The ecology and taxonomy of *Synechococcus* from saltwater lakes in the Vestfold Hills, Antarctica.

by Lynne M. Rankin B Sc (Hons)

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Lynne M. Rankin

School of Agricultural Science

University of Tasmania
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Abstract

In 1992, a marine *Synechococcus* was discovered in a meromictic lake in the Vestfold Hills, Antarctica. This thesis describes the ecology and taxonomy of this organism.

Ace Lake is a saltwater, meromictic that was isolated from the marine environment approximately 6000 years ago. In 1992, The lake was 25 m deep, the top 12 m was oxygenated and the lake had a salinity range of 16 to 40 g kg^{-1} salt. The recent discovery of *Synechococcus* in Ace Lake was aided by flow cytometric methods. In Ace Lake, *Synechococcus* occured in the highest densities below the pycnocline with maximum numbers occurring just above the oxic/anoxic interface. *Synechococcus* bloomed in spring with numbers declining again in early January. At the peak of the bloom in 1992, a density of 8×10^6 cells ml^{-1} was recorded at 11 m in the lake. No diel periodicity in the growth of *Synechococcus* was detected.

Synechococcus was also present in two of ten other meromictic lakes and basins. The organism occured throughout the aerobic zone in Pendant Lake, in densities of approximately 10^7 cells ml^{-1}, and below the pycnocline in Lake Abraxas in densities of 1.4×10^7 cells ml^{-1}. It is possible that salinity restricts the distribution of *Synechococcus* in the meromictic lakes of the Vestfold Hills.

Synechococcus strains were isolated from Ace Lake, Pendant Lake and Lake Abraxas for further characterisation. The three strains were similar in size and had the same lipid soluble pigment signature, with two unknown carotenoid

pigments present in addition to the chlorophyll *a*, zeaxanthin and ββ- carotene. The three strains had phycoerythrin as their principle accessory light harvesting pigment. They were genetically similar (99.7 % similarity in the 16S rRNA sequence) and had a G + C content of between 57 and 58 mol %. They were also genetically similar (95.7 % similarity in the 16S rRNA sequence) to another marine picocyanobacteria, *Prochlorococcus marinus*. Based on the square root temperature dependence model, the minimum and maximum theoretical growth temperatures of the Ace Lake *Synechococcus* strain was -8° C, and 29.8° C. The optimal theoretical growth temperature was 19.7° C.

In-situ growth rates of the Ace Lake *Synechococcus* strain at 6 m, 8 m and 10 m in Ace Lake were determined. These rates were -0.118 d^{-1}, 0.072 d^{-1} and 0.341 d^{-1} respectively. An increase in water temperature and a reduction in light intensity increased the *in-situ* growth rate of the Ace Lake *Synechococcus* population. The grazing pressure on *Synechococcus* in Ace Lake was not determined. It is probable, however, that the distribution and abundance of *Synechococcus* in Ace Lake, Pendant Lake and Lake Abraxas is controlled by grazing.

Chapter 1 summarises and reviews the current ecological and taxonomic research that has been undertaken on Ace Lake. Chapter 2 describes the flow cytometric techniques that were developed to study *Synechococcus* in Antarctic Lakes. Chapter 3 discusses the ecology of *Synechococcus* in Ace Lake and chapter 4 the distribution of *Synechococcus* in meromictic lakes in the Vestfold Hills. Chapter 5 describes the taxonomic characteristics of three Antarctic *Synechococcus* strains and chapter 6 discusses controls of *Synechococcus* growth in Ace Lake.

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Chapter 1

The Chemical Stratification and Microbial Communities of Ace Lake, Antarctica

A review of chemical and microbial characteristics of a marine derived meromictic lake.

1.1 Abstract

Ace Lake is one of many, marine derived, meromictic lakes in the Vestfold Hills, Antarctica. The lake was isolated from the ocean over 6000 years ago through a process of isostatic uplift. Since that time the lake has undergone substantial change to reach its current stratified state. The physical structure of the lake is dependent on the local climatic conditions and the chemical structure is being maintained in part by the wide variety of microbial communities that inhabit the lake. The lake is 25 m deep with the mixolimnion separated from the monimolimnion by a sharp pycnocline. The lake is anoxic below 12 m. The euphotic zone supports a plankton community of low diversity relative to temperate and tropical lakes with only one reported metazoan zooplankton species. There are five dominant phytoplankton species, including high numbers of a picocyanobacterium, in Ace Lake. A population of photosynthetic sulfur bacteria exists at the top of the anoxic zone and forms part of the sulfur cycle in the lake. Active methanogenic archaea have been isolated from the bottom of the monimolimnion. The research on communities in Ace Lake has contributed significantly to the current knowledge of Antarctic microbial diversity. However, a number of questions remain especially in relation to the ecology and taxonomy of the heterotrophic bacteria, the microbial loop and technological applications of microorganisms issolated from Ace Lake.

1.2 Introduction

The Vestfold Hills is an ice-free area of approximately 500 km^2 on the coast of Princess Elizabeth Land, East Antarctica. There are close to 300 lakes and ponds in the Vestfold Hills, ranging from glacier fed freshwater lakes (Lake Druzhby, conductivity of 12 $\mu S\ cm^{-1}$) to hypersaline lakes (Deep Lake, conductivity of 192000 $\mu S\ cm^{-1}$). About ten percent of the lakes are meromictic. There has been extensive scientific interest in the lakes of the Vestfold Hills because of the broad diversity in chemistry and hence biology of the lakes. Ace lake has attracted particular attention since research began on the lakes of the Vestfold Hills in 1973 (Johnstone et al. 1973). This is primarily because of its marine origin and its biogeochemistry. It was one of the first known meromictic lakes of the region.

Ace Lake (68°24'S, 78°11'E) is a saline, meromictic lake on Long Peninsula in the Vestfold Hills (Figure 1.1). It is thought that Ace Lake was isolated from the sea some 6100 years ago through the process of isostatic uplift (Bird et al. 1991). Since that time the lake has undergone substantial change to reach the current physical and chemical stratification. The lake has a maximum depth of 25 m, is approximately cone shaped (Figure 1.2) and has a volume of $1.5 \times 10^6\ m^3$ (Table 1.1).

Ace Lake lies in a flat low valley with a small catchment (Table 1.1). The geological setting of the catchment consists of Archean Gneiss cut by younger dolerite dykes (Burton 1981). There is very little soil in the catchment and what is present is mineralogenic rather than organic.

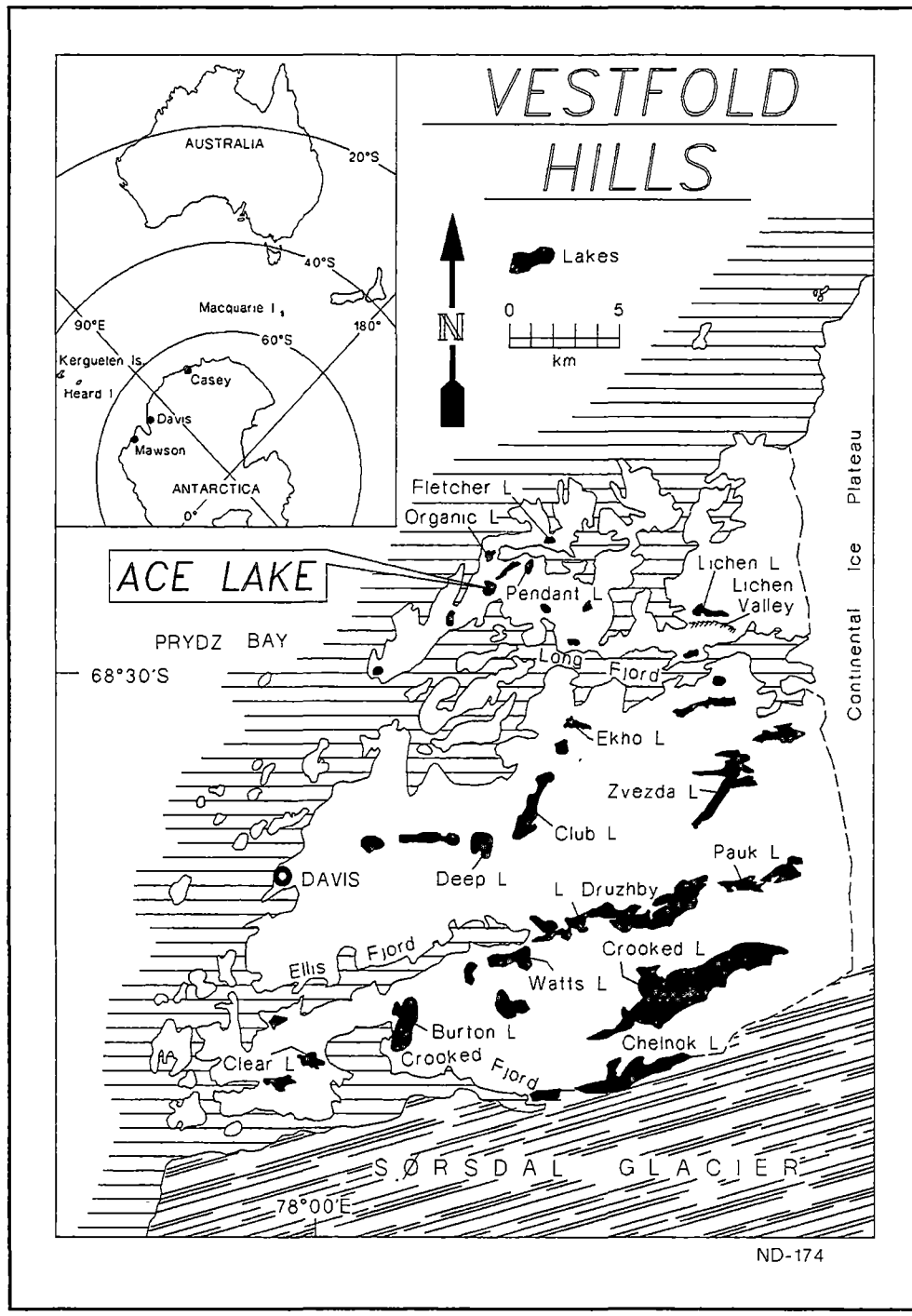


Figure 1.1: Map of the Vestfold Hills showing Ace Lake on Long Peninsula.

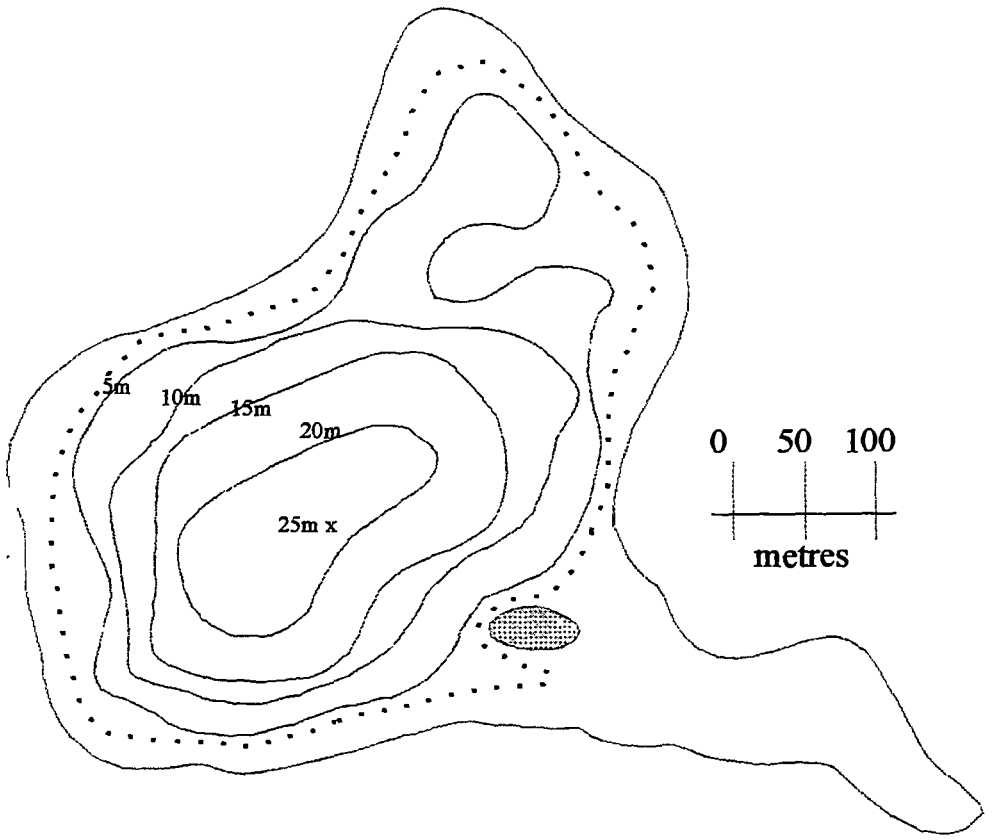


Figure 1.2: Bathymetric map of Ace Lake determined in 1991. The approximate shoreline in 1977 is marked with a dashed line (modified from Dartnall 1992 and Burton 1980). x represents the deepest spot in the lake and the site where most samples are taken.

During winter, small snow banks develop in the catchment. These banks melt during summer leading to some meltwater input. The catchment is devoid of any significant plant growth or permanent bird colonies. Inflow of organic material and nutrients from the catchment would, therefore, be minimal. The contribution of melt water algae to organic input to the lake has not been determined but it is expected to be small.

Table 1.1: Morphometric characteristics of Ace Lake

Maximum depth (Z_{max})	25 m
Volume	1.45×10^6 m^3
Area	1.79×10^5 m^2
Mean depth	8.14 m
Shore line (SL)	2.32×10^3 m
Development of SL	1.55
Total salt	3.020×10^4 tonnes
Average salinity	20. 33 g kg^{-1}
Centre of mass	8.7 m (approx)
Z_{mean}/Z_{max}	0.33
Lake catchment	35.5 ha

The stability of the meromictic lakes in the Vestfold Hills is partially determined by the local climate through its effect on water budget. Compared with other areas in East Antarctica, the weather in the Vestfold Hills is relatively mild by Antarctic standards. This is due to the moderating influence of the ice free land. The area is dry with a relatively low annual wind speed (5.16 m s^{-1} in 1992). The mean annual precipitation is relatively low (7.3 mm in 1992) and the mean annual temperature is mild (-10.4 °C).

Unlike an open ocean Ace Lake provides a stable environment for the study of biological communities and biogeochemical cycles. The ice cover on Ace Lake and the sharp density gradient at the bottom of th mixlimnion increase the stability of the lake, making investigations simpler. A range of biogeochemical cycles, including a sulfur

cycle, occur in Ace Lake and the lake supports large populations of novel bacteria including an active population of methanogenic archaea (Franzmann et al. 1991b). Since isolation, the diversity of phytoplankton in Ace Lake has simplified relative to the marine environment, making it easier to study biological processes. Finally, Ace Lake is relatively easy to reach at most times of the year making it more convenient for scientific study. Because of low phytoplankton diversity, the stability and the physical, chemical and biological stratification, Ace Lake provides an ideal field laboratory for understanding limnological processes that can then be related to the vastly more complex and less stable open ocean systems.

1.3 Isolation and Evolution of Ace Lake

Estimates of the time of separation of the lake from the ocean have been made using a number of methods including sulfur isotope measurement, ^{14}C and fossil dating and sediment core analysis (Bird et al. 1991; Burton and Barker 1979; Fulford-Smith and Sikes 1996). Most of the marine derived lakes of the Vestfold Hills from which sediment cores have been obtained show a marked change from marine conditions to lacustrine within the core. Such a boundary has not been observed in Ace Lake, though this probably means that the sediment cores have not been collected from sufficient depth. Nearby saline lakes, including Highway Lake and Organic Lake show this interface clearly (Bird et al. 1991). Like these lakes, Ace Lake is ringed by relict marine terraces, indicating that it is of marine origin. Further evidence for the marine origin of the lake comes from the observation that major ions are present in similar ratios to those in seawater (Masuda et al. 1988). Bird et al. (1991) used carbon dating of organic material from a sediment core to suggest an age of isolation of 6100 years for Ace Lake.

However because definite marine sediments were not sampled, this might be an underestimation of the lake age. Based on a record of diatom frustules, Fulford-Smith and Sikes (1996), proposed an initial time of isolation of Ace Lake from the sea at approximately 9000 years ago. Nearby Organic and Highway Lakes are thought to have been isolated circa 5820 and 6890 years before present respectively (Bird et al. 1991).

The evolution of Ace Lake since isolation is complex. Fulford-Smith and Sikes (1996). suggested that the lake was subject to two main influences during its time of formation, a freshwater influence from the retreating ice cap and a marine influence. Following the initial isolation of the lake there was a period of partial flushing with freshwater. A second period of sea water inundation occurred some 5000-6000 years ago. The reisolation of the lake from the sea was estimated to have occurred approximately 5500 years ago. According to Fulford-Smith and Sikes (1996), the lake has remained isolated from the sea since this time but has gone through a second period of partial freshwater flushing. The chemical stratification of the lake (Figure 1.3) is consistent with the hypothesis that the lake has undergone periods when evaporation may have lowered the water level considerably, resulting in formation of the hypersaline water that now occurs near the bottom of the lake. The processes that led to the periods of freshwater invasion are not certain. The reduced salt content could have been the result of partial flushing by meltwater from the current lake catchment.

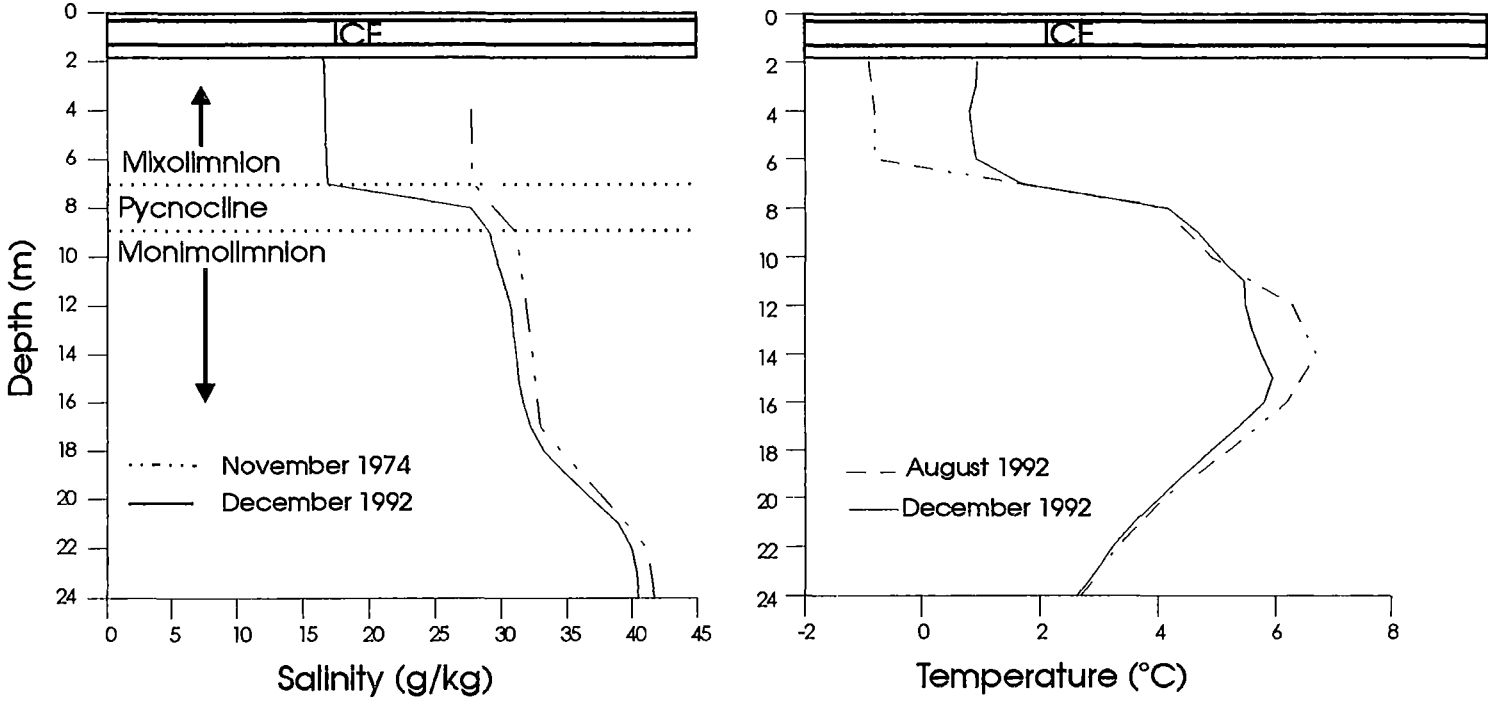


Figure 1.3: Physicochemical stratification of Ace Lake (a) Salinity profiles from 1974 and 1992. The two salinity profiles were taken using different methods. The 1974 salinity profile was calculated from chlorinity data. The salinity in 1992 was calculated from conductivity data. The difference in salinity below 8 m is due to error in the conversion factor from chlorinity to salinity. (b) Summer and winter temperature profile from 1992.

Considering that the catchment is small and that the local area has low precipitation, this is unlikely. It is more likely that flushing occurred during a period when the polar plateau abutted the lake or when flow from the plateau entered the catchment as suggested by Fulford-Smith and Sikes (1996). Similar processes have resulted in the flushing of most of the salt from other lakes in the Vestfold Hills, including Watts and Nicholson Lakes (Pickard et al. 1986), as well as the nearby Highway Lake (which has a salinity of only 5 g kg^{-1}). These lakes do not appear to have been reinvaded by salt water. Clear Lake, on Mule Peninsula, probably had a similar isolation process, including a reinvasion of salt water, to that of Ace Lake (Adamson and Pickard 1986).

Of four meromictic lakes extensively studied in the Dry Valleys (Joyce, Hoare, Vanda and Fryxell) only Lake Joyce has an ionic composition similar to that of seawater (Green et al. 1988). It is thought that other meromictic lakes in this region were formed from glacial retreat and that the salinity of the lakes has increased during periods when evaporation was greater than freshwater input into the lakes (Green et al. 1988; Green et al. 1989). Addition of ions, from erosion of rock surfaces in the Dry Valleys, is also thought to be an important contributor to the salinity increase in the lakes seawater (Green et al. 1988). Matsumoto et al. (1989) argued, however, that the salt composition in Lake Fryxell was similar to seawater but is only one-fifth the salinity of seawater at the lake's highest concentration. Lake Fryxell may therefore have had a marine origin and gone through a flushing process similar to the meromictic lakes in the Vestfold Hills.

The formation of stratification in the meromictic lakes of the Vestfold Hills is thought to be a result of increasing and decreasing water levels in these closed basins, which act as sensitive indicators of local precipitation (Gibson and Burton 1996). As water levels decrease, salinity increases, and the lake becomes totally mixed. Conversely, when levels rise, a layer of fresh water is formed over the surface of the lake. The implications of these processes are discussed below. The salinity profile indicates that at some stage the lake level dropped to such a degree that the lake mixed completely and had a salinity of 41 g kg^{-1} (Gibson and Burton 1996). These authors calculated that this represented a drop in water level of approximately 6 m compared to that at present. Studies of the distribution of sulfur concentrations and sulfur isotopes suggested that, through this process, approximately 78% of the total S in the lake has been lost since it was isolated. The mechanisms that effected this loss are uncertain, but it was possibly due to loss of volatile sulfides during the process of lake mixing. If so, this implies an earlier period of complete mixing.

Between 1974 and 1994 the lake depth has increased from 22.8 m to 25 m. The depth of the oxic/anoxic interface has not changed with respect to the lake bottom, indicating that freshwater inflow on top of the lake. However, recent measurements of lake height above sea level (E. Bell, personal communications) indicated that the water budget in Ace Lake is no longer positive. In the 1970's and 1980's the lake increased in its height above sea level. In 1986 the lake surface was 8.91 m above sea level, in 1989 the lake was 9.15 m above sea level hence the lower salinity in the surface waters in 1992 compared to 1974 (Figure 1.3). By 1996 the surface of the lake had dropped to 8.67 m above sea level. Like other lakes in the Vestfold Hills, for example Deep Lake and

Organic Lake (Gibson and Burton 1996), it appears that the water balance of Ace Lake has recently changed from positive to negative.

1.4 Chemical stratification and maintenance of meromixis

Ace Lake remains permanently stratified because of a strong density gradient and extended ice cover which reduces wind induced mixing (Ace Lake is covered by up to 1.8 m of ice for up to 11 months of the year and in cold years may remain ice covered all year round). The physical stratification provides some insight into the history of, and the processes occurring in, the lake.

1.4.1 Salinity

A summer salinity profile is shown in Figure 1.3. During most of the year, the lake is isohaline from under the ice to a depth of 7 m. Beneath this depth salinity increases, initiated by a sharp pycnocline between 7 m and 8 m, in a number of distinct steps, to the bottom of the lake. The lake is less saline than seawater from the surface to a depth of 18 m, and greater below. Salinity remains essentially constant throughout an annual cycle at all depths except the top 7 m. A lens of fresher water forms during summer at the surface due to the melting of the ice. The top waters also undergo some wind mixing at this time. When ice begins to form again at the end of summer, the salinity of the water under the ice increases again as brine is excluded from newly formed ice.

1.4.2 Temperature

Temperature profiles of Ace Lake also indicate strong stratification (Figure 1.3). Water temperature is a balance of heat transfer from solar heating and loss of heat by conduction, through the ice, to the atmosphere and to the sediment (Wetzel 1983). In Ace Lake, solar heating of the water occurs to a depth of approximately 14 m. During winter, the isohaline layer under the ice is at its freezing point. As the ice cover thickens and the water increases in salinity this layer becomes colder. In spring and summer, when solar radiation increases, this layer is warmer, reaching temperatures of approximately 5° C. Between 7 m and 8 m the temperature rises sharply and the temperature below 10 m is warmer during the winter months than over summer. A mid-water maximum of approximately 10°C occurs in February. The temperature of this region then begins to fall reaching a minimum of approximately 5° C in December. Below 14 m the temperature decreases to the bottom of the lake. Temperature at the bottom of the lake has increased by approximately 1.5°C over the past 20 years, but shows minimal seasonal variation.

1.4.3 Stratification

Stratification of Ace Lake is maintained by the strong salinity gradient and the absence of wind induced mixhing throughout the year. The lake can be divided into three zones (Figure 1.3). At the top of the lake, the mixolimnion, mixes freely and contains oxygen. Convective mixing would occur in the mixolimnion during winter due to exclusion of brine from the forming ice. A convective current of 1 cm s^{-1} has been recorded in the mixed layer of Lake Vanda (Ragotzkie and Likens 1964). The depth of the

mixolimnion can change from year to year depending on local climatic conditions. The mixolimnion is separated from the monimolimnion by a sharp pycnocline. Due to the strong density gradient, transport of heat energy and salt in the monimnolimnion would occur by mostly by molecular diffusion (Canfield and Green 1985). This process explains the midwater temperature maximum, where solar heat is trapped. Beneath the pycnocline is the monimolimnion. The top 5.2 m of the monimolimnion contains oxygen possibly due to the deep mixing of the mixolimnion in the past (Gibson and Burton 1996). Below 12.2 m the monimolimnion is anoxic. Advection in the lake probably occurs quite freely.

Gibson and Burton (1996) have suggested that the steps in the salinity profile (Figure 1.3) reflect depths to which the lake mixed at some stage in the past. If lake levels were to decrease, the salinity of the surface waters would increase, and deeper epilimnetic mixing would be expected. In Figure 1.3, a small salinity step occurs at 12 m coincident with the depth of the interface between oxic and anoxic water (Gibson and Burton 1996). It appears that mixing of oxygenated water to this depth has occurred at some time in the past. Deep mixing of oxygenated water currently occurs in nearby Pendant Lake (L. Rankin, unpublished data). Even though mixing does not occur to this depth at present, it appears that heterotrophic processes have been unable to remove oxygen produced by photosynthesis in the stagnant water between 7 m to 12.2 m. The salinity at the bottom of Ace Lake suggests that the maximum salinity during extended periods of negative water balance was at least 41 g kg^{-1}.

Other meromictic lakes in the Vestfold Hills and in the Dry Valleys are similarly stratified although each lake has individual physical and chemical characteristics

(Gibson and Burton 1996; Simmons et al. 1993). Lake Fryxell in the Dry Valleys, for example, is 19 m deep, has a salinity range of approximately 1 - 4 g kg^{-1}, has a midwater temperature maximum of 3.6° C and an oxic/anoxic interface at approximately 10 m (Vincent 1981). Lake Vanda in the Dry Valleys, is 68 m deep and has a temperature maximum of 23.5° C at the bottom of the lake where the salinity is three times that of seawater (Vincent and Vincent 1982). Ekho Lake in the Vestfold Hills is 40 m deep, has an oxic/anoxic interface at 23 m, a salinity range of 30 - 160 g kg^{-1} and a temperature maximum of 18° C at 18 m (L. Rankin, unpublished).

1.4.4 Light

Like all high-latitude locations, the climate of the Vestfold Hills is dominated by the extreme contrasts in the seasonal inputs of solar radiation (Campbell and Aarup 1989). For approximately six weeks during winter (June-July) there is no light in the Vestfold Hills and for six weeks during summer (Dec-Jan) there is continuous light. Optical properties of Ace Lake are described in detail by Burch (1988). Phytoplankon in Antarctic lakes need to adapt to dramatic seasonal changes in solar radiation. In the surface waters of ice free lakes phytoplankton may be affected by photoinhibition due to the intense summer light (Wright and Burton 1981). During the winter months and also at the bottom of the euphotic zone in ice covered lakes, phytoplankton must be adapted to survive at very low light intensities. Further, the incident light reaching the water column of ice covered lakes is altered by the opaqueness of ice, ice depth and ice age (Kirk 1994). Snow cover on ice affects total light penetration and penetration of certain wavelengths (Burch 1988). Due to the differences in ice thickness, there was only 1 %

penetration of incident light through the ice in Lake Fryxell as compared to 15 % in Lake Vanda (Vincent 1981; Vincent and Vincent 1982). Phytoplankters at the deep chlorophyll maximum (DCM) in these lakes conduct photosynthesis with less than 1% of the surface light intensity. During 1992, in Ace Lake, light measured as photosynthetically active radiation (PAR), was detected to a depth of 2 m in August (detection limit of 1 μmol photons m^{-2} s^{-1}), due to low levels of incident radiation. Light was detected to a depth of 12 m in December. Lake is thikness ranged from 50 cm in April to 180 cm in October. In December, the vertical attenuation coefficient (K_d) changed from 0.2 m^{-1} in the top 10.5 m to 4.4 m^{-1} between 10.5 m and 11.5 m (Chapter 3, Figure 3.7). This was probably due to absorption of light by a dense band of picocyanobacteria at the DCM.

1.4.5 Dissolved gases

Figure 1.4 shows a vertical profile of dissolved gases in Ace Lake in 1987. These concentration of dissolved gasses are consistent with those published by Hand and Burton (1981). Oxygen concentration in the mixolimnion was high (0.4 mmol l^{-1}) (Franzmann et al. 1991b). In the ice covered, meromictic, lakes of Antarctica the mixolimnion generally has supersaturated oxygen concentrations, because the ice cover prevents oxygen and other dissolved gases from reaching equilibrium with the atmosphere (Hand and Burton 1981; Vincent et al. 1981; Wharton et al. 1987). Meromictic lakes in the Dry Valleys have ice cover almost twice as thick as that on Ace Lake and as a consequence dissolved oxygen concentrations as great as 40 mg l^{-1} have been measured in Lake Hoare (Vincent 1988).

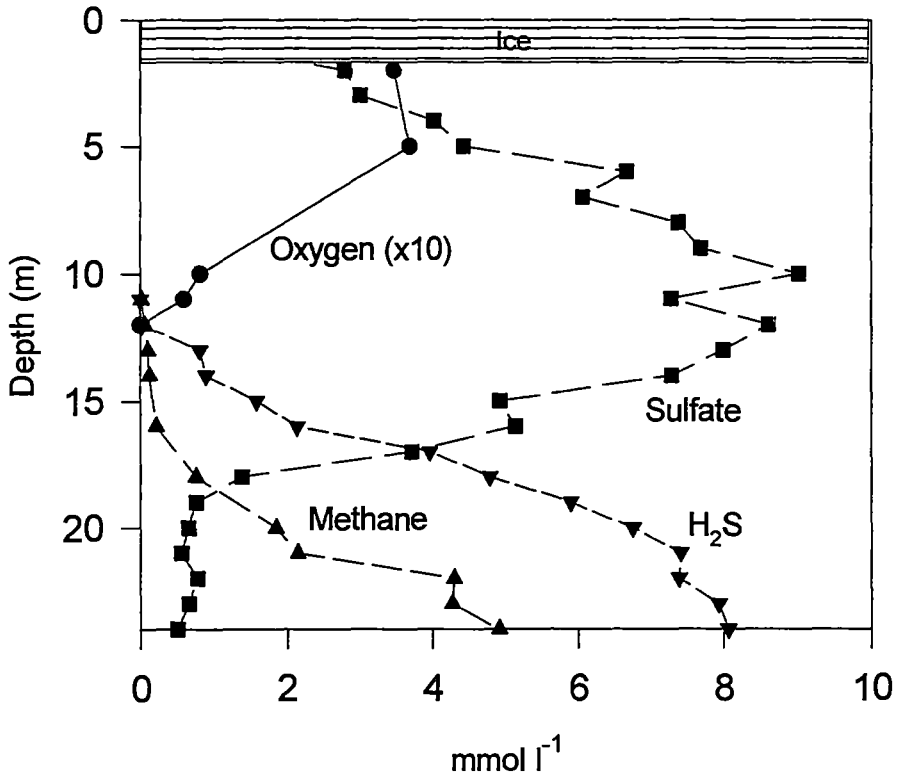


Figure 1.4: Vertical distribution of dissolved gases and sulfate in Ace Lake (from Franzmann et al. 1991b).

During the late 1970's and the 1980's, Burton and Barker (1979); Burch (1988); and Franzmann et al. (1988) recorded a distinct oxycline between the bottom of the mixolimnion and the top of the anaerobic interface in Ace Lake (Figure 1.4). Just above the anaerobic interface oxygen was present in concentrations of less than 0.19 mmol l^{-1}. This reduction in oxygen below the mixolimnion is characteristic of meromictic lakes in Antarctica (Simmons et al. 1993) and throughout the world (Ouellet et al. 1989; Venkateswaran et al. 1993). In recent years high oxygen concentrations (0.7 mmol l^{-1}) have been measured in the top of the monimolimnion of Ace Lake (J. Gibson, unpublished data). Based on this recent information it is proposed that the top of the monimolimnion was oxygenated during a period of deep mixing in the past and that the oxygen is now maintained in this zone through the photosynthetic activity of algae and cyanobacteria. During summer the rates of photosynthesis are probably higher than the rates of respiration by heterotrophs. In Lake Hoare, Dry Valleys, 58 % of the net oxygen production could be accounted for by freeze- concentration of the inflowing meltwater, while 42 % was attributed to net photosynthetic production (Vincent 1988). Respiration rates in Ace Lake have not been measured but this argument is substantiated by measurements of oxygen concentrations taken over a one year period in 1994. Oxygen concentrations at 10 m were higher during the spring and summer months than during the winter months, corresponding to the algal blooms that occur at the D.C.M. during this period (Figure 1.5).

Like oxygen, molecular nitrogen is also supersaturated in the mixolimnion (Figure 1.6). Below 18 m nitrogen falls to undetectable concentrations. It was postulated that below 18 m nitrogen is stripped from the water column by the high methane levels (Hand and Burton 1981).

Hydrogen sulfide and methane in Ace Lake, (Figure 1.4), result from the activity of sulfate reducing bacteria and methanogens in the monimolimnion (Burton and Barker 1979; Franzmann et al. 1988). Along with reduced organic compounds and low valency

metal, the high concentration of hydrogen sulfide below 12 m maintains the reducing conditions in the bottom waters.

1.5 Biota of Ace Lake

The number of eukaryotic and bacterial species recorded in, or newly described from, Ace Lake has increased significantly over the last twenty years. This has resulted from greater taxonomic effort due to increased interest in the lake communities over this time. Community structure is greatly influenced by the physiochemical gradients in stratified ecosystems. Species composition will therefore be discussed according to position in the water column.

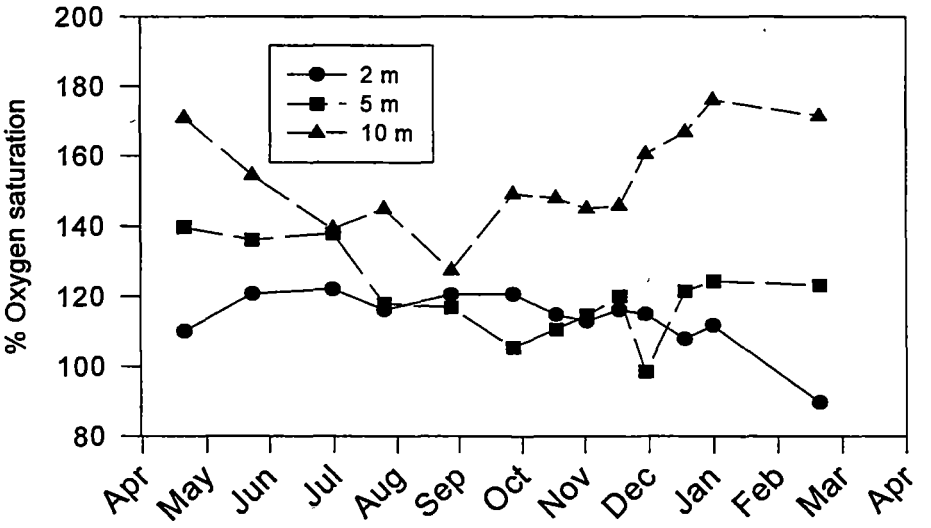


Figure 1.5: Percent oxygen saturation at 2 m, 5 m and 10 m in Ace Lake during 1994 (J. Gibson, unpublished data).

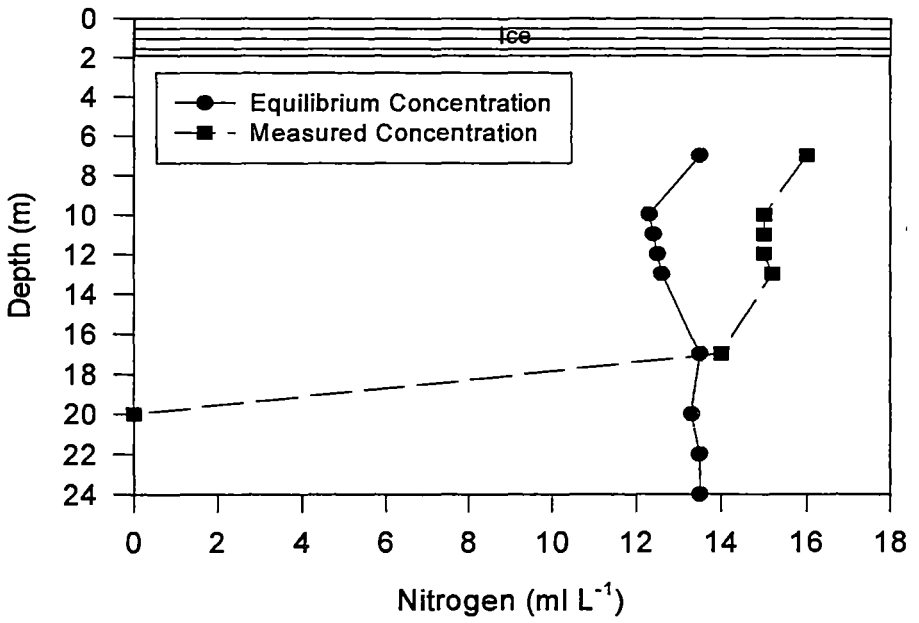


Figure 1.6: Measured nitrogen concentrations and calculated saturated nitrogen in Ace Lake (from Burton 1980).

1.5.1 Oxic Zone

1.5.1.1 Benthic communities

Benthic mats have been reported in the litoral zone of Ace Lake to a depth of approximately 10 m (Wright and Burton 1981). At times, portions of the mats detach and become part of the planktonic community in the lake. These types of algal mats, known as lift-off mats, have been reported in other lakes in the Vestfold Hills (Heath 1988) as well as in the meromictic lakes of the Dry Valleys (Parker and Wharton 1985; Simmons et al. 1993 and references within). The pinnacle mats and prostrate mats that occur in most of the Dry Valley Lakes (Parker and Wharton 1985) have not been reported in Ace Lake but this is probably due to the lack of research effort in this area. The algal mats have a high species diversity. Some of the organisms in the benthic communities from the edges of Ace Lake include mats of brown alga *Ectocarpus* sp., as well as *Urospora penicilliformis*, cf *Rhizoclonium implexium* and many small diatoms have been described (Dartnall 1992). The fauna was similarly rich for there was a small harpacticoid copepod, many ciliates, a large tube dwelling member of the *Folliculinidae* family, an eyed platyhelminyth, nematodes and three species of rotifer (Dartnall 1992; Gibson and Swadling, unpublished data). Twenty three species of diatoms were recorded in the surface sediments of Ace Lake. Many of these undoubtedly live in the thick algal mats (Cragg 1993). Like most benthic algal mats, (Lynch and Hobbie 1988), algal mats in Antarctic Lakes are composed primarily of cyanobacteria, diatoms and heterotrophic bacteria (Parker and Wharton 1985). Nitrogen fixation within the algal mats could provide an important source of organic nitrogen for organisms in the

mixolimnion. Nitrogen fixation occurs in other Antarctic lakes and melt streams (Vincent 1988) and the heterocysts of nitrogen fixing cyanobacteria have been observed in algal mats in Ace Lake (J. Gibson, unpublished data).

1.5.1.2 Zooplankton

The calanoid copepod *Paralabidocera antarctica* (I.C. Thompson), usually associated with sea ice in the marine environment, is the only metazoan zooplanktonic species that inhabits Ace Lake (Bayly 1978). Bayly (1978) also reported a small number of *Acartia* sp. but this organism has not been detected since that time and was possibly the result of sample contamination. A significant difference between the meromictic lakes of the Vestfold Hills and the meromictic lakes of the Dry Valleys is that crustaceous zooplankton are not present in the latter (Parker and Simmons 1985) although Vincent and Howard-Williams (1985) reported the presence of rotifers at the oxic/anoxic interface in Lake Fryxell.

Paralabidocera antarctica exhibits a single generation per year in the lake. Nauplii appeared in autumn and developed to adults in early summer when eggs were produced (K. Swadling, unpublished data). From a detailed study of the distribution of *P. antarctica*, undertaken in January 1982, it was found that there were two density maxima of *P. antarctica*: one just beneath the ice in the depth range of 1.75 m - 3.0 m, and the other near the bottom of the euphotic zone at 10.0 m - 10.5 m (Bayly and Burton 1987). The bottom of the euphotic zone is now at approximately 12 m and the second peak of *P. antarctica* would currently be expected to occur just above this depth. This second peak in numbers of *P. antarctica* occurs at the same depth as a high density of

algal (DCM) and bacteria. High *P. antarctica* numbers just above the anoxic/oxic interface could be sustained by the high numbers of picocyanobacteria and/or the alga *Pyramimonas gelidicola* which also have their maximum cell number in this region (Burch 1988; Rankin et al. 1997). It is considered that the adult stage of Copepods do not feed on picocyanobacteria because the bacteria are too small for the copepod's feeding parts (Johnson et al. 1982). *Paralabidocera antarctica* is however a small copepod (Bayly 1978) and the adult form may be able to feed on small cells as may other copepodic life stages.

1.5.1.3 Eukaryotic phytoplankton

Ace Lake has been subjected to three annual surveys (Burch 1988; Gibson et al. 1997); T. Pitman, unpublished data) and several summer surveys (Laybourn-Parry and Perriss 1995; Mancuso et al. 1990; Perriss et al. 1995; Volkman et al. 1988; Wright and Burton 1981) of phytoplankton ecology using techniques including microscopy, flow cytometry, lipid and pigment chemistry. The general conclusions from these investigations were that the phytoplankton of Ace Lake show low species diversity, strong vertical zonations of species and vertical migration of flagellated forms according to light intensity. Flagellated phytoplankton have also been found to dominate other Antarctic meromictic lakes (Vincent 1988). It has been suggested (Burch 1988; Gibson et al. 1997) that phytoplankton use mechanisms including low carbohydrate metabolism as a survival strategy over winter when light is limiting. Two comprehensive reviews on plankton diversity in Antarctic lakes and streams have recently been written (Ellis-Evans 1996; Vincent and James 1996) and although in Ace Lake, species diversity is low, throughout Antarctica as a whole, each of the major phytoplankton classes are well represented.

Phytoplankton identified in Ace Lake include four dominant species. In 1979 *P. gelidicola* reached a maximum density (3.5 x 10^3 cells ml^{-1} in 1979) in January, just above the oxic/anoxic interface. It is thought that the nutrient rich conditions in this zone support the growth of the shade adapted *P. gelidicola* (Burch 1988). A cryptophyte of the genus *Cryptomonas* began to bloom in September, when low but sufficient light returned. Initially the species was at its highest density just below the ice but migrated downwards with increasing light intensity. It reached its maximum density (700 cells ml^{-1}) in November at 6 m (Burch 1988; Gibson et al. 1997). Recently the autotrophic ciliate, *Mesodinium rubrum* (Lohmann), was identified in Ace Lake (Perriss et al. 1995). Earlier studies (Burch 1988) probably observed *M. rubrum* but did not positively identify the organism. *Mesodinium rubrum* is commonly found in marine phytoplankton assemblages, including Antarctic waters and probably has occurred in Ace Lake since its isolation from the sea (Laybourn-Parry and Perriss 1995). Like other phytoplanktonic species *M. rubrum* was found to survive in low numbers over winter but bloomed in spring and summer (Gibson et al. 1997) and it was suggested that summer concentrations of *M. rubrum* may have been regulated by grazing by *P. antarctica* but this is yet to be confirmed. In 1994, *M. rubrum* cell numbers started to increase in November, reached their maximum (3 x 10^2 cells ml^{-1}) in December at 5 m and then fell dramatically (Gibson et al. 1997). Perriss et al. (1995) indicated that nutrient levels did not appear to be an important factor controlling *M. rubrum* distribution in saline lakes of the Vestfold Hills as no significant relationship was detected between the abundance of *M. rubrum* and phosphorus and nitrogen levels in a range of meromictic lakes. Phospholipid fatty acid profiles confirm that microeukaryotes are the major microbial group in the aerobic zone of the lake (Mancuso

et al. 1990). Pigment and lipid signatures determined by Volkman et al. (1988) confirmed the major planktonic assemblage of Ace lake. The recent identification of *M. rubrum* is also confirmed by previous lipid and pigment chemistry. Phototrophic nanoflagellates (PNAN) have been observed but not positively identified in Ace Lake. In December 1993, Laybourn-Parry and Perriss (1995), found PNAN distributed throughout the euphotic zone, with a peak at just above the pycnocline, in densities of 3 - 5 x 10^5 cells l^{-1}.

Heterotrophic, unarmoured dinoflagellates have been observed in low numbers in the mixolimnion (S. Perriss, personal communication). Laybourn-Parry and Perriss (1995), recorded densities of between less than 1 cell ml^{-1} and 2 x 10^3 cells l^{-1} in December 1993. At this time the greatest densities occurred just below the ice and the smallest densities at the bottom of the euphotic zone. In the preceding month, maximum densities were recorded near the bottom of the euphotic zone. This highlights the dynamic nature of plankton communities in the lake and the need for detailed seasonal information on distribution and abundance in the lake. Heterotrophic nanoflagellates (HNAN) have also been reported to occur in the lake and once again remain to be identified (Laybourn-Parry and Perriss 1995). Heterotrophic nanoflagellate densities of between 1 x 10^5 cells l^{-1} and 2 x 10^5 cells l^{-1}, again with a peak at 6 m, were recorded in euphotic zone of the lake in December 1993. The significance of the HNAN in regulating bacterial cell numbers is yet to be determined but it is anticipated that they form an important link in the predator/prey relationship between bacteria and the larger zooplankton in the lake. Nanoflagellate grazing in freshwater lakes of the Vestfold Hills only removed between 0.1 and 9.7 % of bacterial production per day (Laybourn-Parry et al. 1995) and bacterial abundance in these lakes have been predicted to be controlled by

bottom up processes. However, as the plankton community structure, chemical and physical conditions in the saltwater meromictic lakes is very different, direct comparisons between the two systems are not truly valid.

1.5.1.4 Prokaryotic plankton

A population of picocyanobacteria was located in Ace Lake in 1992, using flow cytometric techniques (Rankin et al. 1997). Based on phylogenetic and chemotaxonomic evidence the cyanobacterium was characterised as a member of the *Synechococcus* group (Chapter 5). It occurred in low numbers (10^3 to 10^4 cells ml^{-1}) throughout euphotic zone over winter. *Synechococcus* cell numbers increased over spring and cell numbers peaked at 11 m in early December (a density of 8 $x10^6$ cells ml^{-1} was measured in December 1992). Cell numbers started to fall again in January. The occurrence of picocyanobacteria had not been previously reported in Ace Lake even though during November and December it occurs in high numbers just above the anoxic interface. In 1974 when the anoxic interface was at 10 m it was reported that there was a high concentration of bacterial cells (bacillus and coccoid shaped) between 7 m and 10 m (Burton and Barker 1979). It was assumed that these cells were associated with the sulfur cycle. It is likely that Barker and Burton had seen *Synechococcus* cells in 1974 but had not identified it as a photosynthetic bacterium. The 'bacillus shaped' bacteria were likely to have been *Chlorobium* sp. which occur in high numbers at the top of the anoxic zone. Other studies since 1974, including lipid analysis (Mancuso et al. 1990; Volkman et al. 1986; Volkman et al. 1988) and direct microscopic observations of phytoplankton (Burch 1988) did not detect these small but abundant cyanobacteria. This may have been due to the time at which samples were taken (*Synechococcus* may

only be detected by lipid chemistry when it bloomed in December), the depths from which samples were taken, filter sizes used for filtration or the microscopic techniques employed. *Synechococcus*, could easily be identified as a heterotrophic bacterium if not observed by fluorescence microscopy or flow cytometry. The other possibility is that *Synechococcus* is a relatively new inhabitant of Ace Lake. Changes in species composition have been reported in other Antarctic meromictic lakes (Spaulding et al. 1994). *Synechococcus* is not common in the Southern Ocean (Letelier and Karl 1989; Marchant et al. 1987), nor in Antarctic coastal waters (Walker and Marchant 1989) and has been reported to occur in low numbers (less than 10 ml^{-1}) in some Antarctic freshwater lakes (Andreoli et al. 1992; Laybourn-Parry and Marchant 1992).

1.5.1.5 Marine Synechococcus distribution

Detailed ecological and taxonomic discussions of *Synechococcus* have been made in chapters three to six. This review concentrates on the distribution and abundance of the marine *Synechococcus* in the oceans of the world. The distribution and abundance of *Synechococcus* in lake environments has been discussed in chapter four. Several reviews on the ecology and physiology of *Synechococcus* were published in 1986 (Fogg 1986; Glover 1985; Joint 1986; Stockner and Antia 1986; Waterbury et al. 1986). The information provided in these reviews is still current. Since 1986 research has added further to the knowledge of the distribution, abundance and contribution to primary productivity of *Synechococcus* in the oceans of the world.

Cyanobacteria belonging to the group *Synechococcus* were first described in samples from the ocean in 1979 (Johnson and Sieburth 1979 242; Waterbury et al. 1979).

Waterbury et al. (1979) reported *Synechococcus* from the Arabian Sea (24° N), the coast of Peru (13° S), north of the Gulf Stream (40° N) and periodically in Woods Hole Harbor (40° N). The Arabian Sea and the waters off the coast of Peru were relatively nutrient rich and *Synechococcus* occurred in densities between 10^4 and 10^5 cells ml^{-1} throughout the euphotic zone. The greatest numbers were observed in the surface 20 m. In comparison, *Synechococcus* densities from the slope water north of the Gulf Stream were an order of magnitude lower. There was also two orders of magnitude variation in cell densities in Woods Hole Harbor, where numbers ranged from 10^3 to 10^5 cells ml^{-1}. Reported cell sizes were 0.9 - 1.3 x 1.8 - 2.2 μm. Johnson and Sieburth (1979) made their initial observations of *Synechococcus* in the Sargasso Sea (12° - 40° N). Cell densities were between 10^3 and 10^4 cells ml^{-1}. Reported cell sizes were 0.5 - 1.0 x 1.0 μm. Further, (Johnson and Sieburth 1979) found *Synechococcus* densities of 10^5 cells ml^{-1} in the productive waters over Georges Bank (40° N) and 10^4 cells ml^{-1} in the upper 50 m of offshore waters off the coast of Iceland (64° N). It was estimated that *Synechococcus* contributed approximately 6 % to the total biomass of plankton in open ocean waters and 15 % in eutrophic waters such as Georges Bank.

Since the initial discovery of marine *Synechococcus*, ecological studies have focused on the distribution and abundance of the picocyanobacteria throughout the oceans of the world. An understanding of the environmental controls of the organism in these regions has also been sought. The latitudinal distribution of marine *Synechococcus* in oceanic, coastal and an eustarine habitat is summarised in Table 1.2. Generally speaking *Synechococcus* densities are lower in the southern polar seas (< 100 cells ml^{-1}) than the northern polar seas (Gradinger and Lenz 1995; Marchant et al. 1987; Smith et al. 1985)

although both Gradinger and Lenz (1995) and Marchant et al. (1987) only sampled surface waters. Densities found in northern polar waters are no lower than some of the *Synechococcus* cell densities found in tropical and temperate oligotrophic waters (Ishizaka et al. 1994; Shimada et al. 1993). Letelier and Karl (1989) and Marchant et al. (1987) found probable correlations between *Synechococcus* cell numbers and temperature in the southern oceans as did Murphy and Haugen (1985) in the northern polar seas. Neuer (1992) and Smith et al. (1985), however, found no correlation between temperature and *Synechococcus* densities. Although there might be correlations between temperature and *Synechococcus* density in some polar regions Vincent (1998) indicated that factors other than temperature are actually controlling the abundance of *Synechococcus* in these regions.

Marine *Synechococcus* abundance appears to be consistently higher, often by an order of magnitude or more, in coastal areas than in oceanic regions at equivalent latitudes (Burkill et al. 1993; El Hag and Fogg 1986; Gradinger et al. 1992; Murphy and Haugen 1985). Even in Antarctica, *Synechococcus* cell densities at a coastal site were higher than those in the Southern Ocean (Marchant et al. 1987; Walker and Marchant 1989). In artificially fertilised lake studies, *Synechococcus* occurs in higher densities in mesotrophic and eutrophic lakes than it does in oligotrophic lakes (Shortbreed and Stockner 1990; Stockner and Shortbreed 1988). This could also be true for coastal marine waters compared to the oligotrophic oceans although El Hag and Fogg (1986) indicated that exposure to higher light intensities, due to shallow mixing depths, may actually be a significant controlling factor in these regions.

The vertical distribution of *Synechococcus*, as summarised in Table 1.2, indicates that the greatest abundance of this picocyanobacteria is in the mixed, surface waters, of the oceans (Jochem 1988; Waterbury et al. 1979). However, in regions that are strongly stratified subsurface maxima in *Synechococcus* cell densities are common at the level of the DCM (Iturriaga and Marra 1988; Jochem 1995). The DCM in oceanic environments forms during stratification of the water column (Anderson 1969). In the permanently stratified tropical waters, the DCM is expected to occur all year round whilst in temperate and polar waters it is only expected to occur during summer with the warming of surface waters (Takahashi and Hori 1984). With the onset of stratification, nutrients are quickly depleted from the surface mixed zone. The DCM usually forms at the bottom of the thermocline where a nitracline occurs because of stratification (Takahashi and Hori 1984). The success of *Synechococcus* at the DCM is thought to be related to its ability to photosynthesis at low light levels (less than 1 % surface irradiance) whilst taking advantage of the higher nitrogen concentrations (Li et al. 1983; Murphy and Haugen 1985).

In temperate oceanic and coastal regions, marine *Synechococcus* densities have been reported to be lower during winter than in summer (Gradinger and Lenz 1995; Krempin and Sullivan 1981; Shapiro and Haugen 1988; Waterbury et al. 1986). Waterbury et al. (1986) found winter minima of 10^2 cells ml^{-1} and summer maxima of 10^5 cells ml^{-1} in the Woods Hole Harbor. El Hag and Fogg (1986) also found winter minimums of 4 x 10^4 cells ml^{-1} and summer maximums of 16 x 10^4 cells ml^{-1} in the Menai Straits, Irish Sea. The one exception to this winter minimum summer maximum cycle in *Synechococcus* abundance was at an Antarctic coastal site (Walker and Marchant 1989). They found that *Synechococcus* densities were at a maximum (140 cells ml^{-1}) in August

and were low in summer (less than 10 cells ml^{-1}). The winter maximum, at 5 m ,was thought to have occurred due to the accumulation of cells lost from surface waters during ice formation. Waterbury et al. (1986) found there to be little seasonal variation in *Synechococcus* numbers in tropical regions.

In some temperate regions, the summer peak in *Synechococcus* cell numbers occurs as a short term bloom (El Hag and Fogg 1986; Glover 1985; Shapiro and Haugen 1988). In other regions the density of *Synechococcus* cells remains high, relative to winter densities, throughout summer (Krempin and Sullivan 1981; Waterbury et al. 1986). El Hag and Fogg (1986) reported that the seasonal cycle of *Synechococcus* was different from the net plankton in the Irish Sea in that the net plankton increased in numbers over spring and summer but their numbers remained constant over summer. The summer increase in *Synechococcus* numbers have been correlated to temperature (Murphy and Haugen 1985; Waterbury et al. 1986), light (El Hag and Fogg 1986) and a change in grazing pressure (Shapiro and Haugen 1988).

Table 1.2: Abundance of *Synechococcus*, during spring or summer, in various geographical regions. *This *Synechococcus* density occurred during winter.

Location	Latitude	Habitat	Depth sampled (m)	Temp.(°C)	Cells ml^{-1}	Highest Densities	Reference
	78-89°N	Oceanic	0	< 0	541		(Gradinger and Lenz 1995)
Foxe Basin, Arctic	68° N	Coastal	5 - 55	-1 - 0	2×10^3	15	(Smith et al. 1985)
Norwegian Sea, Iceland	64° N	Oceanic	0-50		10^4	Throughout this zone	(Johnson and Sieburth 1979)
North Atlantic	60° N	Oceanic	N.G.	5.4 - 6.1	2×10^3	25 m	(Murphy and Haugen 1985)
North Sea	56° N	Oceanic	0 - 80	10 - 15	$(0.2 - 25) \times 10^4$	Surface	(Howard and Joint 1989)
North Pacific	45° N	Oceanic	0 - 150	15 - 5	$(5 - 10) \times 10^2$	Surface	(Ishizaka et al. 1994)
North Atlantic	40° N	Oceanic	N.G.	16.5 - 17.2	7.5×10^3	10 m	(Murphy and Haugen 1985)
Gulf of Maine	40° N	Coastal	N.G.	11.6 - 20.9	9×10^4	25 m	(Murphy and Haugen 1985)
Southern California	34.5° N	Coastal	0 - 40	0 - 100	$(0.1 - 7) \times 10^4$	Surface	(Johnson and Sieburth 1979)
Yangtze River, China	32° N	Eustarine	0 - 40	33	$(5 - 30) \times 10^3$	Surface mixed zone	(Vaulot and Xiuren 1988)
Kiel Bight	27° N	Coastal	0 - 16	22 - 0.7	$(1.3 - 15) \times 10^4$	Surface	(Jochem 1988)
West Pacific	20° N	Oceanic	0 - 150	20 - 28	$(1 - 10) \times 10^2$	Surface	(Shimada et al. 1993)
Arabian Sea	18° N	Oceanic	0 - 80		$(1 - 6) \times 10^4$	Upper mixed layer	(Jochem 1995)
Central Pacific	0°	Oceanic	0 - 150	31 - 13	1×10^3	0 - 50	(Ishizaka et al. 1994)
Central Pacific	8° S	Oceanic	0 - 150	30 - 12	$(0.5 - 10) \times 10^3$	Surface	(Ishizaka et al. 1994)
Southern Ocean	44° 58 S	Oceanic	0	13.9	3.4×10^4		(Marchant et al. 1987)
Drake Channel	56° S	Coastal	0	7.5	2.2×10^3		(Letelier and Karl 1989)
Southern Ocean	56	Oceanic	0	0.1	10		(Marchant et al. 1987)
Southern Ocean	61° S	Oceanic	0	-1	2.4		(Marchant et al. 1987)
Antarctica	68° S	Coastal	5	-2	140*		(Walker and Marchant 1989)

Little research has been carried out on the interactions between species in the aerobic zone of Ace Lake, especially with regard to grazing pressure by ciliates, nanoflagellates and copepods. Unlike the nearby oceanic environment, the highest consumer in Ace Lake is *P. antarctica*. Further, the abundance of viruses and the control exerted by viruses on community structure in any Antarctic lakes has not been investigated. Preliminary experiments showed that light and temperature significantly increased the growth of *Synechococcus* in Ace Lake (Chapter 6). Due to the slow *in-situ* growth rates of *Synechococcus*, grazing rates by heterotrophic plankton and zooplankton could not be determined. It is probable that a combination of factors including grazing, nutrient limitation, light and temperature are controlling the concentration of plankton in the lake.

Apart from viruses, which remain unstudied, the heterotrophic bacteria are the least studied group of organisms in the euphotic zone of Ace Lake. To date very few bacteria from Ace Lake have been fully characterised and their ecological significance determined. This is due to two factors: the difficulty in culturing bacteria, especially those from the anoxic waters; and the lengthy and costly process required to describe organisms. Population density and seasonal variation in cell numbers are known but little is known about species composition, organic carbon cycling by the heterotrophic bacteria or the effect of grazing pressure on the bacterial population. In the euphotic zone of Ace Lake heterotrophic bacteria cell numbers were approximately 5×10^5 cells ml^{-1} over winter (L. Rankin, unpublished data). Over spring and summer, heterotrophic

bacterial cell numbers increased. At 2 m there were peaks in abundance in early spring and again in late summer where cell numbers increased approximately two fold (J. Gibson, unpublished data; L. Rankin, unpublished data). At 10 m there was a ten fold increase in cell numbers over summer. Bacterial cell numbers were highest at the anoxic interface where numbers reached more than 10^7 cells ml^{-1}. High densities of the sulfur bacterium *Chlorobium* sp. have been identified at this depth (see below). Numbers remained high in the anoxic zone of Ace Lake ranging between 5 x 10^6 and 10 x 10^6 cells ml^{-1}. These densities and distribution are consistent with heterotrophic bacterial densities in other meromictic lakes in the Vestfold Hills and the Dry Valleys (Smith and Howes 1990; Vincent 1988) but are higher than densities recorded in Crooked Lake a freshwater lake in the Vestfold Hills (Laybourn-Parry et al. 1992) and freshwater lakes of Schirmacher Oasis, Antarctica (Ramaiah 1995).

Polyclonal antibody staining (James et al. 1994) and flow cytometric techniques have been developed to observe changes in heterotrophic bacteria community structure in a range of meromictic lakes in the Vestfold Hills, including Ace Lake, but to date results have not been conclusive.

1.5.2 Anoxic zone

1.6.2.1 Photosynthetic bacteria

A layer of photosynthetic sulfur bacteria occurs at the top of the anoxic zone, between approximately 12.2 m and 12.8 m (Burke and Burton 1988b). The major species are the green sulfur bacteria *Chlorobium vibrioforme* and *Chlorobium limnicola* (Burke and

Burton 1988a). These anaerobic bacteria use bacteriochlorophylls for photosynthesis, and reduced sulfur compounds such as H_2S or simple organic compounds as electron donors. Species of photosynthetic purple bacteria (Rhodospirillaceae) and *Chromatium* sp. have also been reported from Ace Lake (Hand 1980) but it is thought that the *Chlorobium* spp. dominate because of their fast growth at low temperature and light, and their ability to survive long periods of low light levels (Burke and Burton 1988b). In 1992 the population was at its lowest between May and July, it increased from August and reached a maximum of 6×10^7 cells ml^{-1} in December/January (L. Rankin, unpublished data).

1.5.2.2 Anaerobic Heterotrophs, Sulfate Reducing Bacteria and Methanogens

To date four anaerobic bacteria and one Archaeon have been characterised from the bottom waters (24 m) of Ace Lake (Franzmann and Dobson 1992; Franzmann and Rohde 1992). These include two psychrotrophic species belonging to the genus *Carnobacterium,* an anaerobic wall-less spirochete an obligate anaerobic, coiled bacterium and a methanogen. It is thought that these bacteria are important for anaerobic organic carbon degradation.

Due to their ability to grow, albeit suboptimally, in aerobic conditions it is thought that *Carnobacterium funditum* and *Carnobacterium alterfunditum* may have played a role in the initial establishment of a reduced environment in the lake and the supply electron donors for the sulfate-reducing bacteria that co-exist in the monimolimnion (Franzmann et al. 1991a). These two species produce lactic acid as an end product of carbon metabolism and have a generation time of between 17 h to 19 h at 1 °C, the temperature

of the water column from where they were isolated. They have an optimal growth temperature significantly higher (23° C).

Unlike the carnobacteria, conclusive statements regarding the taxonomy of the Ace Lake coiled bacterium await phylogenetic analysis (Franzmann and Rohde 1991). It is presumed that this bacterium has an intermediate role in the breakdown of biomass, fermenting peptides and a limited number of sugars and producing hydrogen, formic, acetic and butyric acids, the precursors for methanogenesis and acetogenesis.

The wall-less spirochete was initially considered a mycoplasma-like organism (Franzmann and Rohde 1992) until phylogenic analysis placed the organism within the Spirochaetales (Franzmann and Dobson 1992). The organism is a psychrophile, with a optimal growth temperature of between 12° C and 13° C.

Lipid analysis of water column particulates suggests that large populations of methanogenic Archaea are present in the monimolimnion (Mancuso et al. 1990). Phospholipid-derived ether lipids (PLEL), cell membrane lipids that are unique to Archaea (Langworthy et al. 1982; Tornabene and Langworthy 1979), were used to determine the biomass and activity of methanogens in the monimolimnion of Ace Lake. In the water column, methanogenic archaea were present below 17 m in depth at concentrations of between 1 - 7 x 10^5 cells ml^{-1}. Methanogen biomass was higher in the sediment (Mancuso et al. 1990). A new species of methanogen, *Methanococcoides burtonii*, was characterised from the bottom waters of lake. The organism utilised methylamines and methanol as precursors to methanogenesis (Franzmann et al. 1992). In addition, a H_2:CO_2 utilizing methanogen was obtained in an enrichment culture,

prepared with anoxic Ace Lake water. This organism remains to be characterised. It is not known which methanogen type is predominant in Ace Lake, but given that methanogenesis is largely limited to the region of the lake that is depleted in sulfate, it is probable that the H_2:CO_2 methanogen is the major contributor to methanogenesis.

Although limited taxonomic data is available on bacterial species capable of sulfate reduction in Ace Lake, biomass of sulfate reducing bacteria (SRB) in the water column of Ace Lake has been estimated by lipid analysis (Mancuso et al. 1990). It has been estimated that SRB comprised 25% of the microbial population at 23m even though at this depth sulfate reduction is rate limited by low sulfate concentrations (see below). Phospholipid-derived fatty acid profiles (PLFA) indicated that bacteria belonging to the genera *Desulfobacter* and *Desulfovibrio* were present in the monimolimnion and in the sediments of Ace Lake.

The characterisation of other bacteria from the monimolimnion continues. No doubt the metabolic requirements and products of new species from Ace Lake will shed more light on the complex interactions between bacterial species and chemical cycles.

1.6 Nutrient cycles in Ace Lake

The cycling of organic carbon and nutrients in Ace Lake has received little attention. The information available on nutrient concentrations in Ace Lake is not detailed and it is difficult to predict the fate of nutrients in the lake from this information especially in light of the changed oxygen conditions below the pycnocline. Most nutrient studies have only looked at a few depths in the euphotic zone of the lake (Burch 1988; Perriss et al. 1995) and only recently has an annual survey of nutrients in this zone been completed (J. Gibson, unpublished data). It is acknowledged that sulfur in the anoxic zone of the lake can interfere with nutrient analysis (Alpkem Corp 1992) and that the variation in salinity may make analysis more time consuming. However, it would be of great benefit if a detailed study of the distribution of organic carbon and nitrogen and phosphorus species over space and time in Ace Lake was conducted. Sulfur, nitrogen and phosphorus cycling in Lake Vanda have been studied using nutrient concentration gradients (Canfield and Green 1985). This proved to be an effective method for determining rates of nutrient cycling in Lake Vanda and it could form the basis of a similar study on Ace Lake. The information that is available on carbon production and consumption and on nutrient cycling in Ace Lake is presented below.

1.6.1 Aerobic carbon production and decomposition

Ace Lake has a catchment of about 35.5 ha consisting of low hills devoid of life except for a few mosses, lichens and nesting sea birds. Ace Lake is also visited by the occasional Adelie Penguin that uses the lake as a corridor between Prydz Bay and Long

Fjord, but the closest rookery is approximately 5 km to the southwest of the lake. Unlike lakes that are close to penguin rookeries (eg: Rookery Lake, Long Peninsula) there is no evidence that organic matter from birds has a significant input into the carbon cycle of Ace Lake. The input of organic carbon and nutrients, into Ace Lake, from higher plants is also insignificant as the density of mosses and lichens in the area is very low (Hand and Burton 1981). Matsumoto et al. 1984 found no identifiable organic compounds derived from plants but many derived from microbial material in Lake Vanda.

Due to the meromixis in Ace Lake organic carbon is decomposed both aerobically and anoxically. In the euphotic zone, where oxygen is present in the water, carbon would be decomposed through aerobic processes. Dissolved organic carbon (DOC) concentrations in the oxygenated waters ranges from approximately 0.50 mM in the mixolimnion to 0.95 mM at the bottom of the euphotic zone. These concentrations are high compared to the DOC concentration (0.10 mM) at a marine site off the coast of the Vestfold Hills (L. Rankin, unpublished data). The concentrations are, consistent with those of the bottom waters of Dry Valley. In Lake Fryxell microbial activity in the euphotic zone, especially in the DCM results in a downward flux of organic carbon and a accumulation of DOC in the sediment pore water (McKnight et al. 1993). Dissolved organic carbon can be produced by grazing animals, from cell damage and death, and by the production of extracellular carbon by phytoplankton (Lynch and Hobbie 1988). Parker et al. (1977) found that in Lake Hoare, 75 % of the total photosynthetically fixed organic matter appeared as extracellular products. The thick algal mats probably make a significant contribution to the DOC in Ace lake as occurs in other meromictic lakes in Antarctica, (Parker et al. 1977).

Due to the stability of the water column in Ace Lake, dissolved organic carbon (DOC) produced by primary productivity in the euphotic zone would probably be cycled in this region. It is thought that the rate of molecular diffusion is too slow to be of any significance as a mechanism of carbon transportation to the monimolimnion (Yusa 1979). Periods of deep entrainment and mixing (eg: intermittent convection) will also move substances into the monimnolimnion (J. Gibson., personal communications). Particulate organic carbon (POC) in the form of fecal pellets from *P. antarctica* and from sinking bacteria, heterotrophic and phototrophic plankton provides the substrate for carbon cycling in the anoxic zone (Hand 1980). Lipid, pigment and flow cytometric data confirm the presence of cells from the euphotic zone in the anoxic zone of Ace Lake (Mancuso et al. 1990; Volkman et al. 1988; L. Rankin, unpublished data).

Wright and Burton (1981) reported primary productivity rates in Ace Lake of 0.163 mg C m^{-3} h^{-1} at 5 m and 0.472 mg C m^{-3} h^{-1} at 10 m, indicating that the water above the oxic/anoxic interface is more productive than the surface waters. This is probably due to a combination of physical (higher temperature) and chemical factors, for example the availability of ammonium, phosphorus and trace metals, favouring the growth of some phytoplankton species. In November 1993 total primary production rates at 2 m and 8 m were 0.68 mg C m^{-3} h^{-1} and 0.47 mg C m^{-3} h^{-1} respectively (Laybourn-Parry and Perriss 1995). These rates of primary production in Ace Lake are similar to rates measured in other Antarctic meromictic lakes (Parker et al. 1982; Spaulding et al. 1994; Vincent 1981; Vincent and Vincent 1982), in the Southern Ocean (Weber and El-Sayed 1987) but are low in comparison to rates measured in tropical and temperate lakes (Wetzel 1983). They differ from the rates measured by Wright and

Burton (1981) in that they indicate that productivity is higher in the upper, oligotrophic, waters although the latter did not measure rates at 10 m where there is known to be greater numbers of photosynthetic organisms. In Antarctica, primary productivity is seasonal, occurring at detectable rates only through the spring and summer months. Primary productivity may also vary from year to year depending on the physical dynamics of the mixolimnion. Except for *M. rubrum*, the contribution of individual species to primary production in the aerobic zone of the lake is not known, and even with *M. rubrum*, the contribution is only an estimate. Primary productivity measurements in Ace Lake indicate that *M. rubrum* may contribute between 15% and 40% of carbon fixation in the phytoplankton during the bloom period (Laybourn-Parry and Perriss 1995). Due to its small size (1.8 μm x 0.5μm) *Synechococcus* may not contribute significantly to primary productivity although because of the high cell numbers the total surface area estimates indicate otherwise (Chapter 3). Like *Synechococcus* all algal species bloom at specific depths at different times over summer and hence the contribution of each species to primary productivity would be temporarily and spatially variable.

1.6.2. Marine *Synechococcus* and primary production

The contribution of *Synechococcus* to biomass and primary productivity in the oceans varies with vertical distribution, with trophic status and with seasonal abundance of the picocyanobacterium (Fogg 1995; Glover 1985; Waterbury et al. 1986). Although *Synechococcus* is generally more abundant in coastal waters than it is in the oligotrophic oceans, its contribution to biomass and productivity is usually higher in the oligotrophic waters than in coastal waters where larger phytoplankton species are more abundant

(Jochem 1988). Nutrient concentrations in the tropical oceans are usually low, with nitrogen and phosphorus concentrations of < 0.2 μM and < 0.1 μM respectively (Joint 1986; Joint and Pomroy 1986). Due to its small size, *Synechococcus*, along with other picoplankton, is thought to be more competitive than larger phytoplankton species in oligotrophic regions (Fogg 1986). This pattern also applies to the contribution of *Synechococcus* to biomass in freshwater lakes (Burns and Stockner 1991; Shortbreed and Stockner 1990). In vertical stratified waters, the contribution of *Synechococcus* to primary productivity generally increases with depth (Waterbury et al. 1986). It is usually highest in the vicinity of the DCM.

Joint (1986) summarised the contribution of the less than 1 μm picoplankton (principally *Synechococcus*) to primary productivity based on latitudinal distribution. He suggested that in the Arctic during summer there was between a 10 % and 25 % contribution, in the temperate oceans during summer they contributed between 20 % and 30 % and in the tropical oceans they contributed more than 50 % to primary production. However, although the contribution of *Synechococcus* to primary production is high in the tropical, oligotrophic oceans, total biomass estimates, based on chlorophyll *a* concentrations, are usually low. Chlorophyll *a* concentrations of between 0.25 $\mu g\ l^{-1}$ and 2 $\mu g\ l^{-1}$ are typical in tropical oligotrophic oceans (Iturriaga and Mitchell 1986; Jochem 1995). In arctic waters, Gradinger and Lenz (1995) found the contribution of *Synechococcus* to overall picoplankton abundance increased from less than 1 % in summer to 70 -80 % in late autumn. This was, however, due to a decrease in the abundance of eukaryotic picoalgae. In this region, the contribution of *Synechococcus* to biomass and primary productivity is of minor importance. In sub arctic seas, the

contribution of *Synechococcus* to primary production in the summer surface waters was estimated to be 8 %. In early autumn, when *Synechococcus* was more abundant deeper in the euphotic zone (1 % surface irradiance), its contribution to primary production at this depth was estimated to be 68 % (Neuer 1992). During summer, in the temperate waters of Wilkinson's Basin, Northwest Atlantic Ocean (42° N) *Synechococcus* contributed 46 % to the *in-situ* photosynthesis integrated over the water-column (Glover et al. 1986). In the oligotrophic subtropical north Pacific Ocean (35° N), *Synechococcus* contributed approximately 64 % to primary productivity during spring when the mixed layer was between 60 and 80 m and a DCM occurred between 95 m and 105 m (Iturriaga and Mitchell 1986). *Synechococcus* growth rates and loss rates were greatest at the DCM. This pattern holds for the meromictic lakes in the Vestfold Hills (Chapter 3, 4 and 6).

1.6.3 Anoxic carbon production and consumption

The *Chlorobium spp.* that occur at the top of the anoxic zone use H_2S as an electron donor for anoxic bacterial photosynthesis (Burke and Burton 1988b). However, Hand and Burton (1981) suggest atmospheric CO_2 would not contribute significantly to this process as molecular diffusion would be too slow to replenish fixed CO_2. Instead, the *Chlorobium spp.* fix CO_2 and small organic molecules released by sulfate reducers and other heterotrophs occurring in the immediate vicinity. *Chlorobium spp.* are therefore important in the cycling of sulfur and carbon in the anoxic zone of the lake. Hand and Burton (1981) estimated that anoxic photosynthetic bacteria could account for 45% of photosynthetic activity in Ace Lake assuming the band of activity was a metre wide. When *Chlorobium spp.* peaked in Ace Lake during December and January of 1992/93

they were concentrated in a band approximately 50 cm thick. As is the case for aerobic photosynthetic bacteria and algae, the contribution of *Chlorobium* spp. to carbon productivity is expected to vary throughout the year.

The carbon source for anoxic decomposition in Ace Lake presumably comes from the aerobic zone as POC and also from decaying bacteria in the anoxic zone (POC and DOC). Between 12.2 m and 24 m in Ace Lake carbon is decomposed anoxically by sulfate reducing bacteria, (Burton and Barker 1979; Franzmann et al. 1988; Mancuso et al. 1990) fermentative heterotrophic bacteria (Franzmann and Dobson 1992) and by methanogens that utilise short chain organic acids, hydrogen and carbon dioxide, the products of fermentation, to produce methane (Burton 1980; Franzmann et al. 1991b).

1.6.4 Sulfur cycling

The photosynthetic sulfur bacteria (PSB) and sulfate reducing bacteria (SRB) contribute significantly to the sulfur cycle in Ace Lake. The PSB at the anoxic interface use H_2S as an electron donor and oxidise it to sulfate and elemental sulfur. The SRB use sulfate as an electron acceptor reducing it back to sulfide. Carbon dioxide which is used by the PSB and also by methanogens in the anoxic zone is produced during sulfate reduction and fermentation. Dissolved and particulate organic carbon produced during this cycling supports the populations of heterotrophs in the near vicinity of the oxic/anoxic interface (Hand and Burton 1981). SRB and methanogens may also be co-metabolising small chain organic acids in the bottom waters of the monimolimnion where sulfate is limiting (Franzmann et al. 1991b).

1.6.5 Methanogenesis

Below 19 m sulfate reduction is limited by low sulfate concentrations (Figure 1.6) and hence methanogenesis becomes the major terminal mineralisation process. Where sulfate is not limiting the sulfate reducing bacteria may out compete the methanogens for substrates (Franzmann et al. 1991b). It is presumed that sulfate reducing bacteria and other anoxic heterotrophic bacteria such as the C-shaped bacteria (Franzmann and Dobson 1992) break down particulate and high molecular weight dissolved forms of carbon to low molecular weight forms for methanogenesis (Hand and Burton 1981). Burton and Qiang (1988) showed that in Ace Lake concentrations of butyric acid decreased at depths where methane producers were active.

There appears, therefore, to be a strong interaction between photosynthetic sulfur bacteria, sulfate reducing bacteria, anoxic heterotrophic bacteria and methanogenic archaea in the anoxic zone that eventually reduce complex organic compounds to methane. Bryant et al. (1977) indicated that normally methanogens are not known to utilize lactic acid but a symbiotic consortia of sulfate reducing bacteria and methanogens can co-metabolise lactic acid in the absence of sulfate through hydrogen transfer (Franzmann et al. 1991b). A number of organic intermediates have been detected in Ace Lake that would be formed as part of this interaction (Roberts and Burton 1994).

Methanogenesis occurs in the water column and sediments of Ace lake at very slow rates. In the water column, maximum rates of methanogenesis of 2.5 μmol kg^{-1} day^{-1}

from $NaH^{14}CO_3$ were measured at 20 m (Franzmann et al. 1991b). The recorded rates were at the limit of detection for the experimental method used. As substrate (short chain organic acids) was not limiting (Franzmann et al. 1991b) the low rates of methanogenisis from CO_2 reduction were probably caused the low temperatures at these depths. Methanogenic bacteria usually have a optimal growth temperature between 30°C and 40°C (Franzmann et al. 1991b). Recently a psychrophilic methanogen was isolated from the bottom waters of Ace Lake (David Boone, personal communication). However, this organism still has a very slow growth rate. It is probable, from the age of Ace Lake and from culturing experiments, that the methanogens in the lake have not yet evolved to grow maximally at the low temperatures experienced at these depths. Further to this evidence, bacterial biomass estimates of methanogens (Mancuso et al. 1990) implies that current microbial degradation of organic carbon in Ace Lake may be occurring at extremely slow rates.

Despite the fact that methanogenesis occurs at a very slow rate, the bottom waters of Ace Lake (below 19m) are saturated with methane and the ebullition of methane gas has stripped the N_2 from the water in this region (Figure 6). H_2S does not appear to be stripped in the same way and remains at high concentrations in the bottom waters of the lake (Burton 1980). The high concentrations of H_2S in the bottom waters of Ace Lake does not appear to be inhibiting methanogenesis at this depth. However, due to their very slow growth rates in culture the exact controls of growth of the methanogens in the bottom waters of Ace Lake are yet to be determined.

Methanogenesis has not been reported to occur in any other meromictic lakes in the Vestfold Hills (Franzmann et al. 1991b). Methane has been detected in low

concentrations at the bottom of Lake Vanda in the Dry Valleys (Vincent 1988) but no rates have been reported. Rates of methanogenis have been reported for some of the freshwater lakes of Signy Island in maritime Antarctica (Ellis-Evans 1984).

1.6.6 Cycling of other ionic species

Nutrient data and trace metal analysis has allowed biological mechanisms responsible for the distribution of iodine (Butler et al. 1988) and other ions to be postulated (Hand and Burton 1981; Masuda et al. 1988). Butler et al. (1988) noted a close correlation between bacterial numbers and total iodine. They proposed that the distribution of iodine might be the result of a long term interaction with micro-organisms, where the element is assimilated by phytoplankton, which are grazed by zooplankton (copepods). Iodine then sinks in fecal pellets, and other detritus, to be remineralized by anoxic heterotrophic bacteria at the top of the anoxic zone or in near-bottom and sediment pore waters. Reductive dehalogenation of organic compounds has been observed in cultures of methanogenic archaea and sulfate-reducing bacteria (Tandoi et al. 1994).

Masuda et al. (1988) measured high concentrations of trace elements, including iron, magnesium and aluminium, in Ace Lake compared to open ocean water and suggested that aerosol particles and weathering from nearby rocks were probably an important source of trace elements in the lake. It is therefore unlikely that trace elements are limiting microbial growth in the lake.

A similar scheme could also be postulated for the distribution of nitrogen species in Ace Lake. Oxidised nitrogen in summer melt water (not a rich source), nitrogen fixation by

cyanobacteria in algal mats and rapid recycling by heterotrophic bacteria may be the only oxidised nitrogen source in the oligotrophic surface waters of the lake. Total oxidised nitrogen (measured as nitrogen + nitrite- N) concentrations are low (< 0.35 μM) throughout the aerobic waters (Burch 1988; Burton 1980; Perriss et al. 1995). When measured in 1979 reduced nitrogen (NH_4^+ plus amino acids) was not detected (<0.001 mM in the mixolimnion of Ace Lake (Figure 1.7), but concentrations increased below the pycnocline and reached a maximum concentration of 0.06 mM at 15 m (Burton 1980). Reduced nitrogen concentrations have not been measured in Ace Lake since this time. The increase of NH_4^+ and amino acid concentrations is probably due to the deamination of proteins which accumulate due to the slow rates of organic matter mineralization. These reduced forms of nitrogen may also diffuse up from the top of the anoxic zone.

Phosphate-P in the mixolimnion of Ace Lake is at a concentration of approximately 0.65 μM (Burton 1980, J. Gibson, unpublished data; L. Rankin, unpublished data). Phosphate-P concentrations increase below the pycnocline reaching a concentration of approximately 9.7 μM at the bottom of the euphotic zone (Figure 1.7).

