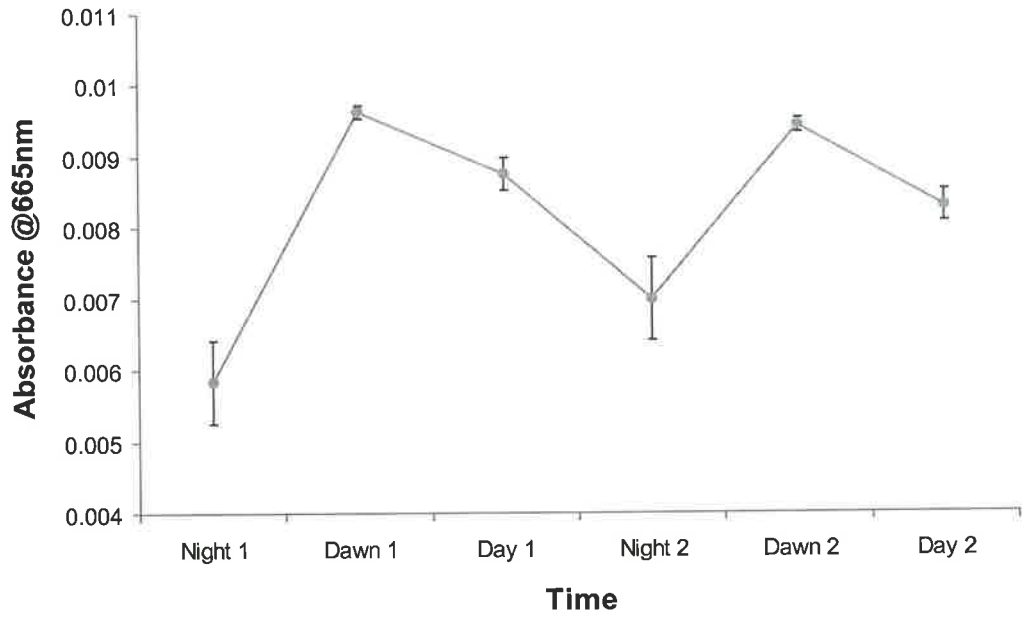


Figure 6. 1: Changes in absorbance at 665nm during a 48-hour winter study at the deep site of the Warren Reservoir (p=0.02).

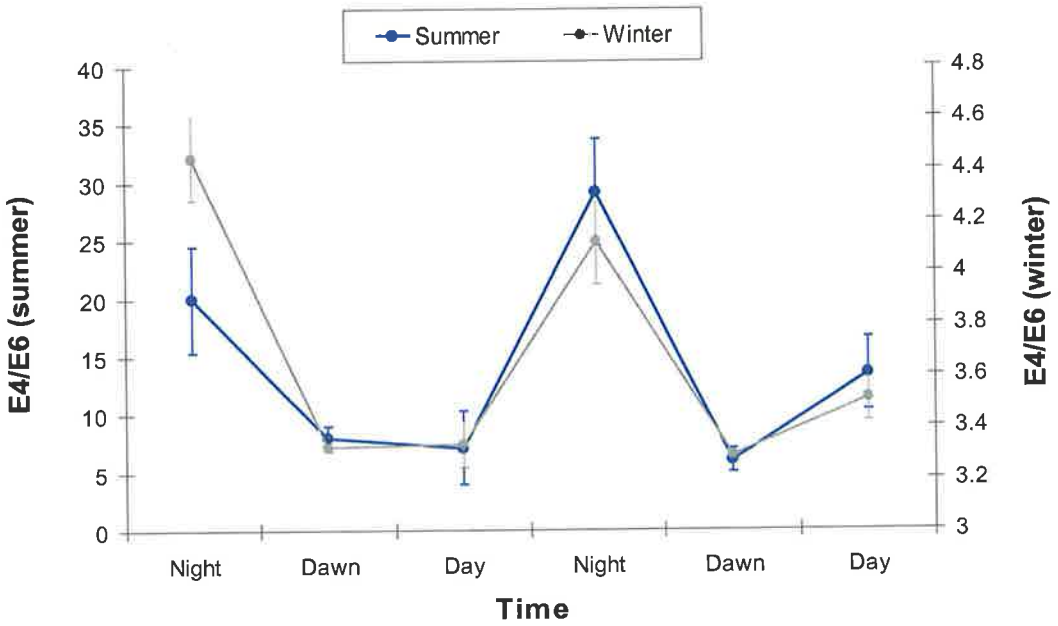


Figure 6. 2: Changes in E_4/E_6 absorbance ratio during a 48-hour summer and winter study at the deep site of the Warren Reservoir.

During the four diel studies, SUVA fluctuated by 5±1% to 16±2%. ANOVA (α=0.05) showed no significant differences between diurnal and nocturnal values during the 48-hour summer or winter studies at either shallow or deep sites (S1 summer p=0.8; D2 summer p=0.8; S1 winter p=0.5; D2 winter p=0.6) (data not shown).

6.3.3 BACTERIAL ABUNDANCE AND BIOMASS

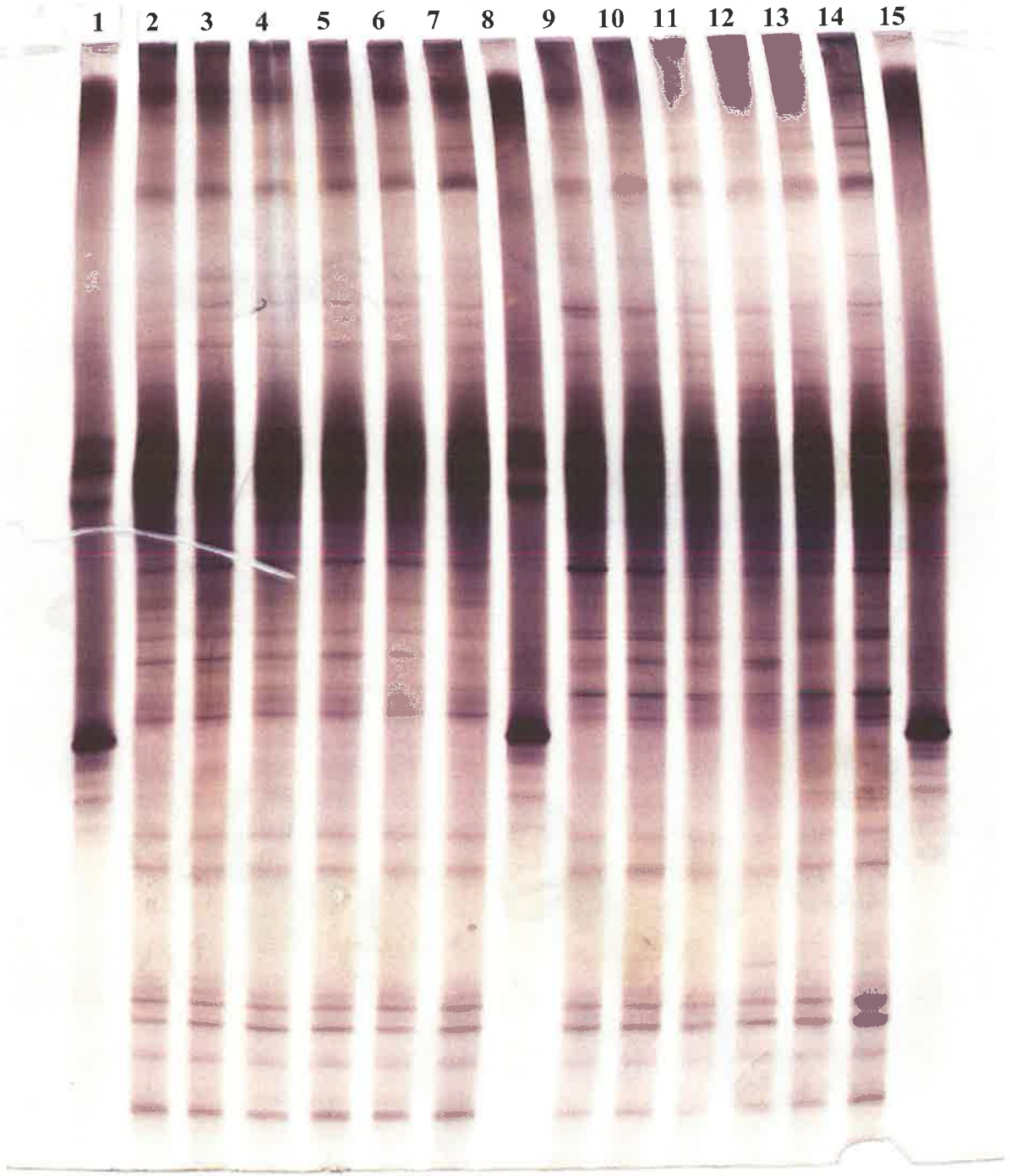
Changes in bacterial numbers and biomass were measured during the summer and winter diel cycles. Summer bacterial numbers during the 48-hour studies ranged between 2.1-2.8 x10^6cells/mL at the shallow site and 1.2-1.9 x10^6cells/mL at the deep site. Winter bacterial numbers at the shallow site were between 1.5-2.4x10^6cells/mL and at the deep site 0.8-1.1 x10^6cells/mL.

Summer bacterial biomasses were between 341-546ngC/mL at the shallow site and 113-277ngC/mL at the deep site. Winter bacterial biomasses ranged between 175-345ngC/mL at the shallow site and 120-163ngC/mL at the deep site.

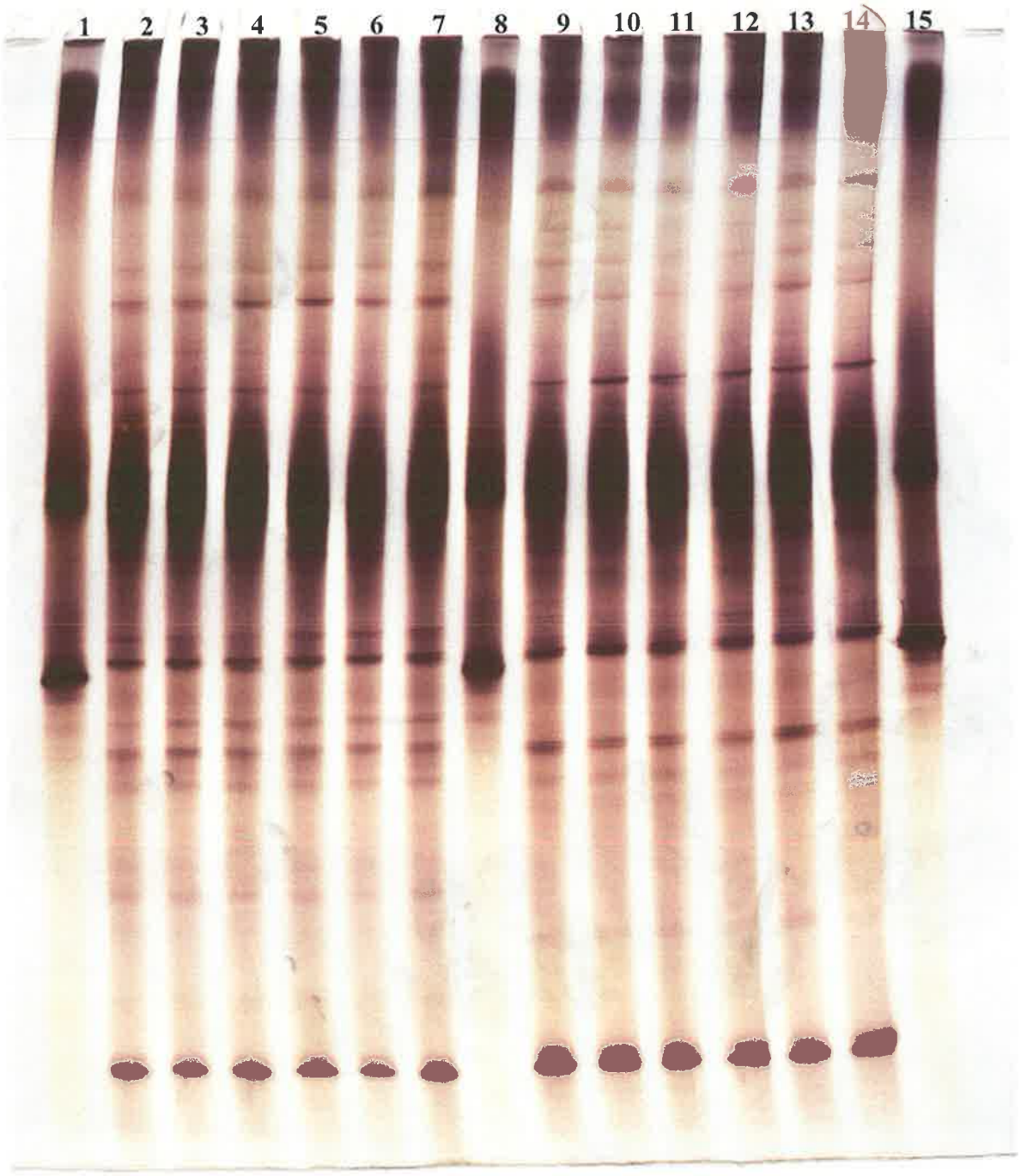
While cell numbers and biomasses changed during the 48-hour diel studies, they did not conform to a diel pattern.

6.3.4 BACTERIAL POPULATIONS

DGGE analysis was performed on resident bacterial populations at the shallow and deep sites of the reservoir, during the summer and winter field studies in order to determine whether bacterial community composition changes according to the diel cycle (Figure 6.3).



(A) Summer diel study

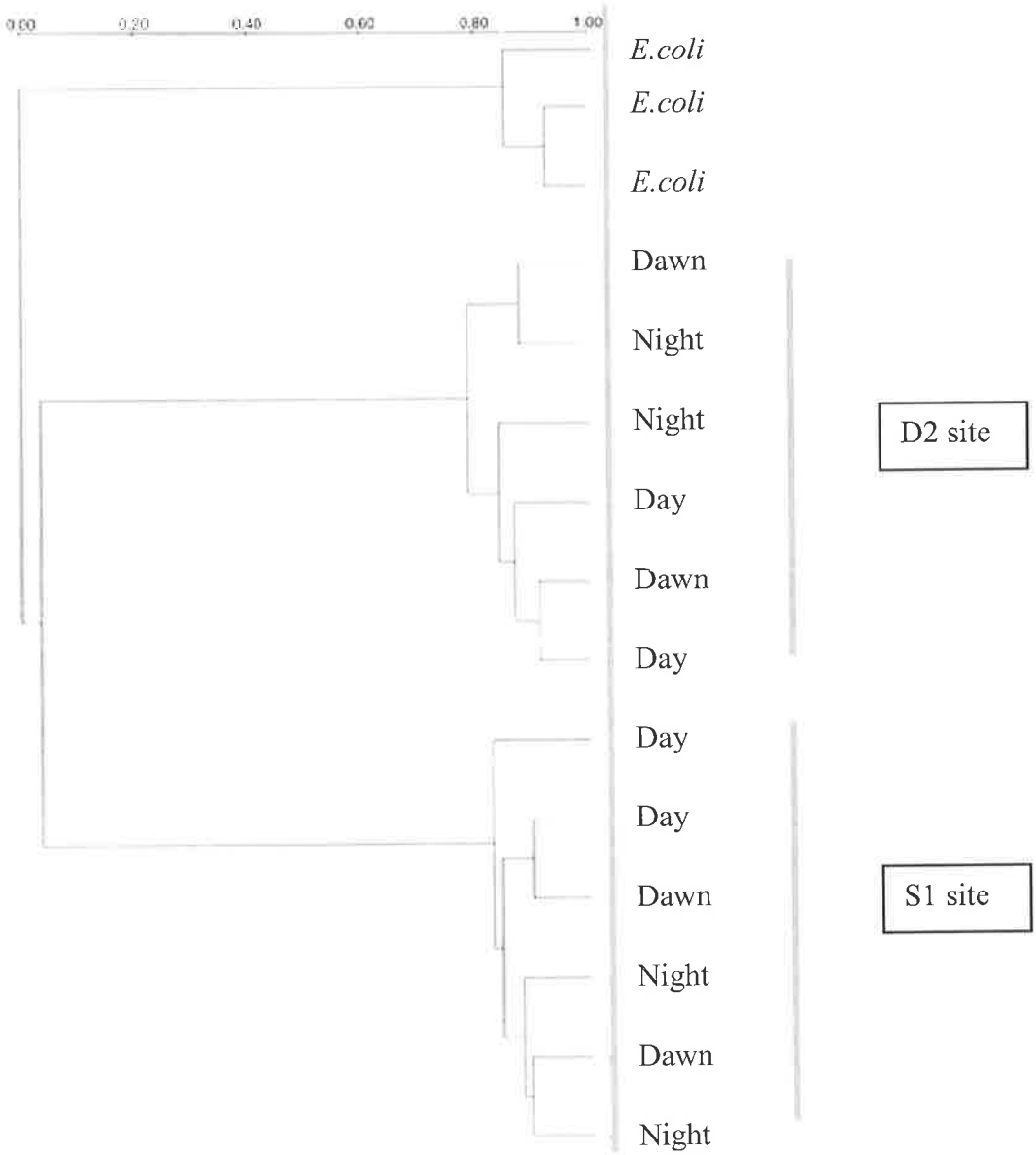


(B) Winter diel study

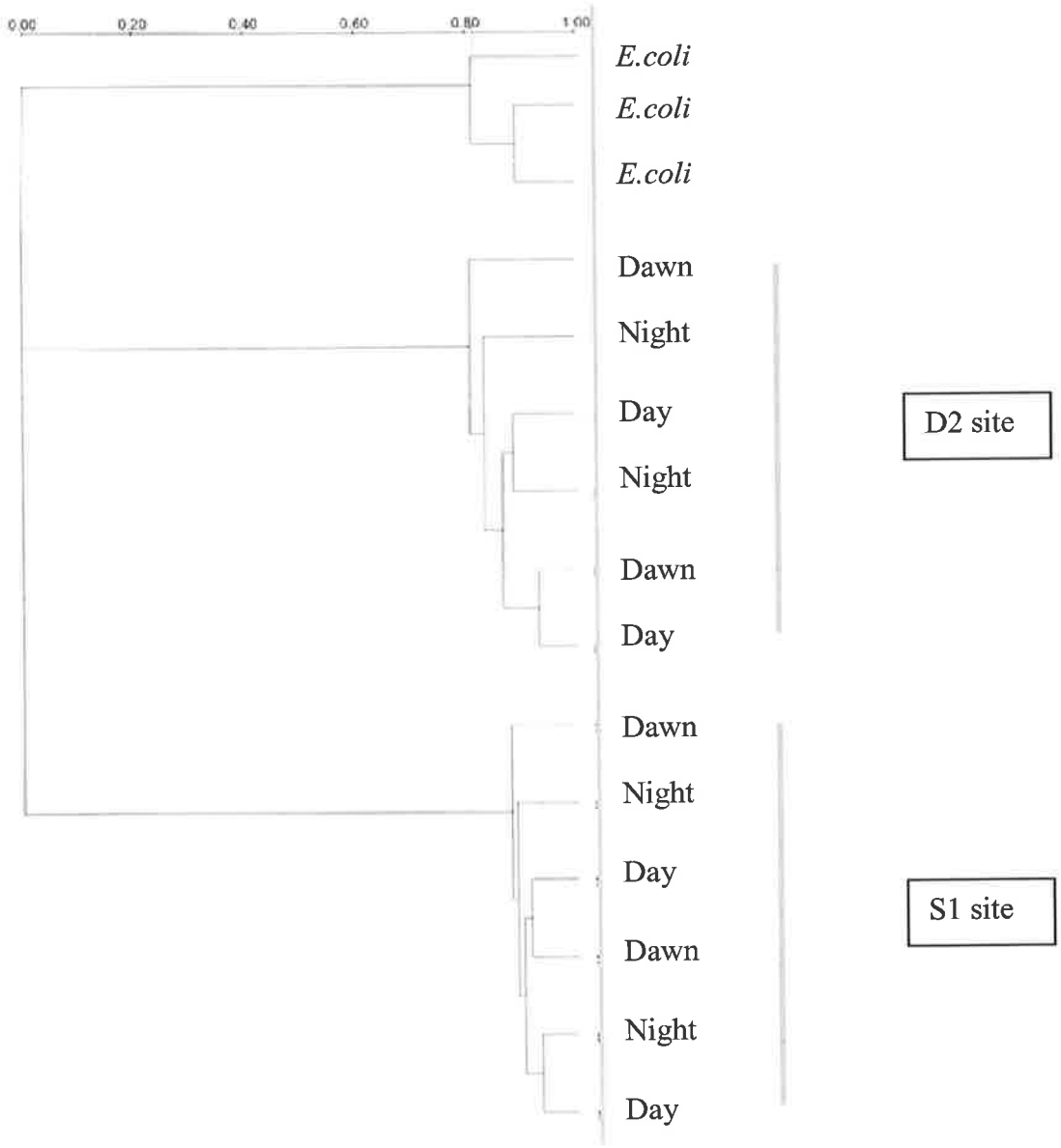
Figure 6. 3: 8% polyacrylamide DGGE gels, showing the separation of 520bp PCR products amplified from genomic DNA that was extracted from (A) summer and (B) winter reservoir water samples collected from shallow-S1 and deep-D2 reservoir sites, during the 48-hour diel study. Lanes 1-E.coli; 2-S1 night1; 3-S1 dawn1; 4-S1 day1; 5-S1 night2; 6-S1 dawn 2; 7- S1 day2; 8-E.coli; 9-D2 night1; 10-D2 dawn1; 11-D2 day1; 12-D2 night2; 13-D2 dawn2; 14-D2 day2; 15-E.coli.

Based on the banding pattern information of samples in the DGGE gels, phylogenetic analysis was performed to determine whether diel bacterial populations at each site differed (Figure 6.4). Samples were grouped based on bacterial population similarity. Except for the *E.coli* markers, both summer and winter studies showed distinct separation of samples belonging to shallow and deep sites, where less than 5% relatedness was found between the communities inhabiting these two furthest points in the reservoir. The diel relatedness of species within each of the two communities ranged from 79% to 91% in summer, and 81% to 93% in winter (Figure 6.4A and B).

In terms of population changes during the diel cycle, there was no clear segregation of populations due to diurnal and nocturnal cycles during either summer or winter.



(A) Summer diel study



(B) Winter diel study

Figure 6. 4: Phylogenetic tree showing relatedness among bacterial populations inhabiting the shallow and deep sites of the reservoir and the relatedness among diel bacterial populations at each site, during the 48-hour summer (A) and winter (B) studies.

Principal component analysis (PCA) was further used to study changes in bacterial community composition (Figure 6.6). PCA of diel samples was based on the band presence as well as the differences in peak intensities of each band (Figure 6.5) in the DGGE gel

(Figure 6.3). Data on band intensity were used to generate a two dimensional matrix indicating sample separation along the horizontal and vertical axes (Figure 6.6). The horizontal axis in Figure 6.6 had a stronger influence on the separation of the presented data (37%) when compared to the vertical axis (17%) suggesting a stronger separation of populations along the horizontal axis than along the vertical axis. The PCA agreed with the results of the phylogenetic tree analysis (Figure 6.4), also suggesting separation of populations in relation to spatial but not time (diurnal and nocturnal) scale.

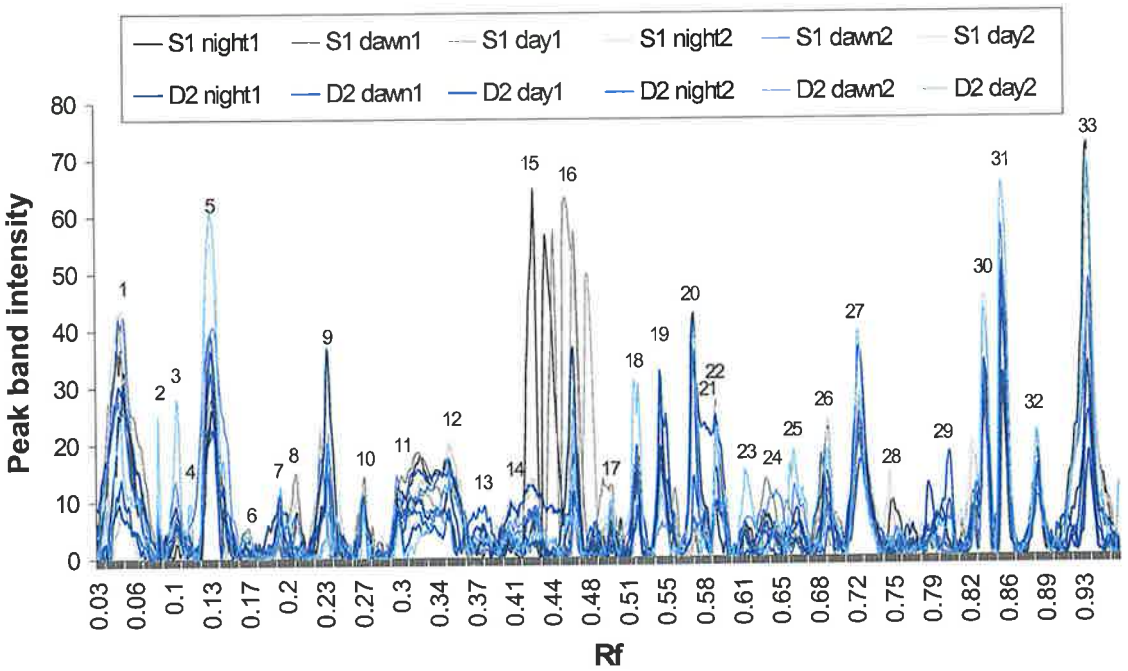


Figure 6. 5: Peak intensity of bands produced by DGGE of diel shallow and deep reservoir summer samples. Shown are a total of 33 bands of varying intensities depending on sample type and time.