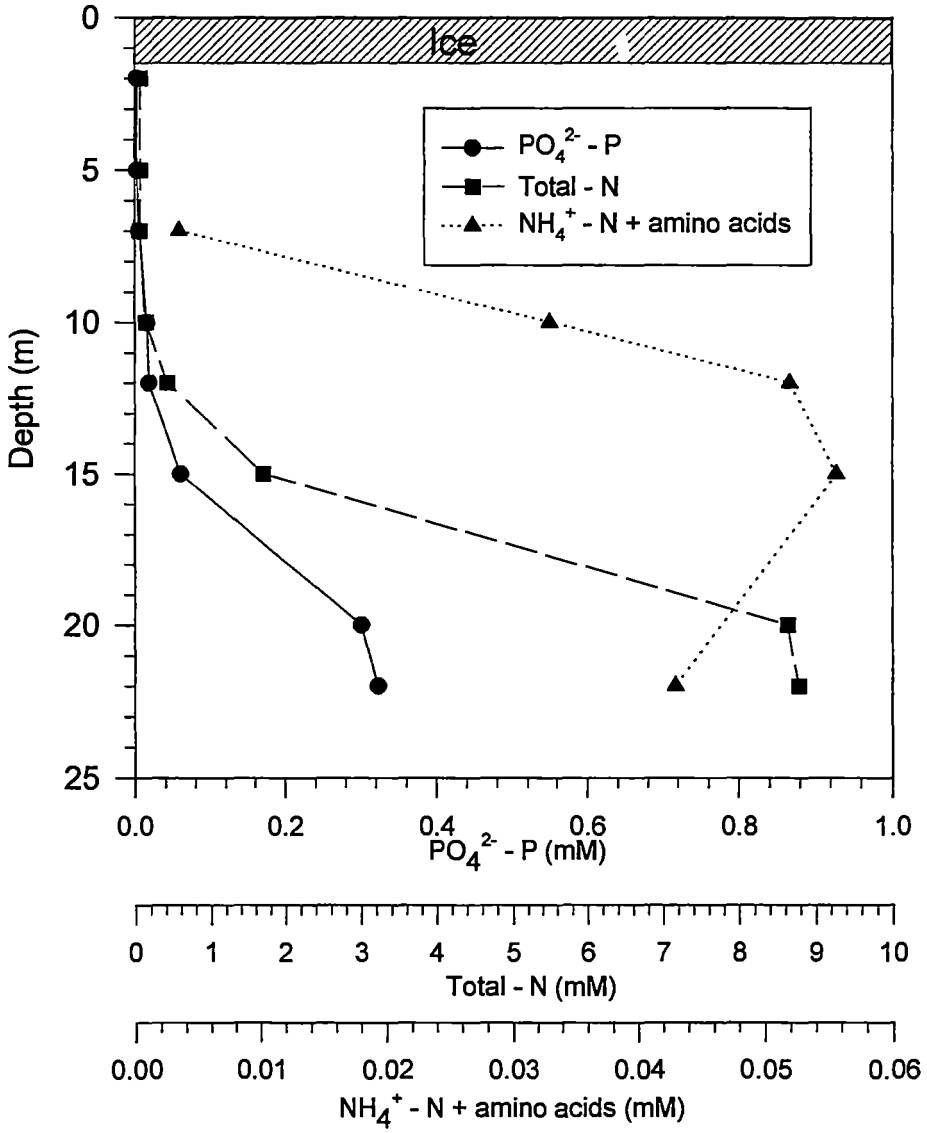


Figure 1.7: Vertical distribution of nutrients in Ace Lake (from Burton 1980)

There is a reduction in the phosphate-P concentration at the anoxic interface but according to Burton (1980) the phosphate-P concentration then continues to increase to the bottom of the lake where a concentration of 0.32 mM was measured.

Like in other meromictic lakes in Antarctica, (Canfield and Green 1985; Lawrence and Hedry 1985), it is probable that diffusion from the nutrient rich anoxic waters contributes to the increased phosphorus and ammonia concentrations below the pycnocline in Ace Lake. Further, it is proposed that the nitrogen and phosphorus concentrations in the mixolimnion are low because the stratification prevents nutrients from the more nutrient rich bottom water from mixing with mixolimnic water. Because the catchment is virtually devoid of plant and animal material and because there are no significant melt streams in the catchment, allochthonous input of nutrients into Ace Lake would not contribute significantly to the nutrient concentrations in the mixolimnion. It is possible that some nitrogen fixation occurs in the thick algal mats in the litoral zone of the lake but, to date, nitrogen fixing species have not been detected in the planktonic community.

It is unlikely that phosphorus limits primary production in Ace Lake (Table 1.3). Hand and Burton (1981) indicated that nitrogen was the limiting nutrient in Ace Lake as it is in Lake Hoare and Lake Fryxell (Simmons et al. 1993; Vincent 1981). The very low phosphorus concentrations above the anoxic interface in Lake Vanda (Table 1.3) are thought to be caused by the formation of an insoluble phosphorus compound in the anoxic zone of the lake (Canfield and Green 1985). In this lake, phosphorus is thought to be limiting phytoplankton productivity (Vincent and Vincent 1982).

Table 1.3: A comparison between nutrient and chlorophyll *a* concentrations in two meromictic lakes in the Vestfold Hills and two in the Dry Valleys. The two depths from each lake represent the epilimnetic concentrations and concentrations at the DCM in each lake. [a]J. Gibson, unpublished data, the ranges given were for a one year period in 1994; [b]L. Rankin, (unpublished data), sampled in November 1993; [c](Perriss et al. 1995), sampled in November 1993; [d](Vincent and Vincent 1982), sampled in December 1980; [e](Vincent et al. 1981), sampled in December 1980; [f](Vincent 1981), sampled in December 1979.

Lake	Depth(m)	Phosphate-P (μM)	NO_3 - N (μM)	Chlorophyll *a* ($\mu g\ l^{-1}$)
Ace	2[a]	0.1 - 0.3	0.07 - 0.45	0.75 - 5.29
Ace	10[a]	2 - 11	0.03 - 0.20	0.9 - 4.1
Abraxas	10[b]	0.07	< 0.02	0.1[c]
Abraxas	19[b]	0.20	< 0.02	N.D.
Vanda	35[d]	6×10^{-4}	0.3	< 0.1[d]
Vanda	57[d]	3×10^{-3}	15	0.9[d]
Fryxell	6[f]	0.05	0.04	4
Fryxell	9[f]	0.19	0.44	28

1.7 Future Outlook

Like other meromictic lakes in the Vestfold Hills, Ace Lake has a dynamic physical and chemical stratification which is dependent on the water budget and local climatic conditions. Within the last two years it appears that the water budget of Ace Lake has shifted from positive to negative, hence the recent fall in lake height above sea level. If this trend continues then it is possible that the mixolimnion will become more saline and the meromictic system less stable. The lake may start mixing to a greater depth and the aerobic section of the monimlimnion may be incorporated into the mixolimnion. Nearby Organic Lake and Highway Lake have also recently had a shift in the water balance from positive to negative and the depth of these lakes is decreasing. It remains to be seen if microbial communities, such as the picocyanobacteria that live just above the anoxic interface, will adapt to these changes in lake structure and become distributed throughout the mixolimnion or whether future physico-chemical conditions of the mixolimnion will not support these communities.

Lake Fryxell in the Dry Valleys, Antarctica, is the only other Antarctic, meromictic, lake reported to have similar biogeochemical cycles to Ace Lake (Green et al. 1989). Both lakes have stable water columns due to strong density gradients. It is not, however, the same as Ace Lake in every way. Lake Fryxell has a perennial ice cover of approximately 4 m and the lake is less saline (Vincent 1988). Further, unlike Ace Lake, Lake Fryxell is fed by many summer melt streams (Green et al. 1988). The two lakes have similar phytoplankton community structures with flagellates and cyanobacteria dominating. The dominant cyanobacterial population in Lake Fryxell is the fillamentous *Oscillatoria* rather than a picocyanobacteria (Spaulding et al. 1994). It is highly probable that all meromictic lakes in Antarctic differ significantly in structure and microbial composition because of their dependency on ancient evolutionary processes and local climatic conditions. There are many other limnologically diverse meromictic lakes in the Vestfold Hills and in other areas of Antarctica most of which have not been studied in any detail. Like Ace Lake these lakes have interesting, dynamic, geochemical cycles and support a wide diversity of bacterial and algal species. The continued study of Ace Lake, as well as other meromictic lakes in the Vestfold Hills will reveal many more biogeochemical mechanisms and possibly provide an insight into ancient climatic conditions. Organisms isolated from unique Antarctic ecosystems, including the meromictic lakes, may have important biotechnological applications in water and wastewater treatment, and in medicine (Ashbolt 1990). No doubt the biodiversity of Ace lake will continue to be unraveled through the continued use of DNA, lipid, and possibly antibody technology.

Chapter 2

Flow Cytometry and Antarctic Aquatic Microbiology

2.1 Abstract

Flow cytometry has become a useful tool in the analysis of aquatic microbial communities. During the 1992 winter at Davis Station, flow cytometric methods were developed for the analysis of bacterial and algal communities in the meromictic lakes of the Vestfold Hills. As the flow cytometer used in these investigations (Becton Dickinson FACScan) can not directly count particles, methods were developed to count bacterial and phytoplankton cells. Due to the density gradients within the meromictic lakes, the effect of salinity on flow cytometric analyses was also determined. In the analysis of cell abundance, the flow cytometer compared well with the microscope. The error in counting obtained using flow cytometric (3 %) was less than the error obtained using microscopic techniques (6 %). Sample salinity had an effect on the forward angle light scatter (FSC) signal. Following the development of methods, the flow cytometer was routinely used for the analysis of lake plankton communities. A population of picocyanobacteria belonging to the genus *Synechococcus* was discovered in three meromictic lakes using flow cytometric methods. With the aid of the flow cytometer, physiological and taxonomic characteristics of these *Synechococcus* populations have now been made. This chapter details the principles of flow cytometry, describes the methods developed for analysis of plankton communities in the meromictic lakes of the Vestfold Hills and discusses the advantages and disadvantages of using flow cytometry for this purpose.

2.2 Introduction

Flow cytometry has been used to study phytoplankton populations since the late 1970's (Legendre and Yentsch 1989; Olson et al. 1983; Pauu et al. 1978; Trask et al. 1982). Initially, flow cytometry was only used to study phytoplankton in culture but since the early 1980's flow cytometry has been used to study phytoplankton community structure in the oceanic environment. Flow cytometers are now commonly employed on marine science cruises (Hofstraat et al. 1991; Hofstraat et al. 1994; Hofstraat et al. 1990; Yentsch et al. 1986).

Although flow cytometers were originally designed for biomedical research (Muirhead et al. 1985; Ryan et al. 1988), the principles of flow cytometry make it an ideal technology for the analysis of bacteria and algae. A flow cytometer uses one or more lasers to emit light at specific wavelengths. When a cell passes through the path of the laser beam, the laser light is scattered producing a signal that corresponds to the relative size and complexity of the cell. Furthermore, if a cell carries a fluorescent tag or if photosynthetic pigments cause the cell to autofluoresce, the emitted fluorescent signal passes through a series of optical filters and is detected by photomultiplier tubes (Burkill 1987). A flow cytometer could almost be described as an automated fluorescence microscope with the photomultiplier tubes acting as an eye.

Commercially available flow cytometers have been designed and optimised for the analysis of human blood cells. The sample stream is optimal for cells between 10 μm and 25 μm in diameter and the filter configuration is optimal for the detection of

FITC and phycoerythrin, fluorescent stains that are commonly used in medical pathology (Hofstraat et al. 1994; Shapiro 1988). There are however many other fluorescent stains, as well as the natural fluorescence from phytoplankton cells, that can be detected by this filter configuration (Burkill 1987; Sosik et al. 1989).

In recent years, flow cytometers have been specifically designed for the analysis of phytoplankton (Balfoort et al. 1992; Frankel et al. 1990; Peeters et al. 1989). Features that have changed include an option of two or three lasers that allows excitation of phytoplankton pigments at wavelengths other than 488 nm. For example a system with three lasers with excitation wavelengths of 442 nm, 529 nm and 633 nm has been successfully used to optically separate phytoplankton populations (Hofstraat et al. 1994). Furthermore, wider sample streams have been designed to account for phytoplankton colonies and fillamentous cells. Larger sample volumes, which allow for the analysis of oceanic samples that contain low numbers of phytoplankton, can also be used (Hofstraat et al. 1994). However, the more optical features that are built into a flow cytometer the greater the chance that the optical system may not be robust. Presumably due to their stability and robustness, the single laser flow cytometers such as the FACScan (Beckton Dickinson) remain popular choices for shipboard flow cytometry (Chisholm et al. 1988a; Li 1989).

There are several reasons as to why flow cytometry has now become a valuable tool in phytoplankton community analysis. The individual fluorescent signatures and the variability in phytoplankton cell size allows for the optical separation of populations based on intrinsic cellular properties (Hofstraat et al. 1991; Olson et al. 1989). This

makes the analysis of phytoplankton communities by flow cytometry a quick and efficient process, allowing for many more samples to be processed than could be achieved by traditional methods. Also, each cell is analysed individually providing accurate information on community structure (Chisholm et al. 1986; Yentsch et al. 1986). A large number of cells can be analysed in a short period of time allowing for more objective analysis and statistical accuracy (Chisholm et al. 1988a; Hofstraat et al. 1994). A flow cytometer can be used to monitor relative changes in phytoplankton community structure and physiological status (Cucci et al. 1985; Demers et al. 1989). Without calibration, however, flow cytometers can not provide quantitative information on the fluorescence or morphological characteristics of phytoplankton cells nor do they have the facility to directly count phytoplankton cells. The machines can, however, be calibrated to obtain this information (Hofstraat et al. 1994).

Prior to this study the flow cytometer had not been used for studies in aquatic microbiology in Australia. This meant that there was no expertise that could be easily accessed in Australia. Flow cytometric methods used in this Antarctic study were therefore based on literature that was available at the time but were primarily developed at Davis Station, Antarctica. The flow cytometer was routinely used for: the determination of bacterial cell numbers (Chapter 3 and 4); for the analysis of *Synechococcus* distribution and abundance (Chapter 3 and 4 and 6) and for the analysis of other phytoplankton communities in the meromictic lakes of the Vestfold Hills (Chapter 4). The flow cytometer was useful in the characterisation of the Antarctic *Synechococcus* (Chapter 5), and for monitoring of *Synechococcus* enrichment cultures.

2.3 Methods

Methods that were developed for the use of flow cytometry included: the production and filtration of sheath fluid; the calibration of the instrument using fluorescent beads; the staining and counting of bacteria by flow cytometry; the counting of *Synechococcus* and other phytoplankton populations by flow cytometry; and determining the effect of salinity on relative cell size.

2.3.1 The flow cytometer

A FACScan flow cytometer (Beckton Dickinson) equipped with a 488 nm air cooled argon laser and the filter configuration outlined in Figure 2.1 was used for the analysis of water samples. The optical filter configuration included: a 530/30 nm band pass filter; a 585/42 nm band pass filter and; a 650 nm long pass filter. This allowed detection of fluorescence emission in three wave length ranges. The high flow rate setting allowed samples to pass through the flow cell at approximately 60 $\mu l\ s^{-1}$ where as the low flow rate allowed the sample to pass through the flow cell at approximately 12 $\mu l\ s^{-1}$. The ejection tube was 180 μm and the sample stream was approximately 12 μm in diameter on low flow rate and 28 μm in diameter on high flow rate

(G. Bauchop, personal communications). The alignment of the optical bench was checked before and after transportation of the flow cytometer to Antarctica, using 'Autocomp' software provided by Becton Dickinson.

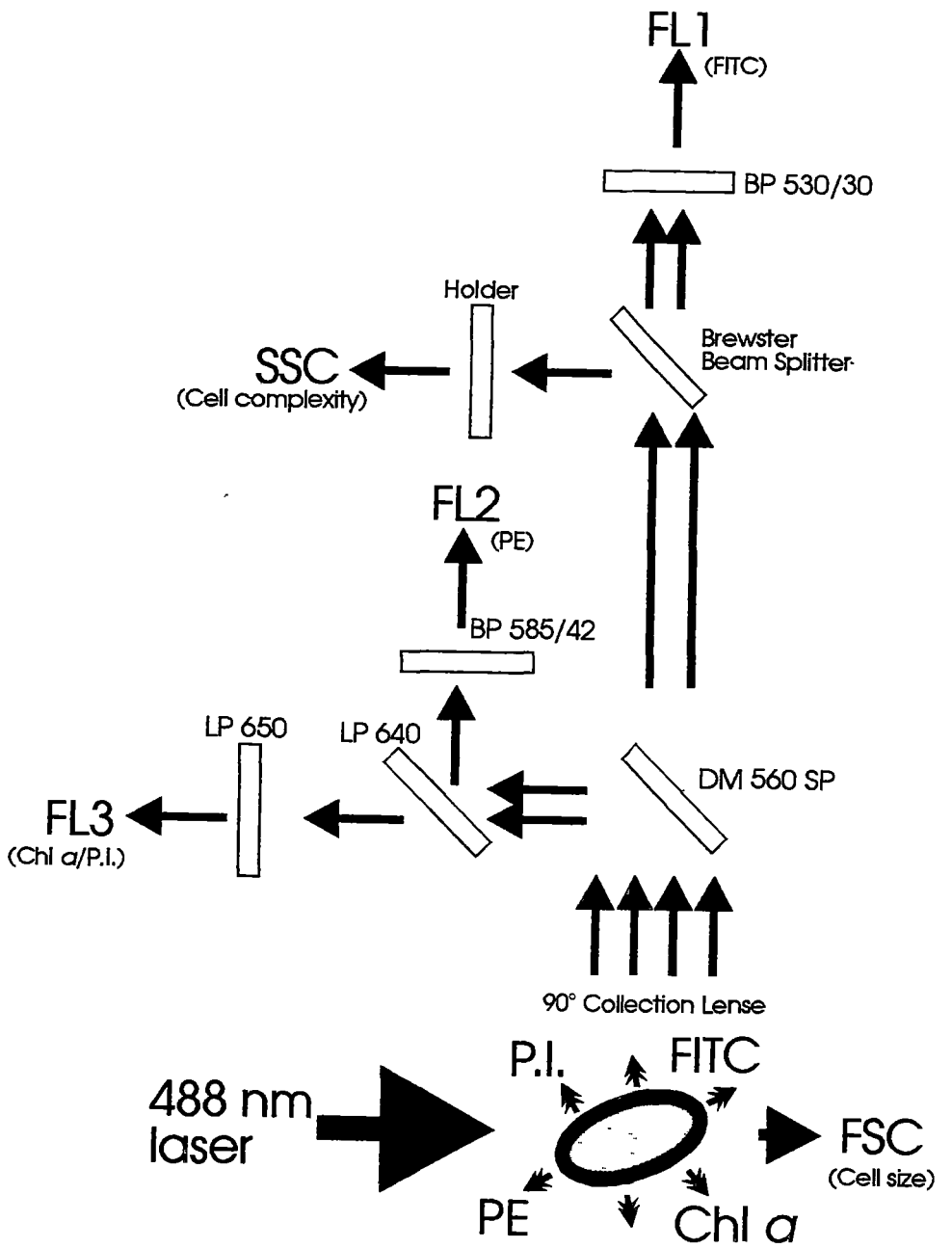


Figure 2.1: The optical configuration of the FACScan flow cytometer. DM = dichroic mirror; BP = band pass filter; LP = long pass filter; PE = phycoerythrin; PI = propidium iodide and FITC = fluoresceinisothiocyanate. (Figure modified from 'Introduction to the FACScan - Flow Cytometry made easy' Becton Dickinson Training Manual - 1991)

Fluorescent beads (1.98 μm diameter) were added to all samples as an internal standard and to assess the alignment of the optical bench. Data were collected in list mode and analysed using the 'Lysis II' software.

2.3.2 Sheath filtration

Initially sheath fluid was prepared using the following protocol. Phosphate buffered saline was prepared, in 20 l volumes, according to the method of (Brayton and Colwell 1987). To 800 ml of deionised water was added: 8.5 g NaCl, 9.1 g Na_2HPO_4 and 1.5 g KH_2PO_4. The solution was then adjusted to pH 7.2-7.3 and filtered through a filter bank series ranging from 0.8 μm, 0.45 μm, 0.2 μm and 0.1 μm. The 0.45 μm filter needed to be changed routinely. The final filtrate passed through a 0.45 μm filter, attached to a filter bell, into a 20 l pre-cleaned plastic drum. Davis Station was dusty and it was important to reduce the risk of contamination of the sheath fluid by dust particles. Once available, commercially prepared sheath fluid was used and filtered through a 0.05 μm filter using a pressure bomb (Millipore).

2.3.3 Bead Calibration

Calibration grade 'Fluoresbrite' 1.98 μm microspheres (Polysciences Inc.) were used in the determination of bacteria and phytoplankton cell numbers. They were also used to check laser alignment and instrument settings.

One drop of the concentrated bead suspension was added to 10 ml of 0.1 μm filtered PBS. The diluted bead suspension was stored in a 10 ml polyethylene falcon tube with 0.1 % sodium azide solution added to inhibit bacterial growth. The beads were kept in the dark and stored below 4° C.

The bead density was determined after 48 h of storage. The beads were sonicated for five minutes before the density was determined. A known volume of the bead suspension (0.1 ml) was added to PBS and filtered onto a prestained irgalan black 0.25 μm, 25 mm filter, at low pressure. The filter was then mounted for microscopic observation at 1000 x magnification. This procedure was repeated in triplicate, 10 fields of view were counted on each slide and the final bead density determined. The tube was sonicated for five minutes before use. This was to dislodge beads that had become stuck to the side of the 'Falcon' tube routinely before use. Beads were then further diluted in PBS or added to a sample, as required.

2.3.4 Propidium iodide staining for bacterial cell counts

Lake water samples previously preserved with 0.2 μm filtered formalin (1 % final concentration) and stored at 4°C in the dark were used to determine the bacterial cell numbers.

A propidium iodide staining method previously described by Wallen et al., (1980) was modified according to the following protocol. Samples (1 ml) were centrifuged at 6000 rpm, with no break, at 4° C, in a Beckman J2-21 centrifuge for 30 minutes.

The supernatant was discarded and the sample washed once in phosphate buffered saline (PBS). The sample was then resuspended in 90 µl PBS. 10 µl of 70 µg ml^{-1} propidium iodide (Sigma Chemicals) was added and the samples incubated at 4° C overnight. The samples were then washed twice with PBS and resuspended in a known volume (1 ml) of PBS. A known volume of the 1.98 µm calibration bead suspension (usually 10 µl) was added to each sample. Bacterial cell numbers were calculated from the ratio of cells to beads as determined by the flow cytometer.

On the FACScan, PI was detected in FL3 where fluorescence emissions greater than 650 nm were measured. The instrument settings used to determine bacteria cell numbers are given in Table 2.1. No compensation was used. These instrument settings were optimised using PI stained, cultured bacterial cells.

Table 2.1: Flow cytometric instrument settings used in the determination of bacterial cell numbers.

Parameter	Settings
FSC	E01
SSC	407
FL1	437
FL2	514
FL3	563
Threshold	80

The bacterial populations and bead suspensions were gated on a FL3/SSC dot plot and cell numbers were determined as follows: Cells ml^{-1} = Number of cells x (Bead density)/(Number of beads). To gate a population in the analysis of flow cytometric data means to set a region around a specific population(s). Data which

falls outside the analysis gate remains in memory but is not included in the analysis (Flow Cytometry, Glossary of Terms, Becton Dickinson 1992).

2.3.5 Analysis of bacteria by fluorescence microscopy

An epifluorescence microscope (Leitz Laborlux -12) equipped with a 100 W mercury vapor lamp and filters to accommodate excitation and emission wavelengths for DAPI (4`, 6-diamidino-2-phenylindol) was used to measure the numbers of bacteria in cultures and lake water samples. A 100 µl volume of 1 % formalin (0.2 µm filtered) fixed bacterial culture was added to 10 ml of 0.2 µm filtered phosphate buffered saline. Pre-filtered (0.2 µm) DAPI (Sigma Chemicals) was added to give a final concentration of 2.5 µg ml^{-1}. Samples were stained overnight at 4°C in the dark. Samples were then filtered, at low pressure, onto 0.2 µm, 25 mm, prestained membrane filters (Millipore) and mounted for microscopic observation at 1000 x magnification (Porter and Feig 1980). Lake water samples were prepared and cells were counted in the same way as described for cultured bacteria samples. Flow cytometric and microscopic counts were then compared (Sigmastat 1992).

2.3.6 Analysis of fresh lake water samples by flow cytometry.

The flow cytometer was used routinely to monitor changes in autofluorescent cell populations, primarily in Ace Lake but also in a selection of other meromictic lakes in the Vestfold Hills. Lake water samples, which had been kept in the dark at 4° C, were analysed within four hours of collection. A known number (usually 10 µl) of

calibration beads was added to 1 ml of lake water and the sample was then analysed directly.

Populations were detected on the flow cytometer according to their fluorescent characteristics. Forward angle light scatter (relative cell size), side angle light scatter (cell complexity), FL1 (Green fluorescence), FL 2 (Orange fluorescence) and FL 3 (Red Fluorescence) were used to identify populations. Populations were gated on two parameter dot plots using the Becton Dickinson Lysis software. Individual populations were analysed to determine cell numbers. Due to the spectral properties and size differences of autofluorescent populations, samples were analysed using several groups of instrument settings. Some populations were not evident when the instrument was set to a threshold on red fluorescence (FL3) but were distinct if the instrument was set to a threshold on green fluorescence (FL 1). The side angle light scatter (SSC) also varied according to the populations that were analysed. If small cells were being analysed the SSC setting was higher than if large cells were being analysed. Two commonly used sets of instrument settings are given in Table 2.2. No compensation was used. These instrument settings were optimised using lake water samples.

The analysis of *Synechococcus* cell numbers was compared to microscopic analysis in the same way that bacterial numbers were checked. A known volume of *Synechococcus* cells were filtered, at low pressure, onto a 0.1 μm membrane filter. An epifluorescence microscope (Leitz Laborlux -12) equipped with a 100 W mercury vapor lamp and green filter set (M2) was used to count the *Synechococcus* cells. For flow cytometric analysis, a known volume of fluorescent calibration beads was added

to 1 ml of sample and the low sample rate was used for counting. The first set of flow cytometric instrument settings in Table 2.2 was routinely used for the analysis of *Synechococcus* populations. Microscopic and flow cytometric data were compared (Sigmastat 1992).

Table 2.2: Flow cytometric instrument settings used in the analysis of phytoplankton communities.

Parameter	Set 1	Set 2
FSC	E00	E01
SSC	323	250
FL1	437	437
FL2	514	514
FL3	459	459
Threshold	FL3 -80	FL1 - 64

2.4 Results

2.4.1 Sheath Fluid Preparation

The sheath fluid was used to carry the sample through the flow cell pass the laser beam. Traditionally, sheath fluid used in flow cytometers has been a buffered saline solution that had a salinity and pH equivalent to human blood. Phosphate buffered saline (PBS) is the standard sheath fluid used and has a salinity of 0.9 %. PBS was originally used for the flow cytometric analysis of Antarctic Lake water samples. PBS (0.9 %) was chosen for two reasons. Firstly, because it was required for work with anti-bacterial polyclonal antibodies that had been made in rabbits, the effect of high salt solutions (for instance seawater) on antibody stability was not known. Secondly, PBS was the recommended standard sheath fluid for flow cytometry.

Traditionally 0.2 μm filtered sheath fluid is used in flow cytometry but this has been to study human cells that are approximately 5 μm or larger. In the current applications, the cell sizes were up to ten fold smaller than this. It was therefore decided to use

0.1 μm filtered sheath. Also, due to the isolation in Antarctica, it was decided to prepare the PBS according to the method outlined above rather than using commercially available sheath fluid.

From the end of the winter season, when the first supplies arrived, commercially produced PBS (OSMISOL) was used. OSMISOL 0.2 μm filtered by the supplier. The OSMISOL was further filtered using a 20 l pressure bomb (Millipore) through a 0.2 μm and a 0.05 μm filter. For analysis of phytoplankton cells from the meromictic lakes and the marine site, as an alternative to using OSMISOL, 0.05 μm filtered sea water was also used.

2.4.2 Calibration of fluorescent beads

1.98 μm microspheres, were used routinely in flow cytometric analysis. Bead numbers per ml were determined using the fluorescent microscope as outlined in the methodology. Immediately before use, the beads were sonicated for five minutes. This step was crucial because the beads would adhere to the side of the storage tube and they would also clump. Although the flow cytometer was effective at breaking

up weakly clumped beads or cells the number of beads added to each sample would not be accurate if the beads clumped or adhered to the side of the storage tube.

2.4.3 The effect of salinity on the flow cytometric image

When fresh lake water samples were analysed on the flow cytometer there was sometimes a large salinity difference between the sample and the sheath fluid. Sample salinity ranged from about 1 % in Clear Lake up to about 20 % at the bottom of Organic Lake. The high salinity gradients caused distortion of the forward angle light scatter (FSC) signal.

A calibration using a variety of salt concentrations in the sample tube was performed. This calibration was carried out using latex beads (Coulter Electronics), a *Dunaliella* sp. culture and a heterotrophic bacterial culture. Data were collected over three forward scatter settings E0-1, E00 and E01, on low and high sample rate. The low flow rate caused the sample stream to narrow. This restricted the position of the cells to a smaller area, and increased resolution because the illumination was more uniform (FACScan users manual).

The three fluorescent signals (FL1, FL2 and FL3) as well as the 90° (SSC) signals were not effected by the salinity gradient, but FSC was altered when there was a large salinity gradient (Chapter 4, Figure 4.10, Burton Lake). Figure 2.2 is a histogram plot that shows the effect of salinity on the forward scatter (FSC) signal from a bead suspension. The primary effect of the high salinity samples on the FSC signal was to increase the spread of the signal from the beads around the mean and also to shift the

signal to the right on the FSC axis (an apparent increase in cell volume). Salinity had the greatest effect on the small beads and the effect was greater when the flow cytometer was run on high sample rate.

Bacteria were effected by salinity in the same way as the beads. There was a shift to the right, on the axis, in the FSC signal with an increase in sample salinity (Figure 2.3). The mean FSC channel number and the standard deviations of the signal from the bacterial population in three samples of different salinity is given in Table 2.3. The *Dunaliella* cells were not effected to the same extent as the bacteria (Figure 2.4). There was a small shift in the signal, to the left on the FSC axis, when there was a large salinity gradient between the sample and the sheath. This was probably due to the large size of the *Dunaliella* cells (approximately 10 μm). The large beads were not effected by the salinity gradients to the same extent as the small beads.

Table 2.3: The effect of sample salinity on the forward angle light scatter signal of bacterial cells.

Parameter	1 % NaCl	3 % NaCl	5 % NaCl
Mean channel	7.74	28.81	84.58
S.D.	4.28	15.68	75.63

As indicated, forward angle light scatter is a measure of the relative difference in cell volume. In the analysis of lake water samples, 90° light scatter and fluorescence were routinely used for the determination of population density. Data was acquired with the sample rate on low. This reduced the effect of the salinity gradient on the FSC signal. If detailed information is required about cell size, sheath fluid of a similar salinity to the sample should be used.

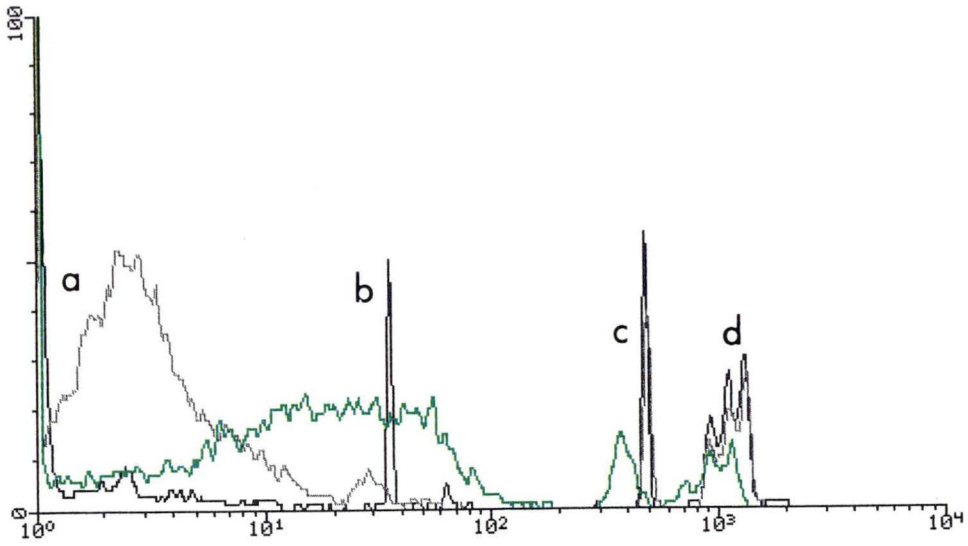


Figure 2.2: Flow cytometric histogram showing the effect of salinity on the relative cell size (FSC) of calibration beads. FSC (X axis) was set on E00, the Y axis represents relative abundance. The black line represents a bead mix in a 1 % NaCl solution; the gray line represents a bead mix in a 3 % NaCl solution and the green line represents a bead mix in a 5 % NaCl solution. The bead mix was composed of a) 0.5 μm beads; b) 1.98 μm beads; c) 5 μm beads and d) 10 μm beads. The sheath fluid was a 0.9 % PBS solution and the acquisition rate was low.

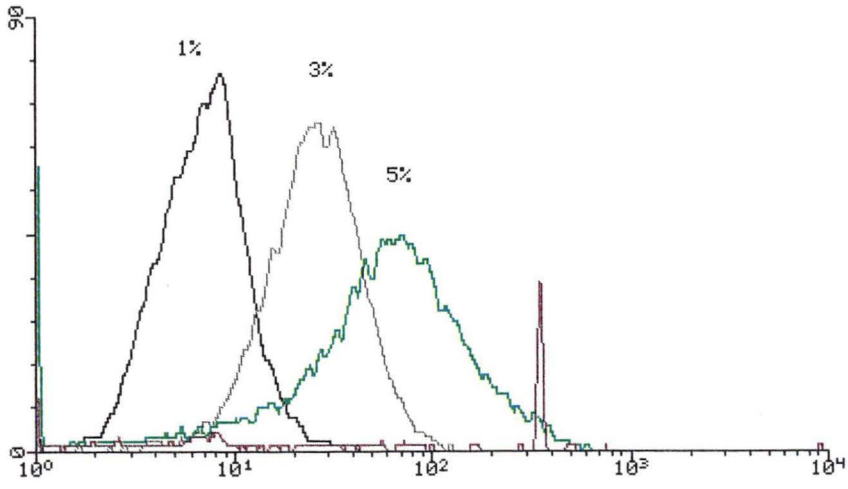


Figure 2.3: The effect of salinity on the relative size of bacteria. The X axis is FSC and was set on E01. In microscopic observations, the bacteria were approximately 0.5 μm in diameter. The Y axis represents relative abundance. The black line represents bacteria in a 1 % NaCl solution, the gray line represents bacteria in a 3 % NaCl solution and the green line represents bacteria in a 5 % NaCl solution. The red line represents 1.98 μm beads in a 1 % NaCl solution.

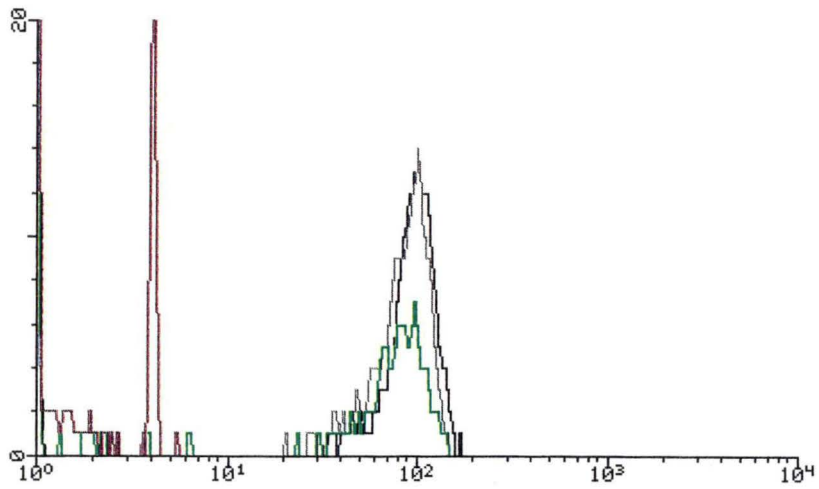


Figure 2.4: The effect of salinity on the relative size of *Dunaliella*. FSC (X axis) was set on E0-1. The black line represents *Dunaliella* in a 1 % NaCl solution, the gray line represents *Dunaliella* in a 3 % NaCl solution and the green line represents *Dunaliella* in a 5 % NaCl solution. The red line was 1.98 μm beads.

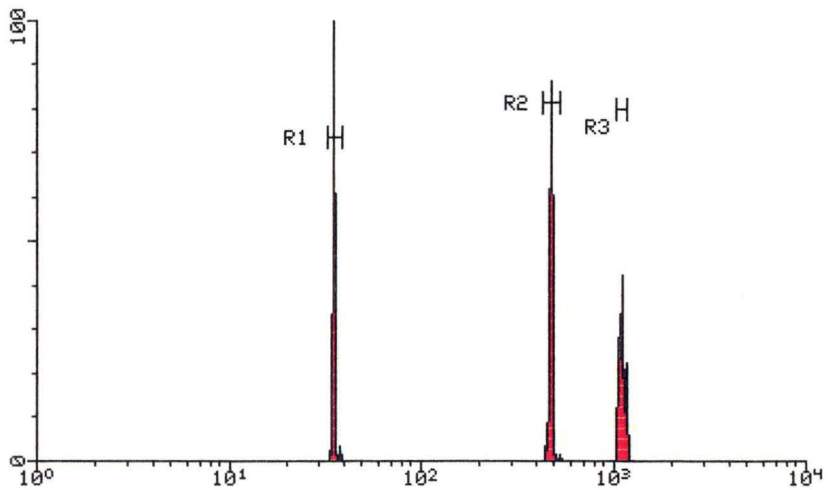


Figure 2.5: Forward angle light scatter (FSC) histogram of a bead mix. FSC was set on E01. R1 represents 1.98 μm beads; R2 represents 5μm beads and R3 represents 10 μm beads.

This was difficult to achieve in the analysis of lake water from the meromictic lakes as these lakes had large salinity gradients. It was therefore not practical to use a sheath fluid with a salinity that exactly matched the salinity of the sample. Filtered seawater was used as the sheath fluid for the analysis of phytoplankton populations in the meromictic lakes of the Vestfold Hills (Chapter 4). FSC was the only flow cytometric parameter effected by salinity, and the effect on FSC was reduced when data was acquired at a slow sample rate compared to a fast sample rate. It was therefore assumed that the change in cell volume and the broad spread in the FSC signal, caused by a difference in salinity between the sheath fluid and the sample, were optical effects rather than a morphological change of the cells.

When 10 % salt was used as sheath fluid for the analysis of Organic Lake samples, it caused a high background noise level for several days after it had been run through the machine. This effect remained even after distilled water had been run through for several hours to clear the lines. Warm water was run through the lines as this was more effective at dissolving salt crystals that may have been lodged in the line. Due to the residual background noise caused by high salt solutions it was decided that salt solutions of salinity greater than seawater salinity would not be used as sheath fluid.

The forward angle light scatter reading (FSC) is a relative measure of cell size. In Figure 2.5, peaks labelled R1, R2 and R3 represent beads of 2, 5 and 10 μm diameter respectively. The photodiode that detects FSC signals and the photomultiplier tubes that detect SSC and the three fluorescence signals can be adjusted to detect signals of different intensities (FACScan users manual). The peaks in Figure 2.5 represented

the relative cell sizes when the photo diode that detects FSC signals was set on the E00 setting. The photodiode can be set at one of four logarithmic settings. On E01, the apparent sizes moved a log unit to the right of the E00 setting and on E0-1 they moved a log unit to the left of the E00 . A setting of E0-1 is therefore designed for the analysis of large particles. FSC is more accurately a measure of cell volume (Olson et al. 1989). In Figure 2.5, the three spherical bead populations had volumes of 2.03 μm^3, 32.7 μm^3 and 261.8 μm^3 respectively.

2.4.4 Propidium iodide staining for total bacterial cell counts

Bacteria stained with propidium iodide fluoresced in FL3 (red fluorescence). The threshold setting of FL3 - 80 helped to eliminate background fluorescence caused by small particles in the sample. Figure 2.6 is a FL3 histogram of a PI stained bacteria culture. The population, was better delineated in a two parameter dot plot (Figure 2.7). SSC and FL3 were used to gate the bacterial populations so that cell numbers could be determined. This was especially important in the analysis of lake samples. In a culture, the cells have a uniform size and the flow cytometric image is therefore more uniform. In a natural bacterial population, however, cell sizes and shapes are variable and hence the dot plot population was not as uniform as a population of cultured cells.

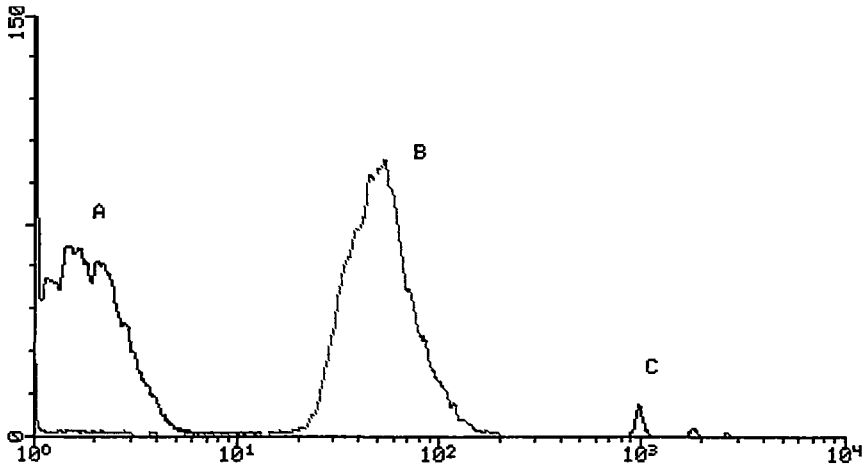


Figure 2.6: Flow cytometric histogram of propidium iodide (PI) stained bacteria. The X axis represents relative red (FL3) fluorescence. The Y axis represents relative cell numbers. A) unstained cells; B) PI stained cells and C) fluorescent beads.

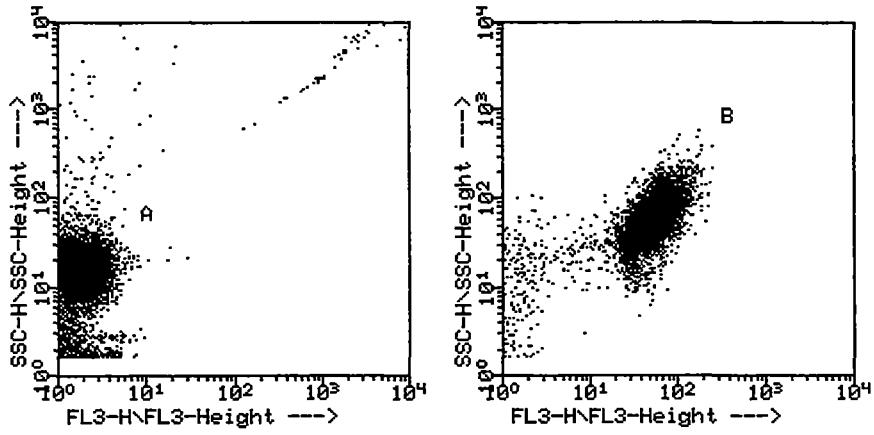


Figure 2.7: Flow cytometric two dimensional dot plots of propidium iodide stained bacteria. A) unstained cells; B) stained cells.

A comparison of counting methods using the flow cytometer and the fluorescent microscope, showed that the two methods did not produce a significant difference in cell numbers, using a bacterial culture (Mann-Whitney Rank Sum Test, $p = 0.496$, $n = 10$). A similar result was obtained when the two methods were compared using lake water samples. There was not a significant difference in cell numbers between the two methods at 8 m (t-test, $p = 0.6555$, $n = 5$) nor at 12 m (t-test, $p = 0.9728$, $n = 5$). In both cases 10000 cells per sample were counted by flow cytometry and 2000 cells per sample by microscopy.

2.4.5 Analysis of fresh lake water samples

The flow cytometer was routinely used for the analysis of lake phytoplankton populations and also for the analysis of *Synechococcus* cultures. As with the analysis of total bacterial numbers, the method of analysis for *Synechococcus* numbers was compared to the traditional method of fluorescence microscopy. There was no significant difference in the numbers of *Synechococcus* using the two different methods of analysis (t-test, $p = 0.7112$, $n = 10$). Furthermore, the counting error on using flow cytometric techniques was 3 % as compared to 6 % using microscopic counting techniques. No comparison was done between the two methods using lake water samples. However, because *Synechococcus* formed discrete populations, that could be easily gated on the flow cytometer, there was no obvious reason why the results would be different to those obtained from cultures. It is probable that the main source of error in the flow cytometric method of counting cells came from the uneven distribution of fluorescent beads in the sample tube. The sample tube has no mixing facility and there is a possibility that the beads were not evenly mixed.

The cultured *Synechococcus* population exhibited the same morphological characteristics on the flow cytometer as did the population obtained from the Ace Lake sample. Figure 2.8 shows a flow cytometric image of a *Synechococcus* culture relative to 1.98 μm microspheres. The relative fluorescent intensity of a *Synechococcus* culture depends on the culture conditions (Chapter 5). A water sample from 10 m in Ace Lake showed the fluorescence characteristics of *Synechococcus* relative to 1.98 μm beads and relative to another population of photosynthetic cells that were between 2 and 3 μm in diameter and that had higher relative red fluorescence (FL3) but lower relative orange fluorescence (FL2) than *Synechococcus* (Figure 2.9). As with all phytoplankton populations, the *Synechococcus* image was more tightly clustered on a two parameter dot plot than in a single parameter histogram. This was because the population had to conform to two morphological or fluorescence characteristics rather than just one. The FSC versus SSC plot gave a relative indication of cell size and complexity. FL 1 showed any green fluorescence emitted from a population when excited by the 488 nm laser light. FL2 showed the orange fluorescence emitted and FL3 showed the red fluorescence emitted.

A depth profile of the phytoplankton communities in a meromictic lake is shown in Figure 2.10. A combination of cell size and fluorescent characteristics were used to gate specific populations. At Davis Station, the flow cytometer was primarily used to detect changes in cell numbers in each phytoplankton population. Without microscopic confirmation, phytoplankton populations could not be identified by flow cytometric analysis.

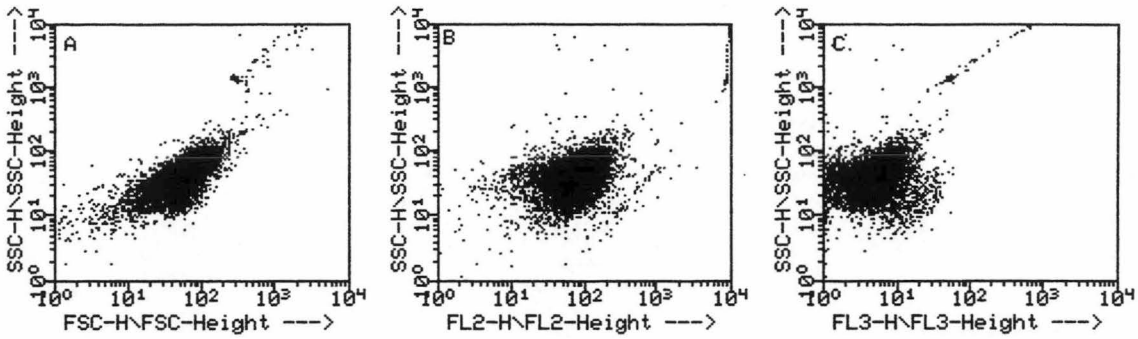


Figure 2.8: Flow cytometric dot plots of a *Synechococcus* culture. All axes are in relative log units. A. The relative cell size and complexity of *Synechococcus* cells. B. Relative cell complexity and orange fluorescence. C. Relative cell complexity and red fluorescence.

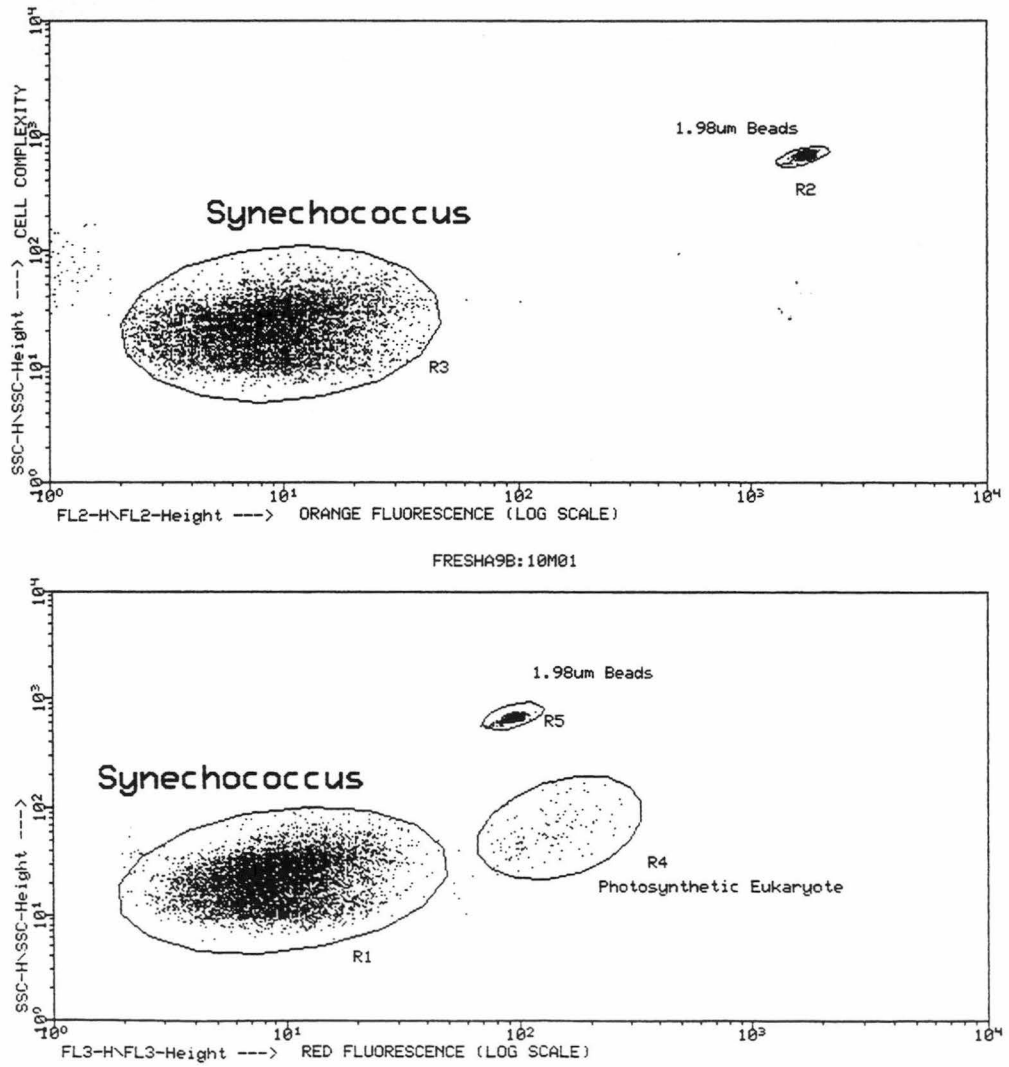


Figure 2.9: A natural *Synechococcus* population relative to 2 μm calibration beads and to a population of photosynthetic eukaryotic phytoplankton.

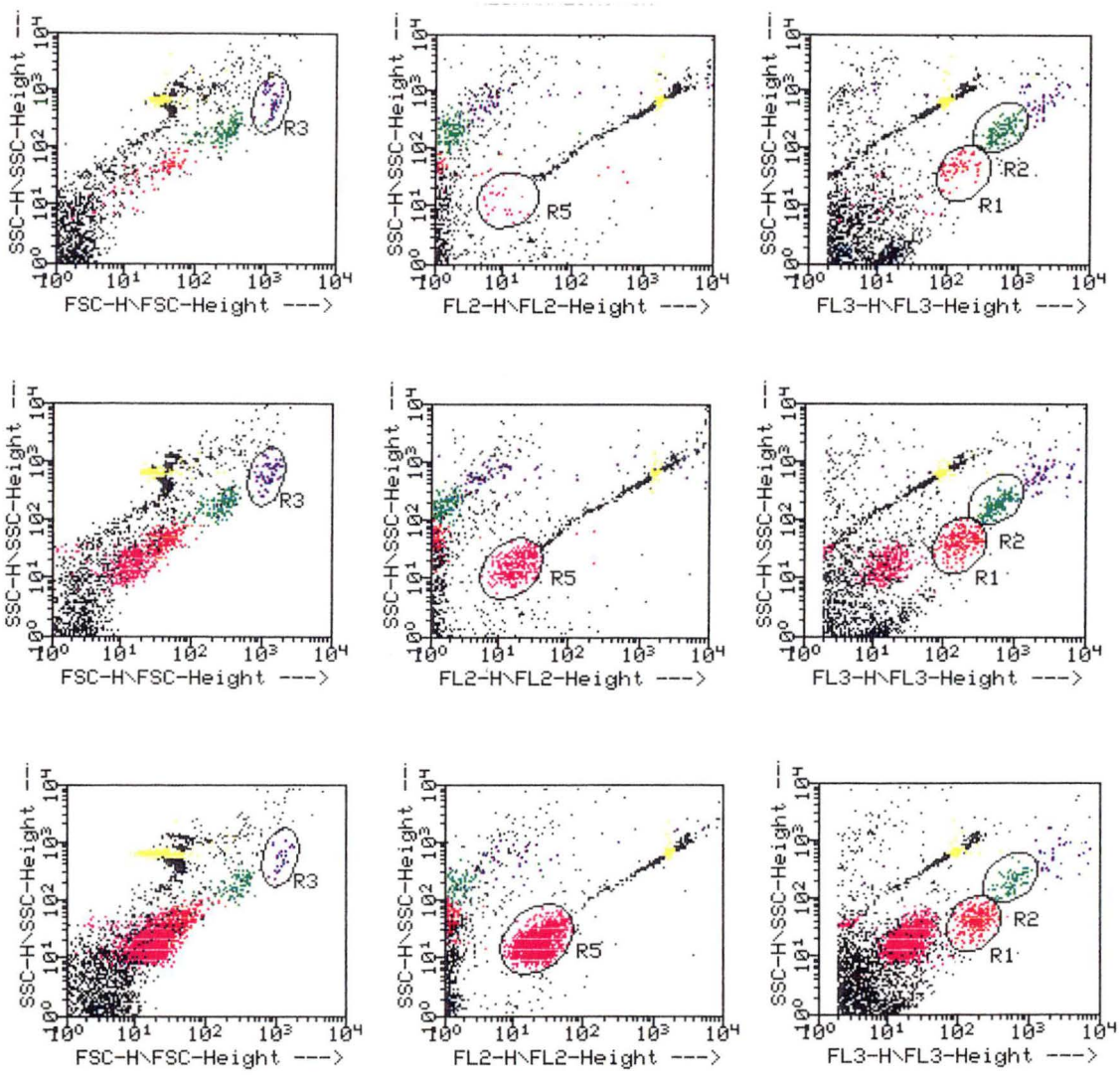


Figure 2.10: Flow cytometric images from three depths (6, 8 and 10 m) in a meromictic lake in the Vestfold Hills, Antarctica. Regions' R1, R2, R3 and R5 represent phytoplankton populations. R4 is the 1.98 μm beads. All axes are in relative log units. FSC is forward angle light scatter; SSC is side angle light scatter; FL2 is orange fluorescence and FL3 is red fluorescence.

In Figure 2.10, population R1 was identified on a dot plot of SSC and FL3. The cells within this population were approximately 3 μm in diameter. The population had low orange fluorescence and the cells increased in number with depth (Table 2.4). Population R2 was also identified on a SSC/FL3 dot plot. The cells within this population had a diameter of approximately 5 μm and had low orange fluorescence. Cell numbers were highest at the middle depth (8 m) in the lake (Table 2.4). Population R3 was identified on a FSC versus SSC dot plot. The cells were approximately 10 μm in diameter and cell numbers were greatest at the middle depth in the lake (Table 2.4). This is an example of the use of the flow cytometer for the study of phytoplankton community structure in the lakes. With *Synechococcus*, the flow cytometer was also used to detect relative changes in cell fluorescence over depth and time (Chapter 3).

Table 2.4: Phytoplankton cell numbers ($x10^3$ cells ml^{-1}) in each of the regions identified on the flow cytometric image of lake water samples taken from a meromictic lake in the Vestfold Hills.

Depth	R1	R2	R3	R5
A	1.82	3.46	0.62	3.25
B	6.47	4.03	1.06	11.2
C	9.15	3.04	0.70	68.1

A, B and C are three depths 6, 8 and 10 m in the lake. R1, R2, R3 and R5 represent four different phytoplankton populations at the three depths

2.5 Discussion

Flow cytometry has proven to be an effective method for the analysis of bacterial and phytoplankton populations (Boye and Lobner-Olesen 1990; Legendre and Yentsch 1989). Extensive calibration of individual machines is, however, needed for quantitative data (Hofstraat et al. 1994).

In the analysis of meromictic lake bacterial communities the flow cytometer gave comparable results to the fluorescent microscope. Monfort and Baleux, (1992) found no significant difference between the two methods when comparing counts of bacteria from aquatic environments using DAPI stained cells. Robertson and Button, (1989) also found that there was no difference between results obtained from the use of the microscope and flow cytometer when aquatic bacteria were DAPI stained. Monger and Landry, (1993), however, found that when cells were DAPI stained, the flow cytometer measured 12 % greater numbers of bacteria than did the microscope. When the cells were stained with Hoechst 33342 there was a 2 % difference in cell numbers between determinations from the flow cytometer and microscope. In the current comparison, cells analysed by microscopy were DAPI stained. DAPI was used as this was the stain routinely used in other studies of meromictic lakes (James et al. 1994; Laybourn-Parry and Perriss 1995) and is also routinely used for the analysis of marine bacterial populations (Porter and Feig 1980). This stain, could not however be used on the FACScan because the excitation wavelength for DAPI is below the emission wavelength (488 nm) of the Argon Laser. DAPI, with an excitation wavelength between 300 nm and 400 nm fluoresces after excitation with

UV light (Monfort and Baleux 1992). Propidium Iodide (PI) was used to stain bacterial cells for analysis by flow cytometry. Both DAPI and PI are DNA stains (Shapiro 1988) but PI has excitation wavelengths between 450 nm and 580 nm (Waggoner 1990) which is compatible with the FACScan. The emission wavelengths of PI are between 580 nm and 690 nm (Waggoner 1990) and it was detected in the FL3 channel (> 650 nm) on the flow cytometer. Propidium iodide could not be used in the microscopic analysis of bacterial cells as the fluorescence faded too quickly for accurate cell counting. Although two different DNA stains were used there was no significant difference ($P > 0.001$) between the microscopic analyses of bacterial cell numbers and those obtained by flow cytometric analyses.

The commercially built flow cytometers were designed to analyse cells with diameters between 10 μm and 25 μm (Burkill 1987). The analysis of bacterial cells is, therefore, at the lower limit of detection for these machines. Using the current method, stained bacterial cells were easily detected by flow cytometry. It was, however, important to remove as many particles as possible from the sheath fluid. The pressure bomb method was the most effective method for the filtration of sheath fluid. As the preparation of bacteria for the analysis by flow cytometry is time consuming, the method is probably most useful for large numbers of samples. Samples could be stored, preserved, for short periods of time and analysed in bulk. Microscopic techniques are probably more appropriate for the analysis of small numbers of samples. In this way, morphological characteristics of the bacterial population can also be observed.

A further consideration when analysing bacterial cell numbers by flow cytometry was the ratio of cells to beads added as an internal standard. For accurate analysis Phinney and Cucci, (1989) recommended a ratio of approximately 10 to 1, cells to beads. If the density of beads was too high, the population of bacteria was swamped and did not form a discrete population. If bacterial numbers were uniform throughout the water column this requirement would not be difficult to achieve. In the meromictic lakes of the Vestfold Hills, where bacterial numbers changed dramatically with depth and between lakes (Chapter 4), the requirement was more difficult to fulfill.

The analysis of bacteria by flow cytometry is now used for a variety of reasons. Analysis of bacterial cell viability has been achieved using fluorescent stains such as Rhodamine 123 (Kaprelyants and Kelt 1992), bis-(1,3-dibutylbarbituric acid)trimethine oxonol (Jepras et al. 1995) and carboxyfluorescein diacetate (Porter et al. 1995). Bacterial cell cycle analysis and growth studies have been conducted based on the DNA content of cells (Boye and Lobner-Olesen 1991). Fluorescent labelled monoclonal or polyclonal antibodies have been used to detect specific bacteria in natural populations (Boye and Lobner-Olesen 1990; Pinder and McClelland 1993) as have 16S rRNA -targeted oligonucleotide probes (Amann et al. 1990). Flow cytometry is therefore well suited for: monitoring pathogenic bacteria in receiving waters (Edwards et al. 1993); for monitoring the growth of bacteria in bioreactors (Bankes and Richard 1993; Boye and Lobner-Olesen 1991) and; for the characterisation of natural bacterial populations (Button and Robertson 1989).

In flow cytometry, the scatter of the laser light when the beam comes in contact with a particle (FSC) gives a relative measure of cell size (Shapiro 1988). Demers et al., (1989) found that FSC correlated well with cell volume as measured by a Coulter counter. Phinney and Cucci, (1989) derived a linear relationship for cell volume versus FSC using spherical calibration beads. For calibration beads, which are uniform in size and shape, any flow cytometer could be calibrated to give quantitative information on cell volume and hence cell size. Further, except for pennate diatoms, Olson et al., (1989), obtained a good relationship between FSC and coulter volume for cultures of phytoplankton. In a sample of water from a lake or from the ocean, however, where phytoplankton cells are not uniform in size or shape the relationship between FSC and cell volume is not as straightforward (Burkill 1987; Hofstraat et al. 1994). At best, cell volume can only be a relative measure when compared with standard beads. Further, due to the laminar flow of the sheath fluid (Phinney and Cucci 1989), it could be assumed that a particle will pass through the flow cell along its most fluid dynamic axis. In this case, the FSC parameter would be an estimate of length (Hofstraat et al. 1994) for a cylindrical or a conical shaped cell and a measure of diameter for a spherical shaped cell. Due to the width of the sample stream (12 to 28 μm), small rod shaped cells such as heterotrophic bacteria and *Synechococcus* may pass through the flow cell at a variety of angles, hence the broad spread in FSC (Figure 2.8 and 2.10). When the flow cytometer is run on low sample rate (12 μl s^{-1}), cells pass through the flow cell in a more uniform manner and hence the FSC signal produces a tighter image (FACScan users manual 1992). A calibration of size using uniform spherical calibration beads can therefore provide relative information on length of cells but accurate information on cell size and shape is best obtained using a microscope.

A difference in salinity between the sample and the sheath fluid effected the flow cytometric analysis of meromictic lake water samples. Phinney and Cucci, (1989) indicated that the sample salinity should differ from the salinity of the sheath fluid by no more than 0.5 %. In the analysis of seawater or freshwater samples this was easy to achieve. In meromictic lakes, where there were large salinity changes with depth, this requirement was more difficult to achieve. The fluorescent parameters and the side angle light scatter were not effected by the salinity differences between the sample and the sheath fluid. Forward angle light scatter was however effected and the greatest effect was observed with the bacterial sized cells. The reason for this is not clear but it probably resulted from an interaction between the sheath fluid and the sample core.

For use in microbial ecology, the greatest value in flow cytometric technology is in the analysis of phytoplankton communities (Hofstraat et al. 1994; Platt 1989; Yentsch et al. 1986). Due to the natural fluorescence of phytoplankton cells, no manipulation was required for sample analysis. However, unless the species composition of the phytoplankton community is known, microscopic analysis of samples is still needed. Once a species list was established, flow cytometry provided an accurate and efficient mechanism to monitor changes in phytoplankton population.

When a phytoplankton population is easily distinguished by flow cytometry, as was the case with *Synechococcus*, changes in distribution, abundance and physiological status can be monitored with relative ease. *Synechococcus* can be separated from

other phytoplankton populations by its small cell size and orange fluorescence emitted by pycoerythrin (Yentsch et al. 1983). Other phytoplankton that emit orange fluorescence when exited at 488 nm are the Cryptomonads and *Mesodinium rubrum* (Yentsch and Phinney 1985). Although these organisms exist in some of the meromictic lakes (Burch 1988; Perriss et al. 1995), the cells are much larger than *Synechococcus* cells. The difficulty in distinguishing phytoplankton populations by flow cytometry arises when there is a large species diversity and no one species dominates. This situation occurred in Taynaya Bay (Figure 4.5, Chapter 4) where there was a continuous spectrum in relative cell size and cell fluorescence. In the meromictic lakes, for example Ace Lake (Figure 2.10), flow cytometric monitoring of phytoplankton populations was ideal because of the low species diversity (Burch 1988). No difference in *Synechococcus* cell abundance was found when cultured samples were analysed using the flow cytometer or using the fluorescent microscope. Hofstraat et al., (1994) also found that when the two methods were compared, they gave consistent measures of phytoplankton cell numbers.

Difficulties that arose during the analysis of phytoplankton populations were caused by the low density of cells in some samples. This has been a common problem especially when using the commercially designed flow cytometers (Hofstraat et al. 1994; Yentsch et al. 1986). These instruments operate optimally at 10^5 - 10^6 cells ml^{-1} (Burkill 1987) and can only analyse two to three millilitres of sample. The Optical Plankton Analyser (Hofstraat et al. 1990) has been designed to analyse larger volumes and the sample is continuously stirred throughout the analysis. Because phytoplankton community structure varied with depth and between lakes, it was difficult to achieve an ideal cell to bead ratio. When the bead density was too high,

or when one phytoplankton population occurred in high densities, other populations were masked. This occurred in Pendant Lake because of the high densities of *Synechococcus* (Figure 4.3b, Chapter 4). When a phytoplankton population occurred in low densities, problems with its detection when it was associated with populations that occurred in high densities, could usually be overcome by manipulation of the flow cytometer instrument settings. The limit of detection for the flow cytometer was therefore dependent on sample dilution and on the instrument settings of the machine. The capacity to manipulate instrumental settings and to gate particular regions makes flow cytometric analysis a powerful tool. To do this, however, it is best if the analyst has a prior knowledge of the populations to expect. It also means that analyses is more time consuming because the process is an investigative one rather than one of routine monitoring.

In the meromictic lakes of the Vestfold Hills, flow cytometry was predominantly used for the analysis of *Synechococcus* communities. With regard to *Synechococcus*, flow cytometry has been used for pigment characterisation (Wood et al. 1985) and to monitor changes in distribution and abundance in several oceanic regions (Desenberry and Frankel 1994; Olson et al. 1988; Vaulot and Xiuren 1988). Andreoli et al., (1992) analysed water samples from Antarctic Lakes of Northern Victoria Land using flow cytometry. In this instance, however, samples were not analysed in Antarctica. Based on the excitation spectra (Chapter 5) the 488 nm wavelength of the Argon laser in the FACScan is not ideal for the detection of phytoplankton cells such as *Synechococcus*. A laser combination that gave, for example, excitation wavelengths of 529 nm, 442 nm and 633 nm (Hofstraat et al. 1994) would provide a stronger fluorescence signal for *Synechococcus* and would

also allow for the clearer separation of other phytoplankton groups based on chlorophyll, phycobiliprotein and size differences. The 488 nm excitation wavelength did, however, give a good separation of phytoplankton communities in the meromictic lakes.

Flow cytometry has provided ecologists with a powerful tool to study microbial systems. With little effort, flow cytometry can give a qualitative view of phytoplankton community structure based on the autofluorescence of phytoplankton cells. Further, with the aid of fluorescent stains, heterotrophic plankton can also be analysed. Much information can be gained and many samples can be processed in a short space of time. With more careful calibration of an instrument, quantitative information, on cell abundance and physiological status, can be gained. The value of flow cytometry is further increased if it is used in combination with fluorescence microscopy. The microscope provides valuable information on species identity and cell sizes where as the flow cytometer provides an objective view of community structure in aquatic systems.

Chapter 3

Seasonal distribution and abundance of *Synechococcus* in Ace Lake, a marine derived Antarctic lake

3.1 Abstract

Recently a picocyanobacterium belonging to the group *Synechococcus* was identified in Ace Lake, a marine derived meromictic lake in the Vestfold Hills, Antarctica. At one depth in the lake, it occurred in numbers forty thousand times greater than reported for southern polar oceans. Ace Lake is 24 m deep and is usually ice covered for 11 months of the year. In 1992, the top seven metres of Ace Lake was mixed and contained very low concentrations of oxidised nitrogen and dissolved phosphorus. The mixed zone was separated from the rest of the water column by a sharp pycnocline between 7 and 8 m. Between 8 m and 12 m there were high concentrations of phosphorus (5 - 9 μM PO_4^{2-} - P) and enough light, over summer, for phytoplankton growth (> 5 μmol photons m^{-2} s^{-1}). Over summer, light was not detected below 12 m. The greatest number of *Synechococcus* cells occured at 11 m in December when numbers reached 8 x 10^6 ml^{-1}. This population density is higher than any previously reported for marine *Synechococcus*. At this depth the salinity (30 g kg^{-1}) remained constant throughout the year and the temperature ranged from 4.5° C in October to 10.5° C in February. The *Synechococcus* bloom at 10 m began in September when the water temperature was at its minimum. Diel periodicity in *Synechococcus* growth in Ace Lake was not detected. *Synechococcus* numbers and fluorescence were monitored by flow cytometry. The distinct orange fluorescence emitted from phycoerythrin and the small cell size (0.8 μm x 1.5 μm) distinguished the *Synechococcus* population from populations of other photosynthetic cells. As temperature is considered to be an important control on *Synechococcus* numbers in this region, the existence of a population of *Synechococcus* in a marine derived Antarctic lake may now provide an insight into controls on cell numbers in the Southern Ocean.

3.2 Introduction

Synechococcus is a small unicellular cyanobacterium that occurs abundantly in temperate and tropical marine and freshwater environments (Chapter 1). The contribution of *Synechococcus* to primary production in these regions is variable but often high (Fogg 1986; Stockner and Antia 1986; Waterbury et al. 1986). The measured contribution of *Synechococcus* to primary productivity in the oceans ranges from 5 % to 65 % depending on location and techniques used (Waterbury et al. 1986). Cell numbers range between 10^2 cells ml^{-1} to almost 10^6 cells ml^{-1} in tropical and temperate oceans and coastal regions. *Synechococcus* is not confined to the marine environment, it can also be a dominant phytoplankter in freshwater lakes (Caron et al. 1985; Venkateswaran et al. 1993; Voros et al. 1991) and its contribution to primary productivity can be high (Caron et al. 1985; Voros et al. 1991).

Unlike temperate and tropical waters, it has been reported that *Synechococcus* occur in low numbers in polar oceans (Letelier and Karl 1989; Marchant et al. 1987; Murphy and Haugen 1985). There is an apparent direct relationship between temperature and the abundance of *Synechococcus* in polar oceans (Gradinger and Lenz 1989; Murphy and Haugen 1985; Walker and Marchant 1989). It may, however, be some other factor or factors other than temperature that limits the development of an appreciable biomass of the picocyanobacteria in these regions. In coastal Antarctic waters *Synechococcus* numbers have been reported to be less than 200 cell ml^{-1} (Walker and Marchant 1989).

Although several lakes in Antarctica have deep chlorophyll maxima that may be dominated by cyanobacteria (Spaulding et al. 1994; Vincent 1988) the numbers of

Synechococcus measured in Ace lake, Vestfold Hills, Antarctica during the 1992/93 summer, to my knowledge, are greater than any previously reported for marine *Synechococcus*. Ace Lake was isolated from the marine system less than 7000 years ago (Burton and Barker 1979). The ratio of major cations to chloride is typical for marine waters (Masuda et al. 1988) and the copepods and eukaryotic phytoplankton in Ace Lake are of marine origin (Bayly and Burton 1987; Burch 1988). The discovery of *Synechococcus* in Ace Lake provides a unique opportunity to characterise an Antarctic population of marine *Synechococcus* and to investigate, in a stable water body, environmental constraints that may limit their abundance in polar regions.

3.3 Materials and Methods

3.3.1 Sample collection and storage

The location, and characteristics of Ace Lake have been described in Chapter 1. Water samples were collected from Ace Lake using a Kemmerer Bottle and water sampling was always carried out at the deepest spot in the lake. Samples were collected monthly between February 1992 and August 1992 and fortnightly between September 1992 and January 1993. Samples were not collected in June due to low light and bad weather. Samples were collected at two metre intervals between 2 m and 12 m with an additional sample taken at 11m. Samples were refrigerated at 4° C in the dark and flow cytometric analysis (Chapter 2) was conducted within 4 hours of sampling. Sub-samples were stored in 0.2 μm filtered, pH adjusted, formalin (1.5% final concentration) for future analysis by epifluorescence microscopy. A submersible data logger (Platypus

Engineering) was used for the collection of temperature and conductivity data. Conductivity data was converted to estimates of salinity using the formula of (Fofonoff and Millard 1983; Gibson et al. 1990).

Light readings were collected with a Digital Scalar Irradiance Meter and an underwater quantum sensor. Light readings were taken as close to solar noon as possible. Vertical attenuation coefficients, K_d ($\log_e$ units m^{-1}) were calculated as the linear regression coefficient of the plot of $\log_e$ (PAR) versus depth (Kirk 1994). Inspection of the plot determined whether the light penetration characteristics could be represented by a single value of K_d, or whether separate K_d values were required for different portions of the water column (Burch 1988). The euphotic depth (Z_{eu}) was calculated as 1 % of the incident light because of the ice cover on the lake.

A laboratory pH meter (Corning Scientific) was used to measure the pH of water samples within two hours of collection.

Samples used for dissolved organic carbon analysis were filtered twice, through a prewashed 0.2 μm polypropylene membrane filter (Millipore), within four hours of collection. Milli- Q blanks were filtered in the same way to ensure that the membrane filters did not comtaminate samples. Filtered samples were stored frozen in glass bottles, that had been heat treated to remove extraneous carbon, until analysis. A dissolved organic carbon analyser SK12 (Skalar) was used to measure the DOC content in the filtered lake water samples. In this process, the sample was pumped through a series of chemical reactions (as described in the Skalar users manual) in which the

carbon dioxide was blown off. Any remaining carbon in the sample was then converted to CO_2 and then methane which was detected by a flame ionisation detector.

Samples for dissolved phosphate-P and oxidised nitrogen analysis were filtered through prewashed glass fiber GF/F filters (Whatman) within four hours of collection. Filtered samples were stored frozen in acid washed, sample rinsed, polyethylene bottles and analysed on return to Australia. Samples were analysed on an Alpkem 'Flow Solution' Autoanalyser. Phosphate-P analysis was based on the formation of a phosphoantimonylmolybdenum blue species (Alpkem Corp 1992). The limit of detection for phosphate analysis was 0.04 μM. Nitrate plus nitrite-N was analysed using an Imidazole buffer chemistry, (Grasshof 1976), with an 12" Open Tubular Cadmium Reductor for quantitative reduction of nitrate to nitrite. The limit of detection for nitrate plus nitrite-N analysis was 0.02 μM.

3.3.2 Analysis of bacteria in water samples

Lake water samples were analysed using a Becton Dickinson FACScan fitted with an argon laser emitting light of 488 nm. Forward light scatter (FSC), side angle light scatter (SSC) and fluorescence emission from phycoerythrin (FL2, BP 585/42) and from chlorophyll *a* (FL3, LP650) were used to detect *Synechococcus* populations (Chapter 2). The instrument was set with a FL3 threshold and a low acquisition rate. A known number of 1.98 μm fluoresbrite calibration grade micro-spheres (Polysciences, Inc) was added to each sample to act as an internal calibration standard. Relative changes in *Synechococcus* fluorescence were measured over time by comparing

Synechococcus populations to the constant fluorescence of the calibration beads. Heterotrophic bacteria were stained with propidium iodide and counted by flow cytometric methods according to the method outlined in Chapter 2.

3.3.3 Epifluorescence Microscopy

Slides were prepared by filtering 5-10 ml (1.5%) formalin preserved lake water samples onto 0.2 μm membranes that had been prestained with Irgalan Black (Millipore). Samples were analysed using a Zeiss Axioscope epifluorescence microscope with green light (filter block 12, excitation filter G546, dichromatic beam splitter FT 580, barrier filter LP 590). Cells were also examined with blue light (filter block 9, excitation filter BP 450-490, dichromatic beam splitter FT 510, barrier filter LP 520).

3.3.4 Diel Periodicity of *Synechococcus*

Samples were collected from three depths (8 m , 10 m and 11 m), at the deepest spot in Ace Lake. Samples were collected every four hours over a forty eight hour period. Aliquots (1 ml) were preserved with gluteraldehyde (1 % final concentration), super cooled in the vapor phase of liquid nitrogen for four hours and then stored in liquid nitrogen until analysis one week later. Samples were analysed for *Synechococcus* cell numbers using flow cytometric techniques (Chapter 2). Light profiles were measured at the same time as sample collection.

3.3.5 Data analysis

Flow cytometric data was analysed using the 'Lysis II' software on the FACScan. Cell volumes and surface areas were estimated from standard geometric equations. Data was presented using Sigmaplot software (1994).

3.4 Results

3.4.1 Physical characteristics of Ace Lake

Ace Lake can be divided into three zones: the top mixed zone; a zone that is supersaturated with oxygen, but is isolated from the mixed zone by a sharp pycnocline; and an anaerobic zone. The depth of the mixed zone can change slightly from year to year as can the depth of the ice cover. During 1992 - 1993 the top seven metres of the lake was mixed, with a summer temperature range of 2° C to 5.5° C and a winter temperature of approximately -1° C (Figure 3.1). Below 7 m the water did not mix because of a sharp pycnocline between 7 m and 8 m (Figure 3.2). At 10 m there was a temperature range of 6° C. The minimum temperature of 4.5° C occurred between August and November. Between November and February the temperature slowly increased. A maximum of 10.5° C was reached in February before the temperature started to fall again. Below 15 m in Ace Lake the temperature did not vary throughout the year.

The stratification in Ace Lake results from the stable density gradient. The salinity of the top 7 m varied between 13 and 16 g kg^{-1} depending on the thickness of the ice cover.

Below 7 m the salinity remained relatively constant throughout the year (Figure 3.2). Between 7 m and 9 m there was an increase in salinity from approximately 16 g kg^{-1} to 28 g kg^{-1}. At 10 m the salinity was 29 g kg^{-1}. Salinity continued to increase with depth and reached a maximum of 40 g kg^{-1} at 24 m.

The pH in the top 12 m of Ace Lake was between 8 and 8.5 (Figure 3.3). The pH was slightly higher in the summer than the winter. At 10 m the pH range was between 8.1 (November) and 8.5 (May).

Light measurements (PAR) were taken in Ace Lake as close to solar noon as possible. Light measurements could not be collected in some months due to instrument failure in the cold weather. In all months, except February, light readings were taken under the ice. A maximum ice thickness of 180 cm was recorded in September (Table 3.1). The thickness of the snow cover on the ice was variable and depended on the frequency of snow storms and the strength of the wind. Over winter, light was only detected in the top two or three metres of the water column (Figure 3.4). Over summer, when the ice thickness decreased, and incident radiation increased, light penetrated deeper into the water column. The greatest light intensity recorded at 10 m was 35 μmol photons m^{-2} s^{-1} at the end of January 1993 (Figure 3.5). Between April and October, the light intensity at 10 m was below the limit of detection.

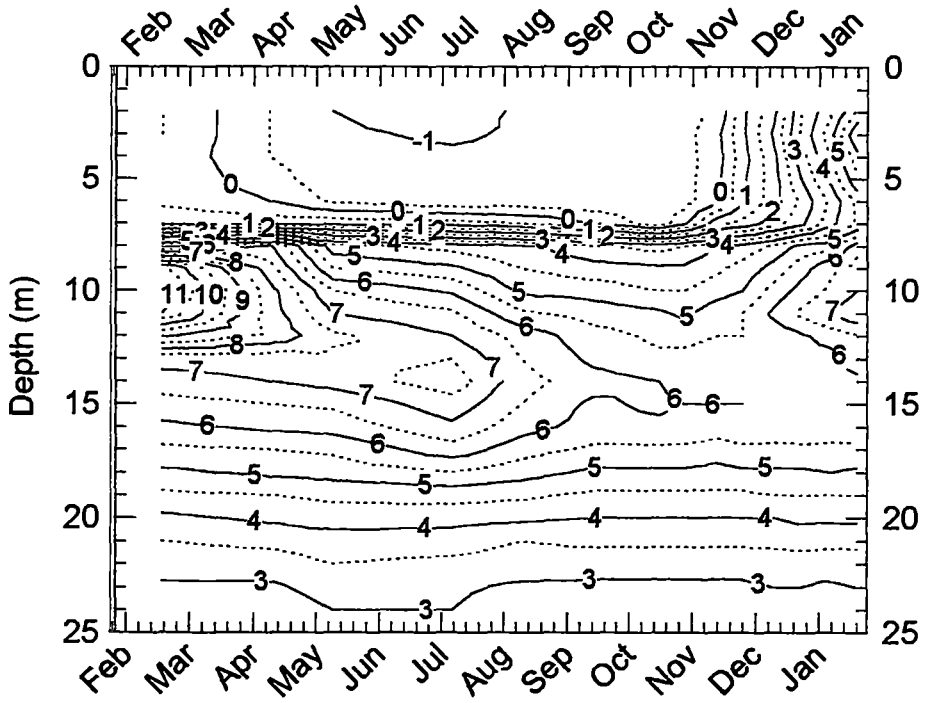


Figure 3.1: Temperature (°C) in Ace Lake during 1992

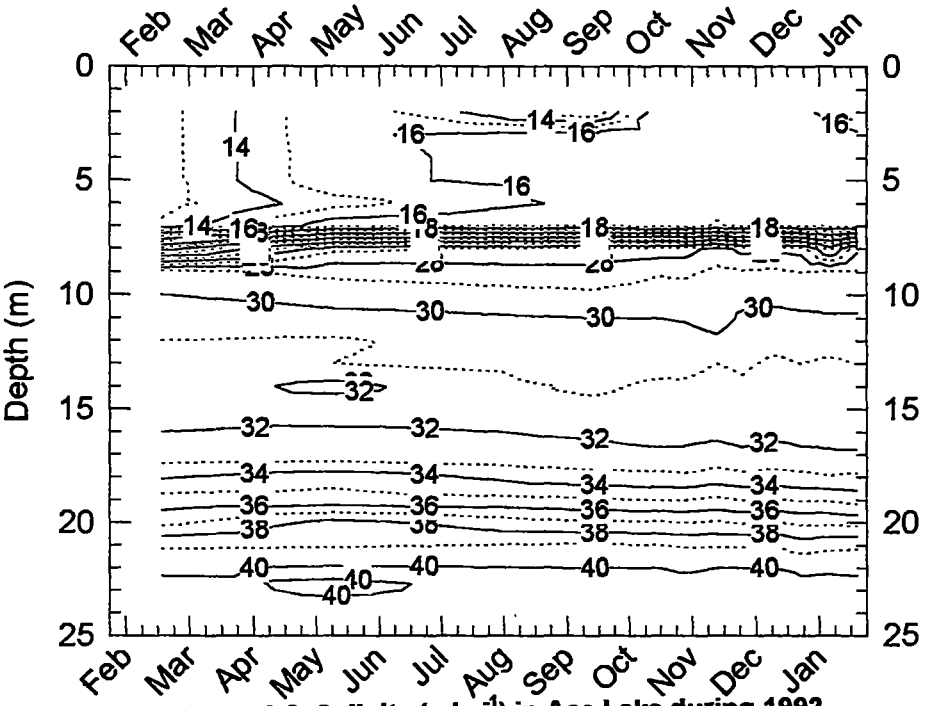


Figure 3.2: Salinity (g kg^{-1}) in Ace Lake during 1992

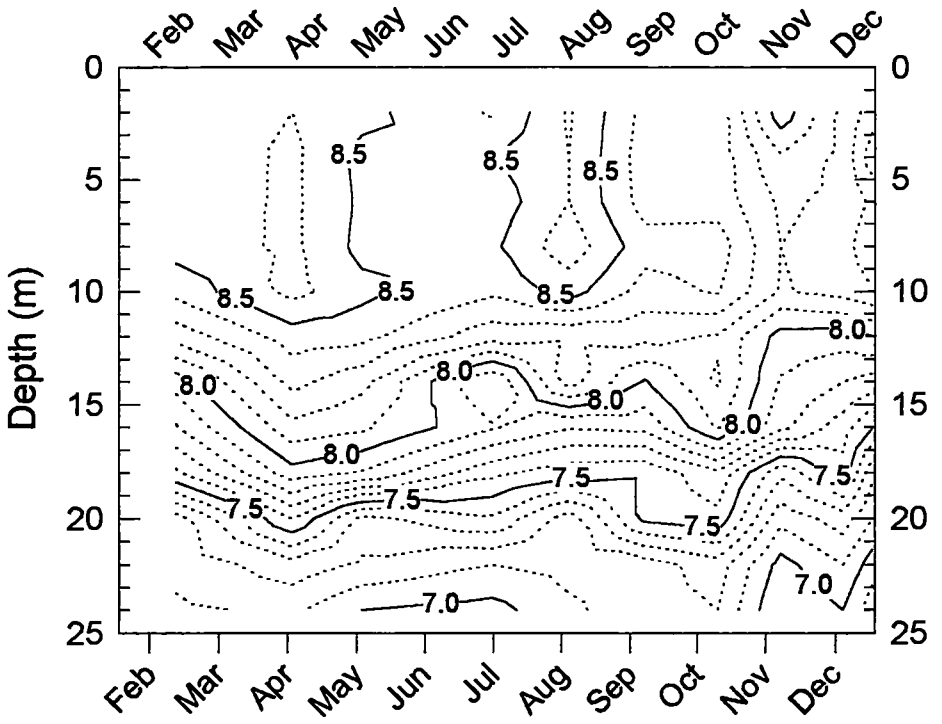


Figure 3.3: pH in Ace Lake during 1992

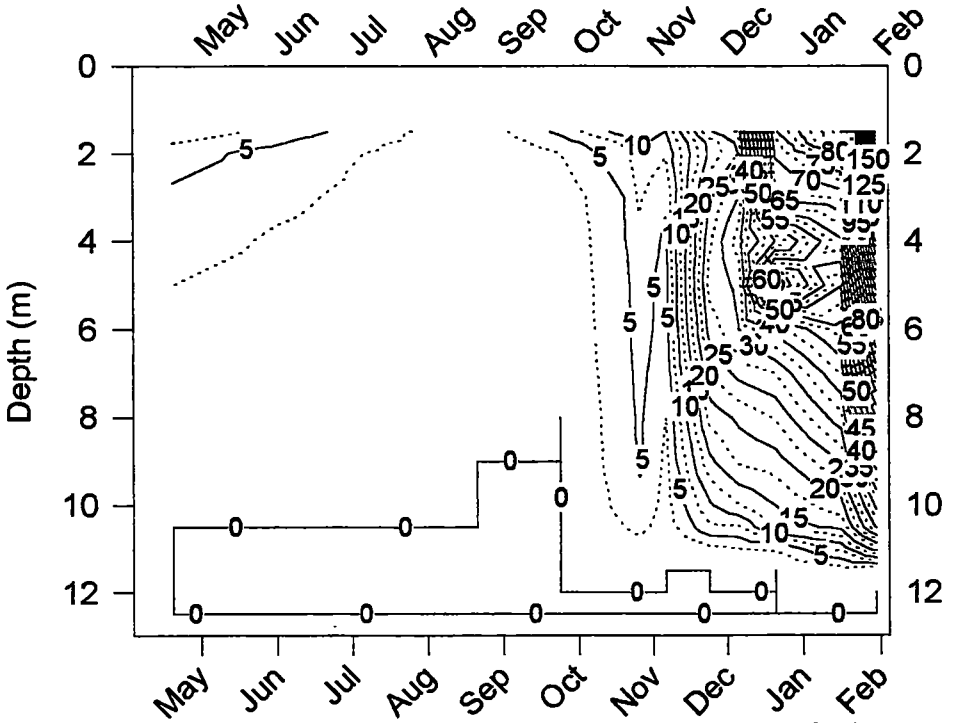


Figure 3.4: Light intensity -PAR (μmol photons m^{-2} s^{-1}) in Ace Lake during 1992.