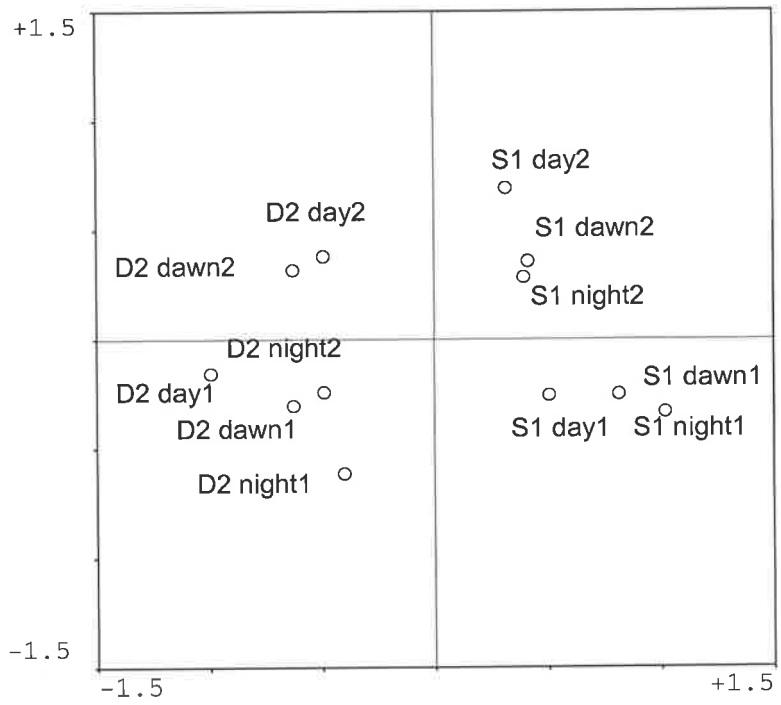
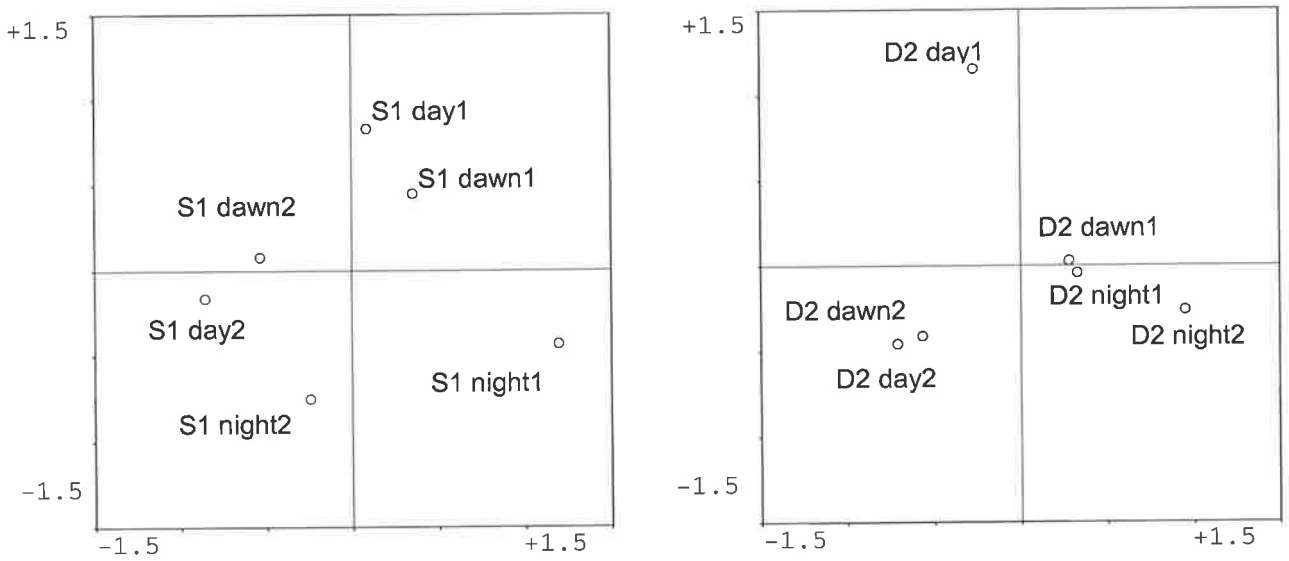


Figure 6. 6: Principal component analysis (PCA) showing differences between diel bacterial populations during the 48-hour summer study at the S1 site and D2 site (Influence on the separation by horizontal axis=37% and by vertical axis=17%).

Since the PCA results agreed with those obtained from the phylogenetic analysis (suggesting separation primarily based on spatial reservoir differences), further PCA was performed on diel samples of each site during both summer and winter to determine whether there were changes to bacterial populations inhabiting the epilimnion of the shallow and deep sites in the Warren Reservoir.

PCA of summer diel samples from the S1 and D2 sites were based on the data of peak intensity of the DGGE bands (Figure 6.5). PCA showed no specific diel pattern to the separation of bacterial populations in the D2 site (Figure 6.7B). In the S1 site however, there was a trend of separation between bacterial populations of diel cycle one and two (Figure 6.7A) but not within the cycles (diurnal and nocturnal scale). Therefore, PCA is in support of the phylogenetic analysis that showed no specific diurnal and nocturnal patterns in the composition of summer bacterial populations in the epilimnion of the shallow and deep sites of the Warren Reservoir.



(A) S1 site *(B) D2 site*

Figure 6. 7: Principal component analysis (PCA) showing differences between diel summer bacterial populations during the 48-hour study of the epilimnion at the (A) S1 and (B) D2 sites of the Warren Reservoir. (A) Influence on the separation by horizontal axis=44% and by vertical axis=28%; (B) Influence on the separation by horizontal axis=33% and by vertical axis=30%.

PCA of diel winter samples was based on the banding pattern and the peak band intensity data (Figure 6.8) obtained from the DGGE gel (Figure 6.3B). The banding patterns representing diel winter populations of S1 and D2 sites were identical (Figure 6.8), thus major contributions toward population separation during PCA were the result of variations in band intensities rather than band presence.

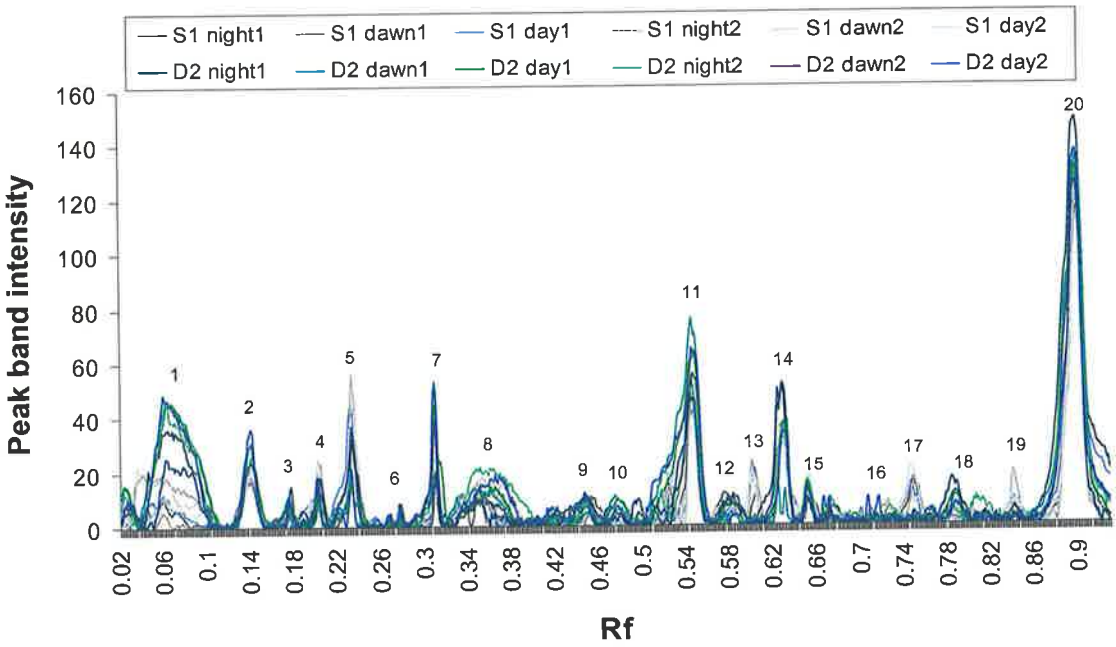
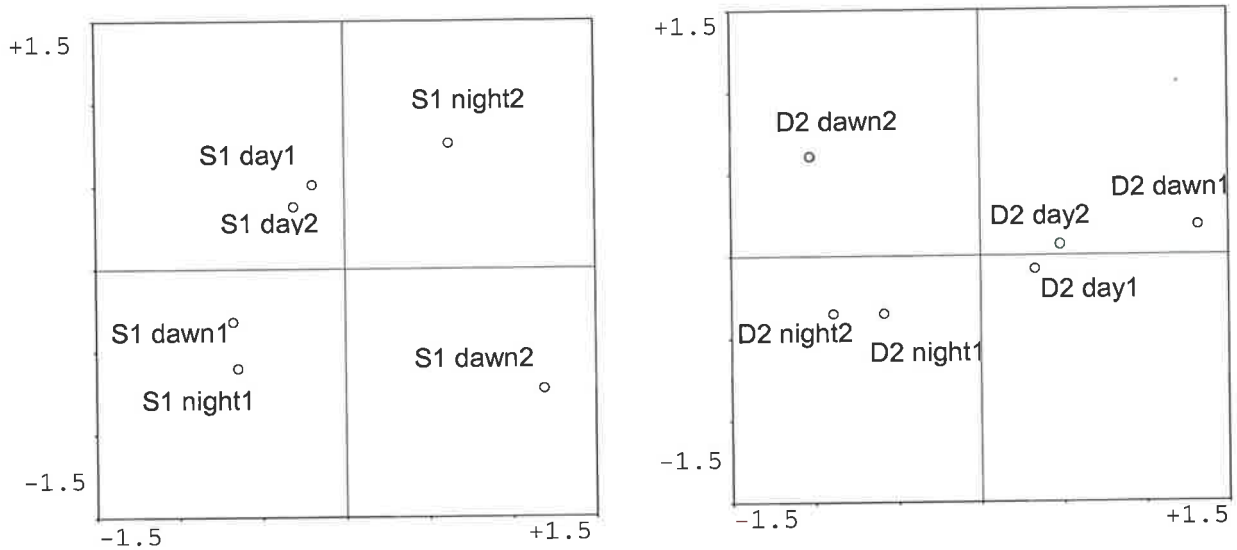


Figure 6. 8: Peak intensity of bands produced by DGGE of diel S1 and D2 reservoir winter samples. Shown are a total of 20 bands of varying intensities depending on sample type and time.



(A) S1 site *(B) D2 site*

Figure 6. 9: Principal component analysis (PCA) showing differences between diel winter bacterial populations during the 48-hour study of the (A) S1 and (B) D2 sites at the Warren Reservoir. (A) Influence on the separation by horizontal axis=47% and by vertical axis=33%; (B) Influence on the separation by horizontal axis=71% and by vertical axis=12%.

Bacterial populations inhabiting the epilimnion of the shallow site during the day showed closest similarity, whereas night and dawn samples did not appear to conform to a group (Figure 6.9A). In the deep site however, both day and night populations appeared to be grouped together, while the dawn populations did not conform to a diel cycle pattern (Figure 6.9B).

6.3.5 CHLOROPHYLL CONCENTRATIONS

During summer, chlorophyll levels at both shallow and deep ends of the reservoir showed a repeated decrease from a dawn high to daytime low (Figure 6.10). During winter, no particular diel pattern was observed at either site. Diel fluctuations during summer were higher than those during winter.

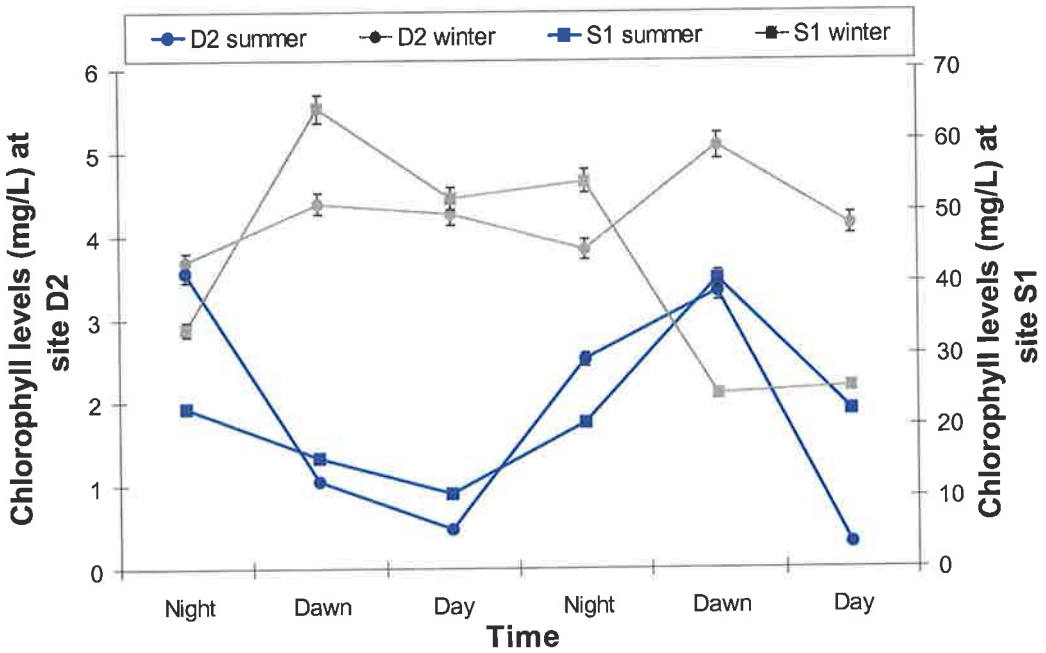


Figure 6. 10: Changes to chlorophyll levels at the S1 and D2 sites of the reservoir during a 48-hour summer and winter study.

6.3.6 PHYSICAL AND CHEMICAL PARAMETERS

There were no significant diel differences in water temperature, conductivity, salinity or nutrients (total phosphorus (TP), soluble reactive phosphorus (SRP) and nitrate (NO_3)) (data not shown). Changes in dissolved oxygen, turbidity and pH measured during the 48-hour diel study showed diurnal and nocturnal patterns.

Dissolved oxygen (DO) was measured during the winter study only and changes were found at the S1 site only. Lowest DO levels at the S1 site were measured at dawn, while equally high levels of DO were obtained at night and day times. Thus, at dawn the DO levels at the S1 site decreased by an average of 10±1%. At the deep site no obvious changes in DO level were detected (Figure 6.11).

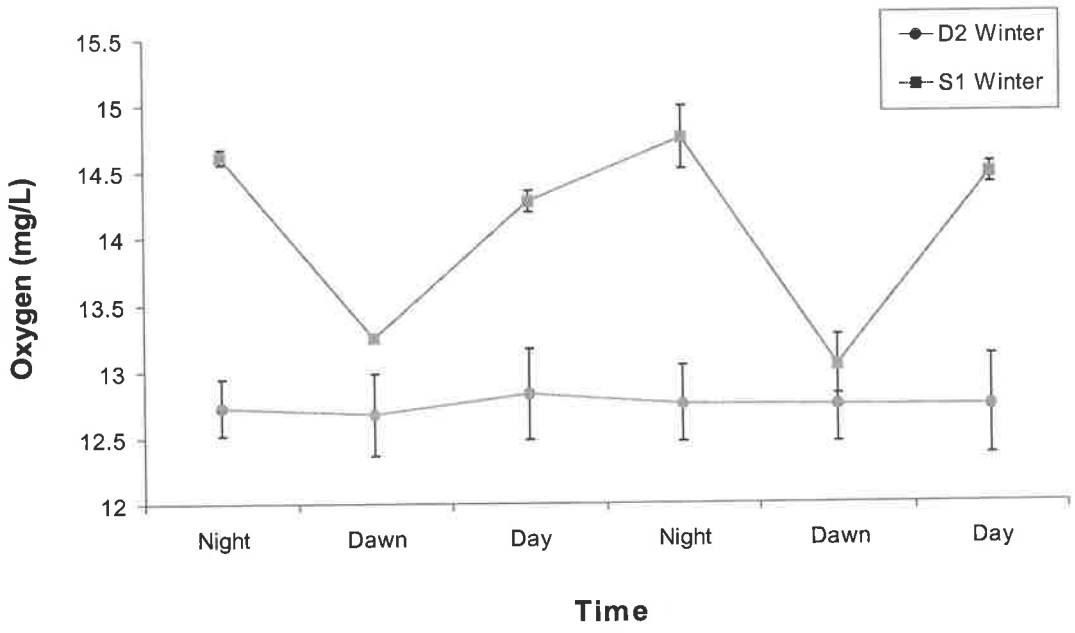


Figure 6. 11: Changes in dissolved oxygen concentration during the 48-hour diel winter study of the shallow and deep reservoir sites.

Turbidity levels at the deep site did not change during the summer or winter diel cycles. At the S1 site turbidity levels showed two different diel patterns, depending on season. In summer, higher turbidity levels were measured at dawn (average increase of 30±7%) when compared to night and day times. Conversely, during winter, lowest turbidity

levels were obtained at dawn (average decrease of 11±2%), with equally high levels measured at night and during the day (Figure 6.12).

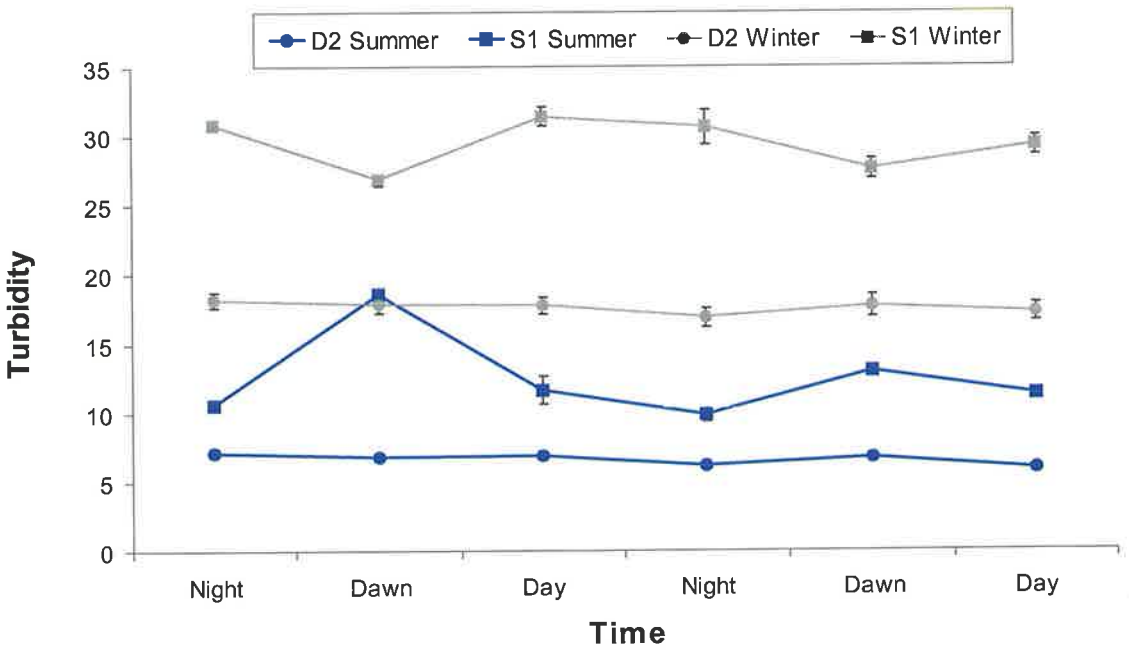


Figure 6. 12: Changes in turbidity levels during the 48-hour diel summer and winter studies of the shallow and deep reservoir sites.

6.4 SHORT-TERM CORRELATIONS BETWEEN DOC CONCENTRATIONS AND VARIOUS WATER PARAMETERS

Correlation and regression analyses were used to examine the short-term relationships between DOC concentration and other water parameters. Trends were observed between DOC concentration and SUVA (Figure 6.13), and DOC concentration and bacterial numbers (Figure 6.14) and biomass (Figure 6.15).

Figure 6.13 shows a strong relationship between DOC concentration and SUVA at the S1 site in summer and at the D2 site in winter. Inverse relationships were found at the S1 site during summer ($R=0.9$, $r^2=0.85$, $p=0.01$) and at the D2 site during winter ($R=0.97$, $r^2=0.94$,

p<0.01). Weaker inverse relationships were found at the D2 site during summer (R=0.4, r^2=0.47, p=0.02), while the S1 winter site showed no significant correlation (R=0.05, r^2=0.005, p=0.8). This relationship suggested two possibilities. One possibility is that the DOC molecules that absorb strongly at 254nm were not dominant during short-term DOC loading in the reservoir (causing reduced SUVA of reservoir water). The second possibility is that during short-term DOC removal from the reservoir, compounds that absorb at 254nm were least likely to be removed from the reservoir water.

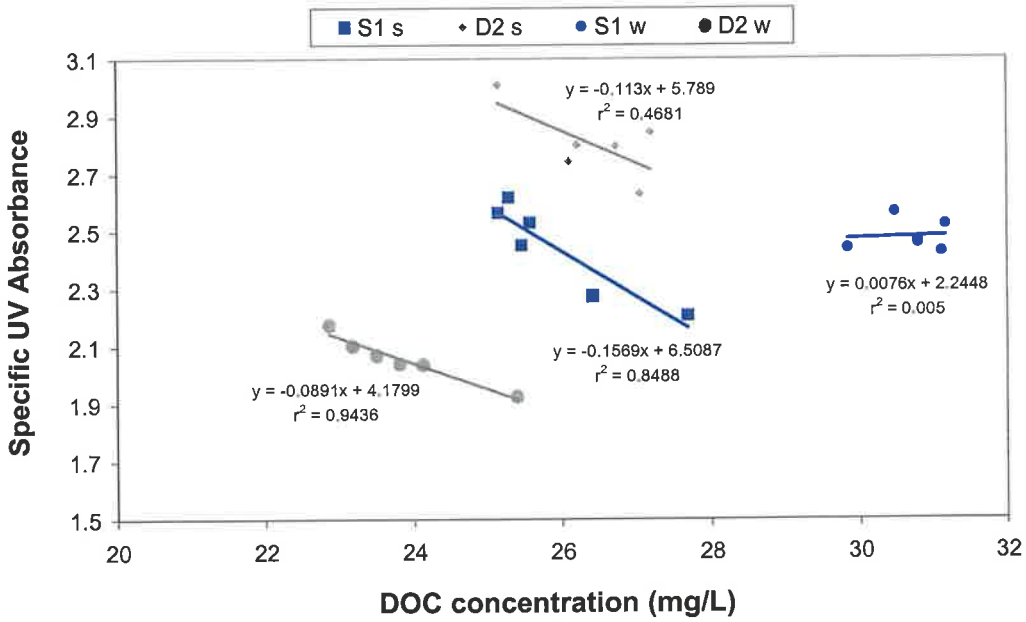


Figure 6. 13: Regression analysis of the short-term relationships between DOC concentration and Specific UV Absorbance (SUVA). S1s=shallow site summer; D2s=deep site summer; S1w=shallow site winter; D2w=deep site winter.

A positive relationship was found between DOC and bacterial numbers at the S1 site during summer (R=0.9, r^2=0.7), while an inverse relationship was found at the same site during winter (R=0.8, r^2=0.7) (Figure 6.14). At the deep site, an inverse relationship was found during both summer (R=0.9, r^2=0.8) and winter (R=0.6, r^2=0.4).

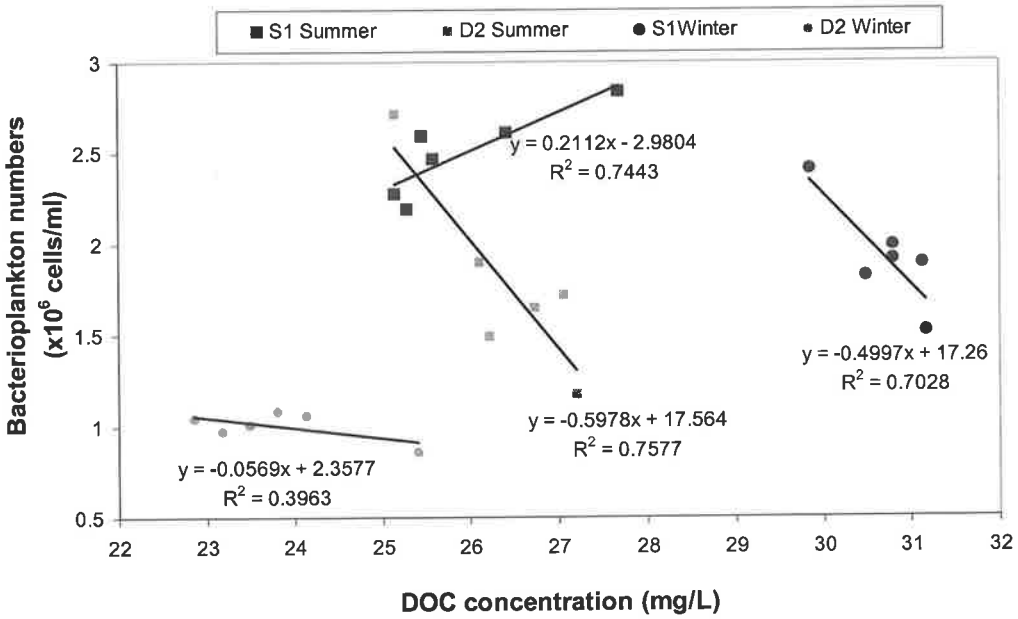


Figure 6. 14: Regression analysis of the short-term relationships between DOC concentration and bacterial numbers.

In terms of the relationship between DOC concentration and bacterial biomass, a positive relationship was found at the shallow site in summer (R=0.9, r^2=0.81), whereas an inverse relationship was found during winter (R=0.9, r^2=0.77) (Figure 6.15). An inverse relationship was found at the deep site in summer (R=0.8, r^2=0.68), while in winter an apparent inverse relationship trend was found to be not significant (R=0.7, r^2=0.48).

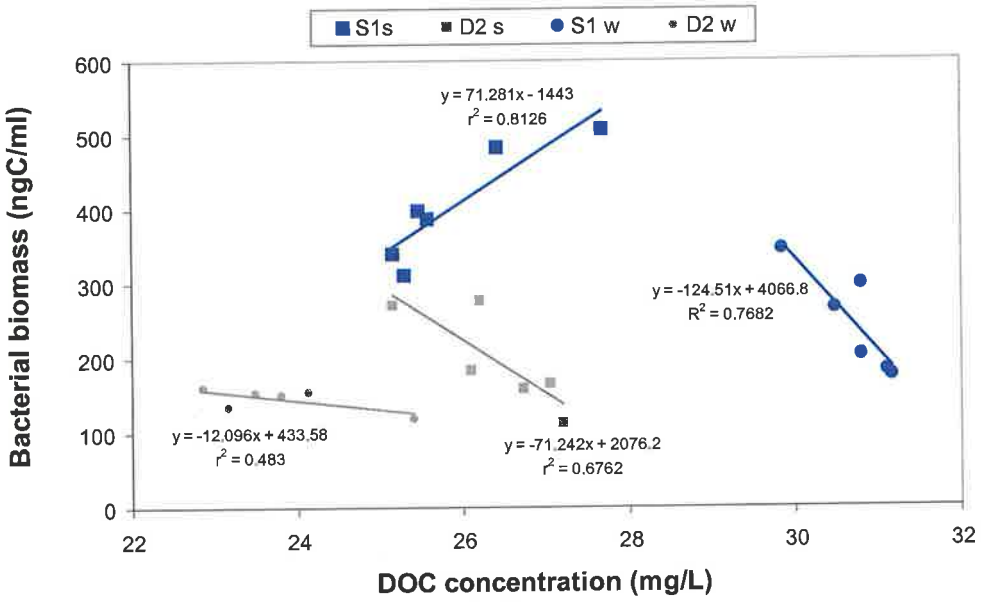


Figure 6. 15: Regression analysis of the short-term relationship between DOC concentration and bacterial biomass.

6.5 SHORT-TERM RELATIONSHIPS BETWEEN DOC NATURE AND WATER PARAMETERS

Changes in SUVA, E_2/E_3 and E_4/E_6 ratios were compared to the changes in chlorophyll levels, bacterial numbers and bacterial biomass in order to determine if there were short-term relationships between changes in DOC quality and changes in biological parameters. Correlations were also made between DOC quality and physical and chemical water parameters, such as temperature, dissolved oxygen and turbidity.

6.5.1 SUVA

No short-term relationships were found between SUVA and chlorophyll at either site during summer or winter. During summer strong relationships were found only at the shallow site between SUVA and bacterial numbers ($R=0.9$, $r^2=0.86$) (Figure 6.16) and biomass ($R=0.99$, $r^2=0.97$) (Figure 6.17).