The euphotic depth (Z_{eu}) was less than 3 m over winter (Figure 3.6, Table 3.1) but increased to between 10 m and 11 m over summer. From October onwards, over the summer months, there was greater than 0.1 % incident light at 10 m. A plot of $\log_e$ (PAR) versus depth gave two distinct gradients (Figure 3.7). Two vertical attenuation coefficients (K_d - PAR) could be calculated from the plot. K_d, which is an indication of the light absorption characteristics of the water (Kirk 1994), was higher in the lower part of the euphotic zone than it was in the upper part of the euphotic zone (Table 3.1).

Table 3.1: Underwater light (PAR) in Ace Lake during 1992. Vertical attenuation coefficients (k_d) were calculated for two depths. The value for K_d was higher for the lower part of the water column than the upper. The 1 % and 0.1 % euphotic depths were calculated from incident light readings rather than surface light readings.

Date	K_d PAR (m) Upper	K_d PAR (m^{-1}) Lower	Z_{eu} (m) 1 %	Z_{eu} (m) 0.1 %	Ice (cm)	Snow (cm)
16 Apr 92	0.32 (2-10 m)		1.25	8	50	8
17 Aug 92	0.12 (3-7 m)	0.35 (8-10 m)	1.25	7	160	13
20 Sep 92	0.10 (4-6 m)	0.70 (6-8 m)	1.5	3	180	0
17 Oct 92	0.06 (3-9 m)	2.30 (10.5-11.5 m)	2	11.75	156	2
22 Oct 92	0.07 (3-9 m)	2.74 (10.5-11.5 m)	3	11	155	2
2 Nov 92	0.16 (3-9 m)	1.50 (10-11 m)	1.5	10.5	155	2
20 Nov 92	0.20 (6-9 m)	1.70 (10-11 m)	10.25	11.5	151	16
17 Dec 92	0.20 (2-10.5 m)	4.90 (10.5-12 m)	10.5	11.5	140	2
12 Jan 93	0.18 (1.5-10 m)	3.00 (10-12 m)	11	11.75	115	3
26 Jan 93	0.17 (1.5-10 m)	4.26 (10-12 m)	11.25	11.75	90	2

Dissolved organic carbon (DOC-C) concentrations were less than 0.83 mM in the aerobic zone of Ace Lake, (Figure 3.8), and increased to over 1.67 mM at 24 m. Based on a profile taken in August (winter) and one taken in November (summer), between 7 m and 12 m, there was a 0.16 - 0.25 mM difference in DOC-C, with a higher concentration recorded in winter. Between 2 m and 7 m there was not a detectable difference in DOC-C concentration. Below 12 m a higher DOC concentration was recorded in summer.

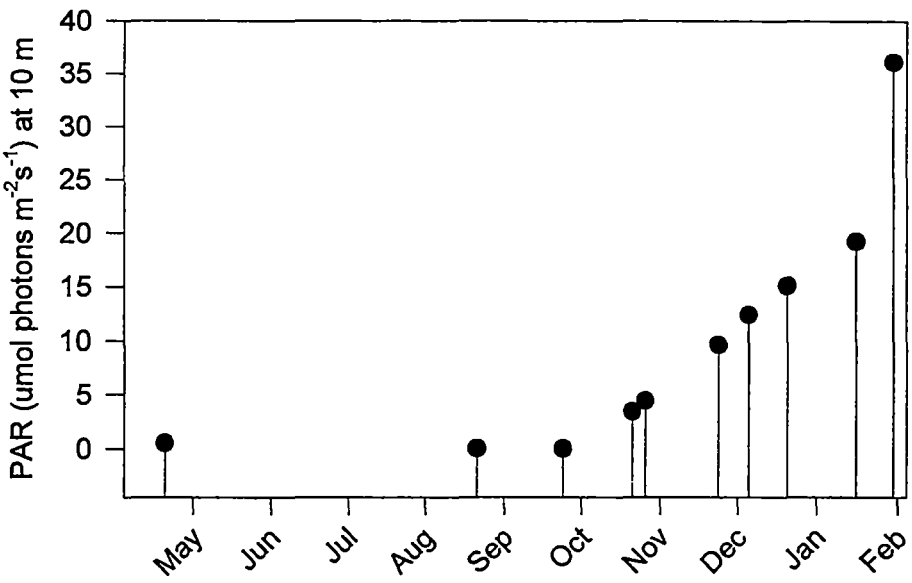


Figure 3.5: Photosynthetically active radiation - PAR (μmol photons m^{-2} s^{-1}) at 10 m in Ace Lake during 1992 at solar noon.

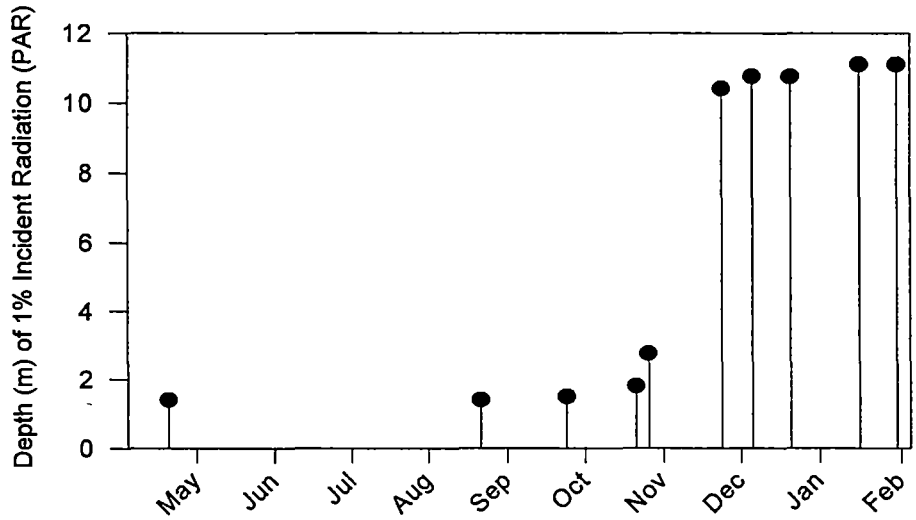


Figure 3.6: Depth to which 1 % of the incident radiation (PAR) reached in Ace Lake during 1992.

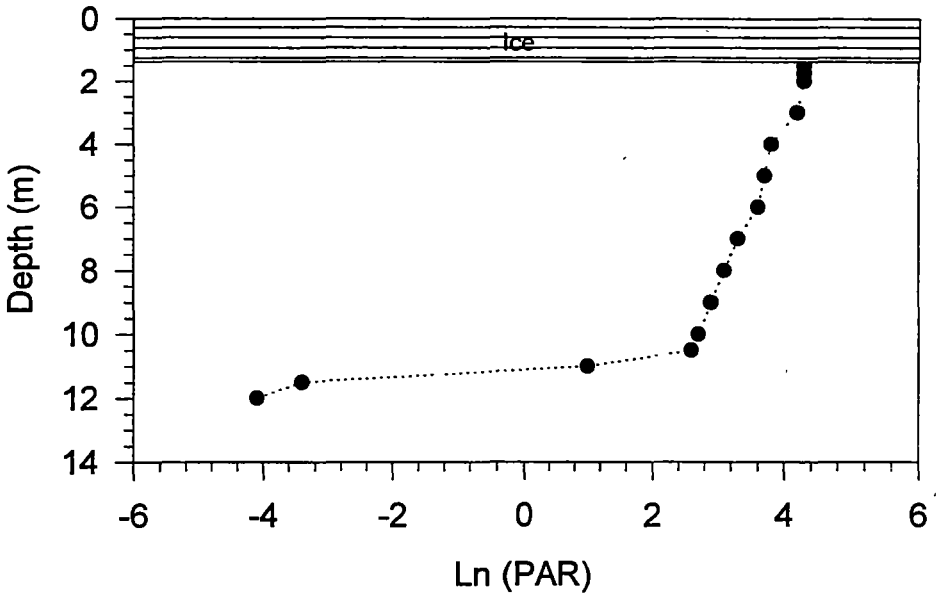


Figure 3.7: Light intensity (PAR) in Ace Lake on 17 December 1992. Light readings were taken at solar noon and have been plotted as ln (PAR). The vertical attenuation coefficients (Kd) reported in Table 3.1 were calculated from the slope of the lines.

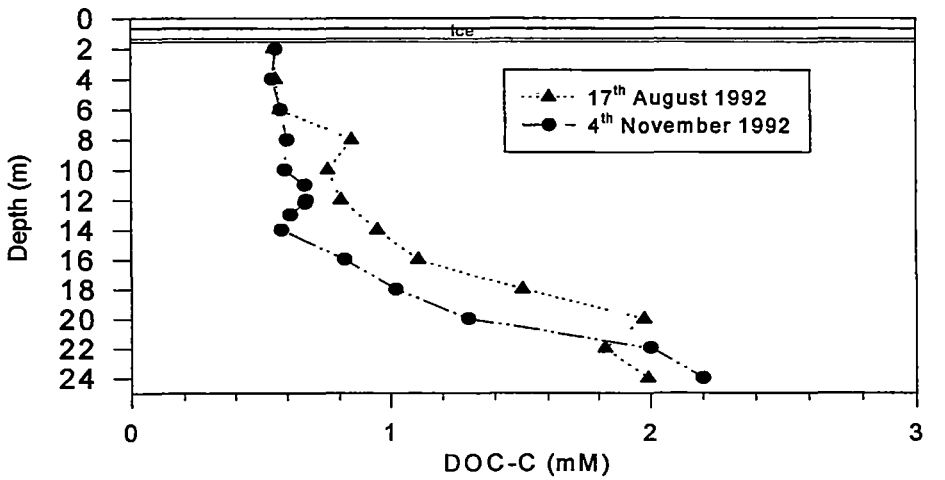


Figure 3.8: Dissolved organic carbon concentration in Ace Lake in August and November 1992.

At 10 m there was a 0.5 mM range in DOC concentration. The highest concentration (0.83 mM) was recorded in August and October and the lowest concentration (0.33 mM) was recorded at the end of summer in April.

Nutrient concentrations in Ace Lake were not measured during 1992. However, phosphate and nitrate plus nitrite -N were measured in lake water samples collected in November 1993. Oxidised nitrogen concentrations were below the limit of detection (0.02 μM) to a depth of 8 m (Figure 3.9). The concentration slightly increased between 10 and 11 m and there was a sharp increase to a concentration 0.25 μM at 12 m.

The phosphate-P concentration in the mixed zone was 0.25 μM and increased to 5.1 μM at 8 m (Figure 3.9). The phosphate-P concentration continued to increase and reached a maximum of 8.8 μM at 11 m. The concentration then fell to 4.2 μM at 12 m.

3.4.2 *Synechococcus* distribution and abundance in Ace Lake

During winter (August) *Synechococcus* cell numbers were less than 10^4 cells ml^{-1} throughout the aerobic zone of Ace Lake (Figure 3.10). During summer (December) cell numbers increased from 5×10^3 ml^{-1} to 2×10^4 ml^{-1} in the mixed zone of the lake. Cell numbers increased from between one and two orders of magnitude below the pycnocline and the maximum cell density (8×10^6 cells ml^{-1}) was recorded at 11 m in December 1992. In figure 3.10, summer and winter *Synechococcus* densities have been plotted with the corresponding summer and winter temperature profiles in the lake.

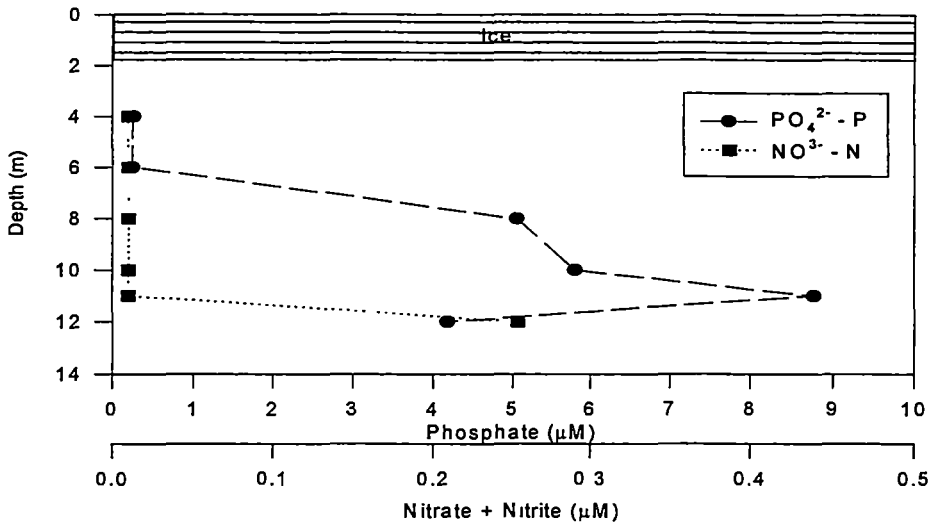


Figure 3.9: Phosphate - P and nitrate plus nitrite - N concentrations in Ace Lake on November 14 1993

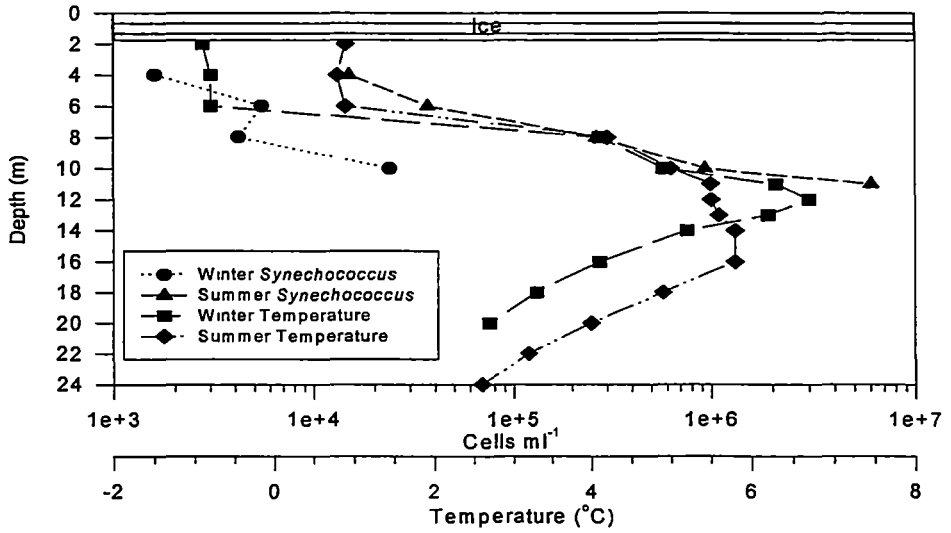


Figure 3.10: *Synechococcus* cell numbers and temperature in Ace Lake on 17 August (winter) and 4 December (summer) 1992. (1e+3 = 1 X 10^3)

In 1992, there was a distinct bloom in *Synechococcus* numbers, at each of the depths that were routinely monitored, below the pycnocline (Figure 3.11). The bloom below the pycnocline occurred during December and numbers quickly fell again in January. The *Synechococcus* bloom at 11 m was larger than the 8 m and 10 m blooms. *Synechococcus* cell numbers at 10 m have been plotted against several physical and biological parameters in order to gain an understanding of the controls on the population density at this depth.

The temporal distribution of *Synechococcus* at 10 m did not correspond to that of heterotrophic bacteria. Over winter the density of heterotrophic bacteria was an order of magnitude greater than the *Synechococcus* density (Figure 3.12). There was an increase in the density of heterotrophic bacteria in August and cell numbers remained high over summer, where as, *Synechococcus* numbers started to increase in August, peaked in early December and fell again in January. The DOC concentration at 10 m was highest at the time that the heterotrophic bacteria cell numbers started to increase.

The water temperature started to rise in November and continued to rise over summer, while the *Synechococcus* cell numbers fell (Figure 3.13). It appeared that the initiation of the *Synechococcus* spring bloom in October could be related to the light intensity increase (Figure 3.14). The light intensity at 10 m started to increase in September and at that time there was a small rise in the density of *Synechococcus* cells.

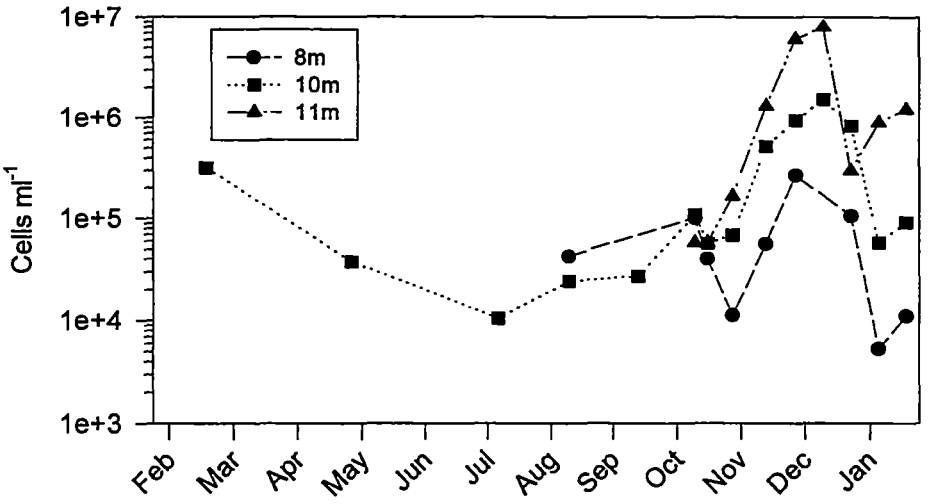


Figure 3.11: *Synechococcus* cell numbers at three depths in Ace Lake in 1992. The *Synechococcus* bloom occurred during November and December. (1e+3 = 1 X 10^3)

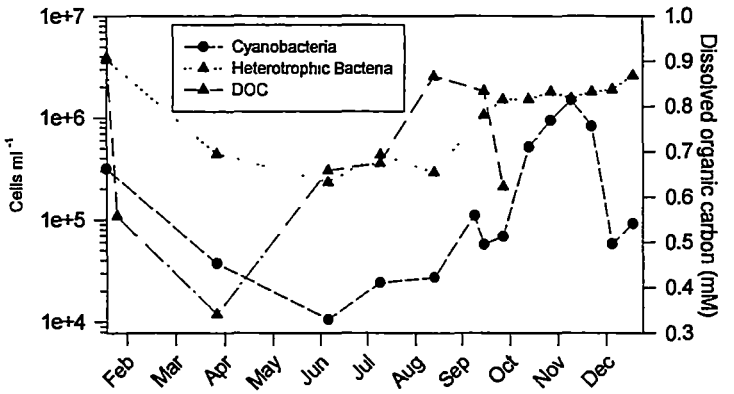


Figure 3.12: *Synechococcus* cell numbers, heterotrophic bacterial cell numbers and dissolved organic carbon concentrations at 10 m in Ace Lake during 1992. (1e+3 = 1 X 10^3)

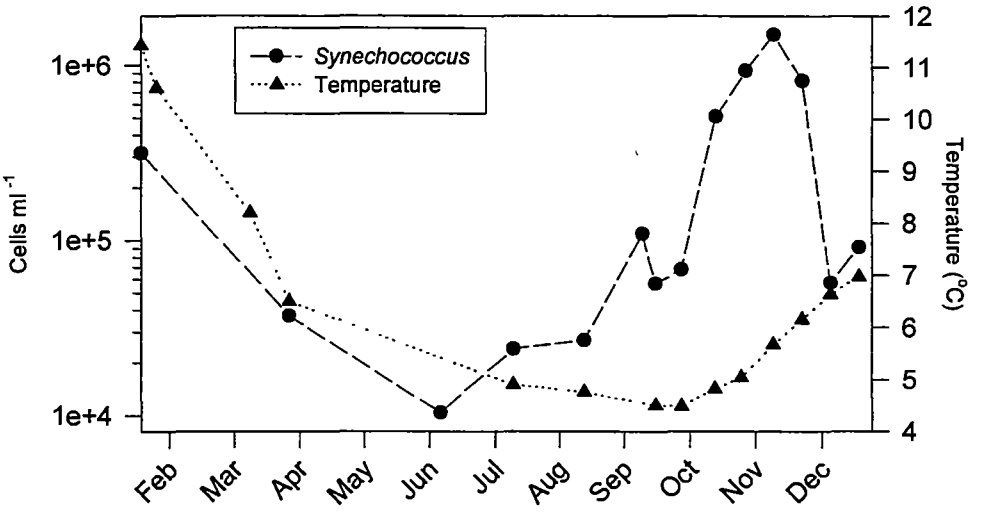


Figure 3.13: *Synechococcus* cell numbers and temperature at 10 m in Ace Lake in 1992. (1e+4 = 1 X 10^4)

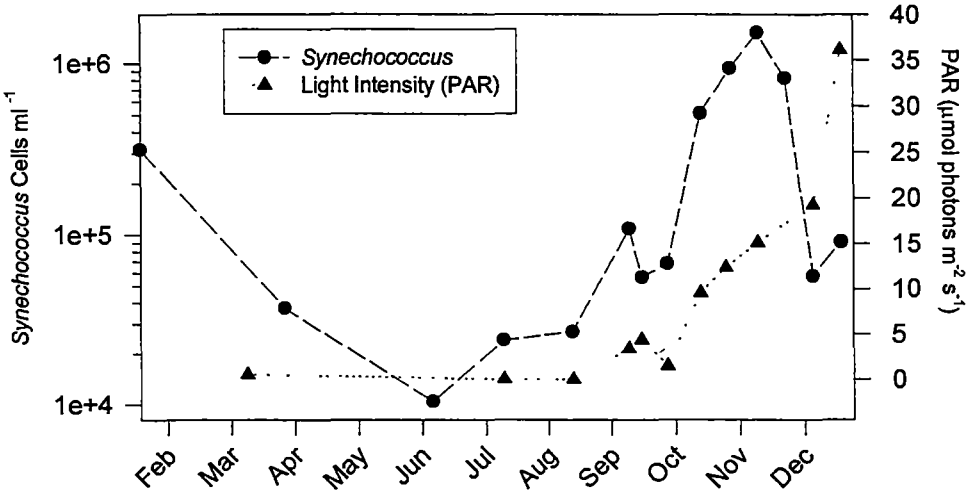


Figure 3.14: *Synechococcus* cell numbers and light intensity (PAR) at 10 m in Ace Lake in 1992. (1e+4 = 1 X 10^4)

In October, when there was a large increase in the PAR at 10 m the bloom in *Synechococcus* occurred. The PAR continued to increase and reached a maximum in December and January, but by this time, the *Synechococcus* cell numbers had declined.

Changes in *Synechococcus* numbers corresponded to changes in dissolved oxygen at 10 m (Figure 3.15). In 1994, at this depth, the winter dissolved oxygen concentration was approximately 13 mg l^{-1}. The oxygen concentration increased over spring as did the number of *Synechococcus* cells.

The contribution of *Synechococcus* to primary productivity in Ace Lake was not measured. There was, however, an increase in total phytoplankton cell surface area through the euphotic zone of Ace Lake (Figure 3.16). The estimated contribution made by *Synechococcus* to total phytoplankton cell number, biovolume and cell surface area in November 1992 is presented in Table 3.2. *Synechococcus* was one of five phytoplankton species identified in the euphotic zone of Ace Lake at this time. *Mesodinium rubrum*, *Pyramimonas gelidicola*, *Cryptomonas* sp, and a phototrophic nanoplankton also contributed to the total phytoplankton cell surface area and biovolume. Although *Synechococcus* constituted a high percentage of the phytoplankton cell numbers, the contribution of the picocyanobacteria to phytoplankton biovolume was small except for at 10 m. At 10 m *Synechococcus* contributed to approximately 44 % of the total phytoplankton biovolume. When measured in terms of cell surface area, *Synechococcus* contributed to 27 % of the phytoplankton surface area at 8 m and 79 % at 10 m (Figure 3.17).

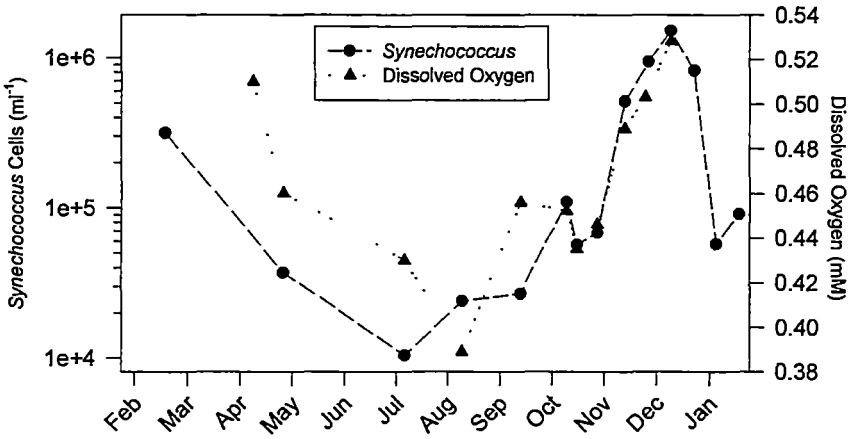


Figure 3.15: *Synechococcus* cell numbers at 10 m in Ace Lake in 1992 (1e+4 = 1 X 10^4). Dissolved oxygen concentrations at 10 m in Ace Lake in 1994 (determined by J. Gibson).

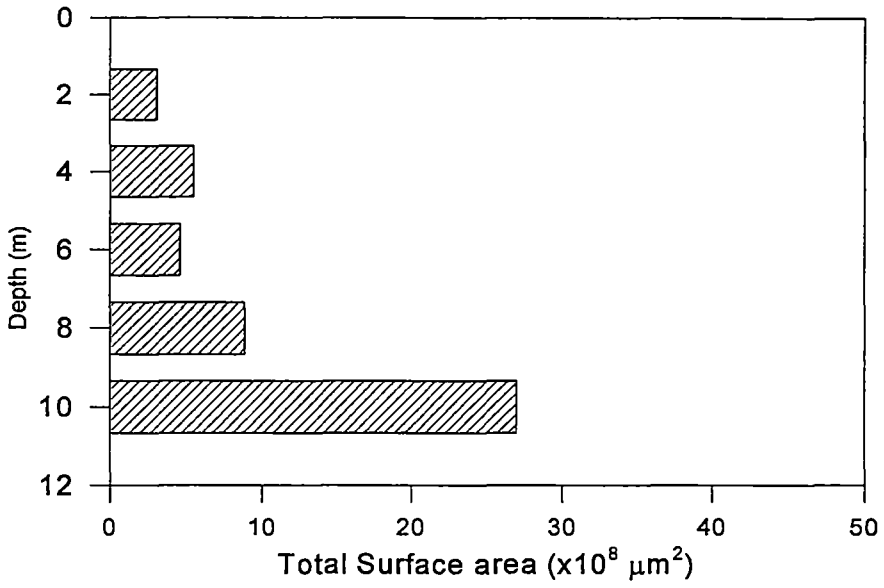


Figure 3.16: Total surface area (μm^2) of phytoplankton at five depths in Ace Lake on 20th November 1992.

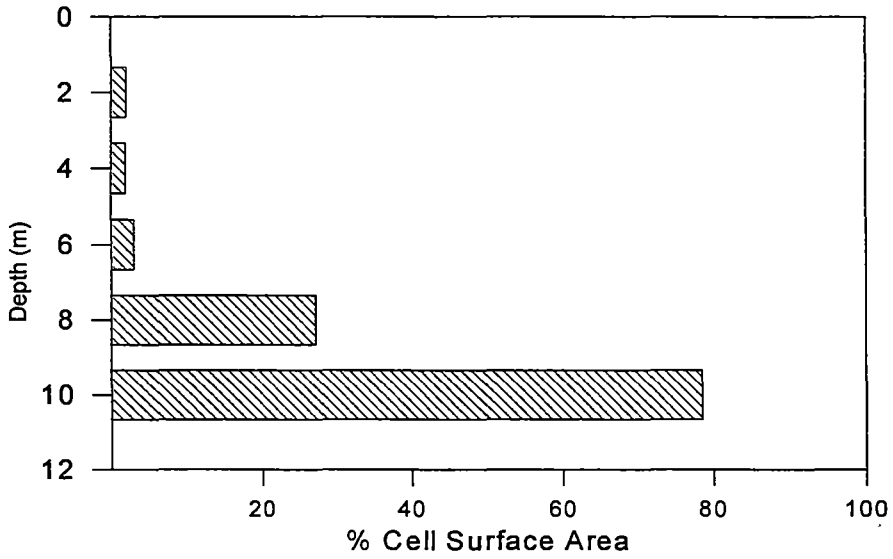


Figure 3.17: Percentage contribution of *Synechococcus* to total phytoplankton cell surface are between 2 m and 8 m on 20th November 1992.

Table 3.2: Percentage contribution of *Synechococcus* to total phytoplankton cell number, biovolume and cell surface area at 5 depths in the euphotic zone of Ace Lake on 20 November 1992. *Synechococcus* cells and the phototrophic nanoplankton were analysed by flow cytometry. Other protists were analysed from Lugol's iodine preserved samples, by inverted microscopy (T. Pitman).

Depth	% of cell number	% of total biovolume	% of total surface area
2	29.10	0.31	2.08
4	35.37	0.24	2.01
6	42.53	0.41	3.09
8	78.83	7.34	27.17
10	97.04	45.38	78.51

As with *Synechococcus*, the phototrophic nanoplankton was analysed by flow cytometric methods (Chapter 2). The nanoplankton, was approximately 2-3 μm in diameter and had a strong chlorophyll fluorescent signal on the flow cytometer. The population occurred in numbers of between 10^3 and 10^4 cells ml^{-1} throughout the photic zone (Figure 3.18).

There was a distinct population bloom at 8 m in December and at 10 m in early January when numbers were greater than 10^5 ml^{-1}. At 2 m the majority of the total phytoplankton surface area was accounted for by *Cryptomonas* sp., *M. rubrum* and the nanoflagellate (Table 3.3). At 4 m and 6 m, *M. rubrum*, *P. gelidicola* and the nanoflagellate dominated and at 8 m and 10 m *Synechococcus* and the nanoflagellate dominated.

Table 3.3: Percentage contribution of phytoplankton species to total phytoplankton cell surface area at 5 depths in Ace Lake on 19 November 1992.

Depth	*Synechococcus*	*M. rubrum*	*Cryptomonas*	*P. gelidicola*	Nanoflagellate
2	2.1	29	38	0.3	31
4	2.0	46	11	24	17
6	3.1	41	13	23	20
8	27	13	9.0	1.3	49
10	79	3.7	0.6	1.0	16

The physiological response of *Synechococcus* in field samples, to the increased light intensity over summer, was monitored using flow cytometric techniques (Chapter 2). Following the period of winter darkness, the increase in light intensity over summer resulted in a decrease in the relative fluorescence of individual *Synechococcus* cells (Figure 3.19). At 10 m in Ace Lake, there was a reduction in both the orange (FL2), phycoerythrin, fluorescence and in the red (FL3), chlorophyll, fluorescence (Table 3.4). The reduction in FL2 was greater than the reduction in FL3 with a decrease in the FL2/FL3 ratio from 1.31 in October to 0.71 in December. An increase in fluorescence intensity was observed in cells taken from deeper in the euphotic zone (10 m) relative to those taken from the surface waters (2-6 m).

Table 3.4: Percentage reduction in orange (FL2) and red (FL3) fluorescence relative to the fluorescence intensity measured on 23 October 1992 of *Synechococcus* at 10 m in Ace Lake. FL2/FL3 is the ratio of orange fluorescence to red fluorescence.

Date	% FL2	% FL3	FL2/FL3
23 Oct 1992			1.31
4 Nov 1992	40	42	1.35
20 Nov 1992	76	70	1.02
4 Dec 1992	97	94	0.71

In December 1993 the diel periodicity of *Synechococcus* was investigated. Although there was twenty four hours of daylight over summer in Antarctica, the intensity of the light varied. During December, the highest light intensity occurred at solar noon (13:00 hours) and the light intensity was lowest between 00:00 and 03:00 hours (Figure 3.20). Although there were slight fluctuations in cell numbers, a distinct diel periodicity in *Synechococcus* cell numbers was not detected at any of the measured depths in Ace Lake (Figure 3.21).

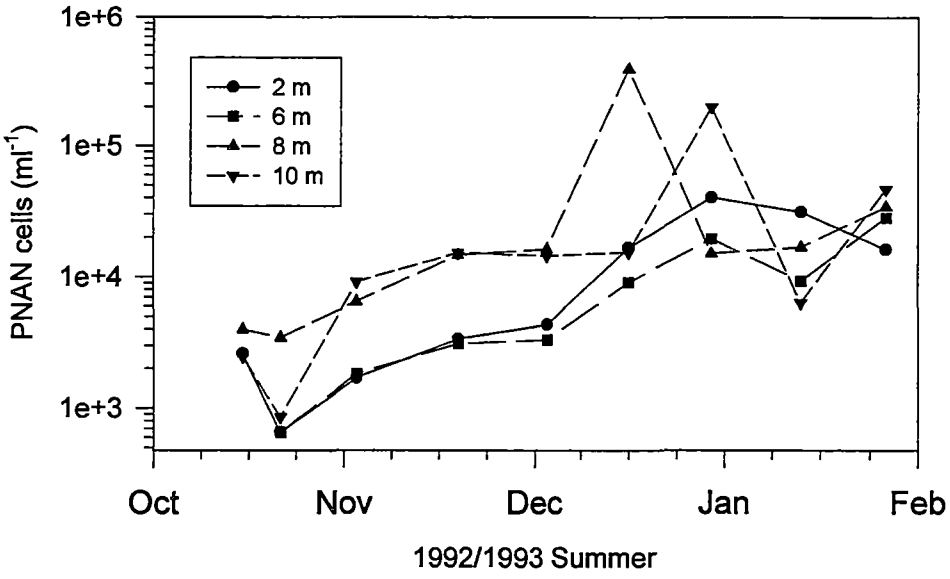


Figure 3.18: Phototrophic nanoflagellate (2-3 μm diameter) (PNAN) at four depths in Ace Lake over the 1992/1993 summer (1e+3 = 1 X 10^3).

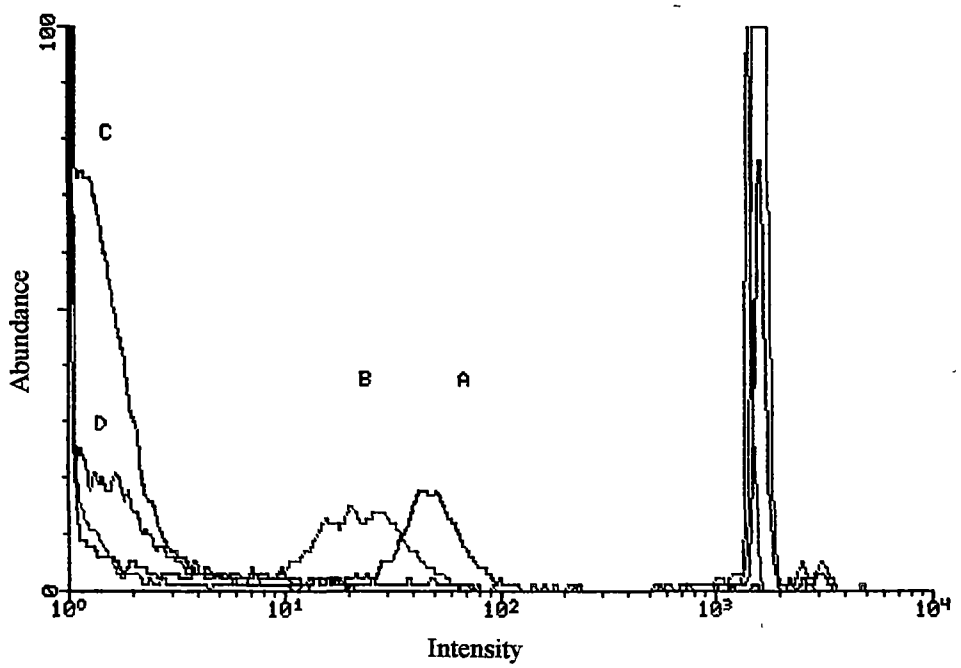


Figure 3.19: Flow cytometric histogram of FL2 (orange) fluorescence in water samples taken from 10 m in Ace Lake. A) 23rd October 1992 , B) 4th November 1992 , C), 20th November 1992 D) 4th December 1992. The Y-axis represents relative abundance and the X- axis is relative log units of fluorescence intensity.

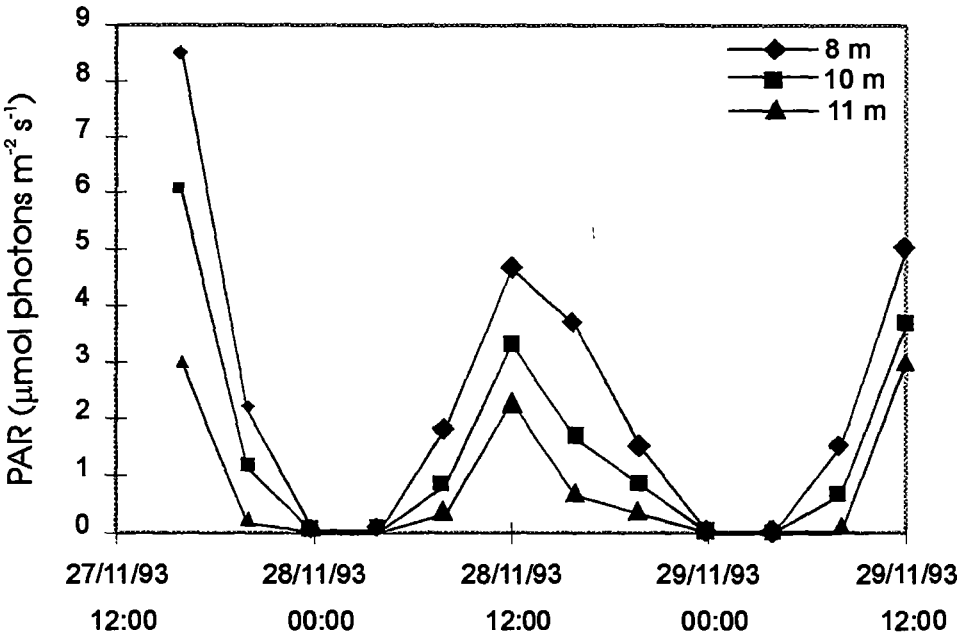


Figure 3.20: Light intensity (PAR) in Ace Lake. Light readings were taken every 4 hours over a 48 hour period.

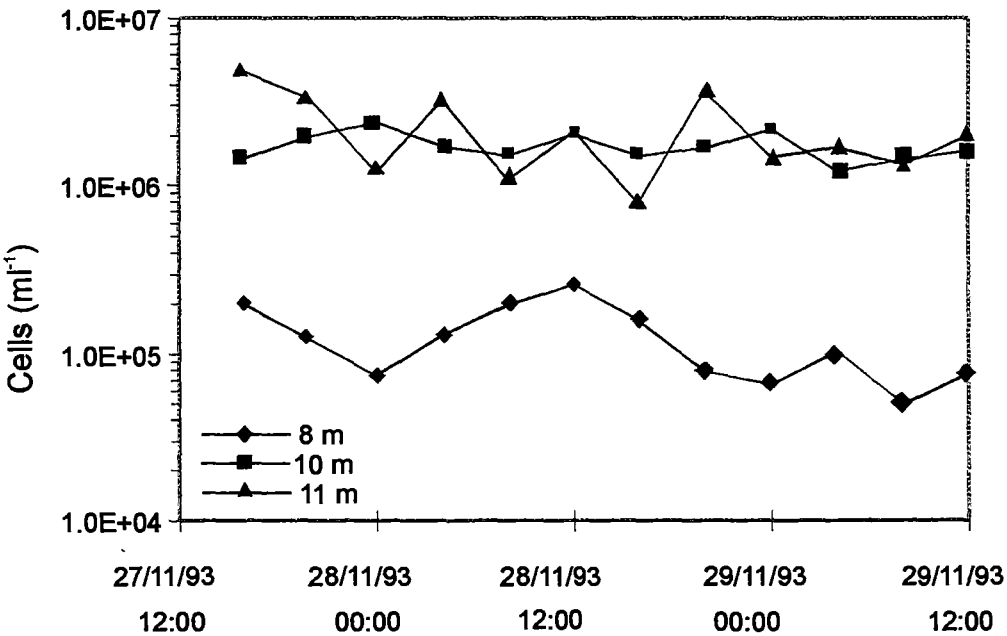


Figure 3.21: *Synechococcus* cell numbers at three depths in Ace Lake. Samples were taken, for analysis by flow cytometry, every 4 hours for 48 hours in December 1993 (1e+3 = 1 X 10^3).

3.5 Discussion

Although Ace Lake has been studied since 1979, (Chapter 1), single celled cyanobacteria have not been reported in the lake until now. It was through the application of flow cytometric methods that the organism was located. The naturally fluorescing *Synechococcus* cells were identified by the distinctive orange fluorescence emitted by the phycobiliproteins. As other organisms of this size do not emit light at this wavelength, *Synechococcus* was clearly distinguished from other photosynthetic organisms by flow cytometry. Phase contrast and epifluorescence photomicrographs confirmed the presence of cells of *Synechococcus* morphology in Ace Lake. The cells were typically 1.5 μm in length and 0.9 μm wide. The photosynthetic pigments in *Synechococcus* caused it to fluoresce orange/red under a green (BP 546 nm) light and yellow/orange under blue (BP 450-490 nm) light.

The physical environment of Ace Lake has been discussed in detail in chapter 1. Throughout most of the year the water temperature below 7 m is warmer than the temperature of the water above 7 m, (Figure 3.1). The stability of Ace Lake and the permanent stratification below 7 m is therefore not based on thermal stratification but is based on salinity (Figure 3.2). Since the ecological studies undertaken in the late 1970's and the 1980's, (Burch 1988; Burton and Barker 1979), the pycnocline between 7 m and 8 m has intensified and the stratification in Ace Lake has become more stable (Figure 3.2). The water below the pycnocline has become more isolated from the water in the mixed layer.

In Ace Lake, the distribution of *Synechococcus* was similar to that seen in other meromictic lakes in the Vestfold Hills (Chapter 4) and in temperate and tropical meromictic lakes (Craig 1987; Maeda et al. 1992; Venkateswaran et al. 1993). The *Synechococcus* density observed in Ace Lake at 11 m during the peak of the spring bloom, (Figure 3.11), was greater than has previously been reported. Due to the negligible sinking rate of *Synechococcus* (Craig 1987; Takahashi and Bienfang 1983), the observation of actively dividing cells at depths below the pycnocline and given that the *Synechococcus* cells at these depths maintained their fluorescent characteristics (Craig 1987) it would indicate that the *Synechococcus* formed an active population rather than a population that had settled from the mixed layer of the lake.

Over winter, when the water temperature in the mixed zone of Ace Lake was approximately -1° C, the density of *Synechococcus* was between 2 x $10^3$and 5 x 10^3 cells ml^{-1} (Figure 3.10). In summer, when the temperature in the mixed zone increased to approximately 1.5° C, the *Synechococcus* density had increased ten fold. A temperature of -1° C is comparable to temperatures experienced south of the Antarctic convergence (Marchant et al. 1987) where *Synechococcus* has been reported to occur in very low numbers (2 to 20 cells ml^{-1}) (Letelier and Karl 1989; Marchant et al. 1987). The *Synechococcus* cell numbers measured in the mixed zone of Ace Lake were comparable to *Synechococcus* cell numbers observed in temperate and tropical oceans where the temperature ranges from 10° C and 30° C (Glover 1985; Joint 1986; Stockner and Antia 1986). Below the pycnocline in Ace Lake, where temperatures were still low relative to tropical and temperate waters, *Synechococcus* reached densities of more than 10^6 cells ml^{-1}. In Ace Lake, temperature does not appear to be the only factor

controlling *Synechococcus* abundance and distribution (Chapter 6). In the Southern Ocean, *Synechococcus* abundance has been correlated to water temperature (Letelier and Karl 1989; Marchant et al. 1987). Temperature would certainly influence the growth and hence the development of biomass of *Synechococcus* in the Southern Ocean but factors including grazing, light intensity and nutrient availability would probably influence the final abundance of *Synechococcus* in this region.

At 10 m, over the winter, (May to August) cell numbers were less than 10^4 cells ml^{-1} (Figure 3.11). Cell numbers increased through September, peaked in December and then started to fall again in January. From this data an estimate of growth rate could be made for the population of *Synechococcus* in the lake. A plot of $\log_e$(cell number) versus time over October, November and December, gave a regression line with an r^2 value of 0.999. A generation time of 14.85 days was determined. This is comparable to growth rates obtained with laboratory cultures of the Ace Lake *Synechococcus* (Chapter 5) and to estimated growth rates of *Synechococcus* in northern polar oceans (Neuer 1992). It is, however, low in comparison to growth rates obtained with natural Ace Lake populations in the spring of 1993 (Chapter 6) and in comparison to growth rates obtained for *Synechococcus* from most temperate and tropical environments. Maximum generation times for *Synechococcus* in tropical and temperate marine environments have been estimated as less than a day (Campbell and Carpenter 1986; Landry et al. 1984; Waterbury et al. 1986); thus this long generation time in Ace Lake is likely to be due, at least partially, by low temperature. However, the spring bloom of *Synechococcus* occurred when the water temperature was at a minimum, (Figure 3.13). Further, the *Synechococcus* population did not continue to increase over summer when the water temperature started to rise. Other factors, such as light, grazing or nutrient

availability, were also controlling the population growth of *Synechococcus* in Ace Lake. The rapid loss of 20% of the total population, which was in an exponential growth phase, between December and January, would suggest grazing had an important controlling influence on cell numbers until May. Potential grazers such as ciliates, heterotrophic nanoflagellates and small copepods occur in Ace Lake and both ciliates (Laybourn-Parry and Perriss 1995) and copepods (Bayly and Burton 1987) have population peaks at approximately 11 m where the *Synechococcus* population also peaked at 8 x 10^6 cells ml^{-1}. This density of biota indicates that the microbial loop is active in Ace Lake.

A consequence of the increased stability of the water below the pycnocline in Ace Lake is the high concentrations of oxygen that have built up in the zone (Figure 3.15). Between the bottom of the mixed layer and the oxic/anoxic interface in Ace Lake Burch, (1988), Burton and Barker, (1979) and Franzmann et al., (1991) measured a decrease in the concentration of oxygen. Burton and Barker, (1979) referred to this as an oxycline. In ice covered lakes, the concentrations of gasses such as oxygen and nitrogen reach supersaturated concentrations (Wharton et al. 1987). In Ace Lake oxygen appears to be trapped in the zone below the pycnocline. It is probable that oxygen from primary production in this zone has accumulated because overall production is greater than consumption. In Lake Hoare, Dry Valleys, 42 % of the oxygen production was attributed to net photosynthetic production as opposed to the outflow of oxygen into the water column during ice formation (Vincent 1988). The high concentrations of *Synechococcus* in this region could be responsible for the accumulation of oxygen. At 10 m, the oxygen concentration throughout the year appeared to be related to the numbers of *Synechococcus* (Figure 3.15). *Synechococcus*

has been reported to have a light dependent sensitivity to oxygen, preferring a low oxygen environment especially under high light conditions (Glover and Morris 1981; Morris and Glover 1981). The carboxyzomes (or polyhedral bodies) observed in the ultrastructure of *Synechococcus* (Chapter 5) are thought to be used in the protection of the photosynthetic enzymes from oxidative damage (Joint 1986). *Synechococcus* growing below the pycnocline in Ace Lake do not appear to be inhibited by the high oxygen concentrations experienced at these depths (140 % - 170 % saturation at 10 m). It is also thought that at high oxygen concentrations, photosynthesis might be inhibited by low carbon dioxide concentrations. Based on the pH in the euphotic zone of Ace Lake (Figure 3.3) the majority of inorganic carbon would probably be in the form of the bicarbonate ion (Goldman and Horne 1983). Dissolved inorganic carbon (DIC) concentrations in the euphotic zone of Ace Lake (> 2000 mmol) are higher than concentrations in the sea (J. Gibson, personal communication; Burton 1980). It is unlikely that DIC concentrations would limit photosynthesis in *Synechococcus* or in other phytoplankton in Ace Lake (J. Gibson, personal communication).

The high numbers of *Synechococcus* below the pycnocline are depicted in the vertical attenuation coefficient, K_d (PAR), at these depths (Table 3.1). The vertical attenuation coefficient is a measure of the absorption qualities of a water body (Kirk 1994; Wetzel 1983). Low productivity water bodies, for example oligotrophic oceans, are expected to have low K_d values. Kirk, (1994) reported a K_d (PAR) of 0.03 m^{-1} for the Sargasso Sea. On the other hand, water bodies that are highly productive, coloured by organic acids, or turbid, are expected to have high K_d values. Lake Pedder in Tasmania had a reported K_d (PAR) of 2.39 m^{-1} because of the high concentration of organic acids in the lake and the highly turbid, tropical Georgetown Billabong had a reported K_d (PAR) of 8.5 m^{-1}

(Kirk 1994). In Ace Lake the absorption characteristics of the euphotic zone could be described using two values for K_d (Figure 3.7). In the upper mixed zone of Ace Lake, K_d (PAR) values of between 0.06 m^{-1} and 0.2 m^{-1} were obtained, indicating that there was relatively little absorption of light by suspended inorganic matter or by phytoplankton. Between 10 m and 12 m, however, where there were high numbers of *Synechococcus*, values of between 1.5 m^{-1} and 4.9 m^{-1} were obtained.

There is uncertainty as to whether *Synechococcus* is adapted to growth under low light conditions (Stockner and Antia 1986). Its vertical distribution in the oceans of the world is variable with most reports of peak numbers in surface waters (Chapter 1; Murphy and Haugen 1985; Venkateswaran et al. 1993) and other reports of peak numbers at the bottom of the euphotic zone (Caron et al. 1985; Waterbury et al. 1979). Joint, (1986) indicated that *Synechococcus* is capable of growth throughout a wide range of light intensities, but due to its small size, its shape and its combination of light harvesting pigments, it has a competitive advantage at the bottom of the euphotic zone where light is limited. An alternative explanation for the broad vertical distribution of *Synechococcus* is that different strains are adapted to different light conditions (Li and Wood 1988). The Ace Lake *Synechococcus* occurs in the greatest abundance at the bottom of the euphotic zone where there is a light intensity range of less than 1 μmol photons m^{-2} s^{-1} in winter to 35 μmol photons m^{-2} s^{-1} at the end of summer (Figure 3.4 and 3.5). In Antarctica, light intensity in aquatic environments is dependent on the time of year (Campbell and Aarup 1989), the meteorological conditions, the thickness and quality of ice and the thickness of snow cover on the lake ice (Burch 1988; Wharton et al. 1993). Over winter, when solar radiation was negligible, the depth of 1 % incident radiation in Ace Lake was less than 2 m (Figure 3.6). This increased to between 10 and

11 m over summer. At 10 m in Ace Lake *Synechococcus* numbers started to increase in September when the light intensity at this depth was less than 5 μmol photons m^{-2} s^{-1}. It has been reported that in the class Cyanophyceae, the minimum light intensity for growth is 5 μmol photons m^{-2} s^{-1} (Richardson et al. 1983). In most aquatic environments low in organic acids and suspended sediment (Herring et al. 1990; Kirk 1994), such as occurs in Ace Lake, green light (500 nm) is the dominant wavelength at the bottom of the euphotic zone (Burch 1988). When grown at low light intensities, the Ace Lake *Synechococcus* contained high concentrations of phycoerythrin (Chapter 5), a phycobiliproteins that absorbs light at 550 nm (Rowan 1989). Further, the Ace Lake *Synechococcus* had a faster growth rate at low light intensities (Chapter 6), and after a period of winter darkness, it appeared that *Synechococcus* growth was initiated by a light intensity of between 1 and 5 μmol photons m^{-2} s^{-1} (Figure 3.14). This light induced spring bloom has also been seen in other phytoplankton species in Ace Lake (Burch 1988), and other Antarctic and subantarctic lakes (Hawes 1985; Vincent 1981; Vincent and Vincent 1982). In 1992, the *Synechococcus* bloom at 10 m in Ace Lake commenced in September when the light intensity started to increase (Figure 3.14). At this time, the temperature at this depth was at a minimum (Figure 3.13). The maximum temperature at 10 m in Ace Lake occurred in February, when *Synechococcus* cell numbers were low. In some temperate waters, the *Synechococcus* spring bloom has been correlated with an increase in water temperature rather than light (Caron et al. 1985; Glover 1985; Shapiro and Haugen 1988).

At 8 m, 10 m and 11 m, the *Synechococcus* bloom peaked in late December (Figure 3.11). At all three depths *Synechococcus* cell numbers decreased in January. The exact

cause of this decline is not known but it is possible that grazing by nanoflagelles may be responsible. Laybourn-Parry and Perriss, (1995) found higher numbers of heterotrophic and autotrophic nanoflagellates in December in Ace Lake than in November. Further, between 8 m and 11 m, the population of suspected autotrophic nanoflagellates, monitored in Ace Lake by flow cytometry (Figure 3.18), bloomed at approximately the same time as the population of *Synechococcus*. The heterotrophic nanoflagellates could not be monitored by flow cytometry because of the lack of fluorescent pigments. However, (Hall et al. 1993), has shown that autotrophic nanoflagellates are capable of feeding heterotrophically, and the autotrophic population in Ace Lake could be grazing on *Synechococcus*.

The identity of the nanoflagellate monitored by flow cytometry was not confirmed by microscopy. However, the size (Burch 1988) and fluorescence characteristics of the population is consistent with that of the autotrophic nanoflagellates (S. Perriss, personal communication), and Laybourn-Parry and Perriss, (1995) has observed both heterotrophic and autotrophic nanoflagellates in the lake. Further, no other photosynthetic organism of this size (3 μm diameter) has been observed in Ace Lake. It is therefore reasonable to assume that the population observed by flow cytometery was the autotrophic nanoflagellate observed by others (T. Pitman, unpublished data) and (Laybourn-Parry and Perriss 1995).

The nutrient concentrations in Ace Lake in December 1993 were consistent with those measured by Burch, (1988) and Burton, (1980). They are also similar to those measured in 1994 (J. Gibson, personal communications). It is probable that the water below the pycnocline has higher nutrient concentrations because of nutrient diffusion

from the anaerobic zone as commonly occurs in meromictic lakes (Canfield and Green 1985). Ammonia concentrations in the lake have not been measured in recent years but Burton, (1980) found concentrations of between 0.007 and 0.29 mM below the pycnocline. Ammonia that diffuses up from the anaerobic zone in Ace Lake would be rapidly used by phytoplankton and heterotrophic bacteria, possibly accounting for the deep chlorophyll maxima that occurs just above the anaerobic interface (Burch 1988; Vincent 1988). In November 1992, total phytoplankton cell surface area increased with depth indicating an increase in productivity just above the anaerobic interface (Figure 3.16).

The mixed zone of the lake is low in oxidised nitrogen (below the limit of detection) and phosphate-P (0.25 μM). This is probably because it is isolated from the more nutrient rich waters by a sharp pycnocline. Further, unlike the meromictic lakes studied in the Dry Valleys (Canfield and Green 1985), there are no melt streams contributing nutrients to Ace Lake during the summer thaw period. There is also very little vegetation in the catchment of Ace Lake. The erosion of rock, caused by the strong catabatic winds, is thought to contribute to lacastrine phosphorus concentrations in Antarctica (Canfield and Green 1985; Masuda et al. 1988) but nitrogen concentrations in Ace Lake are probably controlled by internal recycling both within the aerobic zone and between the anaerobic and aerobic zone.

In the mixed zone of Ace Lake, *Synechococcus* occurred in numbers comparable to the numbers in oligotrophic oceans (Hagstrom et al. 1988; Platt et al. 1983; Waterbury et al. 1986). It is probable that in the mixed zone of the lake, a rapid recycling of nutrients occurs and because of its small cell size, *Synechococcus* survives and competes (Fogg

1986). Despite its capacity to survive in nutrient poor conditions, *Synechococcus* is known to proliferate when nutrient concentrations are increased (Stockner and Shortbreed 1988). Under these conditions, however, *Synechococcus* does not dominate in terms of algal biomass. Larger phytoplankton species are more competitive when nutrient concentrations are higher, for example in coastal waters (Jochem 1988; Krempin and Sullivan 1981). In terms of biovolume and cell surface area, *Synechococcus* dominates the phytoplankton population at 8 m and 10 m in Ace Lake, despite the increase in phosphorus and ammonia.

Estimates of primary productivity in Ace Lake over summer indicated that productivity is low relative to Antarctic coastal waters. Over a three year period, Moline and Prezelin, (1996), obtained integrated primary production rates between 1.08 and 6.58 g C m^{-2} d^{-1} in coastal waters near Palmer Station, Antarctica. In November 1993 Laybourn-Parry and Perriss, (1995) obtained a primary productivity measure of between 0.47 and 0.68 μg C l^{-1} h^{-1} and predicted that *M. rubrum* contributed up to 26 % of productivity at 2 m and 15 % at 8 m in Ace Lake. This is consistent with the percentage contribution of *M. rubrum* to total phytoplankton surface area in November 1992 (Table 3.3). According to Voros et al., (1991), surface area estimates are a more accurate indicator of contribution to primary productivity by individual phytoplankton species than are cell numbers or biovolume estimates. In the case of *Synechococcus*, cell number over estimates the contribution of the picocyanobacteria to primary productivity and biovolume underestimates the contribution (Voros et al. 1991). Based on surface area estimates, *Synechococcus* contributes significantly to primary productivity below the pycnocline in Ace Lake especially during the time of the spring bloom (Table 3.2, Figure 3.17). At this time, 20 November 1992, *M. rubrum* contributed between 29 %

and 46 % of the total phytoplankton surface area in the mixed zone of the lake but only between 3 % and 12 % below the pycnocline (Table 3.3). *Synechococcus* contributed to 2 % to total phytoplankton surface area in the mixed zone but between 26 % and 78 % below the pycnocline. The phototrophic nanoflagellate contributed 16 % and 47 % to surface area throughout the euphotic zone. The relationship between biovolume, surface area and primary productivity should be clarified in Ace Lake and a more detailed study of size fractionated primary productivity over space and time is needed.

Apart from *Synechococcus* and *Chlorobium spp.*, no other autotrophic eukaryotic or prokaryotic picoplankton have been observed in Ace Lake. There has been a suspected reporting of a Prochlorophyte in an Antarctic freshwater lake (Andreoli et al. 1992) but apart from this *Procholorococcus* has not been observed in Antarctic Lakes or in the southern ocean (Ellis-Evans 1996; Vincent and James 1996; Weber and El-Sayed 1987). Eukaryotic picoplankton occur in the southern oceans (Weber and El-Sayed 1987) and have been identified in other continental Antarctic lakes (Simmons et al. 1993) as have other picocyanobacteria (Simmons et al. 1993; Vincent 1988). It is possible that other autotrophic picoplankton occur in Ace Lake but in numbers too low and with fluorescent characteristic that are unable to be detected by flow cytometry (Chapter 2).

At 10 m in Ace Lake over winter, heterotrophic bacterial numbers were an order of magnitude greater than *Synechococcus* numbers (Figure 3.12). *Synechococcus* generally occurs in lower densities than heterotrophic bacteria (Caron et al. 1985; Fogg 1986; Li et al. 1983). In Ace Lake, heterotrophic bacterial numbers started to increase at approximately the same time as the increase in *Synechococcus* numbers. Only at the

peak of the *Synechococcus* spring bloom where the numbers of heterotrophic bacteria the same as the numbers of *Synechococcus*. Burns and Stockner, (1991) found greater *Synechococcus* numbers than heterotrophic bacterial numbers over summer in freshwater lakes in New Zealand. It is probable that at this time, the conditions of growth were better for *Synechococcus* than for the heterotrophic bacteria. At the end of December, however, heterotrophic bacterial numbers stayed high (2×10^6 ml^{-1}) whereas the *Synechococcus* numbers fell dramatically. The reason for this fall in *Synechococcus* numbers, but not in heterotrophic bacterial numbers, is unclear except that it was possibly either due to self shading of the *Synechococcus* population at the peak of the bloom (1×10^6 ml^{-1}) or selective grazing of *Synechococcus*.

Dissolved organic carbon (DOC) concentrations in Ace Lake were consistent with concentrations found in other Antarctic meromictic lakes (Matsumoto et al. 1989; McKnight et al. 1993) and were higher than concentrations measured at a coastal Antarctic site (Chapter 4). The dissolved organic carbon concentrations were lowest in the mixed zone of the lake, increased with depth and reached a maximum (25 mg l^{-1}) at the bottom of the lake (Figure 3.8). The high DOC concentration in the bottom waters result from either the degradation of particulate organic carbon sinking from the euphotic zone or from the diffusion of organic carbon from the sediment and algal mats (Matsumoto et al. 1989). At 10 m, the highest DOC concentrations were observed in August and October, approximately the same time that heterotrophic bacterial numbers started to increase (Figure 3.12). In the euphotic zone of Antarctic meromictic lakes, it is thought that the majority of DOC available to heterotrophic bacteria comes from extracellular carbon produced from phytoplankton (Fogg 1986; Parker et al. 1977) rather than from sloppy feeding by zooplankton. It is possible that the spring increase in

heterotrophic bacteria at 10 m in Ace Lake may have been stimulated by an increase in *Synechococcus* activity.

Like many other phytoplankton species (Rowan 1989), *Synechococcus* is capable of changing its content of photosynthetic pigments to suit the light intensity (Barlow and Albert 1985; Vernet et al. 1990). Under high light intensities, such as in the surface water of oceans, *Synechococcus* cells usually contain a lower concentration of photosynthetic pigments than cells that are grown at a low light intensity near the bottom of the euphotic zone (Li and Wood 1988; Shimada et al. 1993; Vaulot and Xiuren 1988). Over the summer, photoadaptation of *Synechococcus* cells was observed at 10 m in Ace Lake. In early spring, when the light intensity at 10 m was less than 5 μmol photons m^{-2} s^{-1}, *Synechococcus* had a higher concentration of orange, phycoerythrin, fluorescence (FL2, Figure 3.19) and red, chlorophyll fluorescence, FL3, relative to cells at the same depth in early summer. At this time, the light intensity had increased to approximately 15 μmol photons m^{-2} s^{-1}. In phytoplankton, photoadaptation can be achieved either through a change in the number of photosynthetic units per cell or by altering the concentration of accessory pigments but not photosynthetic units (Barlow and Albert 1985; Rowan 1989). In this case it appears that there has been a decrease in the number of photosynthetic units (Table 3.4) but there has also been a decrease in the concentration of accessory pigments (phycoerythrin) relative to photosynthetic units, hence the reduction in the ratio of FL2/FL3. Photoadaptation by *Synechococcus* was also observed with depth in Ace Lake.

Diel periodicity in both growth and photosynthesis has been observed in many *Synechococcus* populations in temperate and tropical environments (Campbell and

Carpenter 1986; Carpenter and Campbell 1988; Glover et al. 1985). In environments where *Synechococcus* generation times of approximately one day have been observed, diel periodicity in growth was easily detected (Fahnenstiel et al. 1991; Waterbury et al. 1986). However, Fahnenstiel et al., (1991b) found that at low growth rates (less than 0.3 d^{-1}) diel periodicity in growth was difficult to detect. *Synechococcus* in Ace Lake did not show a distinct diel periodicity in growth. Due to the fluctuation in light intensity (Figure 3.20), it is probable that the Ace Lake *Synechococcus* would show a diel periodicity in photosynthetic rate (P). Rivkin and Putt, (1987) detected diel periodicity in photosynthesis of marine phytoplankton communities near McMurdo Sound, Antarctica. They found that during spring, when there was a distinct day/night cycle in incident radiation, diel periodicity in photosynthesis was similar to that in temperate and tropical environments. During December and January, when the number of day light hours was distinctly longer than the number of hours of darkness, the P_{max} shifted to midnight and P_{min} shifted to mid-day. Maximum growth rates and photosynthetic rates of *Synechococcus* in the Sargasso Sea, the Northwest Atlantic Ocean and Long Island Sound, New York occurred in the late afternoon, and minimum growth rates generally occurd in the early morning (Campbell and Carpenter 1986; Carpenter and Campbell 1988; Waterbury et al. 1986). Due to the long *in-situ* generation time of *Synechococcus* at 10 m in Ace Lake, it was not surprising that a diel periodicity in growth was not observed.

Diel periodicity in growth has been measured by changes in cell numbers (Waterbury et al. 1986), as was the case in this instance, or by a measurement of the frequency in dividing cells (Campbell and Carpenter 1986; Carpenter and Campbell 1988; Glover et al. 1985). Measurement of the frequency of dividing cells was not compatible with

flow cytometric techniques but this method appears to be more accurate in determining diel periodicity in growth (Waterbury et al. 1986). The periodicity in ^{14}C uptake by *Synechococcus* in Ace Lake should be investigated. The fluctuations in *Synechococcus* cell numbers observed (Figure 3.21) were possibly from experimental error. Samples were collected using a Kemmerer Bottle which has a sampling chamber of approximately 50 cm. As *Synechococcus* has an uneven distribution in the lake a change in the sampling interval of only a few centimetres could introduce a significant error. This situation holds not only for the diel periodicity sampling but for all samples taken throughout the year. A fine interval sampler is required to obtain a more accurate understanding of the vertical and temporal distribution of *Synechococcus* in Ace Lake.

The discovery of *Synechococcus*, in a marine derived Antarctic lake, provided a unique opportunity to investigate factors that control the abundance and distribution of a natural population of the picocyanobacterium in southern polar regions. One of the advantages of doing ecological studies in lake environments is that the population is captive, and thus not removed by currents. It is generally considered that temperature limits the abundance of *Synechococcus* in southern polar waters (Gradinger and Lenz 1989). In Ace Lake, between 8 m and 11 m, where *Synechococcus* was abundant and temperature remained between 5° C and 11° C, it was not temperature alone which controlled the population's abundance. It is probable that *Synechococcus* has evolved to survive under the low light conditions at the bottom of the euphotic zone in Ace Lake where the nutrient conditions are more favourable for growth. Throughout the euphotic zone a combination of factors such as light, temperature, nutrient availability and grazing pressures probably controlled the abundance of *Synechococcus* in the lake. Understanding what controls the abundance of *Synechococcus* in Ace Lake will give

more insight into the factors that are limiting the abundance of the organism in the Southern Ocean.

Chapter 4

A survey of meromictic lakes, in the Vestfold Hills, for the occurrence of *Synechococcus*

4.1 Abstract

Flow cytometric methods were used to survey the phytoplankton populations in meromictic lakes and a marine site in the Vestfold Hills. *Synechococcus* occured in high numbers in three meromictic lakes including Ace Lake. *Synechococcus* also occured in Lake Abraxas and Pendant Lake both of which are in close proximity to Ace Lake on Long Peninsula. *Synechococcus* was distributed throughout the euphotic zone in Pendant Lake with densities of 10^7 cells ml^{-1} recorded in November 1993 and 10^5 cells ml^{-1} recorded in January 1993. In Lake Abraxas, *Synechococcus* occured in low numbers in the mixolimnion but below the pycnocline a density of 10^7 cells ml^{-1} was recorded in November 1993. *Synechococcus* was not found at the marine sites but this was probably because it occured in numbers too low to be detected using flow cytometric techniques. It is probable that *Synechococcus* became part of the lake phytoplankton community at the time the three lakes were isolated from the fjord environment. There was a weak negative correlation between *Synechococcus* abundance and salinity and it is probable that salinity limits the growth of *Synechococcus* in the hypersaline lakes. Ace Lake, Pendant Lake and Lake Abraxas had water of similar salinity (16 to 30 g kg^{-1}) but water temperature was lower in Pendant Lake and Lake Abraxas than it was in Ace Lake. There was no correlation between water temperature and *Synechococcus* cell numbers. Further, there was no correlation between nutrient concentrations and *Synechococcus* abundance. In Ace Lake and Lake Abraxas the highest density of *Synechococcus* occured just above the anoxic/oxic interface where nutrient diffusion from the anoxic/oxic interface might favoured their growth. *Synechococcus* strains were isolated from the three lakes for taxanomic comparison.

4.2 Introduction

There are approximately thirty meromictic lakes in the Vestfold Hills. They represent approximately fifteen percent of the total number of lakes in the 400 square kilometre ice free area. There are also several known meromictic basins in the fjords around the Vestfold Hills (Gallagher et al. 1989). Apart from measurement of temperature and salinity profiles, the majority of these lakes and meromictic basins have not been studied in detail. Ace Lake is the most extensively studied meromictic lake in the Vestfold Hills (Chapter 1) although Burton Lake, Fletcher Lake, Organic Lake and Lake Abraxas have received some attention (Bayly and Eslake 1989; Burke and Burton 1988a; Eslake et al. 1991; Franzmann et al. 1990; Franzmann et al. 1987; van den Hoff and Franzmann 1986). Information on the phytoplankton communities in the other meromictic lakes is starting to emerge. Perriss et al., (1995) recently surveyed a selection of the meromictic lakes for the presence of *Mesodinium rubrum.*

Meromictic Lakes are not common around the world and the majority are located in polar regions where wind induced mixing is reduced by lake ice cover (Walker and Likens 1975). Apart from the Vestfold Hills, a concentration of meromictic lakes occurs in The Dry Valleys, South Victoria Land, Antarctica. These lakes are thought to have been formed from either entrapment of seawater and or from glacial retreat (Green et al. 1988; Green et al. 1989; Matsumoto et al. 1989). Some of the lakes in the Dry Valleys have a permanent cover of ice, and wind driven mixing occurs only in a small moat that forms around the edges of the lakes in summer. Lake Vanda, Lake Bonney and Lake Fryxell are amictic because the entire water column remains

unmixed (Goldman and Horne 1983; Wetzel 1983). Like Ace lake, it is probable that the other saltwater meromictic lakes in the Vestfold Hills have formed from the isostatic uplift of the Antarctic continent and the trapping of seawater in pockets (Adamson and Pickard 1986; Gallagher et al. 1989).

In temperate regions, lake meromixis has formed from mechanisms other than an increase in stability due to ice cover. This has occurred through the intrusion of saline water under freshwater lakes (Croome and Tyler 1988), or in lakes that are deep enough to prevent wind driven mixing in the bottom waters (Goldman and Horne 1983). Many lakes on the Gordon River in Tasmania were meromictic up until the time of the construction of the Gordon River Dam. Intrusion of saline waters from Macquarie Harbour was thought to be responsible for the meromixis (Croome and Tyler 1988). The changed hydrological conditions resulted in the meromixis in some of the lakes being replaced by holomixis, although when saline water was allowed back in, meromixis was restored (Tyler and Bowling 1990).

Meromictic lakes from tropical, temperate and polar regions appear to have many planktonic community characteristics in common. The phytoplankton are often dominated by flagellates and picoplankton (Burch 1988; Croome and Tyler 1985; Spaulding et al. 1994; Vincent 1988). Diatoms are not a dominant part of the plankton community (Vincent 1988) and there is often a deep chlorophyll maxima (DCM) just above the anoxic/oxic interface in these lakes (Parker et al. 1982; Vincent 1988). Many of the lakes have a band of photosynthetic sulfur bacteria at the interface of oxic and anoxic zones. Meromictic lakes also have active populations of sulfur reducing bacteria that produce hydrogen sulfide in the anoxic

zone of the monimnolimnion (Baker et al. 1985; Craig 1987; Howes and Smith 1990; Venkateswaran et al. 1993).

In this study, a selection of meromictic lakes in the Vestfold Hills were surveyed for the presence of *Synechococcus*. The aim of the survey was to determine the distribution and abundance of *Synechococcus* in these lakes and to investigate the factors that might be controlling the distribution of *Synechococcus* in the meromictic lakes.

4.3 Materials and methods

4.3.1 Sample collection and analysis

Nine meromictic lakes in the Vestfold Hills, a fjord site and one coastal site in Davis Bay were sampled (Figure 4.1, Table 4.1). The lakes sampled were chosen because of the broad salinity and temperature range between the lakes. The depths sampled within each lake and the two marine sites were selected from salinity and temperature profiles that had been taken prior to the survey (J. Gibson, unpublished data; L. Rankin, unpublished data). Each lake was sampled at its deepest known site and Taynaya Bay was sampled in a site where there was a known meromictic basin. Each lake was sampled once, at solar noon. A 12 inch hole was drilled through the lake ice using a Jiffy ice drill (Feldmann Engineering, Wisconsin).

Table 4.1: Description of lakes and marine sites that were surveyed for the presence of *Synechococcus* during 1992 and 1993.

Lake	Latitude	Longitude	Max Depth (m)	Anoxic/oxic Interface (m)	Ice Thickness (cm)	Date Sampled
Ace L.	68°28'	78°10'	25	12	184	14-Nov-93
L. Abraxas	68°29'	78°18'	21	20	166	23-Nov-93
Pendant L.	68°27.5'	78°15'	20	12.8	193	24-Nov-93
Ekho L.	68°31'	78°15'	40	23	0	25-Jan-93
Organic L.	68°27'	78°12'	7	5.2	0	29-Dec-92
Fletcher L.	68°26.75'	78°16'	11	7	1.5	10-Dec-92
L. McCallum	68°37.5'	78°01'	27	20	165	18-Nov-93
Clear L.	68°38.5'	77°59'	62	33	165	8-Dec-92
Burton L.	68°38'	78°06'	16	?	150	22-Nov-93
Tanaya Bay	68°27'	78°15'	23	16	205	16-Nov-93
Davis Bay	68°34.5'	77°15'	20		192	17-Nov-93

Samples were collected using a Kemmerer Bottle and stored in acid washed polyethylene bottles, at 4°C in the dark, for up to four hours before analysis. Conductivity, temperature and light readings were taken as described in Chapter 3. In this instance, due to the ice cover on the lakes, the euphotic depth was defined as the depth to which 1 % of the incident light penetrated, rather than 1 % of the surface light. Conductivity readings were converted to salinity using the formulas of (Fofonoff and Millard 1983; Gibson et al. 1990).

All samples were analysed for the presence of *Synechococcus* using flow cytometric techniques (Chapter 2). Flow cytometric instrument settings used in the detection of *Synechococcus* are given in Table 4.2. Heterotrophic bacterial concentrations were also determined using flow cytometric methods (Chapter 2).

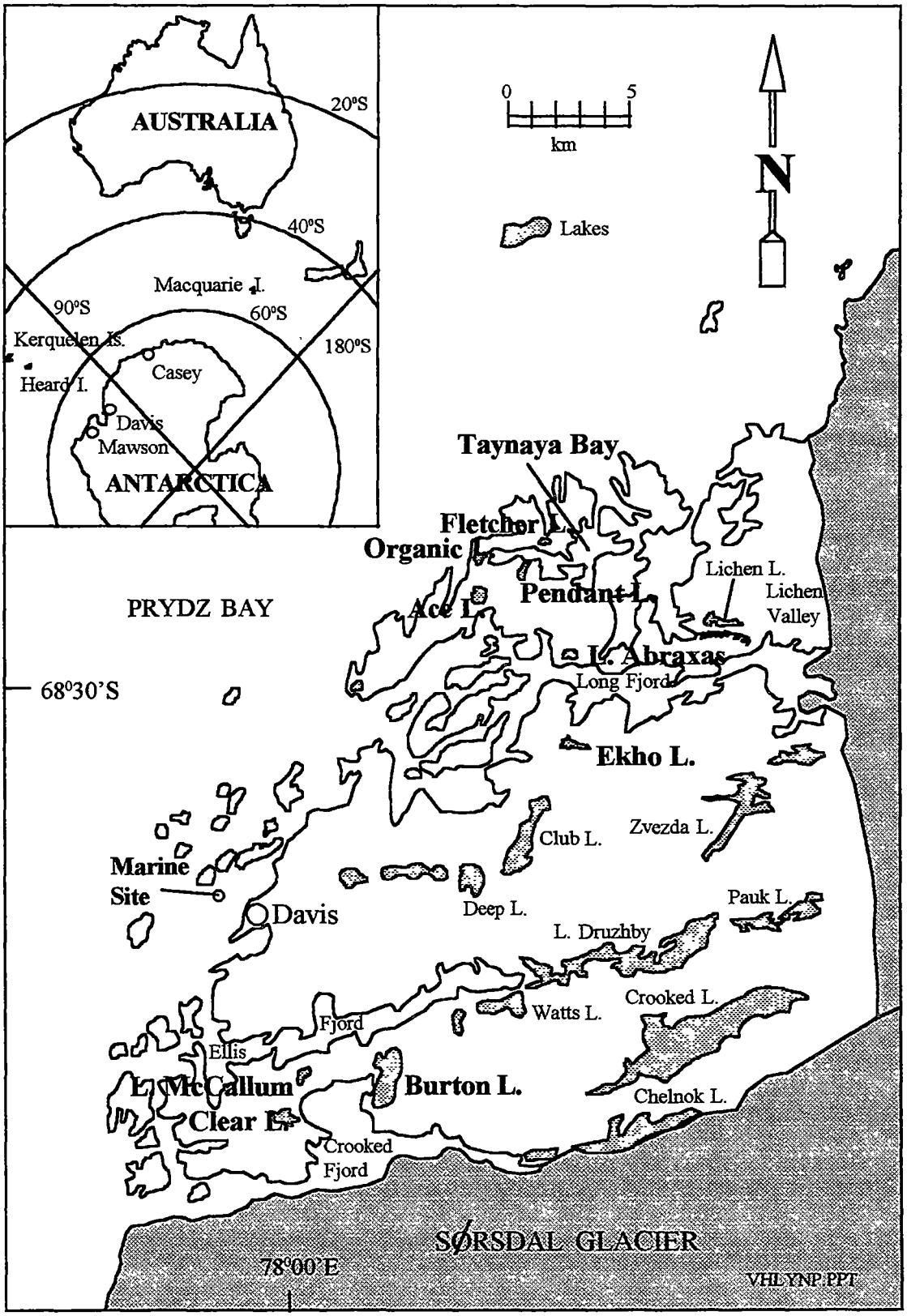


Figure 4.1: Map of the Vestfold Hills showing the meromictic lakes (bold) that were sampled for the presence of *Synechococcus*.

Table 4.2: Flow cytometric settings used in the identification of *Synechococcus* in water samples from meromictic lakes and marine sites in the Vestfold Hills.

Parameter	Setting
FSC	E00
SSC	326
FL1	437
FL2	514
FL3	459
Threshold	FL3 - 80

Samples for DOC analysis were filtered twice through prewashed 0.2 μm polycarbonate membrane filters (Millipore). The filters were washed three times with deionised water and twice with lake water prior to sample filtration. A new filter was used for each sample. Samples were stored in premuffed glass bottles at -20° C in the dark for analysis up to four months later. Samples were analysed using a TOC-5000 total organic carbon analyser (Shimadzu).

Samples were analysed for nutrient concentrations as described in Chapter 3. A series of standards were prepared to determine the effect of the salinity on the nutrient analysis. The standard nutrient series, recommended by (Alpkem Corp 1992), was prepared in a 3.5 % NaCl solution. Nutrient standards were also prepared in 1 %, 2.5 % and 5 % NaCl solutions. The concentration of the nutrient standards are given in Table 4.3. Data was analysed using 'Sigmastat' statistical software (1992). The effect of salinity on the analytical methods was determined prior to sample analysis.

Table 4.3: Standard nutrient series used in the determination of the effect of salinity on nutrient concentrations and in the determination of nutrient concentrations in the meromictic lake and marine water samples.

	1	2	3	4	5
PO_4^{2-} (μM)	0.6	1.2	1.8	2.4	3.0
NO_3^- (μM)	0.7	1.4	2.1	2.8	3.5

4.3.2 Lipid soluble pigment analysis

Lipid soluble pigment analysis was performed on the lake water samples. A known volume of lake water was filtered through 25 mm diameter, GF/F filters (Millipore) under low pressure (< 15 Kpa) in subdued light. Filters were stored in liquid nitrogen until analysis in Australia five months later. Lipid soluble pigments were extracted according to (Wright et al. 1991). Before analysis the filters were cut into small pieces. Buffered methanol (98:2 methanol: ammonium acetate 0.5M pH 7.1) was added (3 ml) and the filter was sonicated for 30 seconds using a Braun Labsonic 1510 equipped with a 4 mm diameter probe, operated at 50 W. The samples were then centrifuged (2000 rpm) at 4°C to remove filter debris and immediately before HPLC analysis the extracts were filtered (Millex - SR 0.5 μm). Samples were analysed on a Spectraphysics HPLC, comprising: a SP8800 ternary pump; Spectra Focus detector; and a Gilson 231 autosampler (with samples refrigerated at -10°C), using a Spherisorb OD52 column 250 x 4.6 mm and the ternary gradient system of (Wright et al. 1991). Chromatograms were analysed using Specta Physics software. Scientific Committee for Oceanic Research (SCOR) cultures from (Wright et al. 1991) were extracted and analysed at the same time as the lake water samples to aid in the identification of pigments (Chapter 5).

4.3.4 Isolation of *Synechococcus* from meromictic lakes

The isolation of *Synechococcus* from all lake water samples, from the fjord site and from the site in Davis Bay, was attempted. An aliquot (5 ml) of each sample was added to 45 ml of SNAX media and F/2 media (Appendix 5) and incubated at 6° C under continuous light (35 μmol photons m^{-2} s^{-1}). Cultures were checked by flow cytometry at two weekly intervals for three months.

4.3.5 Data Analysis

Physical and chemical characteristics at each of the sample sites were correlated with *Synechococcus* cell numbers, using stepwise linear regression and backward elimination (SAS Institute 1989), to determine the controls on *Synechococcus* distribution in the meromictic lakes of the Vestfold Hills.

4.4 Results

4.4.1 Flow cytometric analysis

The flow cytometric instrument settings used to detect *Synechococcus* at all of the sample sites were not optimal for the detection of other phytoplankton species (Table 4.2). Phytoplankton cells greater than approximately 20 μm in diameter were not detected using these instrument settings.

Three distinct phytoplankton populations were detected in the euphotic zone of Ace Lake (Table 4.4). The highest density of *Synechococcus* cells (R4) occurred at 11 m. A population of cells approximately 3 μm in diameter which had a FL3 fluorescence intensity five times greater than that of *Synechococcus* was detected throughout the euphoic zone (Figure 4.2). The highest density occurred at 6 m. Although no microscopic observations were made at the time, it is predicted that this population (R3) was the phototrophic nanoflagellate (PNAN) that Laybourn-Parry and Perriss, (1995) and T. Pitman (personal communication) described from Ace Lake (Chapter 3). The third population (R1) also occurred throughout the euphotic zone, with the greatest density recorded at 6 m. Based on flow cytometric analysis, cells in this population were approximately 5-7 μm in diameter. They had a FL3 fluorescence intensity fifty times that of the *Synechococcus* population (Figure 4.2). It is probable that this was a population of *Pyramimonas geledicola* (Burch 1988). The *Cryptomonas* sp. population known to occur in Ace Lake (Burch 1988) was not clearly detected using the described instrument settings. The cells in R7 (Figure 4.2) may have been cryptomonads as they were slightly larger than the cells in R1 and they had a more intense FL2 fluorescence. *Mesodinium rubrum* (Perriss et al. 1995) was not detected using the described instrument settings.

Table 4.4: Phytoplankton populations (cells ml^{-1}) in Ace Lake on 14 November 1993, detected using flow cytometric techniques. R1 was possibly *P. geledicola*, R2 was possibly a PNAN and R4 was *Synechococcus*.