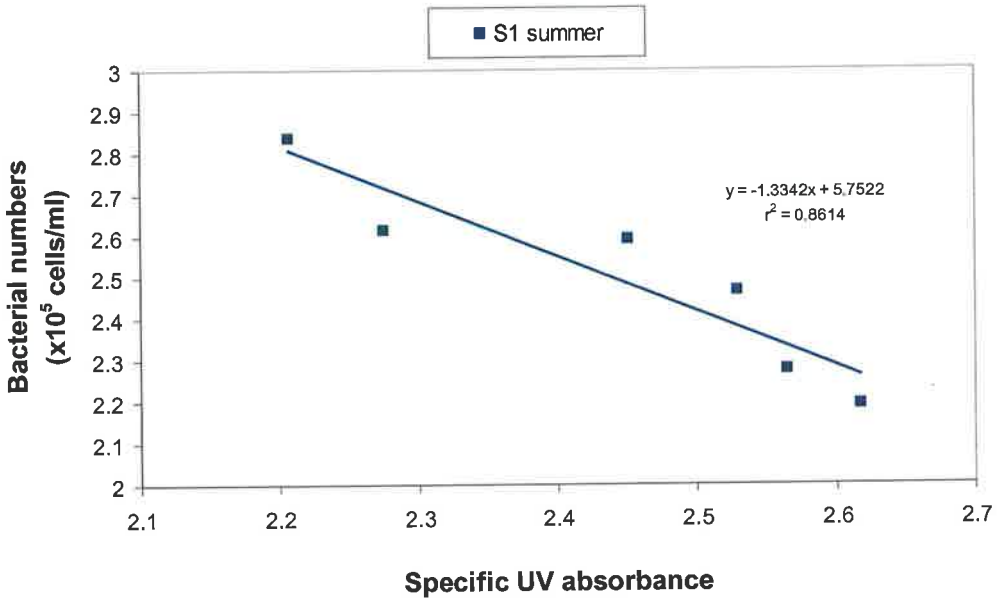


Figure 6. 16: Regression analysis of the short-term relationship between bacterial numbers and SUVA at the shallow site of the Warren Reservoir during the summer.

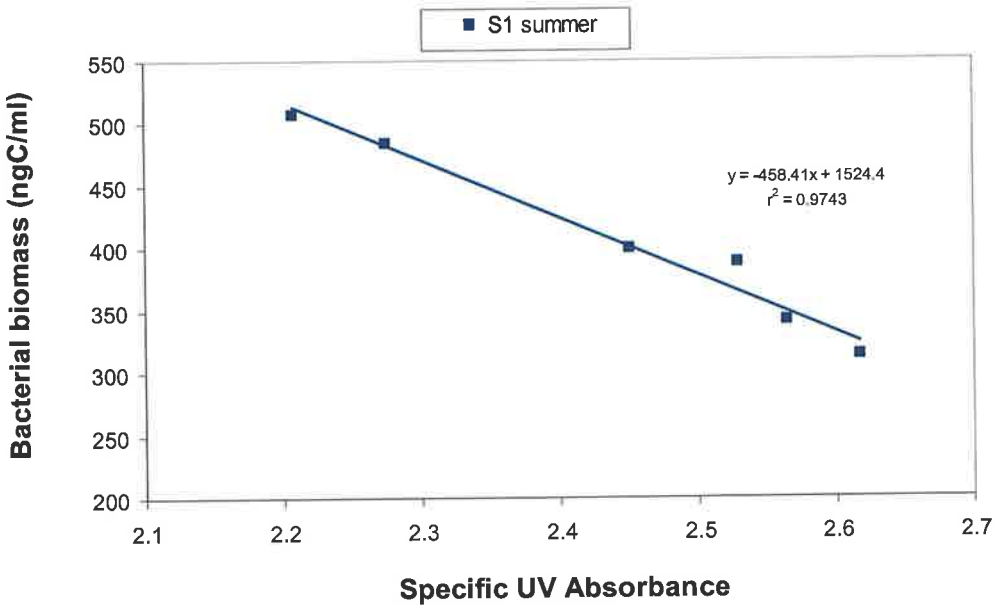


Figure 6. 17: Regression analysis of the short-term relationship between bacterial biomass and SUVA at the shallow site of the Warren Reservoir during summer.

6.5.2 E_2/E_3 ABSORBANCE RATIO

There was no diel relationships between E_2/E_3 absorbance ratios and chlorophyll levels at the S1 site during summer or winter, whereas inverse relationships were observed at the D2 site during both summer (R=0.9, r^2=0.8) and winter (R=0.7, r^2=0.5) (Figure 6.18).

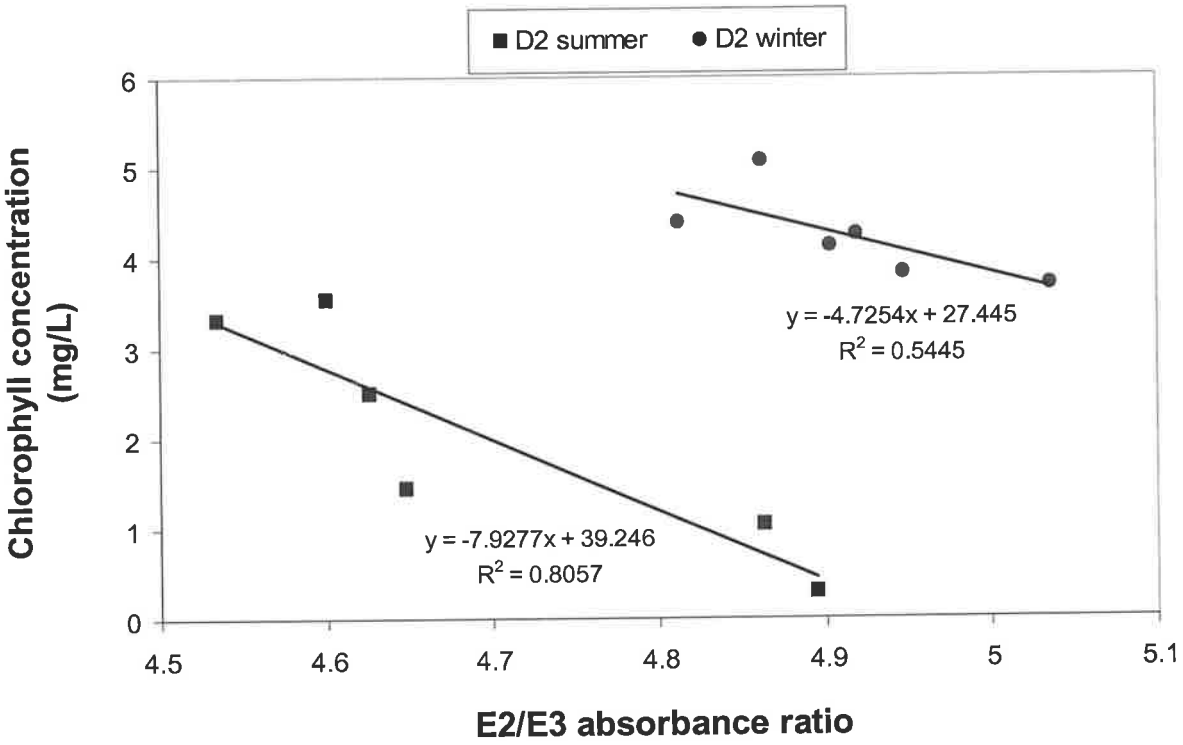


Figure 6. 18: Regression analysis of the short-term relationships between chlorophyll concentration and E_2/E_3 absorbance ratios at the deep site of the Warren Reservoir during both summer and winter.

Positive short-term relationships were also found between E_2/E_3 absorbance ratios and bacterial numbers and biomass (Figure 6.19 and 6.20 respectively) at the S1 site in summer (abundance R=0.8, r^2=0.68; biomass R=0.74, r^2=0.78) and at the D2 site in winter (abundance R=0.9, r^2=0.77; biomass R=0.85, r^2=0.82), whereas no significant relationships were found at the D2 site in summer (abundance R=0.26, r^2=0.07; cell biomass R=0.06, r^2=0.004) or the S1 site in winter (abundance R=0.46, r^2=0.22; biomass R=0.22, r^2=0.05) (data not shown).

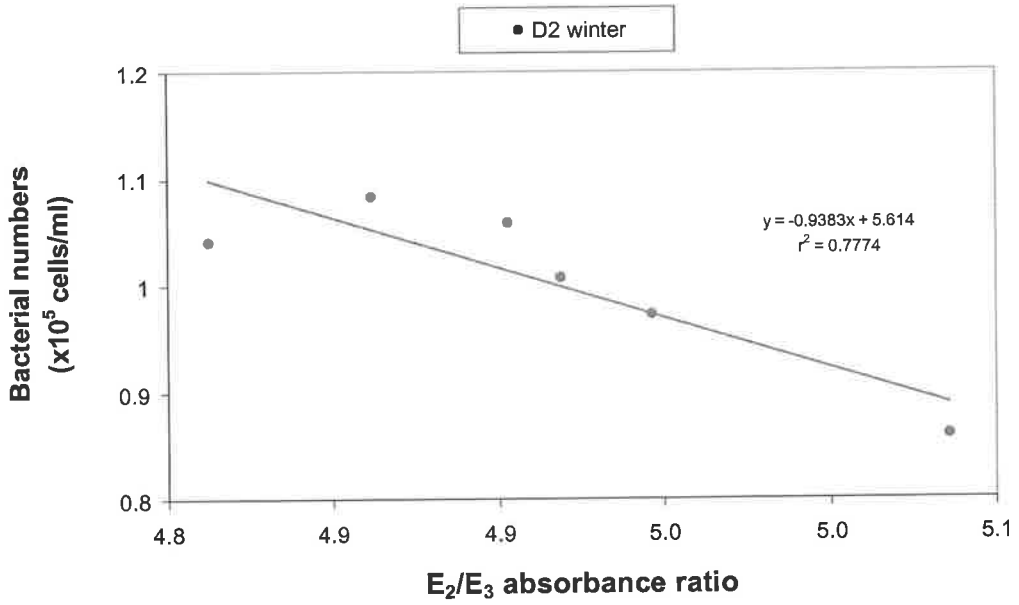


Figure 6. 19: Regression analysis of the short-term relationships between E_2/E_3 absorbance ratios and bacterial numbers.

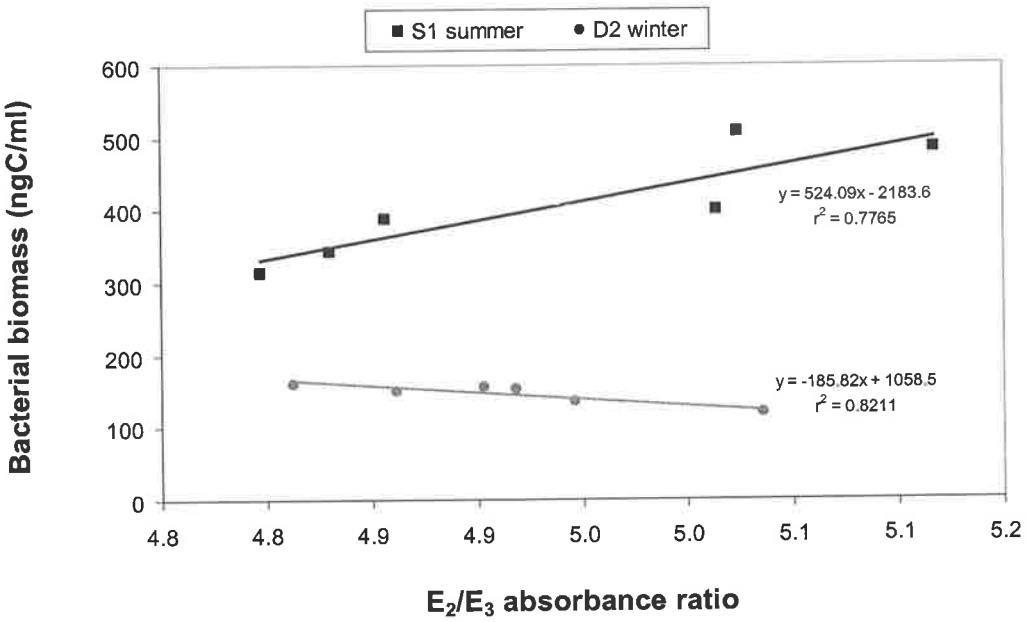


Figure 6. 20: Regression analysis of the diel relationship between E_2/E_3 absorbance ratio and the bacterial biomass. S1 summer R=0.74, r^2=0.78; D2 winter R=0.85, r^2=0.82.

6.5.3 E_4/E_6 ABSORBANCE RATIO

The relationship between E_4/E_6 ratio and chlorophyll levels is shown in Figure 6.21. An inverse relationship was obtained at the deep site during winter (R=0.8, r^2=0.65), while no obvious relationships were found at other times or sites (data not shown) (S1 summer R=0.3, r^2=0.07; D2 summer R=0.36, r^2=0.08; S1 winter R=0.5, r^2=0.27).

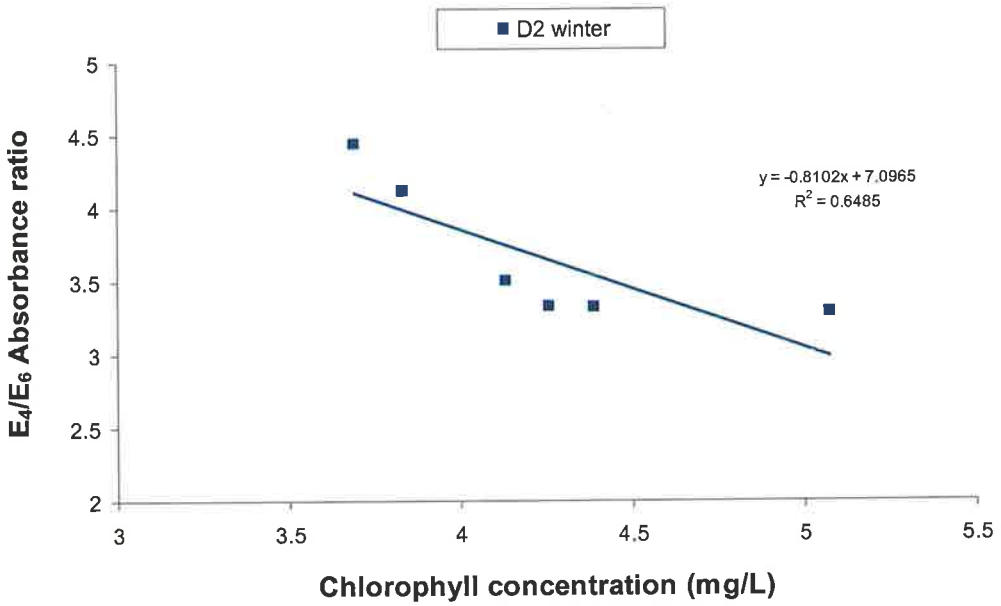


Figure 6. 21: Regression analysis of the short-term relationship between Chlorophyll levels and E4/E6 absorbance ratio at the deep site of the Warren reservoir during the winter diel study. R=0.8; r^2=0.7.

There were no apparent relationships between E_4/E_6 absorbance ratios and bacterial numbers at the S1 site in summer or winter and at the D2 site in summer (D2 summer R=0.2, r^2=0.04; S1 summer R=0.5, r^2=0.25; S1 winter R<0.1, r^2=0.04). An inverse relationship was obtained at the D2 site during winter (R=0.9, r^2=0.8) (Figure 6.22). The same observations were made for the relationship between E_4/E_6 absorbance ratio and bacterial biomass. No relationships were obtained at either site in summer (shallow site R=0.57, r^2=0.32; deep site R=0.1, r^2=0.01) or at the S1 site in winter (R=0.23, r^2=0.05). At the D2 site in winter, a

negative correlation was obtained between E_4/E_6 absorbance ratio and bacterial biomass ($R=0.95$, $r^2=0.9$) (Figure 6.23).

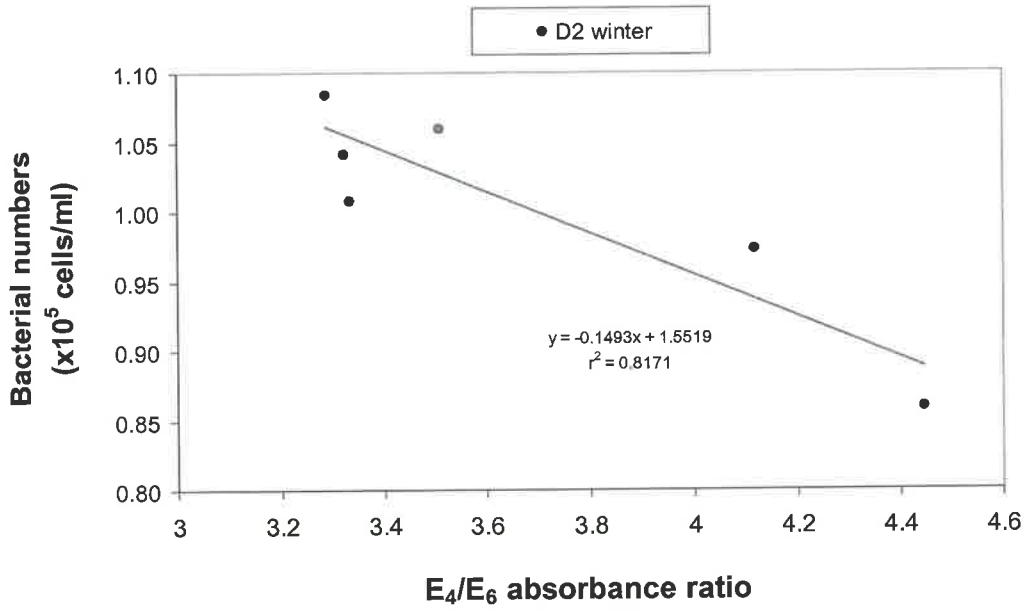


Figure 6. 22: Regression analysis of the short-term relationship between E_4/E_6 absorbance ratio and bacterial numbers.

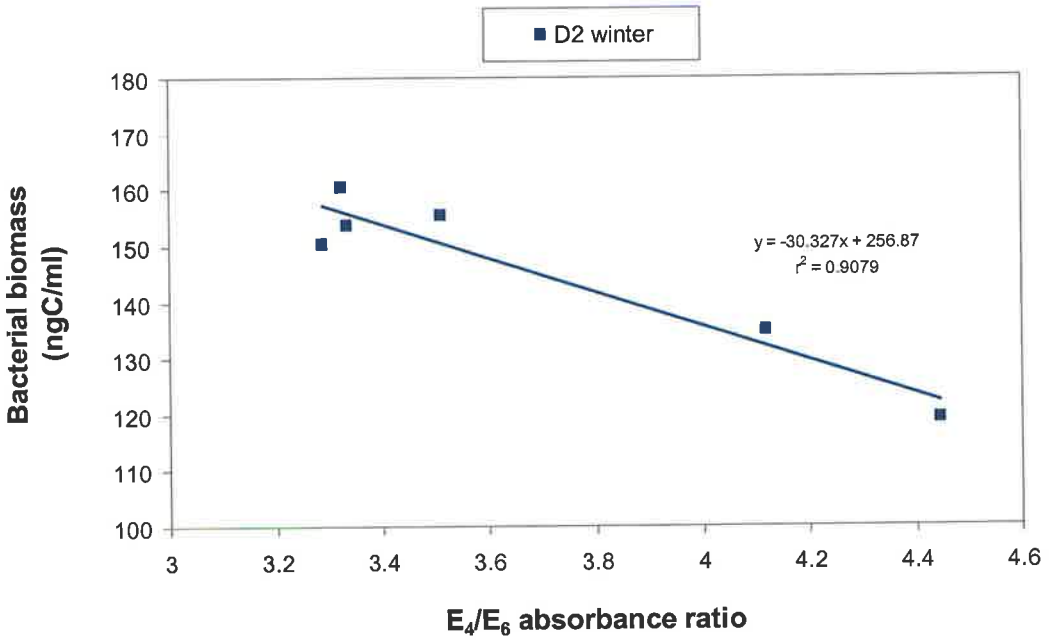


Figure 6. 23: Regression analysis of the short-term relationships between E_4/E_6 absorbance ratio and bacterial biomass.

6.6 SHORT-TERM RELATIONSHIPS BETWEEN CHLOROPHYLL AND WATER PARAMETERS

No apparent short-term relationships were found between chlorophyll levels and other water parameters such as bacterial numbers ($R<0.5$, $r^2<0.4$), biomass ($R<0.3$, $r^2<0.1$), nutrient levels ($R<0.5$, $r^2<0.3$), DOC levels ($R<0.3$, $r^2<0.1$), SUVA ($R<0.3$, $r^2<0.2$), temperature ($R<0.2$, $r^2<0.1$), oxygen ($R<0.1$, $r^2<0.1$), and turbidity ($R<0.2$, $r^2<0.1$) (data not shown).

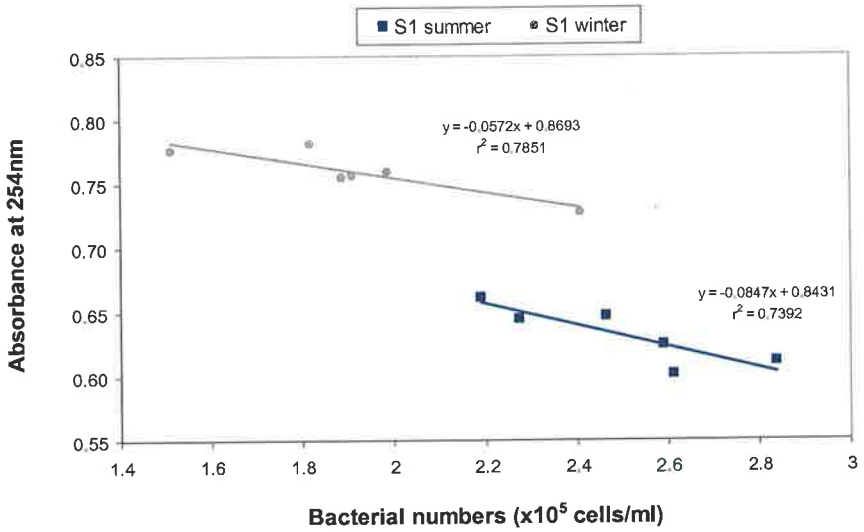
6.7 SHORT-TERM RELATIONSHIPS BETWEEN BACTERIAL NUMBERS AND WATER PARAMETERS

6.7.1 ABSORBANCE

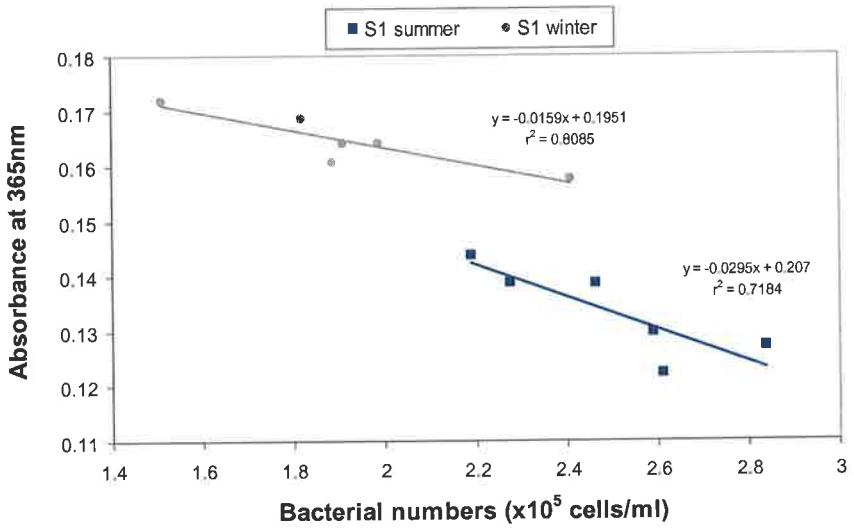
During the diel studies, a number of correlations were established between bacterial numbers and absorbance readings (Table 6.3). Scatter graphs were used to view the direction, form and strength of the relationships presented in Table 6.3, between bacterial numbers and absorbances (Figure 6.24A-D). Significant negative correlations were measured at the shallow site at all times whereas positive correlations were obtained at the deep sites. Decreasing correlations were obtained with increasing wavelengths at the shallow site. At the deep site in summer there was no relationship between absorbance at any wavelength and bacterial numbers, whereas in winter, increasing correlation was measured with increasing wavelengths. Although replication was applied during the study, care should be taken when establishing relationships with A_{465} and A_{665} as the absorbance values were low.

Table 6. 3: Correlation (R) and regression (r^2) analyses between bacterial numbers and absorbance readings at 254nm, 365nm, 465nm and 665nm. Bold data indicate significant relationships (at 95% confidence limit).

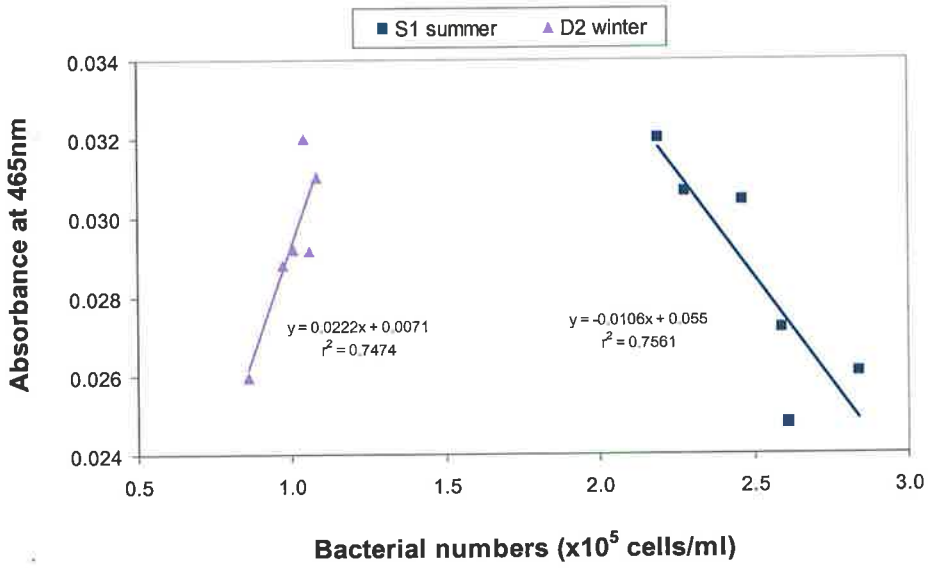
	Summer				Winter			
	Shallow		Deep		Shallow		Deep	
	R	r^2	R	r^2	R	r^2	R	r^2
A_{254}	**-0.86**	**0.74**	-0.08	0.01	**-0.89**	**0.79**	0.13	0.02
A_{365}	**-0.83**	**0.69**	-0.17	0.03	**-0.90**	**0.81**	0.68	0.47
A_{465}	**-0.85**	**0.72**	-0.29	0.08	-0.58	0.36	**0.86**	**0.75**
A_{665}	**-0.76**	**0.58**	0.03	0.01	-0.36	0.13	**0.90**	**0.81**



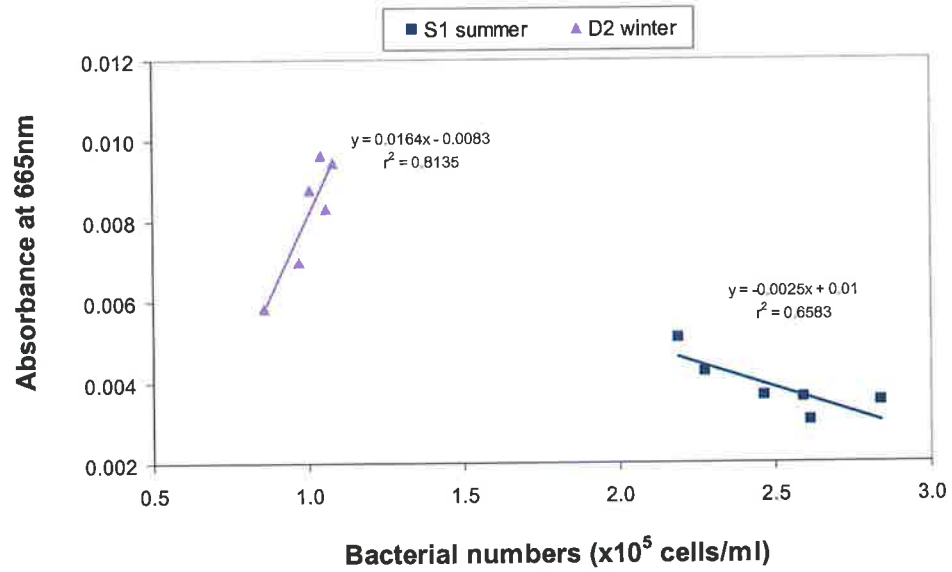
(A) A_{254}



(B) A_{365}



(C) A_{465}



(D) A_{665}

Figure 6. 24: Regression analysis of the short-term relationships between bacterial numbers and absorbances at 254, 365, 465 and 665nm. R and r^2 values are shown in Table 6.4.

6.7.2 ABSORBANCE RATIOS

An inverse relationship was found between bacterial numbers and E_2/E_3 (Figure 6.25) and E_4/E_6 (Figure 6.26) absorbance ratios at the deep site only during winter.

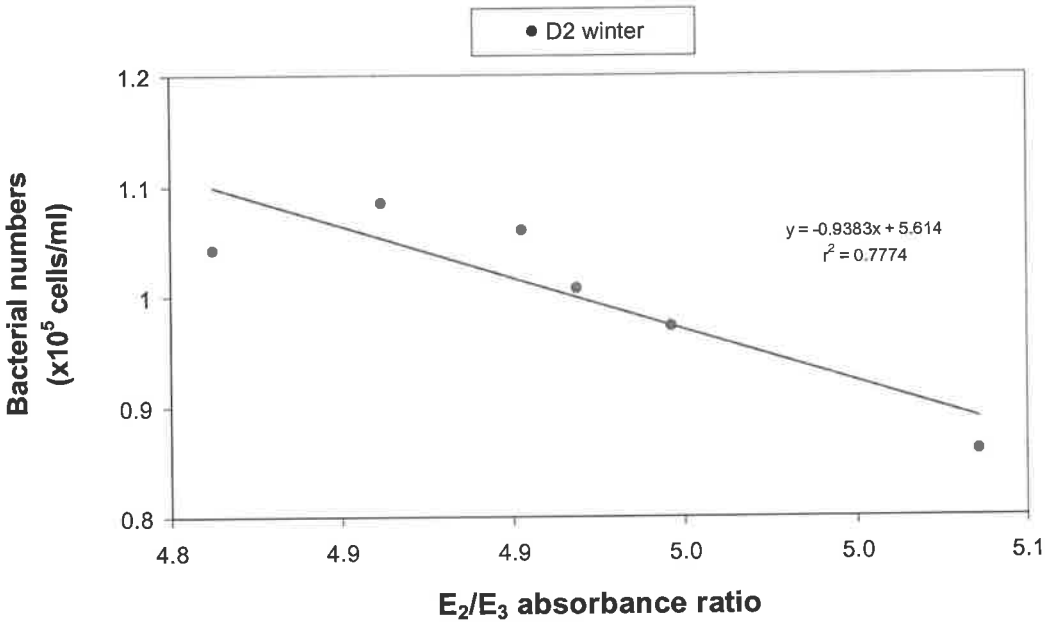


Figure 6. 25: Regression analysis of the short-term relationship between bacterial numbers and E_2/E_3 absorbance ratio (A_{250}:A_{365}) at the deep (D2) reservoir site during winter (R=0.88, r^2=0.78).

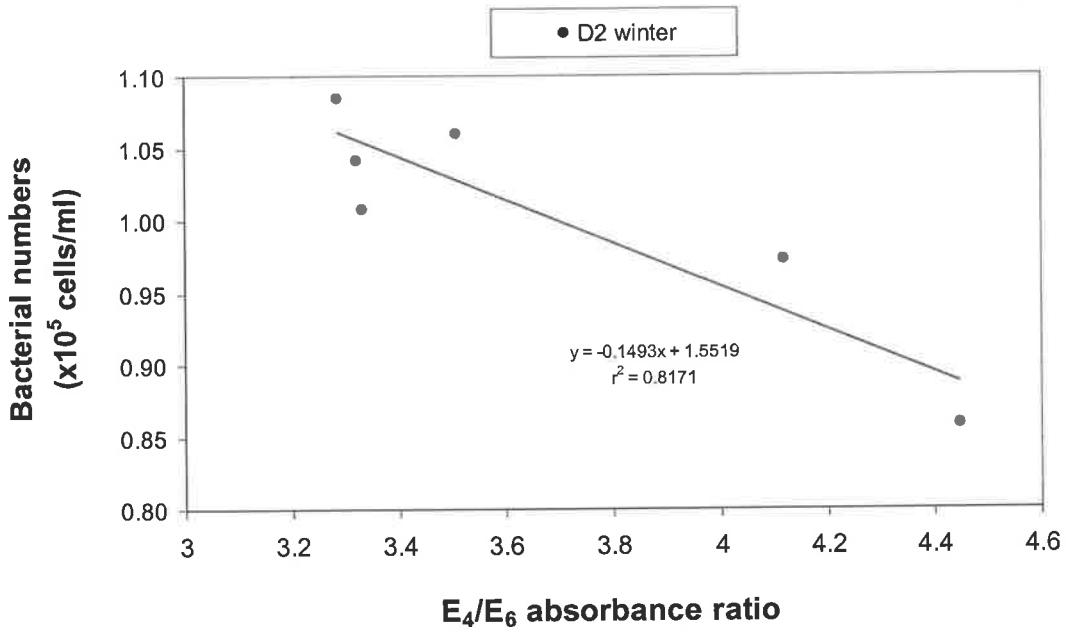


Figure 6. 26: Regression analysis of the short-term relationship between bacterial numbers and E_4/E_6 absorbance ratio (A_{465}:A_{665}) at the deep (D2) reservoir site during winter ($R=0.90$, $r^2=0.82$).

6.7.3 SUVA

An inverse relationship was found between bacterial numbers and specific UV absorbance (SUVA) at the shallow site only during summer (Figure 6.27). The same correlation at the shallow site in winter and at the deep site in summer and winter showed no obvious trend, with correlation coefficient values of $R<0.5$ and $r^2<0.3$ (data not shown).

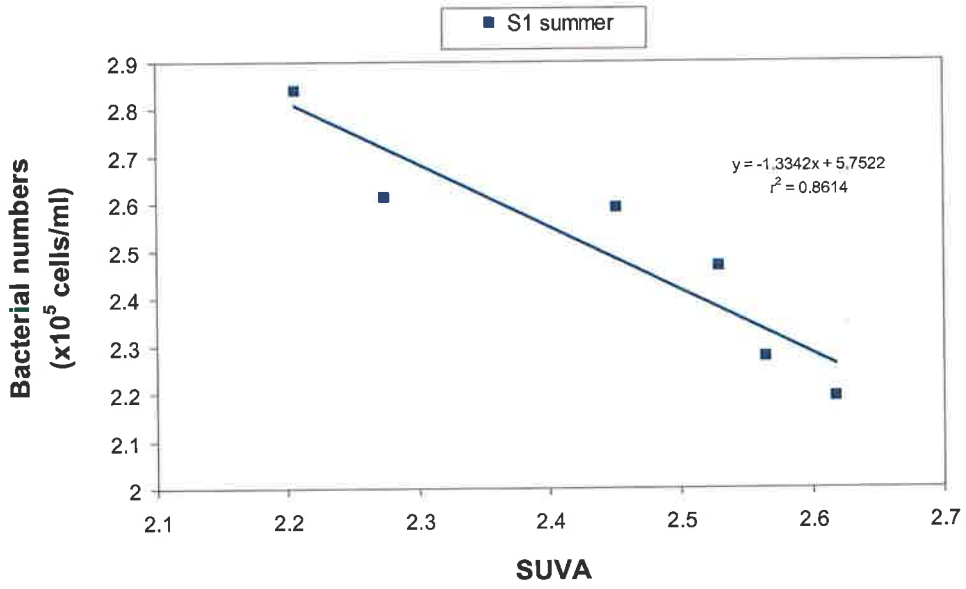


Figure 6. 27: Regression analysis of the relationship between bacterial numbers and SUVA at the shallow site in summer (R=0.90, r^2=0.86).

6.8 SHORT-TERM CHANGES IN BACTERIAL COMMUNITY COMPOSITION AND CORRELATIONS TO VARIOUS WATER PARAMETERS

A multivariate canonical correspondence analysis (CCA) was used to relate changes in bacterial community composition to changes in environmental variables. Figure 6.28A shows the separation of shallow samples during the diel summer study. This separation was based on changes in species composition caused by changing environmental variables. Figure 6.28B shows the environmental variables of that site.

CCA enabled all environmental variables to be ranked on the basis of the fit for each separate variable. The ranking implies the importance of each variable in determining the species composition and/or relative abundance as determined by the band intensities. The importance of each variable is summarised by Eigen values of the CCA (Table 6.4), aiding the data interpretation of ordination diagrams. The statistical significance of the effect of each variable was tested by a Monte Carlo permutation test.

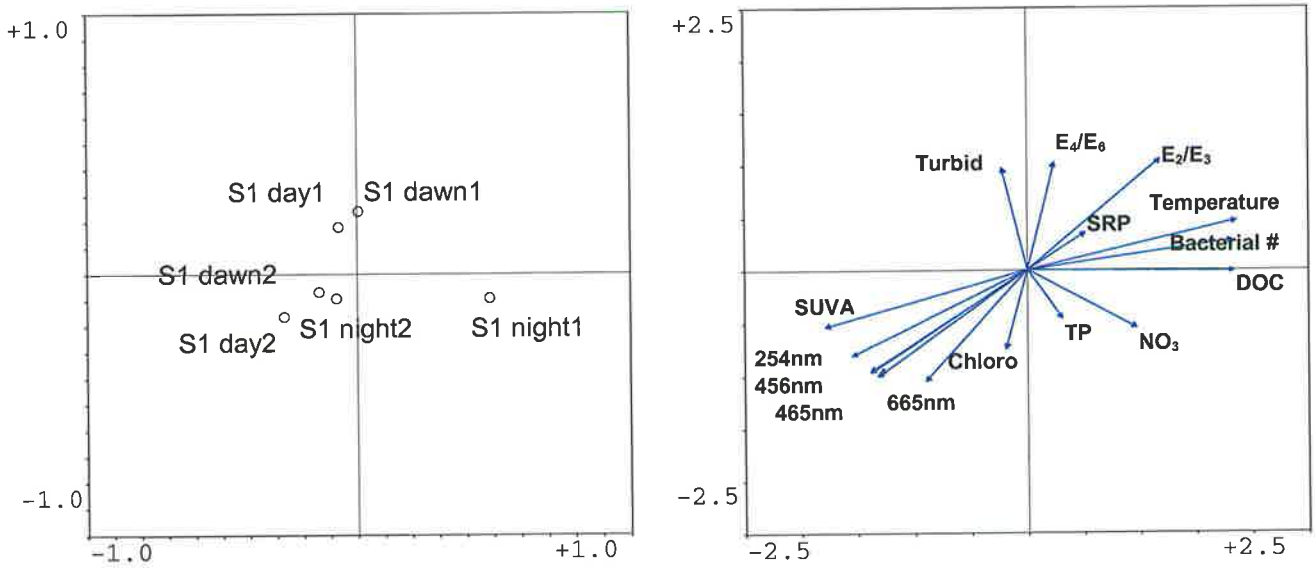


Figure 6. 28: Canonical correspondence analysis ordination diagram showing distribution of samples based on differences in bacterial populations during the diel summer study of the shallow site (weighted X axis = 51%; Y axis=20%). Differences between bacterial populations (A) were based on changes in environmental variables (B).

Analysis of bacterial populations during the 48-hour study suggested changes in the amount of individual species present (as loosely indicated by the banding intensity data) and not so much the type of species (as indicated by the band presence/absence information in Figure 6.5). The banding differences accounted for the separation of samples shown in Figure 6.28A, with no diel pattern found. Figure 6.28B and Table 6.4 suggest that bacterial numbers (competition) and DOC quality were the main driving forces behind the changes in community composition. Therefore, during summer, species competition was one of the parameters likely to control the amount and/or type of species within the S1 site. The amount of organic compounds that absorb light at 254nm was likely to be a second important parameter (as indicated by SUVA and A_{254}). Although temperature was also suggested to be an important parameter (as indicated by the vector position and direction in Figure 6.28B), its effects did not prove statistically significant (Table 6.4). Parameters such as turbidity,

nutrients and chlorophyll did not appear to play a significant role in altering species composition or abundance.

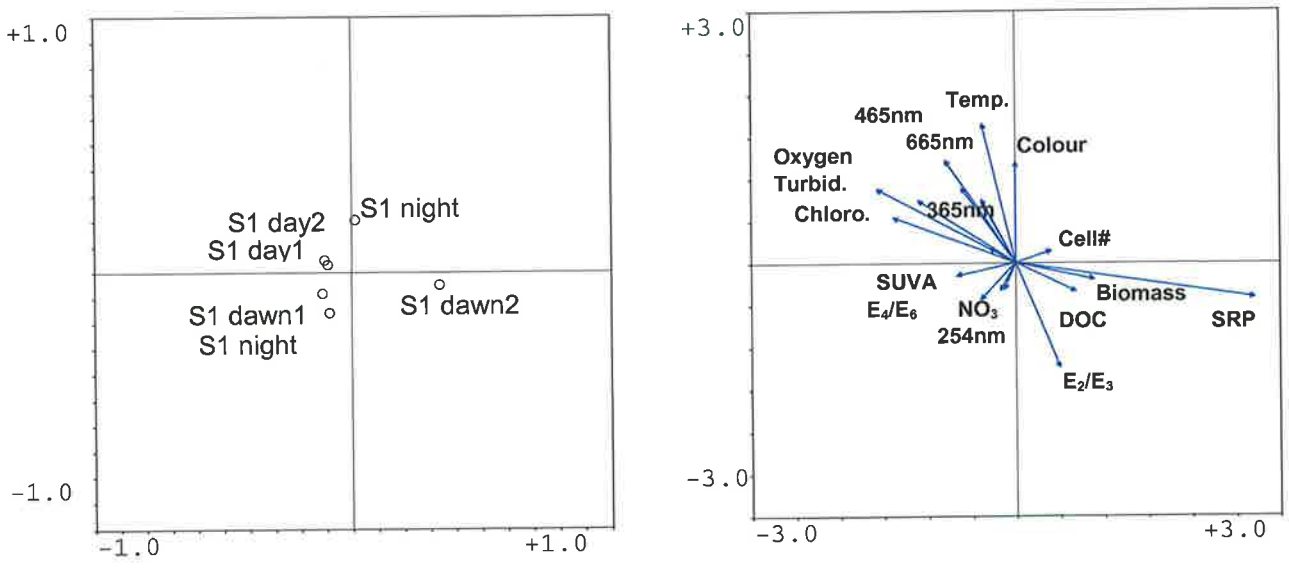
Table 6. 4: Eigen values ranking the importance of the correlation of environmental variables and bacterial community structure at the shallow site in summer. The P values showing statistical significance were based on the 95% significance level of the Monte Carlo permutation test. Significant variables are highlighted.

Environmental variables	Eigen	P
Bacterial numbers	**0.05**	**0.01**
DOC concentration	0.05	>0.1
SUVA	**0.05**	**0.005**
Temperature	0.05	>0.1
A_{254}	**0.04**	**0.04**
A_{465}	0.04	>0.05
A_{665}	0.03	0.06
E2/E3	0.03	>0.1
E4/E6	0.02	>0.1
Nutrients (TP,SRP,NO_3^-)	0.01	>0.1
Turbidity	0.01	>0.1
Chlorophyll	0.01	>0.1

The CCA ranking of winter environmental variables at the S1 site is presented in Table 6.5. Although SRP had the highest Eigen value, it did not prove statistically significant. Changes in temperature were the only significant parameter behind the short-term changes in bacterial community structure at the S1 site in winter. Parameters not presented in Table 6.5 had Eigen values of less than 0.01 and $p>0.05$. PCA showed daytime bacterial populations at the S1 site in winter to be more divergent from dawn and night populations (Figure 6.9A). CCA suggests that short-term changes in environmental variables did not generate diel patterns in species abundance and/or composition at the S1 site in winter (Figure 6.29A). Changes in temperature were the only likely influence behind the changes in species abundance and/or composition during daytime (Table 6.5 Figure 6.9A, Figure 6.29B).

Table 6. 5: Eigen values ranking the importance of the correlation of environmental variables and bacterial community structure at the shallow site in winter. The P values showing statistical significance were based on the 95% significance level of the Monte Carlo permutation test. Significant variables are highlighted.

Environmental variables	**Eigen**	**P**
SRP	0.03	0.14
Temperature	**0.03**	**0.03**
Oxygen	0.01	0.1
Chlorophyll	0.01	0.36
Turbidity	0.01	0.1
SUVA	0.01	0.76
A_{254}	0.01	0.12
A_{465}	0.01	0.5
E2/E3	0.01	0.6



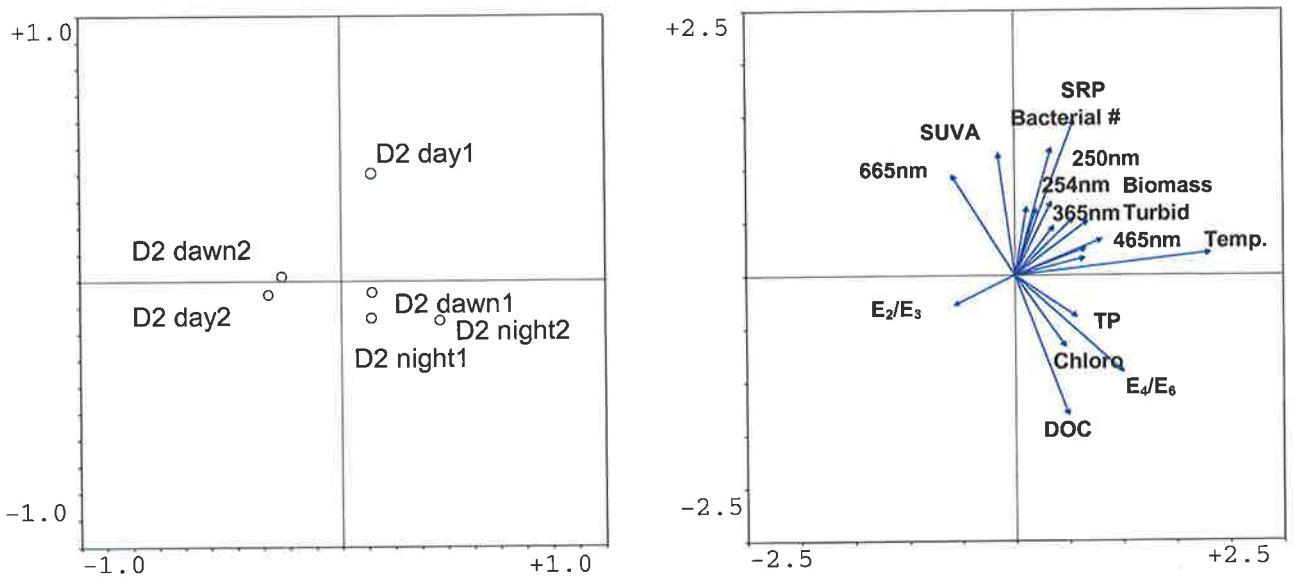
(A) *(B)*

Figure 6. 29: Canonical correspondence analysis ordination diagram showing distribution of samples based on differences in bacterial populations during the diel winter study of the shallow site (weighted X axis = 51%; Y axis=26%). Differences between bacterial populations (A) are based on changes in environmental variables (B).

The CCA ranking of summer environmental variables at the deep site is presented in Table 6.6. Short-term changes in environmental variables did not generate diel patterns in species abundance and/or composition at the deep site in summer. Temperature was the only variable that statistically supported short-term changes in bacterial populations at the deep site in summer (Table 6.6) without conforming to diel community patterns (Figure 6.30). Data on remaining variables studied are not shown as they had low Eigen values and $p>0.05$.

Table 6. 6: Eigen values ranking the importance of the correlation of environmental variables and bacterial community structure at the deep site (D2) in summer. The P values showing statistical significance were based on the 95% significance level of the Monte Carlo permutation test. Significant variables are highlighted.

Environmental variables	Eigen	P
Temperature	**0.04**	**0.02**
SRP	0.03	0.15
DOC	0.03	0.98
Bacterial numbers	0.02	0.27
SUVA	0.02	0.64
Chlorophyll	0.02	0.86
A_{254}	0.01	0.92



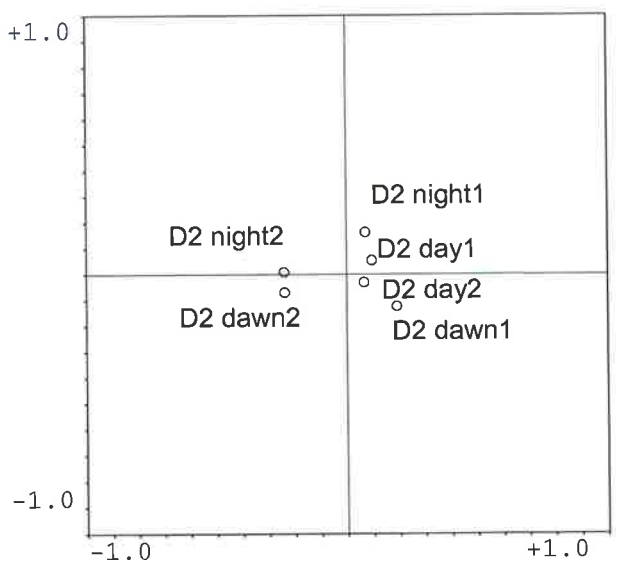
(A) *(B)*

Figure 6. 30: Canonical correspondence analysis ordination diagram showing distribution of samples based on differences in bacterial populations during the diel summer study of the deep site (weighted X axis = 39%; Y axis=25%). Differences between bacterial populations (A) are based on changes in environmental variables (B).

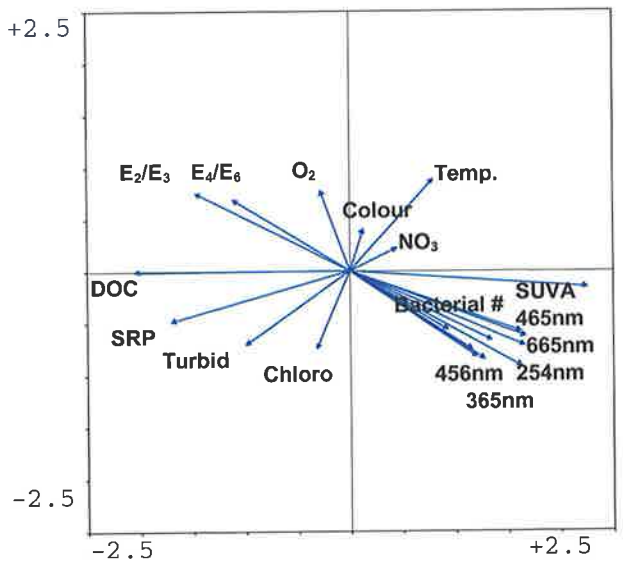
The CCA ranking of winter environmental variables at the deep site is presented in Table 6.7. Short-term changes in environmental variables did not generate diel patterns in species abundance and/or composition at the deep site in winter (Figure 6.31A). SUVA and DOC concentration were the only statistically significant variables causing shifts in community composition and/or abundance (Figure 6.31B, Table 6.7). Data on remaining variables studied are not shown as they had low Eigen values and $p>0.05$.

Table 6. 7: Eigen values ranking the importance of the correlation of environmental variables and bacterial community structure at the deep site in winter. The P values showing statistical significance were based on the 95% significance level of the Monte Carlo permutation test. Significant variables are highlighted.

Environmental variables	Eigen	P
SUVA	**0.02**	**0.05**
DOC concentration	**0.02**	**0.05**
SRP	0.02	0.22
A_{254}, A_{365}, A_{465}, A_{665}	0.01	>0.05



(A)



(B)

Figure 6. 31: Canonical correspondence analysis ordination diagram showing distribution of samples based on differences in bacterial populations during the diel winter study of the deep site (weighted X axis = 63%; Y axis=14%). Differences between bacterial populations (A) are based on changes in environmental variables (B).

6.9 DISCUSSION

The study presented in this chapter was designed to establish:

1. diel water quality patterns, with the main emphasis on DOC, bacteria and phytoplankton during summer and winter.
2. short-term relationships between various physical, chemical and biological parameters of the reservoir water.

6.9.1 DIEL WATER QUALITY PATTERNS

The first objective of the study was to determine whether the physical, chemical and biological water parameters, in summer and winter, form diel patterns. The main focus was on changes in DOC, phytoplankton and bacterial concentrations. Diurnal and nocturnal patterns were only measured for chlorophyll concentrations (representing phytoplankton biomass) during summer. In summer, high chlorophyll concentrations were always recorded during the night/dawn periods, irrespective of the reservoir sampling site. Thus diel chlorophyll patterns were present at both sites during the two summer diel cycles. Primary (algal) productivity is normally positively correlated with light levels required for photosynthesis, as well as temperature, thus the maximum photosynthetic activity is often obtained during daylight (DiSiervi et al. 1995; Kaplan and Bott 1982). Because chlorophyll levels are a measure of algal biomass, not productivity, it is probable that the time of maximum chlorophyll concentration (and thus biomass) measured in this study was displaced from the time of maximum productivity (which was not measured in this study), as suggested by Kaplan and Bott (1982) and DiSiervi et al. (1995).

Bacterial numbers fluctuated during each 48-hour cycle, irrespective of spatial or time factors, however they generated no apparent diel pattern. Such a finding is in agreement with the results reported by Straskrabova and Fuksa (1982) who studied changes in bacterial numbers and relative growth rates in the surface layers of a reservoir during several 24 hour cycles, conducted during summer and winter. These authors reported no difference between the mean values for diurnal and nocturnal periods; maximum and minimum values occurred sometimes during the night and sometimes during the day. Riemann and Sondergaard (1984) measured bacterial secondary production during 13 diel studies at various times of the year, in

marine and lake environments. They reported that no specific time periods during 24 hours showed significantly different production rates, supporting the idea that bacterial activities in natural assemblages are controlled by a variety of events. As the abundance of both summer and winter bacterial populations inhabiting the Warren Reservoir did not show a diel pattern, it appears that they are not inhibited or stimulated by UV-B radiation. The bacterial community composition study further supported the abundance data, suggesting no divergence in populations based on sampling time. Neither stimulatory nor inhibitory effects on bacterial species were observed *in situ* on the diurnal scale.

Diel DOC patterns were not observed, irrespective of the spatial and seasonal factors. These findings were in contrast to the results from a spring time study reported by Kaplan and Bott (1982). They showed significant changes in DOC concentrations that form a pattern of predawn minimum to a late afternoon maximum with a gradual decrease after sunset. They proposed changes in DOC concentrations to be related to changes in algal primary (autochthonous) production. Furthermore, the DOC patterns were concomitant with diel patterns of light and temperature. Based on the chlorophyll patterns observed in this diel study, DOC fluctuations during summer should have correlated to the diel patterns (as reported by Kaplan and Bott, 1982). It is possible that while diel patterns in algal biomass were observed (based on chlorophyll data), the levels of DOC released were not sufficient to cause significant changes to total DOC levels in the water body. Another explanation could be that DOC products originating from phytoplankton production were rapidly metabolised by bacteria at the same time that they were being produced by the algae (Cole et al., 1982; Banes and Pace, 1991). Short-term (<8 hours) DOC increases may not show a diel pattern if their consumption rapidly follows their production. Diel DOC results of the Warren Reservoir study, however, were in agreement with the reports by Dawson et al. (2001). These authors studied diurnal variations in dissolved and gaseous forms of carbon in two streams of a Scottish draining catchment, during spring, summer, autumn and winter. They found no statistically significant differences in DOC levels between day and night times in any of the sampling periods or sites. Dawson et al. (2001) related the lack of diel DOC pattern to the lack of temperature fluctuations. In the Warren Reservoir, there was no diel temperature pattern during either summer or winter.