Depth (m)	R1 (x 10^3)	R3 (x 10^3)	R4 (x 10^3)
4	3.88	6.34	0.25
5	3.90	6.36	1.30
6	11.9	16.0	1.61
7	3.02	6.10	1.51
8	0.87	1.29	28.3
10	0.27	0.46	231
11	0.26	0.72	807

Synechococcus was also detected in Pendant Lake. In November 1993, only two phytoplankton populations were detected by flow cytometry (Figure 4.3b). This was because in November, *Synechococcus* occurred in high densities (R1, Table 4.5). In the flow cytometric analysis, phytoplankton species that occurred in low densities were masked by the high concentrations of *Synechococcus*. The phytoplankton community in Pendant Lake has not been previously described. Population R3 was probably the same as the PNAN described in Ace Lake.

Table 4.5: Phytoplankton populations (cells ml^{-1}) in Pendant Lake on 24 November 1993, detected using flow cytometric techniques. R1 was *Synechococcus* and R3 was probably a PNAN

Depth (m)	R1 (x 10^6)	R3 (x 10^4)
5	8.71	8.96
10	9.63	9.9
11	15.0	1.53
11.8	10.2	1.22

In the previous January, five phytoplankton populations were detected (Table 4.6). The density of *Synechococcus* cells (R4) was between one and two orders of magnitude lower in January 1993 than it was at the end of November 1993. Other phytoplankton populations detected in Pendant Lake in January 1993 included one

that had flow cytometric characteristics similar to those of the suspected P. *geledicola* that occurs in Ace Lake (R1), an unidentified population (R3) that was slightly larger than the R1 population, and two populations (R5 and R6) that were approximately 10 μm in diameter and had relatively more intense FL2 fluorescence than population R1. Population R5 had a FL2 fluorescence intensity approximately 10 times that of population R1 and population R6 had a FL2 fluorescence intensity approximately 10 000 times that or R1 (Figure 4.3a). One of these populations was probably a cryptomonad. The population of PNAN was not detected in the Pendant Lake sample collected in January

Table 4.6: Phytoplankton populations (cells ml^{-1}) in Pendant Lake on 5 January 1993, as detected using flow cytometric techniques. R1 was probably *P. geledicola*, R3, R5 and R6 were unidentified phytoplankton populations and R4 was *Synechococcus*.

Depth (m)	R1 (x 10^3)	R3 (x 10^3)	R4 (x 10^5)	R5 (x 10^3)	R6 (x 10^3)
2	30.2	1.51	0.122		
4	3.15	1.18	1.66		
6	3.17	1.78	1.76	1.10	
8	6.83	2.34	1.41	1.85	1.07
9	3.86	1.83	1.45	4.77	
10	5.08	2.09	1.39	1.64	0.13
11.5	1.11	2.11	1.77	9.15	0.31
12.5	3.19	0.90	1.37	9.22	0.14

The third lake in which *Synechococcus* was detected was Lake Abraxas (Figure 4.4). *Synechococcus* (R5) occurred in low numbers in the mixed zone of the lake but was detected at high densities at 19 m (Table 4.7). It was also detected at 21 m, where the dominant population was a photosynthetic sulfur bacterium. The proposed PNAN was detected in Lake Abraxas (R1), as was a population that was suspected to be *P. geledicola* (R3). A fourth population (R4) was of a similar size to that of R3 but had a greater SSC and a ten times greater FL2 fluorescence intensity.

Table 4.7: Phytoplankton populations (cells ml^{-1}) in Lake Abraxas on 23 January 1993, detected using flow cytometric techniques. R1 was probably be a PNAN, R3 was probably a P. *geledicola*, R4 was unidentified and R5 was *Synechococcus*.

Depth (m)	R1 (x 10^4)	R3 (x 10^3)	R4 (x 10^3)	R5
3	0.59	0.36	0.20	52
10	0.65	0.51	0.25	104
19	81.3	69.1	10.9	1.48×10^7
21	N.D.	N.D.	N.D.	2×10^5

Synechococcus was not detected at any of the other sites using flow cytometric techniques. In Taynaya Bay the phytoplankton density was generally low. Unlike the lake samples, the phytoplankton community appeared as a broad non discrete population possibly indicating that there is a greater diversity of phytoplankton species each occurring in low densities. Three discrete populations were detected (Figure 4.5) each occurring in densities of approximately 10^2 - 10^3 cells ml^{-1}. The coastal marine site had low densities of eukaryotic phytoplankton. Three populations were detected in densities of 10^3 cells ml^{-1}.

Lake McCallum, Clear Lake and Ekho Lake all had low densities of phytoplankton (10^1 to 10^3 cells ml^{-1}). Three populations were detected in Lake McCallum all having a cell diameter of between 5 and 10 μm (Figure 4.6). Population R1 was potentially a cryptomonad. A population of photosynthetic sulfur bacteria (R4) was detected between 19 and 20 m. Two phytoplankton populations were detected in Clear Lake (Figure 4.7) and Ekho Lake (Figure 4.8). These populations were between

5 and 10 μm in size. The photosynthetic sulfur bacterium, *Chlorobium* spp. was detected in Clear Lake but not in Ekho Lake.

Fletcher Lake had three phytoplankton populations (Figure 4.9). Population R4 had characteristics similar to the proposed PNAN population detected in Ace Lake, Pendant Lake and Lake Abraxas. It occurred at densities of 10^3 - 10^4 cells ml^{-1} throughout the euphotic zone of Fletcher Lake. The other two populations were composed of cells with diameters between 5 and 15 μm, and occurred in densities of 10^3 to 10^4 cells ml^{-1}. High densities of a photosynthetic sulfur bacterium occurred below 6.8 m in Fletecher Lake (9.81×10^7 cells ml^{-1} at 6.8 m). No eukaryotic phytoplankton were detected in Burton Lake probably because their presence was masked by the high densities of photosynthetic sulfur bacteria (2.17×10^6 cells ml^{-1} at 4 m, Figure 4.10).

Organic Lake had three distinct phytoplankton populations at 1 m (Figure 4.11) but only one population at the other depths in the lake. The populations occurred in densities of between 10^4 and 10^5 cells ml^{-1}. It was difficult to determine the size of the cells due to the effect of the high salinity water on the flow cytometer (Chapter 2).

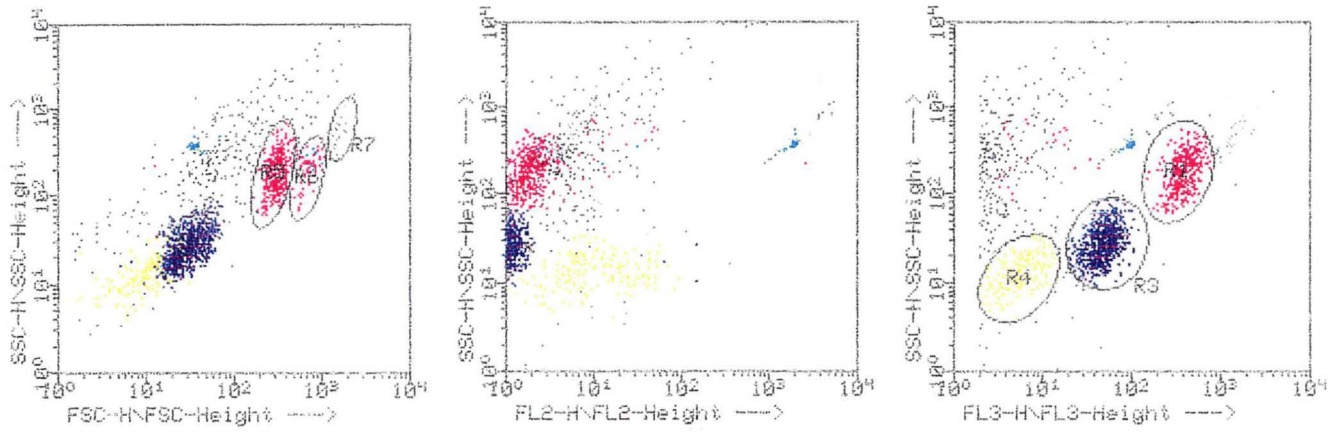


Figure 4.2: Flow cytometric image of a water sample from 7 m in Ace Lake on the 29th November 1993. Axis are in relative log units. R1 (R5 + R6) was possibly *P. geledicola*; R3 was possibly a phototrophic nanoflagellate (PNAN); R4 was the *Synechococcus* population and R7 was possibly a Cryptomonad.

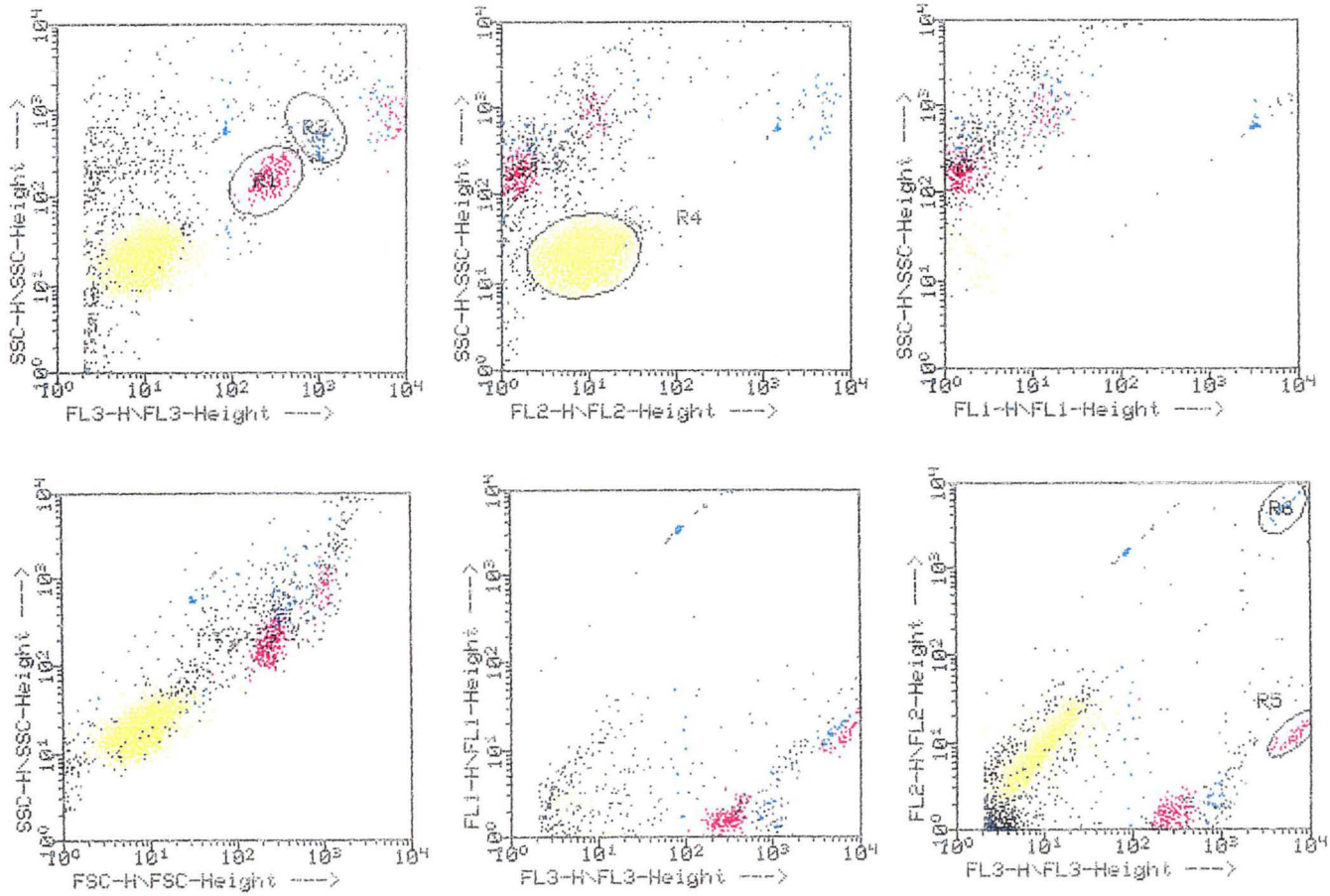


Figure 4.3a: Flow cytometric image of a water sample from 8 m in Pendant Lake on the 5th January 1993. Axis are in relative log units. R1 was possibly *P. geledicola* (PNAN); R4 was the *Synechococcus* population and R6 was possibly a Cryptomonad.

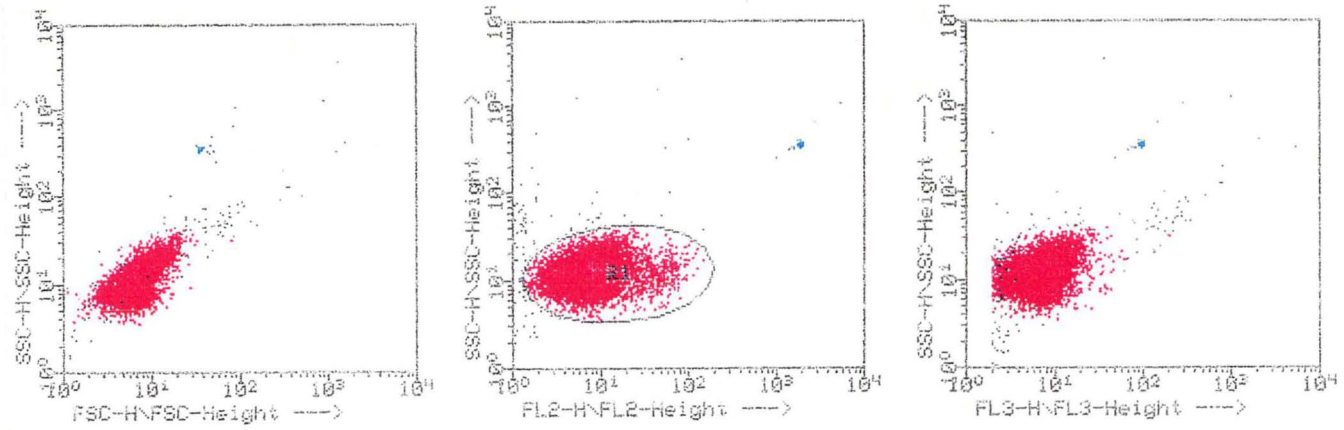


Figure 4.3b: Flow cytometric image of a water sample from 10 m in Pendant Lake on the 24th November 1993. Axis are in relative log units. R1 was the *Synechococcus* population.

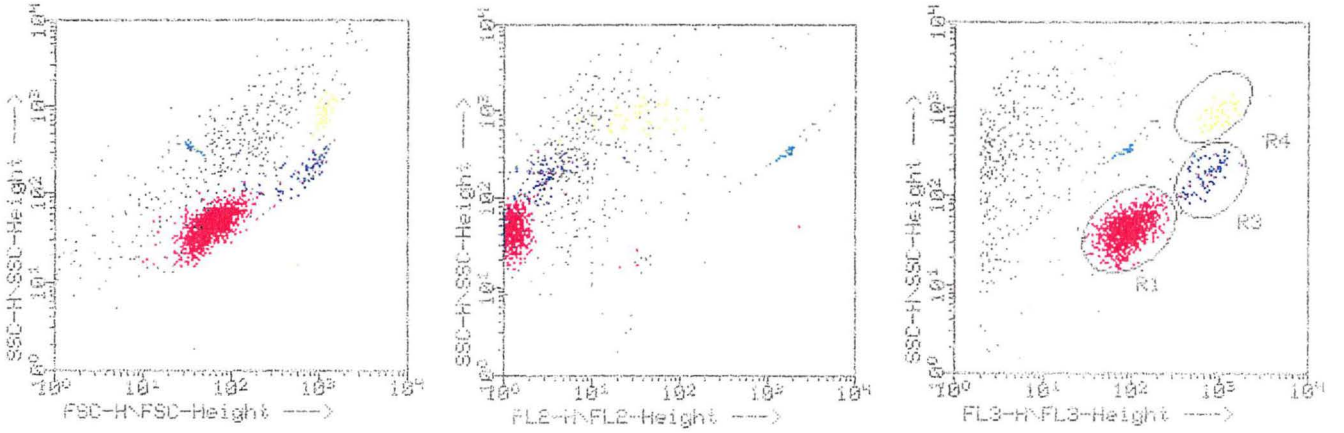


Figure 4.4: Flow cytometric image of a water sample from 3 m in Lake Abraxas on the 23rd November 1993. Axis are in relative log units. R1 was a proposed PNAN population and R3 was a proposed *P. geledicola*.

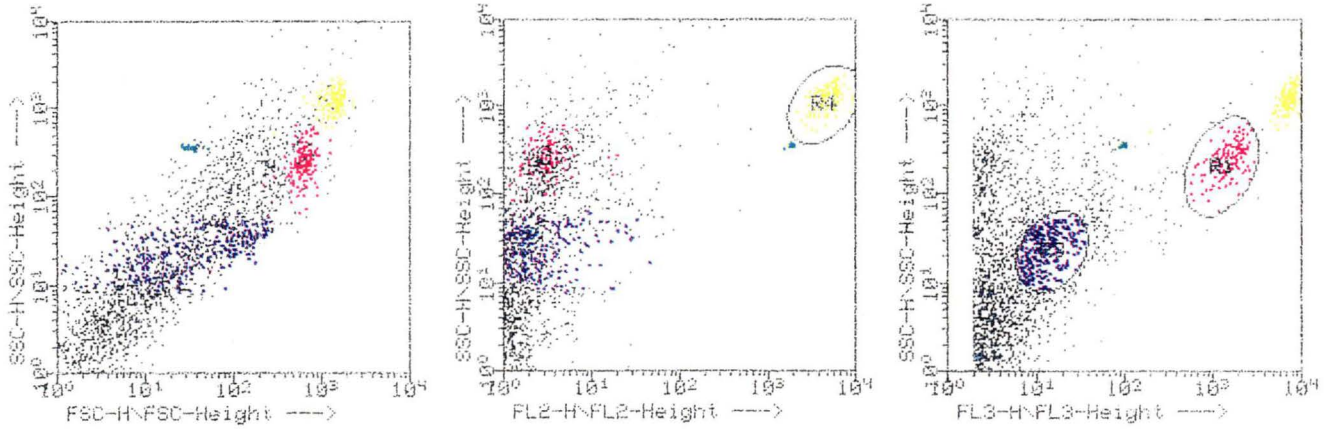


Figure 4.5: Flow cytometric image of a water sample from 5 m in the meromictic basin in Taynaya Bay on the 26th November 1993. Axis are in relative log units. Population R4 differed from R1 in that population R4 had an approximately 1000 X more intense relative orange fluorescence. The identity of these two phytoplankton populations is not known.

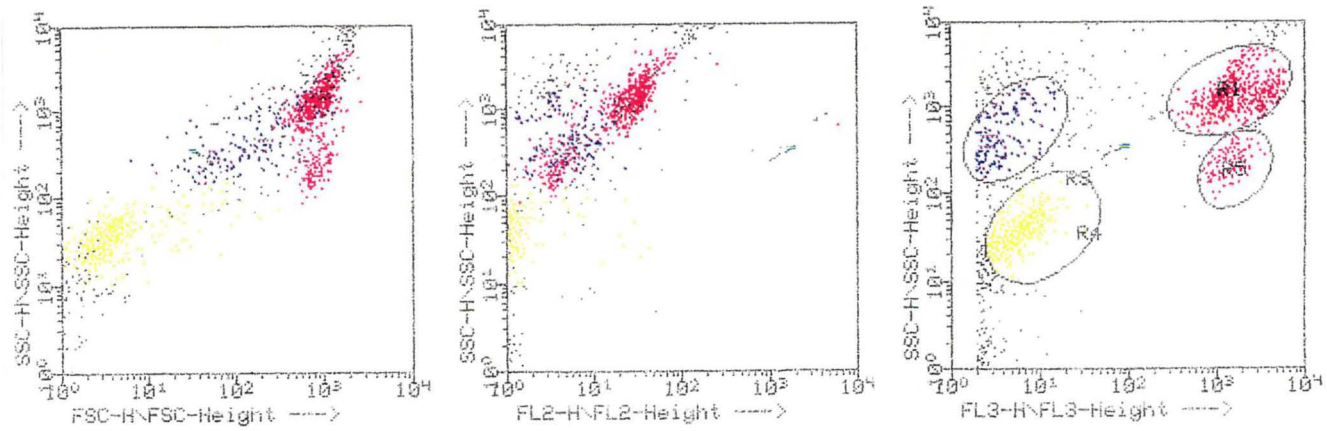


Figure 4.6: Flow cytometric image of a water sample from 19 m in Lake McCallum on the 20th November 1993. Axis are in relative log units. Population R4 was a photosynthetic sulfur bacterium.

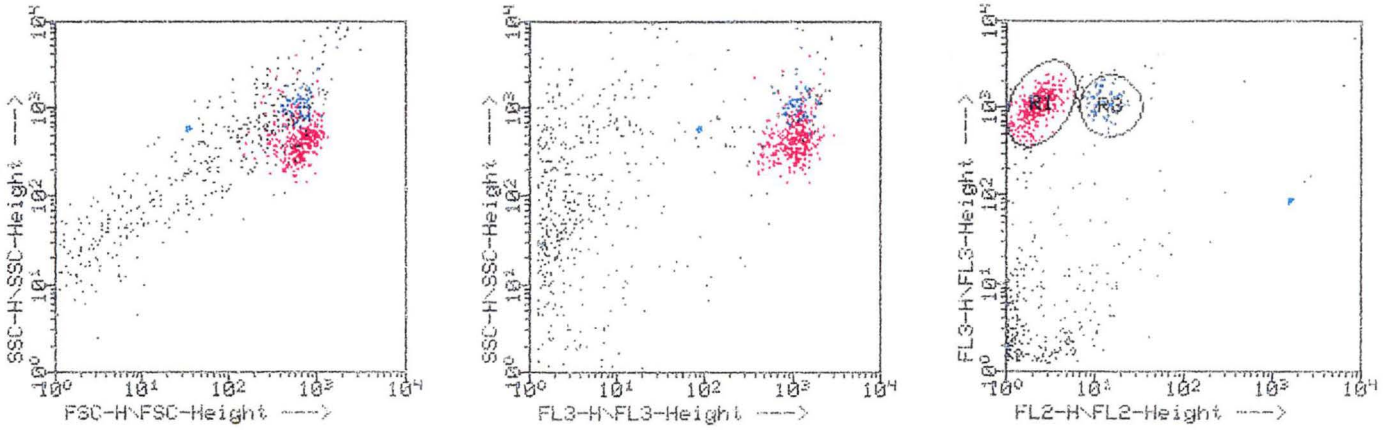


Figure 4.7: Flow cytometric image of a water sample from 30 m in Clear Lake on the 8th December 1992. Axis are in relative log units.

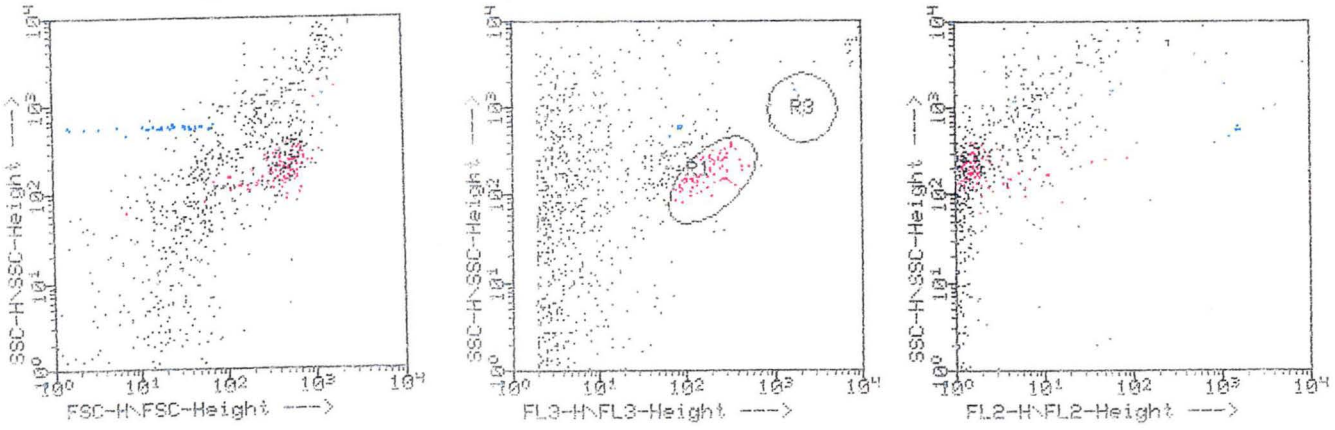


Figure 4.8: Flow cytometric image of a water sample from 6 m in Ekho Lake on the 25th January 1993. Axis are in relative log units.

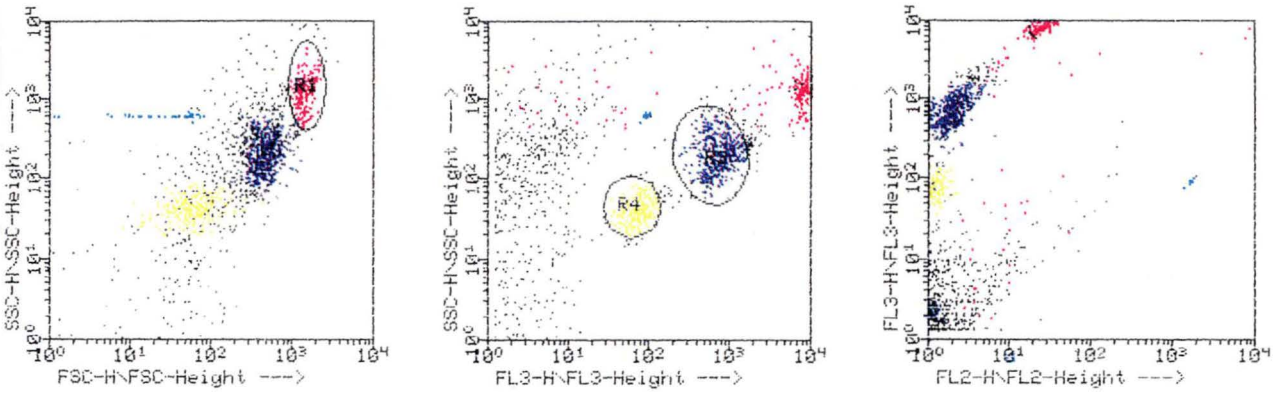


Figure 4.9: Flow cytometric image of a water sample from 6 m in Fletcher Lake on the 10th December 1992. Axis are in relative log units. Population R1 was possibly a Cryptomonad and population R4 was possibly a PNAN.

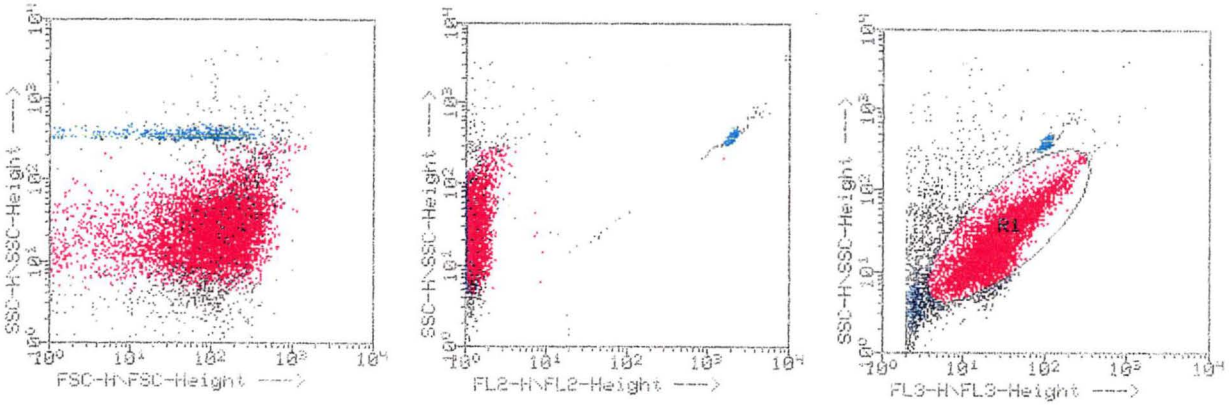


Figure 4.10: Flow cytometric image of a water sample from 12 m in Burton Lake on the 22nd November 1993. Axis are in relative log units. Population R1 was *Chlorobium* spp., a photosynthetic sulfur bacterium (Burke and Burton 1988).

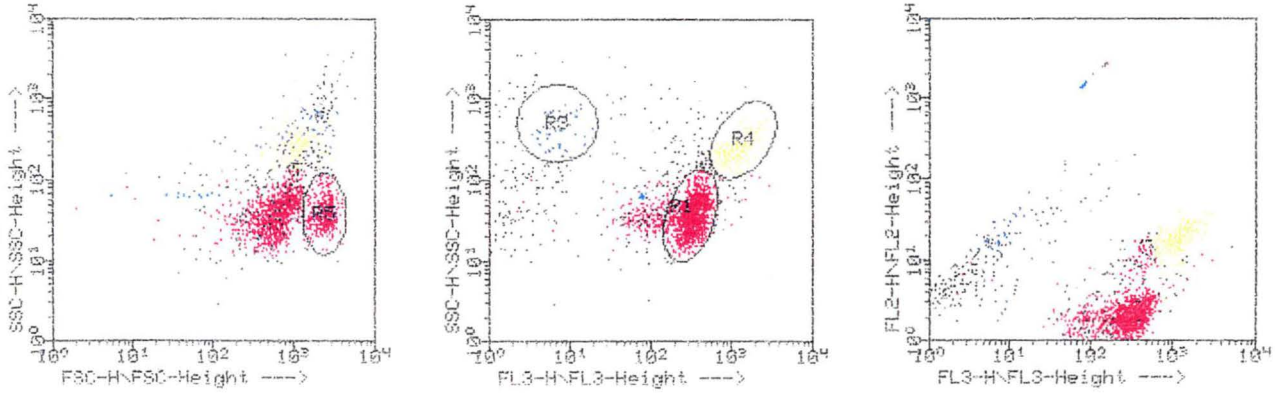


Figure 4.11: Flow cytometric image of a water sample from 1 m in Organic Lake on the 23rd November 1993. Axis are in relative log units. Population R1 was probably the alga *Dunaliella* sp.

4.4.2 Pigment analysis

Samples from Ace Lake, Lake Abraxas, Pendant Lake, Taynaya Bay, Lake McCallum, Burton Lake and the coastal marine site were analysed for the lipid soluble pigment signature of *Synechococcus* (Chapter 5). The *Synechococcus* signature, which is composed of ββ - carotene, chlorophyll - *a*, zeaxanthin and two unknown carotenoids, was detected in Ace Lake, Lake Abraxas and Pendant Lake (Figure 4.12). In Ace Lake the signature was detected in the 8 m, the 10 m and the 11.8 m samples. The signature was not detected in the 4 m sample. The signature was detected in the 5 m, the 10 m and the 11 m samples from Pendant Lake and in the 19 m Lake Abraxas sample. The signature was not detected in the 3 m or the 10 m Lake Abraxas samples nor in any of the other lake, fjord or coastal marine sites that were sampled (Appendix 4).

4.4.3 Isolation of *Synechococcus* from lake water samples

Synechococcus was successfully enriched from Ace Lake, Pendant Lake and Lake Abraxas (Chapter 5). *Synechococcus* was not enriched from any of the other lakes, the fjord site or the coastal marine site.

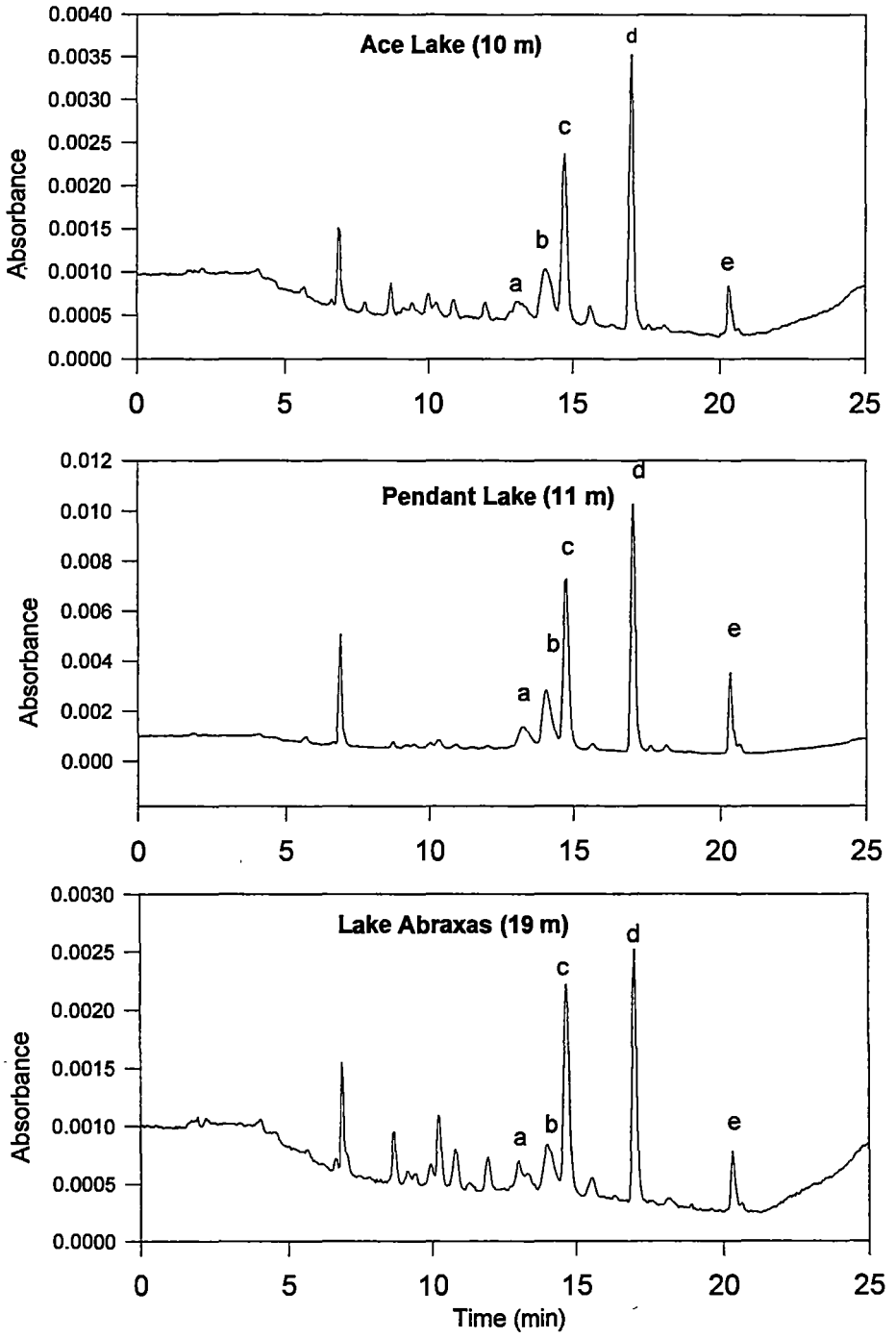


Figure 4.12: Lipid soluble pigment profile of particulates filtered from Ace Lake (10 m), Pendant Lake (11m) and Lake Abraxas (19 m). a = unknown carotenoid; b = unknown carotenoid; c = zeaxanthin; d = chlorophyll *a*; e = ββ - carotene.

4.4.5 Physical characteristics of lakes

There was a broad range of water salinity, temperature and light attenuation with depth amongst the nine meromictic lakes and two marine sites that were surveyed for the presence of *Synechococcus*. Organic lake had the highest salinity, with a range of 105 to 207 g kg^{-1} (Figure 4.13). The temperatures in Organic Lake at the time of sampling were also the lowest of the surveyed sites ranging from -11.5 to -7° C. Light was rapidly attenuated with depth in Organic Lake (Table 4.8). The depth to which 1 % of incident light penetrated (euphotic depth) was less than a metre at a time when there was no ice cover on the lake.

Ekho Lake had the next highest salinity (Figure 4.14). The salinity range in Ekho Lake was between 40 and 150 g kg^{-1}. Although the salinity of Ekho Lake was high, the low temperatures experienced in Organic Lake were not experienced in Ekho Lake. A temperature range of 8° C in the surface water to 18° C at 23 m, at the anoxic/oxic interface, was recorded in January 1993. This was the highest temperature recorded during the survey. At this time the lake was ice free, due to the high salinity and euphotic depth extended to approximately 20 m.

Fletcher Lake had a salinity range of 56 to 102 g kg^{-1} and a temperature range of -2.5 to 4° C (Figure 4.15). The highest temperature and salinity were recorded at the bottom of the lake (8 m). The depth of 1 % I in Fletcher Lake was between 6.5 and 7m (Table 4.8). There was a dense band of photosynthetic sulfur bacteria at the

anoxic/oxic interface in Fletcher Lake as there was throughout Burton Lake. Both Fletcher Lake and Burton Lake still have an occasional connection to the sea. Burton Lake had a salinity of between 43.2 and 43.9 g kg^{-1} and a temperature range of between -2.3 and -1° C (Figure 4.16). The lake was slightly warmer in the surface waters (-1° C) and in the bottom waters (-1.8° C, 16 m). Light attenuated rapidly with depth in Burton Lake (Table 4.8). The euphotic depth was between 2 and 2.5 m. This was probably due to the high densities of photosynthetic sulfur bacteria observed in the lake.

The meromictic basin in Taynaya Bay, had a salinity range of 35 and 53 g kg^{-1} (Figure 4.17). There was a slight salinity gradient between 2 m and 6 m, the salinity was then constant to a depth of 14.8 m. Below this depth there was a sharp increase in salinity to the bottom of the basin at 23 m. There was a temperature range of -1.85° C in the surface waters to -1.35° C at the bottom of the basin. The euphotic depth in Taynaya Bay was at 16 m. There was a band of photosynthetic sulfur bacteria at this depth. The coastal marine site in Davis Bay had a salinity of 34.5 g kg^{-1} and a temperature of -2° C (Figure 4.18). The salinity was slightly less just below the ice, probably due to ice melt. At the time of sampling, light was quickly attenuated at this site. There was a thick cover of compacted snow on the ice and less than 0.5 % of the incident radiation penetrated the snow and ice cover.

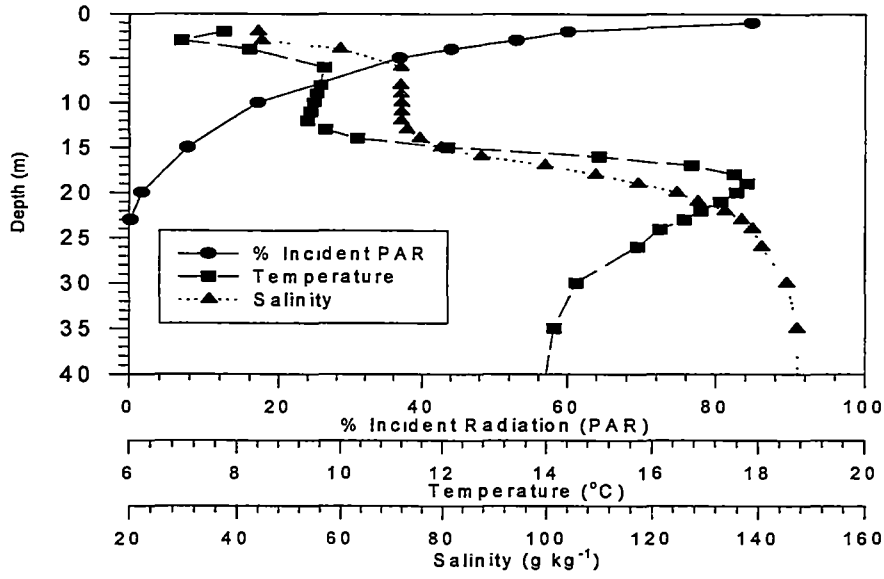


Figure 4.13: Physical characteristics of Organic Lake on the 23rd November 1992.

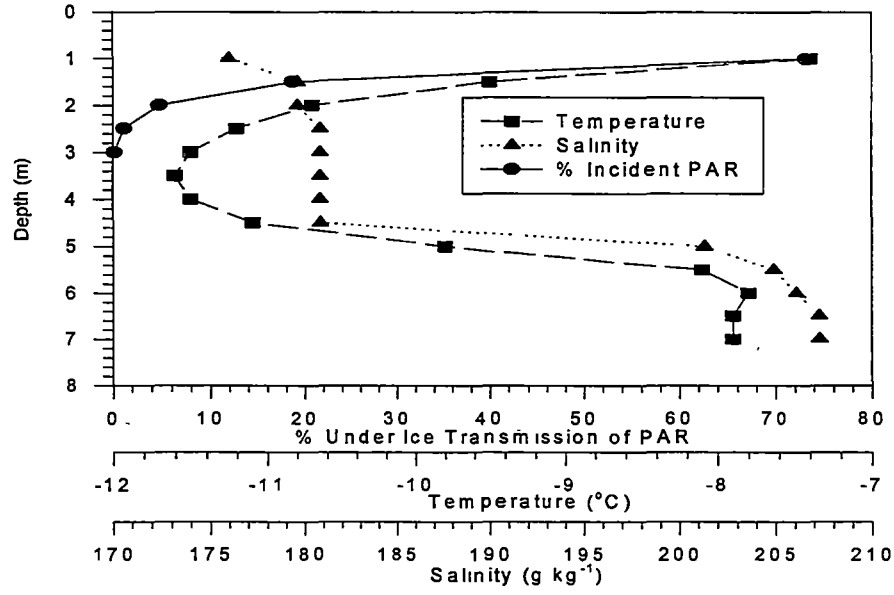


Figure 4.14: Physical characteristics of Ekho Lake on the 25th January 1993.

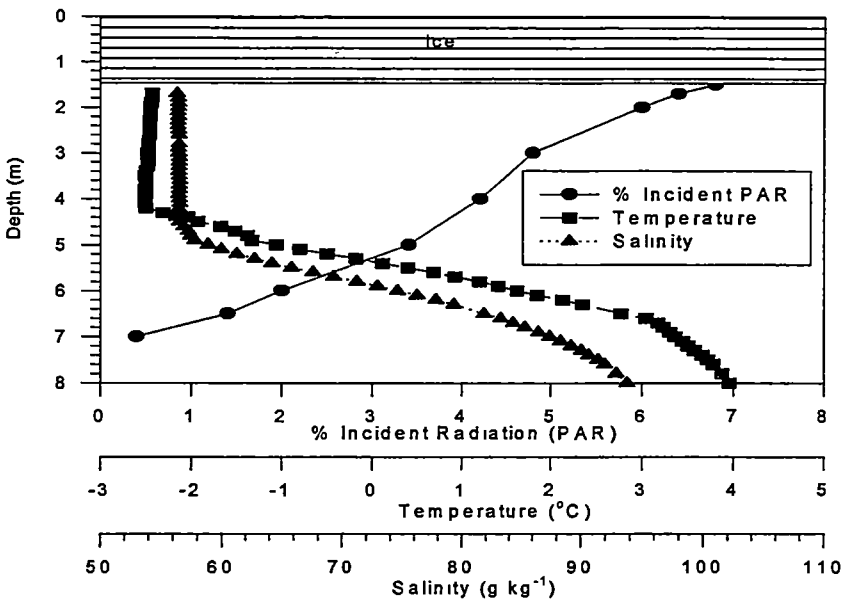


Figure 4.15: Physical characteristics of Fletcher Lake on the 10th December 1992.

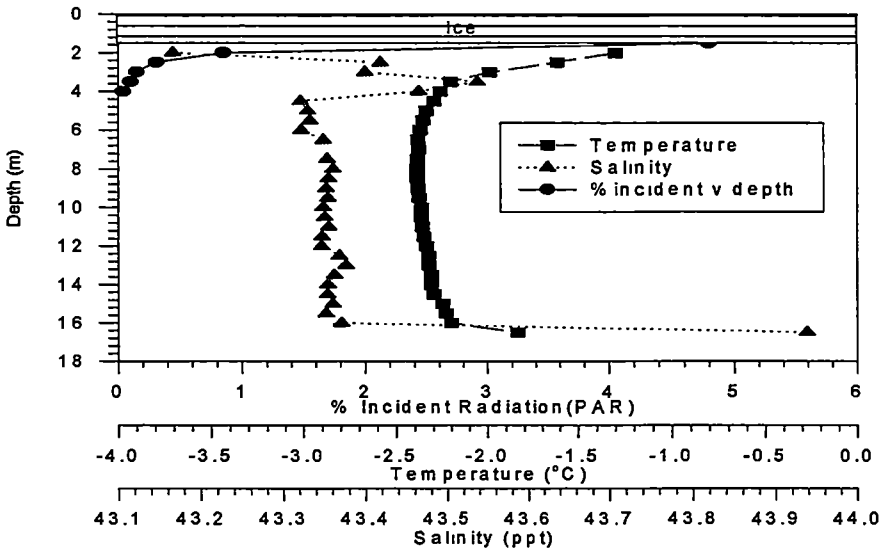


Figure 4.16: Physical characteristics of Burton Lake on the 22nd November 1993.

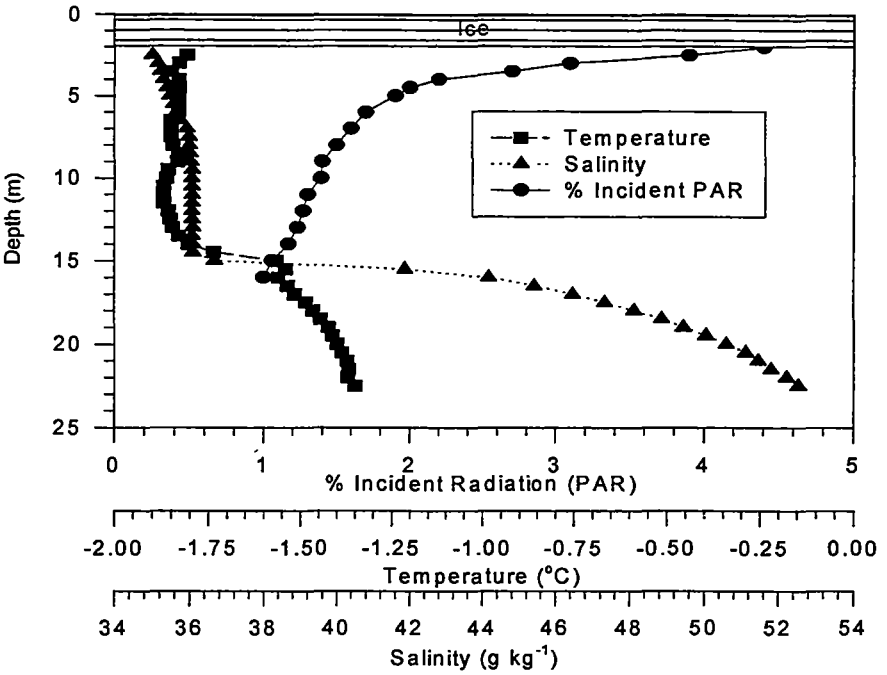


Figure 4.17: Physical characteristics of the meromictic basin in Taynaya Bay on the 26th November 1993.

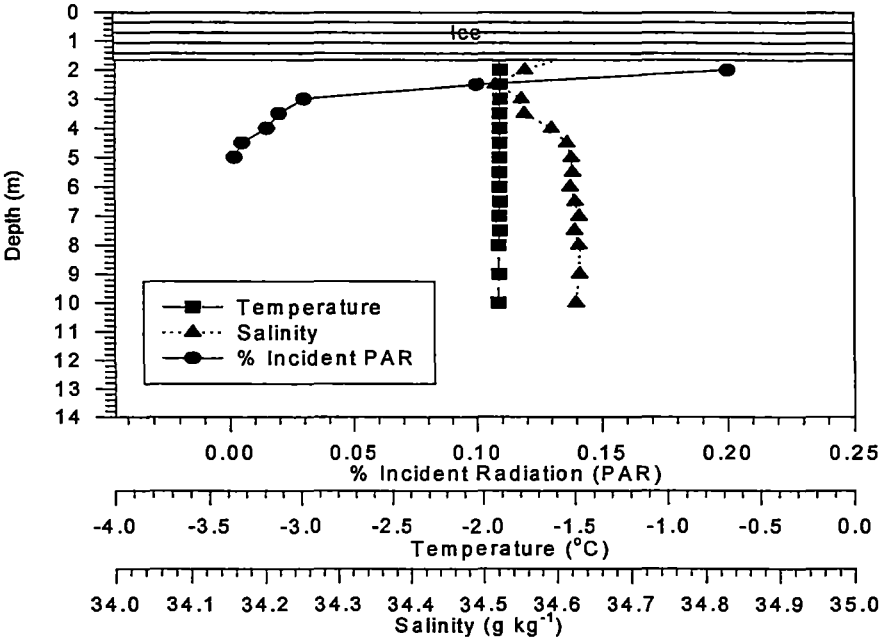


Figure 4.18: Physical characteristics of the marine site in Davis Bay on the 7th November 1993.

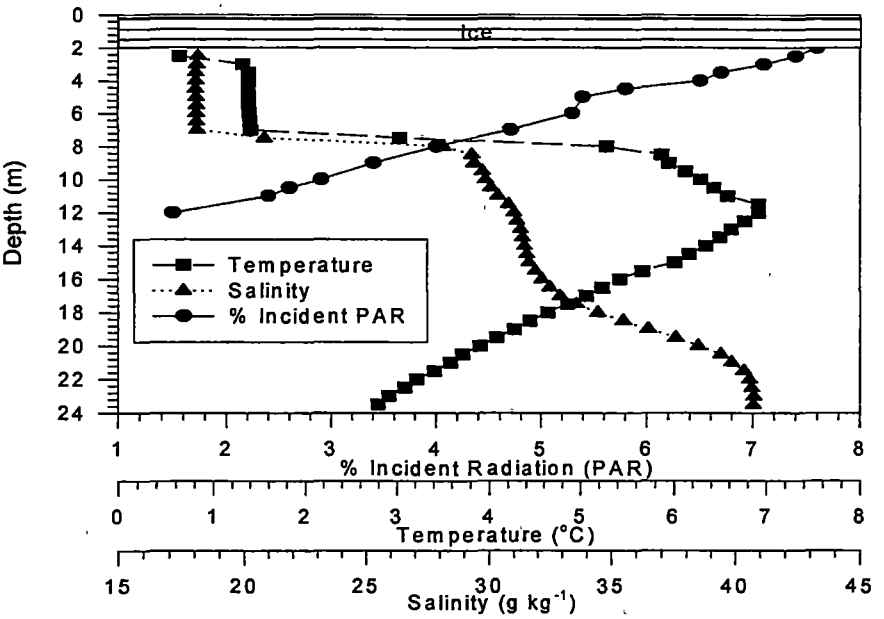


Figure 4.19: Physical characteristics of Ace Lake on the 29th November 1993.

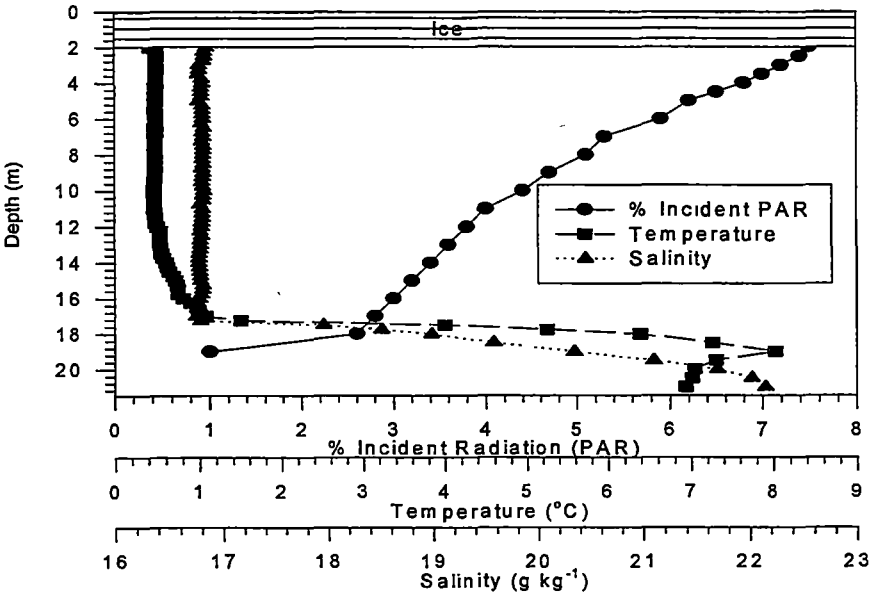


Figure 4.20: Physical characteristics of Lake Abraxas on the 23rd November 1993.

Ace Lake, Pendant Lake and Lake Abraxas contained water of similar salinity. Ace Lake had a salinity range of 18 to 40 g kg^{-1} (Figure 4.19), Lake Abraxas had a salinity range of 16.6 to 22 g kg^{-1} (Figure 4.20) and Pendant Lake had a salinity range of 16 to 48 g kg^{-1} (Figure 4.21). The mixed zone in Pendant Lake (11 m) and Lake Abraxas (17 m) was deeper than it was in Ace Lake (7 m). In Lake Abraxas there was a sharp salinity gradient between 17 m and the bottom of the of the lake at 21 m. The temperature maximum in Lake Abraxas (8° C) was at 19 m. The temperature maximum in Ace Lake (7° C) was at 11 m and in Pendant Lake the temperature maximum (1.6° C) occurred at the bottom of the lake. Each of these lakes had a band of photosynthetic sulfur bacteria at the interface between the anoxic and oxic stratified waters. This was the region in which 1 % euphotic depth was recorded (Table 4.8).

Lake McCallum and Clear Lake had the lowest salinity of the meromictic lakes in the survey, although the salinity of Lake McCallum was similar to that of the mixed zones in Ace Lake, Pendant Lake and Lake Abraxas. Lake McCallum had a salinity range of 14.8 to 24 g kg^{-1} (Figure 4.22). There were two distinct pycnoclines in the lake one at 5 m and the other at 10 m. Below 19 m, there was a gradual increase in salinity. The temperature also increased in discrete steps, reaching a maximum of 7° C at 20 m and then decreasing to 4° C at the bottom of the lake. The euphotic depth in Lake McCallum was at approximately 19 m where there was a band of photosynthetic sulfur bacteria. Clear Lake had a band of photosynthetic sulfur bacteria at a depth of 33 m just below the 1 % euphotic depth (Table 4.8). The temperature range in the lake was 0 to 7.2 ° C with the maximum temperature

occurring at 30 m (Figure 4.23). The salinity range in the lake was 10.32 to 13.6 g kg^{-1}.

Table 4.8: Irradiance (PAR) characteristics of the nine meromictic lakes and two marine sites that were surveyed for the presence of *Synechococcus*. Kd, the extinction coefficient, was calculated from a plot of ln(PAR) versus depth (Kirk 1994); 1% I is the depth (m) to which 1 % of the incident radiation penetrated.

Lake	Date	K_d PAR (m)	1% of I (m)	Ice (cm)	Snow (cm)
Lake Abraxas	23 Nov 93	0.068/1.18	19-20	166	-
Ace Lake	29 Nov 93	0.114/0.355	12	179	-
Burton Lake	22 Nov 93	1.43	2-2.5	150	-
Clear Lake	08 Dec 92	0.092/?	24-30	165	-
Ekho Lake	25 Jan 93	0.192/2.10	10-15	-	-
Lake McCallum	20 Nov 93	0.149/1.5	17	165	-
Pendant Lake	24 Nov 93	0.554/0.061	>13	193	-
Organic Lake	23 Nov 92	2.92	<1	-	-
Fletcher Lake	10-Dec-92	0.19/1.73	6.5-7	150	-
Taynaya Bay	26 Nov 93	0.349/0.048	>16	195	2
Marine Site	07 Nov 93	0.991/0.023	3	179	19

There was no correlation between log (*Synechococcus* cell number +1) and temperature ($r = 0.0814$, $p = 0.6082$, $n = 42$), nor between % incident radiation ($r = -0.1421$, $p = 0.3693$, $n = 42$). The correlation between log (*Synechococcus* cell number +1) and salinity was negative but not significant ($r = -0.2800$, $p = 0.0704$, $n = 42$). A plot of log (*Synechococcus* cell number +1) versus salinity showed that once the salinity exceeded 31.01 g kg^{-1} *Synechococcus* was not present (Figure 4.24). *Synechococcus* was not present in the low salinity samples of Clear Lake and Lake McCallum and preliminary investigations of the salt tolerance of the Ace Lake *Synechococcus* strain indicated that it did not grow as well at low salinity (Chapter 5). Salinities greater than that of seawater were not tested.

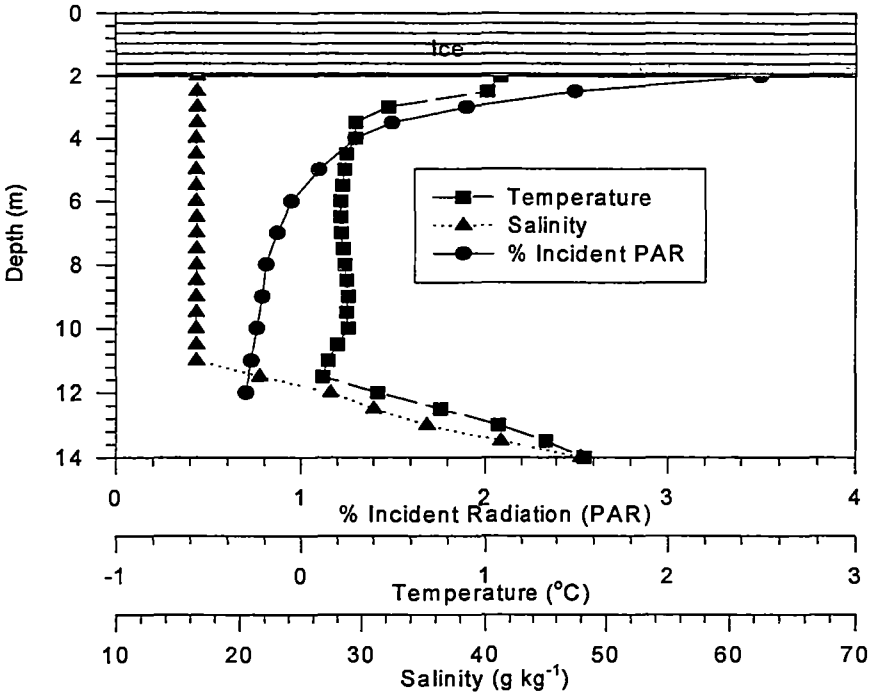


Figure 4.21: Physical characteristics of Pendant Lake on the 24th November 1993.

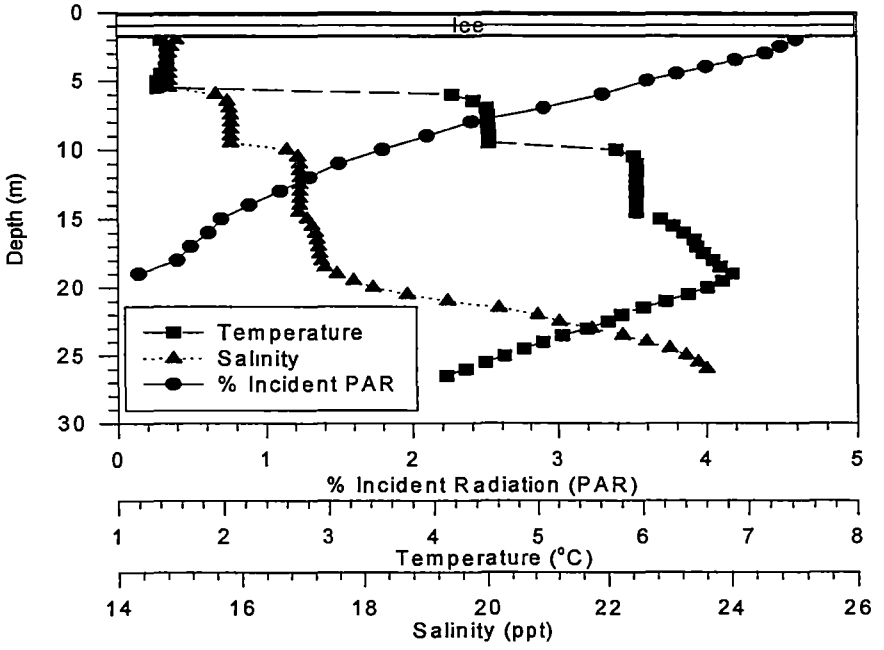


Figure 4.22: Physical characteristics of Lake McCallum on the 20th November 1993.

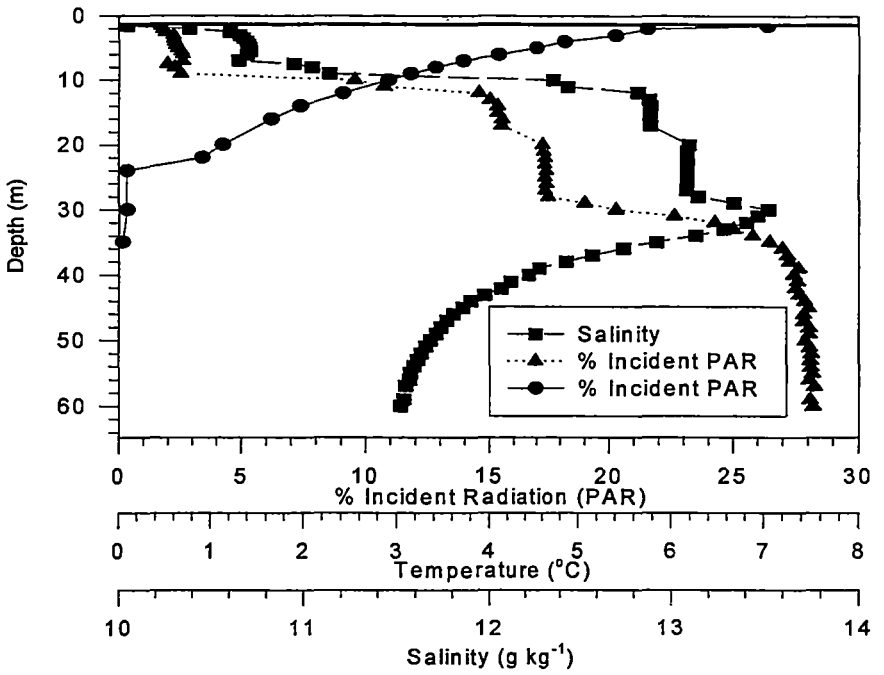


Figure 4.23: Physical characteristics of Clear Lake on the 8 December 1992.

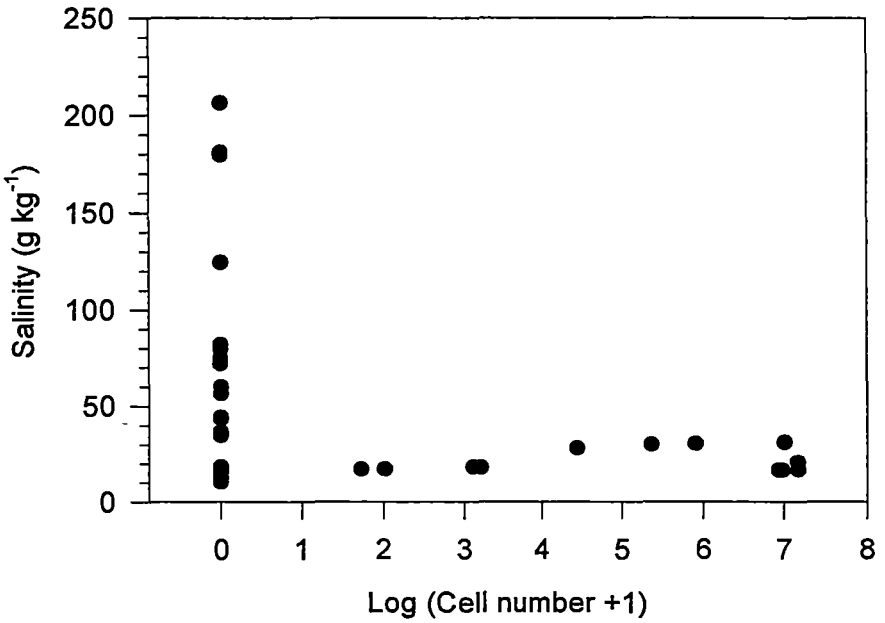


Figure 4.24: Salinity versus Log (*Synechococcus* cell number +1). *Synechococcus* cells were detected in a narrow salinity range.

4.4.6 Nutrient Analysis

A series of nitrate standards were prepared using water of different salinities. This was to determine the effect of salinity on the analytical method of nutrient determination. There was no significant difference in nitrate concentration due to salinity at 2 % NaCl and 5 % NaCl (ANOVA, $p > 0.01$). There was, however, a significant difference in the measured nitrate concentrations at salinities of 3.5 % NaCl and 5 % NaCl (t-test, $p = 0.0002$). The measured concentrations of the standards prepared in 5 % NaCl were 17 % greater than the concentrations prepared in 3.5 % NaCl. As there was not a significant difference between nitrate concentrations prepared in 1 % NaCl and those prepared in 5 % NaCl standard (Mann-Whitney Rank Sum Test, $P = 0.1333$), nor between those prepared in 1 % NaCl and those prepared in 3.5 % NaCl (t-test, $P = 0.7294$), it appears that the difference in nitrate concentrations between the 3.5 % NaCl solution and the 5 % NaCl solution may have been an anomaly and that salinity, in the range 1 % and 5 % does not significantly effect the analysis of nitrate.

The effect of salinity on phosphate determination was more varied. There was a significant effect of salinity on measured phosphate concentration for each of the standards (ANOVA, $p < 0.01$). For the 0.6 μM phosphate standard the two low salinities (1 % and 2.5 %) were 7 % higher than the 3.5 % and the 5 % salinity. There was no significant difference in measured phosphate concentrations between the 1 % and the 2.5 % salinity (t-test, $p = 0.6828$) nor between the 3.5 % and the 5 % salinity (Mann- Whitney Rank Sum Test, $p = 0.2695$). At phosphate concentrations of 1.4 μM, 2.1 μM and 2.8 μM the two low salinities had slightly higher phosphate

concentrations than the two high salinities (5 % at 1.4 μM PO_4^{2-}, 3 % at 2.1 μM PO_4^{2-} and 4 % at 2.8 μM PO_4^{2-}. At 3.5 μM PO_4^{2-} there was no significant difference between the two low salinities (Mann-Whitney Rank Sum test, P = 0.05542) nor between the 1 % and the 3.5 % salinity (P = 0.0993). The was a small (1 %) but significant (P = 0.0034) difference between the 2.5 % and the 3.5 % NaCl solutions and a 21% decrease in the PO_4^{2-} concentration at 5 % NaCl. It therefore appears that phosphate measurements are affected to a larger degree at higher phosphate concentrations (3.5 μM) and at high salinities (5 % and probably greater).

The samples measured for phosphate and nitrate concentrations all had salinities below 5 % and above 1 % and the effect of salinity on the measurement would, therefore, not have been significant. Nitrate concentrations were variable (Table 4.9). In the euphotic zones of the meromictic lakes the nitrate concentrations were usually below the limit of detection (0.02 μM). The marine site and the fjord site had high nitrate concentrations relative to the meromictic lakes. There was a wide range of phosphate concentrations, both within the meromictic lakes and between the meromictic lakes and the marine sites. The highest concentrations were recorded at the bottom of the euphotic zone in Ace Lake (8.77 μM) and in Taynaya Bay (14.36 μM) and also throughout the water column in Burton Lake (10 - 27 μM). There was no correlation between phosphate concentration and *Synechococcus* cell numbers (r = -0.1776, p = 0.3659, n = 28), nor between nitrate concentration and *Synechococcus* cell numbers (r = - 0.3698, p = 0.0527, n = 28).

Dissolved organic carbon (DOC) concentrations were measured in seven of the eleven sites that were surveyed for the presence of *Synechococcus*. These seven sites had a broad range of physical characteristics. Dissolved organic carbon ranged in concentration from 0.58 mM in Ace Lake and McCallum Lake down to approximately 0.17 mM in the marine sites and Burton Lake (Table 4.9). There was no correlation between *Synechococcus* cell numbers and DOC in these lakes. Heterotrophic bacterial numbers ranged from 10^4 cells ml^{-1} to 10^7 cells ml^{-1} (Table 4.9). There was a positive correlation ($r = 0.4951$, $p = 0.001$, $n = 41$) between log(*Synechococcus* cell number +1) and log(heterotrophic bacteria cell number).

4.5 Discussion

The advantages and disadvantages of using flow cytometric methods for the analysis of phytoplankton communities has been discussed in Chapter 2. In this instance the flow cytometer was effective in locating *Synechococcus* populations in three out of eleven sites sampled. The presence of *Synechococcus* in Lake Abraxas, Pendant Lake and Ace Lake was confirmed by lipid soluble pigment analyses of the lake water samples and by presence of *Synechococcus* in enrichment cultures from these three lakes. The morphological and fluorescence characteristics of the Ace lake *Synechococcus* had been confirmed by microscopy (Chapter 3). The flow cytometric image of *Synechococcus* from Pendant Lake and Lake Abraxas was the same as the image of the population in Ace Lake. Unlike *Synechococcus* and also the photosynthetic bacterium *Chlorobium* spp., other phytoplankton populations that appeared on the flow cytometric images could not be easily identified. The phytoplankton community in Ace Lake and Organic Lake have been studied (Burch,

Table 4.9: *Synechococcus* and heterotrophic bacteria cell numbers, temperature, salinity, light and nutrient characteristics at selected depths in nine meromictic lakes, a fjord site and a coastal marine site in the Vestfold Hills, Antarctica. '-' = not determined, 'N.D.' = not detected and 'B.L.D.' = below the limit of detection.

Lake	Depth (m)	Cell Number (cells ml^{-1})	Temperature (°C)	Salinity (g kg^{-1})	% of incident radiation	Phosphate (μM)	Nitrate (μM)	DOC (mM)	Heterotrophs cells ml^{-1}
Ace Lake	4	1300	1.38	18.07	6.45	0.263	B.L.D.	0.45	6.31E+05
	6	1610	1.39	18.09	5.27	0.243	B.L.D	0.54	8.30E+05
	8	2.83E+04	5.28	28.15	3.95	5.05	B.L.D.	0.60	1.27E+06
	10	2.31E+05	6.28	29.85	2.9	5.73	B.L.D	0.61	5.37E+06
	11	8.07E+05	6.58	30.35	2.37	8.773	B.L.D	0.64	5.32E+06
Lake Abraxas	3	5.26E+01	0.49	16.78	7.23	0.065	B.L.D	0.26	1.21E+05
	10	1.04E+02	0.46	16.82	4.39	0.064	B.L.D	0.30	1.03E+05
	15	-	0.72	16.79	3.16	0.07	B.L.D	0.34	1.05E+05
	19	1.48E+07	8.03	20.35	0.99	0.204	0.209	0.27	2.40E+05
Lake McCallum	5	N.D.	1.37	14.84	3.6	0.089	B.L.D	0.54	4.25E+04
	10	N.D.	5.75	16.75	1.8	0.089	B.L.D	0.57	3.48E+04
	12	N.D.	5.94	16.97	1.26	0.111	B.L.D	0.62	5.10E+04
	15	N.D.	6.16	17.08	0.71	0.089	B.L.D	0.56	7.31E+04
	19	N.D.	6.85	17.57	0.14	0.096	B.L.D	0.62	4.86E+04
	20	N.D.	6.61	18.16	0.12	0.134	B.L.D	0.60	8.58E+04
Pendant Lake	5	8.71E+06	0.24	16.5	1.06	0.8	B.L.D	0.43	6.44E+06
	10	9.63E+06	0.26	16.5	0.76	0.815	B.L.D	0.33	6.26E+06
	11	1.50E+07	0.15	16.55	0.73	0.858	B.L.D	0.39	6.67E+06
	12.8	1.02E+07	0.76	31.01	0.68	1.3445	0.364	0.44	4.80E+06
Taynaya Bay	3	N.D.	-1.85	35.15	3.13	0.927	5.105	0.20	6.90E+05
	5	N.D.	-1.83	35.46	1.9	0.88	8.565	0.17	5.19E+05
	10	N.D.	-1.86	36.05	1.39	1.962	21.9	0.17	5.03E+05
	15	N.D.	-1.57	36.66	1.06	3.147	21.7	0.15	4.62E+05
	16	N.D.	-1.56	44.17	0.99	14.355	17.725	0.17	8.06E+05

Table 4.9 continued:

Lake	Depth (m)	Cell Number (cells ml^{-1})	Temperature (°C)	Salinity (g kg^{-1})	% of incident radiation	Phosphate (μM)	Nitrate (μM)	DOC (mM)	Heterotrophs cells ml^{-1}
Coastal Marine	5	N.D.	-1.91	34.61	0.09	2.04	14.88	0.14	8.58E+04
Burton Lake	4	N.D.	-2.26	43.47	0.04	17.358	0.945	0.20	-
	8	N.D.	-2.39	43.36	0.01	27.28	1.089	0.22	1.15E+06
	12	N.D.	-2.33	43.35	0.01	10.48	0.591	0.21	1.58E+06
Clear Lake	5	N.D.	1.42	10.32	16.96	-	-	-	9.43E+04
	15	N.D.	5.77	12.04	6.8	-	-	-	1.17E+05
	25	N.D.	6.17	12.31	1.8	-	-	-	1.00E+05
	30	N.D.	7.04	12.7	0.35	-	-	-	1.13E+05
Ekho Lake	4	N.D.	8.23	59.84	44	-	-	-	7.06E+04
	10	N.D.	9.48	71.94	17.1	-	-	-	3.55E+05
	15	N.D.	12.09	79.62	7.6	-	-	-	3.28E+05
	20	N.D.	17.587	124.65	1.7	-	-	-	5.85E+06
Organic Lake	2	N.D.	-10.7	179.6	2.3	-	-	-	5.30E+06
	4	N.D.	-11.5	180.85	0.02	-	-	-	5.10E+06
	6	N.D.	-7.8	206.11	0.02	-	-	-	1.36E+07
Fletcher Lake	2	N.D.	-2.45	56.44	6	-	-	-	1.97E+05
	6	N.D.	1.62	74.57	2	-	-	-	4.51E+05
	6.5	N.D.	2.77	81.81	1.4	-	-	-	2.33E+06

1988; Franzmann et al. 1987; van den Hoff and Franzmann 1986) and the flow cytometric populations could therefore be identified. The phytoplankton communities of the other lakes and marine sites are poorly characterised. Morphological and fluorescence characteristics of other populations could be described, but in order for the populations that appeared on the flow cytometric images to be positively identified, a thorough microscopic investigation of these lakes and marine sites is needed.

Synechococcus occurred in high densities in the three lakes in which it was identified. It is possible that *Synechococcus* occurred at some of the other sites but in numbers too low to be detected by flow cytometry. Walker and Marchant, (1989) observed *Synechococcus* in coastal marine samples from Davis Bay. The greatest densities of *Synechococcus* occurred at this site in August (140 cells ml^{-1}). Over summer, numbers were less than 10 cells ml^{-1} (Walker and Marchant 1989). Only one millilitre of water was used in flow cytometric analysis. It is possible that if large volumes of water were filtered for fluorescent microscopic observation, *Synechococcus* may have been detected in samples from sites such as Taynaya Bay, Lake McCallum and Clear Lake. The absence of *Synechococcus* in enrichment cultures from these sites and the absence of the *Synechococcus* lipid soluble pigment signature in samples from these lakes confirmed that *Synechococcus* were not a dominant part of the phytoplankton community in these lakes and marine sites.

Synechococcus has been reported in a small number of Antarctic saline lakes but never at densities that occurred in Ace Lake, Pendant Lake and Lake Abraxas. Goldman et al., (1967) reported that *Synechococcus* occurred between 36 and 40 m in Lake Vanda, Dry Valleys. *Synechocystis* rather than *Synechococcus* was reported to

occur at the DCM (50 m) in Lake Vanda (Vincent and Vincent 1982). *Synechococcus* was also reported to occur in Moss Lake, Alexander Island (Heywood 1977). Spaulding et al., (1994) reported *Synechococcus* to occur in Lake Fryxell in 1991 and 1992 but not in the three preceding years. *Synechococcus* cell densities were not reported but "at least one cell was observed in the counting chamber". *Synechococcus* does not dominate the phytoplankton in Lake Fryxell but instead the filamentous cyanobacterium *Oscillatoria* dominates at the DCM (McKnight et al. 1993; Spaulding et al. 1994). Finally, Parker and Wharton, (1985) reported that *Synechococcus* occurred in the algal mat community of Lake Hoare, Dry Valleys. These lakes have salinity ranges similar to Ace Lake, Pendant Lake and Lake Abraxas. Based on the formula of Fofonoff and Millard, (1983), that converts conductivity to salinity, and using the data of Vincent et al., (1981) Lake Vanda has a salinity range of approximately 0.5 g kg^{-1} to 60 g kg^{-1}. Between 36 m and 40 m the salinity is approximately 1.8 g kg^{-1}. Using the same formula and based on conductivity data from Matsumoto et al., (1989), Lake Fryxell has a salinity range of approximately 1.1 to 4 g kg^{-1}. Moss Lake has a salinity of approximately 3.3 g kg^{-1} which makes it less saline than Ace Lake. Nutrient availability and light intensity are thought to control phytoplankton production in Lake Vanda and Lake Fryxell (Vincent 1981; Vincent and Vincent 1982). It is possible that, in the saline meromictic lakes *Synechococcus* has a competitive advantage in a salinity range of 15 to 35 g kg^{-1}.

Marine *Synechococcus* spp. are phylogenetically distinct from strains of *Synechococcus* isolated from freshwater environments (Mullins et al. 1995; Urbach et al. 1992) and the strains isolated from the meromictic lakes in Antarctica were closely related to other marine strains (Chapter 5). *Synechococcus* has been detected,

in low densities, in some freshwater Antarctic Lakes. Andreoli et al., (1992) found *Synechococcus* in low numbers (40 cells ml^{-1}) in lakes in northern Victoria Land. They predicted that nutrients or temperature might limit the growth and cell density of picoplankton in these lakes. Tell et al., (1995) reported that *Synechococcus* occurred in four lakes in Hope Bay. Cell densities were not given but the lakes ranged in trophic status from oligotrophic to eutrophic. The main focus of Tell's research was to determine the origin of phytoplankton in the freshwater lakes of the Hope Bay region. It was thought that the most likely origin of phytoplankton in these lakes was freshwater temperate lakes and that aerosol dispersion was the main mechanism for colonisation. Laybourn-Parry and Marchant, (1992), in a survey of freshwater lakes in the Vestfold Hills found chroococcalean cyanobacteria in numbers of less than 50 ml^{-1}. These organisms were not identified but it was proposed that they belong to the genus *Synechocystis*. It is however, possible that the picocyanobacteria were *Synechococcus* cells. Observation of cells undergoing cell division would clarify the identification. *Synechococcus* divide in one plain only where as *Synechocystis* divide in two plains (Waterbury and Rippka 1989). It was also proposed that phytoplankton cell growth in the freshwater lakes of the Vestfold Hills was nutrient limited (Laybourn-Parry and Marchant 1992) and that the grazing pressure was low (Laybourn-Parry et al. 1995). It would be of interest to isolate *Synechococcus* from nearby freshwater environments and to determine the phylogenetic relatedness of the meromictic lake *Synechococcus* strains to freshwater strains.

High *Synechococcus* cell densities have been reported from the maritime Antarctic lakes of Signy Island (Hawes 1990). The number detected in 'The Wallows' (1.4 x 10^8 cells ml^{-1}) was 10 fold higher than the densities of marine *Synechococcus* found

in the meromictic lakes in the Vestfold Hills. This lake is eutrophic (13.7 mg l^{-1} total nitrogen and 0.8 mg l^{-1} total phosphorus) and extremely turbid. Other freshwater lakes in the area had *Synechococcus* cell numbers ranging from 4×10^4 to 1.5×10^6 cells ml^{-1}. Trophic status was thought to be a controlling factor in *Synechococcus* cell densities in these lakes. *Synechococcus* also dominated freshwater lakes in the Arctic (Vezina and Vincent 1997). In these systems, nutrient concentrations were consistently low.

Stockner, (1991) has summarised the distribution and abundance of autotrophic picoplankton, (APP), dominated by *Synechococcus*, in a range of freshwater lakes in New Zealand, Canada and throughout Europe. Cell densities of between 10^4 and 10^5 cells ml^{-1} were common. Cell densities were correlated with trophic status, the highest densities occurred in mesotrophic systems. The contribution of *Synechococcus* to production and biomass was, however, greatest in the ultraoligotrophic lakes. This was primarily due to the absence of larger phytoplankton species (Stockner 1991). It was predicted that in the freshwater lakes, the role of grazing in controlling APP densities was not as important as the role of nutrient concentrations. Peak densities of 7×10^5 cells ml^{-1} were detected in Lake Ontario, Canada with the greatest densities occurring in the surface waters (Caron et al. 1985). *Synechococcus* contributed to approximately 38 % of the total primary productivity during times of peak abundance. *Synechococcus* cell numbers were correlated with water temperature ($r = 0.89$). Temperature may not, however, have been the controlling factor in determining abundance. Although the grazing pressure was not determined, *Synechococcus* cells were observed in the food vacuoles of heterotrophic microflagellates. The control on *Synechococcus* distribution and abundance by grazing needs greater attention in both freshwater and saltwater lakes.

In Lake Constance, the growth rate of autotrophic picoplankton and the grazing rate on the organisms were relatively equal (Weisse 1988). Growth rates of between 0.006 and 0.051 h^{-1} and grazing rates of between 0.002 and 0.053 h^{-1} were recorded. Cilliates, heterotrophic nanoflagellates and rotifers were identified as the dominant grazers in Lake Constance (Weisse 1988). Voros et al., (1991) reported that nitrogen availability and grazing by zooplankton significantly effect the abundance of *Synechococcus* in freshwater lakes in Hungary.

The distribution of *Synechococcus* within the meromictic lakes of the Vestfold Hills was consistent with the distribution of this organism in meromictic lakes in other regions of the world. As occurs in the three meromictic lakes in the Vestfold Hills, *Synechococcus* occurred in the highest density at the DCM of temperate and tropical meromictic lakes. Jellyfish Lake is a tropical marine meromictic lake in Palau, Pacific Ocean. The lake had a salinity range of 26 to 31 g kg^{-1} (Venkateswaran et al. 1993), was 30 m deep and the anoxic/oxic interface was at 14 m. Below this depth there were high concentrations of hydrogen sulfide. *Synechococcus* densities of between 10^4 and 10^6 cells ml^{-1} were detected in this lake and the peak occurred just above the anoxic/oxic interface as occurred in the Antarctic meromictic lakes. The most striking difference between the Antarctic lakes and Jellyfish Lake was in the water temperature. Jellyfish Lake had a water temperature between 27° C and 31° C. It is less surprising that *Synechococcus* cell numbers are high in this lake as growth rate would be higher (Chapter 5).

Little Round Lake in southeastern Ontario, Canada was a 16 m deep meromictic lake with a salinity of approximately 0.14 to 0.53 g kg^{-1} (Craig 1987). The lake was mesotrophic with respect to phosphate concentration and oligotrophic to mesotrophic

with respect to phytoplankton and zooplankton densities. The lake had a temperature of approximately 24° C in the surface waters over summer but could be ice covered in the winter. Below 12 m the temperature was between 4° C and 5° C. There was a band of purple sulfur bacteria at the anoxic/oxic interface. This differs from the meromictic lakes in the Vestfold Hills that were studied in this survey. Seven of the nine lakes had a band of green photosynthetic sulfur bacteria at the anoxic/oxic interface. Watts Lake, a freshwater lake in the Vestfold Hills, is known for its purple sulfur bacteria (Heath 1988). At 10 m in Little Round Lake, the light intensity was 1 % of surface irradiance but below this there was almost complete light extinction (Craig 1987). *Synechococcus* occurred in high numbers below 9 m in Little Round Lake. The greatest densities occurred in Autumn just above the chemocline when densities of 2.5×10^6 cells ml^{-1} were measured. *Oscillatoria*, the fillamentous cyanobacterium that has been observed in Lake Fryxell, Dry Valleys (Spaulding et al. 1994), was observed in Little Round Lake. Its seasonal distribution differed from *Synechococcus* in that the greatest densities of *Oscilatoria* seemed to occur when the densities of *Synechococcus* were low (Craig 1987).

Two out of four saline meromictic lakes in Å land, Finland, had populations of *Synechococcus* at the bottom of the euphotic zone (1 % surface irradiance). In Inre Verkviken, *Synechococcus*-like cells were found in densities of 0.8×10^6 cells ml^{-1}. The two lakes in which *Synechococcus* was not present were eutrophic shallow lakes that had high densities of larger phytoplankton species (Craig 1987). It was proposed that algal competition and nutrient concentration controlled the distribution of *Synechococcus* in these meromictic lakes. From these accounts of *Synechococcus* abundance and distribution in both fresh and saltwater environments, it appears that *Synechococcus* is adapted to a wide range of physical and chemical conditions.

The correlation between *Synechococcus* densities, in the meromictic lakes of the Vestfold Hills, and a number of physical, chemical and biological factors was tested. The correlation between *Synechococcus* numbers and heterotrophic bacterial numbers was the strongest. This possibly reflects a tight coupling between production and consumption in these lakes. It could however mean that the conditions that favour the growth of *Synechococcus*, for example reduced grazing pressure, also favour the growth of heterotrophic bacteria.

The weak negative correlation between *Synechococcus* numbers and salinity helps to explain why *Synechococcus* may not occur in the more saline meromictic lakes. It does not, however, explain why *Synechococcus* was not observed in the coastal marine or fjord sites. The Ace Lake *Synechococcus* is capable of growth in open ocean sea water (Chapter 5). The nitrate concentration at the marine sites was higher than it was in Ace Lake, Lake Abraxas and Pendant Lake. It is possible that competition with other phytoplankton species restricted the growth of *Synechococcus* at the sea water sites. Nanoplankton are more dominant than picoplankton in the Southern Ocean (Weber and El-Sayed 1987) and it is possible that the same holds for coastal marine sites.