While there was no diel pattern to DOC levels, DOC quality at the deep site (as demonstrated by A_{665} and E_4/E_6 absorbance ratios) showed a diel pattern. During winter, A_{665} was always minimal at night whereas the E_4/E_6 ratio during both summer and winter was at its

peak at the dusk, when compared to dawn and daylight. Changes in E_4/E_6 absorbance ratios are used as an indication of the changes in the degree of humification and changes to molecular weight distribution (Hautala et al., 2000). Thus it appears that following 10-12 hours of daylight and during dusk, higher levels of lower molecular weight humic organic material were present in the water compared to dawn and daylight. Hautala et al. (2000) showed that UV-Visible absorbance of humic organics can vary as a function of pH, but there was no fluctuation in pH at the deep site during this study. The magnitude of the ratio can also be related to the degree of condensation of the aromatic humic components (Simpson et al., 1997; Hautala et al., 2000). Low E_4/E_6 ratios have been associated with increased condensation of aromatic carbon and a greater degree of humification (Gressel et al., 1995). For example, humic acids have ratios between 3 and 5, whereas fulvic acids have ratios between 5.5 and 8 (Stevensen, 1982). As such, the E_4/E_6 ratio suggested higher condensation of aromatic carbon and increased structural complexity during winter than summer. These diel changes to the nature of DOC, based on the A_{665} and E_4/E_6 data should be interpreted cautiously, as the measured absorbances at these wavelengths were low during both summer and winter studies.

In terms of other water parameters studied, only O_2, turbidity and pH diel patterns were observed at the shallow site during summer and/or winter. In winter, both oxygen and turbidity levels were always higher during daytime and night than at dawn while the same was true for pH during summer. These patterns were only obtained at the shallow site where wind induced mixing of the shallow water column caused rapid stirring up of the sediment, which resulted in varying turbidity and dissolved oxygen levels.

6.9.2 SHORT-TERM WATER QUALITY RELATIONSHIPS

The second objective of this study was to analyse short-term relationships between DOC, chlorophyll, bacteria and other water parameters and to determine which parameters, if any, are responsible for short-term DOC accumulation and/or removal, and short-term bacterial growth inhibition and/or stimulation.

During seasonal studies of different water bodies, chlorophyll levels (reflecting the algal biomass) have been shown to be an important parameter for DOC loading in aquatic systems (Coveney and Wetzel, 1995; DiSiervi et al., 1995). During the diel study reported

here there was no short-term relationship between chlorophyll and DOC levels during either summer or winter, suggesting that organic carbon products from primary productivity were not responsible for short-term DOC accumulation in the Warren Reservoir. Observed short-term fluctuations in chlorophyll levels reflected the growth and death cycles of algae. At least some of the algal photosynthetic material is likely to be released into the water as DOC (as suggested by Cole et al. 1982). As there was no correlation between measured DOC and algal levels, it is possible that this DOC was rapidly utilised by bacteria. In one laboratory study, Hobbie (1992) demonstrated bacterial mineralisation of algae-derived DOC to occur within hours of release. This may suggest that products of algal primary production in the Warren Reservoir were rapidly removed from the water soon after their release from primary production.

In terms of the association between chlorophyll and bacteria, bacterial biomass and production can be coupled with phytoplankton biomass and production in many lakes but the intensity and the extent of coupling varies across trophic gradients (Lind et al., 1997). Phytoplankton can provide organic carbon to bacteria while bacteria, in their role as mineralisers, can provide phytoplankton with nutrients (DiSiervi et al., 1995). The important factor in this relationship, however, is the time scale of the coupling (Fuhrman et al., 1985; DiSiervi et al., 1995). As such, this association does not necessarily hold on smaller temporal scales (Coveney and Wetzel, 1995), and some studies have also shown this association not to hold on longer time scales (Findlay et al., 1998; Drakare et al., 2002). Diel fluctuations in bacterial abundance and biomass were observed during this study, but there was no correlation between either of these two parameters and chlorophyll levels. Long-term studies (>week) have shown strong correlations between algal primary and bacterial secondary production due to autochthonous release of DOC (Coveney and Wetzel, 1995; DiSiervi et al., 1995). However, during a seasonal study of the Warren Reservoir (Chapter 3), only a weak correlation was measured between algal and bacterial concentrations. A seasonal study by Bulon and Paveleva (1998) showed increasing numbers of bacterial cells per unit phytoplankton mass with decreasing chlorophyll levels. Conversely, a short-term study by DiSiervi et al. (1995) showed no diel correlation between maximum values of the two parameters. The results of this short-term diel study also showed no short-term correlation between the two parameters. Furthermore, there was no short-term correlation between chlorophyll levels and any of the physical or chemical parameters studied, such as temperature, dissolved oxygen, nutrients and pH.

In terms of DOC levels, a negative correlation was found between DOC levels and SUVA at the shallow site in summer, and the deep site in winter. This relationship suggested that during the short-term removal of DOC from water, organic molecules that absorb at 254nm were possibly more resistant to degradation and/or mineralisation. As SUVA is a measure of the relative aromaticity of organic matter, it appeared that the lower the aromaticity, the higher the DOC removal from water. Short-term correlations were established between DOC levels and bacterial biomass at the shallow end of the reservoir during both summer and winter, while no correlations were observed at the deep end of the reservoir. More data are required in order to determine the nature of this relationship, as DOC can be associated with bacteria in two ways: acting as a substrate and a source of energy (Findlay et al., 1998; Baines and Pace, 1991), or as a product released during bacterial turnover (Murray and Hodson, 1985). Thus it is possible that the nature of the relationship observed during summer was not the same as that in winter.

SUVA, E_2/E_3 and E_4/E_6 absorbance ratios were used to study the relationships between DOC character and other water parameters. Bacterial numbers and biomass showed strong correlations with SUVA at the shallow site in summer. The inverse relationships suggested that the lower SUVA values were associated with higher bacterial abundance and biomass. Thus, the lower the aromaticity, the greater the DOC removal (as shown by the DOC concentration data) by bacteria. Correlations were also established between E_2/E_3 ratios and bacterial abundance and biomass at the shallow end of the reservoir during summer. This relationship suggested that the higher the ratio, the higher the abundance and biomass. As the increase in E_2/E_3 ratio implies decreased aromaticity and/or molecular weight, the E_2/E_3 data were therefore in support of the SUVA data. While these correlations were obtained during summer, no correlations were obtained at the shallow site during winter. During summer the water was relatively calm facilitating the detection of potential changes in water quality. During winter, however, the shallow site was greatly influenced by wind conditions that stirred up the sediment, and this may have concealed changes in water parameters that may otherwise have been detected. Similar reasoning applies for the deep site. During summer no correlations were observed at the deep site as the artificial aeration was applied during daytime, causing stirring up of the sediment and mixing of the water body, thus affecting the detection of potential quality changes in the surface water. During the winter study period, however, the artificial aeration was not applied, and any short-term wind-induced stirring in the surface water would have caused lower degree of mixing (as the depth of the column is

>14m) (in comparison to that obtained during artificial mixing) which could explain the observed correlations. Inverse relationships between E_2/E_3 and E_4/E_6 ratios and chlorophyll were obtained, implying that the decrease in algal biomass was accompanied by increases in organic carbon with reduced aromaticity and reduced humification. This is consistent with the character of DOC generated by algae (Bell and Sakshaug, 1980; Amon and Benner, 1996). In addition, negative correlations were also established between E_2/E_3 and E_4/E_6 ratios and bacterial abundance and biomass. This suggests that decreasing bacterial abundance and biomass were accompanied by lower aromaticity and lower condensation of aromatic carbon and/or humification. Two interpretations are possible depending on which of the parameters (DOC quality or bacterial abundance and biomass) were the causal or dependent variables. Because primary production has been associated with lower aromaticity of organic products, the first possibility is that during winter, at the deep end of the reservoir, bacteria did not depend on primary production but relied on the organic carbon from allochthonous sources even on short-term scales. Some longer-term studies have indicated this to be the case (Findlay et al., 1998; Drakare et al., 2002). An implication of the second possibility is that the turnover of bacteria resulted in release of organic carbon of lower aromaticity and lower humic component. Based on the available data, it is not possible to determine the exact nature of the short-term association between bacteria and the nature of the DOC. The data indicate only an association between algal biomass, DOC and bacterial abundance and biomass. Further studies are required in order to make plausible suggestions into these relationships, as the complexities involved in determining the causal and dependent variables and comparing short and longer-term measurements are considerable.

Further correlation analyses also showed bacterial abundance to be positively related to bacterial biomass, suggesting a close coupling between the process of growth, productivity and cell division. Changes in bacterial abundance were also correlated with changes in UV-Vis absorbances at all wavelengths studied, mainly at the shallow site during summer and winter, and the deep site during winter only, again suggesting an association between bacterial growth and DOC character.

In terms of bacterial community structure, the main association appeared to be with temperature, and DOC nature and concentration. The canonical correspondence data indicated DOC quality to be one of the parameters involved in altering the type and/or abundance of individual species present. Much research has been conducted in recent years on the seasonal patterns of bacterial community composition in marine and freshwater

environments, and on studying differences in bacterial community composition between different trophic systems (Lindstrom, 2000; Diez et al., 2001; Baldy et al., 2002; Carlson et al., 2002; Christoffersen et al., 2002; Gasol et al., 2002; Muylaert et al., 2002; Schauer et al., 2003; Schultz Jr et al., 2003; Selje and Simon, 2003; Simek et al., 2003; Zwart et al., 2002; Zwisler et al., 2003). However, there is little data available regarding *in situ* diel changes in bacterial community composition, and the diel relationships with other water parameters. Schauer et al. (2003) compared changes in bacterial community composition between weekly and monthly sampling intervals, and found that the forces that shape bacterial community structure operate on monthly scales. These authors showed temperature and DOC source as the main factors affecting bacterial composition. These findings are in agreement with the findings from the Warren Reservoir seasonal study (Chapter 3), as well as this diel study, which also implicated changes in diel temperature and DOC character as important factors in structuring bacterial populations.

In one diurnal study, Gasol et al. (2002) performed a transplant experiment in a eutrophic reservoir to assess the factors that control bacterial abundance, activity, growth rate and community composition. They incubated samples from different parts of the reservoir in dialysis bags, placed *in situ* and transplanted to other parts of the reservoir. During this study sampling was conducted at 12 hour intervals, and the authors found that some bacterial species were inhibited by predators, while others were stimulated. As such, presence of predators caused strong changes in bacterial assemblage composition within 24 hours. In the same study, nutrient supply was also found to affect bacterial community composition. Predation was not assessed during the Warren Reservoir study, but nutrient levels did not appear to be important variables in structuring bacterial communities.

Other, non-diel studies, have shown relationships between bacterial community composition and various water parameters. For example, a study by Muylaert et al. (2002) showed seasonal changes in bacterial community composition to be related to bottom-up (resources) and top-down (grazers) variables. That is, the bacterial community structure was dependent on the dominant substrate source (thus the character of DOC) as well as on the food web structure. Zwisler et al. (2003) studied seasonal patterns of bacterial community composition in a mesotrophic lake and found distinct bacterial clusters to be associated with phytoplankton blooms. Lindstrom (2000) studied bacterial composition in five lakes differing in trophic status and humic content, and found the nutrient content of the lakes as well as biomasses of micro-zooplankton, cryptophytes and chrysophytes to be strongly correlated

with changes in community composition. Carlson et al. (2002) showed changes in organic and inorganic pools to affect bacterial community composition. Although various water parameters are known to affect bacterial community composition (as indicated here), no determinations have been made on a short-term basis and *in situ.*

Based on short-term scale correlations, the results from the study of the Warren Reservoir provide basis for designing further studies to evaluate the possibility of altering species composition in reservoirs in order to maximise DOC removal.

6.10 CONCLUSION

Short-term DOC accumulations were not observed during either summer or winter. Because fluctuations in algal biomass were measured between the 8 hour sampling intervals, internal input of DOC probably did occur but was not detected. DOC products of algal growth were probably rapidly utilised by the bacterial populations and did not cause accumulation to any measurable levels. Although correlations were not observed between DOC levels and either phytoplankton or bacteria, short-term relationships were established between DOC nature and these parameters, implying that relationships exist on a short-term scale between phytoplankton biomass, DOC and bacteria. Algal biomass was possibly responsible for altering the nature of the DOC pool by releasing autochthonous DOC that lowered the relative amount of aromatic compounds in the total DOC pool. The association of bacteria and DOC is a more complex issue. Correlations were found between DOC character and bacterial abundance and biomass. Whether DOC character alters the abundance and biomass of bacteria, or whether bacterial turnover alters the overall character of the DOC pool, or both, remains unknown. The former is a likely possibility as the community composition data has shown DOC quality to be a significant parameter in changing the type and/or amount of the bacterial species in the reservoir.

CHAPTER 7 GENERAL CONCLUSION

Colour, taste and odour arising from DOC and recurrent algal blooms are a major water quality problem, causing significant treatment costs in South Australia (Hines and Bursill, 1987). Warren Reservoir is one such water body affected by high levels of total and coloured DOC and recurrent algal blooms. The study of the Warren Reservoir was primarily implemented to establish the patterns in the DOC variation, both in terms of quality and quantity. The aim was to determine which sources, autochthonous or allochthonous, influence changes in the concentration and nature of reservoir DOC, and ultimately the aesthetic properties of the reservoir. Seasonal and annual variations in the concentration and nature of DOC in the Warren Reservoir were observed over the period of study. Furthermore, the contribution of autochthonous and allochthonous DOC was found to undergo strong seasonal variation. Internal production of DOC by the reservoir phytoplankton communities contributed to increased summer loading of the DOC, while the rainfall events, driving the catchment hydrological processes, contributed to the winter increases in DOC concentrations. The winter increases in the reservoir DOC were mainly caused by the high inflow of water from the two catchment streams, C-S1 and C-S2, located at the shallow end of the reservoir. This area is dominated by pine plantation that has, in a previous study by Recknagel et al. (1998), been shown to release relatively high DOC and SRP concentrations through the catchment run-off. In addition to the observed influences of these two naturally occurring processes, and during the course of the study, the inflow of water from the Murray River pipeline became a third source that caused variations in DOC concentrations. As the level of Murray River water entering the reservoir varied during the study, the contribution of the Murray River waters to the annual DOC budgets also differed between years. In 1998, 4584 kg of DOC was added to the reservoir via the Murray River inflow, when the Murray River pipeline was open for three months. This contribution increased to 29369 kg during 1999, 22448 kg during 2000, and 28160 kg during 2001. In comparison, the annual contributions of catchment stream DOC were estimated as 12000 kg, approximately half the input from the Murray River water.

The continuous loading of DOC from the Murray River pipeline from 1999 onward was concurrent with the annual increases in reservoir DOC levels, and as such may have been behind the cause of DOC 'accumulation' in the reservoir. However, algal blooms between

1999 and 2001 were also concurrent with the increased annual concentrations of DOC in the reservoir, showing a strong temporal correlation. It appears that a combination of algal blooms and inflow of Murray River water were responsible for observed annual increases in reservoir DOC levels.

The specific colour of water, although not being a direct measure of the chemical structure of DOC, was an indirect measure of the allochthonous DOC input to the reservoir. Specific colour conformed to a seasonal pattern, increasing during the winter inflow of stream water from the catchment into the reservoir, and decreasing during the dry summer months when autochthonous DOC loading was dominating. Furthermore, decreases in the mean annual specific colour during the course of the study were concurrent with the increases in algal growth and the inflow of Murray River water. Aesthetic properties of the Warren Reservoir water, (namely the total colour), were also found to deteriorate during winter and appeared to be caused mainly by the presence of allochthonous DOC brought into the reservoir by weathering and erosion and via two main streams from the catchment. Depending on whether the priority of the water industry is to control the aesthetic issues of water quality, such as colour and/or control the DOC levels in the Warren Reservoir, focus should be placed on a combination of factors such as the catchment runoff, Murray River inflow or phytoplankton growth. Reducing overall DOC levels of the reservoir could potentially be achieved by implementing management strategies that would vary seasonally. For example, summer management may involve copper sulphate treatments with or without implementing artificial aeration (which was shown by Recknagel et al. (1998) to be a successful algal bloom management tool) to control phytoplankton levels. Copper sulphate treatment alone did not completely manage the algal bloom in 2001, thus a combination of the two strategies would probably prove more effective. On the other hand, winter strategies could include change of land use in the catchment, pre-treatment of run-off water by riparian wetlands and/or DOC fixation in catchment soils by, for example, lime and gypsum. The winter management strategies, although potentially useful, would need to be applied on a per reservoir basis, to ensure that a suitable strategy is applied to this particular reservoir because different types of catchments will respond differently to different types of management.

In terms of assessing the natural processes that impact on DOC levels in the Warren Reservoir, this study applied the use of UV-B radiation to simulate the natural solar UV-B conditions. This was done to test the ability of solar UV-B radiation to remove reservoir

DOC or to increase its bioavailability and thus its subsequent removal from the Warren Reservoir by bacteria.

The summer reservoir DOC pool was found to be more susceptible to direct UV-B radiation leading to mineralisation, whereas the winter reservoir DOC pool was more susceptible to UV-B degradation and thus structural alteration, generating better substrates for bacterial growth. The different responses of summer and winter DOC pools to UV-B radiation, however, are believed to be due to the fact that summer DOC pool had already been exposed to significant levels of natural solar UV-B radiation prior to the study, thus the structural changes that were observed in the winter DOC pool may already have taken place *in situ*. In any case, a combination of solar UV-B radiation and bacterial mineralisation are expected to contribute toward DOC removal from the Warren Reservoir during both summer and winter. Furthermore, both the field and laboratory data indicates that changes in the nature of DOC caused changes to reservoir bacterial community structures. The DGGE analysis proved to be a useful tool to study changes in bacterial populations, as a starting point for future studies into interactions of DOC and bacteria (which are the primary DOC consumers in pelagic waters). DGGE analysis indicated that bacterial populations of the Warren Reservoir changed between seasons, and these changes were found to be mainly parallel to the changes in the nature of DOC. Likewise, the laboratory data indicated that bacterial populations of the Warren Reservoir changed following UV-B induced structural alterations in the DOC pool. Thus, the use of the DGGE technique (extended to band sequencing for species determination) could be applied in correlation with any further studies into the DOC chemistry, in order to determine which bacterial species have more affinity toward which types of DOC molecules. Ultimately, there is potential for bacterial populations in the Warren Reservoir to be manipulated to enhance the DOC removal. Further studies should focus on structural identifications of the DOC in the Warren Reservoir in order to identify the least UV-B or bio-degradable components of the total DOC pool. By adopting this approach, there is potential for future reductions in DOC levels in the Warren Reservoir via a combination of solar and bio-manipulations.

Because UV-B radiation and bacterial growth are two parameters that occur simultaneously in the natural environment, this study also tested the potential for UV-B inhibition of bacterial growth and ultimately bacterial mineralisation of the DOC in the Warren Reservoir. Based on the findings of this study, the total bacterial population mortality due to UV-B irradiation is unlikely to result in DOC accumulation in the Warren Reservoir

during either summer or winter, as a result of either reduced bacterial consumption of DOC or as a result of contribution to the reservoir DOC pool via bacterial turnover.

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APPENDIX 1

Hourly measurements of water entering from C-S1 into the Warren Reservoir during winter 1999.

Date	Time	Volume (L)
14-Jul-99	14:00:00	0
14-Jul-99	15:00:00	0
14-Jul-99	16:00:00	1903
14-Jul-99	17:00:00	1866
14-Jul-99	18:00:00	1866
14-Jul-99	19:00:00	1866
14-Jul-99	20:00:00	1830
14-Jul-99	21:00:00	1830
14-Jul-99	22:00:00	1794
14-Jul-99	23:00:00	1794
15-Jul-99	00:00:00	1794
15-Jul-99	01:00:00	1758
15-Jul-99	02:00:00	1758
15-Jul-99	03:00:00	1758
15-Jul-99	04:00:00	1758
15-Jul-99	05:00:00	1758
15-Jul-99	06:00:00	1758
15-Jul-99	07:00:00	1758
15-Jul-99	08:00:00	1758
15-Jul-99	09:00:00	1723
15-Jul-99	10:00:00	1758
15-Jul-99	11:00:00	1758
15-Jul-99	12:00:00	1723
15-Jul-99	13:00:00	4101
15-Jul-99	14:00:00	4101
15-Jul-99	15:00:00	4102
15-Jul-99	16:00:00	4101
15-Jul-99	17:00:00	1720
15-Jul-99	18:00:00	1389
15-Jul-99	19:00:00	1389
15-Jul-99	20:00:00	1389
15-Jul-99	21:00:00	1389
15-Jul-99	22:00:00	1389
15-Jul-99	23:00:00	1360
16-Jul-99	00:00:00	4080
16-Jul-99	01:00:00	4081
16-Jul-99	02:00:00	4080
16-Jul-99	03:00:00	4167
16-Jul-99	04:00:00	4167
16-Jul-99	05:00:00	4080
16-Jul-99	06:00:00	4081
16-Jul-99	07:00:00	4080
16-Jul-99	08:00:00	1360
16-Jul-99	09:00:00	1360
16-Jul-99	10:00:00	1360
16-Jul-99	11:00:00	1332
16-Jul-99	12:00:00	1331
16-Jul-99	13:00:00	1332
16-Jul-99	14:00:00	1304
16-Jul-99	15:00:00	1331
16-Jul-99	16:00:00	1332
16-Jul-99	17:00:00	1303
16-Jul-99	18:00:00	1303
16-Jul-99	19:00:00	1304
16-Jul-99	20:00:00	1303
16-Jul-99	21:00:00	1303
16-Jul-99	22:00:00	1303
16-Jul-99	23:00:00	1303
17-Jul-99	00:00:00	1304
17-Jul-99	01:00:00	1303
17-Jul-99	02:00:00	1303
17-Jul-99	03:00:00	1303
17-Jul-99	04:00:00	1303
17-Jul-99	05:00:00	1304
17-Jul-99	06:00:00	1303
17-Jul-99	07:00:00	1303
17-Jul-99	08:00:00	1738
17-Jul-99	09:00:00	1701
17-Jul-99	10:00:00	1701
17-Jul-99	11:00:00	2377
17-Jul-99	12:00:00	2430
17-Jul-99	13:00:00	2430
17-Jul-99	14:00:00	2430
17-Jul-99	15:00:00	2430
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17-Jul-99	17:00:00	2430
17-Jul-99	18:00:00	2430
17-Jul-99	19:00:00	2430
17-Jul-99	20:00:00	2430
17-Jul-99	21:00:00	2430
17-Jul-99	22:00:00	2430
17-Jul-99	23:00:00	2430
18-Jul-99	00:00:00	2430
18-Jul-99	01:00:00	2430
18-Jul-99	02:00:00	2430
18-Jul-99	03:00:00	2430
18-Jul-99	04:00:00	1545
18-Jul-99	05:00:00	1545
18-Jul-99	06:00:00	1545
18-Jul-99	07:00:00	1545
18-Jul-99	08:00:00	1579
18-Jul-99	09:00:00	1545
18-Jul-99	10:00:00	1545
18-Jul-99	11:00:00	1545

Date	Time	Volume (L)
18-Jul-99	12:00:00	1961
18-Jul-99	13:00:00	2021
18-Jul-99	14:00:00	1801
18-Jul-99	15:00:00	2789
18-Jul-99	16:00:00	3080
18-Jul-99	17:00:00	3080
18-Jul-99	18:00:00	3079
18-Jul-99	19:00:00	2482
18-Jul-99	20:00:00	2117
18-Jul-99	21:00:00	2343
18-Jul-99	22:00:00	3577
18-Jul-99	23:00:00	4374
19-Jul-99	00:00:00	34282
19-Jul-99	01:00:00	93664
19-Jul-99	02:00:00	209113
19-Jul-99	03:00:00	317429
19-Jul-99	04:00:00	293876
19-Jul-99	05:00:00	256928
19-Jul-99	06:00:00	191415
19-Jul-99	07:00:00	177897
19-Jul-99	08:00:00	147286
19-Jul-99	09:00:00	176588
19-Jul-99	10:00:00	215357
19-Jul-99	11:00:00	218260
19-Jul-99	12:00:00	269172
19-Jul-99	13:00:00	427394
19-Jul-99	14:00:00	333181
19-Jul-99	15:00:00	316896
19-Jul-99	16:00:00	274372
19-Jul-99	17:00:00	258937
19-Jul-99	18:00:00	229431
19-Jul-99	19:00:00	213947
19-Jul-99	20:00:00	182341
19-Jul-99	21:00:00	195343
19-Jul-99	22:00:00	202511
19-Jul-99	23:00:00	279513
20-Jul-99	00:00:00	316359
20-Jul-99	01:00:00	365558
20-Jul-99	02:00:00	317750
20-Jul-99	03:00:00	280014
20-Jul-99	04:00:00	305179
20-Jul-99	05:00:00	261950
20-Jul-99	06:00:00	208659
20-Jul-99	07:00:00	223104
20-Jul-99	08:00:00	210526
20-Jul-99	09:00:00	225932
20-Jul-99	10:00:00	184158
20-Jul-99	11:00:00	191614
20-Jul-99	13:00:00	155639
20-Jul-99	14:00:00	153091
20-Jul-99	15:00:00	142249

Date	Time	Volume (L)
20-Jul-99	16:00:00	138985
20-Jul-99	17:00:00	130561
20-Jul-99	18:00:00	121396
20-Jul-99	19:00:00	106822
20-Jul-99	20:00:00	104880
20-Jul-99	21:00:00	99687
20-Jul-99	22:00:00	104660
20-Jul-99	23:00:00	101875
21-Jul-99	00:00:00	101115
21-Jul-99	01:00:00	94882
21-Jul-99	02:00:00	92296
21-Jul-99	03:00:00	91358
21-Jul-99	04:00:00	84103
21-Jul-99	05:00:00	78651
21-Jul-99	06:00:00	78920
21-Jul-99	07:00:00	74966
21-Jul-99	08:00:00	75524
21-Jul-99	09:00:00	69933
21-Jul-99	10:00:00	64387
21-Jul-99	11:00:00	65432
21-Jul-99	12:00:00	62843
21-Jul-99	13:00:00	61148
21-Jul-99	14:00:00	52706
21-Jul-99	15:00:00	52869
21-Jul-99	16:00:00	52309
21-Jul-99	17:00:00	47533
21-Jul-99	18:00:00	47816
21-Jul-99	19:00:00	47151
21-Jul-99	20:00:00	43086
21-Jul-99	21:00:00	43894
21-Jul-99	22:00:00	41943
21-Jul-99	23:00:00	40756
22-Jul-99	00:00:00	40119
22-Jul-99	01:00:00	38437
22-Jul-99	02:00:00	37048
22-Jul-99	03:00:00	34214
22-Jul-99	04:00:00	33031
22-Jul-99	05:00:00	33351
22-Jul-99	06:00:00	32731
22-Jul-99	07:00:00	27900
22-Jul-99	08:00:00	28700
22-Jul-99	09:00:00	33029
22-Jul-99	10:00:00	28708
22-Jul-99	11:00:00	28169
22-Jul-99	12:00:00	28282
22-Jul-99	13:00:00	27656
22-Jul-99	14:00:00	25897
22-Jul-99	15:00:00	25609
22-Jul-99	16:00:00	26333
22-Jul-99	17:00:00	23562
22-Jul-99	18:00:00	23294

Date	Time	Volume (L)
22-Jul-99	19:00:00	23220
22-Jul-99	20:00:00	24480
22-Jul-99	21:00:00	21549
22-Jul-99	22:00:00	20549
22-Jul-99	23:00:00	21527
23-Jul-99	00:00:00	20186
23-Jul-99	01:00:00	21013
23-Jul-99	02:00:00	19527
23-Jul-99	03:00:00	19461
23-Jul-99	04:00:00	20683
23-Jul-99	05:00:00	19254
23-Jul-99	06:00:00	19254
23-Jul-99	07:00:00	19178
23-Jul-99	08:00:00	18281
23-Jul-99	09:00:00	16761
23-Jul-99	10:00:00	16548
23-Jul-99	11:00:00	17118
23-Jul-99	12:00:00	17118
23-Jul-99	13:00:00	16589
23-Jul-99	14:00:00	16677
23-Jul-99	15:00:00	15300
23-Jul-99	16:00:00	16156
23-Jul-99	17:00:00	15795
23-Jul-99	18:00:00	13113
23-Jul-99	19:00:00	15288
23-Jul-99	20:00:00	12789
23-Jul-99	21:00:00	14505
23-Jul-99	22:00:00	15085
23-Jul-99	23:00:00	14307
24-Jul-99	00:00:00	12701
24-Jul-99	01:00:00	13970
24-Jul-99	02:00:00	14199
24-Jul-99	03:00:00	12946
24-Jul-99	04:00:00	13176
24-Jul-99	05:00:00	13450
24-Jul-99	06:00:00	12995
24-Jul-99	07:00:00	12724
24-Jul-99	08:00:00	12277
24-Jul-99	09:00:00	11742
24-Jul-99	10:00:00	10918
24-Jul-99	11:00:00	12234
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24-Jul-99	14:00:00	10866
24-Jul-99	15:00:00	10994
24-Jul-99	16:00:00	11376
24-Jul-99	17:00:00	10833
24-Jul-99	18:00:00	11086
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24-Jul-99	20:00:00	10052
24-Jul-99	21:00:00	9680
24-Jul-99	22:00:00	10272
24-Jul-99	23:00:00	10515
25-Jul-99	00:00:00	10272
25-Jul-99	01:00:00	10120
25-Jul-99	02:00:00	9759
25-Jul-99	03:00:00	10240
25-Jul-99	04:00:00	9376
25-Jul-99	05:00:00	9732
25-Jul-99	06:00:00	9234
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25-Jul-99	08:00:00	8649
25-Jul-99	09:00:00	8883
25-Jul-99	10:00:00	8403
25-Jul-99	11:00:00	8633
25-Jul-99	12:00:00	8633
25-Jul-99	13:00:00	8614
25-Jul-99	14:00:00	8614
25-Jul-99	15:00:00	8256
25-Jul-99	16:00:00	8367
25-Jul-99	17:00:00	7921
25-Jul-99	18:00:00	7578
25-Jul-99	19:00:00	7578
25-Jul-99	20:00:00	6479
25-Jul-99	21:00:00	7674
25-Jul-99	22:00:00	7350
25-Jul-99	23:00:00	7999
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26-Jul-99	09:00:00	6177
26-Jul-99	10:00:00	7927
26-Jul-99	11:00:00	5869
26-Jul-99	12:00:00	5868
26-Jul-99	13:00:00	0
26-Jul-99	14:00:00	6002
26-Jul-99	15:00:00	6089
26-Jul-99	16:00:00	6178
26-Jul-99	17:00:00	6243
26-Jul-99	18:00:00	5982
26-Jul-99	19:00:00	5029
26-Jul-99	20:00:00	4768
26-Jul-99	21:00:00	5809
26-Jul-99	22:00:00	5704

Date	Time	Volume (L)
26-Jul-99	23:00:00	5789
27-Jul-99	00:00:00	5789
27-Jul-99	01:00:00	5790
27-Jul-99	02:00:00	5790
27-Jul-99	03:00:00	5789
27-Jul-99	04:00:00	5685
27-Jul-99	05:00:00	5518
27-Jul-99	06:00:00	5518
27-Jul-99	07:00:00	5350
27-Jul-99	08:00:00	5417
27-Jul-99	09:00:00	5417
27-Jul-99	10:00:00	5417
27-Jul-99	11:00:00	5418
27-Jul-99	12:00:00	5417
27-Jul-99	13:00:00	5335
27-Jul-99	14:00:00	5238
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27-Jul-99	17:00:00	4674
27-Jul-99	18:00:00	4673
27-Jul-99	19:00:00	4349
27-Jul-99	20:00:00	4508
27-Jul-99	21:00:00	4665
27-Jul-99	22:00:00	4507
27-Jul-99	23:00:00	4422
28-Jul-99	00:00:00	4344
28-Jul-99	01:00:00	4422
28-Jul-99	02:00:00	4267
28-Jul-99	03:00:00	1862
28-Jul-99	04:00:00	2948
28-Jul-99	05:00:00	2968
28-Jul-99	06:00:00	4795
28-Jul-99	07:00:00	4795
28-Jul-99	08:00:00	4702
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28-Jul-99	11:00:00	4702
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28-Jul-99	16:00:00	3660
28-Jul-99	17:00:00	3660
28-Jul-99	18:00:00	2224
28-Jul-99	19:00:00	3229
28-Jul-99	20:00:00	4090
28-Jul-99	21:00:00	3305
28-Jul-99	22:00:00	3093
28-Jul-99	23:00:00	3094
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29-Jul-99	05:00:00	2756
29-Jul-99	06:00:00	2757
29-Jul-99	07:00:00	2757
29-Jul-99	08:00:00	2701
29-Jul-99	09:00:00	2701
29-Jul-99	10:00:00	2701
29-Jul-99	11:00:00	2646
29-Jul-99	12:00:00	2646
29-Jul-99	13:00:00	1985
29-Jul-99	14:00:00	1985
29-Jul-99	15:00:00	1944
29-Jul-99	16:00:00	1943
29-Jul-99	17:00:00	1943
29-Jul-99	18:00:00	2462
29-Jul-99	19:00:00	2655
29-Jul-99	20:00:00	2655
29-Jul-99	21:00:00	2600
29-Jul-99	22:00:00	2600
29-Jul-99	23:00:00	2600
30-Jul-99	00:00:00	2600
30-Jul-99	01:00:00	2600
30-Jul-99	02:00:00	2545
30-Jul-99	03:00:00	2544
30-Jul-99	04:00:00	2545
30-Jul-99	05:00:00	2545
30-Jul-99	06:00:00	3289
30-Jul-99	07:00:00	2048
30-Jul-99	08:00:00	2483
30-Jul-99	09:00:00	2483
30-Jul-99	10:00:00	2483
30-Jul-99	11:00:00	1800
30-Jul-99	12:00:00	1761
30-Jul-99	13:00:00	3888
30-Jul-99	14:00:00	3888
30-Jul-99	15:00:00	3645
30-Jul-99	16:00:00	3767
30-Jul-99	17:00:00	1902
30-Jul-99	18:00:00	1901
30-Jul-99	19:00:00	1902
30-Jul-99	20:00:00	1902
30-Jul-99	21:00:00	1859
30-Jul-99	22:00:00	1859
30-Jul-99	23:00:00	1859
31-Jul-99	00:00:00	1989
31-Jul-99	01:00:00	1989
31-Jul-99	02:00:00	1875

Date	Time	Volume (L)
31-Jul-99	03:00:00	1875
31-Jul-99	04:00:00	1833
31-Jul-99	05:00:00	1834
31-Jul-99	06:00:00	1833
31-Jul-99	07:00:00	1833
31-Jul-99	08:00:00	1834
31-Jul-99	09:00:00	1833
31-Jul-99	10:00:00	1834
31-Jul-99	11:00:00	1833
31-Jul-99	12:00:00	1833
31-Jul-99	13:00:00	1875
31-Jul-99	14:00:00	1875
31-Jul-99	15:00:00	1875
31-Jul-99	16:00:00	1875
31-Jul-99	17:00:00	1875
31-Jul-99	18:00:00	1875
31-Jul-99	19:00:00	1833
31-Jul-99	20:00:00	1834
31-Jul-99	21:00:00	3167
31-Jul-99	22:00:00	3167
31-Jul-99	23:00:00	3095
01-Aug-99	00:00:00	3095
01-Aug-99	01:00:00	3095
01-Aug-99	02:00:00	1140
01-Aug-99	03:00:00	1575
01-Aug-99	04:00:00	1574
01-Aug-99	05:00:00	1575
01-Aug-99	06:00:00	1574
01-Aug-99	07:00:00	1575
01-Aug-99	08:00:00	1220
01-Aug-99	09:00:00	1220
01-Aug-99	10:00:00	1220
01-Aug-99	11:00:00	1219
01-Aug-99	12:00:00	1220
01-Aug-99	13:00:00	1220
01-Aug-99	14:00:00	1220
01-Aug-99	15:00:00	1220
01-Aug-99	16:00:00	1219
01-Aug-99	17:00:00	1220
01-Aug-99	18:00:00	1220
01-Aug-99	19:00:00	1220
01-Aug-99	20:00:00	1220
01-Aug-99	21:00:00	1167
01-Aug-99	22:00:00	1166
01-Aug-99	23:00:00	1167
02-Aug-99	00:00:00	1166
02-Aug-99	01:00:00	1167
02-Aug-99	02:00:00	1167
02-Aug-99	03:00:00	1166
02-Aug-99	04:00:00	1167

Date	Time	Volume (L)
02-Aug-99	05:00:00	1166
02-Aug-99	06:00:00	1140
02-Aug-99	07:00:00	1140
02-Aug-99	08:00:00	1139
02-Aug-99	09:00:00	1140
02-Aug-99	10:00:00	1139
02-Aug-99	11:00:00	1140
02-Aug-99	12:00:00	3160
02-Aug-99	13:00:00	3235
02-Aug-99	14:00:00	3235
02-Aug-99	15:00:00	3235
02-Aug-99	16:00:00	4031
02-Aug-99	17:00:00	4031
02-Aug-99	18:00:00	4031
02-Aug-99	19:00:00	4031
02-Aug-99	20:00:00	4031
02-Aug-99	21:00:00	4031
02-Aug-99	22:00:00	4030
02-Aug-99	23:00:00	4031
03-Aug-99	00:00:00	4031
03-Aug-99	01:00:00	4031
03-Aug-99	02:00:00	4031
03-Aug-99	03:00:00	3937
03-Aug-99	04:00:00	3937
03-Aug-99	05:00:00	3937
03-Aug-99	06:00:00	3937
03-Aug-99	07:00:00	3937
03-Aug-99	08:00:00	3937
03-Aug-99	09:00:00	3937
03-Aug-99	10:00:00	3844
03-Aug-99	11:00:00	3844
03-Aug-99	12:00:00	3844
03-Aug-99	13:00:00	3938
03-Aug-99	14:00:00	3937
03-Aug-99	15:00:00	3844
03-Aug-99	16:00:00	3844
03-Aug-99	17:00:00	3937
03-Aug-99	18:00:00	3937
03-Aug-99	19:00:00	3937
03-Aug-99	20:00:00	3937
03-Aug-99	21:00:00	3937
03-Aug-99	22:00:00	3844
03-Aug-99	23:00:00	3844
04-Aug-99	00:00:00	3844
04-Aug-99	01:00:00	3844
04-Aug-99	02:00:00	3844
04-Aug-99	03:00:00	3844
04-Aug-99	04:00:00	3844
04-Aug-99	05:00:00	3844
04-Aug-99	06:00:00	3844

Date	Time	Volume (L)
04-Aug-99	07:00:00	3844
04-Aug-99	08:00:00	3751
04-Aug-99	09:00:00	3751
04-Aug-99	10:00:00	3751
04-Aug-99	11:00:00	3751
04-Aug-99	12:00:00	3751
04-Aug-99	13:00:00	3750
04-Aug-99	14:00:00	3750
04-Aug-99	15:00:00	3659
04-Aug-99	16:00:00	3660
04-Aug-99	17:00:00	3750
04-Aug-99	18:00:00	3659
04-Aug-99	19:00:00	3751
04-Aug-99	20:00:00	3751
04-Aug-99	21:00:00	3751
04-Aug-99	22:00:00	3750
04-Aug-99	23:00:00	3750
05-Aug-99	00:00:00	3660
05-Aug-99	01:00:00	3660
05-Aug-99	02:00:00	3659
05-Aug-99	03:00:00	3659
05-Aug-99	04:00:00	3659
05-Aug-99	05:00:00	3660
05-Aug-99	06:00:00	3660
05-Aug-99	07:00:00	3659
05-Aug-99	08:00:00	3570
05-Aug-99	09:00:00	3570
05-Aug-99	10:00:00	3571
05-Aug-99	11:00:00	3570
05-Aug-99	12:00:00	1691
05-Aug-99	13:00:00	1734
05-Aug-99	14:00:00	1733
05-Aug-99	15:00:00	1692
05-Aug-99	16:00:00	1734
05-Aug-99	17:00:00	1691
05-Aug-99	18:00:00	1691
05-Aug-99	19:00:00	1692
05-Aug-99	20:00:00	1691
05-Aug-99	21:00:00	1691
05-Aug-99	22:00:00	1691
05-Aug-99	23:00:00	1691
06-Aug-99	00:00:00	1691
06-Aug-99	01:00:00	1733
06-Aug-99	02:00:00	1692
06-Aug-99	03:00:00	1691
06-Aug-99	04:00:00	1692
06-Aug-99	05:00:00	1733
06-Aug-99	06:00:00	1733
06-Aug-99	07:00:00	1734
06-Aug-99	08:00:00	1734

Date	Time	Volume (L)
06-Aug-99	09:00:00	1733
06-Aug-99	10:00:00	1733
06-Aug-99	11:00:00	1638
06-Aug-99	12:00:00	1396
06-Aug-99	13:00:00	1396
06-Aug-99	14:00:00	1629
06-Aug-99	15:00:00	1589
06-Aug-99	16:00:00	2504
06-Aug-99	17:00:00	2504
06-Aug-99	18:00:00	2504
06-Aug-99	19:00:00	2504
06-Aug-99	20:00:00	2503
06-Aug-99	21:00:00	2566
06-Aug-99	22:00:00	2567
06-Aug-99	23:00:00	1431
07-Aug-99	00:00:00	2566
07-Aug-99	01:00:00	1628
07-Aug-99	02:00:00	1876
07-Aug-99	03:00:00	1875
07-Aug-99	04:00:00	1875
07-Aug-99	05:00:00	1875
07-Aug-99	06:00:00	1875
07-Aug-99	07:00:00	1875
07-Aug-99	08:00:00	1876
07-Aug-99	09:00:00	1875
07-Aug-99	10:00:00	1678
07-Aug-99	11:00:00	1720
07-Aug-99	12:00:00	1720
07-Aug-99	13:00:00	1719
07-Aug-99	14:00:00	1761
07-Aug-99	15:00:00	1761
07-Aug-99	16:00:00	1761
07-Aug-99	17:00:00	1846
07-Aug-99	18:00:00	1889
07-Aug-99	19:00:00	1931
07-Aug-99	20:00:00	2033
07-Aug-99	21:00:00	2731
07-Aug-99	22:00:00	2790
07-Aug-99	23:00:00	2778
08-Aug-99	00:00:00	3586
08-Aug-99	01:00:00	4655
08-Aug-99	02:00:00	6726
08-Aug-99	03:00:00	6828
08-Aug-99	04:00:00	7590
08-Aug-99	05:00:00	7223
08-Aug-99	06:00:00	7772
08-Aug-99	07:00:00	6817
08-Aug-99	08:00:00	7029
08-Aug-99	09:00:00	9960
08-Aug-99	10:00:00	9525

Date	Time	Volume (L)
08-Aug-99	11:00:00	12086
08-Aug-99	12:00:00	15665
08-Aug-99	13:00:00	32445
08-Aug-99	14:00:00	88873
08-Aug-99	15:00:00	129534
08-Aug-99	16:00:00	130154
08-Aug-99	17:00:00	305691
08-Aug-99	18:00:00	279602
08-Aug-99	19:00:00	220650
08-Aug-99	20:00:00	179072
08-Aug-99	21:00:00	152130
08-Aug-99	22:00:00	181827
08-Aug-99	23:00:00	218383
09-Aug-99	00:00:00	265908
09-Aug-99	01:00:00	339532
09-Aug-99	02:00:00	447585
09-Aug-99	03:00:00	490256
09-Aug-99	04:00:00	492398
09-Aug-99	05:00:00	663001
09-Aug-99	06:00:00	1066534
09-Aug-99	07:00:00	1228321
09-Aug-99	08:00:00	1057199
09-Aug-99	09:00:00	866923
09-Aug-99	10:00:00	701931
09-Aug-99	11:00:00	700119
09-Aug-99	12:00:00	584494
09-Aug-99	13:00:00	544156
09-Aug-99	14:00:00	406458
09-Aug-99	15:00:00	365148
09-Aug-99	16:00:00	300111
09-Aug-99	17:00:00	265550
09-Aug-99	18:00:00	228524
09-Aug-99	19:00:00	200514
09-Aug-99	20:00:00	167850
09-Aug-99	21:00:00	164499
09-Aug-99	22:00:00	142722
09-Aug-99	23:00:00	130971
10-Aug-99	00:00:00	115801
10-Aug-99	01:00:00	113270
10-Aug-99	02:00:00	100000
10-Aug-99	03:00:00	92182
10-Aug-99	04:00:00	88785
10-Aug-99	05:00:00	85759
10-Aug-99	06:00:00	79361
10-Aug-99	07:00:00	75309
10-Aug-99	08:00:00	65791
10-Aug-99	09:00:00	65135
10-Aug-99	10:00:00	56797
10-Aug-99	11:00:00	55243
10-Aug-99	12:00:00	55841

Date	Time	Volume (L)
10-Aug-99	13:00:00	54546
10-Aug-99	14:00:00	49665
10-Aug-99	15:00:00	47615
10-Aug-99	16:00:00	46887
10-Aug-99	17:00:00	42415
10-Aug-99	18:00:00	43548
10-Aug-99	19:00:00	40174
10-Aug-99	20:00:00	38352
10-Aug-99	21:00:00	34113
10-Aug-99	22:00:00	35499
10-Aug-99	23:00:00	33794
11-Aug-99	00:00:00	32260
11-Aug-99	01:00:00	30895
11-Aug-99	02:00:00	30533
11-Aug-99	03:00:00	29143
11-Aug-99	04:00:00	29148
11-Aug-99	05:00:00	28490
11-Aug-99	06:00:00	28169
11-Aug-99	07:00:00	27118
11-Aug-99	08:00:00	25690
11-Aug-99	09:00:00	25404
11-Aug-99	10:00:00	22484
11-Aug-99	11:00:00	22176
11-Aug-99	12:00:00	24077
11-Aug-99	13:00:00	21993
11-Aug-99	14:00:00	21549
16-Aug-99	15:00:00	7722
16-Aug-99	16:00:00	7001
16-Aug-99	17:00:00	6486
16-Aug-99	18:00:00	7596
16-Aug-99	19:00:00	2228
16-Aug-99	20:00:00	2228
16-Aug-99	21:00:00	2228
16-Aug-99	22:00:00	2228
16-Aug-99	23:00:00	2191
17-Aug-99	00:00:00	7569
17-Aug-99	01:00:00	2291
17-Aug-99	02:00:00	7072
17-Aug-99	03:00:00	2092
17-Aug-99	04:00:00	2155
17-Aug-99	05:00:00	2057
17-Aug-99	06:00:00	2057
17-Aug-99	07:00:00	2057
17-Aug-99	08:00:00	2023
17-Aug-99	09:00:00	6645
17-Aug-99	10:00:00	6644
17-Aug-99	11:00:00	7030
17-Aug-99	12:00:00	5971
17-Aug-99	13:00:00	6344
17-Aug-99	14:00:00	6722

Date	Time	Volume (L)
17-Aug-99	15:00:00	6344
17-Aug-99	16:00:00	6344
17-Aug-99	17:00:00	6235
17-Aug-99	18:00:00	6235
17-Aug-99	19:00:00	6235
17-Aug-99	20:00:00	5578
17-Aug-99	21:00:00	5944
17-Aug-99	22:00:00	5578
17-Aug-99	23:00:00	5395
18-Aug-99	00:00:00	5578
18-Aug-99	01:00:00	6019
18-Aug-99	02:00:00	5211
18-Aug-99	03:00:00	5930
18-Aug-99	04:00:00	5480
18-Aug-99	05:00:00	5571
18-Aug-99	06:00:00	5301
18-Aug-99	07:00:00	5295
18-Aug-99	08:00:00	5296
18-Aug-99	09:00:00	6090
18-Aug-99	10:00:00	5115
18-Aug-99	11:00:00	5723
18-Aug-99	12:00:00	5202
18-Aug-99	13:00:00	4768
18-Aug-99	14:00:00	4855
18-Aug-99	15:00:00	5023
18-Aug-99	16:00:00	5363
18-Aug-99	17:00:00	4938
18-Aug-99	18:00:00	4682
18-Aug-99	19:00:00	4933
18-Aug-99	20:00:00	4843
18-Aug-99	21:00:00	4596
18-Aug-99	22:00:00	4514
18-Aug-99	23:00:00	4512
19-Aug-99	00:00:00	4432
19-Aug-99	01:00:00	4432
19-Aug-99	02:00:00	4432
19-Aug-99	03:00:00	4033
19-Aug-99	04:00:00	4033
19-Aug-99	05:00:00	4349
19-Aug-99	06:00:00	4112
19-Aug-99	07:00:00	4191
19-Aug-99	08:00:00	4112
19-Aug-99	09:00:00	4428
19-Aug-99	10:00:00	3957
19-Aug-99	11:00:00	4189
19-Aug-99	12:00:00	4189
19-Aug-99	13:00:00	4189
19-Aug-99	14:00:00	4190
19-Aug-99	15:00:00	4034
19-Aug-99	16:00:00	4186
19-Aug-99	17:00:00	3879
19-Aug-99	18:00:00	4267
19-Aug-99	19:00:00	4186
19-Aug-99	20:00:00	3577
19-Aug-99	21:00:00	4033
19-Aug-99	22:00:00	4110
19-Aug-99	23:00:00	4031
20-Aug-99	00:00:00	4031
20-Aug-99	01:00:00	3657
20-Aug-99	02:00:00	3294
20-Aug-99	03:00:00	3294
20-Aug-99	04:00:00	3441
20-Aug-99	05:00:00	3440
20-Aug-99	06:00:00	3440
20-Aug-99	07:00:00	3440
20-Aug-99	08:00:00	3368
20-Aug-99	09:00:00	3444
20-Aug-99	10:00:00	3444
20-Aug-99	11:00:00	3445
20-Aug-99	12:00:00	3444
20-Aug-99	13:00:00	3445
20-Aug-99	14:00:00	3444
20-Aug-99	15:00:00	3085
20-Aug-99	16:00:00	3375
20-Aug-99	17:00:00	3094
20-Aug-99	18:00:00	3307
20-Aug-99	19:00:00	2343
20-Aug-99	20:00:00	2342
20-Aug-99	21:00:00	3170
20-Aug-99	22:00:00	2549
20-Aug-99	23:00:00	2549
21-Aug-99	00:00:00	2549
21-Aug-99	01:00:00	2634
21-Aug-99	02:00:00	2904
21-Aug-99	03:00:00	2904
21-Aug-99	04:00:00	2903
21-Aug-99	05:00:00	2904
21-Aug-99	06:00:00	2634
21-Aug-99	07:00:00	2634
21-Aug-99	08:00:00	2580
21-Aug-99	09:00:00	2580
21-Aug-99	10:00:00	2579
21-Aug-99	11:00:00	2633
21-Aug-99	12:00:00	3175
21-Aug-99	13:00:00	2778
21-Aug-99	14:00:00	2778
21-Aug-99	15:00:00	2779
21-Aug-99	16:00:00	2712
21-Aug-99	17:00:00	2397
21-Aug-99	18:00:00	2655

Date	Time	Volume (L)
21-Aug-99	19:00:00	2461
21-Aug-99	20:00:00	2410
21-Aug-99	21:00:00	2409
21-Aug-99	22:00:00	2410
21-Aug-99	23:00:00	2410
22-Aug-99	00:00:00	2727
22-Aug-99	01:00:00	2727
22-Aug-99	02:00:00	2727
22-Aug-99	03:00:00	2092
22-Aug-99	04:00:00	2664
22-Aug-99	05:00:00	2663
22-Aug-99	06:00:00	2663
22-Aug-99	07:00:00	2606
22-Aug-99	08:00:00	2607
22-Aug-99	09:00:00	2606
22-Aug-99	10:00:00	2606
22-Aug-99	11:00:00	2551
22-Aug-99	12:00:00	2552
22-Aug-99	13:00:00	2066
22-Aug-99	14:00:00	2066
22-Aug-99	15:00:00	2369
22-Aug-99	16:00:00	2126
22-Aug-99	17:00:00	2308
22-Aug-99	18:00:00	2258
22-Aug-99	19:00:00	2258
22-Aug-99	20:00:00	2258
22-Aug-99	21:00:00	2258
22-Aug-99	22:00:00	2080
22-Aug-99	23:00:00	2258
23-Aug-99	00:00:00	2139
23-Aug-99	01:00:00	2139
23-Aug-99	02:00:00	2139
23-Aug-99	03:00:00	1961
23-Aug-99	04:00:00	1961
23-Aug-99	05:00:00	1961
23-Aug-99	06:00:00	1962
23-Aug-99	07:00:00	3922
23-Aug-99	08:00:00	2020
23-Aug-99	09:00:00	2033
23-Aug-99	10:00:00	2034
23-Aug-99	11:00:00	2033
23-Aug-99	12:00:00	1685
23-Aug-99	13:00:00	1685
23-Aug-99	14:00:00	1686
23-Aug-99	15:00:00	4823
23-Aug-99	16:00:00	1761
23-Aug-99	17:00:00	1705
23-Aug-99	18:00:00	1705
23-Aug-99	19:00:00	1705
23-Aug-99	20:00:00	1704

Date	Time	Volume (L)
23-Aug-99	21:00:00	1705
23-Aug-99	22:00:00	1705
23-Aug-99	23:00:00	1704
24-Aug-99	00:00:00	1705
24-Aug-99	01:00:00	1743
24-Aug-99	02:00:00	1743
24-Aug-99	03:00:00	1744
24-Aug-99	04:00:00	1743
24-Aug-99	05:00:00	1743
24-Aug-99	06:00:00	1744
24-Aug-99	07:00:00	1743
24-Aug-99	08:00:00	1705
24-Aug-99	09:00:00	1704
24-Aug-99	10:00:00	1704
24-Aug-99	11:00:00	1705
24-Aug-99	12:00:00	1705
24-Aug-99	13:00:00	1743
24-Aug-99	14:00:00	1743
24-Aug-99	15:00:00	1744
24-Aug-99	16:00:00	1743
24-Aug-99	17:00:00	1743
24-Aug-99	18:00:00	1743
24-Aug-99	19:00:00	1743
24-Aug-99	20:00:00	1744
24-Aug-99	21:00:00	1743
24-Aug-99	22:00:00	1743
24-Aug-99	23:00:00	1859
25-Aug-99	00:00:00	1685
25-Aug-99	01:00:00	1685
25-Aug-99	02:00:00	1686
25-Aug-99	03:00:00	1685
25-Aug-99	04:00:00	1686
25-Aug-99	05:00:00	1685
25-Aug-99	06:00:00	1685
25-Aug-99	07:00:00	1685
25-Aug-99	08:00:00	1686
25-Aug-99	09:00:00	1685
25-Aug-99	10:00:00	1685
25-Aug-99	11:00:00	1685
25-Aug-99	12:00:00	1685
30-Aug-99	12:00:00	1575
30-Aug-99	13:00:00	1538
30-Aug-99	14:00:00	1538
30-Aug-99	15:00:00	1538
30-Aug-99	16:00:00	1538
30-Aug-99	17:00:00	1538
30-Aug-99	18:00:00	1538
30-Aug-99	19:00:00	1591
30-Aug-99	20:00:00	1591
30-Aug-99	21:00:00	1591