No correlation was found between *Synechococcus* numbers, temperature, light intensity or nutrient concentrations. Unfortunately, the density and distribution of potential grazers was not measured in this survey so no comment can be made on a correlation between *Synechococcus* distribution and grazing pressure. Heterotrophic nanoflagellates (HNAN), the grazers that are thought to be the greatest consumers of *Synechococcus* (Estep et al. 1986; Hall et al. 1993; Johnson et al. 1982), occurred in

both Ace Lake and Lake Abraxas (Laybourn-Parry and Perriss 1995). It is probable that HNAN also occur in Pendant Lake and that *Synechococcus* is grazed by the HNAN in all of these lakes. In a survey of *M. rubrum* in brackish and saline lakes of the Vestfold Hills (Perriss et al. 1995) indicated that salinity and water column temperature appeared to be the most important factors controlling the distribution and abundance of this organism. They found that *M. rubrum* was not present in the hypersaline lakes (salinity greater than 3.5 %) and that the greatest densities occurred in the brackish lakes. This holds for the distribution of *Synechococcus*. (Perriss et al. 1995) found that there was no correlation between *M. rubrum* numbers and numbers of potential grazers. This may not be true for *Synechococcus*. *M. rubrum* is a large organism (approximately 20 μm diameter) and may not be under the same grazing pressure as the 0.5 μm diameter *Synechococcus* cells.

Perriss et al., (1995) also found no correlation between *M. rubrum* numbers and nutrient concentrations. In Ace Lake and Lake Abraxas, *M. rubrum* occurred in greatest densities in the mixolimnion where nitrate concentrations were low. In contrast, *Synechococcus* occurred in greatest numbers below the pycnocline, just above the layer of *Chlorobium* spp.. Nutrients diffusing from the anoxic zone in the meromictic lakes are thought to be responsible for the occurrence of the DCM in these lakes (Vincent 1988; Vincent and Vincent 1982). Nutrient diffusion from the anoxic zone may promote the growth of *Synechococcus* in this region. In Pendant Lake, there was a fairly even distribution of *Synechococcus* throughout the euphotic zone. This is probably because, in Pendant Lake, the mixolimnion extended almost to the interface with the anoxic zone and water from the mixed zone comes into contact with the nutrient rich anoxic waters. In Lake Abraxas, there was deep mixing but there was a sharp pycnocline between 17 m and 18 m that isolated the

mixolimnion from the nutrient rich anoxic waters. The nitrate concentration in the mixed zone of Pendant Lake was less than 0.02 μM. It is probable that, in this region, nutrients are rapidly recycled.

Ace Lake, Pendant Lake and Lake Abraxas are all in close proximity on Long Peninsula. Pendant Lake and Lake Abraxas would have become isolated from the marine environment in the same way that Ace Lake was isolated (Bird et al. 1991). Ace Lake and Lake Abraxas would have become isolated from Long Fjord where as Pendant Lake would have been isolated from Taynaya Bay. It is probable that *Synechococcus* was part of the phytoplankton community of the fjords at the time of isolation and that stability of the lake environments has favoured the proliferation of *Synechococcus*. It is thought that *M. rubrum* became part of the lake phytoplankton community in the same way (Laybourn-Parry and Perriss 1995). It would be of value to isolate *Synechococcus* from the nearby marine environment and compare its phylogeny to that of the three lake isolates.

The distribution and abundance of *Synechococcus* in meromictic, monomictic, saltwater and freshwater lakes is probably controlled by number of physical, chemical and biological factors. In Ace Lake, Lake Abraxas and Pendant Lake the densities of marine *Synechococcus* are greater than has previously been reported. In Lake Abraxas, as in Ace Lake (Chapter 3) *Synechococcus* would contribute significantly to primary production below the pycnocline. In Pendant Lake, *Synechococcus* would probably contribute significantly to primary production throughout the euphotic zone. As Vincent, (1998) indicated for cyanobacteria in polar regions, the distribution and abundance of *Synechococcus* would be controlled by a balance between rates of gain, for example faster growth rates at warmer

temperatures, and loss rates, for example grazing by heterotrophs or nutrient limitations. In the meromictic lakes of Finland, trophic status and competition with other phytoplankton species appeared to control which lakes *Synechococcus* dominated. In the meromictic lakes of the Vestfold Hills, it appears that salinity may limit the distribution of *Synechococcus*. This is a probable explanation for the absence of *Synechococcus* in Burton Lake, Fletcher Lake, Ekho Lake and Organic Lake. This could be verified in the laboratory using *Synechococcus* isolated from Ace Lake, Pendant Lake and Lake Abraxas (Chapter 5). In meromictic lakes of Antarctica, northern polar regions and in the tropics, the vertical distribution of *Synechococcus* is similar with the maximum cell density at the DCM just above the anoxic/oxic interface (Craig 1987; Venkateswaran et al. 1993). In the oligotrophic lakes, it is probably nutrient availability that controls this distribution however the reduced light intensity and warmer temperatures experienced at the DCM may also influence the distribution and abundance. The importance of grazing in the control of *Synechococcus* densities in the meromictic lakes in the Vestfold Hills remains to be determined.

Chapter 5

Phenotypic and phylogenetic characterisation of picocyanobacteria strains from moderately saline lakes of the Vestfold Hills.

5.1 Abstract

Cultures of *Synechococcus* were enriched from Ace Lake in December 1992, from Pendant Lake in January 1993 and a from Lake Abraxas in November 1993. The cultures contained no other photosynthetic organisms but were not axenic. Throughout this chapter these unicyanobacterial cultures are referred to as strains. The three Antarctic strains of *Synechococcus* are now in the algal culture collection of the CSIRO Marine Laboratories, and await strain number designation.

Morphologically, the *Synechococcus* cells from Ace Lake, Pendant Lake and Lake Abraxas were similar to one another, and to other strains in this group. Cells were similar in size (0.91-1.08 μm wide), fluorescence characteristics, *in-vivo* absorption and emission spectra and lipid soluble pigment content. Cells of the three strains had two unknown zeaxanthin like pigments that were present in high concentrations. The Ace Lake strain had an ultrastructure and lipid signature consistent with other strains of *Synechococcus* group. The cells contained phycoerythrin (c-PE) as their main light harvesting pigment, had a G + C content between 57 and 58.2 mol % of their DNA, and required saltwater in culture. These characteristics were consistent with strains accommodated within "Marine Cluster A" (Burgey's Manual of Systematic Bacteriology). The 16S rDNA sequences from the Ace Lake and Pendant Lake strains had 99.8% similarity. Except for *Synechococcus* strain NIVA-CYA 328, which was isolated from Oslofjord, Norway, the three Antarctic strains were more closely related to *Prochlorococcus marinus* than to other marine *Synechococcus* strains sequenced. The *Synechococcus* group appears to be polyphyletic, with one of the branches clustering

with *P. marinus*. 16S rDNA sequences of further strains from this group of organisms are required to determine their taxonomy more clearly.

Based on the square root temperature dependence model, the Ace Lake strain of *Synechococcus* had a theoretical t_{min} of -8° C, a t_{opt} of 19.7° C and a t_{max} of 29.8° C. In culture, the Ace Lake strain could not sustain growth at a light intensity of 5 μmol photons m^{-2} s^{-1} white light, and growth did not appear to be inhibited at high light intensities (200 μmol photons m^{-2} s^{-1}). All three strains were incapable of growth in freshwater.

Morphological and physiological characteristics of the Antarctic *Synechococcus* strains are discussed and compared with other marine strains previously assigned to the genus *Synechococcus* and the species *P. marinus.*

5.2 Introduction

Traditionally cyanobacteria have been classified according to morphological and physiological characteristics (Bourrelly 1985; Drouet 1981; Geitler 1932). This was largely true for all prokaryotes until the advent of molecular biological techniques (Fox et al. 1980). Unlike other prokaryotes, the cyanobacteria have a wide variety of morphological characteristics that has made their classification simpler than the classification of the heterotrophic bacteria. Even so, the taxonomy of the group has been plagued with problems and is now undergoing considerable change based on phylogenetic characteristics (Castenholz 1992).

Classification within the *Synechococcus* group has not escaped taxonomic revision (Stanier et al. 1971). Due to their morphological similarity, species distinction within this group have probably been more difficult than species distinction in other genera of cyanobacteria. This is illustrated by the name changes of strains over time, and the assignment of different names or strain numbers (depending on the culture collection) to the one *Synechococcus* strain. As an example, the wrongly characterised *Anacyctis nidulans* is now known as PCC6301 or ATCC27144 (Waterbury and Rippka 1989) but has also been known as *Synechococcus leopoliensis* (Rigby et al. 1980), *Synechococcus* 6301 (McKie et al. 1981) or simply as *Synechococcus* (Lawry and Jensen 1979). Taxonomic problems have been exacerbated by the assignment of species names based on limited taxonomic evidence. This is highlighted by the sixty species names, once used in the classification of the genus (Drouet and Daily 1956; Komarek 1976).

The taxonomic system described by Waterbury and Rippka, (1989) which is the one currently being used for the genus *Synechococcus* is based on a combination of morphological (cell size), biochemical (pigment content), physiological (salinity tolerance) and limited genetic (G + C ratio) characteristics. In addition, new strains are not given species names, they simply retain a strain designation. This system is a modification of the 'Stanier system' (Stanier et al. 1971) and classifies the genus *Synechococcus* into one of six clusters, each having a reference strain. It is anticipated that, with the collection of more phylogenetic information, each of these clusters will represent a genus. The clusters are currently named Cyanobacterium, *Synechococcus*, Marine Cluster C, Marine Cluster A, Marine Cluster B and Cyanobium. It appears, however, that this is not a universally accepted taxonomic system for the *Synechococcus* group. Organisms from this group are still being given species names based on morphological and physiological characteristics (Bailey-Watts 1991).

It is estimated that only about 5% of *Synechococcus* strains have been described (Castenholz 1992). The above classification system will grow, and probably be modified based on 16S rRNA information, as more organisms from within this group and from within other genera of single celled cyanobacteria, such as *Prochlorococcus*, are characterised.

Synechococcus are classified as picoplankton according to the size classification system of Sieburth et al. (1978). Picoplankton are between 0.2 and 2 μm wide, nanoplankton between 2 and 20 μm and microplankton are greater than 20 μm. Since the discovery of marine picoplankton in the late 1970's (Johnson and Sieburth 1979; Waterbury et al.

1979), *Synechococcus* has been shown to be widely distributed and important for primary productivity in most oceans of the world. The isolation of strains of the *Synechococcus* group from Antarctic saltwater lake environments has provided a unique opportunity to investigate the taxonomic similarities between these polar *Synechococcus* strains to their tropical and temperate counterparts.

In this chapter, the culture history and the morphological, biochemical, phylogenetic and physiological characteristics of three *Synechococcus* strains isolated from marine derived, meromictic, lakes in the Vestfold Hills, Antarctica is described. These taxonomic characteristics are discussed in relation to other strains of *Synechococcus* and to the closely related *P. marinus.*

5.3 Materials and Methods

5.3.1 Isolation of *Synechococcus* sp. from Antarctic meromictic lakes

The series of steps used to obtain the unicyanobacterial culture of the Ace Lake *Synechococcus* strain is given below.

5.3.1.1 Initial isolation of Synechococcus from Ace Lake

SNAX, SOX, F/2 and FE media prepared in filtered lake water were used for the initial isolation of *Synechococcus* from Ace Lake (Appendix 5 for media). One millilitre of fresh inoculum taken from each of several depths in Ace Lake was cultured in 25 ml of media (Falcon 50 ml culture flasks) at 4° C on a 12h dark/light cycle using cool white

light (20 μmol photons m^{-2} s^{-1}). At this stage, it was important to make the media in water taken from the same depth from which the inoculum came. This is because the lake is meromictic and the chemistry, in particularly the salinity, changes with depth. Cultures were left for one month before being checked for growth. The flow cytometer and fluorescent microscope were used to examine cultures for the growth of *Synechococcus* cells.

Following the initial isolation, *Synechococcus* cells from 11 m grown in SNAX media made with 11 m Ace Lake water were used for manipulation of the culture. Unless stated otherwise, cultures were grown at 10° C, 30 μmol photons m^{-2} s^{-1} on a 12 h day/night cycle.

5.3.1.2 Purification by serial dilution

As *Synechococcus* was dominant in the cultures taken from 11 m, serial dilutions were prepared to try to obtain axenic cultures of the cyanobacteria. An initial serial dilution was set up as follows:

The serial dilution was prepared in a series of ten flasks. Twenty-five millilitres of culture media (SNAX) was added to each flask. Flask one was inoculated with 100 μl of the original culture. The flask was mixed thoroughly and used as the inoculant for the next flask in the series. One millilitre was used as the inoculant for subsequent culture flasks. Between each inoculation the flask containing the inoculant was mixed thoroughly. Inoculant transfer was done using sterile 1 ml syringes. A new syringe was

used for each inoculation. Cultures were monitored for growth by both flow cytometry and visual observations.

5.3.1.3 Purification by cycloheximide treatment

In parallel to a repeat serial dilution, cycloheximide was used to remove the *Cryptomonas* sp. and any other eukaryotes that contaminated the *Synechococcus* cultures. Cycloheximide is a specific inhibitor of protein synthesis in eukaryotes but does not effect prokaryotes (Newell et al. 1983). Cycloheximide (Sigma Chemicals) was prepared as an aqueous solution at a concentration of 10 mg ml^{-1} (Xiuren and Vaulot 1992). The solution was filter sterilised through a 0.2 μm filter (Millipore). This was used as a 100 X stock solution and was stored at 2° C in the dark. Cycloheximide was added, at a final concentration of 0.1 mg ml^{-1}, to a subculture of the originally enriched Ace Lake 11 m culture. The culture was incubated, as above, until growth occurred. Following evaluation of the culture by microscopy and flow cytometry, the culture was used in a third serial dilution. The last culture to show growth in the dilution series was used as an eukaryote free, unicyanobacterial culture. This culture was further manipulated to remove heterotrophic bacteria.

5.3.1.4 Culturing on AGAR plates

While cultures were being put through serial dilution and antibiotic treatment, an attempt was made to grow the organism on solid media so as to allow physical separation of the cyanobacterial cells. Agar plates using SNAX medium and Ace Lake 11 m water were prepared (Appendix 5) and cells from the originally enriched culture were streaked over five plates. Two pour plates were also prepared. Plates were incubated as above.

5.3.1.5 Removal of heterotrophic bacteria

By this stage, *Synechococcus* cultures were free of eukaryotic organisms but were contaminated with a heterotrophic bacterium. The removal of heterotrophic bacteria from the Ace Lake *Synechococcus* culture was attempted using two antibiotic treatments.

Synechococcus cultures that were in stationary phase of growth were incubated in SNAX, containing either 200 mg l^{-1}, 300 mg l^{-1}, or 400 mg l^{-1} imipenem, for 40 hours in the dark at 10°C. Imipenem (Merck), 100 mg, was dissolved in 20 ml SNAX media and filter sterilized using a 0.2 μm filter (Millipore). This 100 X stock solution was used in the antibiotic treatments and was stored in small aliquots at -70° C. Imipenem is a broad spectrum β-lactam antibiotic that inhibits bacterial peptidoglycan biosynthesis (Kropp et al. 1985).

Synechococcus is an autotrophic organism and therefore will not grow and divide during dark incubation. Heterotrophic bacteria will grow independent of the light intensity. If a *Synechococcus* culture is incubated in the dark with a carbon source such as glycerol, then the heterotrophic bacteria, if present, will grow and divide where as the *Synechococcus* cells will remain in stationary phase. As imipenem inhibits bacterial peptidoglycan biosynthesis the heterotroph should be killed where as the *Synechococcus* cells should survive (Ferris and Hirsch 1991).

Following a 40 h incubation in media containing imipenem the cultures were centrifuged at 11,000 rpm, 4° C for 30 minutes, washed twice with sterile SNAX medium and resuspended in 25 ml of SNAX medium. The cultures were then incubated, as above, to allow the *Synechococcus* cells to recover from the imipenem treatment. A small volume of the washed culture was used as an inoculant for a glycerol-containing culture. This was to determine if the heterotrophic bacteria had been killed by the imipenem. The antibiotic treatment was repeated twice.

Alternatively, an antibiotic cocktail (1, 1.5 or 2 ml) was added to 25 ml of *Synechococcus* cultures. The antibiotic cocktail consisted of 100 mg penicillin and 100 mg streptomycin dissolved in 10 ml water along with 10 mg chloramphenicol dissolved in 1 ml 95% ethanol. The cocktail was filter sterilized and stored at 4° C. These cultures were incubated at 10° C in the dark.

After either a 24 h or 36 h period, cultures were centrifuged twice at 11 000 rpm, 4° C for 30 minutes and the cells were resuspended in SNAX medium to allow the *Synechococcus* cells to recover. Alternatively a 1/100 dilution was taken from the antibiotic treatment and incubated in SNAX media. This alternative procedure was performed in case the centrifugation step introduced further contamination.

5.3.1.6 Culturing in seawater

For long term maintenance of the Ace Lake *Synechococcus* strain, cells needed to be grown in sea water rather than lake water. At 11 m in Ace Lake the salinity is close to that of sea water. Cultures were grown in SNAX medium using open ocean sea water. Other growth conditions remained constant.

5.3.2 Isolation of Synechococcus strains from Pendant Lake and Lake Abraxas

Strains of *Synechococcus* were isolated from Pendant Lake (6 m) and Lake Abraxas (19 m) using cycloheximide containing SNAX media based on Ace Lake 11 m water. The cultures were incubated at 10° C, 30 μmol photons m^{-2} s^{-1}, with a 12 h day-night cycle. Following treatment with cycloheximide, both strains were serially diluted and the last cultures to show growth were used as the unicyanobacterial cultures.

5.3.3 Taxonomic characteristics of Antarctic *Synechococcus*.

5.3.3.1 Epifluorescence microscopy

A Leitz DMRBE epifluorescence microscope with filter block M2 (excitation filter BP 546/14, dichromatic mirror RKP 580, suppression filter LP 580) was used for cell counts, morphological characterisation and photography. Slides of cultures or lake water samples were prepared by filtering cells onto 0.2 μm polycarbonate filters, pre-stained with Irgalan Black (Millipore). Cell sizes were calculated using a pre-calibrated eyepiece micrometer. Twenty cells of each strain were measured and the average cell size of each strain calculated. For photomicroscopy cells were applied to glass slides pre-coated with agar (Noble Agar, Difco) and photographed under oil immersion, 1000 X magnification, using green fluorescence.

5.3.3.2 Electron microscopy

The Ace Lake strain of *Synechococcus* was examined by transmission electron microscopy (TEM). Cultures grown in SNAX medium based on Ace Lake 11 m water, were grown at 30 μmol photons m^{-2} s^{-1} continuous light (dark red/brown culture). Cells were harvested in late exponential phase and prepared for TEM analysis according to the following method. Cells were fixed at 4° C in the dark for 1 hr with 4 % gluteraldehyde and then centrifuged at 3000 r.p.m. for 10 min, at 4° C. Cells were transferred to Eppendorf tubes and post-fixed in 2 % OsO_4 at room temperature for 1 hr in growth medium. Cells were centrifuged, as above, and dehydrated for 15 min in 10

%, 30 %, and 50 % acetone. Cells were left overnight at 4° C in 70 % acetone. The following day the dehydration series continued (70 %, 80 %, 90 %, 95 %, 3 x 100 %). Cells were then suspended in 50:50 dry acetone: Spurr's resin for 1 hr. Cells were spun at 10 000 r.p.m. 5 minutes and the pellet suspended in 100 % Spurr's resin overnight at 70° C. After cooling, serial sections were cut with a diamond knife and stained with uranyl acetate and lead citrate. Sections were examined using a JOEL 1200 EX transmission electron microscope.

5.3.3.3 In-vivo absorption, emission and excitation spectra

Cultures used in the analysis of *in-vivo* absorption and emission spectra were grown at 4° C at a constant light intensity of 10 μmol photons m^{-2} s^{-1}, which resulted in dark red/brown cultures due to the low light conditions. Dense culture of each of the three strains was used for the scans. A GBC UV/VIS 916 spectrophotometer with an integrating sphere and disposable cuvettes (Biorad) were used for *in-vivo* absorption spectra over the range 400 nm to 750 nm (5 nm intervals, 2 nm slit width). The integrating sphere eliminated the effects of scattering and gave scans with well-defined peaks. A baseline scan was performed on filtered seawater. The *in-vivo* emission and excitation spectra were obtained on a Perkin Elmer Luminescence Spectrometer using disposable cuvettes (Biorad). The emission spectra were collected over a wavelength range of 500 - 700 nm with an excitation wavelength of 450 nm (Ex bp 15 nm, Em bp 10 nm). The excitation spectra were collected over a wavelength range of 400-665 nm with an emission wavelength of 680 nm (Ex bp 10 nm, Em bp 10 nm). Baseline scans were performed with filtered seawater.

5.3.3.4 Extraction and analysis of lipid soluble pigments

Pigment analysis was performed on the Ace Lake and Pendant Lake *Synechococcus* strains. Cultures were grown in SNAX medium based on 11 m Ace Lake water, at 10° C, under continuous cool white light, at 20 μmol photons m^{-2} s^{-1}. The "high light cultures" were grown at 100 μmol photons m^{-2} s^{-1}.

Lipid soluble pigments were extracted according to Wright et al., (1991). Cells in late exponential phase, were filtered onto GF/F filters (Millipore) under low pressure (< 15 Kpa) in subdued light. The filters were frozen in liquid nitrogen, and before analysis, were cut into small pieces. Buffered methanol (98:2 methanol: ammonium acetate 0.5M pH 7.1) was added (3 ml) and the filter was sonicated for 30 seconds using a Braun Labsonic 1510 equipped with a 4 mm diameter probe, operated at 50 W. The samples were then centrifuged (2000 rpm) at 4°C to remove filter debris and immediately before HPLC analysis the extracts were filtered (Millex - SR 0.5 μm). Samples were analysed on a Spectraphysics HPLC, comprising: a SP8800 ternary pump; Spectra Focus detector; and a Gilson 231 autosampler (with samples refrigerated at - 10°C), using a Spherisorb OD52 column 250 x 4.6 mm and the ternary gradient system of Wright et al., (1991). Chromatograms were analysed using Specta Physics software.

Scientific Committee for Oceanic Research (SCOR) cultures from Wright et al., (1991) were extracted and analysed at the same time as the Antarctic *Synechococcus* culture to aid in the identification of pigments. SCOR cultures included: *Amphidinium carterae*

(CS 212); *Chroomonas salina* (CS 174); *Dunaliella tertiolecta* (CS 175); *Micromonas pusilla* (CS 86); *Pavlova lutheri* (CS 182); *Porphyridium cruentum* (CS 25); *Pycnococcus provasolii* (CS 185) and *Synechococcus* strain (DC-2, CS 197).

5.3.3.5 Extraction and analysis of lipids

Lipid analysis was performed on the Ace Lake *Synechococcus* strain. Cultures were grown as for lipid soluble pigment analysis. Culture A was harvested at the beginning of exponential phase and culture B at the end of exponential phase for lipid analysis. Cultures were filtered onto GF/F glass fibre filters and extracted by the modified one-phase $CHCl_3$-MeOH-H_2O Bligh Dyer method (Bligh and Dyer 1959; White et al. 1979). Two filters were analysed for each culture. Filters were shredded before addition of solvents. After phase separation, lipids were recovered from the lower $CHCl_3$ layer, concentrated and stored at -20° C under N_2.

A portion of the total lipid extract was analysed with an Iatrascan Mk. III TH-10 thin layer chromatography-flame ionization detector (TLC-FID) analyser (Volkman and Nichols 1991). The solvent system used was C_6H_{14}-$(C_2H_5)O$-CH_3CO_2H (60:17:0.5 v/v/v). Peak areas of the individual lipid classes were quantified with chromatography software.

A subunit of the total lipid extract (30%) was saponified (Nichols et al. 1991). The saponified fatty acid fraction was treated with MeOH-HCl-$CHCl_3$ (10:1:1 v/v/v 3 ml) to produce the corresponding fatty acid methyl esters (FAMEs) which were then extracted with C_6H_{14}-$CHCl_3$ (4:1 v/v). Gas chromatographic analyses were performed using a

Hewlett Packard 5890 gas chromatograph (GC) fitted with a 50 m x 0.32 mm i.d. HP1 (0.17 μm film thickness) cross-linked methyl silicone fused-silica capillary column with H_2 as the carrier gas. The GC was fitted with a flame ionisation detector (310° C) and a purged split/splitless injector (290° C). Samples were injected using an Hewlett Packard 7673A autosampler. Oven temperature was programmed from 50°C to 150°C at 30° C min^{-1}, then at 2° C to 250° C at 5° C min^{-1} to 300° C. Identification of compounds was performed by gas chromatography-mass spectrometry (FISONS MD800 GC-MS) using conditions as described by Barrett et al., (1995) for the hydrogenated samples and by comparison of retention time and the mass spectral data with data obtained from authentic and laboratory standards. Double bond positions were determined by the method described by Skerratt et al., (1991).

5.3.3.6 Phylogenetic analysis

5.3.3.6.1 16S rDNA Analysis

DNA was extracted from the strains of *Synechococcus* from Ace Lake and the Pendant Lake and Lake Abraxas according to a method modified from Marmur and Doty, (1962); Fang et al., (1992) and C. Bolch, (personal communications). Cells were grown until they formed a dense culture. Cells were allowed to settle from 5 ml of culture. Cells were mixed with saline-EDTA (0.1 M NaCl and 0.1 M EDTA, pH 8.1) to make a final volume of 80 μl. One-hundred microlitres of Lysozyme (100 mg ml^{-1}) was added and the cells incubated at 60° C for 30 minutes Then 20 μl SDS (0.2 % w/v) and 20 μl Proteinase K (25 mg ml^{-1}) were added, followed by a second incubation at 60° C for 30 minutes. A further 60 μl of 0.2 % SDS was added and a 30 minute

incubation at 60° C followed. A chloroform: IAA (isoamylalcohol) extraction was performed followed by and ethanol precipitation of DNA.

The majority of cells were not lysed using this method and a more rigorous method used. Unlysed cells, taken from the interface between the aqueous layer and the chloroform layer in the above procedure, were mixed with saline EDTA to a final volume of 200 µl and 20 µl Lysozyme, incubated at 60° C for 5 hours, followed by an overnight incubation with 20 µL 0.2% SDS. Proteinase K (60 µl) was then added and incubated at 37° C for 3 hours. Another 60 µl 0.2% SDS was added and incubated at 37°C for 30 minutes. Sodium perchlorate 60 µl (5 M) was added and a chloroform : IAA extraction was performed. An ethanol precipitation (2 volumes of absolute ethanol, -20° C for 2 hours, centrifuged at 14000 rpm for 30 minutes), a salt precipitation (1 volume of 4 M NaCl followed by 2 volumes of absolute ethanol, -20° C for 10 minutes, centrifuged at 14000 r.p.m. for 30 minutes, pellet washed with 70 % EtOH) and a PEG (13 % PEG (6000) in 1.6 M NaCl) precipitation followed (1 volume of PEG, centrifuged at 14000 for 30 minutes). Measurement on the absorption spectrophotometer indicated a successful extraction of DNA although genomic DNA was not detected by gel electrophoresis. This genomic DNA was used successfully in the following PCR reactions.

The 16S rDNA from the two strains were amplified by PCR using primers A and H (Lane 1991). The sequences for these two primers is given in Table 5.1. Each PCR mixture (100 µl) contained each deoxynucleotide at a concentration of 50 µM, 2.5 µM magnesium chloride, PCR buffer IV (25mM ammonium sulfate, 75 mM Tris- HCl, (pH 9.0), 0.01 % Tween 20), 50 pmol of each primer, 5 % (v/v) dimethyl sulfoxide, 80 or

560 ng genomic DNA, and 2.5 U of thermostable DNA polymerase (Advanced Biotechnologies, Surrey, UK). The PCR were performed in a Corbett Research model FTS-960 thermocycler. The reaction parameters included an initial 5 minute incubation at 94° C, followed by 30 cycles consisting of 94° C for 1 minute, 50° C for 1 minute and 72° C for 5 minutes PCR products were purified using a QIA quick gel extraction kit (Qaigen, Inc., Chatsworth, Cal). The PCR products were sequenced directly to obtain the 16S rDNA sequence. An Applied Biosystems model 3738A automated sequencer using a fluorescent -dye terminator cycle sequencing kit (Perkin Elmer) and primers A, H, 20, 785r, 23 and 19 (Lane 1991) were used for the sequencing (Table 5.1).

Table 5.1: Primer sequences used in the PCR amplification of the 16S rDNA from the Ace Lake strain and Pendant Lake strain of *Synechococcus*. Sequences from (Lane 1991).

Primer	Sequence	Position
A	5`AGAGTTTGATCCTGGCTCAG	8-28
20	5`GTAGCGGTGAAATGCGTAGA	684-704
785r	5`TCTACGCATTTCACCGCTAC	785-765
H	5`AAGGAGGTGATCCAGCCGCA	1542-1522
23	5`AAACTCAAAGGAATTGACGG	908-928
19	5`CAGCAGCCGCGGTAATAC	518-536

The 16S rDNA sequences were aligned with other sequences downloaded from the GeneBank internet site. PHYLIP (v3.5) (Felsenstein 1993) was then utilized for phylogenetic analysis, and evolutionary distances were determined with the maximum likelihood algorithm by using the DNADIST program. Rooted phylogenetic trees were obtained using the Neighbor program. Phylogenetic trees were generated using DRAWGRAM. The strain numbers and GenBank accession numbers for the 16S rDNA sequences utilized for comparison in this study are given in Table 5.2

Table 5.2: Names, strain numbers, GenBank accession numbers and environment, from which strain was isolated, for the strains used for comparison in the phylogenetic tree produced for the Ace Lake and Pendant Lake strains of *Synechococcus* .

Strain	Strain No.	GenBank No.	Isolated from:	Reference
Prochlorococcus marinus	SSW5	X63140	Temperate marine, Sargasso Sea	(Urbach et al. 1992)
Synechococcus elongatus	no data	D83715	Freshwater, thermophilic	(Kobayashi et al. 1996)
Synechococcus lividus	Y-7b7c-S	X67091-X67093	Thermal springs, U.S.A.	(Ward et al. 1990)
Unknown marine bacterioplankton	SAR6	X52169	Temperate marine, Sargasso Sea	(Giovannoni et al. 1990)
Unknown marine bacterioplankton	SAR7	X52171	Temperate Marine, Sargasso Sea	(Giovannoni et al. 1990)
Synechococcus leopoliensis	PCC6301 or NIVA-CYA 20	Z82780	Freshwater, Texas	(Rudi et al. 1997)
Synechococcus sp.	PCC6301	X03538, X01296, K01982	Freshwater, Tesas	(Kumano et al. 1986)
Synechococcus sp.	NIVA-CYA 328	Z82779	Marine, Oslo Fjord, Norway	(Rudi et al. 1997)
Synechococcus sp.	PCC7002	D88289	Marine Sediment, Perto Rico	(Tronsmo et al. 1996)
Synechococcus sp.	PCC7942	D88288	Freshwater	(Tronsmo et al. 1996)
Prochloron sp.	no data	X63141	Tropical, marine	(Urbach et al. 1992)
Prochlorothrix hollandica	NIVA 5/89	Z82782	Freshwater, The Netherlands	(Rudi et al. 1997)
Phormidium ectocarpi	CCAP 1462/5	X62080-X62082	Marine	(Wilmotte et al. 1993)
Phormidium minutum	D5	X62685, X62686	Marine	(Wilmotte et al. 1993)
Microcystis holstatica		U40336	Freshwater	(Neilan et al. 1997)
Microcystis elabens		U40335	Freshwater	(Neilan et al. 1997)

5.3.3.6.2 DNA Base Composition (mol % G+C) analysis using the thermal denaturation procedure:

DNA was extracted from the three Antarctic strains of *Synechococcus*, using a method adapted from (Marmur and Doty 1962). Cells were centrifuged at 10 000 x g for 10 minutes at 4° C and pellets were resuspended in 5 ml 1 M NaCl- 0.1 M sodium EDTA buffer (pH 8.1) in 15 ml polypropylene tubes. Lysozyme was added to the cell suspensions to a concentration of 1 mg ml^{-1} and incubated at 60° C for 1 hour. The cell suspension was then treated with pronase E (Sigma) added at 10 mg ml^{-1} and incubated at 37° C for 30 minutes. Sodium dodecyl sulfate (20% solution) was added to obtain a concentration of 2 % and the suspensions were incubated at 60° C for 10 minutes. Sodium perchlorate (5 M solution) was added to a concentration of 1 M and the suspensions were vigorously shaken. An equal volume of isoamylalcohol:chloroform (1:24) was then added (approximately 7 ml) and the suspensions were vigorously shaken. The emulsion was then centrifuged at 4000 X g in a bench centrifuge for 5 minutes. The aqueous layer was then transferred to a clean glass beaker. DNA was precipitated by addition of 1 to 2 volumes of chilled absolute ethanol. Precipitated DNA was spooled onto a pasteur pipette and air dried. The air dried DNA was then redissolved into 2 ml 0.1 x SSC (SSC, saline-sodium citrate buffer, 0.15 M NaCl-0.015 M trisodium citrate, pH 7.0). Ribonuclease A was then added at 1 mg ml^{-1} and the solution was incubated at 37° C for 1 hour. An equal volume of isoamylalcohol:chloroform (1:24) was then added (2 ml) and the suspensions were vigorously shaken. The emulsion was then centrifuged at 4000 x g in a bench centrifuge for 5 minutes. The aqueous layer was then transferred to a clean glass beaker. DNA was precipitated by addition of 1-2 volumes of chilled isopropanol and 0.1 volume of 3

M sodium acetate-10 mM sodium EDTA (pH 7.0). Precipitated DNA was spooled onto a pasteur pipette washed with briefly with isopropanol and air dried. The air dried DNA was then redissolved into 1-2 ml 0.1 X SSC (SSC, saline-sodium citrate buffer, 0.15 M NaCl-0.015 M trisodium citrate, pH 7.0).

The DNA base composition was determined by a method adapted from Sly et al., (1986). DNA solutions were adjusted to a concentration of approximately 30 $\mu g\ ml^{-1}$ in 0.1 SSC. Quartz cuvettes containing 1 ml of the DNA solution were then placed in a GBC 916 spectrophotometer (GBC Scientific, Adelaide, SA) and held at 50 C. The temperature was then ramped at 2.5° C min^{-1} (up to 95° C) using water circulating through a water-jacketed cuvette holder via a controlled temperature waterbath. Absorbance readings at 260 nm were taken automatically at 0.5 min. intervals for each sample using a linear module. DNA from strain *Pseudolateromonas haloplanktis* ACAM 547 (mol % G+C 41.0 mol %) was used as a reference strain. Triplicate melting curves of the test strains and reference strain were plotted (absorbance versus temperature) and the Tm (DNA melting temperature) was determined. The mol % G+C of the test strains were then determined from the following equation adapted from (Meinkoth and Wahl, (1984 and Sly et al., (1986): Tm(reference) - Tm(test) = 53.1 - 0.41 (G+C% (reference) - G+C% (test)).

5.3.4 Physiological characteristics of Antarctic *Synechococcus* strains.

5.3.4.1 Determination of cardinal growth temperature

Cultures were grown in SNAX medium, based on Ace Lake 11 m water, in 15 ml culture tubes (Falcon), under cool white light at 20 µmol photons m^{-2} s^{-1}, on a 12 hour day/night cycle. A gradient incubator was used with constant slow agitation, with a temperature range of -1° C to 30° C, and with the light source at the back was used. A electronic temperature data logger was kept in one of the ports of the incubator throughout the experiment to ensure that temperatures remained constant. Cultures were incubated at each temperature in duplicate or triplicate over a seven week period. Samples (0.25 µl) were taken from the cultures, aseptically, and analysed for changes in cell number by flow cytometry at intervals which varied depending on the growth temperature.

Growth rate (GR) at each temperature was determined during exponential phase of growth, according to the equation $GR = k = (\log_{10} C_2 - \log C_1)/ (t_2 - t_1)$, where C_1 was the cell concentration (cells ml^{-1}) at time (t_1) and C_2 was the cell concentration at t_2. Theoretical minimum, optimal and maximum growth rates for the Ace Lake *Synechococcus* strain were determined over the entire biokinetic temperature range using the square root model (Ratkowsky et al. 1983; Ratkowsky et al. 1982). The equation for the square root model was $\sqrt{k} = b(T-T_{min})(1-\exp(c(T-T_{max})))$, where k = bacterial growth rate, b = a parameter to be fitted, T = growth temperature in Kelvin, T_{min} = theoretical temperature at which k is zero, T_{max} = upper theoretical temperature at

and which no growth is possible and c = parameter to be fitted. Data was also fitted to Rosso's model (Rosso et al. 1993) in order to confirm the cardianal temperatures of the Ace Lake *Synechococcus*. The equation for Rosso's model was $k = \mu_{opt}(T-T_{max})(T-T_{min})^2/(T_{opt}-T_{min})\{(T_{opt}-T_{min})(T-T_{opt})-(T_{opt}-T_{max})(T_{opt}+T_{min}-2T)$. The SAS statistical program (SAS Institute 1989) was used to fit data to the model.

5.3.4.2 Effect of light intensity on growth

Cultures of the Ace Lake *Synechococcus* strain were grown in SNAX medium based on Ace lake 11 m water at 15 (± 2)° C under the light regimes outlined in Table 5.3.

Table 5.3: Light regime to which *Synechococcus* cells were exposed. The light source was cool white light. Light measurements are in μmol photons m^{-2} s^{-1}. Numbers in brackets indicate hours per day that cells were exposed.

Treatment	Light
Control	20 (12)
A	20 (24)
B	5 (24)
C	300 (24)

Light was measured with a Li-Cor Quantum/Ratiometer/Photometer Li -185. The light intensity in the gradient incubator was measured by inserting a perspex rod into a slot where a culture tube would normally sit. This was used to carry the light from the source to the light meter at the front of the incubator. The cultures were monitored over time, for changes in fluorescence intensity and cell numbers, by flow cytometry.

5.3.4.3 Effect of salinity on the growth of Synechococcus cells.

Strains of *Synechococcus* from Ace Lake, Pendant Lake and Lake Abraxas were grown in SNAX medium, based on Ace Lake 11 m water, in 50 ml culture flasks (Falcon). Cells in exponential growth were used in the salinity tolerance experiment. Cells were grown at 10°C, with 30 μmol photons m^{-2} s^{-1} continuous cool white light. Cells from each of the three lakes were subjected to four treatments:

- SNAX mineral medium made with 100 % 11 m Ace Lake water (Salinity ~ 30 ppt).
- SNAX mineral medium made with two thirds Ace Lake water and one third Milli-Q water (Salinity ~ 20 ppt).
- SNAX mineral medium made with one third Ace Lake water and two thirds Milli-Q water (Salinity ~ 10 ppt).
- SNAX mineral medium made with 100% Milli-Q water (Salinity ~ 0 ppt).

Flow cytometric methods (Chapter 2) were used to monitor changes in cell numbers over time and data was subjected to ANOVA (Sigmastat 1992) and pairwise multiple comparison procedures (Student-Newman-Keuls (SNK) Test) to determine the effect of salinity on the growth of the organism.

5.5 Results

5.5.1 Isolation of *Synechococcus* from Antarctic meromictic lakes

5.5.1.1 Initial isolation from Ace Lake

When *Synechococcus* cells were initially enriched from Ace Lake, waters from four depths were used to inoculate four types of media. The microbiota that developed in the initial enrichments are summarised in Table 5.4.

Table 5.4: Organisms that dominated cultures from the initial isolation of *Synechococcus* from Ace Lake in December 1992. The numbers in brackets indicate the depth from which water was collected for preparation of the medium.

Medium	Inoculum	Dominant Phototroph
F/2 (2m)	2m	Predominantly *Cryptomonas* sp but mixed
FE (2m)	2m	Predominantly *Cryptomonas* sp but mixed
F/2 (2m)	6m	*Synechococcus* (low numbers), *Cryptomonas* sp.
FE(2m)	6m	*Synechococcus* (low numbers), *Cryptomonas* sp.
F/2 (8m)	8m	*Synechococcus* but mixed culture
FE (8m)	8m	*Synechococcus* but mixed culture
F/2 (11m)	11m	*Synechococcus* dominant but mixed culture
SNAX (11m)	11m	*Synechococcus* dominant but mixed culture
SOX (11m)	11m	No growth obvious

Synechococcus cells were not observed in cultures inoculated with water from 2 m in Ace Lake. Cultures predominantly consisted of *Cryptomonas* sp. The cultures were pink which is consistent for enrichments containing *Cryptomonas* sp. cells. The orange fluorescence emitted from the cryptomonad phycoerythrins was also evident with flow cytometric analysis.

It was possible to use media made up in water collected from 2 m for the 6 m inoculation, as the lake was mixed between 2 m and 7 m and the water chemistry was the same (Chapter 3). *Synechococcus* cells were observed in cultures from 6 m but they were not dominant. The dominant species was *Cryptomonas* sp.. In Ace Lake *Synechococcus* has been observed at 6 m but other organisms dominate at this depth (Chapter 3).

At 8 m *Synechococcus* was dominant in most cultures but the cultures contained a high number of eukaryotic organisms.

In Ace Lake, *Synechococcus* cells were in the greatest numbers at 11 m, (Chapter 3), and growth of the organism in cultures from this depth was the most successful. *Synechococcus* did not grow in SOX medium, even when actively growing cells were transferred to SOX medium. At 11 m growth of *Synechococcus* cells was greatest in the SNAX medium, although the F/2 medium also supported the growth of the picocyanobacteria. When cells were transferred between the two types of media *Synechococcus* cells grew equally as well in both types of media. The cultures initially inoculated with water from 11 m in Ace Lake contained *Synechococcus* cells in high numbers but they also contained other organisms. The cultures were light orange-brown in colour. Diatoms, that adhered firmly to the bottom and sides of culture flasks, were the first organism to appear in the cultures. The diatoms appeared after five weeks incubation at 4° C. The diatoms produced small brown colonies and were bright red (under a green light), elongated cells approximately 10 μm in length, when observed by fluorescence microscopy. *Synechococcus* cells were often associated with the diatom colonies A coccoid shaped unidentified microflagellate (also about 10 μm in diameter)

was observed. *Pyramamonas gelidicola* and *Cryptomonas* sp. were also observed in some cultures.

Synechococcus was always more abundant than the eukaryotic organisms in enrichments inoculated with water from 11 m. For this reason enrichments inoculated with water from 11 m were used for the production an axenic culture of *Synechococcus*. SNAX medium, based on 11 m Ace Lake water, was used as a standard medium for growing the Ace Lake *Synechococcus* strain.

5.5.1.2 Serial dilutions and cycloheximide treatment

A dilution series was performed using the enrichment culture that had been inoculated with 11 m Ace Lake water. After five weeks, growth was evident in the 10^{-4} dilution flask of the first dilution series. No growth was observed in the 10^{-5} dilution flask. Although *Synechococcus* was dominant in the inoculum the *Cryptomonas* sp. dominated the 10^{-4} dilution flask. When each of the flasks in the series was examined by flow cytometry, the change in ratio of *Synechococcus* cells to the *Cryptomonas* sp. cells over the series was evident. The serial dilution, was repeated and the same result was obtained.

When a sample of the enrichment culture inoculated with 11 m Ace Lake water was exposed to cycloheximide the growth of *Synechococcus* cells was not inhibited but neither the *Cryptomonas* sp., nor the diatom, grew. The final culture from a further dilution series was used for biochemical analysis and physiological characterisation of the Ace Lake *Synechococcus* strain. This culture was orange-brown in colour.

5.5.1.3 Culturing on agar media

Growth of *Synechococcus* cells on agar media was not consistent. Growth was obtained on 2 of the 5 streak plates and one of the pour plates. Isolated colonies were not visible on any plates. The colonies merged together. The cells growing on the plates were dark red-brown in colour and were slimy. Cells from agar media were restreaked, but colonies did not develop.

There may have been too many impurities in the agar used and *Synechococcus* may have grown more successfully on molecular grade agarose plates (A. Davidson, personal communications). Agarose medium (0.5%), made from SNAX medium based on water collected from 11 m in Ace Lake, was prepared. Two plates were inoculated with *Synechococcus* cells from the original enriched culture and two plates were inoculated with cells from the agar plates. *Synechococcus* cells did not grow on any of the agarose media.

5.5.1.4 Removal of heterotrophic bacteria from cultures

Glycerol was initially added to cultures to determine if cells could be preserved under liquid nitrogen. Heterotrophic bacteria always grew instead of *Synechococcus* upon retrieval of stored stocks. SNAX medium has no added carbon source. A heterotrophic bacteria were surviving in the *Synechococcus* stored stocks and was using glycerol as an energy and carbon source. The heterotroph only grew when a carbon source was added

to the medium. In order to continue characterisation of the Ace Lake *Synechococcus* strain attempts were made to eliminate the heterotroph.

The antibiotic, imipenem, and an antibiotic cocktail were used to try to eliminate the heterotroph. After exposure to imipenem, followed by an incubation period of 72 hours in antibiotic free medium, the heterotrophic bacterium was not observed by phase contrast microscopy. Heterotrophic bacteria appeared in the cultures after two weeks. Following antibiotic treatment, it may have taken a longer time period for the heterotroph to recover. The imipenem treatment was not successful and the antibiotic cocktail was tested.

Following exposure to the antibiotic cocktail, the heterotrophic bacterium had grown in all treatments except the 36 h (2 ml) antibiotic treatment. Heterotrophic bacteria were observed in this treatment after three weeks.

Neither antibiotic treatment was successful in removing the heterotrophic bacterium from Synechococcus cultures. In medium to which a carbon source had not been added, *Synechococcus* cells dominated and heterotrophic bacteria were present in background levels (<1 %).

5.5.1.5 Growth of Synechococcus in seawater

The Ace Lake *Synechococcus* strains did not grow in SNAX medium based on coastal seawater collected at a pristine site on the Tasman Peninsula, Tasmania. When open ocean seawater, collected during marine science cruises, was used the Ace Lake *Synechococcus* strain grew well.

5.5.2 *Synechococcus* from other locations

In January 1993 a second strain of *Synechococcus* was isolated from Pendant Lake and, in November 1993, a third strain was isolated from Lake Abraxas. Both strains grew well in the presence of cycloheximide producing cultures free from other phototrophs. The cultures were the same colour as the culture of the Ace Lake strain. They ranged from light brown to dark brown-red depending on the light intensity under which they were grown. Like the Ace Lake strain the cultures were not axenic but without an external carbon source heterotrophic bacteria occurred in low numbers (< 1%).

5.5.3 Taxonomic characteristics of Antarctic *Synechococcus* strains

5.5.3.1 Epifluorescent microscopy.

Cells of all three strains appeared as orange-red fluorescent coccoid cells under green light (Figure 5.1). Cells were uniform in shape and size (Table 5.5). The Ace Lake strain and the Lake Abraxas strain were the same size and cells were distributed evenly

on the filter. The Pendant Lake strain was slightly larger and cells were clump loosely together on the filter. *Synechococcus* cells in Pendant Lake water samples also clumped loosely on the filter.

Table 5.5: Cell size of the three strains of *Synechococcus* isolated from meromictic lakes in the Vestfold Hills. Numbers in brackets are standard deviations based on ten replicates.

Strain	Width (μm)	Length (μm)
Ace Lake	0.91 (0.13)	1.50 (0.11)
Pendant Lake	1.1 (0.14)	1.81 (0.11)
Lake Abraxas	0.91 (0.13)	1.51 (0.11)

5.5.3.2 Electron Microscopy

Cells of the Ace Lake *Synechococcus* strain contained a cell wall, cell membrane, thylakoid membranes, ribosomes, phycobilisomes and polyhedral bodies (Figure 5.2). The polyhedral bodies were approximately 85 nm in diameter and the phycobilisomes were 20.3 ± 5.7 nm in diameter.

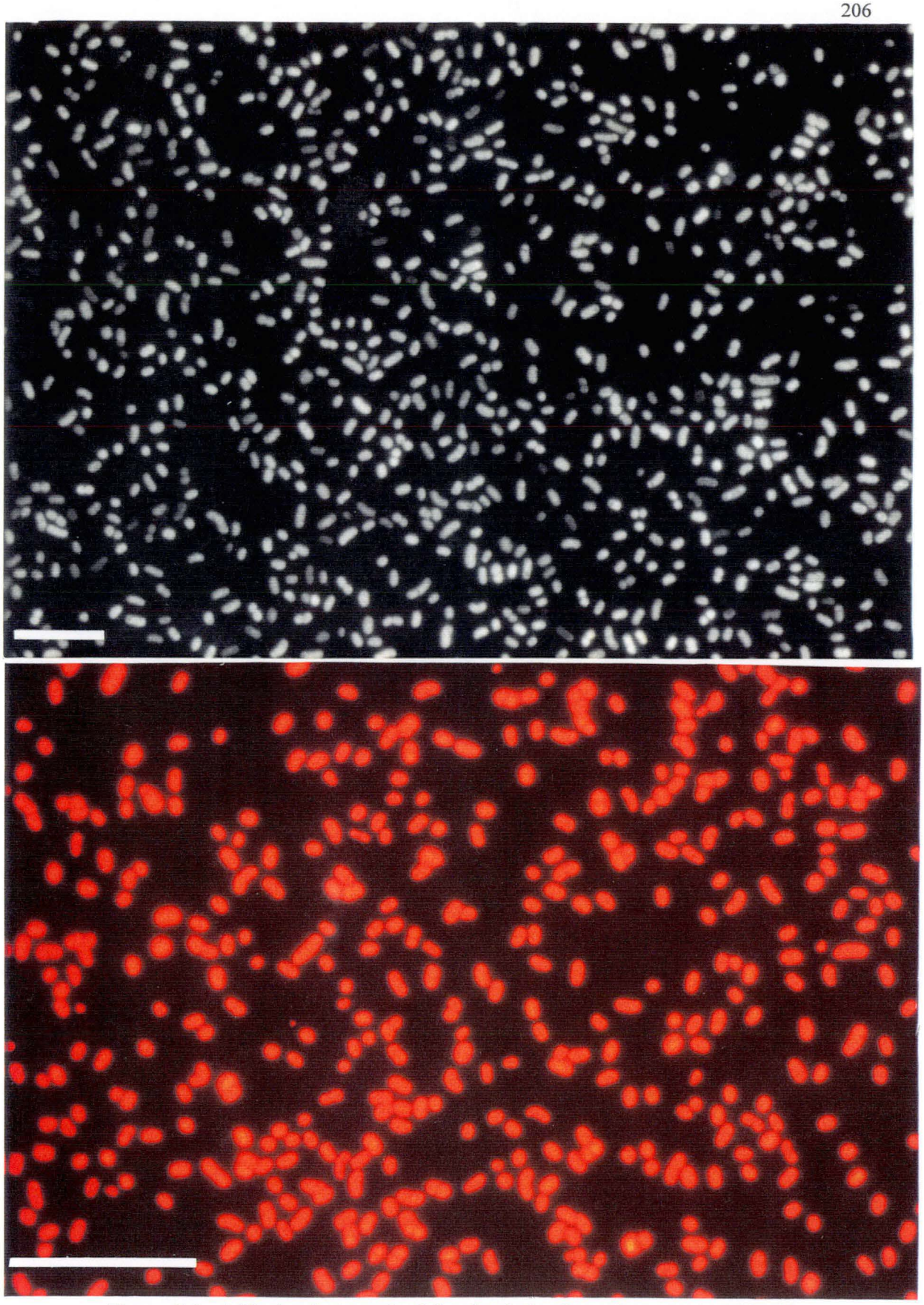


Figure 5.1: a) Photomicrograph of the Ace Lake *Synechococcus* strain. b) Photomicrograph of the Ace Lake *Synechococcus* strain subjected to green light fluorescence. Bar = 10 μm.

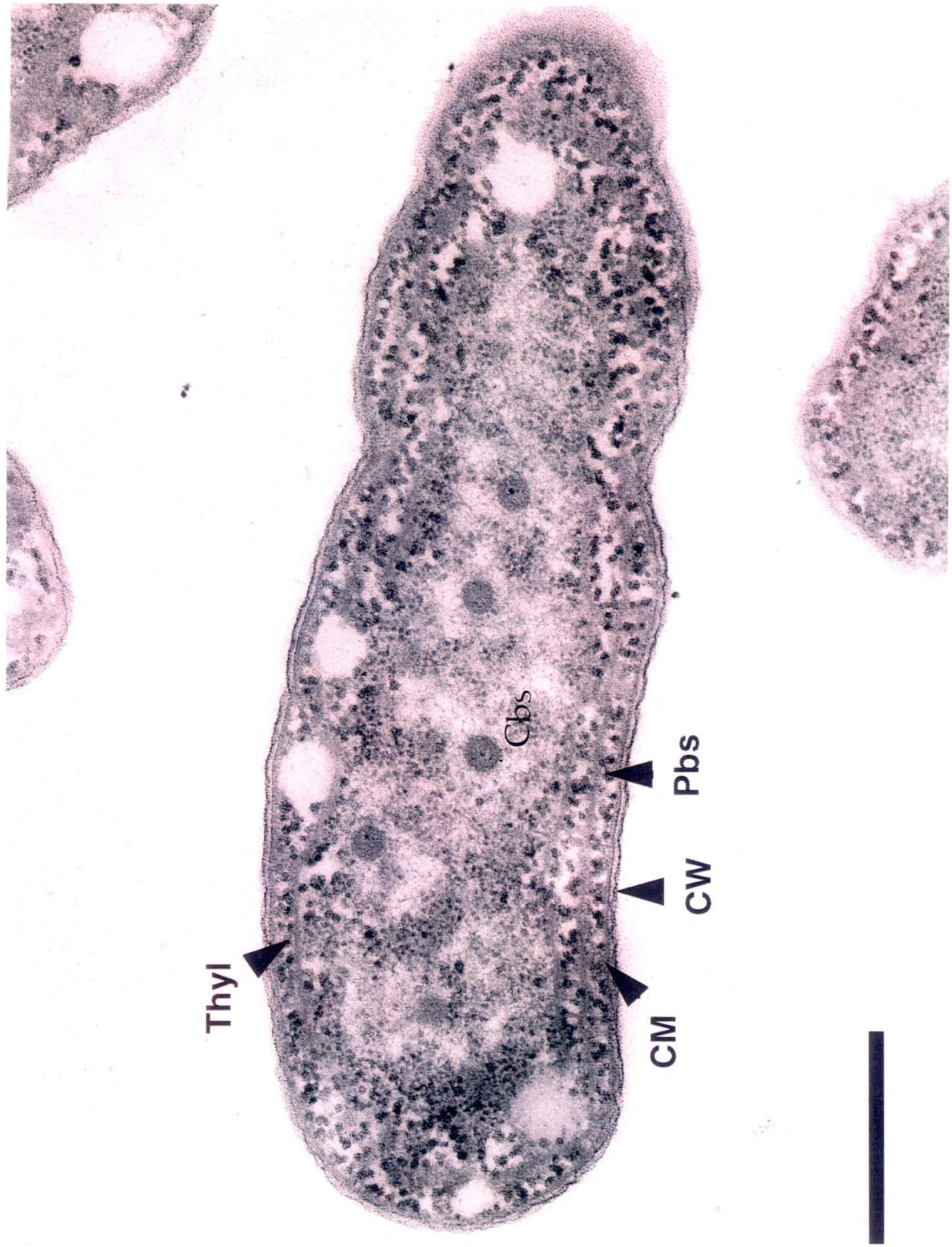
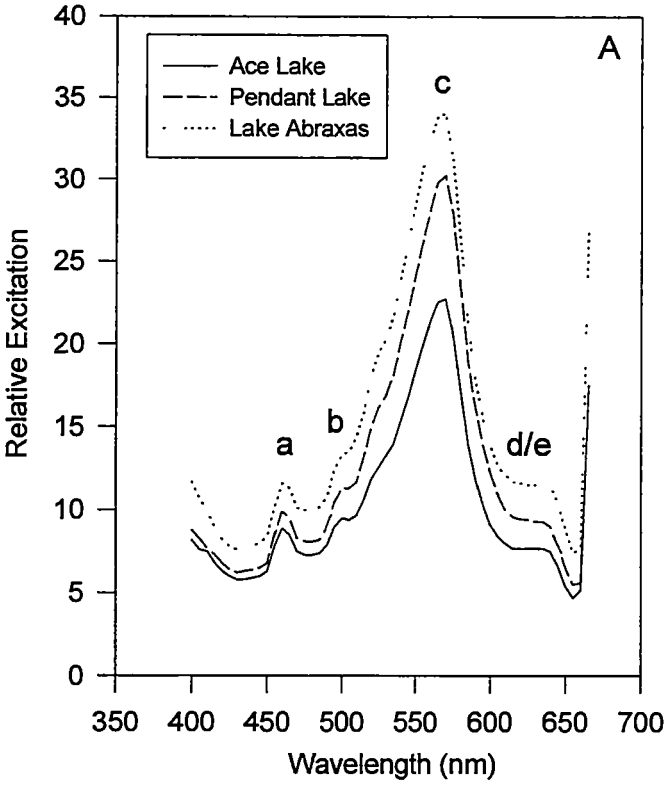


Figure 5.2: Electron micrograph of Ace Lake *Synechococcus* strain. Cells were grown under low light conditions (30 μmol photons m^{-2} s^{-1}) at 10° C. CW = cells wall; CM = cell membrane; Thyl = thylakoid membrane; Pbs = phycobilisomes; Cbs = carboxysomes. Bar = 540 nm.


A
Ace Lake
Pendant Lake
Lake Abraxas
a
b
c
d/e
Relative Excitation
Wavelength (nm)
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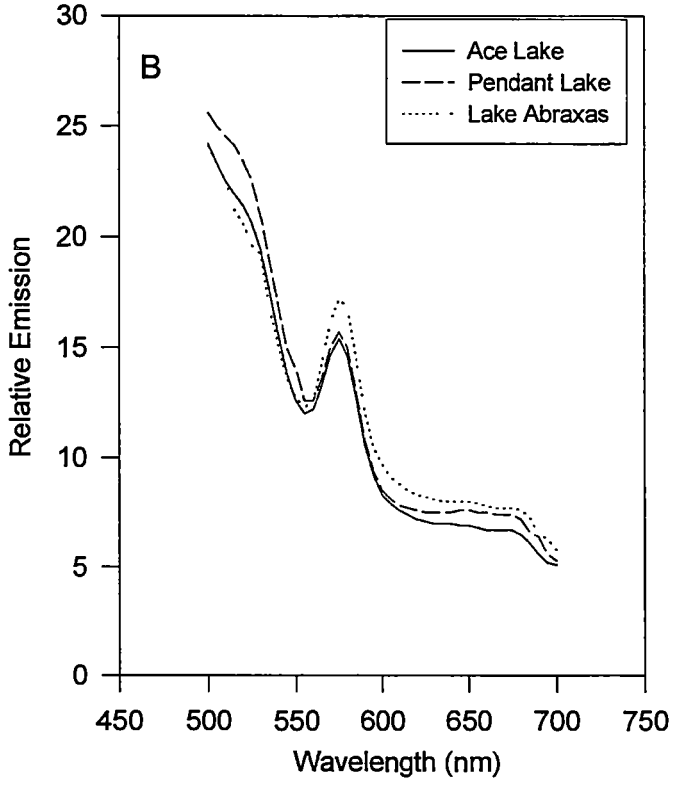
5
10
15
20
25
30
35
40
350
400
450
500
550
600
650
700


B
Ace Lake
Pendant Lake
Lake Abraxas
Relative Emission
Wavelength (nm)
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5
10
15
20
25
30
450
500
550
600
650
700
750

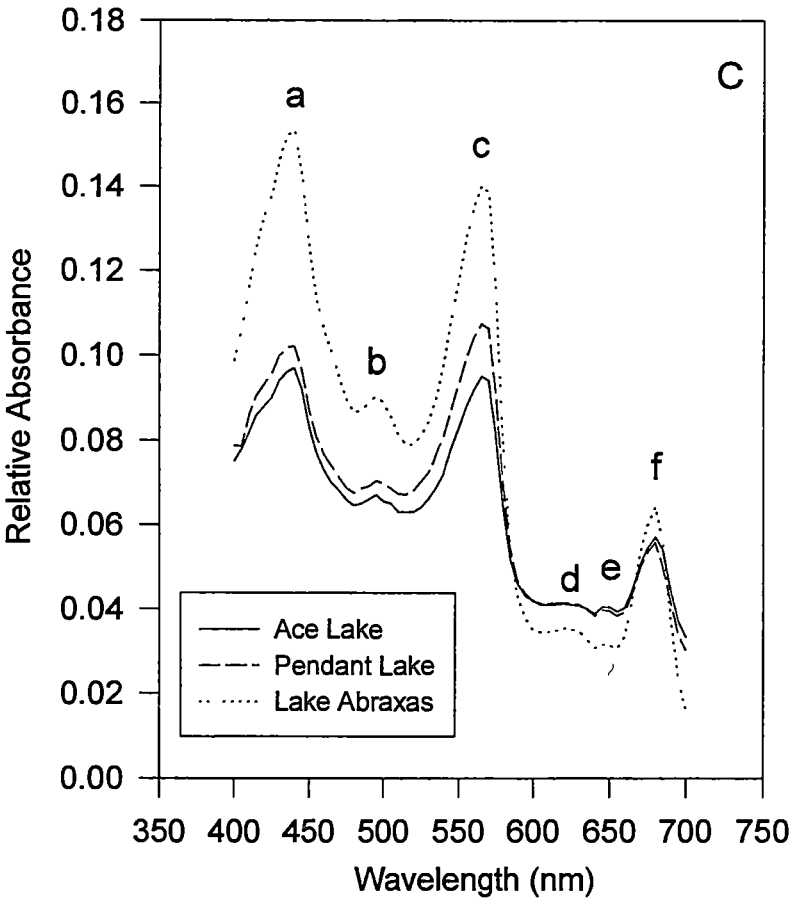

Figure 5.3: *In-vivo* A) Excitation spectra; B) Emission spectra and C) Absorption spectra from the three Antarctic *Synechococcus* strains. a) chlorophyll *a*; b) ββ-carotene; c) c- phycoerythrin; d) phycocyanin; e) allophycocyanin and f) chlorophyll *a*.

5.5.3.3 In-vivo excitation, emission and absorption spectra

The *in-vivo* spectra (excitation, emission and absorption) were the same for the three Antarctic strains of *Synechococcus* (Figure 5.3). The excitation spectra contained peaks from chlorophyll *a* (460 nm and 670 nm), phycoerythrin (570 nm), a shoulder, possibly from ββ - carotene or phycoeurobilin (500 nm), and a plateau from phycocyanin and allophycocyanin (615-635 nm).

The emission spectra contained a phycoerythrin peak at 575nm. The absorption spectra contained peaks from chlorophyll *a* (440 nm and 680 nm), possibly ββ- carotene (495 nm), a phycoerythrin peak (565 nm), a phycocyanin peak (620 nm) and an allophycocyanin peak (650 nm).

5.5.3.4 Lipid soluble pigment composition.

The Antarctic *Synechococcus* strains contained chlorophyll *a* (absorption maximum 431 nm), ββ- carotene and zeaxanthin as well as two unidentified, zeaxanthin like, carotenoids (Figure 5.4). The absorption spectra of the two unknown were similar. The retention time of the two pigments differed by 0.75 minutes. Unknown 'B' had absorption maxima at 454 nm and 483 nm and Unknown 'A' had absorption maxima at 455 nm and 485 nm (Figure 5.5).

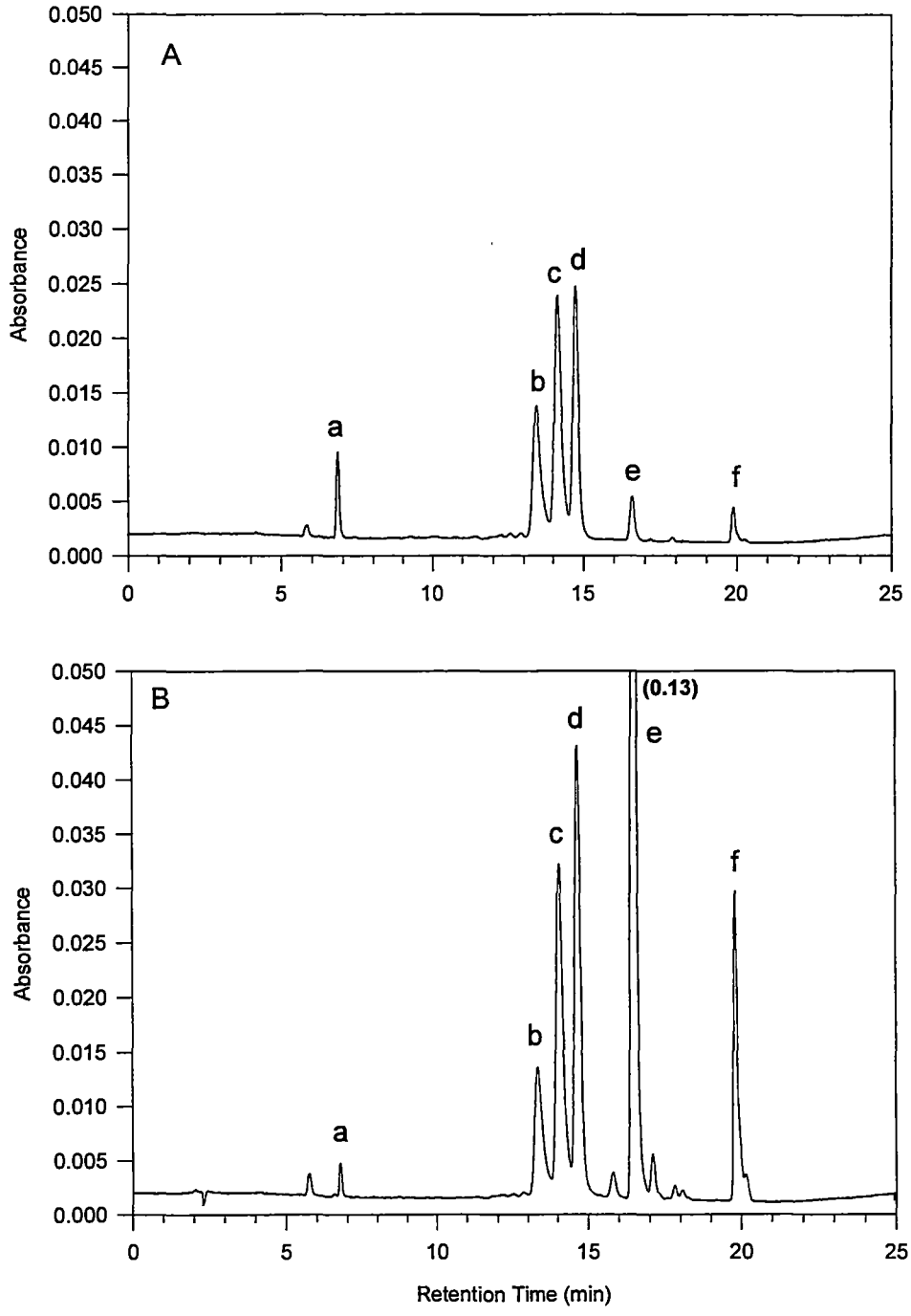

Figure 5.4: HPLC trace of lipid soluble pigments separated from the Ace Lake *Synechococcus* strain grown under A) high light intensity and B) low light intensity. a) me-chlorophyllide *a*; b) = unknown a; c = unknown a; d = zeaxanthin; e= chlorophyll *a* and f = ββ - carotene.

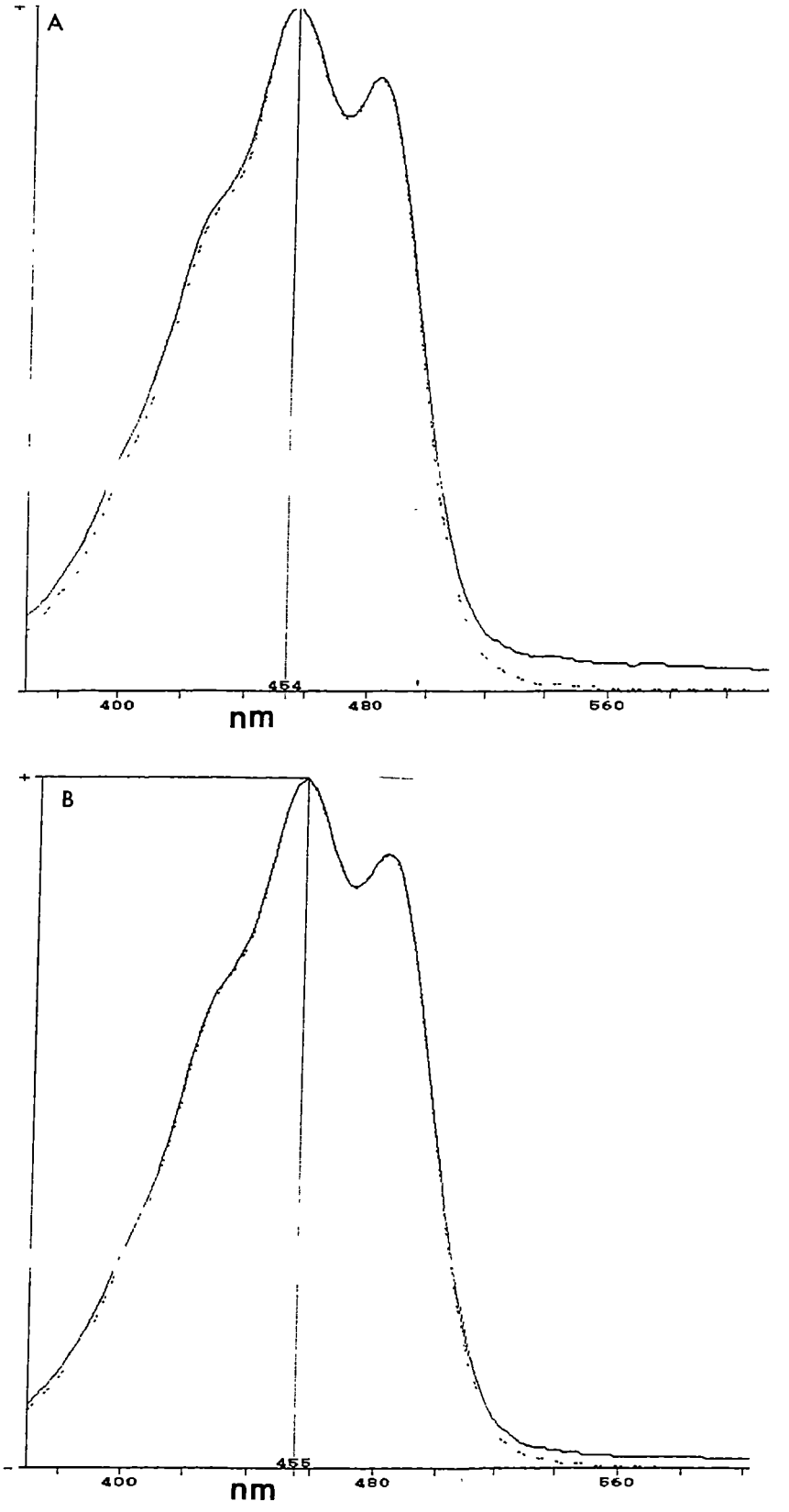

Figure 5.5: Absorption spectra from the unknown lipid soluble pigments isolated from the Ace Lake *Synechococcus* strain. A) Unknown a had a retention time of 13.435 minutes and an absorbance maximum of 454 nm; B) Unknown b had a retention time of 14.184 minutes and an absorbance maximum of 455 nm.

When grown under high light conditions (100 μmol photons m^{-2} s^{-1}), cells from the Ace Lake *Synechococcus* strain expressed a different lipid soluble pigment ratios to those expressed when cells were grown under low light (20 μmol photons m^{-2} s^{-1}), (Figure 5.4). In cells grown at the high light intensity, the zeaxanthin:chlorophyll *a* ratio was 4.5 where as in the low light grown cells the ratio was 0.34 (Table 5.6). In contrast the ratio of ββ- carotene:chlorophyll *a* was 0.4 in the high light grown cells and 0.23 in the low light grown cells. Zeaxanthin, unknown 'A' and unknown 'B' represented 36, 35 and 21 % respectively of the total carotenoids in the high light grown cells and 37, 27 and 11 % in the low light grown cells.

Table 5.6: Lipid soluble pigment composition (percent of total) in Ace Lake *Synechococcus* strain grown under high (100 μmol photons m^{-2} s^{-1}) and low light (20 μmol photons m^{-2} s^{-1}) intensities.

Pigment	High Light Intensity	Low Light Intensity
Chlorophyllide *a*	11	2
Unknown B	19	5
Unknown A	29	13
Zeaxanthin	30	17
Chlorophyll *a*	7	51
ββ - carotene	6	20

The Pendant Lake strain of *Synechococcus*, when grown under a low light intensity (20 μmol photons m^{-2} s^{-1}), had the same lipid soluble pigments as the Ace Lake strain, albeit in different ratios (Figure 5.6). It contained a chlorophyll *a* derivative (abs. max. 429 nm) but no obvious chlorophyll *a*, and it also had a high ratio of a chlorophyll *a* epimer (abs. max. 432 nm). Cis-ββ- carotene, a chlorophyll *a* allomere (abs. max. 423 nm) and a second chlorophyll *a* derivative (abs. max. 417 nm) were present in low concentrations.

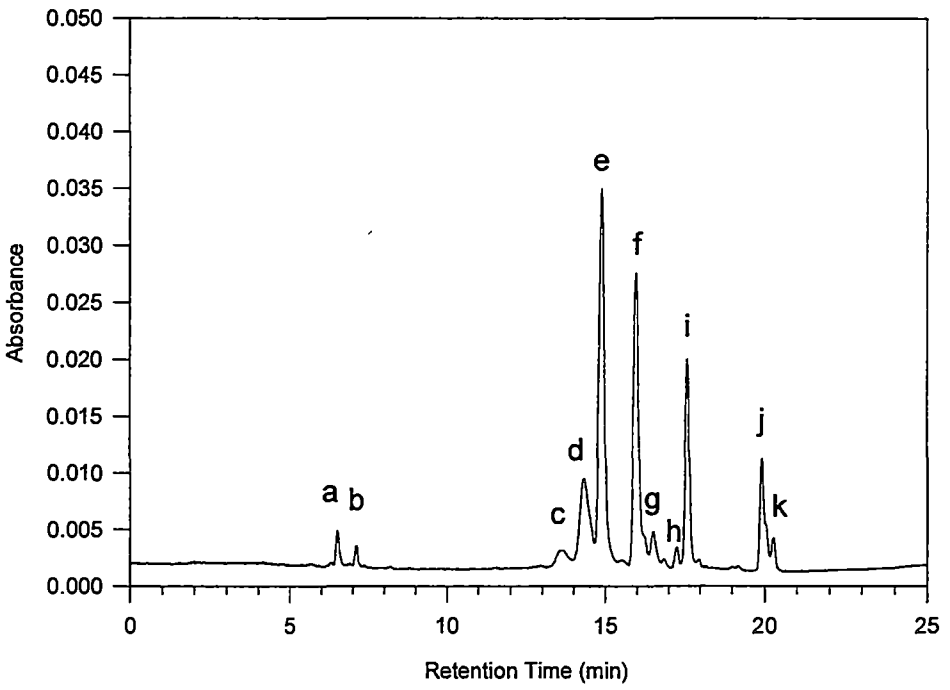

Figure 5.6: HPLC trace of the lipid soluble pigments extracted and separated from the Pendant Lake *Synechococcus* strain grown under a low light intensity. a) chlorophyllide *a*; b) me - chlorophyllide *a*; c) Unknown a; d) Unknown b; e) zeaxanthin; f) chlorophyll *a* derivative; g) chlorophyll *a* allomere; h) chlorophyll *a* derivative; i) chlorophyll *a* epimere; j) ββ- carptene and k) cis - ββ - carotene.

5.5.3.5 Lipid composition

Polar lipids comprised 99% (± 1%) of the total lipids in the two cultures of the Antarctic strain of *Synechococcus*. The fatty acid classes of the Ace Lake *Synechococcus* strain are presented in Table 5.7.

Table 5.7. Fatty acid composition of the Ace Lake strain of *Synechococcus*. Culture A was harvested during early exponential growth and culture B during late exponential growth. Fatty acids are designated x:yωz where x is the number of carbon atoms, y is the number of double bonds and z is the position of the last double bond from the terminal methyl group.

Fatty acid	Culture A	Culture B
14:1	4.7 (1.3)	7.1 (0.3)
14:0	18.1 (4.5)	28.0 (3.5)
15:1	-	0.2 (0.2)
15:0	0.4 (0.4)	0.6 (0.6)
16:1w7c	49.5 (2.4)	42.9 (0.5)
16:0	11.8 (1.0)	10.0 (2.0)
17:1	1.2 (0.1)	0.9 (0.9)
17:0	0.7 (0.7)	0.2 (0.2)
18:1w9c	1.4 (0.4)	0.5 (0.3)
18:1w7c	3.1 (0.9)	1.3 (0.1)
18:0	5.5 (3.0)	1.9 (1.2)
Unknown 1	1.0 (1.0)	0.3 (0.3)
Unknown 2	0.6 (0.6)	0.3 (0.3)
Unknown 3	Tr	-

tr = trace (< 0.1 %). Each data series is the average (SD in parentheses) of duplicate cultured strains of *Synechococcus*.

16:1ω7 was the dominant fatty acid (49.5% in culture A and 42.9% in culture B) followed by 14:0 (18.1% in culture A and 28% in culture B). Polyunsaturated fatty acids (PUFA's) were not detected in the cultures. There were minor differences in fatty acid composition between culture A, harvested during early log growth and culture B, harvested during late log growth. The percentage of 14:0 was higher and that of 16:1ω7

was lower in cells from culture B. 18:1ω9, 18:1ω7 and 18:0 comprised 10% of fatty acids in culture A and only 3.5% in culture B. The ratio of 14:1 to 14:0 and 16:1ω7 to 16:0 did not change with culture age. Two novel lipids were detected at low concentration in the Antarctic strain of *Synechococcus* (Figure 5.7a). These compounds were analysed by GC-MS. The two novel compounds had similar ring structures. Unknown 1 was tentatively identified as 2,4,6,-tri(dimethyl(benzyl)phenol and unknown 3 as 2,4-bis(dimethylbenzyl)-6-t-butylphenol (Figure 5.7b). Unknown '2' was not identified.

5.5.3.6 Phylogenetic analysis

The Ace Lake, Pendant Lake and Lake Abraxas strains of *Synechococcus* had DNA G + C contents of 57.0 ± 0.6 %, 58.2 ± 0.5 % and 57.7 ± 0.3% respectively.

The 16S rDNA genes of the Ace Lake and Pendant Lake strains were partially sequenced (Table 5.8). The sequences were 1456 base pairs long and were aligned from nucleotide position 17 to nucleotide position 1473 (*Escherichia coli* numbering). The phylogenetic tree (Figure 5.8) shows the evolutionary relationship between these three Antarctic strains of *Synechococcus*, other *Synechococcus* strains, *Prochlorococcus* spp., *Prochlorophytes*, *Phormidium* spp. and two strains from the Sargasso Sea. The Ace Lake strain and Pendant Lake strain were 99.7 % similar (Table 5.9). These two strains were 97 % similar to *Synechococcus* strain NIVA-CYA 328 and 96 % similar to *P. marinus*.

There was no evidence of heterozygosity in the sequences, meaning that there was only one strain per culture and that there was no contamination of the DNA by heterotrophic bacteria. The Pendant Lake strain was sequenced twice for confirmation. The 16S rRNA from the Lake Abraxas *Synechococcus* strain has been partially sequenced and it was closely related to the Ace Lake and Pendant Lake *Synechococcus* strains (Figure 5.8).

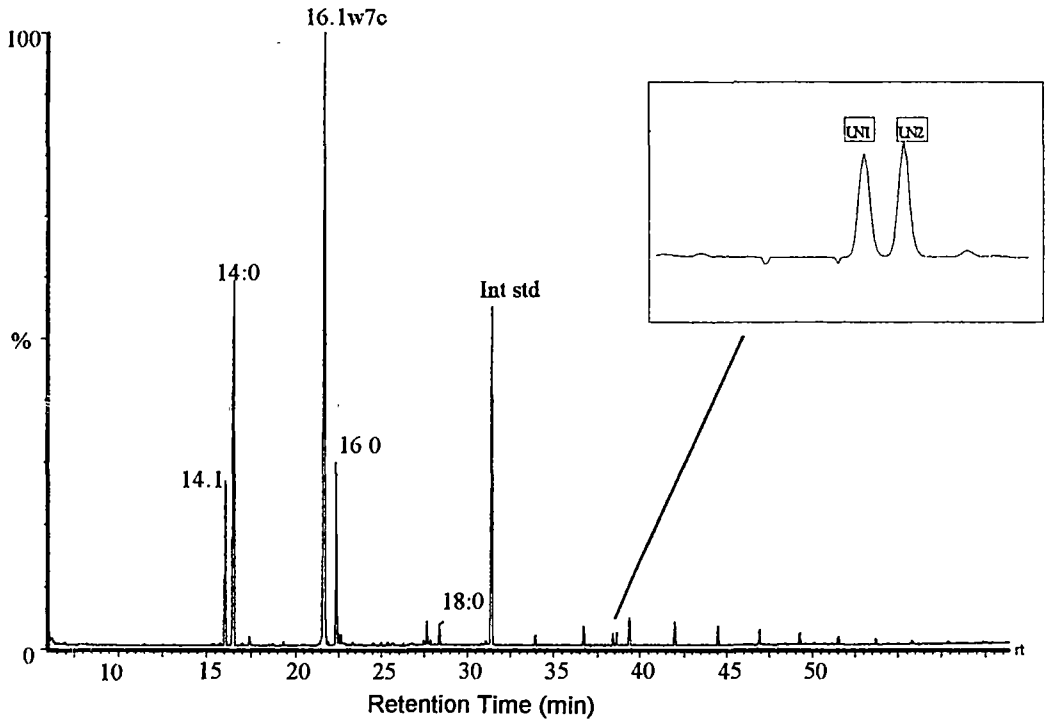

Figure 5.7a: Partial gas chromatogram of fatty acids from the Ace Lake *Synechococcus* strain. Int Std denotes internal standard. Abbreviations, fatty acid nomenclature is as defined in Table 5.7.

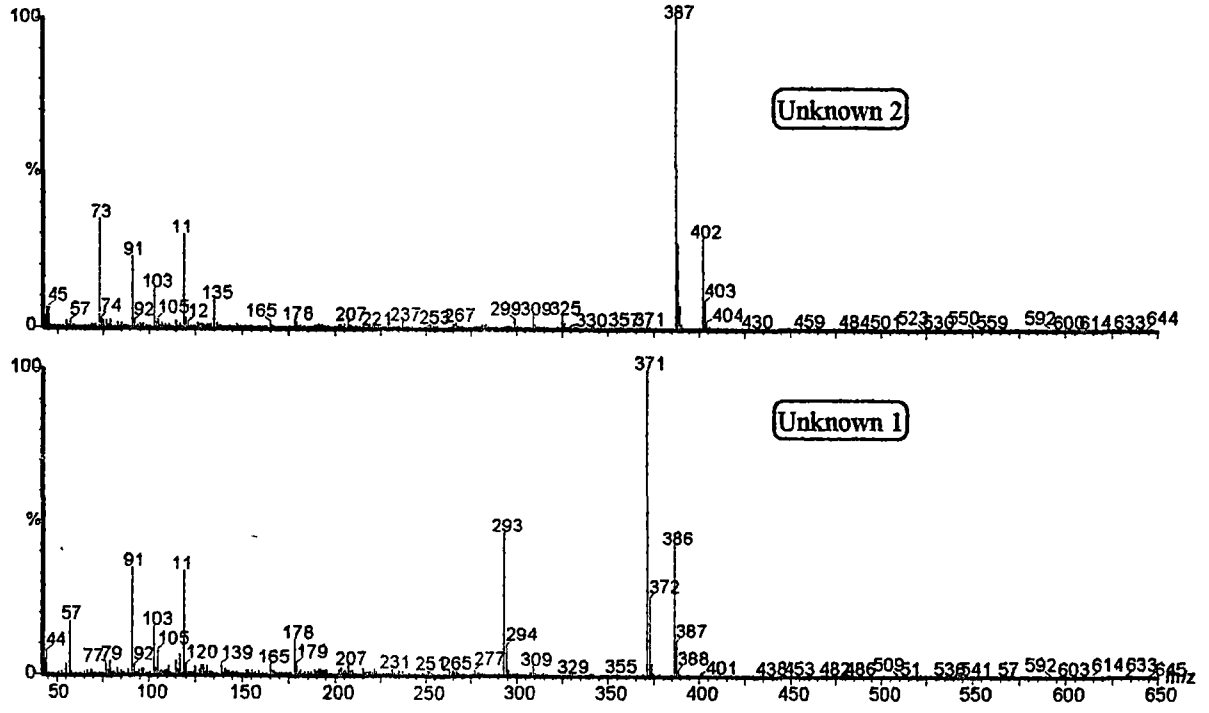

Figure 5.7b: Mass spectra of Unknown 1 and Unknown 2 in a fatty acid fraction isolated from the Ace Lake *Synechococcus* strain. Unknown 1 was tentatively identified as 2,4 -bis (dimethylbenzy) - 6 - butylphenol.

Table 5.8: Nucleotide sequence for 16S rDNA from the Ace Lake and Pendant Lake strains of *Synechococcus*. The Pendant Lake strain was sequenced twice (hence Pendant2).