Date	Time	Volume (L)
30-Aug-99	22:00:00	1591
30-Aug-99	23:00:00	1555
31-Aug-99	00:00:00	1555
31-Aug-99	01:00:00	2331
31-Aug-99	02:00:00	2331
31-Aug-99	03:00:00	2332
31-Aug-99	04:00:00	2331
31-Aug-99	05:00:00	2332
31-Aug-99	06:00:00	2331
31-Aug-99	07:00:00	2331
31-Aug-99	08:00:00	2332
31-Aug-99	09:00:00	2331
31-Aug-99	10:00:00	2332
31-Aug-99	11:00:00	2276
31-Aug-99	12:00:00	2276
31-Aug-99	13:00:00	2276
31-Aug-99	14:00:00	2277
31-Aug-99	15:00:00	2276
31-Aug-99	16:00:00	2276
31-Aug-99	17:00:00	2276
31-Aug-99	18:00:00	2276
31-Aug-99	19:00:00	2221
31-Aug-99	20:00:00	2220
31-Aug-99	21:00:00	2221
31-Aug-99	22:00:00	2220
31-Aug-99	23:00:00	2220
01-Sep-99	00:00:00	2221
01-Sep-99	01:00:00	2221
01-Sep-99	02:00:00	2221
01-Sep-99	03:00:00	2220
01-Sep-99	04:00:00	2220
01-Sep-99	05:00:00	2221
01-Sep-99	06:00:00	2276
01-Sep-99	07:00:00	2023
01-Sep-99	08:00:00	2023
01-Sep-99	09:00:00	2024
01-Sep-99	10:00:00	1974
01-Sep-99	11:00:00	1974
01-Sep-99	12:00:00	1974
01-Sep-99	13:00:00	2023
01-Sep-99	14:00:00	2023
01-Sep-99	15:00:00	1974
01-Sep-99	16:00:00	1416
01-Sep-99	17:00:00	1416
01-Sep-99	18:00:00	1416
01-Sep-99	19:00:00	1416
01-Sep-99	20:00:00	1416
01-Sep-99	21:00:00	1416
01-Sep-99	22:00:00	1416
01-Sep-99	23:00:00	1416
02-Sep-99	00:00:00	1416
02-Sep-99	01:00:00	1416
02-Sep-99	02:00:00	2883
02-Sep-99	03:00:00	2883
02-Sep-99	04:00:00	2883
02-Sep-99	05:00:00	2883
02-Sep-99	06:00:00	2953
02-Sep-99	07:00:00	2883
02-Sep-99	08:00:00	2953
02-Sep-99	09:00:00	1871
02-Sep-99	10:00:00	1917
02-Sep-99	11:00:00	1871
02-Sep-99	12:00:00	1872
02-Sep-99	13:00:00	1871
02-Sep-99	14:00:00	1871
02-Sep-99	15:00:00	1871
02-Sep-99	16:00:00	1871
02-Sep-99	17:00:00	1871
02-Sep-99	18:00:00	1826
02-Sep-99	19:00:00	1826
02-Sep-99	20:00:00	1825
02-Sep-99	21:00:00	1826
02-Sep-99	22:00:00	1826
02-Sep-99	23:00:00	1872
03-Sep-99	00:00:00	1872
03-Sep-99	01:00:00	1618
03-Sep-99	02:00:00	1618
03-Sep-99	03:00:00	1618
03-Sep-99	04:00:00	1618
03-Sep-99	05:00:00	1618
03-Sep-99	06:00:00	1618
03-Sep-99	07:00:00	1618
03-Sep-99	08:00:00	1618
03-Sep-99	09:00:00	1618
03-Sep-99	10:00:00	1618
03-Sep-99	11:00:00	1619
03-Sep-99	12:00:00	1619
03-Sep-99	13:00:00	1579
03-Sep-99	14:00:00	1579
03-Sep-99	15:00:00	1579
03-Sep-99	16:00:00	1580
03-Sep-99	17:00:00	1579
03-Sep-99	18:00:00	1579
03-Sep-99	19:00:00	1826
03-Sep-99	20:00:00	1826
03-Sep-99	21:00:00	1781
03-Sep-99	22:00:00	1781
03-Sep-99	23:00:00	1782
04-Sep-99	00:00:00	1685
04-Sep-99	01:00:00	1685

Date	Time	Volume (L)
04-Sep-99	02:00:00	1727
04-Sep-99	03:00:00	2606
04-Sep-99	04:00:00	2853
04-Sep-99	05:00:00	2980
04-Sep-99	06:00:00	2964
04-Sep-99	07:00:00	4270
04-Sep-99	08:00:00	10487
04-Sep-99	09:00:00	28638
04-Sep-99	10:00:00	58450
04-Sep-99	11:00:00	74723
04-Sep-99	12:00:00	90659
04-Sep-99	13:00:00	136777
04-Sep-99	14:00:00	211632
04-Sep-99	15:00:00	222119
04-Sep-99	16:00:00	186048
04-Sep-99	17:00:00	206205
04-Sep-99	18:00:00	206241
04-Sep-99	19:00:00	217363
04-Sep-99	20:00:00	196204
04-Sep-99	21:00:00	182065
04-Sep-99	22:00:00	154218
04-Sep-99	23:00:00	166157
05-Sep-99	00:00:00	197136
05-Sep-99	01:00:00	217628
05-Sep-99	02:00:00	297078
05-Sep-99	03:00:00	443438
05-Sep-99	04:00:00	616052
05-Sep-99	05:00:00	606690
05-Sep-99	06:00:00	702144
05-Sep-99	07:00:00	846131
05-Sep-99	08:00:00	798219
05-Sep-99	09:00:00	659340
05-Sep-99	10:00:00	719698
05-Sep-99	11:00:00	598752
05-Sep-99	12:00:00	505247
05-Sep-99	13:00:00	425078
05-Sep-99	14:00:00	400091
05-Sep-99	15:00:00	366069
05-Sep-99	16:00:00	312125
05-Sep-99	17:00:00	289823
05-Sep-99	18:00:00	229287
05-Sep-99	19:00:00	210968
05-Sep-99	20:00:00	171837
05-Sep-99	21:00:00	168476
05-Sep-99	22:00:00	166222
05-Sep-99	23:00:00	138880
06-Sep-99	00:00:00	118132
06-Sep-99	01:00:00	115816
06-Sep-99	02:00:00	102264
06-Sep-99	03:00:00	94252
06-Sep-99	04:00:00	87629
06-Sep-99	05:00:00	87164
06-Sep-99	06:00:00	81336
06-Sep-99	07:00:00	76336
06-Sep-99	08:00:00	75155
06-Sep-99	09:00:00	64959
06-Sep-99	10:00:00	67289
06-Sep-99	11:00:00	62875
06-Sep-99	12:00:00	61908
06-Sep-99	13:00:00	59568
06-Sep-99	14:00:00	58003
06-Sep-99	15:00:00	54344
06-Sep-99	16:00:00	54225
06-Sep-99	17:00:00	47943
06-Sep-99	18:00:00	47989
06-Sep-99	19:00:00	46908
06-Sep-99	20:00:00	45977
06-Sep-99	21:00:00	45650
06-Sep-99	22:00:00	42503
06-Sep-99	23:00:00	42300
07-Sep-99	00:00:00	40936
07-Sep-99	01:00:00	40383
07-Sep-99	02:00:00	36632
07-Sep-99	03:00:00	35869
07-Sep-99	04:00:00	33030
07-Sep-99	05:00:00	32035
07-Sep-99	06:00:00	32512
07-Sep-99	07:00:00	30023
07-Sep-99	08:00:00	29782
07-Sep-99	09:00:00	26190
07-Sep-99	10:00:00	26837
07-Sep-99	11:00:00	25636
07-Sep-99	12:00:00	24156
07-Sep-99	13:00:00	23801
07-Sep-99	14:00:00	24007
07-Sep-99	15:00:00	21296
07-Sep-99	16:00:00	22439
07-Sep-99	17:00:00	23071
07-Sep-99	18:00:00	22438
07-Sep-99	19:00:00	21990
07-Sep-99	20:00:00	19566
07-Sep-99	21:00:00	20259
07-Sep-99	22:00:00	19013
07-Sep-99	23:00:00	19261
08-Sep-99	00:00:00	16600
08-Sep-99	01:00:00	18458
08-Sep-99	02:00:00	17589
08-Sep-99	03:00:00	18054
08-Sep-99	04:00:00	16307
08-Sep-99	05:00:00	16391

Date	Time	Volume (L)
08-Sep-99	06:00:00	16170
08-Sep-99	07:00:00	15811
08-Sep-99	08:00:00	15738
08-Sep-99	09:00:00	14112
08-Sep-99	10:00:00	13642
08-Sep-99	11:00:00	14274
08-Sep-99	12:00:00	13942
08-Sep-99	13:00:00	14011
08-Sep-99	14:00:00	13813
08-Sep-99	15:00:00	12365
08-Sep-99	16:00:00	13877
08-Sep-99	17:00:00	12527
08-Sep-99	18:00:00	13039
08-Sep-99	19:00:00	11086
08-Sep-99	20:00:00	12597
08-Sep-99	21:00:00	12162
08-Sep-99	22:00:00	12411
08-Sep-99	23:00:00	11617
09-Sep-99	00:00:00	11127
09-Sep-99	01:00:00	10482
09-Sep-99	02:00:00	11927
09-Sep-99	03:00:00	10088
09-Sep-99	04:00:00	9495
09-Sep-99	05:00:00	10169
09-Sep-99	06:00:00	9468
09-Sep-99	07:00:00	9584
09-Sep-99	08:00:00	10475
09-Sep-99	09:00:00	10244
09-Sep-99	10:00:00	9747
09-Sep-99	11:00:00	8591
09-Sep-99	12:00:00	9595
09-Sep-99	13:00:00	10434
09-Sep-99	14:00:00	9555
09-Sep-99	15:00:00	9225
09-Sep-99	16:00:00	7999
09-Sep-99	17:00:00	8863
09-Sep-99	18:00:00	8538
09-Sep-99	19:00:00	9295
09-Sep-99	20:00:00	8617
09-Sep-99	21:00:00	8723
09-Sep-99	22:00:00	8297
09-Sep-99	23:00:00	7765
10-Sep-99	00:00:00	6702
10-Sep-99	01:00:00	8792
10-Sep-99	02:00:00	7431
10-Sep-99	03:00:00	8164
10-Sep-99	04:00:00	9787
10-Sep-99	05:00:00	8539
10-Sep-99	06:00:00	8346
10-Sep-99	07:00:00	7367

Date	Time	Volume (L)
10-Sep-99	08:00:00	10129
10-Sep-99	09:00:00	11749
10-Sep-99	10:00:00	11084
10-Sep-99	11:00:00	11294
10-Sep-99	12:00:00	11666
10-Sep-99	13:00:00	12849
10-Sep-99	14:00:00	13294
10-Sep-99	15:00:00	12399
10-Sep-99	16:00:00	12399
10-Sep-99	17:00:00	12969
10-Sep-99	18:00:00	14137
10-Sep-99	19:00:00	16830
10-Sep-99	20:00:00	20307
10-Sep-99	21:00:00	19261
10-Sep-99	22:00:00	19178
10-Sep-99	23:00:00	17747
11-Sep-99	00:00:00	18054
11-Sep-99	01:00:00	17515
11-Sep-99	02:00:00	15943
11-Sep-99	03:00:00	14360
11-Sep-99	04:00:00	16596
11-Sep-99	05:00:00	12701
11-Sep-99	06:00:00	15451
11-Sep-99	07:00:00	13265
11-Sep-99	08:00:00	15613
11-Sep-99	09:00:00	15392
11-Sep-99	10:00:00	13747
11-Sep-99	11:00:00	13166
11-Sep-99	12:00:00	13550
11-Sep-99	13:00:00	12849
11-Sep-99	14:00:00	11543
11-Sep-99	15:00:00	11372
11-Sep-99	16:00:00	11738
11-Sep-99	17:00:00	10964
11-Sep-99	18:00:00	10087
11-Sep-99	19:00:00	10753
11-Sep-99	20:00:00	11220
11-Sep-99	21:00:00	10052
11-Sep-99	22:00:00	10519
11-Sep-99	23:00:00	8172
12-Sep-99	00:00:00	10014
12-Sep-99	01:00:00	9861
12-Sep-99	02:00:00	8727
12-Sep-99	03:00:00	8591
12-Sep-99	04:00:00	8926
12-Sep-99	05:00:00	8786
12-Sep-99	06:00:00	9006
12-Sep-99	07:00:00	8972
12-Sep-99	08:00:00	9404
12-Sep-99	09:00:00	8539

Date	Time	Volume (L)
12-Sep-99	10:00:00	8404
12-Sep-99	11:00:00	8404
12-Sep-99	12:00:00	9211
12-Sep-99	13:00:00	10048
12-Sep-99	14:00:00	8030
12-Sep-99	15:00:00	7899
12-Sep-99	16:00:00	7900
12-Sep-99	17:00:00	7370
12-Sep-99	18:00:00	7968
12-Sep-99	19:00:00	7444
12-Sep-99	20:00:00	7445
12-Sep-99	21:00:00	7319
12-Sep-99	22:00:00	5971
12-Sep-99	23:00:00	5971
13-Sep-99	00:00:00	6723
13-Sep-99	01:00:00	6722
13-Sep-99	02:00:00	7101
13-Sep-99	03:00:00	6979
13-Sep-99	04:00:00	6235
13-Sep-99	05:00:00	6235
13-Sep-99	06:00:00	7538
13-Sep-99	07:00:00	5852
13-Sep-99	08:00:00	6218
13-Sep-99	09:00:00	6492
13-Sep-99	10:00:00	6035

Hourly measurements of water flow from C-S2 into the Warren Reservoir during winter 1999.

Date	Time	Volume (L)
14-Jul-99	16:00:00	17653
14-Jul-99	17:00:00	31383
14-Jul-99	18:00:00	31383
14-Jul-99	19:00:00	177839
14-Jul-99	20:00:00	177839
14-Jul-99	21:00:00	177838
14-Jul-99	22:00:00	177839
14-Jul-99	23:00:00	49037
15-Jul-99	00:00:00	49037
15-Jul-99	01:00:00	177839
15-Jul-99	02:00:00	177838
15-Jul-99	03:00:00	177839
15-Jul-99	04:00:00	175211
15-Jul-99	05:00:00	805845
15-Jul-99	06:00:00	805844
15-Jul-99	07:00:00	805844
15-Jul-99	08:00:00	805844
15-Jul-99	09:00:00	27054
15-Jul-99	10:00:00	27055
15-Jul-99	11:00:00	34141
15-Jul-99	12:00:00	23834
15-Jul-99	13:00:00	20613
15-Jul-99	14:00:00	21901
15-Jul-99	15:00:00	16748
15-Jul-99	16:00:00	21257
15-Jul-99	17:00:00	33497
15-Jul-99	18:00:00	32208
15-Jul-99	19:00:00	21257
15-Jul-99	20:00:00	21257
15-Jul-99	21:00:00	18680
15-Jul-99	22:00:00	18680
15-Jul-99	23:00:00	18680
16-Jul-99	00:00:00	18681
16-Jul-99	01:00:00	18681
16-Jul-99	02:00:00	18680
16-Jul-99	03:00:00	54754
16-Jul-99	04:00:00	54753
16-Jul-99	05:00:00	54753
16-Jul-99	06:00:00	54754
16-Jul-99	07:00:00	54754
16-Jul-99	08:00:00	54754
16-Jul-99	09:00:00	54753
16-Jul-99	10:00:00	53937
16-Jul-99	11:00:00	53938
16-Jul-99	12:00:00	53938
16-Jul-99	13:00:00	17133
16-Jul-99	14:00:00	18680
16-Jul-99	15:00:00	16104
16-Jul-99	16:00:00	15229
16-Jul-99	17:00:00	15230
16-Jul-99	18:00:00	13960
16-Jul-99	19:00:00	13960
16-Jul-99	20:00:00	13961
16-Jul-99	21:00:00	13960
16-Jul-99	22:00:00	13960
16-Jul-99	23:00:00	13960
17-Jul-99	00:00:00	13960
17-Jul-99	01:00:00	13961
17-Jul-99	02:00:00	13960
17-Jul-99	03:00:00	13960
17-Jul-99	04:00:00	13960
17-Jul-99	05:00:00	32363
17-Jul-99	06:00:00	32363
17-Jul-99	07:00:00	32363
17-Jul-99	08:00:00	31874
17-Jul-99	09:00:00	31875
17-Jul-99	10:00:00	32362
17-Jul-99	11:00:00	31874
17-Jul-99	12:00:00	32362
17-Jul-99	13:00:00	32362
17-Jul-99	14:00:00	24375
17-Jul-99	15:00:00	24747
17-Jul-99	16:00:00	24374
17-Jul-99	17:00:00	24375
17-Jul-99	18:00:00	14374
17-Jul-99	19:00:00	14374
17-Jul-99	20:00:00	14374
17-Jul-99	21:00:00	14374
17-Jul-99	22:00:00	19375
17-Jul-99	23:00:00	19375
18-Jul-99	00:00:00	19375
18-Jul-99	01:00:00	19374
18-Jul-99	02:00:00	19374
18-Jul-99	03:00:00	19375
18-Jul-99	04:00:00	19375
18-Jul-99	05:00:00	164372
18-Jul-99	06:00:00	164372
18-Jul-99	07:00:00	762488
18-Jul-99	08:00:00	961995
18-Jul-99	09:00:00	976859
18-Jul-99	10:00:00	961995
18-Jul-99	11:00:00	686875
18-Jul-99	12:00:00	686876
18-Jul-99	13:00:00	697488
18-Jul-99	14:00:00	686876
18-Jul-99	15:00:00	1211265
18-Jul-99	16:00:00	23749
18-Jul-99	17:00:00	23388

Date	Time	Volume (L)
18-Jul-99	18:00:00	23388
18-Jul-99	19:00:00	24113
18-Jul-99	20:00:00	111803
18-Jul-99	21:00:00	15924
18-Jul-99	22:00:00	87047
18-Jul-99	23:00:00	55963
19-Jul-99	00:00:00	45240
19-Jul-99	01:00:00	60973
19-Jul-99	02:00:00	50950
19-Jul-99	03:00:00	47436
19-Jul-99	04:00:00	39952
19-Jul-99	05:00:00	60894
19-Jul-99	06:00:00	648967
19-Jul-99	07:00:00	662332
19-Jul-99	08:00:00	716354
19-Jul-99	09:00:00	617611
19-Jul-99	10:00:00	595021
19-Jul-99	11:00:00	487790
19-Jul-99	12:00:00	538634
19-Jul-99	13:00:00	453847
19-Jul-99	14:00:00	609494
19-Jul-99	15:00:00	802149
19-Jul-99	16:00:00	802149
19-Jul-99	17:00:00	900451
19-Jul-99	18:00:00	908315
19-Jul-99	19:00:00	800182
19-Jul-99	20:00:00	813945
19-Jul-99	21:00:00	652728
19-Jul-99	22:00:00	725472
19-Jul-99	23:00:00	712644
20-Jul-99	00:00:00	475274
20-Jul-99	01:00:00	699151
20-Jul-99	02:00:00	602273
20-Jul-99	03:00:00	667581
20-Jul-99	04:00:00	589535
20-Jul-99	05:00:00	722737
20-Jul-99	06:00:00	670118
20-Jul-99	07:00:00	681965
20-Jul-99	08:00:00	606506
20-Jul-99	09:00:00	495880
20-Jul-99	10:00:00	528659
20-Jul-99	11:00:00	418742
20-Jul-99	12:00:00	440797
20-Jul-99	13:00:00	409443
20-Jul-99	14:00:00	366366
20-Jul-99	15:00:00	347117
20-Jul-99	16:00:00	347266
20-Jul-99	17:00:00	326920
20-Jul-99	18:00:00	310289
20-Jul-99	19:00:00	331949
20-Jul-99	20:00:00	315080
20-Jul-99	21:00:00	288983
20-Jul-99	22:00:00	278473
20-Jul-99	23:00:00	261730
21-Jul-99	00:00:00	258902
21-Jul-99	01:00:00	265388
21-Jul-99	02:00:00	252237
21-Jul-99	03:00:00	242140
21-Jul-99	04:00:00	266583
21-Jul-99	05:00:00	255824
21-Jul-99	06:00:00	262997
21-Jul-99	07:00:00	255824
21-Jul-99	08:00:00	276146
21-Jul-99	09:00:00	257377
21-Jul-99	10:00:00	271365
21-Jul-99	11:00:00	248340
21-Jul-99	12:00:00	219909
21-Jul-99	13:00:00	-227908
21-Jul-99	14:00:00	220603
21-Jul-99	15:00:00	208061
21-Jul-99	16:00:00	223407
21-Jul-99	17:00:00	215892
21-Jul-99	18:00:00	207760
21-Jul-99	19:00:00	200908
21-Jul-99	20:00:00	196300
21-Jul-99	21:00:00	184733
21-Jul-99	22:00:00	191760
21-Jul-99	23:00:00	176537
22-Jul-99	00:00:00	170588
22-Jul-99	01:00:00	156847
22-Jul-99	02:00:00	160935
22-Jul-99	03:00:00	149469
22-Jul-99	04:00:00	147763
22-Jul-99	05:00:00	141354
22-Jul-99	06:00:00	141354
22-Jul-99	07:00:00	136922
22-Jul-99	08:00:00	127040
22-Jul-99	09:00:00	122437
22-Jul-99	10:00:00	126456
22-Jul-99	11:00:00	126455
22-Jul-99	12:00:00	110575
22-Jul-99	13:00:00	110575
22-Jul-99	14:00:00	109254
22-Jul-99	15:00:00	100371
22-Jul-99	16:00:00	107940
22-Jul-99	17:00:00	100920
22-Jul-99	18:00:00	103551
22-Jul-99	19:00:00	110965

Date	Time	Volume (L)
22-Jul-99	20:00:00	92483
22-Jul-99	21:00:00	101902
22-Jul-99	22:00:00	95570
22-Jul-99	23:00:00	93549
23-Jul-99	00:00:00	98560
23-Jul-99	01:00:00	86867
23-Jul-99	02:00:00	87427
23-Jul-99	03:00:00	84695
23-Jul-99	04:00:00	80623
23-Jul-99	05:00:00	77184
23-Jul-99	06:00:00	72360
23-Jul-99	07:00:00	77988
23-Jul-99	08:00:00	76193
23-Jul-99	09:00:00	76987
23-Jul-99	10:00:00	70505
23-Jul-99	11:00:00	73639
23-Jul-99	12:00:00	66588
23-Jul-99	13:00:00	68938
23-Jul-99	14:00:00	64172
23-Jul-99	15:00:00	64172
23-Jul-99	16:00:00	64851
23-Jul-99	17:00:00	62563
23-Jul-99	18:00:00	64852
23-Jul-99	19:00:00	61800
23-Jul-99	20:00:00	60978
23-Jul-99	21:00:00	60978
23-Jul-99	22:00:00	59473
23-Jul-99	23:00:00	62484
24-Jul-99	00:00:00	56446
24-Jul-99	01:00:00	55703
24-Jul-99	02:00:00	54960
24-Jul-99	03:00:00	54217
24-Jul-99	04:00:00	55682
24-Jul-99	05:00:00	52751
24-Jul-99	06:00:00	52752
24-Jul-99	07:00:00	53475
24-Jul-99	08:00:00	50585
24-Jul-99	09:00:00	55643
24-Jul-99	10:00:00	53476
24-Jul-99	11:00:00	48462
24-Jul-99	12:00:00	47750
24-Jul-99	13:00:00	42048
24-Jul-99	14:00:00	49887
24-Jul-99	15:00:00	47036
24-Jul-99	16:00:00	47787
24-Jul-99	17:00:00	44976
24-Jul-99	18:00:00	41462
24-Jul-99	19:00:00	43571
24-Jul-99	20:00:00	43571

Date	Time	Volume (L)
24-Jul-99	21:00:00	43651
24-Jul-99	22:00:00	42266
24-Jul-99	23:00:00	45038
25-Jul-99	00:00:00	40880
25-Jul-99	01:00:00	40187
25-Jul-99	02:00:00	38933
25-Jul-99	03:00:00	38250
25-Jul-99	04:00:00	40299
25-Jul-99	05:00:00	39616
25-Jul-99	06:00:00	38933
25-Jul-99	07:00:00	40299
25-Jul-99	08:00:00	35009
25-Jul-99	09:00:00	38376
25-Jul-99	10:00:00	35683
25-Jul-99	11:00:00	39048
25-Jul-99	12:00:00	37030
25-Jul-99	13:00:00	38376
25-Jul-99	14:00:00	37702
25-Jul-99	15:00:00	37702
25-Jul-99	16:00:00	35166
25-Jul-99	17:00:00	36356
25-Jul-99	18:00:00	34502
25-Jul-99	19:00:00	31185
25-Jul-99	20:00:00	35166
25-Jul-99	21:00:00	33175
25-Jul-99	22:00:00	33839
25-Jul-99	23:00:00	33175
26-Jul-99	00:00:00	33176
26-Jul-99	01:00:00	32691
26-Jul-99	02:00:00	31383
26-Jul-99	03:00:00	30730
26-Jul-99	04:00:00	32691
26-Jul-99	05:00:00	31383
26-Jul-99	06:00:00	28768
26-Jul-99	07:00:00	30275
26-Jul-99	08:00:00	32208
26-Jul-99	09:00:00	30275
26-Jul-99	10:00:00	28987
26-Jul-99	11:00:00	30276
26-Jul-99	12:00:00	30919
26-Jul-99	13:00:00	34141
26-Jul-99	14:00:00	30276
26-Jul-99	15:00:00	28343
26-Jul-99	16:00:00	27699
26-Jul-99	17:00:00	29632
26-Jul-99	18:00:00	27054
26-Jul-99	19:00:00	26017
26-Jul-99	20:00:00	25382
26-Jul-99	21:00:00	27286

Date	Time	Volume (L)
26-Jul-99	22:00:00	27921
26-Jul-99	23:00:00	27920
27-Jul-99	00:00:00	26017
27-Jul-99	01:00:00	26017
27-Jul-99	02:00:00	26017
27-Jul-99	03:00:00	26652
27-Jul-99	04:00:00	26651
27-Jul-99	05:00:00	30625
27-Jul-99	06:00:00	25624
27-Jul-99	07:00:00	27500
27-Jul-99	08:00:00	23750
27-Jul-99	09:00:00	25000
27-Jul-99	10:00:00	26249
27-Jul-99	11:00:00	26466
27-Jul-99	12:00:00	25235
27-Jul-99	13:00:00	28927
27-Jul-99	14:00:00	24999
27-Jul-99	15:00:00	22772
27-Jul-99	16:00:00	22157
27-Jul-99	17:00:00	22157
27-Jul-99	18:00:00	22773
27-Jul-99	19:00:00	20310
27-Jul-99	20:00:00	23388
27-Jul-99	21:00:00	20604
27-Jul-99	22:00:00	19998
27-Jul-99	23:00:00	18180
28-Jul-99	00:00:00	22422
28-Jul-99	01:00:00	21210
28-Jul-99	02:00:00	19998
28-Jul-99	03:00:00	20604
28-Jul-99	04:00:00	20604
28-Jul-99	05:00:00	18786
28-Jul-99	06:00:00	19998
28-Jul-99	07:00:00	20284
28-Jul-99	08:00:00	19091
28-Jul-99	09:00:00	20284
28-Jul-99	10:00:00	20283
28-Jul-99	11:00:00	22073
28-Jul-99	12:00:00	28635
28-Jul-99	13:00:00	22670
28-Jul-99	14:00:00	22073
28-Jul-99	15:00:00	21477
28-Jul-99	16:00:00	19091
28-Jul-99	17:00:00	16704
28-Jul-99	18:00:00	16704
28-Jul-99	19:00:00	17618
28-Jul-99	20:00:00	17618
28-Jul-99	21:00:00	17619
28-Jul-99	22:00:00	17031
28-Jul-99	23:00:00	19380
29-Jul-99	00:00:00	21386
29-Jul-99	01:00:00	17340
29-Jul-99	02:00:00	17341
29-Jul-99	03:00:00	16184
29-Jul-99	04:00:00	16184
29-Jul-99	05:00:00	17918
29-Jul-99	06:00:00	16763
29-Jul-99	07:00:00	16762
29-Jul-99	08:00:00	22542
29-Jul-99	09:00:00	16184
29-Jul-99	10:00:00	20231
29-Jul-99	11:00:00	18496
29-Jul-99	12:00:00	19074
29-Jul-99	13:00:00	20230
29-Jul-99	14:00:00	17918
29-Jul-99	15:00:00	17340
29-Jul-99	16:00:00	18496
29-Jul-99	17:00:00	15606
29-Jul-99	18:00:00	15028
29-Jul-99	19:00:00	14790
29-Jul-99	20:00:00	14791
29-Jul-99	21:00:00	14790
29-Jul-99	22:00:00	14222
29-Jul-99	23:00:00	14222
30-Jul-99	00:00:00	14221
30-Jul-99	01:00:00	14221
30-Jul-99	02:00:00	13653
30-Jul-99	03:00:00	14791
30-Jul-99	04:00:00	21048
30-Jul-99	05:00:00	13652
30-Jul-99	06:00:00	15359
30-Jul-99	07:00:00	14222
30-Jul-99	08:00:00	13994
30-Jul-99	09:00:00	13994
30-Jul-99	10:00:00	13994
30-Jul-99	11:00:00	15114
30-Jul-99	12:00:00	15674
30-Jul-99	13:00:00	16233
30-Jul-99	14:00:00	16234
30-Jul-99	15:00:00	14554
30-Jul-99	16:00:00	17352
30-Jul-99	17:00:00	13434
30-Jul-99	18:00:00	12314
30-Jul-99	19:00:00	12874
30-Jul-99	20:00:00	11755
30-Jul-99	21:00:00	12314
30-Jul-99	22:00:00	12118
30-Jul-99	23:00:00	11567

Date	Time	Volume (L)
31-Jul-99	00:00:00	11567
31-Jul-99	01:00:00	12117
31-Jul-99	02:00:00	12668
31-Jul-99	03:00:00	12669
31-Jul-99	04:00:00	12669
31-Jul-99	05:00:00	12668
31-Jul-99	06:00:00	11567
31-Jul-99	07:00:00	13219
31-Jul-99	08:00:00	13004
31-Jul-99	09:00:00	11921
31-Jul-99	10:00:00	11921
31-Jul-99	11:00:00	15713
31-Jul-99	12:00:00	14392
31-Jul-99	13:00:00	15714
31-Jul-99	14:00:00	14391
31-Jul-99	15:00:00	13547
31-Jul-99	16:00:00	13005
31-Jul-99	17:00:00	13005
31-Jul-99	18:00:00	22217
31-Jul-99	19:00:00	10837
31-Jul-99	20:00:00	10837
31-Jul-99	21:00:00	10837
31-Jul-99	22:00:00	10837
31-Jul-99	23:00:00	10838
01-Aug-99	00:00:00	10837
01-Aug-99	01:00:00	10837
01-Aug-99	02:00:00	10837
01-Aug-99	03:00:00	10837
01-Aug-99	04:00:00	10838
01-Aug-99	05:00:00	10838
01-Aug-99	06:00:00	10661
01-Aug-99	07:00:00	10661
01-Aug-99	08:00:00	10660
01-Aug-99	09:00:00	10661
01-Aug-99	10:00:00	10661
01-Aug-99	11:00:00	10661
01-Aug-99	12:00:00	13859
01-Aug-99	13:00:00	13859
01-Aug-99	14:00:00	14392
01-Aug-99	15:00:00	19722
01-Aug-99	16:00:00	12260
01-Aug-99	17:00:00	13859
01-Aug-99	18:00:00	13859
01-Aug-99	19:00:00	13859
01-Aug-99	20:00:00	13858
01-Aug-99	21:00:00	13859
01-Aug-99	22:00:00	13859
01-Aug-99	23:00:00	13859
02-Aug-99	00:00:00	13859
02-Aug-99	01:00:00	13858
02-Aug-99	02:00:00	13859
02-Aug-99	03:00:00	13859
02-Aug-99	04:00:00	13859
02-Aug-99	05:00:00	13859
02-Aug-99	06:00:00	13858
02-Aug-99	07:00:00	13859
02-Aug-99	08:00:00	13859
02-Aug-99	09:00:00	13631
02-Aug-99	10:00:00	13631
02-Aug-99	11:00:00	13631
02-Aug-99	12:00:00	13631
02-Aug-99	13:00:00	13631
02-Aug-99	14:00:00	13859
02-Aug-99	15:00:00	14392
02-Aug-99	16:00:00	12793
02-Aug-99	17:00:00	14392
02-Aug-99	18:00:00	14392
02-Aug-99	19:00:00	14392
02-Aug-99	20:00:00	14391
02-Aug-99	21:00:00	14392
02-Aug-99	22:00:00	14392
02-Aug-99	23:00:00	14156
03-Aug-99	00:00:00	14156
03-Aug-99	01:00:00	14392
03-Aug-99	02:00:00	14155
03-Aug-99	03:00:00	14392
03-Aug-99	04:00:00	14392
03-Aug-99	05:00:00	14392
03-Aug-99	06:00:00	14392
03-Aug-99	07:00:00	14392
03-Aug-99	08:00:00	14155
03-Aug-99	09:00:00	14155
03-Aug-99	10:00:00	14155
03-Aug-99	11:00:00	14155
03-Aug-99	13:00:00	18350
03-Aug-99	14:00:00	18350
03-Aug-99	15:00:00	18656
03-Aug-99	16:00:00	18656
03-Aug-99	17:00:00	18656
03-Aug-99	18:00:00	11726
03-Aug-99	19:00:00	11727
03-Aug-99	20:00:00	11534
03-Aug-99	21:00:00	11534
03-Aug-99	22:00:00	11534
03-Aug-99	23:00:00	11534
04-Aug-99	00:00:00	11534
04-Aug-99	01:00:00	11534
04-Aug-99	02:00:00	11534

Date	Time	Volume (L)
04-Aug-99	03:00:00	11534
04-Aug-99	04:00:00	11534
04-Aug-99	05:00:00	11534
04-Aug-99	06:00:00	11534
04-Aug-99	07:00:00	11534
04-Aug-99	08:00:00	11534
04-Aug-99	09:00:00	11534
04-Aug-99	10:00:00	11534
04-Aug-99	11:00:00	11534
04-Aug-99	12:00:00	13107
04-Aug-99	13:00:00	12058
04-Aug-99	14:00:00	17301
04-Aug-99	15:00:00	12582
04-Aug-99	16:00:00	13107
04-Aug-99	17:00:00	14155
04-Aug-99	18:00:00	11534
04-Aug-99	19:00:00	11534
04-Aug-99	20:00:00	11534
04-Aug-99	21:00:00	13631
04-Aug-99	22:00:00	17301
04-Aug-99	23:00:00	17301
05-Aug-99	00:00:00	17301
05-Aug-99	01:00:00	17301
05-Aug-99	02:00:00	17301
05-Aug-99	03:00:00	17301
05-Aug-99	04:00:00	17301
05-Aug-99	05:00:00	17301
05-Aug-99	06:00:00	17301
05-Aug-99	07:00:00	17301
05-Aug-99	08:00:00	17301
05-Aug-99	09:00:00	14953
05-Aug-99	10:00:00	22688
05-Aug-99	11:00:00	23069
05-Aug-99	12:00:00	20625
05-Aug-99	13:00:00	15728
05-Aug-99	14:00:00	25165
05-Aug-99	15:00:00	12583
05-Aug-99	16:00:00	12583
05-Aug-99	17:00:00	12582
05-Aug-99	18:00:00	16777
05-Aug-99	19:00:00	16777
05-Aug-99	20:00:00	16500
05-Aug-99	21:00:00	16500
05-Aug-99	22:00:00	16501
05-Aug-99	23:00:00	16500
06-Aug-99	00:00:00	16500
06-Aug-99	01:00:00	16500
06-Aug-99	02:00:00	16500
06-Aug-99	03:00:00	16777
06-Aug-99	04:00:00	16776
06-Aug-99	05:00:00	16500
06-Aug-99	06:00:00	16500
06-Aug-99	07:00:00	16500
06-Aug-99	08:00:00	16500
06-Aug-99	09:00:00	16500
06-Aug-99	10:00:00	16501
06-Aug-99	11:00:00	16500
06-Aug-99	12:00:00	16500
06-Aug-99	13:00:00	19594
06-Aug-99	14:00:00	19594
06-Aug-99	15:00:00	28875
06-Aug-99	16:00:00	18562
06-Aug-99	17:00:00	18563
06-Aug-99	18:00:00	18563
06-Aug-99	19:00:00	18563
06-Aug-99	20:00:00	18563
06-Aug-99	21:00:00	18562
06-Aug-99	22:00:00	18563
06-Aug-99	23:00:00	17531
07-Aug-99	00:00:00	29907
07-Aug-99	01:00:00	18047
07-Aug-99	02:00:00	23719
07-Aug-99	03:00:00	20626
07-Aug-99	04:00:00	22173
07-Aug-99	05:00:00	18563
07-Aug-99	06:00:00	18563
07-Aug-99	07:00:00	28360
07-Aug-99	08:00:00	22688
07-Aug-99	09:00:00	903917
07-Aug-99	10:00:00	17531
07-Aug-99	11:00:00	14679
07-Aug-99	12:00:00	18350
07-Aug-99	13:00:00	18350
07-Aug-99	14:00:00	18350
07-Aug-99	15:00:00	19922
07-Aug-99	16:00:00	19922
07-Aug-99	17:00:00	19923
07-Aug-99	18:00:00	12793
07-Aug-99	19:00:00	15991
07-Aug-99	20:00:00	18656
07-Aug-99	21:00:00	14554
07-Aug-99	22:00:00	15113
07-Aug-99	23:00:00	19075
08-Aug-99	00:00:00	22316
08-Aug-99	01:00:00	19380
08-Aug-99	02:00:00	18793
08-Aug-99	03:00:00	17340
08-Aug-99	04:00:00	16762

Date	Time	Volume (L)
08-Aug-99	05:00:00	18793
08-Aug-99	06:00:00	19380
08-Aug-99	07:00:00	20604
08-Aug-99	08:00:00	27500
08-Aug-99	09:00:00	35829
08-Aug-99	10:00:00	49888
08-Aug-99	11:00:00	57932
08-Aug-99	12:00:00	88253
08-Aug-99	13:00:00	126455
08-Aug-99	14:00:00	203232
08-Aug-99	15:00:00	190925
08-Aug-99	16:00:00	185920
08-Aug-99	17:00:00	313424
08-Aug-99	18:00:00	792871
08-Aug-99	19:00:00	853266
08-Aug-99	20:00:00	947636
08-Aug-99	21:00:00	898485
08-Aug-99	22:00:00	778555
08-Aug-99	23:00:00	684185
09-Aug-99	00:00:00	686600
09-Aug-99	01:00:00	773745
09-Aug-99	02:00:00	829673
09-Aug-99	03:00:00	835571
09-Aug-99	04:00:00	959432
09-Aug-99	05:00:00	882756
09-Aug-99	06:00:00	1303491
09-Aug-99	07:00:00	1130479
09-Aug-99	08:00:00	1252373
09-Aug-99	09:00:00	1156037
09-Aug-99	10:00:00	1075429
09-Aug-99	11:00:00	1266136
09-Aug-99	12:00:00	1156037
09-Aug-99	13:00:00	880790
09-Aug-99	14:00:00	884722
09-Aug-99	15:00:00	924044
09-Aug-99	16:00:00	802148
09-Aug-99	17:00:00	876858
09-Aug-99	18:00:00	784837
09-Aug-99	19:00:00	683669
09-Aug-99	20:00:00	567454
09-Aug-99	21:00:00	504380
09-Aug-99	22:00:00	548191
09-Aug-99	23:00:00	462354
10-Aug-99	00:00:00	368887
10-Aug-99	01:00:00	377891
10-Aug-99	02:00:00	394726
10-Aug-99	03:00:00	366183
10-Aug-99	04:00:00	327300
10-Aug-99	05:00:00	322796
10-Aug-99	06:00:00	311775
10-Aug-99	07:00:00	283327
10-Aug-99	08:00:00	271365
10-Aug-99	09:00:00	240965
10-Aug-99	10:00:00	241394
10-Aug-99	11:00:00	202942
10-Aug-99	12:00:00	207924
10-Aug-99	13:00:00	205573
10-Aug-99	14:00:00	183809
10-Aug-99	15:00:00	197539
10-Aug-99	16:00:00	186903
10-Aug-99	17:00:00	181637
10-Aug-99	18:00:00	166260
10-Aug-99	19:00:00	168466
10-Aug-99	20:00:00	156622
10-Aug-99	21:00:00	142050
10-Aug-99	22:00:00	141428
10-Aug-99	23:00:00	143684
11-Aug-99	00:00:00	134417
11-Aug-99	01:00:00	138527
11-Aug-99	02:00:00	134127
11-Aug-99	03:00:00	122437
11-Aug-99	04:00:00	114628
11-Aug-99	05:00:00	110574
11-Aug-99	06:00:00	109675
11-Aug-99	07:00:00	105700
11-Aug-99	08:00:00	108818
11-Aug-99	09:00:00	97094
11-Aug-99	10:00:00	95360
11-Aug-99	11:00:00	92482
11-Aug-99	12:00:00	91626
11-Aug-99	13:00:00	94725
11-Aug-99	14:00:00	90497
11-Aug-99	15:00:00	88537

APPENDIX 2

Peak intensity data of DGGE bands (from Fig 3.16 A, B, C & D) used to perform PCA analyses of seasonal differences in bacterial populations at sites (A) S1, (B) M1, (C) D1 and (D) D2 between October 2000 and August 2001.

(A)

S1	24-Oct	5-Dec	2-Jan	15-Jan	1-Feb	14-Feb	13-Mar	2-Apr	23-Apr	7-May	28-May	18-Jun	16-Jul	12-Aug
1	9	7	5	5	4	2	9	7	4	2	6	10	7	5
2	3	8	7	35	8	24	18	23	26	2	7	6	7	35
3	3	3	2	7	8	2	4	13	8	3	33	22	17	31
4	7	9	8	6	7	7	15	14	3	4	17	11	11	12
5	4	17	26	36	35	28	50	24	6	7	9	14	19	16
7	2	5	6	5	4	6	9	12	5	7	7	5	5	6
8	5	17	29	22	17	13	27	20	6	2	9	11	12	13
9	6	14	15	10	8	7	10	5	4	2	4	14	21	23
10	4	9	3	3	5	7	6	3	2	3	1	4	6	3
11	8	19	24	16	13	20	35	13	14	5	38	31	6	7
12	4	7	4	18	3	4	4	6	33	47	30	23	10	5
13	14	7	21	13	14	12	10	17	10	25	13	16	16	11
14	12	16	27	30	21	10	19	29	10	6	12	9	18	15
16	3	6	6	8	10	8	8	20	6	3	2	8	18	3
18	2	1	1	5	2	1	3	1	1	1	3	8	5	3
19	20	37	23	58	18	21	41	9	12	12	33	54	68	56
20	5	9	4	4	4	5	4	1	4	2	4	5	2	3
21	5	18	13	13	10	4	5	5	13	4	14	16	8	5
22	4	5	7	8	6	6	4	3	4	2	6	5	2	11
23	14	27	38	32	32	14	19	5	20	10	21	20	44	14
24	12	21	22	32	26	20	21	13	5	4	6	10	4	11
25	4	5	3	2	3	2	5	2	5	2	2	2	8	7
26	3	2	2	3	2	3	2	2	6	2	2	3	25	10
27	2	10	19	20	12	37	15	33	4	2	1	2	6	2
28	2	29	29	75	42	42	38	47	5	3	9	3	3	4
29	4	9	13	25	10	10	9	9	2	4	4	3	5	7
30	2	29	61	60	36	69	39	41	20	8	83	126	95	115

(B)

M1	24-Oct	5-Dec	2-Jan	15-Jan	1-Feb	14-Feb	13-Mar	2-Apr	23-Apr	7-May	28-May	18-Jun	16-Jul	12-Aug
1	34	7	6	7	2	2	4	8	8	3	5	4	3	35
2	20	12	9	49	11	31	24	15	32	2	32	24	12	25
3	3	2	5	11	14	4	11	10	16	2	16	22	5	12
4	5	2	4	2	7	3	8	5	2	1	10	10	3	3
5	6	6	12	48	38	28	51	13	6	3	5	13	6	14
6	3	4	5	10	10	3	4	2	3	1	1	2	3	3
7	7	4	14	11	6	4	6	7	12	2	6	7	4	5
8	10	9	17	15	17	3	8	14	10	2	6	10	6	8
9	9	3	4	3	4	4	7	2	2	9	3	5	4	4
10	2	4	2	2	1	2	1	1	2	3	1	1	3	3
11	44	25	17	28	24	6	25	17	18	2	21	15	28	26
14	27	11	13	23	24	2	22	18	21	3	19	18	26	24
15	10	3	2	11	6	2	13	1	6	3	9	13	7	10
16	8	1	1	4	6	6	5	8	6	2	7	3	1	1
17	5	4	2	7	3	1	13	31	2	2	3	2	5	19
18	29	34	42	68	43	5	39	24	17	3	2	16	17	34
19	23	27	33	55	33	10	39	24	15	4	7	34	34	47
21	11	9	16	15	7	5	6	48	14	3	13	17	7	15
23	14	15	16	14	9	5	16	2	14	5	11	21	18	20
24	15	10	7	17	10	8	26	11	8	2	2	4	5	10
26	3	3	3	3	2	3	3	4	4	2	4	8	5	5
27	6	7	6	15	5	8	18	17	5	3	4	8	2	2
28	4	5	14	47	33	11	46	27	7	3	4	3	5	3
29	3	4	7	17	6	3	11	5	2	3	2	2	3	3
30	8	18	23	63	30	28	40	25	15	4	32	113	37	110

(C)

D1	24-Oct	5-Dec	2-Jan	15-Jan	1-Feb	14-Feb	13-Mar	2-Apr	23-Apr	7-May	28-May	18-Jun	16-Jul	12-Aug
1	40	17	12	34	18	25	30	7	12	12	2	1	9	10
2	13	16	11	18	17	3	5	8	9	17	22	34	25	22
3	2	1	2	3	10	9	7	3	2	3	9	33	15	17
4	6	11	4	5	13	4	13	6	2	4	8	19	6	14
5	17	30	37	60	67	34	79	35	16	43	17	30	14	3
6	4	8	13	7	17	3	9	1	1	2	2	2	3	22
7	5	8	13	21	5	11	13	24	9	7	17	19	18	6
8	6	8	14	20	10	17	6	7	19	3	4	23	13	6
9	22	11	11	10	3	7	10	14	7	22	8	7	10	22
10	2	1	1	1	1	1	5	2	2	4	1	1	2	1
11	19	20	19	14	11	11	9	17	10	10	24	20	14	21
12	12	9	8	5	4	12	3	4	5	5	12	7	9	4
13	15	9	9	7	8	13	13	11	3	9	19	5	13	2
14	10	11	6	12	6	12	10	12	10	6	14	12	17	12
15	11	17	10	18	13	3	13	3	6	19	35	13	17	14
16	1	3	1	13	11	12	11	6	6	1	1	1	1	1
17	7	2	1	8	5	5	1	2	1	1	2	1	2	1
18	19	16	18	32	18	36	20	30	15	13	3	18	24	7
19	45	47	40	62	25	28	18	46	16	25	19	38	56	56
21	6	5	5	12	8	9	6	47	14	13	23	18	4	13
22	3	3	4	6	4	6	7	6	6	15	11	5	2	5
23	28	13	10	18	17	9	9	5	11	22	28	35	26	33
24	32	30	21	39	42	18	35	31	5	23	7	5	10	20
27	8	18	34	16	29	29	30	55	9	5	6	9	4	2
28	18	32	55	77	80	46	70	73	9	3	21	33	25	7
29	12	13	13	22	30	11	24	18	3	8	3	3	3	3
30	21	29	29	73	74	33	39	46	18	12	31	132	49	130

(D)

D2	24-Oct	5-Dec	2-Jan	15-Jan	1-Feb	14-Feb	13-Mar	2-Apr	23-Apr	7-May	28-May	18-Jun	16-Jul	12-Aug
3	8	35	13	12	18	9	17	14	18	5	20	41	9	38
4	8	15	8	10	21	19	13	10	3	2	4	20	2	8
5	36	37	53	66	67	32	59	33	28	10	22	26	21	45
6	2	3	20	13	15	1	2	1	3	2	7	2	3	2
7	3	6	21	29	11	6	7	21	12	7	19	17	20	10
8	6	18	39	30	11	17	11	17	20	11	12	12	7	7
9	24	16	22	12	1	6	13	17	9	4	6	1	10	14
10	8	4	5	4	4	4	6	8	2	4	1	1	1	2
11	30	32	23	17	17	15	16	16	16	14	17	24	11	23
12	6	14	9	7	5	8	5	12	9	3	6	7	15	5
14	13	11	16	8	15	5	7	5	6	8	14	8	16	9
15	7	7	8	11	17	8	10	5	5	2	5	7	10	4
16	6	1	0	3	1	1	9	21	2	3	2	1	5	4
17	10	6	14	27	24	13	11	11	6	1	2	6	11	10
18	5	7	4	36	27	2	4	23	3	3	9	11	26	19
19	16	14	16	44	35	12	14	38	17	6	21	24	45	43
20	6	3	9	1	2	6	3	2	2	4	2	1	1	3
21	3	5	8	17	14	6	5	9	15	5	12	11	5	9
22	7	5	4	13	18	12	9	9	13	5	9	9	3	9
23	39	19	24	26	21	11	13	6	11	9	64	50	36	38
24	35	36	38	42	37	36	39	42	11	8	8	8	9	18
25	15	4	6	7	6	4	7	9	6	3	8	10	5	14
26	6	7	4	10	8	10	6	2	3	3	12	7	1	5
27	4	15	8	34	21	42	36	48	8	2	5	8	17	2
28	23	35	41	60	73	52	71	73	18	2	15	24	21	6
29	10	8	12	32	27	12	23	15	7	2	3	4	4	2
30	13	21	33	63	47	45	43	39	27	2	30	93	73	105

APPENDIX 3

Colour (HU) measurements of water in streams C-S1 and C-S2 between July and October 1999.

Date	C-S1	C-S2
15/07/1999	120	107
20/07/1999	212	221
26/07/1999	251	182
3/08/1999	220	115
6/08/1999	211	102
9/08/1999	182	189
11/08/1999	206	207
13/08/1999	190	169
16/08/1999	178	135
20/08/1999	140	100
23/08/1999	169	104
27/08/1999	148	163
30/08/1999	154	80
3/09/1999	147	72
6/09/1999	149	190
10/09/1999	207	156
17/09/1999	201	194
21/09/1999	196	180
24/09/1999	176	131
27/09/1999	189	123
29/09/1999	169	113
1/10/1999	168	108
5/10/1999	146	118
8/10/1999	184	134
12/10/1999	249	165
15/10/1999	202	269
19/10/1999	209	218

APPENDIX 4

Nutrient (TP, SRP and NO_3^-) measurements of water in streams C-S1 and C-S2 between July and October 1999.

	TP(mg/L)		SRP(mg/L)		NO_3^-(mg/L)	
Date	C-S1	C-S2	C-S1	C-S2	C-S1	C-S2
15/07/1999	0.017	0.022	0.013	0.004	0.5	0.2
20/07/1999	0.113	0.122	0.017	0.022	0.66	0.62
26/07/1999	0.07	0.05	0.03	0.03	0.8	0.2
3/08/1999	0.07	0.04	0.01	0.02	0.6	0.1
6/08/1999	0.08	0.03	0.01	0.01	0.5	0.2
9/08/1999	0.15	0.19	0.03	0.04	0.5	0.5
11/08/1999	0.1	0.1	0.02	0.03	0.5	0.5
13/08/1999	0.06	0.05	0.06	0.02	1.3	1.9
16/08/1999	0.07	0.04	0.02	0.01	0.7	0.5
20/08/1999	0.08	0.065	0.014	0.01	0.8	0.7
23/08/1999	0.062	0.019	0.014	0.015	0.04	0.1
27/08/1999	0.087	0.067	0.011	0.012	0.1	0.34
30/08/1999	0.071	0.071	0.013	0.011	0.33	0.08
3/09/1999	0.103	0.054	0.02	0.017	0.56	0.08
6/09/1999	0.117	0.112	0.014	0.012	1.24	0.9
10/09/1999	0.03	0.028	0.025	0.021	0.92	0.74
17/09/1999	0.169	0.187	0.018	0.019	0.42	0.31
21/09/1999	0.077	0.083	0.012	0.014	0.53	0.67
24/09/1999	0.075	0.056	0.014	0.01	0.21	0.29
27/09/1999	0.101	0.073	0.005	0.004	0.23	0.3
29/09/1999	0.099	0.063	0.011	0.01	0.31	0.13
1/10/1999	0.08	0.061	0.004	0.005	0.16	0.1
5/10/1999	0.066	0.057	0.02	0.011	1.02	0.98
8/10/1999	0.084	0.079	-	0.005	0.3	0.3
12/10/1999	0.118	0.161	0.04	0.032	0.33	0.33
15/10/1999	0.115	0.118	0.053	0.019	1.54	1.44
19/10/1999	0.092	0.089	0.016	0.014	0.04	0.67

ERRATA

1. Page 94, Figure 3.7: The y-axis should be changed to single or no decimal points.
2. Page 99, Figure 3.11: In the y-axis text 'resrvoir' should read 'reservoir'.
3. Page 120, Figure 3.21: Specific colour units are missing on the y-axis. They should be (HU $mg^{-1}L$).
4. Page 145, Figure 3.32 and Page 165, Figure 4.6: R^2 on the graph should read r^2.
5. Page 161-162, Figure 4.2; Page 182, Figure 4.17; Page 225, Figure 5.25; Page 228-230, Figure 5.27: Y-axis should read "Absorbance (cm^{-1})".
6. Page 169, Figure 4.8: Inoculation time = 336 is missing "hr".
7. Page 227, Figure 5.26 A: Wavelength is missing (nm) units.
8. Page 274, Figure 6.12: Units for turbidity (NTU) are missing on the y-axis.
9. Page 164, Figure 4.5; Page 278, Figure 6.16 and Figure 6.17; Page 288, Figure 6.27. No SUVA units are listed. They should read ($m^{-1}mg^{-1}L$).