Ace NNNNNNNNNNNNNNNNNNNNNNTCAGGATGAACGCTGGCGGCGTGCTTA
Pendant NNNNNNNNNNNNNNNNNNNNNNNNNNNNATGAACGCTGGCGGCGTGCTTA
Pendant2 NNNNNNNNNNAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCTTA

ACACATGCAAGTCGAAGCGGC---TTCG---GCTAGTGGCGGAAGGGTGAGTAACG
ACACATGCAAGTCGAAGCGGC---TTCG---GCTAGTGGCGGAAGGGTGAGTAACG
ACACATGCAAGTCGAAGCGGC---TTCG---GCTAGTGGCGGAAGGGTGAGTAACG

CGTGAGAATCTGCCCTCAGGAGGGGGATAACAGCTGGAAACGGCTGCTAATACCCCATAT
CGTGAGAATCTGCCCTCAGGAGGGGGATAACGGCTGGAAACGGCTGCTAATACCCCATAT
CGTGAGAATCTGCCCTCAGGAGGGGGATAACAGCTGGAAACGGCTGCTAATACCCCATNT

GCCGAGAGGTGAAA-CAATT-TCGCCTGAGGATGAGCTCGCGTCTGATTAGCTAGTTGGT
GCCGAGAGGTGAAA-CAATT-TCGCCTGAGGATGAGCTCGCGTCTGATTAGCTAGTTGGT
GCCGAGAGGTGAAA-CAATT-TCGCCTGAGGATGAGCTCGCGTCTGATTAGCTAGTTGGT

GAGGTAAGGGCTCACCAAGGCATCGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACA
GAGGTAAGGGCTCACCAAGGCATCGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACA
GAGGTAAGGGCTCACCAAGGCATCGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACA

CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAAT
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAAT
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTCCGCAAT

GGGCGCAAGCCTGACGGAGCAACGCCGCGTGAGGGACGAAGGCCTCTGGGCTGTAAACCT
GGGCGCAAGCCTGACGGAGCAACGCCGCGTGAGGGACAAAGGCCTCTGGGCTGTAAACCT
GGGCGCAAGCCTGACGGAGCAACGCCGCGTGAGGGACAAAGGCCTCTGGGCTGTAAACCT

CTTTTGTCAAGGAAGAAGACA-TGACGGTACTTGAGGAATAAGCCACGGCTAATTCCG
CTTTTGTCAAGGAAGAAGACA-TGACGGTACTTGAGGAATAAGCCACGGCTAATTCCG
CTTTTGTCAAGGAAGAAGACA-TGACGGTACTTGAGGAATAAGCCACGGCTAATTCCG

TGCCAGCAGCCGCGGTAATACGGGAGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCG
TGCCAGCAGCCGCGGTAATACGGGAGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCG
TGCCAGCAGCCGCGGTAATACGGGAGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCG

TCCGCAGGCGGCCCAGAAAGTCTGTTGTTAAAAAGTGGAGCTCAACTCCATCCAGGCAAT
TCCGCAGGCGGCCCAGAAAGTCTGTTGTTAAAAAGTGGAGCTCAACTCCATCCAGGCAAT
TCCGCAGGCGGCCCAGAAAGTCTGTTGTTAAAAAGTGGAGCTCAACTCCATCCAGGCAAT

GGAAACTACTGGGCTAGAGTGTGGTAGGGGCAGAGGGAATTCCCGGTGTAGCGGTGAAAT
GGAAACTACTGGGCTAGAGTGTGGTAGGGGCAGAGGGAATTCCCGGTGTAGCGGTGAAAT
GGAAACTACTGGGCTAGAGTGTGGTAGGGGCAGAGGGAATTCCCGGTGTAGCGGTGAAAT

GCGTAGATATCGGGAAGAACACCAGTGGCGAAGGCGCTCTGCTGGGCCATAACTGACGCT
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GCGTAGATATCGGGAAGAACACCAGTGGCGAATGCGCTCTGCTGGGCCATAACTGACGCT

CATGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGTAAAC
CATGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGTAAAC
CATGGACGAAAGCTAGGGGAGCGAAAGGGATTAGATACCCCTGTAGTCCTAGCCGTAAAC

GATGAACACTAGGTGTCGGGAGAATTATCCCTCTCGGTGTCGTAGCCAACGCGTTAAGTG
GATGAACACTAGGTGTCGGGAGAATTATCCCTCTCGGTGTCGTAGCCAACGCGTTAAGTG
GATGAACACTAGGTGTCGGGAGAATTATCCCTCTCGGTGTCGTAGCCAACGCGTTAAGTG

TTCCGCCTGGGGAGTACGCNCGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCAC
TTCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCAC
TTCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAATTGACGGGGGCCCGCAC

AAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACA
AAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACA
AAGCGGTGGAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACA

TCCTGCGAACCCCTGAGAAATCGGGGGGTGCCTTCGGGAACGCAGTGACAGGTGGTGCAT
TCCTGCGAACCTCTGAGAAATCGGAGGGTGCCTTCGGGAACGCAGTGACAGGTGGTGCAT
TCCTGCGAACCTCTGAGAAATCGGAGGGTGCCTTCGGGAACGCAGTGACAGGTGGTGCAT

Table 5.8 continued: Nucleotide sequence for 16S rDNA from the Ace Lake and Pendant Lake strains of *Synechococcus*.

GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCAC
GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCAC
GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCAC

GTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAAAGACCGCCGGTGA
GTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAAAGACCGCCGGTGA
GTCTTTAGTTGCCAGCATTTAGTTGGGCACTCTAAAAAGACCGCCGGTGA

TAAACCGGAGGAAGGTGTGGATGACGTCAAGTCATCATGCCCCTTACGTCCTGGGCTACA
TAAACCGGAGGAAGGTGTGGATGACGTCAAGTCATCATGCCCCTTACGTCCTGGGCTACA
TAAACCGGAGGAAGGTGTGGATGACGTCAAGTCATCATGCCCCTTACGTCCTGGGCTACA

CACGTACTACAATGCTACGGACAAAGGGCAGCAAACTCGCGAGAGCTAGCAAATCCCAT
CACGTACTACAATGCTACGGACAAAGGGCAGCAAACTCGCGAGAGCTAGCAAATCCCAT
CACGTACTACAATGCTACGGACAAAGGGCAGCAAACTCGCGAGAGCTAGCAAATCCCAT

-AAACCGTGGCTCAGTTCAGATCGTAGGCTGCAACTCGCCTGCATGAAGGAGGAATCGCT
-AAACCGTGGCTCAGTTCAGATCGTAGGCTGCAACTCGCCTGCATGAAGGAGGAATCGCT
-AAACCGTGGCTCAGTTCAGATCGTAGGCTGCAACTCGCCTGCATGAAGGAGGAATCGCT

AGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
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AGTAATCGCAGGTCAGCATACTGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG

TCACACCATGGAAGTTGGCCATGCCCGAAGTCGTTACTCCAACCC-GCAAGGGAGGGGC
TCACACCATGGAAGTTGGCCATGCCCGAAGTCGTTACTCCAACCC-GCAAGGGAGGGGC
TCACACCATGGAAGTTGGCCATGCCCGAAGTCGTTACTCCAACCC-GCAAGGGAGGGGC

TCACACCATGGAAGTTGGCCATGCCCGAAGTCGTTACTCCAACCC-GCAAGGGAGGGGC
TCACACCATGGAAGTTGGCCATGCCCGAAGTCGTTACTCCAACCC-GCAAGGGAGGGGC
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("-" = alignment gaps)

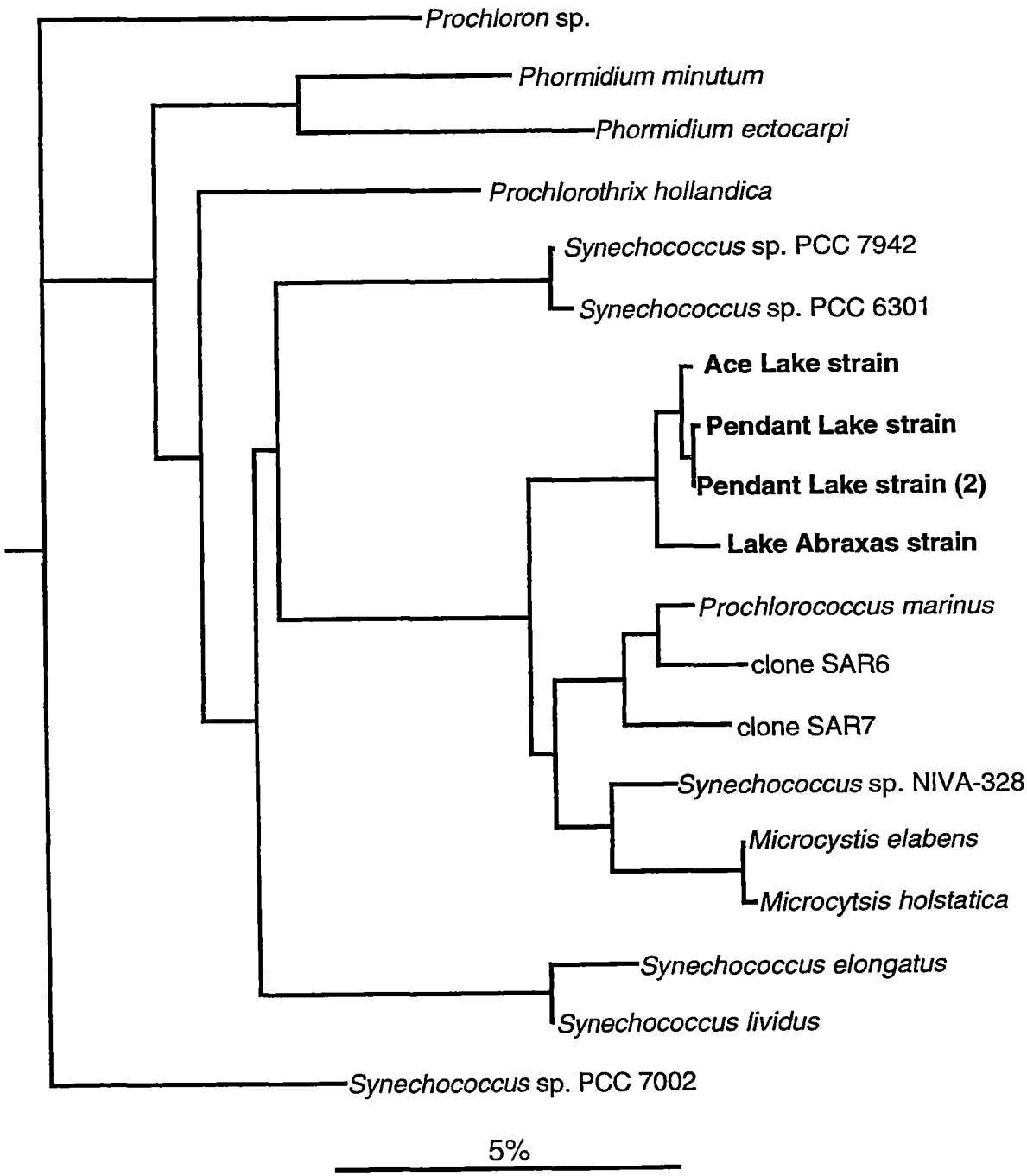

Figure 5.8 Phylogenetic tree showing the evolutionary relationship between the three Antarctic *Synechococcus* strains and other closely related cyanobacteria. The 16S rDNA sequences were aligned with other sequences downloaded from the GeneBank internet site. PHYLIP (v3.5) (Felsenstein, 1993) was utilized for phylogenetic analysis, and evolutionary distances were determined with the maximum likelihood algorithm by using the DNADIST program. Rooted phylogenetic trees were obtained using the Neighbor.

Table 5.9: 16S rDNA sequence similarity between *Synechococcus* strains and *Prochlorophytes*

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
							Sequence Similarity(%):										
1. Ace Lake strain	100																
2. Pendant Lake strain	99.6	100															
3. Pendant Lake strain (2)	99.7	99.9	100														
4. *Synechococcus* sp. NIVA-CYA 328	97.0	96.6	96.6	100													
5. *Prochlorococcus marinus*	95.6	95.7	95.6	95.4	100												
6. Clone SAR7	94.7	94.6	94.7	94.5	97.0	100											
7. Clone SAR6	93.9	94.0	93.9	95.4	98.2	96.9	100										
8. *Synechococcus leopoliensis*	91.0	90.7	90.7	90.0	89.4	88.4	90.0	100									
9. *Synechococcus* sp. PCC6301	89.0	88.9	89.2	88.8	88.6	89.3	89.4	99.3	100								
10. *Synechococcus* sp. PCC7942	89.9	89.9	90.0	89.2	88.9	89.2	89.2	99.8	99.6	100							
11. *Synechococcus lividus*	89.1	89.0	89.0	87.5	88.2	88.5	89.1	90.5	91.1	91.5	100						
12. *Prochlorothrix hollandica*	88.5	88.4	88.8	86.4	87.9	88.2	88.4	92.2	91.0	90.8	90.3	100					
13. *Synechococcus elongatus*	87.6	87.4	87.4	85.0	87.4	87.7	87.7	86.9	89.1	88.9	98.8	89.1	100				
14. *Phormidium minutum*	86.7	86.3	86.6	83.0	85.7	87.2	86.8	88.2	89.0	88.9	88.5	89.5	87.4	100			
15. *Phormidium ectocarpi*	86.6	86.2	86.2	82.6	84.3	84.9	84.8	86.4	87.5	87.6	87.9	89.0	84.3	92.4	100		
16. *Synechococcus* sp. PCC7002	84.9	84.9	84.9	82.9	86.5	86.9	86.8	84.9	88.1	88.7	89.2	89.2	88.1	89.8	86.8	100	
17. *Prochloron* sp.	84.8	84.8	84.9	82.1	83.0	85.1	84.8	95.9	86.7	86.9	88.8	88.1	88.3	87.9	85.5	90.1	100

5.5.4 Physiological characteristics of Antarctic *Synechococcus* strains.

5.5.4.1 Optimal growth temperature

Growth rates for the Ace Lake strain of *Synechococcus* were determined over the range -1° C to 30° C. Growth was detected between 1.7° C and 28° C. The growth rates were slow, even at the optimal growth temperatures. Growth rates and generation times for the Ace Lake *Synechococcus* strain grown between 1.7° C and 28° C are given in Table 5.10.

Table 5.10: Predicted growth rates and generation times based on the square root model for the Ace Lake strain of *Synechococcus* grown between 1° C and 28° C. Actual growth rates and generation times were close to those predicted.

Temperature (° C)	Growth Rate (k) days^{-1}	Generation Time (Days)
1.7	0.038	18.0
3.3	0.040	17.3
5.3	0.049	14.2
7.9	0.068	10.2
9.6	0.088	7.9
11	0.091	7.6
12.1	0.092	7.5
13.7	0.097	7.1
15.1	0.089	7.8
16.2	0.094	7.3
19.8	0.113	6.1
22.3	0.132	5.3
24.6	0.077	8.9
25.6	0.068	10.1
27.4	0.033	20.9

Actual growth rates fitted well to the square root model for predicting theoretical minimum and maximum growth temperatures (Figure 5.9).

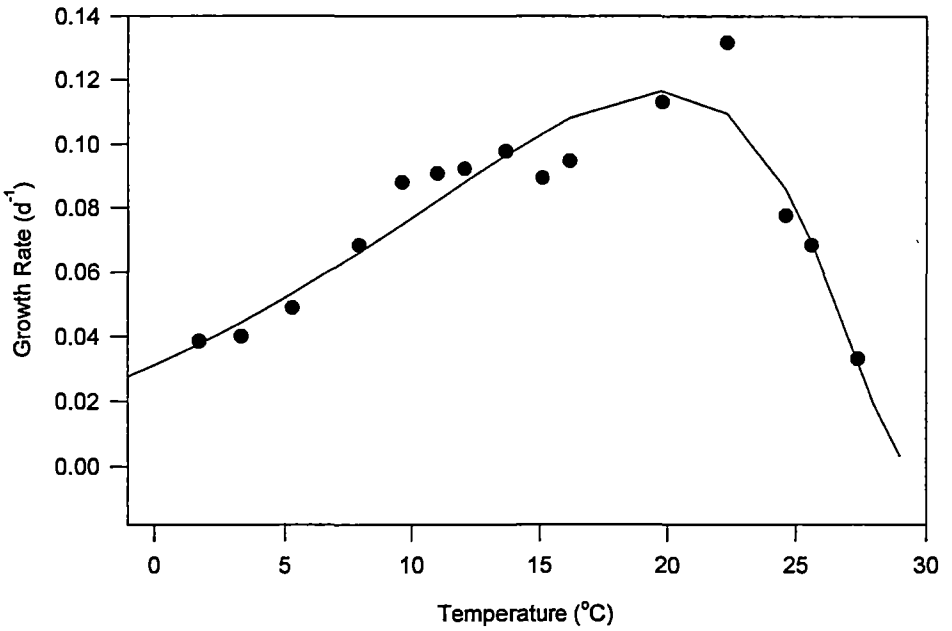

Figure 5.9: Theoretical growth curve, obtained from the square root model (Ratkowsky et al. 1983), for the Ace Lake *Synechococcus* strain. Actual data points obtained in the temperature gradient experiments are shown. Irradiance was 20 μmol photoms m^{-2} s^{-1}.

The equation for the square root model (Ratkowsky et al. 1983) based on growth rates between 1.7° C and 28° C was: $\sqrt{k} = 0.001484(T-256.1)(1-\exp(0.22994(T-302.71)))$. The theoretical minimum, optimum and maximum growth temperatures and their confidence limits are given in Table 5.11.

Table 5.11: Predicted minimum, optimum and maximum growth temperatures for the Ace Lake *Synechococcus* strain, based on the 'square root model' (Ratkowsky et al. 1983).

	Theoretical Temp. (° C)	Confidence Limits (° C)
T (min)	-17.1	-25.6 to -8.6
T (opt)	19.7	-
T (max)	29.55	28.3 to 30.8

Cardinal growth temperatures, based on Rosso's model (Rosso et al. 1993) are given in Table 5.12. Predicted minimum and maximum growth temperatures were similar to those predicted by the 'square root model'.

Table 5.12: Predicted minimum, optimum and maximum growth temperatures for the Ace Lake *Synechococcus* strain, based Rosso's model (Rosso et al. 1993).

	Theoretical Temp. (° C)	Confidence Limits (° C)
T (min)	-13.7	-23.2 to -4.1
T (opt)	19.7	18.0 to 21.5
T (max)	28.2	27.2 to 29.3

5.5.4.2 Effect of Light Intensity on fluorescence emission

Exposure of the Ace Lake *Synechococcus* strain to 'high' and 'low' light intensities altered both the orange and red fluorescence flow cytometric relative to the control (Figure 5.10). Exposure of cells to light of a lower intensity (5 μmol photons m^{-2} s^{-1}) than the control (20 μmol photons m^{-2} s^{-1}) increased both the orange and red fluorescence signals relative to the control (Table 5.13).

Table 5.13: Relative changes (as a percent of the control) in orange (FL2) and red (FL3) fluorescence, and in the ratio of FL2/FL3, in the Ace Lake strain of *Synechococcus* when exposed to 'high' and 'low' light intensities. Arrows indicate an increase (↑) or a decrease (↓) in fluorescence intensity.

*μmol photons m^{-2} s^{-1}	FL2 signal	FL3 signal	FL2/FL3
20 (12h) - Control			11
5 (24 h)	409 % ↑	326 % ↑	13
20 (24 h)	20 % ↓	54 % ↑	6
300 (24 h)	90 % ↓	45 % ↓	2

* numbers in brackets are hours per day that cells were exposed to the lightintensity

Exposure of cells to a high light intensity (300 μmol photons m^{-2} s^{-1}) relative to the control resulted in decreased fluorescence signals. There was no apparent change in cell volume (FSC). Cell numbers in cultures exposed to low (5 μmol photons m^{-2} s^{-1}) light intensity decreased relative to the control (Figure 5.11) (t-test, $P < 0.0001$, $n = 4$).

5.5.4.3 Effect of salinity on growth

Salinity affected the growth of the Ace Lake *Synechococcus* strain (ANOVA, $p<0.0001$, $n = 3$). There was not a significant difference in the growth of cells grown in medium based on water collected from 11 m in Ace Lake (30 g kg^{-1}) water and medium that was 2/3 (20 g kg^{-1}) the salinity of Ace Lake water collected from 11 m (SNT test, $p> 0.05$, $n = 3$). There was however a significant reduction in growth between other treatments (Table 5.14).

Table 5.14: Pairwise multiple comparisons (SNT test, $P < 0.05$, $n = 3$) of changes in cell number, of Ace Lake *Synechococcus* strain, grown at different salinities

Salinity (g kg^{-1})	Reduction in growth
30 v's 20	No
30 v's 10	Yes
30 v's 0	Yes
20 v's 10	Yes
20 v's 0	Yes
10 v's 0	Yes

Based on flow cytometric forward angle light scatter (FSC) and fluorescence emission signals, there was no observed change in relative cell volume or fluorescence emission intensity in FL2 (orange) or FL3 (red) due to the salinity changes.

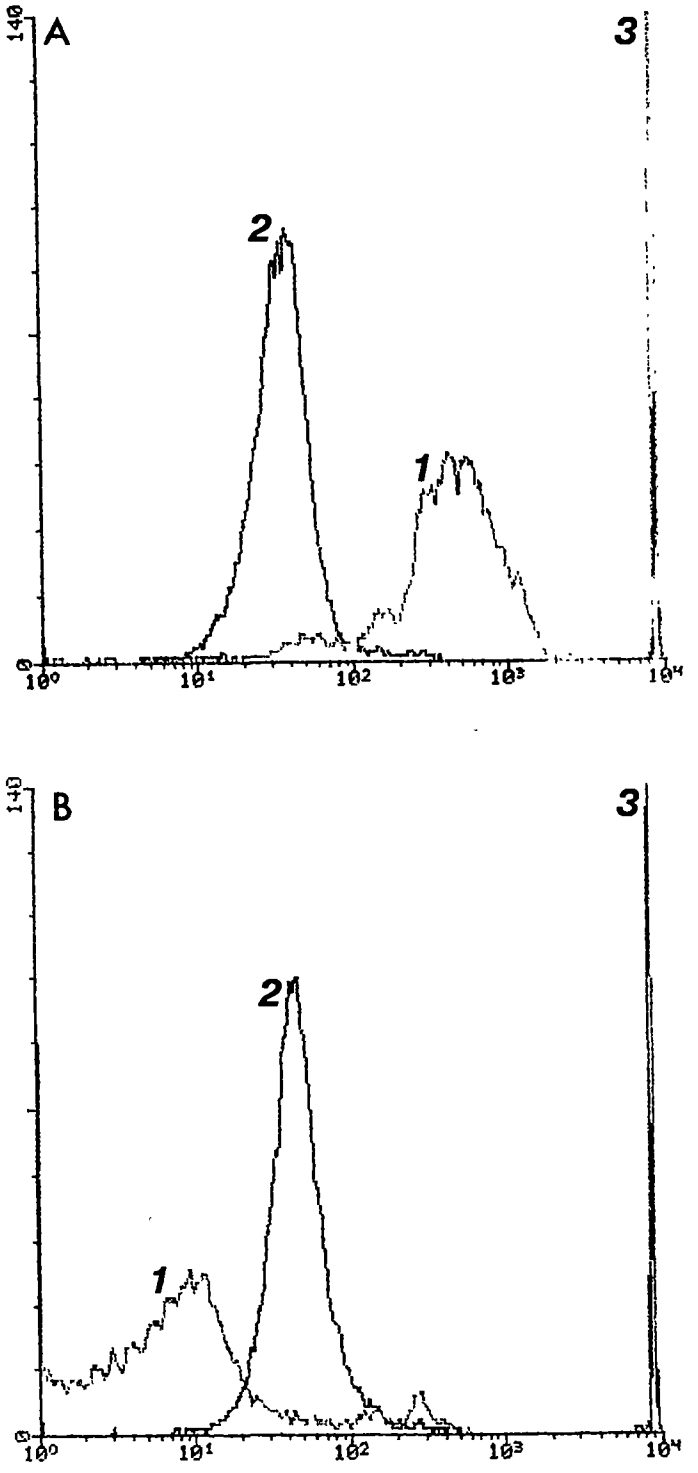

Figure 5.10: Flow cytometric histograms showing the relative change in orange fluorescence (x-axis) between of the Ace Lake *Synechococcus* strain grown under low light intensity, 5 μmol photons m^{-2} s^{-1}, (A) and high light intensity, 300 μmol photons m^{-2} s^{-1}, (B). The X axis is on a log scale and represents FL2. The Y-axis represents the relative number of individual events. 1 = treatment, 2 = control (20μmol photons m^{-2} s^{-1} for 12 h per day) and 3 = calibration beads.

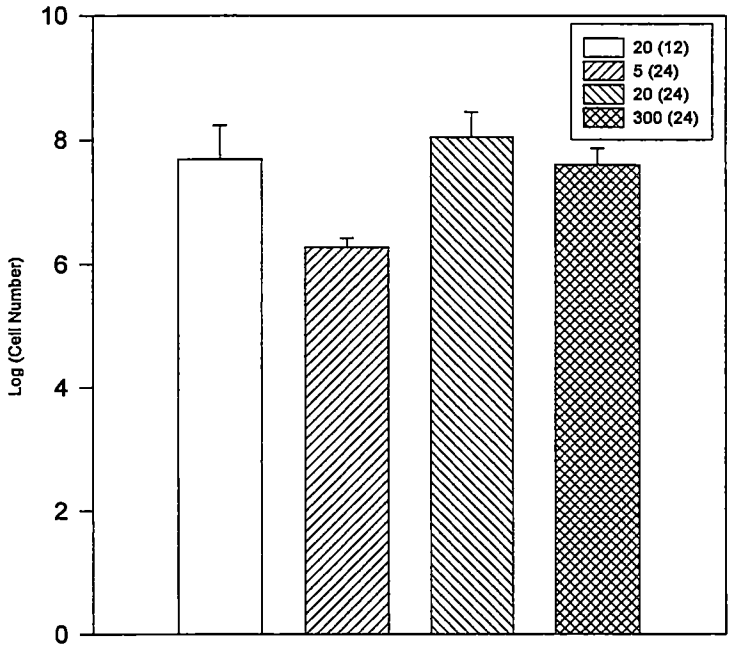

Figure 5.11: Effect of light intensity of the growth, in culture, of the Ace Lake *Synechococcus* strain. Cultures grown at a low light intensity (5 μmol photons m^{-2} s^{-1}) had significantly less cells than cultures grown at 20 μmol photons m^{-2} s^{-1} and 300 μmol photons m^{-2} s^{-1} after thirteen days exposure. Error bars represent 95 % confidence limits. Numbers in brackets indicate the time (hours) per day that cells were exposed to the light intensity indicated.

5.6 Discussion

It was a relatively easy process to obtain the enrichment cultures of the three Antarctic *Synechococcus* strain as they occurred in high numbers in the lakes and cycloheximide treatment was successfully used to eliminate eukaryotic organisms. Eliminating heterotrophic bacteria from cultures was a more difficult process and was in fact only attempted with the Ace Lake strain. Unless an external carbon source was added to the culture, the heterotroph remained at back ground levels (<1 %). It is known (Stanier et al. 1971) that heterotrophic bacteria are harbored in the extracellular slime of many cyanobacteria, which makes purification by serial dilution difficult. The Ace lake strain grew on solid media but growth was inconsistent and it was difficult to isolate single colonies. With further modification and optimisation of this method, it should be possible to obtain axenic cultures of these three strains as has been achieved with other *Synechococcus* strains (Castenholz 1969; El Hag 1986; Stanier et al. 1971; Waterbury et al. 1986).

Not one of the three Antarctic strains would grow in seawater collected from a rocky coastal site, yet all grew well in seawater collected from a deep oceanic site. The reason for this is not known but it could have been due to impurities in the water at the coastal site that may have entered from the local catchment. No attempt was made to analyses the quality of the coastal seawater, but it was routinely used for growing krill which are known to be sensitive to water quality (P. Cramp, personal communications).

The three strains of *Synechococcus* are now stored with the phototroph culture collection at CSIRO marine laboratories, Hobart, Australia. They are growing in G.P. media based on seawater (Appendix E), at 4° C, under 10 μmol photons m^{-1} s^{-1}. They are subcultured at three monthly intervals. At this stage the three strains are referred to as the Ace Lake strain, the Pendant Lake strain and the Lake Abraxas strain. They are awaiting culture collection strain numbers.

Isolation of *Synechococcus* were attempted from the other meromictic sites and the coastal marine site, as *Synechococcus* may have been present in numbers too low for detection by flow cytometry. No cultures were obtained using undiluted lake water samples as the inoculum. It is possible that cultures could be obtained, especially from the marine site where *Synechococcus* has been previously reported (Walker and Marchant 1989), by concentrating the inoculum by filtration or centrifugation.

5.6.1 Taxonomic characterisation

The cell sizes of the three Antarctic strains was consistent with other members of the group *Synechococcus*. Marine cluster A has a size range of 0.6 to 1.7 μm (Waterbury and Rippka 1989).

Fluorescence characteristics indicated the cells contained phycoerythrin. The cells were orange-red under green light and yellow-orange under blue light. As the cultures were not obtained from single celled isolates it was possible that there was some genetic variability in the cultures. Cells from each strain, however, had uniform fluorescence under green light, indicating that the ratio of phycoerythrin to other phycobiliproteins

was consistent (Alberte et al. 1984; Wood et al. 1985). The ratio of phycoerythrin to other phycobilliproteins is supposedly an intrinsic characteristic of a strain that is not affected by culture age or by the light intensity to which cells are exposed (Wood et al. 1985).

The difference in uniformity of size between those cells measured and the cells in Figure 5.1 is probably due to the technique by which the cells were prepared for microscopy. The cells were photographed on agarose coated slides whereas the cells that were used for size measurements were filtered onto irgulan black stained filters.

The Ace Lake strain had ultrustructural features consistent with other strains of the *Synechococcus* group (Edwards et al. 1968; Fogg et al. 1973; Gantt and Conti 1969; Golecki 1979). The thylakoid membranes were loosely arranged around the outside of the cells and the phycobilisoms lined the edges of the thyakoids (Figure 5.2). The phycobilisoms, which are the light harvesting complexes in these organisms, were 20.3 ± 5.7 nm in diameter. This is consistent with the size range (20 - 70 nm) given by Allen, (1984) and Glazer, (1982) for cyanobacteria.

The polyhedral bodies, named because of their polygon shape, were slightly smaller in size than the size reported by others (0.1 - 0.7 μm; Allen 1984). Little is known of the function of these structures but they have also been called carboxysomes and are thought to store the photosynthetic enzyme ribulose-1,5-bisphosphate carboxylase RUBPase (Allen 1984). One hypothesis for their existence is that they protect the enzyme from oxygen radical damage when cells grow in a high oxygen environment (Joint 1986). If this is the case it is not surprising that they would be present in cells

isolated from Ace Lake where the oxygen concentration can be greater than 150 percent saturation.

Like all other cyanobacteria, *Synechococcus* cells contain chlorophyll *a* as their primary photosynthetic pigment and phycobiliproteins as accessory light harvesting pigments (Waterbury and Rippka 1989). The abundance of the phycobiliproteins is responsible for the colour of cyanobacterial cells. Cyanobacteria which contain no phycoerythrin, for example many of the freshwater strains (Stockner and Antia 1986; Vezina and Vincent 1997) and some marine strains of *Synechococcus* (Stockner and Antia 1986; Waterbury et al. 1986), appear blue-green. Cyanobacteria such as the Antarctic strains of *Synechococcus*, which contain relatively high concentrations of phycoerythrin, appear reddish-orange (Kana et al. 1988). *Prochlorococcus marinus* does not contain phycobiliproteins (Goericke and Repeta 1992).

The absorption peak at 500 nm (Figure 5.3) was probably from ββ- carotene (Guillard et al. 1985). (β-carotene, unlike the xanthophyll pigments, is involved in light harvesting and occurs in all strains of *Synechococcus* thus far studied (Kana et al. 1988).

Identical phycobiliprotein absorption, excitation and emission peaks were obtained for the three Antarctic *Synechococcus* strains. In these strains, the phycoerythrin absorbed at 565 nm and emitted at 575 nm. This is typical of c-PE; c standing for cyanobacteria (Cohen-Bazire and Bryant 1982). Many marine *Synechococcus* strains posses phycobiliproteins composed of both phycoerythrobilin (PEB) and phycourobilin (PUB) (Alberte et al. 1984; Olson et al. 1990b). This gives them a wider absorption range in the blue-green range of the spectrum, possibly resulting in a competitive advantage for

photosynthesis at greater depths in the water column (Wood 1985; Yentsch and Phinney 1985) or under the ice in Antarctic lakes (Burch 1988). Even without phycourobilin, cyanobacteria that possess phycoerythrin have an advantage over organisms that only possess chlorophylls and even over those organisms that use phycocyanins as their major light harvesting pigments.

The expression of the phycobiliproteins in marine *Synechococcus* seems to be independent of culture age and light intensity, indicating that it is constitutively expressed. The concentration of phycoerythrin, as well as chlorophyll *a* and ββ-carotene in a cell is, however, dependent on light intensity (Wood et al. 1985). A change in the concentration of phycoerythrin and chlorophyll *a* was observed, through an increase or decrease in fluorescence intensity, in the Ace Lake *Synechococcus* strain when it was grown at different light intensities.

Zeaxanthin is a common pigment found in the *Synechococcus* group and has been used as a chemical signature to identify the picocyanobacteria in environmental samples (Guillard et al. 1985). ββ- carotene and zeaxanthin were the only two carotenoids found in the Scientific Committee for Scientific Research (SCOR) reference strain of *Synechococcus* (DC-2) (Wright et al. 1991). The unknown carotenoid compounds in the Antarctic strains were not found in the SCOR strain or in other *Synechococcus* strains reported in literature. The role of the two unknown carotenoids is not known but they occurred in significant quantities (approximately 30% of total carotenoid for Unknown A and 16 % of total carotenoid for Unknown B).

Zeaxanthin is considered to play a role in protecting cells from photooxidative damage at high light intensities (Siefermann-Harms 1987). The unknown pigments had absorption spectra similar to that of zeaxanthin and could also play a role in protecting cells from damage at high light intensities. ββ - carotene, the chlorophyll molecules and the phycobiliproteins are all part of the light harvesting complex and the concentration of these pigments in the cells is dependent on light intensity and light quality (Kana et al. 1988; Moore et al. 1995). Unlike ββ -carotene, the proportion of zeaxanthin in cells is not dependent on light intensity (Kana et al. 1988). When the Ace Lake *Synechococcus* strain was grown under high light conditions, there was a substantial decrease in the proportion of the light harvesting pigments compared to cells grown at low light intensities. There were small differences in the ratio of zeaxanthin and the unknown carotenoids to total lipid soluble pigments, between the high light and low light grown cells, but they did not appear to be affected by light intensity to the same degree as the light harvesting pigments.

It is not known why the Pendant Lake strain had more chlorophyll *a* derivatives than the Ace Lake strain. The cells were slightly bigger and appeared to produce more mucus, even when grown under the same conditions, which indicates that the strain is physiologically different. It is possible that some of these derivatives are present in the Ace Lake strain of *Synechococcus* and would be detected if the pigments had been extracted from a greater number of cells.

Lipid soluble pigments from the Lake Abraxas strain have not been analysed but it is anticipated that they would be the same as the Ace Lake strain. The lipid soluble pigment signature from Lake Abraxas is similar to that from Ace Lake (Chapter 4).

The Ace Lake strain of *Synechococcus* possessed a fatty acid profile similar to other strains of *Synechococcus* (Kenyon 1972; Merritt et al. 1991). The lack of free fatty acids indicated that lipid degradation had not occurred and the absence of triglyceride reflected that the cyanobacteria were viable and were not storing lipid. Cyanobacteria do not store large amounts of triacylglycerides (Merritt et al. 1991). The fatty acids 16:1 and 14:0 are the dominant fatty acids in other marine and freshwater strains of *Synechococcus* although the ratio of 14:0 to 16:1ω7 varied between strains (Kenyon 1972; Merritt et al. 1991). In one study 14:0 occurred in higher concentrations than 16:1ω7 (Merritt et al. 1991) and in another 16:1ω7 occurred in higher concentrations than 14:0 (Kenyon 1972). Only trace levels of PUFA's (16:2, 18:2, 18:3α and 18:3λ) have been found in some marine and freshwater strains (Kenyon 1972; Merritt et al. 1991).

Culture age did not significantly affect the fatty acid composition in the Ace Lake strain (Table 5.7) which is a finding consistent with other members of the group *Synechococcus* (Merritt et al. 1991). Culture conditions including light, temperature and salinity changed the percentages of fatty acids in photosynthetic eukaryotes (Barrett et al. 1995; Nichols et al. 1991; Volkman and Nichols 1991) and would probably do so in *Synechococcus*.

Three unique phenol containing compounds were detected in low quantities. It is possible that these compounds were artifacts but as they were detected in a field sample from Ace Lake (Chapter 3) and their structural derivatives have been observed in the lake (Skerratt et al. 1991) it is probable that they are not artifacts. The unknowns were

found in higher concentrations in culture A than culture B indicating that they are present in more metabolically active cells. The role of these phenol based compounds remains to be determined.

The DNA base compositions of the Ace Lake, Pendant Lake and Lake Abraxas strains of *Synechococcus* were 57.0, 58.2 and 57.7 mol % respectively. DNA base composition, which is constant for a given organism, has become a fundamental character for classification of bacteria. Waterbury et al., (1986) has determined the base ratios of 27 strains from Marine Cluster A and the base ratios have fallen between 54.9 and 62.4 mol % G + C. According to Bergey's Manual of Systematic Bacteriology, the mol % G + C of the DNA ranges from 55 to 62 for this cluster (Waterbury and Rippka 1989). Therefore, according to characterisation based on G + C ratio, the three Antarctic strains fit into Marine Cluster A. *Prochlorococcus marinus*, has a DNA base composition ranging from 33- 40 mol % (Palenik 1994).

The phylogenetic similarity between marine *Synechococcus* strains and *P. marinus* has been previously reported (Mullins et al. 1995; Palenik 1994; Urbach et al. 1992; Wood and Townsend 1990). The Ace Lake and Pendant Lake strains are most closely related to *Synechococcus* strain NIVA-CYA 328, which was isolated from Oslofjord (60° N), (Rudi et al. 1997). This *Synechococcus* cluster has a 16S rDNA similarity of 95 % with *P. marinus*. The ecological implications of this are not known except that Ace Lake was isolated from a polar fjord more than 2000 years ago (Burton and Barker 1979) and that one isolate came from a region close to the northern polar sea and the others from southern polar marine derived lakes. Based on phylogenetic information (Mullins et al.

1995), the similarity between the Antarctic strains of *Synechococcus* and the reference strain of Marine Cluster A (WH 8103), is approximately 93%.

5.6.2 Physiological characterisation.

Cyanobacteria occupy a wide range of thermal environments and also show a high degree of adaptation to their environments (Fogg et al. 1973). Many of the freshwater strains of *Synechococcus* have t_{opt} of approximately 35° C and a t_{max} greater than 40° C (Kruger and Eloff 1978). Strains of *Synechococcus* from Marine cluster A, which are the organisms that are probably the most closely related to the Antarctic strains of *Synechococcus* had T_{opt} between 20-28° C and T_{max} at 30-35° C (Moore et al. 1995; Waterbury et al. 1986). These organisms were isolated from tropical and temperate oceans and their optimum and maximum growth temperatures are close to that of the Ace Lake strain (Table 5.11). The *P. marinus* strains, SS120 (Sargasso Sea) and MED4 (Mediterranian Sea), had a t_{min} of 12° C, t_{opt} of 24° C and t_{max} of 28° C (Moore et al. 1995).

The maximum growth rate obtained for the Ace Lake *Synechococcus* was 0.12 d^{-1} (generation time = 6.1 days) at 19.7° C and 20 µmol photons m^{-2} s^{-1}. This rate was slow when compared to rates obtained for other strains of marine *Synechococcus* (Stockner and Antia 1986). El Hag, (1986,) obtained a growth rate of 1.4 d^{-1} at 20° C and 30 µmol photons m^{-2} s^{-1}. Campbell and Carpenter, (1986) obtained growth rates of between 0.42 and 0.86 d^{-1} at 25° C and 55 µmol photons m^{-2} s^{-1}. This growth rate was, however, comparable to *in-situ* growth rates of 0.04 to 0.50 d^{-1} found for *P. marinus* (Goericke and Welschmeyer 1993). It is also comparable to growth rates obtained for freshwater

Synechococcus strains, (Fahnenstiel et al. 1991a; Fahnenstiel et al. 1991b, Weisse 1988; Stockner and Antia 1986) and for other polar cyanobacteria. Recently, Tang et al., (1997), determined the growth rate for several cyanobacteria species, none of which belonged to the group *Synechococcus*, isolated from polar freshwater environments. Growth rates were be between 0.12 and 0.14 d^{-1} at a t_{opt} of 19.9 (S.E. 4.85). These growth rates are consistent with that obtained for the Ace Lake *Synechococcus* strain but, they too, are low compared to those predicted by (Eppley 1972) for marine algal growth between 5° and 35° C. Freshwater phytoplankton are known to have lower growth rates than their marine counterparts (Fahnenstiel et al. 1986; Weisse 1988), the physiological reason for this is unclear.

T_{min} is the theoretical minimum temperature at which growth could occur if there were no other physical, chemical or biological constraints (eg: membrane failure) placed on the organism (McMeekin et al. 1993). It is estimated by extrapolation of the regression line from the plot of $\sqrt{k}$ versus temperature (Figure 5.9) to the temperature axis (Ratkowsky et al. 1982). T_{min} for the Ace Lake strain of *Synechococcus* was estimated to be within the range -25° C to -8° C. The range for T_{min} is large because the theoretical t_{min} is much lower than the lowest temperature used to measure growth. In addition, a minimum growth temperature was not observed and the extrapolation was greater than would otherwise be expected if a minimum growth temperature was observed. It is probable that theoretical minimum growth temperature, predicted by the square root model, is closer to -8° C which is at the top end of the range. The square root model has been applied to other Antarctic bacteria, albeit heterotrophic organisms, (McMeekin 1988; McMeekin and Franzmann 1988), and theoretical growth minima for

these strains fit into the range predicted for the Ace Lake *Synechococcus* strain (Table 5.15). Assuming that t_{min} for the Ace Lake strain of *Synechococcus* is approximately -8° C, it would be classed as a psychrotroph according to (Ratkowsky et al. 1982).

T_{opt} and T_{max} for the Ace Lake *Synechococcus* strain are a few degrees below those for other bacteria classed as psychrotrophs. This possibly reflects selection pressure on organisms living in constantly cold environments. However, because of their slow growth rate the optimal growth temperature (20° C) is still above those temperatures experienced in the lake environment.

Table 5.15: Predicted minimum, optimum and maximum growth temperatures for bacterial strains isolated from lakes in the Vestfold Hills, Antarctica.

Organism	Strain	Tmin (Obs)	T_{min}	T_{opt}	T_{max}	Origin	Water Temp.
Flavobacterium sp.[a]	ACAM 755	N.O.	-22	15	20	Ellis Fjord	-2 to 2[c]
Halomonas subglaciescola[b]	ACAM 11	-5.2	-9.2	20	32	Organic Lake (2m)	-14 to 2[d]
Halomonas subglaciescola[b]	ACAM 15	-5.4	-9.2	22	30	Organic Lake (6m)	-8 to -7[d]
Halobacterium sp.[b]	ACAM 32	4.1	2.5	31		Deep Lake	-19 to 8
Synechococcus sp.		N.O.	-25 to -8	20	29	Ace Lake	4 to 10

Strain numbers are from the Australian Collection of Antarctic Microorganisms (ACAM). N.O. indicates that minimum growth temperaturs were not observed, all temperature readings are in degrees celcius (°C), [a] (McMeekin 1988), [b] (McMeekin and Franzmann 1988), [c] (Gallagher and Burton 1988), [d] (Rankin and Pitman 1993).

Although the growth rate of the Ace Lake *Synechococcus* strain was low it is probable that, because of the stable lake environment, with the low diversity of grazers (Burch 1988; Laybourn-Parry and Perriss 1995), the organism can be sustained in the high numbers measured in the lake. Vincent, (1998) proposed this hypothesis to explain why cyanobacteria are more successful in polar lake environments than they are in the polar oceans. Joint, (1986) also indicated that factors, other than temperature, were controlling the distribution and abundance of *Synechococcus* in the marine environment.

In the lakes, over the growing season, the loss rates were probably lower than the production rates and with *Synechococcus*, even over winter, there were high numbers relative to temperate environments, to act as an inoculum for the following summer (Chapter 3). The T_{min} for the Ace Lake *Synechococcus* strain, which is probably of marine origin (Chapter 1 and Chapter 3), indicates that it is capable of growing at temperatures experienced in the southern polar oceans and should therefore be present in this region if there were no other environmental constraints.

The most striking effect of altering the intensity of light to which cultures were exposed was in the change in fluorescence emission of individual cells. The ability of the flow cytometer to monitor quantitative changes in physiological characteristics of individual cells, provides a powerful tool for phytoplankton ecologists (Olson et al. 1990b) At a low light intensity, (5 μmol photons m^{-2} s^{-1}), there was over a 400 % increase in the relative intensity of phycoerythrin (FL2) fluorescence per cell and over 300 % increase in the chlorophyll (FL3) fluorescence per cell in the Ace Lake *Synechococcus* strain. This was relative to cells grown at 20 μmol photons m^{-2} s^{-1}. There was only a small increase in the FL2/FL3 ratio. At the higher light intensities the FL2/FL3 ratio decreased indicating that there was a decrease in phycoerythrin relative to chlorophyll *a*. This seems to imply that at low intensities the organism alters the number of photosynthetic units per cell rather than altering the concentration of accessory pigments (Barlow and Albert 1985). At the very low light intensities the organism is probably putting more energy into producing pigment than it is into other cellular activities such as cell division. Under high light intensities, it is possible that either the cells are producing less photosynthetic units, hence the lower fluorescence or photosynthetic pigments were photo-oxidised (Barlow and Albert 1985) and the phycoerthrin pigment

is affected to a greater extent than chlorophyll *a*. The effect of light intensity on the photosynthetic pigments warrants further investigation because it will provide useful information on photosynthetic strategies in different photic environments.

It is recognised that these are only preliminary investigations and that more detailed growth irradiance experiments are needed for all three strains of *Synechococcus* isolated from the Antarctic meromictic lakes. Growth irradiance curves have been produced for *Synechococcus* strains isolated from a wide variety of habitats (El Hag 1986; Fahnenstiel et al. 1991a; Stockner and Antia 1986; Waterbury et al. 1986), over a light intensity range of 0 - 500 μmol photons m^{-2} s^{-1}. It appears that *Synechococcus* is adapted to low light intensities as maximum growth rates and photosynthetic rates, in cultured or environmental samples, were obtained at light intensities between 45 and 100 μmol photons m^{-2} s^{-1}. At higher light intensities the growth rate plateaued, indicating that high light intensities do not necessarily inhibit growth (Howard and Joint 1989; Joint and Pomroy 1986; Waterbury et al. 1986).

These preliminary investigations indicate that, although the Antarctic strains are probably adapted to growth at the low light intensities, high light intensities may not inhibit their growth. The significant reduction in growth rate of *Synechococcus* at very low light intensities highlights its inability to grow over the winter months. The maximum growth rate obtained at t_{opt} was low compared to other strains of marine *Synechococcus*. It will be important to determine if the growth rate increases when the organism is grown at the optimal temperature and the optimal light.

In Antarctica, lake ice significantly reduces the intensity of light that penetrates the water column, especially in the saline lakes where lake ice is opaque (Burch 1988). Even over summer, during the phytoplankton growth period, in lakes that have year round ice cover, light intensity is low compared to ice free lakes. The light intensity in Ace Lake, where *Synechococcus* grew optimally, ranged from less than 2 μmol photons m^{-2} s^{-1} over the winter to a maximum of 30 μmol photons m^{-2} s^{-1} in February when the lake was ice free (Chapter 3).

By exposing the Ace Lake *Synechococcus* to a salinity range (0 to 30 g kg^{-1}) it has been shown that the organism prefers to grow at a salinity close to seawater (Table 5.14). The upper salinity tolerance of the organism was not obtained and it would be useful to know the entire range of salinity tolerance shown by this organism. Part of the reason for investigating the salinity tolerance of the Ace Lake *Synechococcus* strain was to determine if the organism was of marine or freshwater origin. Although there is not definitive proof, there is mounting evidence that Ace Lake is of marine origin (Burton and Barker 1979). It has an ionic composition close to that of sea water and its eukaryotic algal assemblage, although simplified, resembles that of seawater (Burch 1988; Masuda et al. 1988). It is possible, however, that Ace Lake was seeded by a freshwater strain of *Synechococcus* from one of the nearby freshwater lakes which are also known to have picocyanobacteria (Laybourn-Parry and Marchant 1992). Because the Ace Lake strain appears to be intolerant of low salinities, it is unlikely that it came from freshwater but rather was part of the phytoplankton assemblage when the lake was isolated from the nearby marine environment.

It is possible that the reduction in cell numbers in the lower salinity treatments was caused by the dilution of another chemical species in Ace Lake water, that was needed for the growth of *Synechococcus*, rather than an intolerance to low salinity water. If the Ace Lake *Synechococcus* strain could be successfully grown in artificial sea water where nutrient concentrations could be manipulated, the experiment could be repeated to determine if the Ace Lake *Synechococcus* strain was really intolerant to low salinity water. Further, in this experiment *Synechococcus* cells were not conditioned to the lower salinity water. If over time cells were conditioned to the lower salinity water, it is possible that cells may tolerate the low salinity. *Synechococcus* is distributed widely through out the world in environments ranging from glacial fed freshwater lakes (Burns and Stockner 1991; Laybourn-Parry and Marchant 1992; Vezina and Vincent 1997), river estuaries (Bertrand and Vincent 1994; Jochem 1988; Xiuren et al. 1988), to coastal and marine waters (Iturriaga and Mitchell 1986; Joint 1986; Waterbury et al. 1979). It is possible that there is substantial niche overlap between these environments. Phylogenetically, however, the freshwater species and the saltwater species appear to be quite different (Figure 5.8) although more 16s rDNA sequences are needed for this difference to be clearly defined. Bergey's manual differentiates groups of *Synechococcus* partly based on salinity tolerance. Marine cluster A (reference strain WH8103), for example, is of marine origin and is not tolerant of freshwater (Waterbury and Rippka 1989)

One unique feature of meromictic lakes, especially those in Antarctica, is the salinity ranges found within individual lakes and between lakes. The three meromictic lakes in the Vestfold Hills, from which *Synechococcus* strains were isolated, had salinity ranges of 17-40 g kg^{-1}(Ace Lake), 15-60 g kg^{-1} (Pendant Lake) and 17-23 g kg^{-1} (Lake

Abraxas). Many other meromictic lakes with salinity ranges both higher and lower than these were surveyed for the presence of *Synechococcus* cells (Chapter 4) but none were detected. This is possibly due to factors other than salinity, but it is possible that salinity may play a role in limiting *Synechococcus* to specific lakes.

Characterisation of the three strains of *Synechococcus* isolated from the marine derived meromictic lakes in the Vestfold Hills, Antarctica has provided an opportunity to further understand the taxonomy of this group. It would be useful to isolate picocyanobacteria from other Antarctic lakes, both freshwater and saline, and compare there phylogeny to picocyanobacteria from northern polar regions as well as from temperate and tropical regions. The phylogenetic relationship between *Synechococcus* and *Prochlorococcus* needs clarification. The taxonomic classification of the oxygenic-phototrophic bacteria has recently been questioned (Pinevich et al. 1997). If more strains were isolated from either group, a clearer understanding of the divergence and the ecological implications could be determined.

The close phylogenetic relationship between the marine *Synechococcus* and *P. marinus* has been observed either through 16S rDNA sequencing or through RNA Polymerase gene sequencing (Kane et al. 1997; Mullins et al. 1995; Palenik 1994; Urbach et al. 1992; Wood and Townsend 1990). Phenotypically, however, the two groups are quite different and distribution surveys to date highlight their possible ecological difference (Charpy and Blanchot 1996; Chisholm et al. 1988; Olson et al. 1990a; Partensky et al. 1993).

Prochlorococcus marinus is smaller than *Synechococcus* (0.6-0.8 μm diameter), (Chisholm et al. 1992; Olson et al. 1990a), possibly because it does not have phycobilisomes. *Prochlorococcus marinus* lacks phycobilisomes (Goericke and Repeta 1992). Instead it has divinyl chlorophyll *b*. It also has divinyl chlorophyll *a* instead of chlorophyll *a* and α -carotene instead of ββ - carotene (Goericke and Repeta 1992). It does have zeaxanthin as does *Synechococcus*. *Prochlorococcus marinus* therefore, fluoresces red under green light instead of the orange or orange/red produced by phycoerythrin in *Synechococcus*. The stacking of the thylakoid membranes, used as an identifying characteristic in *P. marinus* (Chisholm et al. 1988), is thought to result from the loss of phycobilisomes (Palenik and Swift 1996). It appears that *P. marinus* and marine *Synechococcus* have evolved slightly different physiological strategies for occupying a similar niche in the marine environment. Gene sequences, responsible for the expression of photosynthetic pigments have, however, been shown to be transferable in bacteria (Pemberton and Harding 1987). The expression of photosynthetic pigments is therefore not a good taxonomic marker on which to describe a new genus.

The taxonomic organisation of the *Synechococcus* group is still incomplete (Castenholz 1992) and some strains within the group are still classified according to morphological and physiological characteristics. The use of phylogenetic tools in taxonomy will help to better understand the taxonomy of the group. It appears, however, that it will be important to use a combination of phylogentetic, physiological and morphological characteristics to classify organism within the group. *Synechococcus* is a polyphyletic group and some strains cluster closely with the *P. marinus*. It was suggested by (Seewaldt and Stackebrandt 1982), that *Prochloron*, another phototrophic prokaryote, is an offshoot of cyanobacteria that has re-invented chlorophyll *b* rather than being a direct

descendent of chloroplasts. If *P. marinus* did in fact recently diverge from strains of *Synechococcus* (Chisholm et al. 1992), by acquiring the ability to produce chlorophyll *b* and loosing the ability to produce the phycobiliproteins, it is possible that the divergence was from the *Synechococcus* cluster which has strain NIV-CYA 328 and the Antarctic strains.

Chapter 6

Controls on the abundance and distribution of *Synechococcus* in Ace Lake.

6.1 ABSTRACT

Two *in-situ* experiments were undertaken to investigate factors controlling the distribution and abundance of *Synechococcus* in Ace. The effect of light intensity, nutrient concentration, salinity and temperature on the growth rate of and grazing rate on *Synechococcus* in Ace Lake was investigated. The metabolic inhibitor technique was used to calculate growth rates and grazing rates. Unfortunately, although the metabolic inhibitor method worked successfully in laboratory experiments, there was no significant difference ($p > 0.05$) between treatments in the field experiments. This may have been due to an inability to detect differences between growth and grazing, at slow *in-situ* growth rates and low grazing pressure. However, specific growth rates of *Synechococcus* (growth - grazing) could be calculated for all treatments. The specific growth rate of *Synechococcus* at 6 m , 8 m and 10 m in Ace Lake was -0.1176 d^{-1}, 0.072 d^{-1} and 0.3408 d^{-1} respectively. The negative growth rate at 6 m may have resulted from over grazing in the sample. An increase in water temperature significantly increased growth rate ($p < 0.0001$) and there was a significant increase in growth rate ($p < 0.001$) from an interaction between higher water temperature and lower light intensity. There were significant increases ($p < 0.05$) in growth rates at lower light intensities. In one instance the growth rate of *Synechococcus* increased from 0.072 d^{-1} to 0.2808 d^{-1} when incubated at the same temperature but with a reduced light intensity (from 12.5 µmol photons m^{-2} s^{-1} to 7.3 µmol photons m^{-2} s^{-1}). The addition of nutrients and an increase in salinity did not increase growth rates ($p > 0.05$) of the Ace Lake *Synechococcus*. At the time when these experiments were conducted, it is probable that between depths 7 m to 11 m, growth rate was greater than loss rates. At other times however, for instance at

the end of the spring bloom, this balance could change. As occured in the top 7 m of Ace Lake, where *Synechococcus* growth rates were low due to low temperatures, it is probable that in the Southern Ocean the loss rates are high and the population density is therefore low.

6.2 INTRODUCTION

Picocyanobacteria, belonging to the *Synechococcus* group, are important contributors to primary productivity in many of the tropical and temperate oceans and lakes of the world (Caron et al. 1985; Craig 1987; Fahnenstiel et al. 1991a; Glover et al. 1986; Iturriaga and Mitchell 1986; Joint et al. 1986; Maeda et al. 1992). In Antarctic waters, however, *Synechococcus* occurs in low numbers (Letelier and Karl 1989; Marchant et al. 1987). It has been assumed that temperature primarily limits numbers in this region, because of a correlation found between temperature and *Synechococcus* cell abundance in north to south transects of the Southern Ocean (Marchant et al. 1987). There is, however, little experimental evidence to substantiate this conclusion and for microbial populations under no other constraints, low temperatures will limit growth rates but will not significantly affect final biomass yields.

Obvious factors that have been shown to control the growth of *Synechococcus* in tropical and temperate regions could also be exerting pressure in polar regions. These factors include combinations of temperature (El Hag and Fogg 1986; Murphy and Haugen 1985; Waterbury et al. 1986), light (El Hag 1986; Morris and Glover 1981; Platt et al. 1983), nutrients (Lignell et al. 1992; Stockner and Shortbreed 1988; Walsh et al. 1994), salinity (Bertrand and Vincent 1994) and grazing pressure by eukaryotic organisms and viruses (Caron et al. 1991; Hagstrom et al. 1988; Hall et al. 1993; Waterbury and Valois 1993). It is probable that a combination of factors is responsible for the distribution and abundance of *Synechococcus* in these regions.

Unlike the open ocean environment, where there is constant movement of water and phytoplankton populations can be patchy (Harris 1986; Weber and El-Sayed 1987), the ice covered, marine derived, meromictic lakes of the Vestfold Hills provide a stable environment for phytoplankton communities. Experimental manipulation of the parameters, outlined above, in a stable lake environment could provide a greater understanding of the controls on *Synechococcus* abundance and distribution in Antarctic lakes, polar and temperate oceans.

The aim of this investigation was to use a factorially designed experiment that manipulated light, temperature, salinity and nutrients, in combination with the specific metabolic inhibitor technique, to gain an understanding of controls of *Synechococcus* abundance and distribution in Ace Lake.

In the specific metabolic inhibitor technique, first used by Fuhrman and McManus, (1984), ampicillin (an inhibitor of prokaryotic cell division) is used to hold the prokaryotic population at a constant level so that the grazing rate (g) can be determined. This inhibitor does not affect eukaryotic grazers. A second treatment uses cycloheximide to inhibit grazing by eukaryotes so that the absolute growth rate (k) can be determined. Cycloheximide is a specific inhibitor of protein synthesis in eukaryotes but does not affect prokaryotes (Sherr et al. 1986; Watanabe 1972). During the experimental period the change in prokaryotic cell numbers is monitored. As a control, both ampicillin and cycloheximide are used to confirm that prokaryote number remains constant in the absence of grazing by eukaryotes. This technique has been used successfully to determine growth and grazing rates of *Synechococcus* in many temperate and tropical oceans (Campbell and Carpenter 1986; Liu et al. 1995; Xiuren and Vaulot

1992). In the calculation of growth rate and grazing rate the technique assumes exponential growth of the prokaryote population (Fuhrman and McManus 1984).

It is proposed that temperature alone is not controlling the distribution and abundance of *Synechococcus* in Ace Lake but that light intensity and grazing pressure by eukaryotes are also significant contributors.

6.3 MATERIALS AND METHODS

Before the field experiment could be carried out, a preservation method for *Synechococcus* and optimal antibiotic concentrations for the metabolic inhibitor technique were required. These experiments were carried out with *Synechococcus* cultures, enriched from Ace Lake in the previous year.

6.3.1 Sample preservation

Due to logistical constraints during the 1993/94 summer at Davis Station, it was necessary to preserve and store all samples collected during the experimental period. A number of preservation protocols were tested to determine the most effective method of preservation and storage of samples. Preservation protocols were modified from Lepesteur et al. (1993) and Vaulot et al. (1989). These protocols are outlined in Table 6.1.

Table 6.1: Protocols used to determine the most effective method of preservation of *Synechococcus* cells for analysis by flow cytometry. Percentages represent final concentrations

Protocol	Preservation method
1	No preserving agent
2	1% Gluteraldehyde
3	1% Gluteraldehyde, 10 % DMSO as a cryoprotectant
4	1 % Gluteraldehyde, 10 % DMSO and glycerol as cryoprotectants
5	1 % Formalin
6	1 % Formalin and 10 % DMSO as a cryoprotectant
7	1 % Formalin, 10 % DMSO and glycerol as cryoprotectants

An actively growing *Synechococcus* culture (approximately 1 x 10^6 cells ml^{-1}) was aliquoted, (500µl), into 'Nunc' cryoprotectant tubes. The exact cell density of the culture was determined by flow cytometric methods (Chapter 2).

Cryoprotectants were used according to the method of Lepesteur et al., (1993). They were added before the fixatives, drop by drop, at 4° C, with constant agitation. The following sterile cryoprotectants were used: dimethylsulfoxide (DMSO) 10%, glycerol 20%, and a mixture of DMSO 10% and glycerol 10% v/v (Sigma Chemicals).

Fixatives were added fifteen minutes after the addition of the cryoprotectants. Electron microscope grade gluteraldehyde (pH 7.51) and formalin were added to a final concentration of 1%. Samples were then incubated at room temperature for ten minutes.

Following preservation, all samples were super-cooled in the vapour phase of the liquid nitrogen for four hours before being plunged into the liquid nitrogen.

Samples were retrieved from the liquid nitrogen at intervals of between one and six weeks. As recommended by Lepesteur et al., (1993), samples were rapidly thawed

(37°C) and the cell densities determined by flow cytometric techniques (Chapter 2). Data were subjected to statistical analysis (Sigmastat 1992).

6.3.2 Optimal antibiotic concentrations

A *Synechococcus* strain and *Cryptomonas* sp. isolated from Ace Lake were used to determine the optimal concentrations of ampicillin and cycloheximide for use in the field experiments. Although no trials with lake samples could be performed prior to the experiment, it was anticipated that analysis of these two organisms would be adequate as the experiment was to be carried out in Ace Lake.

Synechococcus and *Cryptomonas* sp. were grown in batch culture at 6° C under white light (PAR = 30 μmol photons m^{-2} s^{-1}) on a 12 h day/night cycle. These growth conditions are similar to those experienced by the cells at 10 m in Ace Lake. Cells were harvested for the antibiotic trail when they were in exponential growth.

Synechococcus cells were diluted to approximately 10^5 cell ml^{-1} and 50 ml aliquots were dispensed aseptically into 'Whirl Pacs'. The exact cell density in the aliquots was determined by flow cytometry. Initial ampicillin concentrations (Table 6.2) were used according to Xiuren and Vaulot, (1992). The appropriate volume of ampicillin was added to the 'Whirl Pacs'. Each concentration of ampicillin was replicated 5 times. The 'Whirl Pacs' were incubated as described above. Cell densities from all treatments were measured after 24, 48, 72, 120 and 216 h using flow cytometric techniques and subjected to statistical analysis (Sigmastat 1992). A second set of cultures were

incubated and analysed according to the same protocol, except that all the cultures had 100 mg l^{-1} cycloheximide added. This was to determine if cycloheximide inhibited the growth of *Synechococcus* or if it affected the response of *Synechococcus* to the ampicillin (Table 6.3).

The *Cryptomonas* sp. culture was harvested in exponential growth, diluted to 10^4 cells ml^{-1} and aliquoted, (50 ml), aseptically into 'Whirl Pacs'. These subcultures were treated according to the protocol in Table 6.2 and as recommended by Xiuren and Vaulot, (1992). This was to determine if 100 mg l^{-1} cycloheximide would inhibit the growth of *Cryptomonas* sp. without causing cell lysis, and also to see if ampicillin inhibited the growth of the *Cryptomonas* sp.

Table 6.2: Ampicillin and cycloheximide concentrations used in *Synechococcus* and *Cryptomonas* sp. cultures to determine optimal antibiotic concentration.

Phytoplankton Species	Ampicillin (mg l^{-1})	Cycloheximide (mg l^{-1})
Synechococcus	0	0
Synechococcus	0.1	0
Synechococcus	0.25	0
Synechococcus	0.5	0
Synechococcus	1	0
Synechococcus	2.5	0
Synechococcus	5	0
Synechococcus	0	100
Synechococcus	0.1	100
Synechococcus	0.25	100
Synechococcus	0.5	100
Synechococcus	1	100
Synechococcus	2.5	100
Synechococcus	5	100
Cryptomonas sp.	0	0
Cryptomonas sp.	0	100
Cryptomonas sp.	5	0

Table 6.3: Concentrations of ampicillin and cycloheximide used in *Synechococcus* cultures to determine optimal antibiotic concentration for use in the Ace Lake experiment.

Phytoplankton Species	Ampicillin (mg l^{-1})	Cycloheximide (mg l^{-1})
Synechococcus	0	0
Synechococcus	0.005	0
Synechococcus	0.01	0
Synechococcus	0.05	0
Synechococcus	0.1	0
Synechococcus	0	100
Synechococcus	0.005	100
Synechococcus	0.01	100
Synechococcus	0.05	100
Synechococcus	0.1	100

6.3.3 Controls on *Synechococcus* growth Ace Lake.

Four experiments were undertaken over a two week period in December 1993, in Ace Lake. The first three experiments were replicates of the same experimental design. The experiment was replicated in this way, instead of replicates of each treatment within the single experiment, because of the large sample number. The fourth experiment had a separate design. The experimental designs of both experiments are outlined below.

6.3.4 The effect of nutrients, salinity, light and temperature on the growth of the Ace Lake *Synechococcus*

The first experiment was designed to determine the effect of nutrients, salinity, temperature and light on the growth rate of, and grazing pressure on, *Synechococcus* in Ace Lake. The metabolic inhibitor technique was used to determine growth rates and grazing pressures (Campbell and Carpenter 1986; Fuhrman and McManus 1984). The experimental design used is shown in Table 6.4, which has the form of a 2^4 factorial design (i.e.: a design with four factors, each with two levels).

Table 6.4: Tested effects of temperature (depth), light, salinity and nutrients, under field conditions, on the growth of *Synechococcus* in Ace Lake. The implementation of these test conditions is discussed in the text below.

Depth	1	2	3	4	5	6	7	8
6m 6m 6m	10m light 6m sal low nut	10m light 6m sal high nut	10m light 10m sal low nut	10m light 10m sal high nut	11m light 6m sal low nut	11m light 6m sal high nut	11m light 10m sal low nut	11m light 10m sal high nut
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
10m 10m 10m	10m light 6m sal low nut	10m light 6m sal high nut	10m light 10m sal low nut	10m light 10m sal high nut	11m light 6m sal low nut	11m light 6m sal high nut	11m light 10m sal low nut	11m light 10m sal high nut

sal = salinity; nut = nutrients

Lake water from 6 m was collected using a Kemmerer Bottle and aliquoted into polyethylene 'Whirl Pacs' according to the protocol in Table 6.5. The final volume in each 'Whirl Pac' was 50 ml.

6.3.4.1 Experimental temperatures

The effect of a 4° C difference in water temperature on the growth of *Synechococcus* was tested. The temperature treatments were obtained by suspending the 'Whirl Pacs' at two depths in Ace Lake, 6 m (3° C) and 10 m (7° C).

6.3.4.2 Experimental light intensities

In accordance with the factorial design interpretation of this experiment, light intensities equivalent to 10 m and 11 m in Ace Lake were compared. The light intensities used were obtained by measuring the PAR at 10 m (14 % of the under ice light intensity) and 11 m (1% of the under ice light intensity) and then using combinations of gray (neutral density) plastic bags and shade cloth tied around the samples to replicate these light

intensities at 6 m and 10 m respectively. Light intensity was measured according to the method described in Chapter 3.

6.3.4.3 Experimental salinities

In order to test the effect of salinity on the growth of *Synechococcus*, the salinity at 6 m in Ace Lake was compared to a salinity that was equivalent to 10 m in Ace lake. On the day prior to the experiment, the salinity in Ace Lake at 6 m and 10 m was determined using a submersible data logger (Chapter 3). The salinity at 6 m was 18.1 ppt. The salinity at 10 m was 29.9 ppt. To increase the salinity of the 6 m water by 1.2 %, to give a salinity equivalent to water from 10 m, sodium chloride was added. A 12 % stock NaCl solution was used.

6.3.4.4 Experimental nutrient concentrations

The effect of nutrient concentration on the growth of *Synechococcus* was tested by comparing growth of cells exposed to the nutrient levels in 6 m Ace Lake water (low nutrient conditions - Chapter 3) to that of cells when additional nutrients were added to the 6 m Ace Lake water (high nutrient conditions). Ten percent v/v SNAX medium (Appendix 5) was added to the 'Whirl Pacs' for the high nutrient treatment.

Table 6.5: Treatment protocol used for samples . Sample numbers correspond to numbers in table 5.4.

Sample Number	Treatment
1, 5, 9, 13	50 ml lake water
2, 6, 10, 14	45 ml lake water plus 5 ml 10X SNAX
3, 7, 11, 15	45 ml lake water plus 5 ml NaCl (12 g 100 ml^{-1})
4, 8, 12, 16	40 ml lake water plus 5 ml 10X SNAX plus 5 ml NaCl

6.3.4.5 Antibiotic treatments

Within each of the above treatments antibiotics were added according to the following protocol: 1) No antibiotics; 2) Ampicillin (15 µl of a 0.1 mg ml^{-1} stock solution in 50 ml sample = 0.01 mg l^{-1}); 3) Cycloheximide (500 µl of a 10 mg ml^{-1} stock solution in 50 ml sample = 100 mg l^{-1}); 4) Cycloheximide (100 mg l^{-1}) and ampicillin (10 mg l^{-1}) - Control.

6.3.4.6 Incubation of samples in Ace Lake and sample collection.

Once all the additions had been made, initial samples, t = 0, (1 ml aliquot) were taken. The 'Whirl Pacs' were sealed and tied randomly around six armed sample holders (Figure 6.1) Where needed, the neutral density filters were tied around the samples and each sample holder was lowered into the lake, to the desired depth, through 1 of 6 holes drilled through the ice (Figure 6.2). All treatments were re-sampled every 12 h for 48 hours and all procedures was performed in low light conditions. When samples were collected, a thick canvas tent was erected over the holes in the ice. This was to reduce the possibility of photoinhibition, when bringing samples to the surface. The tent was

dismantled at the end of each sampling procedure and moved away from the experimental area. This was to eliminate the possibility of shading during the *in-situ* incubation periods. All 1 ml aliquots were preserved with gluteraldehyde and stored in liquid nitrogen, as outlined above.

Temperature and salinity (Chapter 3) was measured through the top 13 m of the lake at the beginning of each replicate experiment. Light (Chapter 3), was measured every 12 h throughout the experimental period. This was to monitor changes in the physical conditions during the experiment.

The experiment outlined above was replicated three times over a period of two weeks.

6.3.5 The effect of light on the growth rate of the Ace Lake *Synechococcus*.

A second experiment was designed to investigate the effect of light intensity on the growth rate of and grazing pressure on the Ace Lake *Synechococcus* strain.

In this experiment five treatments were incubated *in-situ* for a 48 h period. Each treatment was performed in triplicate. The treatments were as follows: 1) lake water from 6 m in Ace Lake incubated at 6 m with a 6 m light intensity; 2) lake water from 6 m in Ace Lake incubated at 6 m with a 10 m light intensity 3) lake water from 8 m in Ace Lake incubated at 8 m with a 8 m light intensity; 4) lake water from 8 m in Ace Lake incubated at 8 m with a 10 m light intensity; 5) lake water from 10 m in Ace Lake incubated at 10 m with a 10 m light intensity. The required light intensities were

determined using a Digital Scalar Irradiance Meter (Biospherics) and neutral density filters, as outlined above.

All treatments were prepared, incubated and sampled according to the protocol outlined in the experiment above. Samples were preserved with gluteraldehyde and stored in liquid nitrogen until analysis. Stored samples were analysed between two and six weeks after collection using flow cytometric methods.

6.3.6 Data analysis

Cell numbers were determined using the 'Lysis II' software on the flow cytometer (Becton Dickinson). Growth rates (**k**) and grazing rates (**g**) were determined according to the equation:

$$\mathbf{r = k\text{-}g = \ln(N_t/N_0)/t}$$

where N_0 = cell abundance at time zero; N_t = cell abundance at time t and r = net growth rate. Specific growth rate (**k**) was calculated from the rate of increased cell density in the cycloheximide treatment, while specific grazing rate (**g**) was calculated from the disappearance rate of cells in the ampicillin treatment minus control. Data were subjected to analysis of variance. In the factorial designed experiment first order interactions were also tested (SAS Institute 1989).

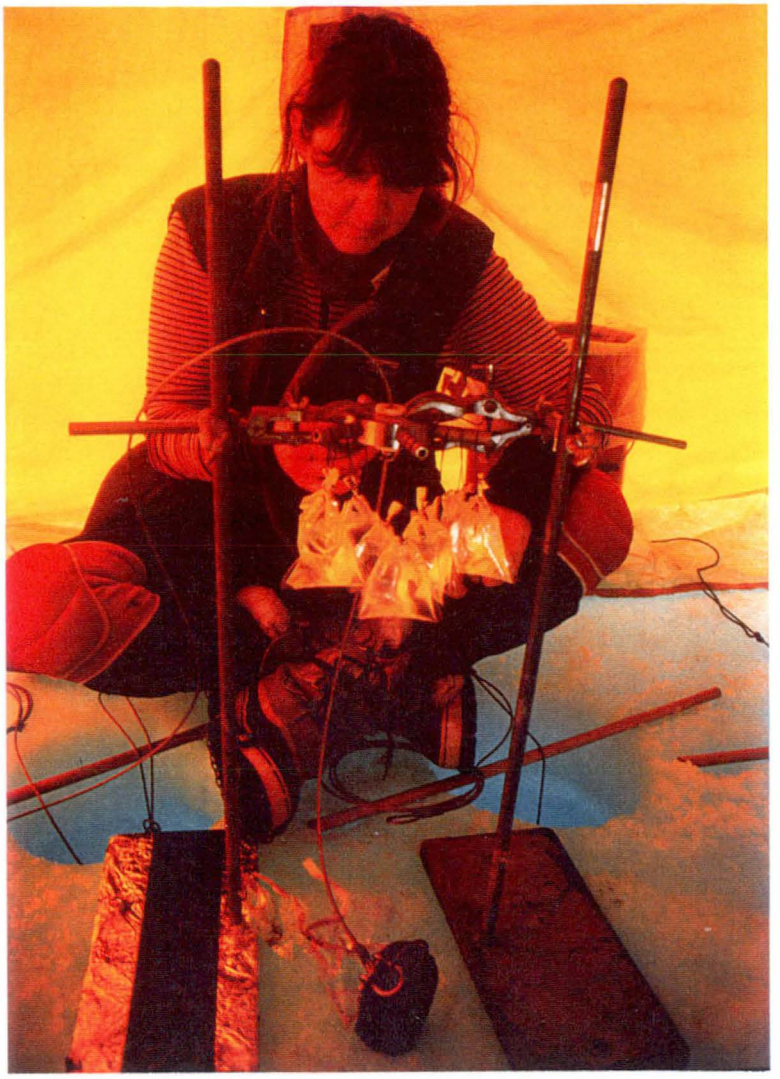

Figure 6.1: Device used to suspend samples in Ace Lake. 'Whirl Pacs' were placed randomly around the device before it was lowered into the lake and suspended at the desired depth.

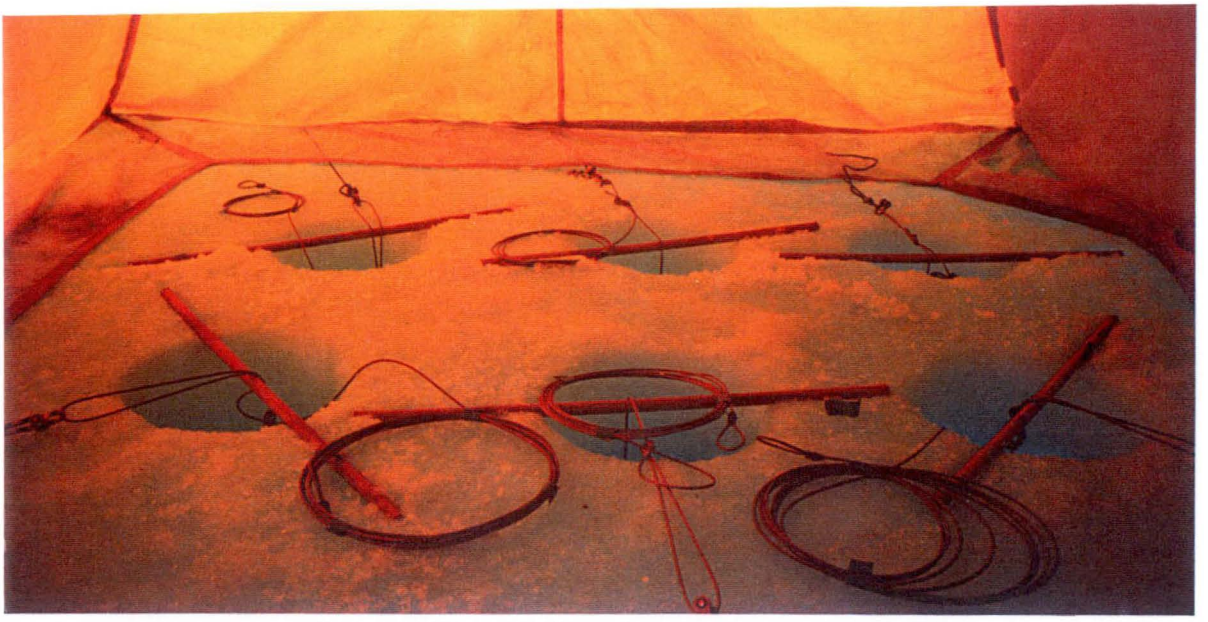

Figure 6.2: Sample treatments were suspended in one of six holes drilled through the ice.

6.4 RESULTS

6.4.1 Optimal preservation method

Seven preservation techniques were tested to determine the method that gave the least cell loss. The cell numbers in the preserved cultures were compared to that of the original fresh culture (control) by determining the ratio of numbers of preserved over numbers of fresh (Figure 6.3). Although sample numbers were small, no preservation protocol gave significantly different numbers of cells in the treatments to the number of cells in the control (Kruskal-Wallis One Way Analysis of Ranks, DF = 6, p = 0.4475). Similar results were also obtained by others (Lepesteur et al. 1993; Vaulot et al. 1989).

Although quantitative changes in fluorescence were not recorded, some general observations were made about the effect of sample preservation on changes in cell fluorescence and morphology. There was an increase in the orange fluorescence (FL2), of cells stored without a preserving agent (protocol 1) and with cryoprotectants and formalin as a preservative (protocols 7, 8 and 9), relative to the fluorescence of the original fresh cells. There was no change in the orange fluorescence of cells preserved in gluteraldehyde or formalin without cryoprotectants (protocols 2 and 6) nor in protocols 3, 4 and 5 where gluteraldehyde was the preservative. Cells that had been stored with a cryoprotectant all increased in size relative to the fresh cells stored without cryoprotectants.

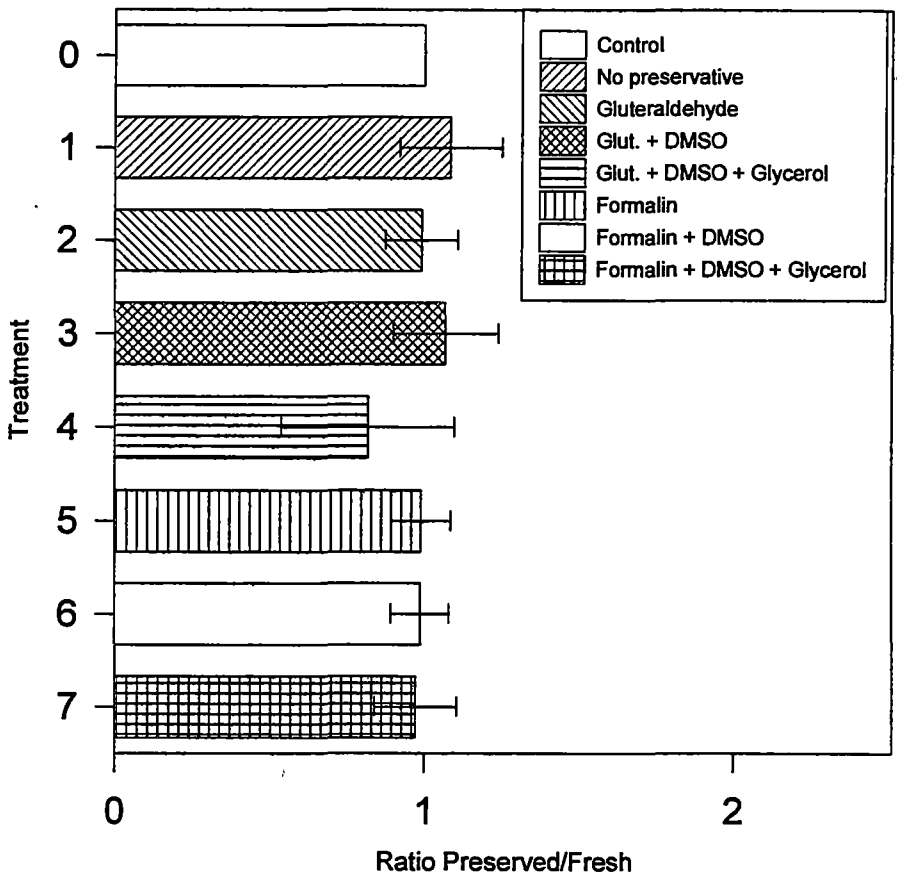

Figure 6.3: Effect of preservation on *Synechococcus* cell numbers. Results are expressed as a ratio of the numbers in the preserved sample divided by the numbers in the fresh sample; a ratio of 1 indicates perfect preservation. Error bars correspond to one standard deviation.

There was a greater spread in cell size and fluorescence in the populations of cells when cryoprotectants were used. There was no change in red fluorescence intensity with increasing storage time. These observations were made on cells that had been stored in liquid nitrogen over a one month period. Significant increases in orange fluorescence were observed by Vaulot et al., (1989) who suggested that the changes in fluorescence could have been caused by an uncoupling of the photosynthetic pigments. Changes in

cell size were also observed by Vaulot et al., (1989) although they observed greater changes in right angle light scatter (refractive index) than in forward angle light scatter (cell size).

Table 6.6: Percentage number of cells preserved compared to that of unpreserved cells using the various protocols.

Protocol	% of cells preserved
1	109 (7.4)
2	99 (3.4)
3	107 (7.6)
4	82 (12.6)
5	99 (2.6)
6	99 (3.5)
7	97 (6.0)

Numbers in brackets are standard errors.

From the above results and from Vaulot et al., (1989) a standard preservation protocol, for samples collected from Ace Lake during the experiment, was developed. Samples (1 ml) were preserved in cryoprotectant tubes with 1% gluteraldehyde (pH 7.5), followed by incubation at approximately 5° C for 30 minutes. Samples were then suspended in the vapour phase of liquid nitrogen for 8 h before immersion in liquid nitrogen for storage for up to one month prior to analysis.

For analysis, the samples were rapidly thawed (but not left) at 37°C. Calibration beads were added to the cryotubes and mixed thoroughly on a vortex mixer before an aliquot (500 µl) was taken for analysis by flow cytometry (Chapter 2).

6.4.2 Optimal antibiotic concentrations

In order for the metabolic inhibitor technique to be used *in-situ*, in Ace Lake, optimal concentrations of ampicillin and cycloheximide, that would inhibit the growth of prokaryotes and eukaryotes respectively, were needed.

From the analysis of growth response, it was determined that a cycloheximide concentration of 100 mg l^{-1} was sufficient to inhibit the growth of the *Cryptomonas* sp. without causing cell loss (t-test, n = 4, p = 0.0011, Figure 6.4). In addition, the concentration of ampicillin used in the *Cryptomonas* sp cultures (5 mg l^{-1}) did not significantly inhibit the growth of the organism (Mann-Whitney Rank Sum Test, n = 4, p = 0.0357, Figure 6.4).

The initial concentrations of ampicillin that were used to inhibit the growth of *Synechococcus* significantly reduced the number of cells (t test, Table 6.7, Figure 6.5). This implied that cell lysis may have occurred (Xiuren and Vaulot 1992). Cycloheximide did not significantly alter the effect of ampicillin on *Synechococcus* (t-test, n = 5, p > 0.05). A second experiment was performed with lower concentrations of ampicillin (Table 6.8).

Table 6.7: Probability that *Synechococcus* cell numbers at t = 120 h were the same as *Synechococcus* cell numbers at t= 0 h for six ampicillin concentrations. In all cases, there were significantly less cells.

Ampicillin (mg l^{-1})	Cell numbers at t=120 h	(n, P value)
0.1	3.2×10^5	4, p = 0.001
0.25	2.9×10^5	4, p = 0.001
0.5	3.6×10^5	5, p = 0.02
1.0	3.2×10^5	5, p = 0.0093
2.5	3.0×19^5	5, p = 0.0076
5.0	3.2×10^5	5, p = 0.0093

Cell number at t = 0 h was 5.2×10^5 cells ml^{-1}

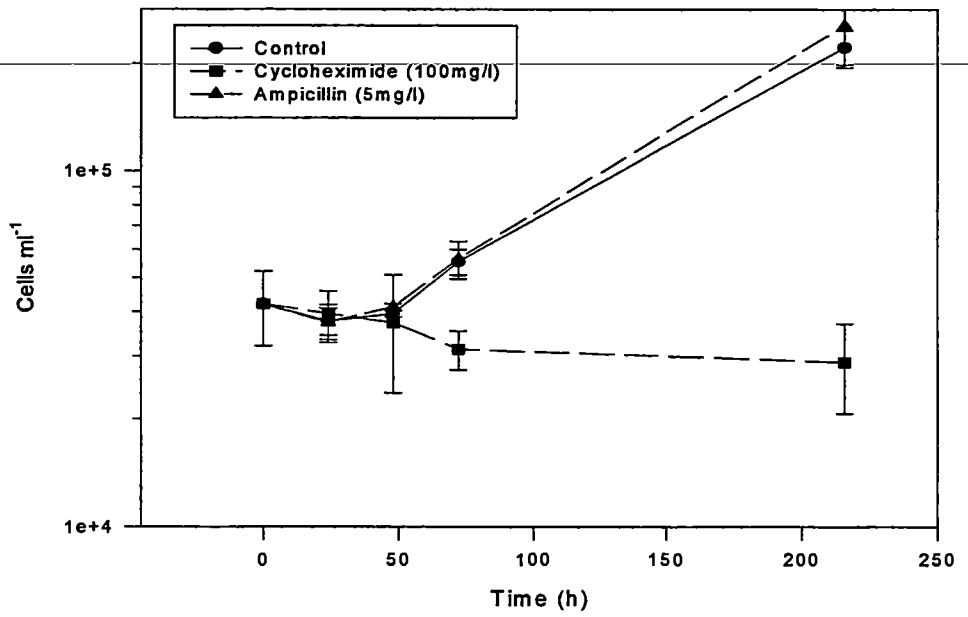

Figure 6.4: Effect of ampicillin and cycloheximide on the growth of an Ace Lake *Cryptomonas* sp. (Error bars correspond to 1 S.D. and 1e+4 = x 10^4)

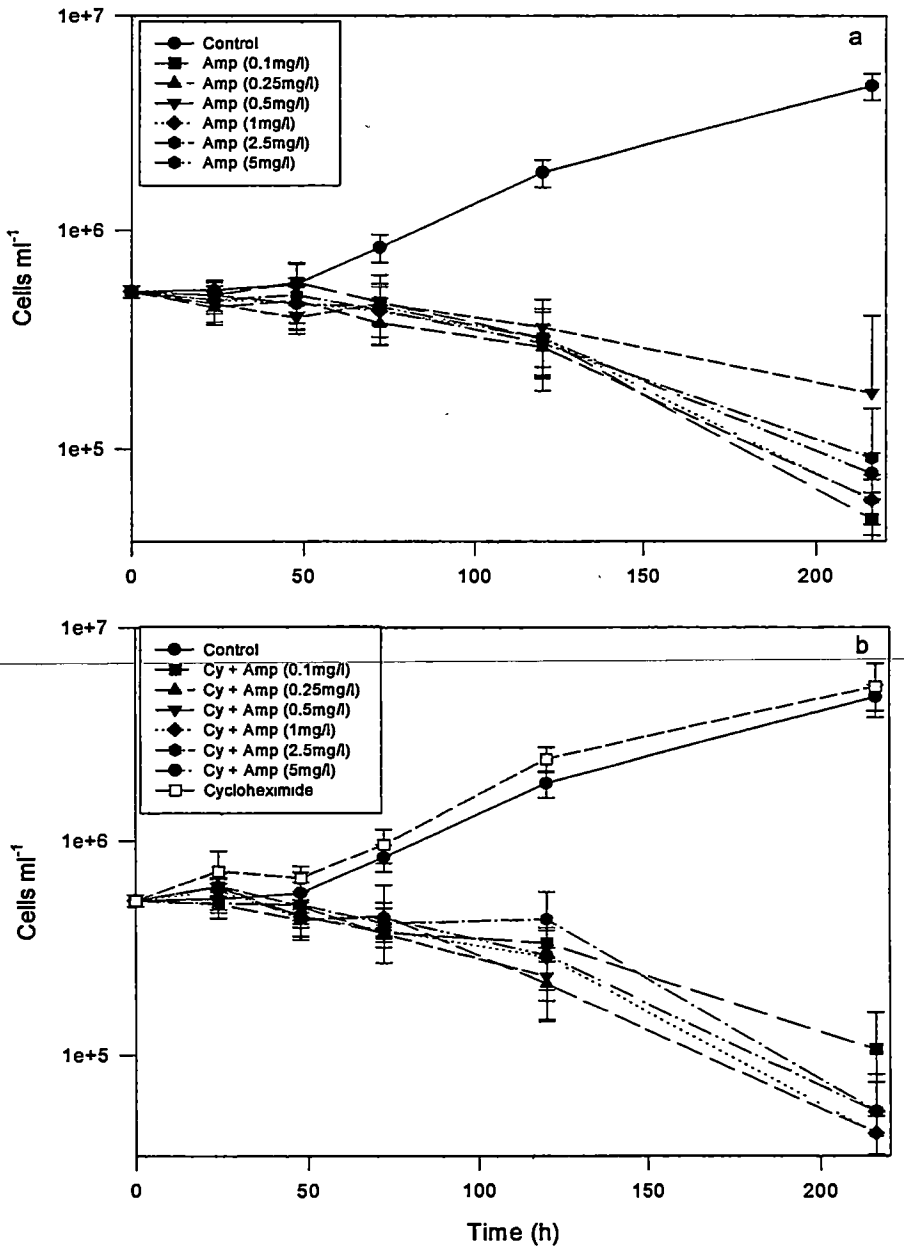

Figure 6.5: Effect of ampicillin (Amp) in the a) absence and b) presence of cycloheximide (Cy) on the growth of the Ace Lake *Synechococcus*. (Error bars correspond to 1 S.D. and 1e+4 = x 10^4)

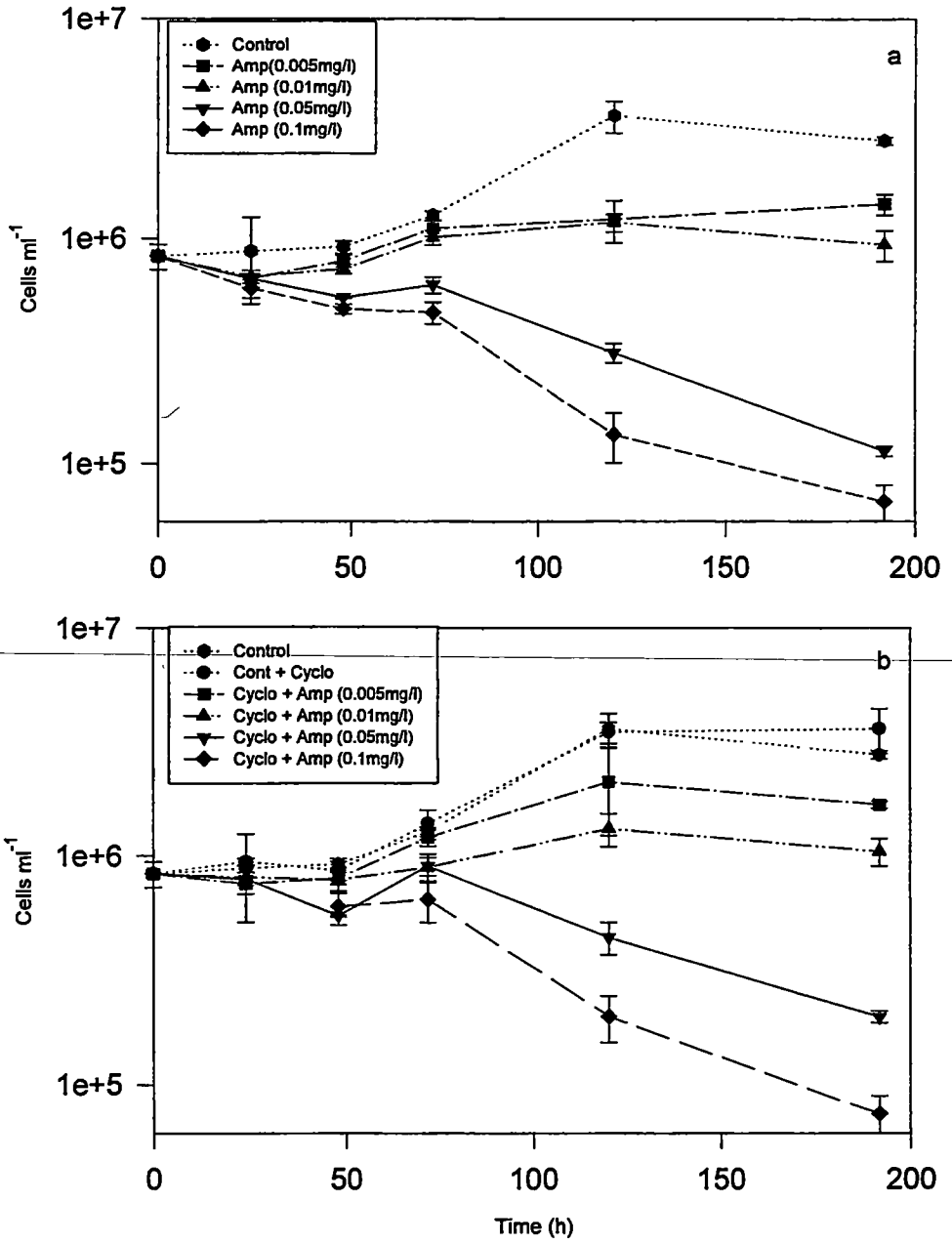

Figure 6.6: Effect of ampicillin (Amp 0.005 - 0.01 mg l^{-1}) in the a) absence and b) presence of cycloheximide (Cy 100 mg l^{-1}) on the growth of the Ace Lake *Synechococcus*. (Error bars correspond to 1 S.D. and 1e+4 = x 10^4)

Following incubaton for 24 hours, *Synechococcus* cell numbers in cultures that contained 0.005 mg l^{-1} and 0.01 mg l^{-1} ampicillin increased marginally but significantly ($p > 0.01$) compared to the control culture at $t = 0$ (t-test, Table 6.8). After 120 h, cell numbers in cultures containing 0.05 mg l^{-1} and 0.1 mg l^{-1} ampicillin decreased significantly ($p \leq 0.002$) compared to the control indicating that cell lysis occurred in these cultures (Figure 6.6). Cycloheximide did not significantly alter the effect of ampicillin on *Synechococcus* (t-test, $n = 5$, $p > 0.05$)

Table 6.8: Probability that *Synechococcus* cell numbers at t = 120 h were the same as *Synechococcus* cell numbers at t = 0 h for four ampicillin concentrations. For the two lower ampicillin concentrations there were significantly more cells and for the two higher ampicillin concentrations there were significantly less cells.

Ampicillin (mg l^{-1})	Cell numbers at t=120 h	(n, p value)
0.005	2.1×10^6	4, 0.0231
0.01	1.3×10^6	3, 0.0125
0.05	4.4×10^5	4, 0.002
0.1	2.0×10^5	4, <0.001

Cell number at t = 0 h was 8.3×10^5 cells ml^{-1}

From these results it was decided to use an ampicillin concentration of 0.03 mg l^{-1} and a cycloheximide concentration of 100 mg l^{-1}. Due to time constraints, the effectiveness of this ampicillin concentration in inhibiting the growth of *Synechococcus*, without causing cell lysis, could not be measured.

6.4.3 Controls of growth in Ace Lake

Experiments were performed in Ace Lake using lake water samples in order to determine the effect of grazing, temperature, light intensity, nutrient concentration and salinity on the growth of *Synechococcus*. Light (PAR), salinity and temperature were measured throughout the experiments. There was a 0.39° C increase in water temperature at 6 m and a 0.14° C increase in water temperature at 10 m during the experimental period. Light intensity changed depending on cloud cover and the time of day. The ice conditions did not change and no fresh snow fell throughout the experimental period. The water temperature of the epilimnion (between 2.5 m and 7 m) was 2.7° C. Between 7 m and 8 m there was a sharp rise in temperature to 6.3° C. At 10 m the temperature was 7° C. The maximum temperature (7.8° C) occurred at 11.5 m (Figure 6.7). The salinity at 6 m was 18.1 g kg^{-1} and at 10 m the salinity was 29 g kg^{-1}. Over the experimental period (12 days) there was a large variation in the amount of cloud cover ranging from a clear sunny sky, to heavy low cloud which greatly reduced the light intensity. Light intensity also depended on the time of day (Chapter 3) and the maximum light intensity occurred at solar noon (approximately 13:00, Davis time). The light measurements given in Figure 6.7 were made at solar noon on a day when there was approximately 10 % high cloud cover. On this occassion light intensity below the ice was low (< 50 μmol photons m^{-2} s^{-1}) and decreased to 8 μmol photons m^{-2} s^{-1} at 10 m . Light was not detected below 12 m.

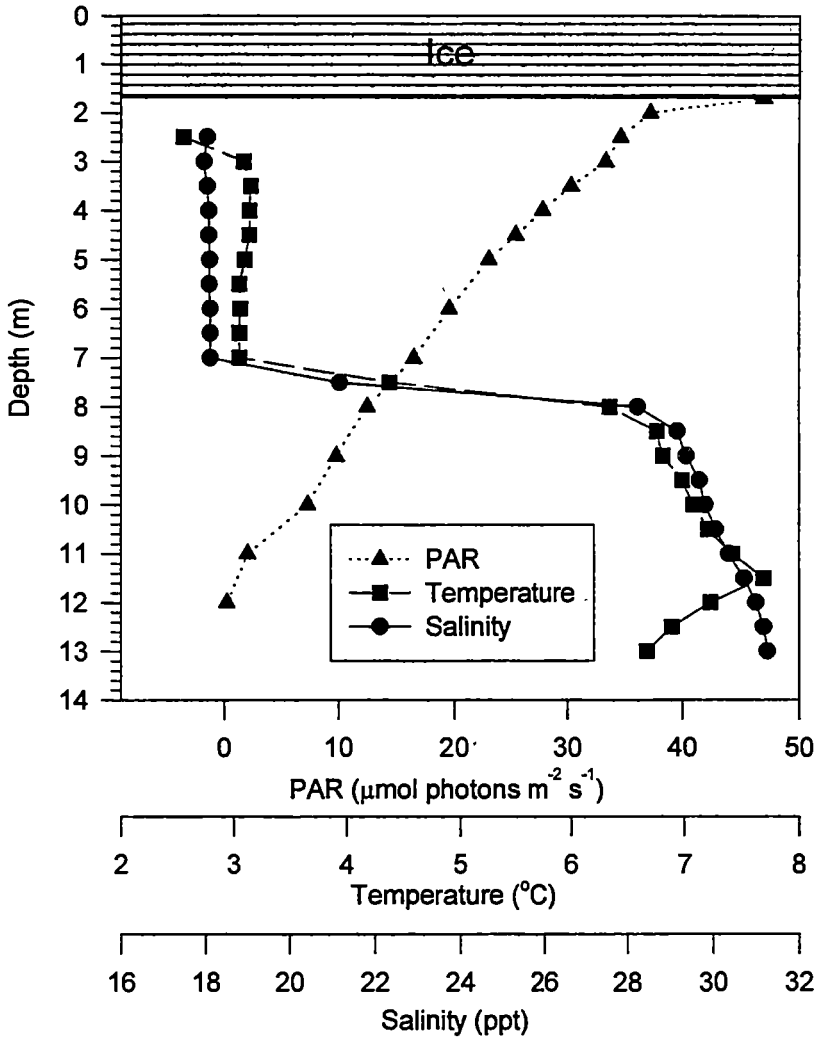

Figure 6.7: Light, temperature and salinity profile of Ace Lake on the 22nd December 1993.

6.4.4 Effect of antibiotic treatment on *Synechococcus* growth rate.

Unfortunately, the metabolic inhibitor method was not sensitive enough for the detection of the difference between *Synechococcus* growth rates and grazing pressure in Ace Lake. There was no significant difference between any of the antibiotic treatments in the factorial experiment (Table 6.9). The mean growth rates for each of the four antibiotic treatments were between 0.10 d^{-1} and 0.17 d^{-1} (Figure 6.8a).

Table 6.9: Interactions between the antibiotic treatments in the factorial field experiment that was designed to determine the effect of light, temperature, salinity and nutrients on growth rate of and grazing rate on *Synechococcus*. Least square means = LSM of growth rate (d^{-1}), standard errors = S.E. of LSM, n= sample number; A = ampicillin; C = cycloheximide; CA = cycloheximide and ampicillin and WA = without antibiotics. The nul hypothesis was that there was no significant difference in growth rate between treatments. Probabilities greater than 0.05 are not deemed to be significant.

				Probability of a difference between treatments				
Treatment	n	LSM	S.E. (LSM)	Treatment	A	C	CA	WA
A	48	0.1682	0.0353	A	-			
C	48	0.1098	0.0348	C	0.2502	-		
CA	48	0.1375	0.0352	CA	0.5392	0.5933	-	
WA	48	0.1349	0.0348	WA	0.5032	0.6278	0.9584	-

A similar result was obtained for the antibiotic interactions in the experiment designed to determine the effect of light on the growth rate and grazing rate of *Synechococcus* (Table 6.10). Mean growth rates obtained for the antibiotic treatments were between 0.06 and 0.19 d^{-1} (Figure 6.8b).

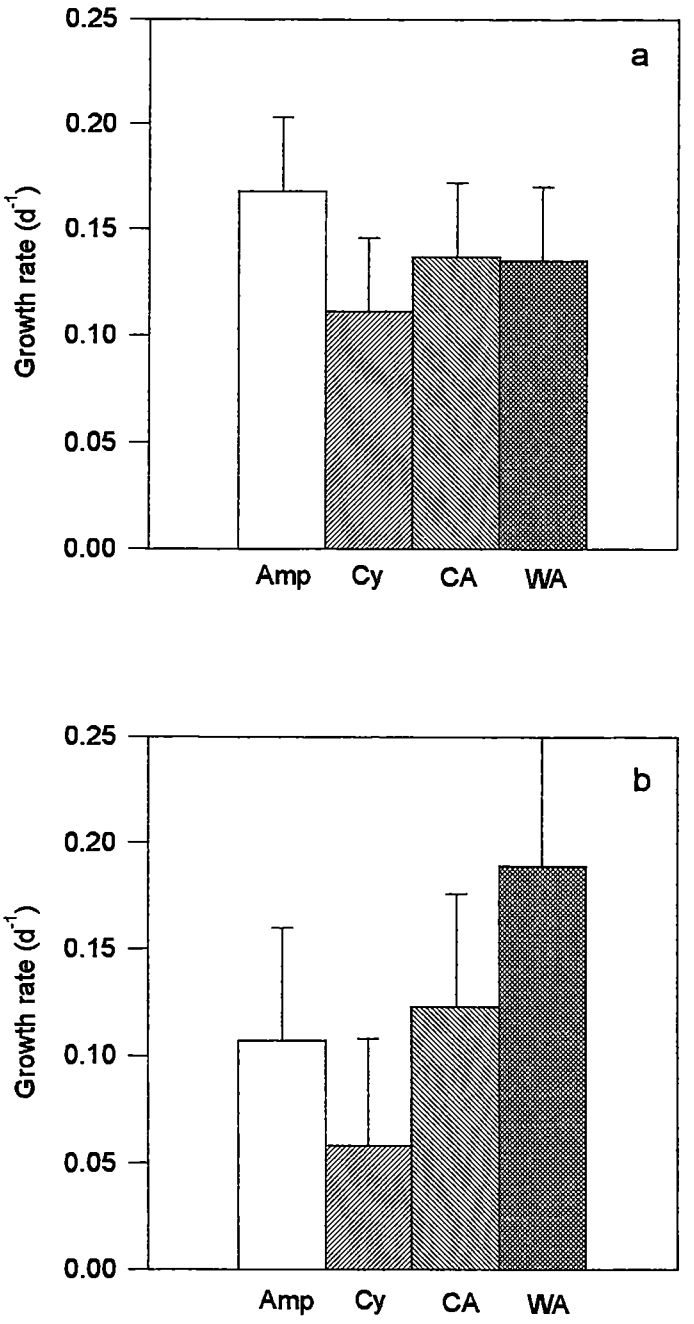

Figure 6.8: Mean growth rates obtained from the four antibiotic treatments in a) the factorially designed experiment and b) the light and depth experiment. Amp = ampicillin, Cy = cycloheximide, CA = both antibiotics and WA = no antibiotics. Error bars are S.E.

Table 6.10: Interactions between the antibiotic treatments in the experiment that was designed to determine the effect of light on the growth rate of and grazing rate on *Synechococcus*. Least square means = LSM of growth rate (d^{-1}), standard errors = S.E. of LSM, n= sample number; A = ampicillin; C = cycloheximide; CA = cycloheximide and ampicillin and WA = without antibiotics. The nul hypothesis was that there was no significant difference in growth rate between treatments. Probabilities greater than 0.05 were deemed to be not significant.

				Probability of a difference between treatments				
Treatment	n	LSM	S.E. (LSM)	Treatment	A	C	CA	WA
A	15	0.1065	0.0532	A	-			
C	15	0.0580	0.0507	C	0.5222	-		
CA	15	0.1234	0.0524	CA	0.8266	0.3717	-	
WA	15	0.1893	0.0622	WA	0.3317	0.1278	0.4361	-

6.4.5 Effect of temperature, light, nutrients and salinity on *Synechococcus* growth rate

As the antibiotic interactions were not significantly different from one another, the grazing rate on *Synechococcus* could not be determined. The antibiotic treatments were pooled in the analysis of depth, light, salinity and nutrient effects on the specific growth rate (r) of *Synechococcus*.

An increase in temperature from 3° C to 7° C (depth increase) increased significantly the growth rate of *Synechococcus* (Table 6.11). The mean growth rate increased from 0.023 d^{-1} at 3° C to 0.2976 d^{-1} at 7° C. There was no significant difference in the growth rate of *Synechococcus* with an increase in the salinity, nor with an increase in nutrient concentration. At 10 m, a 13 % decrease in the light intensity resulted in a higher growth rate (from 0.0.221 d^{-1} to 0.353 d^{-1}) but the difference was not significant ($p = 0.181$). From these results, it was decided to analyse first order interactions but not higher order interactions. If there were significant differences in growth rates from the

effect of salinity, nutrients and light, it would have been worthwhile investigating higher order interactions.

Table 6.11: ANOVA of first order interactions using type III sum of squares.

Treatment	n	Mean Square	f Value	p
Depth	96	0.00737	72.87	0.0001
Nutrients	96	0.00002	0.19	0.6611
Salinity	96	0.00001	0.06	0.7994
Light	96	0.00018	1.81	0.1808

The only first order interaction that produced a significantly greater growth rate ($p < 0.05$) was the interaction between depth and light ($p = 0.0159$). The probability of differences between depth and light combinations are given in Table 6.12. The growth rate of cells grown at 6 m with a 10 m light intensity was lower than the growth rate of cells grown at 6 m with 11 m light intensity (Figure 6.9), although the difference was not significant ($p = 0.4442$). There was a significantly higher growth rate for *Synechococcus* cells grown at 10 m than for those grown at 6 m. The growth rate of *Synechococcus* cells grown at 10 m with a 11 m light intensity (0.35 d^{-1}) was significantly greater ($p = 0.0079$) than cells grown at 10 m with a 10 m light intensity (0.22 d^{-1}, Figure 6.9).

Table 6.12: Least square means (LSM) of growth rate (d^{-1}), standard errors S.E. of LSM, and probabilities of first order interactions between light and temperature. Interaction: 1) 6 m depth, 10 m light; 2) 6 m depth, 11 m light; 3) 10 m depth, 10 m light; 4) 10 m depth, 11 m light. The null hypothesis was that there was no significant difference in growth rate between treatments. Probabilities greater than 0.05 are deemed to be not significant.

				Probability of a difference between treatments				
Treatment	n	LSM	S.E. (LSM)	Treatment	1	2	3	4
1	48	0.0074	0.0348	1	-			
2	48	-0.0309	0.0357	2	0.4442	-		
3	48	0.2213	0.0348	3	0.0001	0.0001	-	
4	48	0.3538	0.0348	4	0.0001	0.0001	0.0079	-

6.4.6 The effect of light and temperature on *Synechococcus* growth rate:

As there were no significant differences between antibiotic interactions and *Synechococcus* growth rate (Table 6.10), antibiotic treatments were pooled to analyse the effect of depth and light on the growth rate of *Synechococcus*. A change in depth from 6 m to 10 m was equivalent to a 4° C temperature increase. Field samples of *Synechococcus*, exposed to 6 m light intensity at 6 m depth (Figure 6.10) had a growth rate that was not significantly different from that of *Synechococcus* at 6 m with a 10 m light intensity (Table 6.13). There were also no significant differences in population numbers of cells grown at 6 m with a 10 m light intensity and those grown at 8 m with a 8 m light intensity. There was, however, a marginally significant difference between growth rate of cells grown at 6 m with a 6 m light intensity and those grown at 8 m with a 8 m light intensity ($p = 0.0351$).

Table 6.13: Least square means (LSM) of growth rate (d^{-1}), standard errors S.E. of LSM, and probabilities of interactions between the following treatments: 1) 6 m depth, 6 m light; 2) 6 m depth, 10 m light; 3) 8 m depth, 8 m light; 4) 8 m depth, 10 m light; 5) 10 m depth, 10 m light. The null hypothesis was that there was no significant difference in growth rate between treatments. Probabilities greater than 0.05 are deemed to be not significant.

				Probability of a difference between treatments				
Treatment	n	LSM	S.E. (LSM)	Treatment	1	2	3	4
1	12	-0.1176	0.0576	1	-			
2	12	0.0408	0.0576	2	0.0876	-		
3	12	0.072	0.0576	3	0.0351	0.7108	-	
4	12	0.2808	0.0576	4	0.0003	0.0152	0.0233	-
5	12	0.3408	0.06	5	0.0001	0.0051	0.0072	0.4929

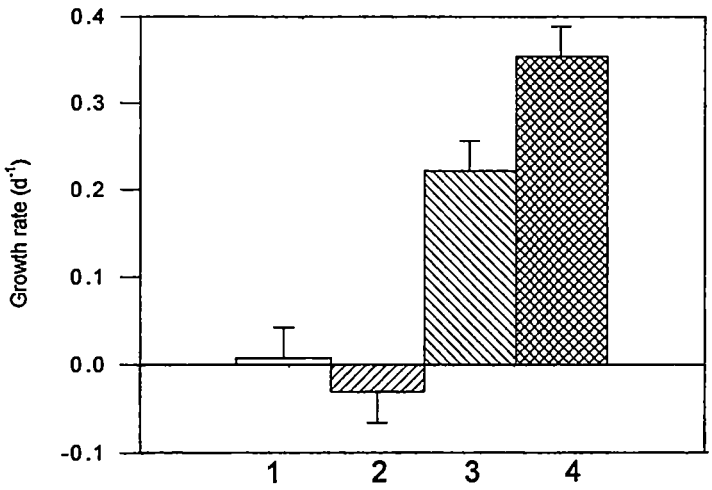

Figure 6.9: Growth rates of *Synechococcus* from first order interactions between depth and light in the 2^4 factorially designed experiment. 1) 6 m depth, 10 m light; 2) 6 m depth, 11 m light; 3) 10 m depth, 10 m light; 4) 10 m depth, 11 m light. Error bars are S.E.

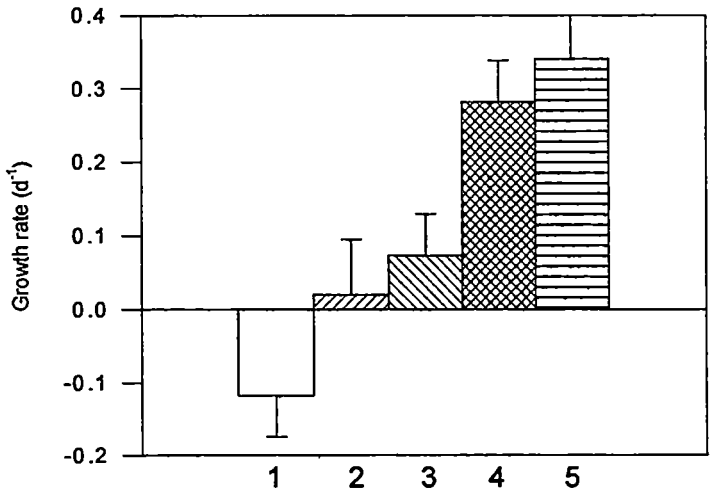

Figure 6.10: Mean growth rate of *Synechococcus* cells grown at three depths and at two light intensities in Ace Lake. 1) 6 m depth, 6 m light; 2) 6 m depth, 10 m light; 3) 8 m depth, 8 m light; 4) 8 m depth, 10 m light; 5) 10 m depth. Error bars are S.E.

Cells grown at 10 m grew significantly faster ($p < 0.05$) than those at 6 m with a 6 m or a 10 m light intensity or at 8 m with an 8 m light intensity. Further, there were no significant differences between cells grown a 8 m with 10 m light, and those grown at 10 m ($p = 0.4929$). The specific growth rate observed for *Synechococcus* grown at 6 m , 8 m and 10 m, without any manipulation of light intensity, was -0.1176 d^{-1}, 0.072 d^{-1} and 0.341 d^{-1} respectively.

6.5 DISCUSSION

The distribution and abundance of *Synechococcus* in the marine and freshwater environment is equivalent to rate of growth (k) minus the loss rate in that environment. In Ace Lake, there is little physical mixing to remove cells. The sinking rate of *Synechococcus* is negligible (Craig 1987; Takahashi and Bienfang 1983) and because of the density gradient in the lake the sinking rate would be lower than that predicted. Low temperatures, nutrient limitations and light intensity limitations may reduce the growth rate of *Synechococcus*. *Synechococcus* is capable of growth in a wide range of nutrient concentrations (Burns and Stockner 1991; Voros et al. 1991), and light intensities (Waterbury et al. 1986). Significant *Synechococcus* cell losses, in Ace Lake, could be from grazing by heterotrophic eukaryotes or possibly by viral attack. Waterbury and Valois, (1993) indicated that *Synechococcus* is more resistant to viral attack than are other picoplankton although this claim needs to be tested in Ace Lake. Heterotrophic microflagellates that are capable of grazing *Synechococcus* (Hagstrom et al. 1988; Waterbury and Valois 1993) have been observed in Ace Lake (Laybourn-Parry and Perriss 1995) and grazing by these organisms is probably the major cause of *Synechococcus* loss in Ace Lake.

Ace Lake has a relatively simple phytoplankton assemblage (Burch 1988). The potential grazing populating on *Synechococcus* in the lake consists of nanoflagellates, both heterotrophic (Laybourn-Parry and Perriss 1995), and possibly autotrophic (Hall et al. 1993) ciliates (Laybourn-Parry and Perriss 1995) and one copepod species (Bayly and Burton 1987). Copepods are considered to have minimal direct impact on picocyanobacteria abundance as they usually graze on particles of sizes larger than *Synechococcus* cells (Johnson et al. 1982) although nauplii and early copepodite stages may have a significant impact.

The metabolic inhibitor method has been used extensively to determine the growth rate of, and grazing rate on, *Synechococcus* cells (Campbell and Carpenter 1986; Liu et al. 1995; Xiuren and Vaulot 1992). This method relies on specific metabolic inhibitors (ampicillin and cycloheximide) to keep either prey or predator populations constant. When ampicillin is added to a sample, the grazing rate (g) of *Synechococcus* can be determined via measurement of the decrease in population size of the non-replicating *Synechococcus* cells. When cycloheximide is added the absolute growth rate (k) can be determined as grazing by eukaryotes is inhibited. The specific growth rate (r) is equivalent to k - g (Xiuren and Vaulot 1992). The primary assumptions of the method is that organisms, *in-situ*, grow exponentially and that this is not changed by manipulation of samples (Campbell and Carpenter 1986). Another assumption is that all eukaryotic growth is inhibited by cycloheximide and that all prokaryote growth is inhibited by ampicillin (Xiuren and Vaulot 1992). Unfortunately, there was no significant difference in the growth of *Synechococcus* between any of the antibiotic treatments. This was unexpected as the antibiotic specificity and optimal concentrations

had been determined on Ace Lake *Synechococcus* cells (Figure 6.6), and an Ace Lake cryptomonad (Figure 6.4) in laboratory experiments prior to the field experiments. Also, these antibiotics had been used by others to predict successfully the growth rates of, and grazing rates on, *Synechococcus* in tropical and temperate environments (Campbell and Carpenter 1986; Xiuren and Vaulot 1992).

There are several possible reasons as to why differences in growth rates were not detected between antibiotic treatments. It may be that the antibiotics were not specific in their action after all, in the laboratory experiments, cycloheximide was only tested against one eukaryote from Ace Lake. However, other studies had achieved general inhibition of a diverse group of grazing eukaryotes by using cycloheximide (Campbell and Carpenter 1986; Xiuren and Vaulot 1992). It is possible that grazing on *Synechococcus* was not great enough to be detected using this experimental design. It is also possible that the effect of cycloheximide on the eukaryotic grazers was too slow to show an effect. Another problem in the experiment may have resulted from logistical constraints. Samples had to be preserved and frozen and although under laboratory conditions these preservation techniques did not result in a significant cell loss (Figure 6.3) the situation may have been different under the field conditions. Ideally, the samples would have been analysed immediately after collection without preservation. The effectiveness of the individual metabolic inhibitors may have affected the experimental outcomes. A final possibility involves the assumption that manipulation of samples did not perturb growth characteristics. In the laboratory, when a *Synechococcus* culture was diluted and antibiotics added, there was a lag phase of approximately two days (Figure 6.5, 6.6). The same lag period occurred in the *Cryptomonas* sp. cultures (Figure 6.4). It is possible that manipulation and dilution of

samples in the field resulted in a perturbation of growth of the microorganisms in the samples.

The metabolic inhibitor technique is one of four methods available to determine the growth rates and grazing loss of *Synechococcus*. The serial dilution technique relies on a reduction in predator concentration and hence grazing pressure (Landry et al. 1984). The assumptions of this technique include the exponential growth of *Synechococcus*, that dilution of samples does not cause a lag phase in exponential growth, and that the predator grazing rates are not affected by *Synechococcus* densities (Campbell and Carpenter 1986; Xiuren and Vaulot 1992). The frequency of dividing cells technique calculates growth rate based on the number of dividing cells in a population (Campbell and Carpenter 1986). The main assumption of this technique is that the duration of cell division is constant with respect to environmental conditions. The last technique, used by Iturriaga and Mitchell, (1986), determines growth rate and grazing loss using cellular carbon incorporation and ^{14}C- labelled sample addition. Assumptions associated with this technique include an estimation of cellular carbon content, determination of a mean cellular volume and no discrimination of labeled cells by grazers. According to Campbell and Carpenter, (1986) and Xiuren and Vaulot, (1992) the metabolic inhibitor technique gives the most reliable and sensitive results especially when *Synechococcus* densities are low. Flow cytometric techniques were also more suited to this technique than the others described.

Even though absolute growth rates (k) and grazing rates (g) could not be calculated, the specific growth rate (r) which incorporates grazing loss, was determined. The effect of

salinity, nutrient addition, temperature and light intensity on the growth of *Synechococcus* could still be ascertained.

At the time of the experiment, the population density of *Synechococcus* cells was low (10^4 ml^{-1}) in the top 7 m of the lake and it increased between 7 m and 11 m. In 1992, when a bloom population was monitored, numbers peaked at 11 m with a maximum density of 8 x 10^6 ml^{-1} (Chapter 3). It should be considered that absolute cell densities and growth rates obtained at any particular time are only relevant to that time. Cell numbers and growth rates will vary from day to day and year to year depending on past and present physical, chemical and biological conditions (Harris 1986) and depending on the balance between population gains and losses at that time (Vincent 1998). Seasonal trends in distribution and abundance of *Synechococcus* in Ace Lake should, however, be relatively constant.

Over the course of the experiments, salinity and temperature in Ace Lake remained relatively constant. The temperature in the lake at 6 m was approximately 3° C and at 10 m the temperature was 7° C. Light intensity varied because of variation in the cloud cover. Waterbury et al., (1986) found that changes in light intensity throughout an experimental period altered the growth rate of *Synechococcus*. With a 50 % increase in cloud cover there was between a 9 % and 20 % decrease in the number of dividing cells in surface water samples collected from Woods Hole Harbour. It is probable, however, that if the light intensity variation between sunny and cloudy condition were averaged, the difference in mean light intensity between each experiment would be small.

Synechococcus growing at 6 m in Ace Lake at a temperature of 3° C had a negative specific growth rate (Figure 6.10). The predicted absolute growth rate for the Ace Lake *Synechococcus* at this temperature, based on laboratory measurements and square root model prediction, was 0.04 d^{-1} (generation time, g.t., 17.5 days, Chapter 5). It is probable that the negative growth rate resulted from grazing during containment. The specific growth rate of *Synechococcus* at 8 m, at which the temperature was 6.3° C, was 0.072 d^{-1} (g.t. 9.6 days). This compares to k = 0.058 d^{-1} (generation time of 11.95 days) as predicted from the square root model (Chapter 5). At 10 m in Ace Lake, 7° C, the specific growth rate was 0.341 d^{-1} (g.t. 2.03 days). This growth rate was much higher than that obtained under laboratory conditions. At 7° C the predicted absolute growth rate was 0.061 d^{-1} (g.t. 11.29 days, Chapter 5). The growth rates obtained under laboratory conditions were from an experiment designed to determine optimal growth temperatures. It is probable that during these experiments, other physical (eg: light intensity), chemical and biological conditions were not optimal for the growth of *Synechococcus* cells. Year long measurement of *Synechococcus* cell numbers at 10 m, taken in 1992, showed that a *Synechococcus* bloom occurred between late October and December (Chapter 3). The growth rate of *Synechococcus* calculated over this bloom period was 0.060 d^{-1} (g.t. 11.6 days) which was very close to that predicted for the temperature experienced at 10 m based on laboratory data fitted to the square root model (μ = 0.061 d^{-1}). It is possible that during a bloom, the increase in abundance of *Synechococcus* could alter the local physical, chemical or biological conditions, thereby altering the growth rate of the population. Self shading, for example, could reduce the light intensity. The other possibility is that the containment of the population produced an artificially high growth rate.

During the 1993/94 summer, the *Synechococcus* bloom at 10 m was not monitored. If it is assumed that the bloom occurred at approximately the same time as it did in 1992 (Chapter 3) then these experiments were carried out when the *Synechococcus* population was almost at its peak and just about to decline. It is probable that during this time the impact of grazing was low and that specific growth rate obtained at 10 m would be close to the absolute growth rate (k) for the population.

The specific growth rates for the 6 m, 8 m and 10 m populations were calculated on populations taken from these three depths respectively. Although the differences in growth rate at the three depths can probably be attributed largely to temperature, other causes, such as nutrient concentration and light intensity, can not be eliminated.

The growth rates of and grazing rates on *Synechococcus* in a variety of marine and freshwater habitats are summarised in Table 6.14. Using the metabolic inhibitor technique, Xiuren and Vaulot, (1992) obtained absolute growth rates ranging between 0.25 and 0.72 d^{-1} and grazing rates ranging between 0.21 and 0.64 d^{-1} for *Synechococcus* in the English Channel. A growth rate of 0.77 ± 0.19 d^{-1} and grazing rate of 0 - 0.35 d^{-1} was obtained by Campbell and Carpenter, (1986) in the Northwest Atlantic Ocean. Liu et al., (1995) found that the growth rate of *Synechococcus* in coastal waters off Hawaii decreased with depth and grazing pressure was variable. The specific growth rates obtained for the Ace Lake *Synechococcus* at the three depths in Ace Lake were consistent with those summarised in table 6.14.

The factorially designed experiment tested the effect of four factors on the growth of the *Synechococcus* population from 6 m in Ace Lake. The growth of *Synechococcus* significantly increased with an increase in water temperature from 3° C at 6 m to 7° C at 10 m (P = 0.0001, Table 6.11). Li and Dickie, (1991) found that growth rate and cell abundance were correlated. This holds for the distribution of *Synechococcus* in Ace Lake. The highest growth rates and cell concentrations were obtained at 10 m where the water was warmer. At 6 m in Ace Lake where *Synechococcus* growth rate was slow, net losses, probably due to grazing, could have easily outweighed net gains. At higher temperatures where the net gains are higher, greater loss rates are required to reduce the population density. Populations grown at 6 m did not respond well to any manipulation. This is probably because growth rate was suppressed by the low temperature at this depth.

Table 6.14: Growth rates (k) of and grazing rates (g) on *Synechococcus* in some coastal, oceanic and lake environments. k = absolute growth rate, g = grazing rate, r = (k - g) = specific growth rate. MI = metabolic inhibitor technique, FDC = frequency of dividing cells technique, ^{14}C = grazing was determined by addition of ^{14}C labelled *Synechococcus* cells, growth rate was determined by changes in *Synechococcus* cell numbers in natural population, DT = dilution technique

Location	Habitat	(k) d^{-1}	(g) d^{-1}	r (d^{-1})	Method	Reference
English Channel	Coastal	0.51	0.44	0.07	MI	(Xiuren and Vaulot 1992)
Celtic Sea	Ocean	0.32	0.41	-0.09	MI	(Xiuren and Vaulot 1992)
NW Atlantic	Ocean	0.77	0.350	0.42	MI	(Campbell and Carpenter 1986)
Hawaii	Coastal	0.64	0.31	0.33	MI	(Liu et al. 1995)
Hawaii	Oceanic	0.42	0.28	0.14	MI	(Liu et al. 1995)
Hawaii	Coastal	1.7	0.23	1.47	DT	(Landry et al. 1984)
North Pacific	Oceanic	1.8	0.3	1.50	^{14}C	(Iturriaga and Mitchell 1986)
Lake Huron	Freshwater			0.34	FDC	(Fahnenstiel et al. 1991b)
Lake Michigan	Freshwater			0.35	FDC	(Fahnenstiel et al. 1991b)
Lake Constance	Freshwater	0.14-1.22	0.05-1.27	0.66	DT	(Weisse 1988)

There are conflicting reports as to the sensitivity of *Synechococcus* to light intensity. In laboratory experiments, El Hag, (1986) and Morris and Glover, (1981) indicated that *Synechococcus* was adapted to growth under low light intensities. Further, the presence

of the phycobiliproteins make it efficient at harvesting light deeper in the water column (Barlow and Albert 1985). Kruger and Eloff, (1978) found that the activation energy of *Synechococcus* PCC6301 was higher at lower low light intensities (25 μmol photons m^{-2} s^{-1}) and (Platt et al. 1983) found that photosynthesis was inhibited at high light intensities. Also, in some ocean and lake environments *Synechococcus* was more abundant deeper in the euphotic zone than in the surface waters (Murphy and Haugen 1985; Takahashi and Hori 1984; Venkateswaran et al. 1993). In other regions, however, *Synechococcus* was more abundant in surface waters than deeper in the water column (Caron et al. 1985; Jochem 1995; Waterbury et al. 1979). Further, in photosynthetic irradiance curves for several cultured strains, although photosynthesis was saturated at low light intensities, a reduction in photosynthesis was not detected at high light intensities (Alberte et al. 1984; Glover and Morris 1981). This investigation has determined that the Ace Lake *Synechococcus* has a higher growth rate at lower light intensities (Figure 6.9 and 6.10). *Synechococcus* populations grown at 8 m but with a 10 m light intensity had a growth rate that was not significantly different from the population grown at 10 m. Phylogenetic analysis of *Synechococcus* strains is now showing a substantial degree of genetic variation within this group of picocyanobacteria (Chapter 5; Palenik 1994; Schmidt et al. 1991). It is probable that there are strains that are adapted to low light conditions and others that are adapted to life in the mixed zone of oceanic surface waters where they are exposed to periods of high light intensity. Most of the reports describing the abundance and distribution of *Synechococcus* in the Southern Ocean have been from surface water samples (Letelier and Karl 1989; Marchant et al. 1987). The possibility that, at certain times throughout the year, *Synechococcus* cells could be more abundant at the bottom of the euphotic zone, should

not be discounted. A detailed understanding of the vertical distribution of *Synechococcus* in the Southern Ocean warrants investigation.

The addition of nutrients and the increase in salinity failed to alter significantly the growth rate of *Synechococcus* (Table 6.11). It was anticipated that increasing the nutrient concentration would increase the growth rate of *Synechococcus*. Although *Synechococcus* is competitive in oligotrophic waters (Cuhel and Waterbury 1984; Iturriaga and Mitchell 1986; Platt et al. 1983), there is ample evidence of an increase in *Synechococcus* abundance in nutrient rich lakes as compared to oligotrophic lakes (Burns and Stockner 1991; Voros et al. 1991) and in coastal waters as compared to the oligotrophic open ocean (Jochem 1995; Li et al. 1983). In Canada, lakes are artificially fertilized to encourage phytoplankton growth. Studies of *Synechococcus* abundance, before and after fertilization, indicate a preference for higher nutrient conditions by *Synechococcus* (Hardy et al. 1986; Shortbreed and Stockner 1990; Stockner and Shortbreed 1988). The top 7 m of Ace Lake had nitrogen and phosphorus concentrations that were below the limit of detection of the analytical techniques used (Chapter 3). Addition of SNAX mineral media to the contained samples resulted in a phosphorus (HPO_4^{2-}) increase of 1.5 mg l^{-1} and a nitrogen (NO_3^- and NH_4^+) increase of 75 mg l^{-1} and 5.3 mg l^{-1}. Based on experimental evidence (Shortbreed and Stockner 1990; Stockner and Shortbreed 1988), *Synechococcus* should have responded with an increased growth rate. It is possible that incubation for 48 h was not long enough to detect this response. It can take more than one generation for phytoplankton to equilibrate to new conditions (Harris 1986). It is also known that some *Synechococcus* strains store nitrogen as phycobiliproteins (Wyman et al. 1985) and that when nutrients

are added to an oligotrophic system, *Synechococcus* may take up nutrients for storage rather than for growth (Glibert et al. 1986).

The Ace Lake *Synechococcus* strain could tolerate a broad salinity range (Chapter 5), although a salinity equivalent to freshwater was not tolerated. Further, *Synechococcus* has been observed in several estuarine and coastal environments where the environment is affected by tidal movement and freshwater inflow (Bertrand and Vincent 1994; Vaulot and Xiuren 1988). Within Ace Lake and within the other meromictic lakes in the Vestfold Hills where *Synechococcus* occurs, salinity is probably not a strong controlling factor in the distribution of *Synechococcus* cells.

It is acknowledged that when carrying out experiments in confined containers over long periods, there is the possibility that effects caused simply by containment could be observed (Harris 1986). These effects could result from nutrient deficiencies, over grazing by organisms trapped in the containers (Prezelin et al. 1986) or simply by the manipulation of the contained planktonic populations. In the current experiments, incubation of the containers *in-situ* and manipulation of samples under subdued light may have mimimised the artificial effects of containment. Containment of samples in small (50 ml) aliquots and addition of nutrients and salt water dilution the cell numbers may have altered the growth of *Synechococcus*. Due to the large number of treatments small sample volumes were necessary. Incubation over 48 h was necessary because of the slow *in-situ* growth rates of Antarctic organisms (Franzmann et al. 1988; McMeekin and Franzmann 1988), including *Synechococcus*, and it is possible that because of the slow growth rates nutrient depletion may not have been a concern.

Of the four factors measured, water temperature had the greatest effect on *Synechococcus* growth rate in Ace Lake. There was almost a one hundred times increase in growth rate between the population grown at 6 m and the population grown at 10 m, with the same light intensity (Table 6.12). However, according to laboratory experiments, Ace Lake *Synechococcus* is capable of growth at 3° C (0.058 d^{-1}) and the low (almost negligible) specific growth rate at 6 m in Ace Lake, (Table 6.13), probably resulted from grazing pressure at this depth. At this low growth rate, an intense grazing pressure would not have been required to keep the abundance of *Synechococcus* cells low. Light intensity also had a significant impact on *Synechococcus* growth rate. *Synechococcus* grew faster at the light intensities experienced deeper in the euphotic zone of the lake than in the surface waters. Cells growing at 10 m in the lake were therefore advantaged by the warmer temperatures and the lower light intensity relative to those growing at 6 m. The pigment complex of the Ace Lake *Synechococcus* (Chapter 5) is optimal for harvesting energy at the wavelengths that penetrate to the bottom of the euphotic zone (Burch 1988).

These experiments were carried out during the spring bloom of *Synechococcus*, (Chapter 3), and it is possible that the grazing population was still responding to the *Synechococcus* bloom. It is probable that, at this time, the grazing pressure on *Synechococcus* was relatively low. The grazing population in Ace Lake requires further characterisation. Currently a mixotrophic ciliate (*Mesodinium rubrum*) (Perriss et al. 1995) and the copepod *Paralabidocera antarctica* (Bayly 1978) have been characterised. Heterotrophic and autotrophic microflagellates have been identified (Laybourn-Parry et al. 1996) but not characterised. The possibility of the autotrophic

nanoflagellates being mesotrophic is yet to be determined and the grazing potential of all of these organisms on *Synechococcus* remains unknown.

In the polar oceans (Gradinger and Lenz 1989; Ishizaka et al. 1994; Letelier and Karl 1989; Marchant et al. 1987; Murphy and Haugen 1985) and freshwater lakes (Caron et al. 1985) *Synechococcus* population densities have been found to be correlated with temperature. The temporal distribution of *Synechococcus* has also been correlated to temperature (El Hag and Fogg 1986; Waterbury et al. 1986). At the low temperatures experienced in the southern and northern polar oceans, the growth rate of *Synechococcus* would probably be low, (0.038 d^{-1} at 1.7° C for the Ace Lake *Synechococcus*). However, if no other factor was controlling the abundance of *Synechococcus* the organism could still reach high densities, it would just take longer. Vincent, (1998) proposed that the abundance of cyanobacteria in polar regions is dependent on a balance between gain and loss rates. If, as is probably occurring in the Southern Ocean, the loss rates (grazing, turbulence) are high, and the gain rates are low (temperature), *Synechococcus* cell abundance will be low.

Conclusion

Ace Lake is one of approximately thirty meromictic lakes and marine basins in the Vestfold Hills Antarctica. The lake was isolated from the sea some 6000 years ago and has undergone substantial change to reach its current stratified state. The discovery and characterisation of the Ace Lake *Synechococcus* strain, has increased the knowledge of biodiversity in this intensively studied lake.

The discovery of *Synechococcus* in Ace Lake and the subsequent ecological and taxonomic investigations were aided by the use of flow cytometric methods. In aquatic microbiology, flow cytometry relies upon the detection of autofluorescence produced by phytoplankton when cells are exposed to specific wavelengths of light. Flow cytometry has become a valuable tool for microbial ecologists and is now routinely used for the rapid detection and analysis of individual phytoplankton cells. The use of flow cytometry to monitor phytoplankton populations in the meromictic lakes of the Vestfold Hills was the first time this technology had been applied in Antarctica.

In Ace Lake, *Synechococcus* occurred in highest numbers just above the anaerobic interface. At the peak of the spring bloom, in 1992, a density of 8×10^6 cells ml^{-1} was measured at 11 m in Ace Lake. The spring bloom of *Synechococcus*, at 10 m in Ace Lake, began in September when the water temperature was at a minimum. It is probable that the spring bloom was induced by an increase in light intensity. At 10 m there was a rapid

decline in *Synechococcus* cell numbers at the end of December. The cause of this decline in cell numbers is not known but it is possible that it resulted from grazing, nutrient limitations or self-shading. No diel periodicity was detected in the Ace Lake *Synechococcus* population.

In a survey of eleven other meromictic lakes and marine basins in the Vestfold Hills, *Synechococcus* was detected in Lake Abraxas and in Pendant Lake. These two lakes are in close proximity to Ace Lake, on Long Peninsula. The organism occurred throughout the aerobic zone in Pendant Lake, in densities of approximately 10^7 cells ml^{-1}, and below the pycnocline in Lake Abraxas in densities of 1.4×10^7 cells ml^{-1}. It is possible that the distribution of *Synechococcus* in the meromictic lakes of the Vestfold Hills is controlled by salinity.

Synechococcus strains were isolated from Ace Lake, Pendant Lake and Lake Abraxas for taxonomic characterisation. The three strains were similar in size and had the same lipid soluble pigment signature, with two unknown carotenoid pigments present in addition to the chlorophyll *a*, zeaxanthin and ββ- carotene. The three strains had phycoerythrin as their principle accessory light harvesting pigment. They were genetically similar (99.7 % similarity in the 16S rRNA sequence) and had a G + C content of between 57 and 58 mol %. They were also genetically similar (95.7 % similarity in the 16S rRNA sequence) to other marine *Synechococcus* strains and to another marine picocyanobacteria, *Prochlorococcus marinus*. The relationship between marine *Synechococcus* and *P. marinus* was investigated and the use of photosynthetic pigments, as a taxonomic characteristic in

which to separate genera of photosynthetic bacteria, was questioned. The minimum, optimal and maximum theoretical growth temperatures of the Ace Lake *Synechococcus* strain, based on the square root model, were -8° C, 19.7° C, and 29.8° C respectively.

In-situ experiments were carried out in Ace Lake to determine growth rates and to determine the factors that controlled the distribution of *Synechococcus* in the lake. *In-situ* growth rates of the Ace Lake *Synechococcus* strain at 6 m, 8 m and 10 m in Ace Lake were determined. These rates were -0.118 d^{-1}, 0.072 d^{-1} and 0.341 d^{-1} respectively. An increase in water temperature and a reduction in light intensity increased the *in-situ* growth rate of the Ace Lake *Synechococcus* population. The grazing pressure on *Synechococcus* in Ace Lake was not determined. However, eukaryotic organisms, known to graze on *Synechococcus*, occur in Ace Lake and it is probable that the distribution and abundance of *Synechococcus* in Ace Lake, Pendant Lake and Lake Abraxas is partially controlled by grazing.

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The ecology and taxonomy of *Synechococcus* from saltwater lakes in the Vestfold Hills, Antarctica.

(Appendices)

Appendix 1

Raw data from Chapter 1

Table A1.1: Summer and winter temperature (° C) profiles in Ace Lake during 1992.

Depth	August	February
2	-0.9	0.45
4	-0.8	0.42
6	-0.8	0.42
7		0.76
8	4.1	
9		10.57
10	4.9	11.42
12	6.3	
13		7.17
14	6.7	
15		6.33
16	6.2	
17		5.42
18	5.2	
19		4.38
20	4.1	
21		3.5
22	3.3	
24	2.7	

Table A1.2: Salinity readings (g kg^{-1})in Ace Lake in December 1992 and 1974.

Depth	Salinity (1992)	Salinity (1974)
1	15.44997	
2	16.57482	
3	16.62506	
4	16.65458	27.70295
5	16.6736	
6	16.68836	
7	16.79381	27.82449
8	27.65842	
9	29.1057	31.11545
10	29.57036	
11	30.16604	
12	30.70317	31.90984
13	30.92374	
14	31.13651	32.37568
15	31.32659	
16	31.64487	
17	32.23695	32.96186
18	33.27002	
19	35.03938	
20	36.89861	
21	38.8994	
22	39.97319	41.11661
23	40.33538	
24	40.47211	41.72948
24.5	33.62832	

Table A13: Dissloved gases and sulfate concentrations (mmol l^{-1}) in Ace Lake (from Franzmann et al., 1991).

Depth	Oxygen	Sulfate	Methane	H2S
1		0.96		
2	3.464	2.79		
3		3		
4		4.02		
5	3.683	4.43		
6		6.67		
7		6.06		
8		7.38		
9		7.69		
10	0.804	9.02		
11	0.585	7.28	0.0158	0
12	0	8.61	0.064	0.018
13		7.99	0.0999	0.797
14		7.29	0.125	0.888
15		4.93		1.571
16		5.14	0.2158	2.132
17		3.71		3.962
18		1.37	0.747	4.794
19		0.75		5.891
20		0.65	1.8408	6.747
21		0.55	2.1429	7.412
22		0.77	4.2946	7.394
23		0.65	4.2679	7.932
24		0.5	4.9252	8.062

Table A1.4: Measured nitrogen concentration and calculated saturated nitrogen concentration in Ace Lake (from Burton, 1980).

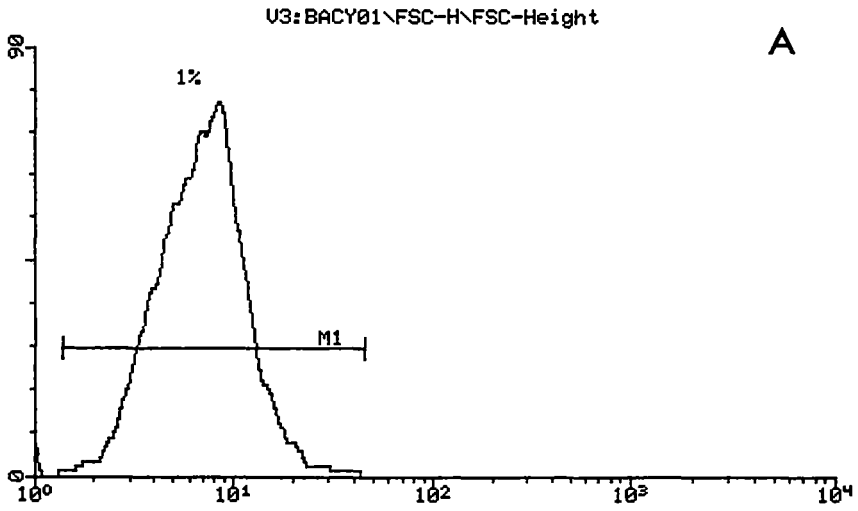
Depth	Equilibrium	Measured
7	13.5	16
10	12.3	15
11	12.4	15
12	12.5	15
13	12.6	15.2
17	13.5	14
20	13.3	0
22	13.5	
24	13.5	

Table A1.5: Vertical distribution of nutrients in Ace Lake (from Burton, 1980)

Depth (m)	PO_4^{2-}	Total N	NH_4- N+ Amino acids
2	0.10	1.0	
5	0.10	1.0	
7	0.20	1.0	0.050
10	0.50	2.0	0.465
12	0.60	6.0	0.730
15	1.95	24.0	0.780
20	9.35	121.0	
22	10.00	123.0	0.695

Appendix 2

Raw data from Chapter 2



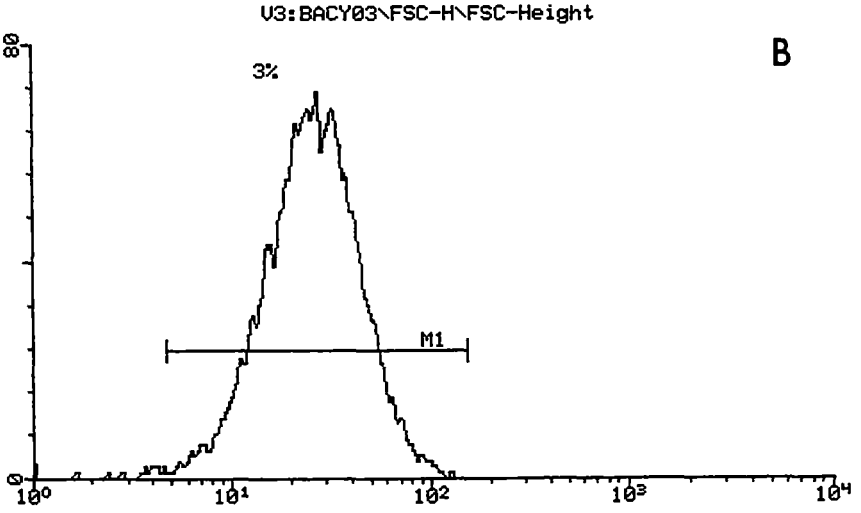
V3:BACY01\FSC-H\FSC-Height

--- Arithmetic Histogram Statistics for V3:BACY01 ---

Selected Preferences: Arithmetic/Linear

Parameter FSC-H FSC-Height Ungated

M	Left,Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
0	1.00, 9910	9995	100.00	78	8.28	7.71	6.98	4.29	55.71
1	1.41,45.32	9951	99.56	78	8.28	7.74	6.98	4.28	55.32



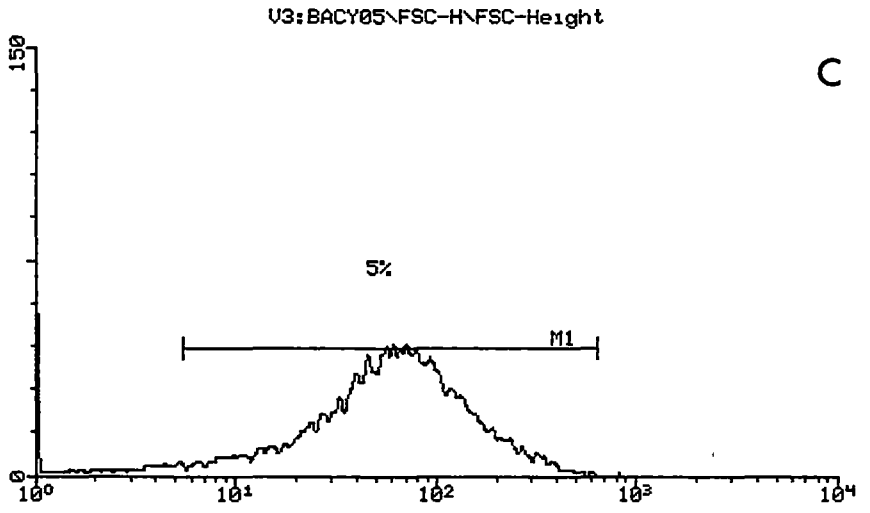
V3:BACY03\FSC-H\FSC-Height

--- Arithmetic Histogram Statistics for V3:BACY03 ---

Selected Preferences: Arithmetic/Linear

Parameter FSC-H FSC-Height Ungated

M	Left,Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
0	1.00, 9910	9972	100.00	71	26.90	28.57	25.48	15.80	55.29
1	4.74, 152	9881	99.09	71	26.90	28.81	25.48	15.68	54.43



V3:BACY05\FSC-H\FSC-Height
--- Arithmetic Histogram Statistics for V3:BACY05 ---
Selected Preferences: Arithmetic/Linear
Parameter FSC-H FSC-Height Ungated

M	Left,Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
0	1.00, 9910	9996	100.00	123	1.00	78.49	58.29	76.27	97.18
1	5.52, 643	9248	92.52	46	60.43	84.58	63.21	75.63	89.43

Figure A2.1: Flow cytometric histograms and histogram statistics showing the effect of salinity on the forward angle light scatter (FSC) image of bacteria. A) bacteria in a 1 % NaCl solution; B) bacteria in a 3 % NaCl solution; C) bacteria in a 5 % NaCl solution.

Table A2.1: Comparison between the flow cytometer and the microscope for the counting of cultured bacterial cells (cells ml^{-1}).

Flow Cytometer	Microscope
3.6×10^8	4.1×10^8
6.4×10^8	4.5×10^8
6.4×10^8	4.2×10^8
4.8×10^8	3.9×10^8
4.6×10^8	4.2×10^8
3.9×10^8	4.6×10^8
4.7×10^8	4.3×10^8
3.4×10^8	4.1×10^8
4.7×10^8	4.5×10^8
3.2×10^8	4.3×10^8

Table A2.2: Comparison between the flow cytometer and the microscope for the counting of bacterial cells in lake water samples (cells ml^{-1}).

Sample	Flow Cytometer	Microscope
Ace Lake 12 m	8.3×10^6	8.7×10^6
	8.5×10^6	8.5×10^6
	8.3×10^6	8.1×10^6
	8.5×10^6	8.3×10^6
	8.4×10^6	8.4×10^6
Ace Lake 8 m	2.1×10^6	1.7×10^6
	2.2×10^6	2.5×10^6
	2.1×10^6	1.9×10^6
	2.1×10^6	1.9×10^6
	1.9×10^6	2.1×10^6

Table A2.3: Comparison between the flow cytometer and the microscope for the counting of cultured *Synechococcus* cells (cells ml^{-1})

Flow Cytometer	Microscope
3.02E+07	2.52E+07
2.16E+07	2.45E+07
2.61E+07	2.63E+07
2.46E+07	2.76E+07
2.60E+07	2.59E+07
2.41E+07	2.57E+07
2.55E+07	2.77E+07
2.97E+07	2.55E+07
2.47E+07	2.69E+07
2.43E+07	2.82E+07

Table A2.4 Counting error in flow cytometer (cells ml^{-1})

A	B	C	D	E
1.48E+08	1.48E+08	1.53E+08	1.21E+08	1.04E+08
1.59E+08	1.26E+08	1.52E+08	1.18E+08	1.05E+08
1.58E+08	1.25E+08	1.48E+08	1.18E+08	8.97E+07

Table A2.5: Flow cytometric data used to calculate cell numbers in Table 2.4 of Chapter 2. The bead concentration used was 1.76 x 10^7 beads ml^{-1}.

Depth	Events	Volume (ml)	Bead Volume (ml)	Bead count	R1	R2	R3	R5
A	10000	1	0.01	6775	70	133	24	125
B	10000	1	0.01	6288	231	144	38	401
C	10000	1	0.01	4811	250	83	19	1861

Appendix 3

Raw data from Chapter 3

Table A3.1: Temperature data (°C) in Ace Lake during 1992

Depth	25-Feb-92	16-Apr-92	17-May-92	14-Jul-92	17-Aug-92	20-Sep-92	23-Oct-92	04-Nov-92	20-Nov-92	02-Dec-92	17-Dec-92	30-Dec-92	13-Jan-93	26-Jan-93
2	0.5	-0.5	-1.0	-1.3	-0.9	-0.9	-0.8	-0.7	-0.4	0.9	1.9	3.5	4.4	5.6
3	0.5	-0.5	-0.9	-1.1	-0.9	-0.9	-0.8	-0.7	-0.1	0.9	2.1	3.6	4.8	6.5
4	0.4	-0.5	-0.8	-0.9	-0.8	-0.9	-0.8	-0.7	-0.2	0.8	1.9	3.3	4.5	5.8
5	0.4	-0.3	-0.8	-0.9	-0.8	-0.9	-0.8	-0.7	-0.1	0.9	1.8	3.0	4.1	5.4
6	0.4	0.0	-0.6	-0.9	-0.8	-0.9	-0.8	-0.7	-0.1	0.9	1.8	2.6	3.6	4.6
7	0.8	0.8	0.8	0.7	0.5	0.0	-0.8	-0.1	1.4	1.7	2.3	2.9	3.8	4.6
8	5.7	7.1	4.7	4.3	4.1	3.6	3.3	3.5	4.0	4.2	4.6	5.2	5.3	6.3
9	10.6	7.7	5.5	5.1	4.5	4.2	4.1	4.1	4.4	4.7	5.2	5.8	6.4	6.7
10	11.4	8.2	6.5	5.9	4.9	4.8	4.5	4.5	4.8	5.0	5.7	6.2	6.6	7.0
11	11.0	8.3	7.0	6.6	5.6	5.3	5.1	4.9	5.3	5.5	6.1	6.6	7.0	7.4
12	9.1	8.4	7.6	7.0	6.3	5.7	5.4	5.4	5.5	5.5	5.7	5.9	6.2	6.4
13	7.2	7.2	7.4	7.5	6.5	5.9	5.6	5.6	5.7	5.6	5.7	5.8	5.8	6.2
14	6.8	7.0	7.2	7.8	6.7	6.2	6.0	5.8	5.9	5.8	5.8	5.9	5.9	5.9
15	6.3	6.5	6.6	7.3	6.5	5.9	6.1	6.0	6.0	6.0	5.9	5.9	5.9	5.9
16	5.9	6.1	6.2	6.9	6.2	5.8	5.9	5.8	5.7	5.8	5.7	5.8	5.7	5.7
17	5.4	5.5	5.6	6.3	5.7	5.4	5.4	5.4	5.3	5.4	5.4	5.4	5.4	5.4
18	4.9	5.1	5.2	5.5	5.2	4.9	4.9	4.9	4.8	4.9	4.9	4.9	5.0	4.9
19	4.4	4.5	4.6	4.7	4.6	4.4	4.4	4.4	4.4	4.4	4.5	4.5	4.5	4.5
20	3.9	4.1	4.3	4.2	4.1	4.0	4.0	4.0	4.0	4.0	4.0	4.1	4.1	4.1
21	3.5	3.6	3.7	3.7	3.5	3.6	3.6	3.6	3.6	3.6	3.6	3.7	3.6	3.6
22	3.3	3.3	3.5	3.4	3.3	3.2	3.2	3.2	3.2	3.2	3.3	3.2	3.3	3.3
23	2.9	2.9	3.2	3.1	2.9	2.9	2.9	2.9	2.9	2.9	3.0	3.0	2.9	3.0
24	2.7	2.7	3.0	3.0	2.7	2.6	2.7	2.6	2.6	2.6	2.7	2.7	2.7	2.7

Table A3.2: Salinity data (g kg^{-1}) for Ace Lake during 1992

Depth	25-Feb-92	17-May-92	20-Sep-92	23-Oct-92	04-Nov-92	20-Nov-92	02-Dec-92	17-Dec-92	30-Dec-92	13-Jan-93	26-Jan-93
2	12.6	15.9	12.1	16.8	16.7	16.7	16.6	16.2	16.2	15.8	14.2
3	12.6	15.9	16.3	16.7	16.7	16.6	16.6	16.6	16.6	16.5	16.4
4	12.6	15.8	16.3	16.7	16.6	16.6	16.6	16.7	16.7	16.6	16.6
5	12.6	15.8	16.3	16.6	16.6	16.6	16.7	16.8	16.7	16.7	16.7
6	12.6	14.6	16.3	16.6	16.6	16.7	16.7	16.8	16.7	16.8	16.8
7	13.2	16.6	17.0	16.6	16.6	17.1	16.8	16.9	16.9	16.8	16.8
8	21.3	26.7	27.4	27.4	27.5	28.5	27.7	27.8	27.7	24.7	27.8
9	29.3	28.7	28.3	28.9	28.8	29.2	29.1	29.2	29.0	29.1	29.1
10	30.0	29.6	29.2	29.4	29.4	29.7	29.6	29.7	29.6	29.6	29.7
11	30.6	30.3	30.0	30.0	29.9	29.5	30.2	30.3	30.2	30.1	30.1
12	31.0	31.1	30.4	30.5	30.6	30.2	30.7	30.8	30.8	30.7	30.8
13	31.3	31.0	30.6	30.6	30.8	31.0	30.9	31.1	31.0	31.1	31.0
14	31.4	32.3	30.8	31.2	31.1	31.2	31.1	31.3	31.2	31.2	31.2
15	31.6	31.4	31.3	31.3	31.3	31.4	31.3	31.5	31.4	31.4	31.3
16	32.0	32.2	31.9	31.6	31.7	31.8	31.6	31.8	31.7	31.7	31.6
17	32.4	32.3	32.2	32.2	32.2	32.3	32.2	32.3	32.2	32.1	32.1
18	33.9	34.6	33.4	33.3	33.2	33.5	33.3	33.4	33.3	33.1	33.2
19	35.4	35.4	35.1	35.0	35.0	35.2	35.0	35.1	34.9	34.9	34.6
20	36.7	38.3	37.1	37.1	37.0	37.1	36.9	37.1	36.8	36.8	36.7
21	38.8	38.9	39.1	39.0	38.9	38.9	38.9	39.0	38.5	38.7	38.8
22	39.9	40.1	40.0	40.0	39.9	40.0	40.0	40.0	39.9	39.9	39.9
23	40.2	39.9	40.3	40.3	40.4	40.3	40.3	40.5	40.2	40.3	40.2
24	40.3	40.3	40.5	40.4	40.5	40.4	40.5	40.4	40.3	40.4	40.3

Table A3.3: pH data for Ace Lake in 1992

	25-Feb-92	16-Apr-92	17-May-92	14-Jul-92	17-Aug-92	20-Sep-92	23-Oct-92	20-Nov-92	17-Dec-92	30-Dec-92
2	8.55	8.60	8.53	8.39	8.61	8.37	8.36	7.94	8.23	8.35
4	8.52	8.62	8.47	8.49	8.60	8.35	8.36	8.11	8.23	8.46
6	8.53	8.62	8.48	8.44	8.60	8.36	8.36	8.18	8.23	8.32
8	8.54	8.62	8.47	8.48	8.66	8.44	8.45	8.20	8.25	8.39
10	8.43	8.63	8.53	8.42	8.54	8.36	8.41	8.19	8.24	8.29
12	8.24	8.45	8.43	8.28	8.22	8.23	8.19	7.96	7.95	8.00
14	7.94	8.33	8.28	7.80	8.24	7.99	8.21	7.89	7.80	7.76
16	7.79	8.17	8.12	7.91	7.81	7.83	8.11	7.83	7.65	7.50
18	7.58	7.96	7.89	7.63	7.54	7.52	7.68	7.32	7.56	7.23
20	7.15	7.60	7.27	7.38	7.15	7.52	7.57	7.13	7.25	7.10
22	7.13	7.26	7.18	7.10	7.13	7.15	7.25	6.96	7.10	6.95
24	7.08	7.12	7.00	6.96	7.08	7.16	7.20	6.90	7.00	6.84

Table A3.4: Light readings (PAR - μmol photons m^{-2} s^{-1}) in Ace Lake during 1992. Io is the incident radiation on that that date.

Depth	16-Apr-92	17-Aug-92	20-Sep-92	17-Oct-92	220ct92	02-Nov-92	20-Nov-92	02-Dec-92	17-Dec-92	12-Jan-93	26-Jan-93
I_o	1197	164	568	759	818	988	745	511	611	632.4	906
1.5	9.5	0.6	5.5	11.7	11.9	9.9	24.4	27.8	74.4	89.1	196.7
1.75	7.6	0.5	3.6	8.1	10.7	8.7	20.9	25.6	71.3	88.9	181.5
2	6.0	0.4	2.4	7.1	9.4	7.7	20.5	25.0	70.4	80.1	169.7
3	4.5	0.3	0.8	5.2	7.8	5.4	24.7	30.1	66.3	68.0	145.4
4	3.3	0.2	0.4	5.1	6.9	4.5	26.9	33.7	45.0	59.3	124.8
5	2.5	0.2	0.4	4.7	6.4	3.9	28.2	30.6	39.6	50.2	106.6
6	2.0	0.2	0.3	4.6	6.2	3.8	25.9	29.9	36.1	43.9	90.6
7	1.7	0.2	0.2	4.4	5.8	3.2	22.3	25.8	27.5	37.8	76.7
8	1.0	0.1	0.0	4.2	5.4	2.5	18.2	20.6	22.8	31.7	60.5
9	0.7	0.0	0.0	4.0	5.3	1.9	14.3	17.9	18.9	25.2	47.3
10	0.5	0.0	0.0	3.4	4.4	1.5	9.6	12.4	15.1	19.2	36.1
10.5	0.0	0.0	0.0	2.6	3.2	1.1	7.0	7.3	13.5	15.8	30.3
11	0.0	0.0	0.0	1.6	1.1	0.3	1.8	2.5	2.8	8.4	16.7
11.5	0.0	0.0	0.0	0.2	0.1	0.0	0.0	0.6	0.0	1.3	0.4
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table A3.5: Dissolved organic carbon concentrations in Ace Lake (mg l^{-1}) during 1992.

Depth	03-Mar-92	16-Apr-92	17-May-92	14-Jul-92	17-Aug-92	23-Oct-92	04-Nov-92
2				7.5	6.6		7.07
4				6.5	6.7		
6				6.4	6.9		6.86
8				10.4	10.2		6.95
10	6.7	4.1	7.9	8.1	9.1	10	7.47
12				9.5	9.7		9.66
14				7.1	11.4		9.64
16				5.9	13.3		10.16
18				7.1	18		13.14
20					23.7		17.73
22					21.9		30.64
24				20.1	23.9		38.04

Table A3.6: Nutrient concentrations in Ace Lake on November 17 1993. All concentrations are measured in μM

Depth (m)	$PO4^{2-}$	Oxidised N	Silicate
4	0.263	N.D.	21.410
6	0.243	N.D.	22.342
8	5.050	N.D.	58.153
10	5.810	N.D.	59.503
11	8.773	N.D.	97.438
12	4.174	0.253	63.252

N.D. = below the limit of detection. Silicate was determined on filtered samples that were not frozen.

Table A3.7: Dissolved oxygen concentrations in Ace Lake during 1994.

Date	2m mmol	2m mg l-1	2m % Sat.	5m mmo	5m mg/l	5m %sat	10m mmol	10m mmol mmol	10m %sat
20-Apr-94	0.483	15.456	110.1	0.487	15.584	139.7	0.51	16.32	170.9
22-May-94	0.527	16.864	120.8	0.478	15.296	136.1	0.462	14.784	154.5
28-Jun-94	0.522	16.704	122.1	0.493	15.776	137.9	0.417	13.344	139.3
22-Jul-94	0.486	15.552	116.2	0.426	13.632	117.9	0.437	13.984	144.9
23-Aug-94	0.506	16.192	120.5	0.432	13.824	116.9	0.389	12.448	127.4
21-Sep-94	0.497	15.904	120.7	0.393	12.576	105.5	0.456	14.592	149.1
12-Oct-94	0.471	15.072	114.8	0.412	13.184	110.6	0.452	14.464	147.9
26-Oct-94	0.453	14.496	113	0.424	13.568	114.7	0.444	14.208	145
10-Nov-94	0.46	14.72	116.3	0.438	14.016	120.1	0.446	14.272	145.9
22-Nov-94	0.442	14.144	115	0.353	11.296	98.5	0.489	15.648	160.5
10-Dec-94	0.405	12.96	107.9	0.425	13.6	121.5	0.503	16.096	166.7
23-Dec-94	0.407	13.024	111.8	0.424	13.568	124.4	0.528	16.896	176
10-Feb-95	0.378	12.096	89.7	0.399	12.768	123.2	0.499	15.968	171.4

***(Measurements were performed by J. Gibson.)**

Table A3.8: Prokaryote cell numbers (cells ml^{-1}) in Ace Lake during 1992. This includes numbers of heterotrophic bacteria, *Synechococcus* and *Chlorobium* sp.

Depth	25-Feb-92	3-Mar-92	17-May-92	14-Jul-92	17-Aug-92	20-Sep-92	17-Oct-92	23-Oct-92	4-Nov-92	20-Nov-92	2-Dec-92	4-Dec-92	17-Dec-92	30-Dec-92
0	3.76E+05	3.08E+05												
2	9.52E+05	3.39E+05	3.39E+05	4.20E+05	3.01E+05	7.91E+05	1.63E+05	6.26E+05	5.26E+05	2.49E+05	2.99E+05	2.21E+05	3.79E+05	6.32E+05
4	3.33E+05	3.05E+05	5.17E+05	1.54E+05	2.89E+05	2.66E+05		2.43E+05	2.29E+05	2.50E+05	2.00E+05	1.89E+05	2.83E+05	3.93E+05
6	2.36E+05	2.15E+05	1.44E+05	1.24E+05	2.43E+05	1.79E+05		2.42E+05	2.17E+05	5.48E+04	1.83E+05	2.49E+05	4.82E+05	3.58E+05
8	3.24E+05	3.30E+05	2.70E+05	8.66E+05	4.91E+05	2.30E+05	2.30E+05	6.75E+05	3.07E+05	2.51E+05	7.05E+05	1.05E+06	9.19E+05	1.11E+06
10	4.06E+06	5.77E+05	4.74E+05	2.44E+05	4.56E+05	3.13E+05		1.11E+06	1.57E+06	2.33E+06	3.96E+06	3.31E+06	1.71E+06	2.61E+06
11							3.03E+05	1.39E+06	1.21E+06	2.51E+06	1.55E+07	6.81E+06	8.75E+06	1.93E+06
12	3.86E+07		2.15E+06	7.78E+06	3.81E+07	4.18E+07	3.50E+07	4.79E+07	**5.35E+07**	2.87E+07	**4.70E+07**	**3.22E+07**	**5.25E+07**	**1.06E+07**
13							7.51E+06	1.10E+07	1.19E+07	8.41E+06		2.56E+07	8.70E+06	6.04E+06
14	1.29E+07		4.95E+06	1.30E+07	5.75E+06	9.92E+06	4.80E+06	8.97E+06	9.80E+06			1.41E+07	8.72E+06	8.73E+06
16	7.46E+06		2.49E+06	7.81E+06	9.18E+06	1.09E+07		1.56E+07	5.81E+06	8.41E+06				
18	1.72E+07			8.84E+06	1.26E+07	2.57E+06	6.11E+06	1.30E+07	7.48E+06	7.05E+06		1.98E+07	1.04E+07	1.03E+07
20	5.34E+06		2.69E+06	8.39E+06	6.06E+06	2.50E+07		1.50E+07	2.29E+07	8.67E+06				
22			4.97E+06	2.53E+07	5.27E+06	3.25E+07		1.72E+07	2.18E+07	8.33E+06				
24			1.05E+07		4.63E+07			4.22E+07	3.05E+07	9.60E+06				1.26E+07

Table A3.9: *Synechococcus* cell numbers (cells ml^{-1}) in Ace Lake during 1992.

Depth	25-Feb-92	17-May-92	14-Jul-92	17-Aug-92	20-Sep-92	17-Oct-92	23-Oct-92	4-Nov-92	20-Nov-92	4-Dec-92	17-Dec-92	30-Dec-92	12-Jan-93	25-Jan-93
2						9.15E+02	1.68E+03	1.56E+03						
4				1.60E+03			1.67E+03	2.66E+03						
6				5.49E+03			4.82E+03	3.25E+03						
8				4.20E+03		1.01E+05	4.00E+04	1.12E+04	5.6E+04	2.67E+05		1.05E+05	5.28E+03	1.10E+04
10	3.15E+05	3.71E+04	1.04E+04	2.40E+04	2.69E+04	1.09E+05	5.67e+04	6.81E+04	5.12E+05	9.36E+05	1.51E+06	8.21E+05	5.74E+04	9.14E+04
11						5.73E+04	5.77E+04	1.65E+05	1.3E+06	6.08E+06	7.98E+06	2.96E+06	8.88E+05	1.20E+06

Table A3.10: Cell numbers (cells l^{-1}) of phytoplankton in Ace Lake on 20 November 1992. PNAN = phototrophic nanoflagellate.

20-Nov-92	*Synechococcus*	*M. rubrum*	*Cryptomonas* sp.	*P. gelidicola*	PNAN
2	1.56E+06	7.00E+04	3.60E+05	1.00E+04	3.36E+06
4	2.66E+06	2.00E+05	1.90E+05	1.27E+06	3.20E+06
6	3.25E+06	1.40E+05	1.70E+05	9.80E+05	3.10E+06
8	5.60E+07	9.00E+04	2.40E+05	1.10E+05	1.46E+07
10	5.12E+08	8.00E+04	5.20E+04	2.70E+05	1.52E+07
Total	5.75E+08	5.80E+05	1.01E+06	2.64E+06	3.95E+07

Cell counts of microplankton were performed by T. Pitman

Table A3.11: Biovolume (μm^3) of phytoplankton in Ace Lake on 20 November 1992.

20-Nov-92	*Synechococcus*	*M. rubrum*	*Cryptomonas* sp.	*P. gelidicola*	PNAN
2	1.49E+06	2.93E+08	1.41E+08	6.55E+05	4.75E+07
4	2.54E+06	8.38E+08	7.46E+07	8.31E+07	4.52E+07
6	3.10E+06	5.87E+08	6.68E+07	6.41E+07	4.38E+07
8	5.34E+07	3.77E+08	9.43E+07	7.20E+06	2.06E+08
10	4.89E+08	3.35E+08	2.04E+07	1.77E+07	2.15E+08
Total	5.49E+08	2.43E+09	3.97E+08	1.73E+08	5.58E+08

Dimentions of phytoplankton were as follows: *Synechococcus* (Cyclindrial, 1.5 X 0.9); *M. rubrum* (spherical, diameter 20 μm) *Cryptomonas* sp. (conical 10 μm X 15 μm), *P. gelidicola* (conical, 5 μm X 10 μm); Dinoflagellate (spherical, diameter 20 μm); PNAN (spherical, diameter 3μm)

Table A3.12: Cell surface are (μm^2) of phytoplankton in Ace Lake on 20 November 1992

20-Nov-92	*Synechococcus*	*M. rubrum*	*Cryptomonas* sp.	*P. gelidicola*	PNAN
2	6.40E+06	8.80E+07	1.18E+08	1.01E+06	9.50E+07
4	1.09E+07	2.51E+08	6.21E+07	1.28E+08	9.05E+07
6	1.33E+07	1.76E+08	5.56E+07	9.86E+07	8.77E+07
8	2.30E+08	1.13E+08	7.85E+07	1.11E+07	4.13E+08
10	2.10E+09	1.01E+08	1.70E+07	2.72E+07	4.30E+08
Total	2.36E+09	7.29E+08	3.31E+08	2.66E+08	4.46E+09

Table A3.13 Autotrophic nanoflagellate numbers (cells ml^{-1}) in Ace Lake during the 1992/1993 summer

Date	2 m	6 m	8 m	10 m
17-Oct-92	2.60E+03		3.97E+03	2.46E+03
23-Oct-92	6.55E+02	6.55E+02	3.44E+03	8.55E+02
04-Nov-92	1.70E+03	1.82E+03	6.47E+03	9.15E+03
20-Nov-92	3.36E+03	3.10E+03	1.46E+04	1.52E+04
04-Dec-92	4.33E+03	3.31E+03	1.62E+04	1.44E+04
17-Dec-92	1.66E+04	9.05E+03	3.92E+05	1.54E+04
30-Dec-92	4.03E+04	1.95E+04	1.51E+04	1.99E+05
13-Jan-93	3.12E+04	9.28E+03	1.68E+04	6.34E+03
26-Jan-93	1.62E+04	2.84E+04	3.42E+04	4.67E+04

Table A3.14: Arithithmetic Hstogram statistics for FRESHA8B.10M01 (23-October- 1992). Selected preferences: Arithmetic/Linear. Parameter FL2 (Height) and FL3 (Height), Ungated.

Parameter	M	Left	Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
Fl2	1	18.11	83.54	2103	21.03	36	37.52	41.38	39.6	13.99	33.81
Fl2	3	1333	2226	6409	64.09	524	1685	1710.42	1700.08	90.47	5.29
Fl3	2	13.46	60.98	2260	22.60	31	27.88	31.59	30.23	10.82	34.27

Table A3.15: Arithmetic Histogram statistics for FRESHA9B.10M02 (20-November- 1992). Selected preferences: Arithmetic/Linear. Parameter FL2 (Height) and FL3 (Height), Ungated.

Parameter	M	Left	Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
Fl2	1	1.73	44.11	7547	75.47	69	8.13	9.76	8.28	6.14	62.98
Fl2	3	1333	2226	1629	16.29	208	1685	1684.04	1684.85	79.16	4.70
Fl3	2	2.74	28.64	7578	75.78	74	10.09	9.59	8.58	4.79	49.96

Table A3.16: Arithmetic Histogram statistics for FRESHA10A2.10M01 (4-December- 1992). Selected preferences: Arithmetic/Linear. Parameter FL2 (Height) and FL3 (Height), Ungated.

Parameter	M	Left	Right	Events	%	Peak	PkChl	Mean	Median	SD	CV %
Fl2	1	1	5	9831	98.31	4357	1	1.34	1.07	0.55	41.43
Fl2	3	1333	2226	11	0.11	3	1499	1587.05	1512.47	207.93	13.10
Fl3	2	7.50	9631	96.31	992	1	1.93	1.64	1.03	53.35	

Table A3.17: Light intensities - PAR (μmol photons m^{-2} s^{-1}) in Ace Lake over a forty eight hour period during December 1993. Light intensities were taken to investigate the diel distribution of *Synechococcus* in Ace Lake.

Depth (m)	27-Nov-93 16:00	27-Nov-93 20:00	28-Nov-93 00:00	28-Nov-93 04:00	28-Nov-93 08:00	28-Nov-93 12:00	28-Nov-93 16:00	28-Nov-93 20:00	29-Nov-93 00:00	29-Nov-93 04:00	29-Nov-93 08:00	29-Nov-93 12:00
1.79	20.58	7.30	0.05	0.08	7.47	13.61	10.46	4.81	0.33	8.30	4.81	10.62
2	19.09	6.97	0.05	0.08	5.81	10.13	9.96	4.65	0.17	0.83	4.48	9.63
2.5	18.43	6.31	0.04	0.07	5.31	9.96	9.63	4.32	0.17	0.66	4.32	9.30
3	17.60	5.81	0.04	0.07	4.98	9.63	9.13	3.98	0.17	0.50	4.15	8.96
3.5	16.43	5.48	0.04	0.07	4.65	9.13	8.63	3.65	0.17	0.50	3.82	8.47
4	15.44	4.98	0.04	0.06	4.32	8.63	8.13	3.32	0.17	0.33	3.65	8.13
4.5	14.44	4.48	0.03	0.06	3.98	8.13	7.64	2.99	0.00	0.33	3.32	7.30
5	13.28	4.15	0.03	0.06	3.65	7.64	7.14	2.82	0.00	0.33	3.15	6.81
6	11.79	3.49	0.03	0.05	2.99	5.81	5.98	2.49	0.00	0.17	2.66	6.64
7	10.46	2.82	0.02	0.04	2.49	4.65	4.98	1.99	0.00	0.17	2.16	5.98
8	8.47	2.16	0.02	0.03	1.83	3.98	3.65	1.49	0.00	0.00	1.49	4.98
9	6.97	1.49	0.01	0.02	1.33	3.32	2.66	1.16	0.00	0.00	1.16	4.32
10	5.98	1.16	0.01	0.01	0.83	2.32	1.66	0.83	0.00	0.00	0.66	3.65
11	2.99	0.17	0.00	0.01	0.33	1.83	0.66	0.33	0.00	0.00	0.00	2.99
12	1.99	0.15	0.00	0.00	0.00		0.00	0.00	0.00	0.00	0.00	1.83

Table A3.18: *Synechococcus* cell numbers (cells ml^{-1}) in Ace Lake over a forty eight hour period in November 1993.

Date	Time	8 m	10 m	11 m
27-Nov-93	16:00	2.0E+05	1.5E+06	4.8E+06
27-Nov-93	20:00	1.2E+05	2.0E+06	3.3E+06
28-Nov-93	00:00	7.4E+04	2.4E+06	1.2E+06
28-Nov-93	04:00	1.3E+05	1.7E+06	3.2E+06
28-Nov-93	08:00	2.0E+05	1.5E+06	1.1E+06
28-Nov-93	12:00	2.6E+05	2.0E+06	2.1E+06
28-Nov-93	16:00	1.6E+05	1.5E+06	7.8E+05
28-Nov-93	20:00	7.8E+04	1.7E+06	3.6E+06
29-Nov-93	00:00	6.6E+04	2.2E+06	1.5E+06
29-Nov-93	04:00	1.0E+05	1.2E+06	1.7E+06
29-Nov-93	08:00	5.1E+04	1.4E+06	1.3E+06
29-Nov-93	12:00	7.6E+04	1.6E+06	2.0E+06

Appendix 4

Raw data from Chapter 4

Table A4.1: Phytoplankton populations (represented by regions - R) in nine meromictic lakes, a fjord site and a coastal marine site in the Vestfold Hills. Measurements were made by flow cytometry.

Pendant Lake 24-Nov-93

Bead concentration 9.5E+06 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R3	Cells ml^{-1}
5	10000	1000	10	106	9721	8.71E+06	33	8.96E+03
10	10000	1000	10	96	9729	9.63E+06	36	9.90E+03
11	10000	1000	10	62	9765	1.50E+07	34	1.53E+04
12.8	10000	1000	10	78	8342	1.02E+07	41	1.22E+04

Pendant Lake 5-Jan-93

Bead concentration 1.76E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R4	Cells ml^{-1}	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R5	Cells ml^{-1}	R6	Cells ml^{-1}
2	5022	1000	0.5	222	307	1.22E+04	761	3.02E+04	38	1.51E+03				
4	5031	1000	0.5	201	3785	1.66E+05	72	3.15E+03	27	1.18E+03				
6	5685	1000	0.5	208	4159	1.76E+05	75	3.17E+03	42	1.78E+03	26	1.10E+03		
8	5274	1000	0.5	214	3417	1.41E+05	166	6.83E+03	57	2.34E+03	45	1.85E+03	26	1.07E+03
9	5160	1000	0.5	212	3484	1.45E+05	93	3.86E+03	44	1.83E+03	115	4.77E+03		
10	6030	1000	0.5	206	3259	1.39E+05	119	5.08E+03	49	2.09E+03	383	1.64E+04	3	1.28E+02
11.5	7140	1000	0.5	254	5105	1.77E+05	32	1.11E+03	61	2.11E+03	264	9.15E+03	9	3.12E+02
12.5	5040	1000	0.5	127	1983	1.37E+05	46	3.19E+03	13	9.01E+02	133	9.22E+03	2	1.39E+02
12.8	10000	1000	25	909	81	3.92E+05								

Ace Lake 14-Nov-93

Bead concentration 2.0E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R4	Cells ml^{-1}	R3	Cells ml^{-1}	R1	Cells ml^{-1}
4	3009	1000	0.1	250	31	2.48E+02	792	6.34E+03	485	3.88E+03
5	6680	1000	0.1	250	163	1.30E+03	792	6.34E+03	487	3.90E+03
6	3009	1000	0.1	558	450	1.61E+03	2004	1.60E+04	1487	1.19E+04
7	2060	1000	0.1	232	175	1.51E+03	762	6.10E+03	378	3.02E+03
8	1500	1000	0.1	59	836	2.83E+04	161	1.29E+03	109	8.72E+02
10	2718	1000	10	981	1132	2.31E+05	58	4.64E+02	34	2.72E+02
11	5040	1000	10	878	3542	8.07E+05	89	7.12E+02	33	2.64E+02

Lake Abraxas

Bead concentration 2.0E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R5	Cells ml^{-1}	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R4	Cells ml^{-1}
3	2300	1000	0.1	418	11	5.26E+01	1231	5.89E+03	75	3.59E+02	42	2.01E+02
10	1600	1000	0.1	231	12	1.04E+02	745	6.45E+03	59	5.11E+02	26	2.25E+02
19	10000	1000	10	110	8145	1.48E+07	447	8.13E+05	38	6.91E+04	6	1.09E+04
21	10000	1000	10	110	110	2.00E+05	ND		ND		ND	

Taynaya Bay 16-Nov-93

Bead concentration 2.0E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R4	Cells ml^{-1}	R5	Cells ml^{-1}
3	3546	1000	0.01	78	213	5.46E+02	459	1.18E+03	8	2.05E+01		
5	2556	1000	0.01	44	149	6.77E+02	323	1.47E+03	173	7.86E+02		
10	2000	1000	0.01	58	95	3.28E+02	143	4.93E+02	32	1.10E+02		
15	2260	1000	0.01	101	148	2.93E+02	107	2.12E+02	42	8.32E+01		
16	10000	1000	10	1739	32	3.68E+03	24	2.76E+03	7	8.05E+02	6685	7.69E+05

Lake McCallum 18-Nov-93
Bead concentration 2.0E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R4	Cells ml^{-1}	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R5	Cells ml^{-1}
5	1960	1000	0.01	441			209	9.48E+01	591	2.68E+02	200	9.07E+01
6	1240	1000	0.01	253			112	8.85E+01	437	3.45E+02	71	5.61E+01
10	1200	1000	0.01	183			217	2.37E+02	341	3.73E+02	105	1.15E+02
15	1020	1000	0.01	153	332	3.82E+02	240	3.14E+02	244	3.19E+02	65	8.50E+01
19	1580	1000	0.01	174	8218	9.67E+04	432	4.97E+02	150	1.72E+02	118	1.36E+02
19.8	10000	1000	0.01	17			30	3.53E+02	75	8.82E+02	18	2.12E+02

Burton Lake 22-Nov-93
Bead concentration 2.0E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}
4	10000	1000	10	665	7226	2.17E+06
8	10000	1000	10	489	8034	3.29E+06
12	10000	1000	10	521	7952	3.05E+06

Ekho Lake 25-Jan-93
Bead concentration 1.76E+07

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R3	Cells ml^{-1}
2	2520	1000	0.05	28	30	9.43E+02	41	1.29E+03
6	1000	1000	0.05	41	97	2.08E+03	8	1.72E+02
10	520	1000	0.05	34	64	1.66E+03	6	1.55E+02
15	520	1000	0.05	34	104	2.69E+03	9	2.33E+02
20	1000	1000	0.05	23	26	9.95E+02	236	9.03E+03
23	520							

Clear Lake 8-Dec-92

Bead concentration 1.76E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R4	Cells ml^{-1}
2	1000	1000	0.1	90	18	3.52E+02	218	4.26E+03		
5	1000	1000	0.1	57	11	3.40E+02	136	4.20E+03		
15	1000	1000	0.1	71	69	1.71E+03	101	2.50E+03		
25	1000	1000	0.1	75	320	7.51E+03	84	1.97E+03		
30	1000	1000	0.1	54	320	1.04E+04	76	2.48E+03		
33	10000	1000	5	219					3206	1.29E+06

Organic Lake 29-Dec-92

Bead concentration 1.76E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R3	Cells ml^{-1}	R4	Cells ml^{-1}
0	3000	1000	0.1	21	2616	2.19E+05				
1	2000	1000	0.1	13	1172	1.59E+05	243	3.29E+04	410	5.55E+04
2	1000	1000	0.1	18	532	5.20E+04				
3	1000	1000	0.1	12	523	7.67E+04				
4	1000	1000	0.1	19	586	5.43E+04				

Fletcher Lake 10-Dec-92

Bead concentration 1.76E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R4	Cells ml^{-1}	R3	Cells ml^{-1}	R1	Cells ml^{-1}	R5	Cells ml^{-1}
2	1000	1000	0.1	22	170	1.36E+04	216	1.73E+04				
4	1000	1000	0.1	28	75	4.71E+03	314	1.97E+04				
6	2000	1000	0.1	39	227	1.02E+04	386	1.74E+04	165	7.45E+03		
6.8	2000	1000	5	435	31	6.27E+03	26	5.26E+03	37	7.49E+03	5263	9.81E+07

Coastal Marine 17-Nov-93

Bead concentration 1.76E+07 ml^{-1}

Depth (m)	Events	Sample (µl)	Beads (µl)	Beads	R1	Cells ml^{-1}	R2	Cells ml^{-1}	R3	Cells ml^{-1}
5	2628	1000	0.1	81	40	8.69E+02	67	1.46E+03	40	8.69E+02

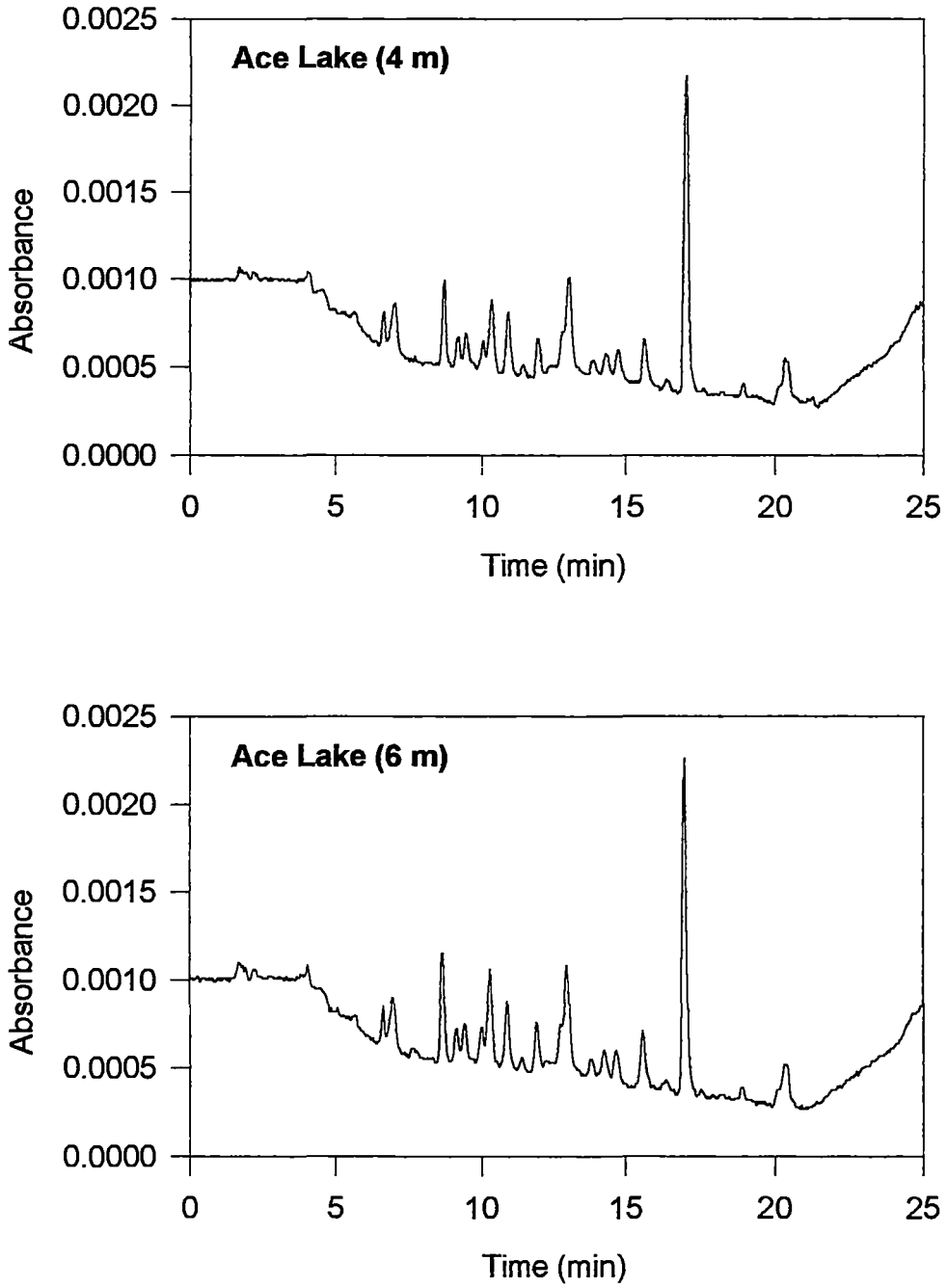


Figure A4.1: Lipid soluble pigment profile of particulates filtered from 4 m and 6 m in Ace Lake on the 14th November 1993

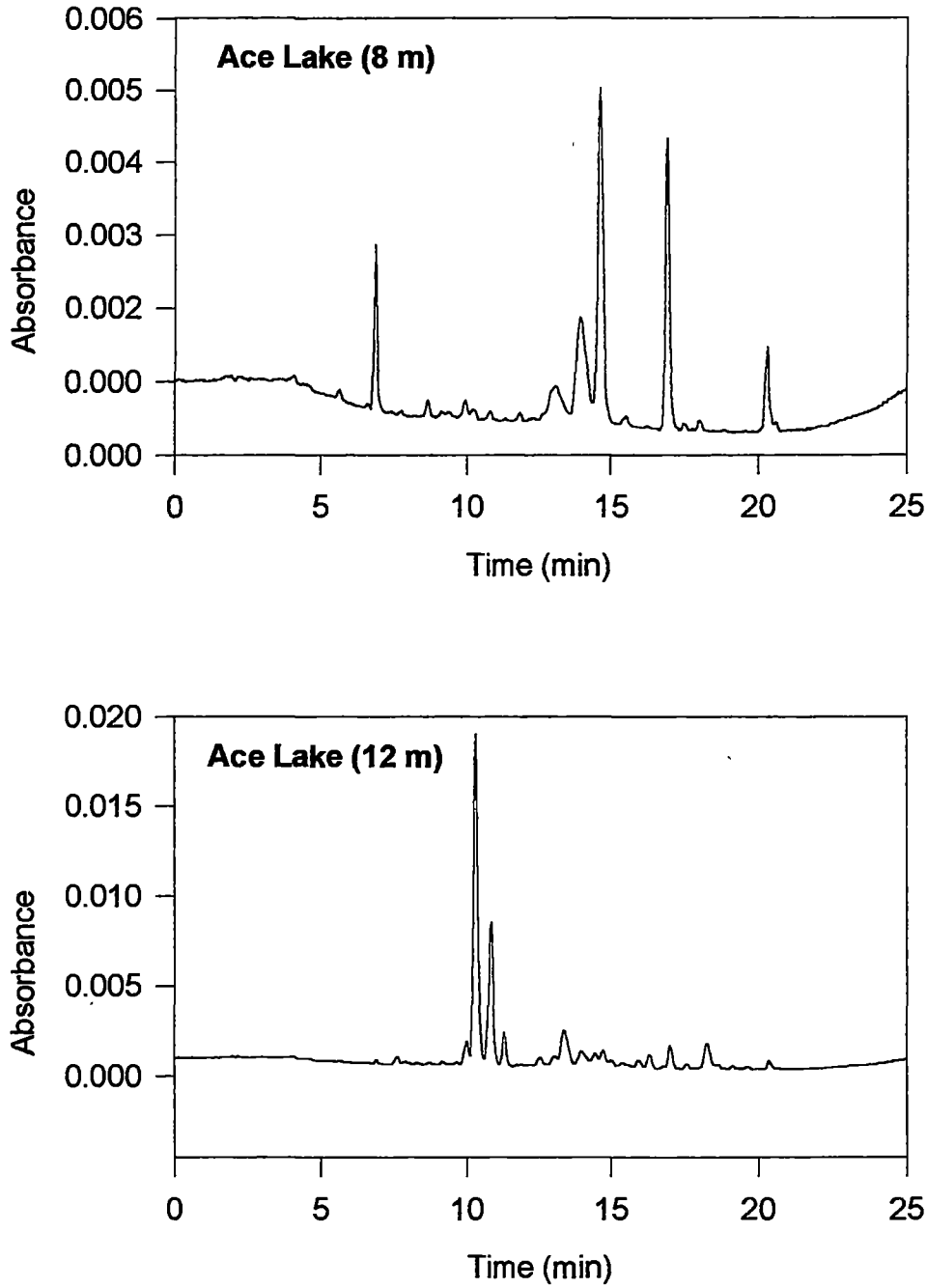
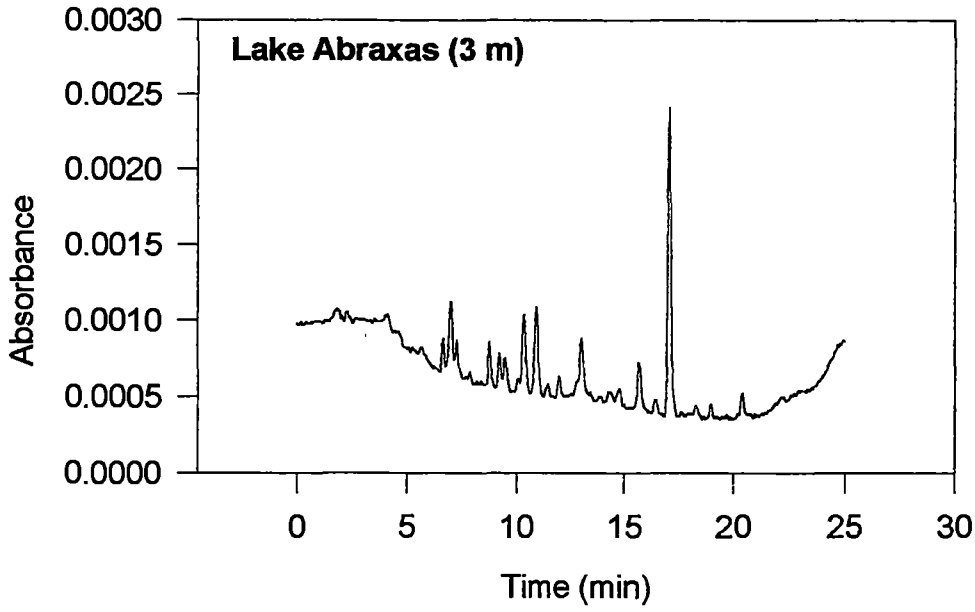


Figure A4.2: Lipid soluble pigment profile of filtered particulates from 8 m and 12 m in Ace Lake on 14 November 1993



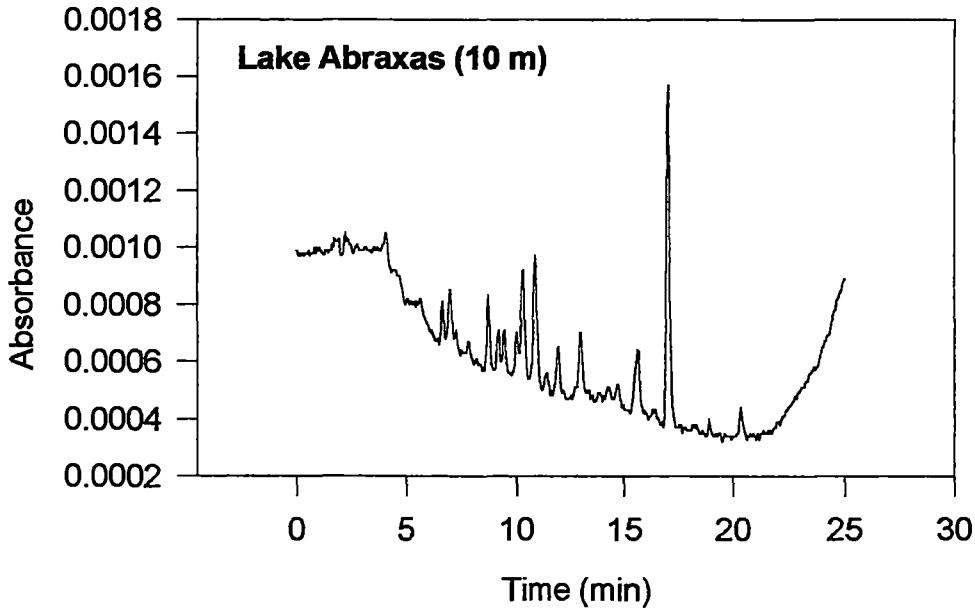


Figure A4.3: Lipid soluble pigment profiles of filtered particulates from Lake Abraxas on 23 November 1993.

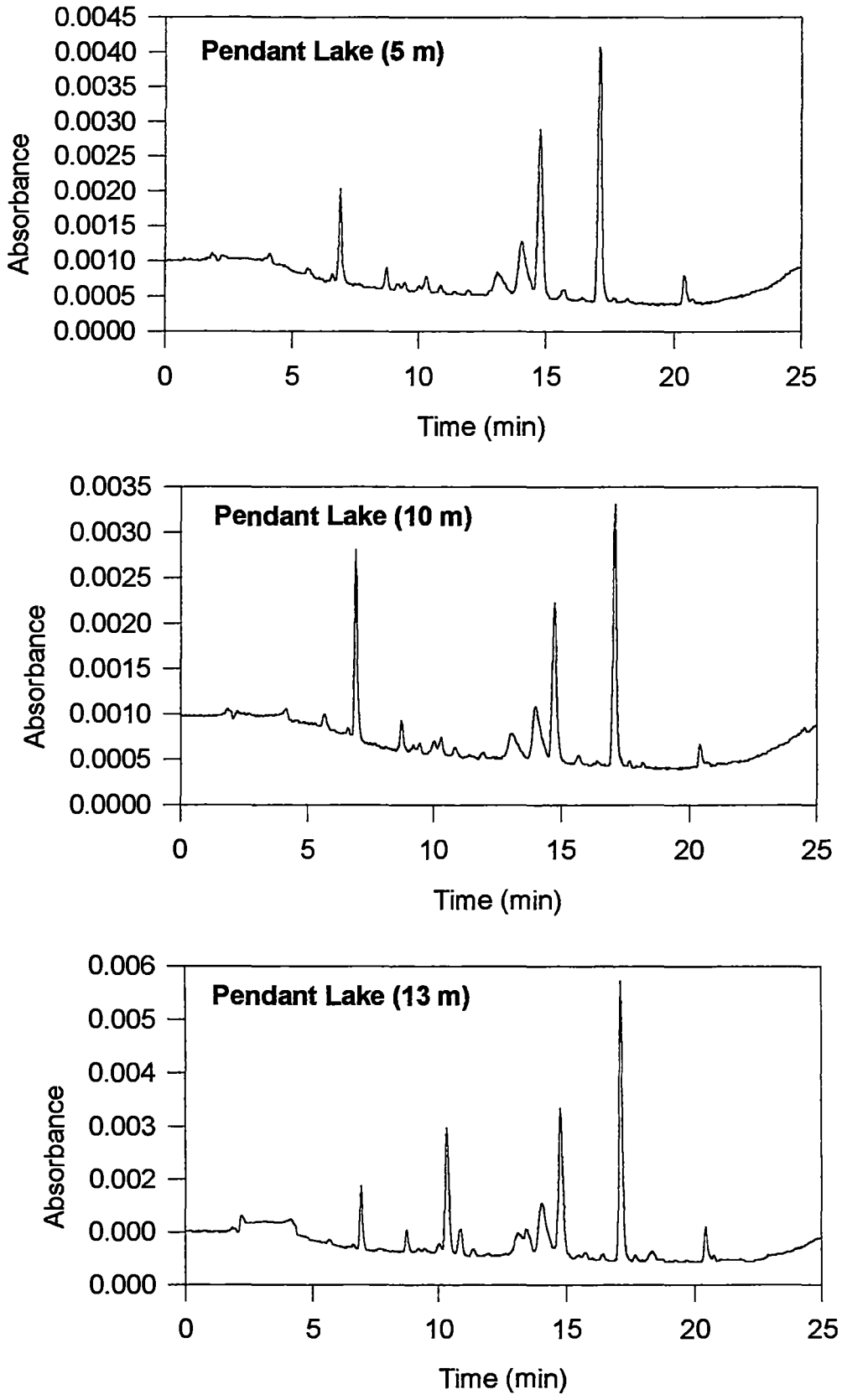


Figure A4.4: Lipid soluble pigment profiles of filtered particulates from 5 m, 10 m and 13 m in Pendant Lake on 24 November 1993

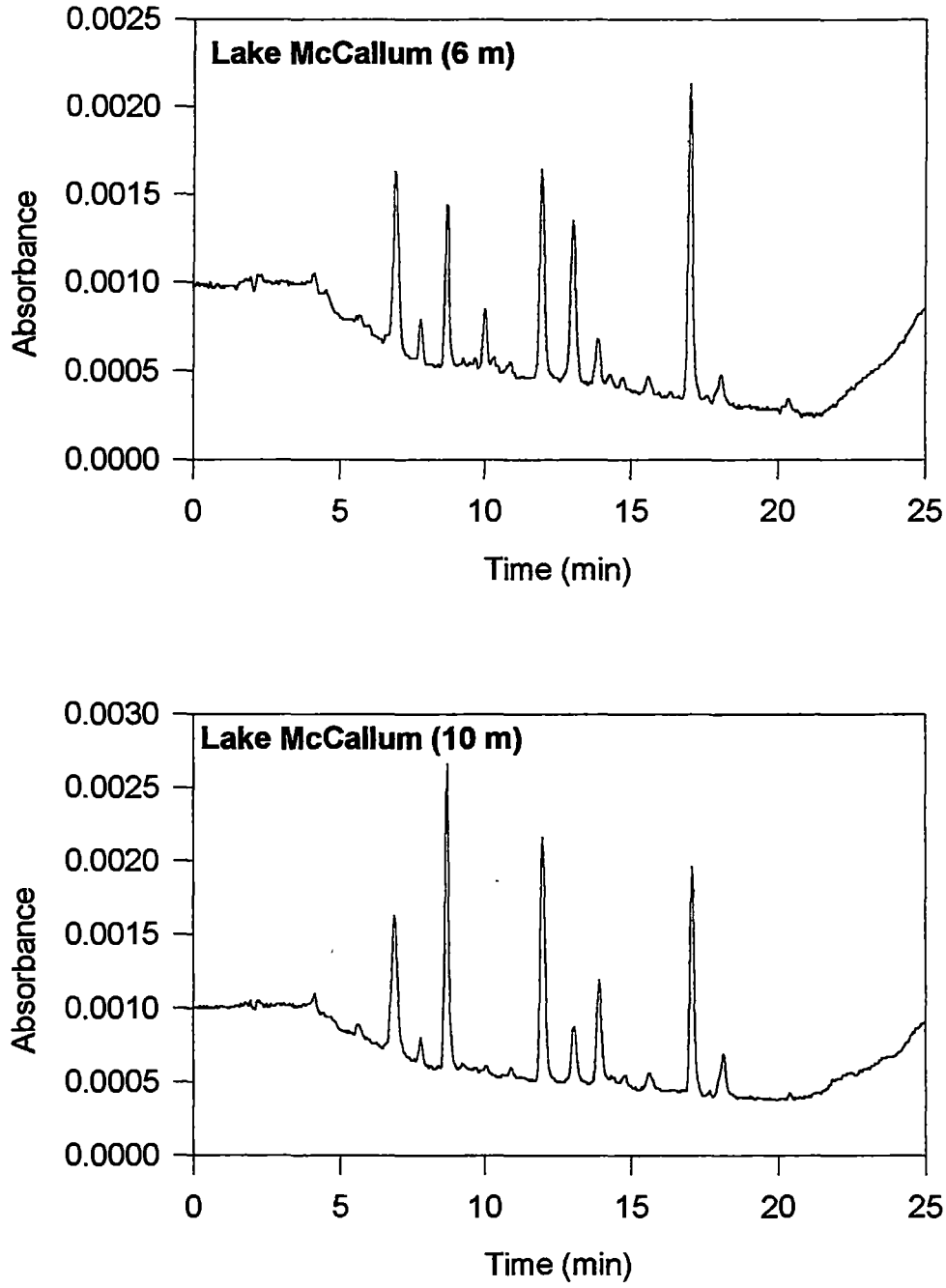


Figure A4.5: Lipid soluble pigment profiles of filtered particulates from 6 m and 10 m in Lake McCallum on 18 November 1993

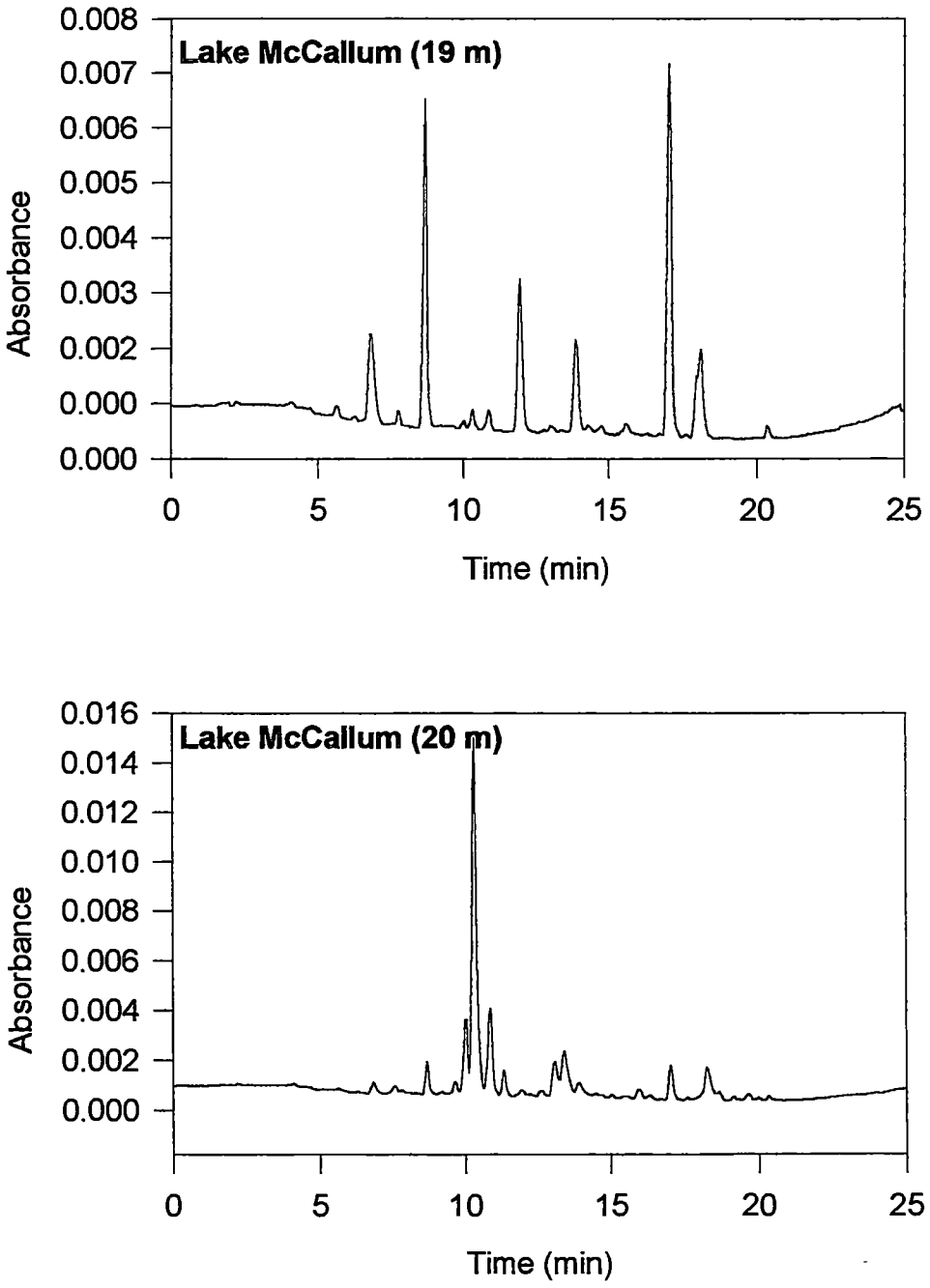


Figure A4.6: Lipid soluble pigment profiles of filtered particulates from 19 m and 20 m in Lake McCallum on 18 November 1993

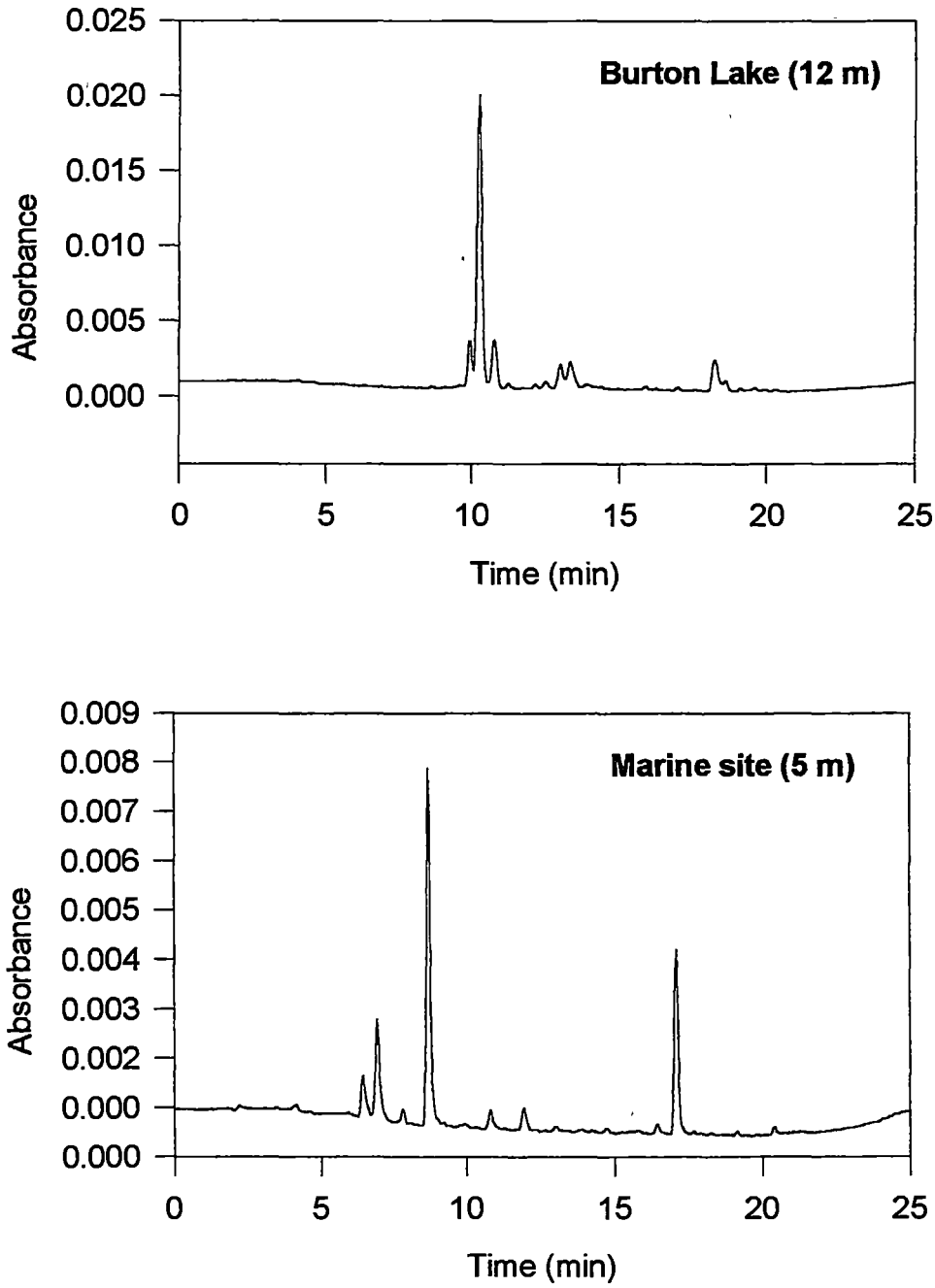


Figure A4.7: Lipid soluble pigment profiles of filtered particulates from Burton Lake on 22 November 1993 and from the marine site in Davis Bay on 17 November 1993

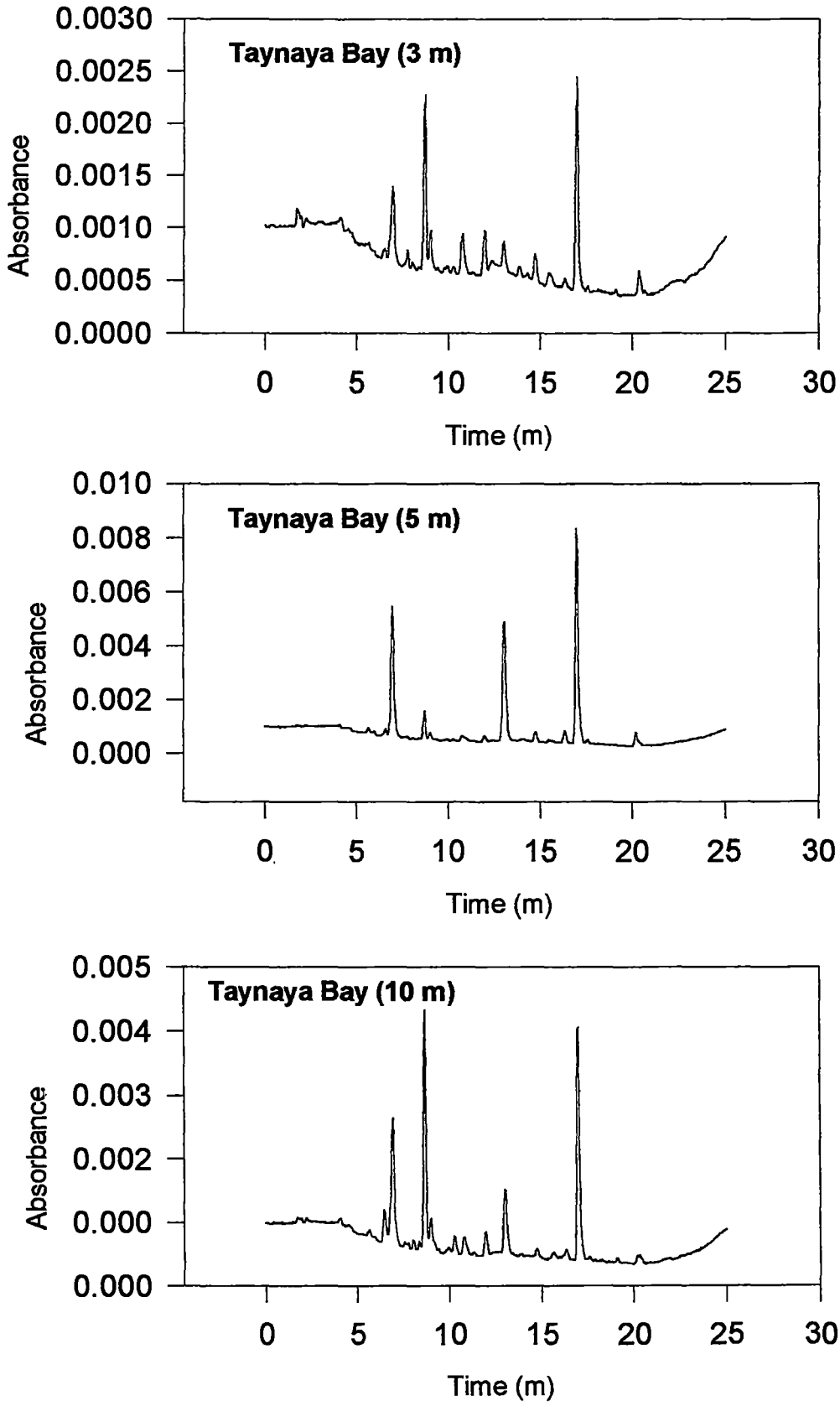
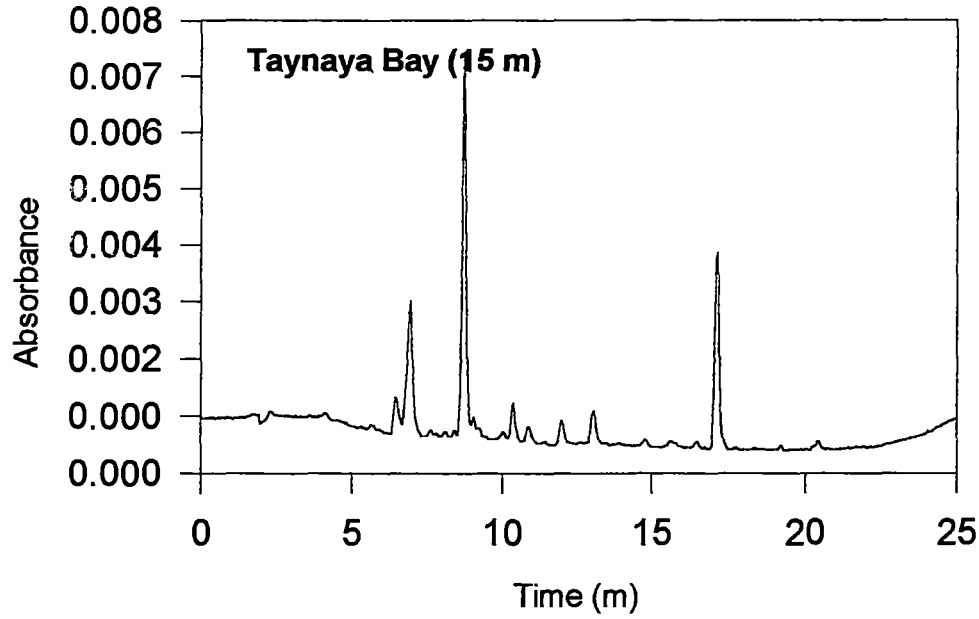


Figure A4.8: Lipid soluble pigment profiles of filtered particulates from 3 m, 5 m and 10 m in Taynaya Bay on 16 November 1993



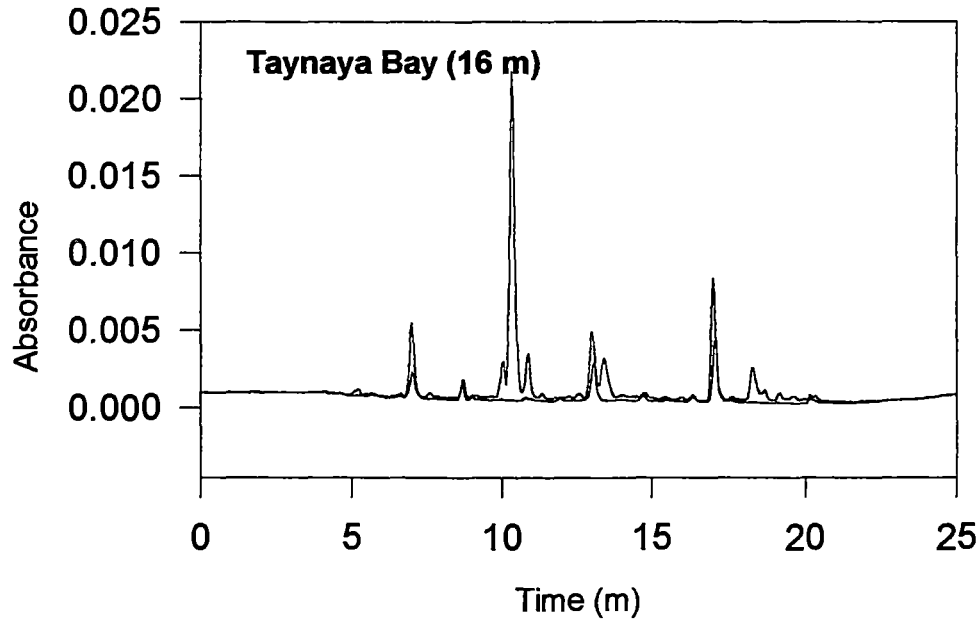


Figure A4.9: Lipid soluble pigment profiles of filtered particulates from 15 m and 16 m in Taynaya Bay on 16 November 1993

Table A4.2: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Lake Abraxas on 23 November 1993

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
2	0.43	16.85	63.71	8	0.48	16.81	43.13	14	0.60	16.77	28.52
2.25	0.49	16.83		8.25	0.48	16.81		14.25	0.62	16.78	
2.5	0.50	16.83	62.71	8.5	0.48	16.82		14.5	0.64	16.78	
2.75	0.50	16.82		8.75	0.48	16.81		14.75	0.69	16.79	
3	0.50	16.78	61.05	9	0.48	16.82	39.81	15	0.72	16.79	26.69
3.25	0.50	16.78		9.25	0.48	16.81		15.25	0.74	16.81	
3.5	0.49	16.77	59.06	9.5	0.47	16.81		15.5	0.75	16.81	
3.75	0.50	16.81		9.75	0.47	16.81		15.75	0.75	16.80	
4	0.50	16.81	57.07	10	0.47	16.82	37.15	16	0.81	16.78	25.20
4.25	0.48	16.79		10.25	0.47	16.81		16.25	0.90	16.77	
4.5	0.49	16.80	55.08	10.5	0.47	16.81		16.5	0.97	16.78	
4.75	0.48	16.80		10.75	0.47	16.79		16.75	0.99	16.77	
5	0.49	16.78	52.75	11	0.47	16.81	34.16	17	1.08	16.75	23.70
5.25	0.48	16.81		11.25	0.48	16.81		17.25	1.50	16.79	
5.5	0.48	16.81		11.5	0.49	16.81		17.5	4.01	17.96	
5.75	0.49	16.81		11.75	0.49	16.80		17.75	5.25	18.51	
6	0.49	16.81	50.10	12	0.51	16.80	32.17	18	6.39	18.99	21.88
6.25	0.49	16.80		12.25	0.54	16.79		18.5	7.27	19.57	
6.5	0.48	16.82		12.5	0.54	16.80		19	8.03	20.35	8.43
6.75	0.49	16.81		12.75	0.54	16.79		19.5	7.31	21.10	0.00
7	0.48	16.81	44.95	13	0.54	16.79	30.18	20	7.05	21.70	
7.25	0.48	16.80		13.25	0.54	16.79		20.5	7.02	22.02	
7.5	0.48	16.82		13.5	0.55	16.78		21	6.94	22.15	
7.75	0.48	16.82		13.75	0.57	16.77					

Table A4.3: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Ace Lake on 14 November 1993

Depth	Temp	Salinity	Light	Depth	Temp	Salinity
2			9.63	13	6.62	31.27
2.5	0.64	18.14	9.30	13.5	6.50	31.36
3	1.32	18.09	8.96	14	6.34	31.44
3.5	1.38	18.06	8.47	14.5	6.16	31.54
4	1.38	18.07	8.13	15	6.01	31.63
4.5	1.38	18.06	7.30	15.5	5.66	31.90
5	1.38	18.08	6.81	16	5.43	32.17
5.5	1.38	18.10		16.5	5.24	32.51
6	1.39	18.09	6.64	17	5.06	32.92
6.5	1.40	18.10		17.5	4.86	33.58
7	1.41	18.10	5.98	18	4.65	34.45
7.5	3.03	20.85		18.5	4.44	35.48
8	5.28	28.15	4.98	19	4.27	36.48
8.5	5.86	29.30		19.5	4.08	37.55
9	5.94	29.39	4.32	20	3.90	38.48
9.5	6.12	29.72		20.5	3.72	39.41
10	6.28	29.85	3.65	21	3.58	39.85
10.5	6.43	30.05	3.32	21.5	3.41	40.34
11	6.58	30.35	2.99	22	3.22	40.58
11.5	6.91	30.77		22.5	3.09	40.67
12	6.91	31.00	1.83	23	2.92	40.75
12.5	6.77	31.13		23.5	2.80	40.74

Table A4.4: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Pendant Lake on 24 November 1993 and in Ekho Lake of 25 January 1993.

Pendant Lake			
Depth	Temp	Salinity	Light
2	1.08	16.58	21.25
2.5	1.01	16.54	15.27
3	0.48	16.58	11.45
3.5	0.30	16.56	9.30
4	0.30	16.49	7.64
4.5	0.25	16.50	
5	0.24	16.50	6.47
5.5	0.23	16.51	
6	0.22	16.52	5.81
6.5	0.22	16.52	
7	0.22	16.52	5.31
7.5	0.23	16.52	
8	0.24	16.52	4.98
8.5	0.25	16.52	
9	0.26	16.50	4.81
9.5	0.25	16.52	
10	0.26	16.50	4.65
10.5	0.20	16.50	
11	0.15	16.55	4.48
11.5	0.12	21.65	
12	0.42	27.41	4.32
12.5	0.76	31.01	
13	1.07	35.30	4.15
13.5	1.34	41.26	
14	1.55	47.99	
14.5	1.71	54.66	
15	1.84	59.88	

Ekho Lake			
Depth	Temp	Salinity	PAR
2	7.75	43.95	462
3	6.95	44.64	407
4	8.23	59.85	339
5			283
6	9.67	71.75	
8	9.61	71.75	
9	9.54	71.87	
10	9.48	71.94	132
11	9.42	71.93	
12	9.36	71.89	
13	9.71	73.07	
14	10.32	75.38	
15	12.09	79.62	58
16	14.99	87.52	
17	16.74	99.60	
18	17.54	109.39	
19	17.78	117.30	
20	17.59	124.65	13
21	17.28	128.65	
22	16.92	133.69	
23	16.61	136.82	1.5
24	16.14	138.91	
26	15.72	140.59	0.11
30	14.57	145.36	0.1
35	14.14	147.24	
42	13.94	147.45	

Table A4.5: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Organic Lake on 29 December 1992 and at the marine site in Davis Bay on 7 November 1993.

Organic Lake

Depth	Temp	Salinity	Light
0.75			33.88
1	-7.4	175.99	15.46
1.5	-9.5	179.63	3.76
2	-10.7	179.63	1.08
2.5	-11.2	180.85	0.3
3	-11.5	180.85	0.04
3.5	-11.6	180.85	0
4	-11.5	180.85	0
4.5	-11.1	180.85	0
5	-9.8	201.33	0
5.5	-8.1	204.91	0
6	-7.8	206.11	0
6.5	-7.9	207.3	0
7	-7.9	207.3	0

Marine Site

Depth	Temp	Salinity	Light
1	-1.90	34.81	
1.5	-1.91	34.63	
1.75			3.11
2	-1.91	34.56	1.18
2.5	-1.91	34.52	0.72
3	-1.91	34.55	0.3
3.5	-1.91	34.56	0.18
4	-1.91	34.59	0.12
4.5	-1.91	34.61	0.04
5	-1.91	34.62	0.02
5.5	-1.91	34.62	
6	-1.91	34.62	0
6.5	-1.91	34.63	
7	-1.91	34.63	
7.5	-1.91	34.62	
8	-1.92	34.63	
9	-1.92	34.63	
10	-1.92	34.63	

Table A4.6: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Organic Lake on 10 December 1992

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
1.5			85	5.4	0.11	64.21	
1.6				5.5	0.39	65.81	
1.7	-2.43	56.35	80	5.6	0.68	67.58	
1.8	-2.43	56.44		5.7	0.92	69.28	
1.9	-2.44	56.46		5.8	1.19	71.23	
2	-2.45	56.44	75.1	5.9	1.4	72.93	
2.1	-2.45	56.43		6	1.62	74.57	25.2
2.2	-2.45	56.46		6.1	1.85	76.22	
2.3	-2.46	56.43		6.2	2.13	77.81	
2.4	-2.46	56.42		6.3	2.35	79.33	
2.5	-2.46	56.47		6.5	2.77	81.81	17.7
2.6	-2.46	56.5		6.6	3.04	83.21	
2.8	-2.47	56.5		6.7	3.17	84.3	
2.9	-2.47	56.45		6.8	3.23	85.34	
3	-2.48	56.46	60.1	6.9	3.3	86.5	
3	-2.47	56.5		7	3.36	87.43	5.2
3.1	-2.48	56.52		7.1	3.44	88.33	
3.2	-2.47	56.5		7.2	3.5	89.21	
3.3	-2.48	56.5		7.3	3.57	90.04	
3.4	-2.5	56.48		7.4	3.65	90.66	
3.5	-2.51	56.51		7.5	3.71	91.42	
3.6	-2.5	56.54		7.6	3.77	91.97	
3.7	-2.5	56.53		7.8	3.87	92.9	
3.8	-2.51	56.51		8	3.95	93.78	
3.9	-2.5	56.49		8.2	4.01	94.51	
4	-2.51	56.53	52.6	8.4	4.04	95.3	
4.1	-2.51	56.55		8.6	4.06	96.23	
4.2	-2.5	56.49		8.8	4.04	96.84	
4.3	-2.31	56.3		9	4.03	97.29	
4.4	-2.04	56.28		9.2	3.98	97.67	
4.5	-1.93	56.52		9.4	3.95	97.97	
4.6	-1.68	56.9		9.6	3.87	98.32	
4.7	-1.52	57.27		9.8	3.82	98.57	
4.8	-1.38	57.45		10	3.7	98.94	
4.9	-1.33	57.8		10.2	3.63	99.18	
5	-1.06	58.88	42.6	10.4	3.49	99.49	
5.1	-0.8	59.96		10.6	3.41	99.7	
5.2	-0.5	61.26		10.8	3.29		
5.3	-0.18	62.76					

Table A4.7: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Lake McCallum on 18 November 1993.

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
2	1.41	14.95	38.68	10.5	5.91	16.93		19	6.85	17.57	1.16
2.5	1.47	14.87	38.18	11	5.94	16.96	12.62	19.5	6.75	17.83	
3	1.47	14.84	36.69	11.5	5.94	16.96		20	6.61	18.16	0.17
3.5	1.47	14.79	35.03	12	5.94	16.97	10.62	20.5	6.43	18.72	
4	1.45	14.83	33.37	12.5	5.94	16.97		21	6.21	19.36	
4.5	1.41	14.84	31.87	13	5.94	16.96	8.96	21.5	6.01	20.19	
5	1.37	14.84	30.54	13.5	5.94	16.97		22	5.80	20.85	
5.5	1.37	14.83		14	5.94	16.97	7.47	22.5	5.67	21.23	
6	4.17	15.59	27.89	14.5	5.94	16.95		23	5.48	21.75	
6.5	4.37	15.78		15	6.16	17.08	5.98	23.5	5.24	22.25	
7	4.50	15.81	24.07	15.5	6.28	17.15		24	5.05	22.63	
7.5	4.51	15.83		16	6.39	17.21	5.15	24.5	4.86	23.01	
8	4.51	15.84	20.42	16.5	6.48	17.24		25	4.67	23.28	
8.5	4.52	15.83		17	6.51	17.26	4.15	25.5	4.49	23.47	
9	4.52	15.83	17.60	17.5	6.57	17.28		26	4.30	23.61	
9.5	4.52	15.84		18	6.66	17.31	3.34	26.5	4.12		
10	5.75	16.75	15.11	18.5	6.73	17.36					

Table A4.8: Temperature (° C), salinity (g kg^{-1}) and PAR (µmol photons m^{-2} s^{-1}) in Clear Lake on 8 December 1992.

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
1.5	0.10	10.23	182.17	29	6.67	12.53	
2	0.77	10.24	148.62	30	7.05	12.70	2.41
2.5	1.22	10.26		31	6.93	13.02	
3	1.32	10.30	139.74	32	6.81	13.23	
3.5	1.36	10.30		33	6.57	13.33	
4	1.39	10.30	125.37	34	6.26	13.44	
4.5	1.41	10.31		35	5.83	13.53	1.16
5	1.42	10.32	116.99	36	5.49	13.60	
5.5	1.43	10.34		37	5.15	13.62	
6	1.39	10.34	105.94	38	4.87	13.64	
7	1.30	10.35	96.06	39	4.56	13.68	
7.5	1.90	10.26		40	4.44	13.66	1.25
8	2.09	10.30	88.26	41	4.23	13.68	
9	2.27	10.34	81.45	42	4.13	13.67	
10	4.72	11.27	75.31	43	3.95	13.69	
11	4.88	11.43		44	3.80	13.72	
12	5.64	11.94	62.60	45	3.72	13.74	
13	5.76	12.00		46	3.61	13.71	
14	5.78	12.04	50.98	47	3.54	13.71	
15	5.77	12.04		48	3.48	13.74	
16	5.77	12.07	42.93	49	3.42	13.74	
17	5.78	12.07		50	3.37	13.72	0.50
20	6.19	12.30	29.39	51	3.31	13.74	
21	6.18	12.30		52	3.27	13.75	
22	6.17	12.31	23.58	53	3.23	13.75	
23	6.18	12.31		54	3.19	13.75	
24	6.17	12.32	19.51	55	3.15	13.75	
25	6.17	12.31		56	3.14	13.74	
26	6.17	12.32		57	3.10	13.77	
27	6.17	12.31		59	3.08	13.74	
28	6.29	12.33		60	3.04	13.76	

Table A4.9: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Burton Lake on 22 November 1993.

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
1.5			14.91	9.5	-2.38	43.35	
2	-1.30	43.17	2.62	10	-2.36	43.35	
2.5	-1.61	43.42	0.96	10.5	-2.37	43.35	
3	-1.99	43.40	0.46	11	-2.36	43.36	
3.5	-2.20	43.54	0.30	11.5	-2.35	43.35	
4	-2.26	43.47	0.13	12	-2.34	43.35	
4.5	-2.30	43.32	0.00	12.5	-2.33	43.37	
5	-2.34	43.33		13	-2.32	43.38	
5.5	-2.35	43.33		13.5	-2.31	43.36	
6	-2.37	43.32		14	-2.31	43.35	
6.5	-2.38	43.35		14.5	-2.30	43.35	
7	-2.38			15	-2.25	43.36	
7.5	-2.39	43.35		15.5	-2.23	43.35	
8	-2.39	43.36		16	-2.20	43.37	
8.5	-2.39	43.36		16.5	-1.84	43.94	
9	-2.38	43.35					

Table A4.10: Temperature (° C), salinity (g kg^{-1}) and PAR (μmol photons m^{-2} s^{-1}) in Taynaya Bay on 16 November 1993.

Depth	Temp	Salinity	Light	Depth	Temp	Salinity	Light
2			13.08	12.5	-1.85	36.06	
2.5	-1.81	35.00	11.59	13	-1.85	36.08	3.62
3	-1.83	35.15	9.26	13.5	-1.83	36.06	
3.5	-1.85	35.22	7.93	14	-1.80	36.09	3.45
4	-1.83	35.30	6.44	14.5	-1.74	36.08	
4.5	-1.83	35.39	5.94	15	-1.57	36.66	3.12
5	-1.83	35.46	5.61	15.5	-1.54	41.86	
5.5	-1.83	35.56		16	-1.56	44.17	2.95
6	-1.83	35.63	4.95	16.5	-1.54	45.41	
6.5	-1.85	35.76		17	-1.52	46.47	
7	-1.85	35.92	4.61	17.5	-1.49	47.34	
7.5	-1.85	35.98		18	-1.47	48.13	
8	-1.84	35.99	4.45	18.5	-1.44	48.87	
8.5	-1.83	36.01		19	-1.42	49.44	
9	-1.83	36.02	4.28	19.5	-1.41	50.05	
9.5	-1.86	36.06		20	-1.40	50.59	
10	-1.86	36.05	4.21	20.5	-1.39	51.12	
10.5	-1.87	36.06		21	-1.37	51.46	
11	-1.87	36.07	3.95	21.5	-1.37	51.81	
11.5	-1.87	36.06		22	-1.37	52.22	
12	-1.86	36.06	3.78	22.5	-1.35	52.54	

Table A4.11: Nitrate concentrations (μM) in a range of salinity standards from 1 % NaCl to 5 % NaCl.

1%b	1% 1	1% 2	1% 3	1% 4	1% 5	2.5%b	2.5% 1	2.5% 2	2.5% 3	2.5% 4	2.5% 5	3.5%b	3.5% 1	3.5% 2	3.5% 3	3.5% 4	3.5% 5	5%b	5% 1	5% 2	5% 3	5% 4	5% 5
0.023	0.608	1.465	2.255	2.776	3.576	0	0.689	1.369	2.101	2.893	3.482	0.013	0.7	1.392	2.179	2.757	3.504	0.077	0.916	1.665	2.464	2.804	3.725
0.023	0.608	1.465	2.255	2.776	4.356	0	0.732	1.428	2.192	3.026	3.701	0	0.661	1.388	2.154	2.777	3.464	0.206	0.774	1.434	2.18	2.927	3.474
0.062	0.784	1.453	2.08	2.721	4.364	0	0.72	1.441	2.23	3.004	3.781	0.016	0.705	1.397	2.123	2.705	3.461		0.773	1.469	2.229	2.986	3.497
0	0.71	1.442	2.287	3.08	4.364						3.831	0.061	0.706	1.396	2.115	2.709	3.609						3.478
0	0.575	1.305	2.043	2.813	4.364							0.061	0.69	1.363	1.881	2.756	4.431						3.497
0	0.59	1.3	2.1	2.86	3.576							0.076	0.69	1.363	1.881	2.756	4.446			.			3.801
0	0.621	1.344	2.118	2.886	3.542							0	0.633	1.317	2.049	2.828	3.09						3.769
0	0.888	1.842	2.845	3.159	3.464							0.13	0.664	1.278	1.941	2.607	3.035						4.085
					3.439							0	0.644	1.345	2.073	2.837	3.524						
					3.434							0	0.727	1.44	2.188	2.947	3.397						
					3.462								0.64	1.308	2.055	2.838	3.405						
					3.914								0.627	1.308	2.052	2.821	3.378						
					3.637								0.671	1.347	2.09	2.883	3.396						
					3.71								0.733	1.448	2.192	2.965	3.415						
					3.742								0.651	1.299	2.037	2.829	3.417						
					3.955								0.697	1.395	2.179	2.945	3.545						
													0.782	1.524	2.335	3.132	3.381						
																	3.397	**3.5% 5 cont**					
																	3.34	3.702					
																	3.352	3.537					
																	3.33	3.763					
																	3.333	3.717					
																	3.445	3.612					
																	3.7	3.612					
																	3.56	3.634					
																	3.445	3.736					
																	3.623	3.626					
																	3.299	3.747					
																	3.585	3.97					
																	3.834	3.966					
																	3.702	3.971					
																	3.622	3.947					
																	3.683	3.971					

Table A4.12: Phosphate concentrations (μM) in a range of salinity standards from 1 % NaCl to 5 % NaCl.

1%b	1% 1	1% 2	1% 3	1% 4	1% 5	2.5%b	2.5% 1	2.5% 2	2.5% 3	2.5% 4	2.5% 5	3.5%b	3.5% 1	3.5% 2	3.5% 3	3.5% 4	3.5% 5	5%b	5% 1	5% 2	5% 3	5% 4	5% 5
0.084	0.62	1.234	1.854	2.511	2.99	0.114	0.622	1.216	1.843	2.438	3.039	0.016	0.589	1.21	1.829	2.39	2.967	0.079	0.579	1.182	1.804	2.402	2.952
0.172	0.624	1.244	1.875	2.469	3.02	0.087	0.626	1.221	1.846	2.452	3.041	0 013	0.575	1.206	1.825	2.406	2.97	0	0.581	1.184	1.808	2.383	2.991
0.162	0.633	1.246	1.862	2.46	2.989	0.056	0.605	1.216	1.822	2.436	3.049	0.017	0.575	1.188	1.803	2.388	3.02						2.954
0.134	0.584	1.237	1.844	2.47	3.02						3.028	0	0.554	1.168	1.96	2.375	3.02						2.988
0.043	0.6	1.226	1.851	2.513	2.993						3.033	0	0.579	1.219	1.796	2.375	2.977						3.021
					3.015							0.004	0.581	1.206	1.802	2.286	2.995						3.037
					2.99							0.033	0.577	1.197	1.81	2.402	3.035						2.978
					3.029							0	0.582	1.192	1.798	2 409	3.09						
					3.078								0.569	1.21	1.794	2.397	2.993						
					3.081								0.578	1.217	1.791	2.403	3.021						
					3.1								0.578	1.194	1.804	2.42	3.022						
					3 084								0.574	1.184	1.78	2.395	3.02						
					3.074								0.551	1.158	1.931	2.397	3.02						
																2.355	2.994						
																	2.995						
																	2.984						
																	3.007						
																	2.956						
																	3.004						
																	3.012						
																	3.004						

Appendix 5

Raw data from Chapter 5

Media for culturing *Synechococcus*

2X SNAX (From Waterbury et al.,1986)
-made as a 10X concentrated stock solution (see quantities in brackets)

Mineral Medium

$NaNO_3$ (mg)	75	(750)
NH_4Cl (mg)	5.3	(53)
K_2HPO_4 (mg)	1.5	(15)
Na_2CO_3 (g)	0.001	(0.01)
$Na_2EDTA.2H_2O$ (mg)	0.5	(5)
Micronutrients (ml)	0.1	(1)

Micronutrients

Deionized water (ml)	1000
$MnC_{l2}.4H_2O$ (g)	1.4
$ZnSO_4.7H_2O$ (g)	0.22
$NaMoO_4.2H_2O$ (g)	0.39
$Co(NO_3)_2.6H_2O$ (g)	0.025
Citric acid.H_2O (g)	6.25
Ferric ammonium citrate (g) (brown crystals)	6.00

To make 1 l of culture medium 100 ml of SNAX 10X stock solution was added to:

Deionized water	250ml
Lake water	750ml

2X SOX (From Waterbury et al., 1986)
-made as a 10X concentrated stock solution (see quantities in brackets)

Mineral Media

$NaNO_3$ (mg)	Nil	
NH_4Cl (mg)	Nil	
K_2HPO_4 (mg)	1.5	(15)
Na_2CO_3 (g)	0.001	(0.01)
$Na_2EDTA.2H_2O$ (mg)	0.5	(5)
Micronutrients (ml) (see SNAX medium)	0.1	(1)

To make 1l of culture media 100ml of SOX mineral medium was added to:

Deionized water	250ml
Lake water	750ml

Water (60 L) was collected from 11m in Ace Lake, filtered through a 0.2μm filter using a millipore pressure bomb apparatus and stored at 4°C in the dark. This lake water was used as a base for culture media and agar plates when growing *Synechococcus sp.* After the initial culturing of *Synechococcus* sp. SNAX media was routinely used for all further culturing of the cyanobacteria.

F/2 (CSIRO modification)
From Guillard, R.R.L. and Ryther J.H. (1962) Can. J. Microbiol 8: 229-239

Stock solns	$NaNO_3$ (g l^{-1})	150
	$NaH_2PO_4.2H_2O$ (g l^{-1})	10
	$NaSiO_3$ (g l^{-1})	5 (Omitted)
	Citric Acid (g l^{-1})	9
	Ferric Citrate (g l^{-1})	9
Trace Metals	$CuSO_4.5H_2O$ (mg l^{-1})	19.6
	$ZnSO_4.7H_2O$ (mg l^{-1})	44
	$CoCl_2.6H_2O$ (mg l^{-1})	20
	$MnCl_2.4H_2O$ (mg l^{-1})	360
	$Na_2MoO_4.2H_2O$ (mg l^{-1})	12.6
Vitamins	Thiamine.HCl (g l^{-1})	0.4
	Biotin (mg l^{-1})	2
	B12 (mg l^{-1})	2

Stock solutions were prepared and stored seperately. All stock solutions except vitamines were heat sterilized. Vitamines were filter sterilized. Stock solutions were stored at 4°C in the dark. Vitamines were not kept for longer than one month.

0.1 ml of all nutrient stocks and 0.05 ml of vitamines were added to 1 l of Ace Lake (2m, 8m or 11m) water and filter sterilized through a 0.05 µm filter (Millipore). F/2 media was stored for short periods of time at 4° C in the dark.

To prepare **FE media** 1ml of $(Na)_2EDTA.2H_2O$ was added to the media at a concentration of 30g l^{-1}.

0.1ml of each stock solution was added to 1 l of Ace Lake Water.

Preparation of agar and agarose plates

Difco Nobel (unwashed) was used to make 1% agar plates. 100 ml SNAX mineral medium and 750ml Ace Lake water was 0.05 µm filter sterilized. 150 ml water was used to prepare the agar. The agar was added to the water and pH balenced to 7.2. The agar was then heat sterilized. The liquid media was warmed to 50°C and the agar was coled to 50°C. The two were combined, gently mixed and agar plates were poured. The plates were allowed to set and dry in the laminar flow cabinet and were then stored at 4°C in the dark.

Agarose plates (0.5%) were made with water collected from 11 m in Ace Lake and SNAX mineral medium according to the above method.

Table A5.1: Size of cultured cells (μm) from Ace Lake, Pendant Lake and Lake Abraxas.

Ace Lake		Pendant Lake		Lake Abraxas	
Length	Width	Length	Width	Length	Width
1.4	0.8	1.7	1.1	1.4	0.9
1.5	0.9	1.9	1.1	1.3	0.8
1.5	0.9	1.9	1.3	1.5	0.8
1.6	1	1.8	1.2	1.5	0.9
1.4	0.8	2	1	1.4	1
1.3	0.7	1.8	1	1.6	0.9
1.65	0.9	1.8	0.9	1.5	0.9
1.5	1.2	1.7	1	1.7	0.7
1.6	0.9	1.9	0.9	1.5	1
1.5	1	1.8	1.1	1.5	1.2
1.4	0.8	1.8	1.3	1.5	0.9
1.3	1.1	2	0.9	1.4	0.8
1.6	0.9	1.7	1.3	1.6	0.9
1.5	0.8	1.8	1.2	1.4	1
1.4	0.9	1.6	0.9	1.3	1
1.5	0.9	1.7	0.9	1.5	0.8
1.6	0.9	1.7	1.1	1.5	0.9
1.7	0.7	2	1.2	1.6	0.9
1.6	1.1	1.8	1.1	1.5	1.2
1.4	1	1.8	1.1	1.7	0.7

Table A5.2: Cell numbers from gradient incubator experiment to determine the cardinal temperatures for the Ace Lake *Synechococcus* strain.

Time (h)	-1.1° C				0.4° C		1.7° C		3.3° C		5.3° C		6.3° C	
	a	b	c	d	a	b	a	b	a	b	a	b	a	b
0	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05
24	2.20E+05	3.00E+05	3.10E+05	2.90E+05	3.20E+05	3.50E+05	3.80E+05	3.20E+05	4.60E+05	3.40E+05	4.30E+05	4.30E+05	3.80E+05	4.60E+05
48	4.70E+05	4.40E+05	4.80E+05	4.40E+05	4.70E+05	5.00E+05	4.70E+05	4.00E+05	4.40E+05	4.20E+05	4.40E+05	4 00E+05	4.50E+05	4.30E+05
96	4.20E+05	3.80E+05	3.70E+05	3.80E+05	4.00E+05	3.40E+05	4.30E+05	4.00E+05	3.70E+05	3.50E+05	3.70E+05	3.80E+05	4.00E+05	3.50E+05
144	2.60E+05	2.60E+05	2.70E+05	2.80E+05	2.50E+05	2.50E+05	2.50E+05	2.70E+05	2.50E+05	2.50E+05	2.60E+05	2.40E+05	2.70E+05	2.30E+05
160	3.70E+05	3.80E+05	3.60E+05	3.30E+05	4.50E+05	3.50E+05	3.70E+05	3.50E+05	3.60E+05	3.60E+05	3.70E+05	3.50E+05	4.10E+05	4.10E+05
184	6.30E+05	6.40E+05	7.10E+05	6.10E+05	6.00E+05	5.70E+05	6.20E+05	5.60E+05	5.40E+05	6.00E+05	6.90E+05	8.40E+05	6.10E+05	7.70E+05
208	7.20E+05	5.80E+05	6.40E+05	7.10E+05	6.10E+05	6.50E+05	6.40E+05	7.00E+05	6.20E+05	6.50E+05	8.20E+05	7.60E+05	7.30E+05	7.30E+05
256	9.10E+05	7.60E+05	8.50E+05	7.60E+05	9.30E+05	8.40E+05	9.00E+05	8.90E+05	1.00E+06	8.50E+05	1.10E+06	1.30E+06	8.70E+05	1.30E+06
304														
352	5.57E+05	5.10E+05	6.25E+05	5.77E+05	5.22E+05	5.56E+05	6.43E+05	6.28E+05	4.77E+05	7.32E+05	1.51E+06	1.06E+06	8.60E+05	1.38E+06
426														
496														
570											1.58E+06	1.52E+06	1.77E+06	3.29E+06
690													2.38E+06	
858											2.09E+06	3.55E+06	2.43E+06	1.62E+06
1002	1.54E+05	3.65E+05	2.73E+05	2.45E+05	4.30E+05	5.84E+05	9.78E+05	1.51E+06	1.30E+06	1.22E+06	5.70E+06	7.78E+06	2.73E+06	5.37E+06
1194					6.88E+04	1.94E+05	1.99E+05	3.71E+05	6.83E+05	6.72E+05	2.82E+06	1.68E+06	1.04E+06	1.77E+06

Table A5.2 continued: Cell numbers from gradient incubator experiment to determine the cardinal temperatures for the Ace Lake *Synechococcus* strain.

Time (h)	7.9° C				9.6° C		11° C		12.1° C		13.7° C	
	a	b	c	d	a	b	a	b	a	b	11a	11b
0	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05
24	5.30E+05	5.40E+05	4.60E+05	5.20E+05	6.00E+05	5.50E+05	7.10E+05	6.50E+05	5.90E+05	6.10E+05	7.60E+05	5.90E+05
48	5.00E+05	4.40E+05	4.80E+05	5.00E+05	5.70E+05	4.30E+05	4.80E+05	4.90E+05	5.10E+05	5.10E+05	4.70E+05	5.00E+05
96	3.60E+05	4.10E+05	4.20E+05	3.60E+05	3.60E+05	3.80E+05	3.90E+05	4.20E+05	4.10E+05	4.10E+05	4.00E+05	3.70E+05
144	2.20E+05	2.60E+05	2.50E+05	2.90E+05	3.20E+05	3.20E+05	2.70E+05	3.90E+05	3.30E+05	3.40E+05	3.40E+05	3.90E+05
160	4.50E+05	4.10E+05	3.70E+05	3.70E+05	5.20E+05	5.10E+05	5.00E+05	5.60E+05	4.20E+05	5.20E+05	4.30E+05	4.50E+05
184	6.70E+05	8.20E+05	6 70E+05	7.50E+05	9.10E+05	9.70E+05	7.70E+05	1.10E+06	9.70E+05	1.20E+06	8.20E+05	9.50E+05
208	6.40E+05	1.00E+06	9.30E+05	6.90E+05	1.30E+06	1.20E+06	1.40E+06	1.30E+06	1.30E+06	2.30E+06	9.20E+05	1.50E+06
256	9.90E+05	1.20E+06	1.40E+06	9.00E+05	1.60E+06	1.90E+06	1.20E+06	1.80E+06	1.80E+06	2.00E+06	1.40E+06	1.60E+06
304	1.10E+06	1.50E+06	1.50E+06	1.00E+06	1.30E+06	1.70E+06	1.20E+06	1.90E+06	1.90E+06	2.80E+06	1.50E+06	1.90E+06
352	1.47E+06	2.07E+06	1.81E+06	1.21E+06	2.34E+06	2.40E+06	1.85E+06	2.99E+06	2.78E+06	3.80E+06	2.18E+06	2.83E+06
426	1.99E+06	5.47E+06	3.05E+06	1.77E+06	3.17E+06	3.52E+06	2.70E+06	4.85E+06	4.08E+06	4.89E+06	3.25E+06	5.99E+06
496	2.20E+06	3.00E+06	2.70E+06	1.30E+06	3.40E+06	3.70E+06	2.70E+06	5.50E+06	5.00E+06	7.50E+06	3.20E+06	5.00E+06
570	2.20E+06	3.20E+06	4.21E+06	1.62E+06	6.21E+06	5.65E+06	3.10E+06	5.89E+06	8.35E+06	1.13E+07	3.93E+06	6.71E+06
690		1.11E+07	5.38E+06		8.01E+06	2.99E+06	2.30E+06	6.57E+06	1.30E+07		2.83E+06	5.55E+06
858		5.92E+06	5.31E+06	1.55E+06	5.09E+06	1.33E+07	4.38E+06	2.49E+07	2.55E+07	3.42E+07	1.20E+07	3.83E+07
1002	2.64E+06	2.47E+07	1.44E+07	4.67E+06	3.03E+07	3.29E+07	8.19E+06	7.22E+07	4.20E+07	5.65E+07	1.43E+07	4.62E+07
1194	6.90E+06	6.17E+06	3.95E+06	1.49E+06	1.04E+07	1.13E+07	6.35E+06	1.27E+07	1.64E+07	1.38E+07	5.86E+06	1.10E+07

Table A5.2 continued: Cell numbers from gradient incubator experiment to determine the cardinal temperatures for the Ace Lake *Synechococcus* strain.

Time (h)	15.1° C				16.2° C		19.8° C		21.1° C		22.3° C	
	a	b	c	d	a	b	a	b	a	b	a	b
0	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05
24	6.30E+05	6.10E+05	6.70E+05	5.50E+05	5.80E+05	5.80E+05	6.00E+05	5.70E+05	6.70E+05	6.40E+05	6.00E+05	6.40E+05
48	4.30E+05	5.30E+05	4.80E+05	4.30E+05	4.40E+05	5.30E+05	4.40E+05	5.30E+05	4.60E+05	4.30E+05	4.90E+05	4.60E+05
96	3.60E+05	4.00E+05	4.00E+05	4.50E+05	3.70E+05	4.00E+05	4.80E+05	3.80E+05	3.90E+05	3.90E+05	3.70E+05	4.20E+05
144	2.80E+05	3.40E+05	3.70E+05	2.60E+05	3.20E+05	3.20E+05	3.30E+05	2.40E+05	5.10E+05	3.80E+05	3.00E+05	2.30E+05
160	4.30E+05	3 90E+05	4.60E+05	3.70E+05	4.70E+05	4.60E+05	4.10E+05	3.80E+05	5.20E+05	4.70E+05	4.70E+05	4 40E+05
184	6.90E+05	8.30E+05	9.80E+05	6.40E+05	9.30E+05	9.80E+05	8.00E+05	5.80E+05	8.70E+05	8.80E+05	6.60E+05	6.00E+05
208	8.00E+05	1.30E+06	1.20E+06	8.00E+05	1.70E+06	1.50E+06	1.10E+06	9.80E+05	1.70E+06	1.20E+06	1.00E+06	9.80E+05
256	1.10E+06	1.30E+06	1.30E+06	7.80E+05	1.40E+06	1.90E+06	1.30E+06	1.20E+06	1.90E+06	1.40E+06	1.40E+06	1.20E+06
304	1.50E+06	1.80E+06	1.80E+06	9.70E+05								
352	1.96E+06	2.94E+06	3.08E+06	1.35E+06	2.96E+06	3.45E+06	3.06E+06	2.07E+06	4.13E+06	2.50E+06	2.99E+06	2.19E+06
426	2.04E+06	3.08E+06	3.59E+06	1.44E+06	3.57E+06	5.43E+06	3.88E+06	2.88E+06	6.09E+06	4.30E+06	4.59E+06	2.86E+06
496	2.50E+06	5.00E+06	4.50E+06	2.00E+06	5.10E+06	7.20E+06	5.40E+06	3.80E+06	9.80E+06	6.90E+06	6.30E+06	3.80E+06
570	2.39E+06	6.29E+06	4.31E+06	2.07E+06	5.01E+06	9.28E+06	5.51E+06	4.40E+06	9.73E+06	5.97E+06	7.71E+06	2.28E+06
690	1.60E+06	2.10E+06	2.68E+06	8.77E+06	7.43E+06	3.96E+06	6.28E+06	3.86E+06	1.05E+07	3.80E+06	4.77E+06	2.08E+06
858	7.19E+07	1.66E+07	1.72E+07	8.60E+06	2.90E+07	3.24E+07	2.43E+07		1.72E+07	1.25E+07	2.26E+07	
1002	3.14E+07	2.24E+07	4.09E+07	4.56E+06	2.37E+07	3.47E+07	1.95E+07	8.00E+06	1.93E+07	2.20E+07		
1194	1.69E+06	6.75E+06	5.64E+06	1.50E+06	1.05E+07	1.23E+07	4.73E+06	2.40E+06	7.15E+06	3.86E+06		

Table A5.2 continued: Cell numbers from gradient incubator experiment to determine the cardinal temperatures for the Ace Lake *Synechococcus* strain.

Time (h)	24.6° C				25.8° C		27.4° C		29.3° C		31.1° C			
	a	b	c	d	a	b	a	b	a	b	a	b	c	d
0	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05	5.30E+05
24	5.30E+05	6.00E+05	5.50E+05	5.70E+05	5.30E+05	5.90E+05	6.80E+05	5.70E+05	5.80E+05	6.20E+05	9.20E+05	6.50E+05	7.10E+05	7.40E+05
48	4.10E+05	4.20E+05	4.30E+05	4.00E+05	4.30E+05	3.90E+05	4.00E+05	4.70E+05	5.00E+05	4.80E+05	4.20E+05	4.30E+05	4.40E+05	3.80E+05
96	3.20E+05	4.00E+05	4.10E+05	3.80E+05	3.30E+05	3.20E+05	3.20E+05	3.40E+05	2.80E+05	2.70E+05	3.40E+05	2.80E+05	2.90E+05	2.70E+05
144	2.10E+05	3.30E+05	2.40E+05	3.50E+05	2.00E+05	2.10E+05	2.00E+05	2.30E+05	1.80E+05	2.00E+05	2.10E+05	2.10E+05	2.00E+05	2.30E+05
160	3.20E+05	3.50E+05	3.80E+05	3.50E+05	3.20E+05	2.90E+05	3.60E+05	3.00E+05	3.60E+05	3.00E+05	3.00E+05	5.20E+05	3.10E+05	3.90E+05
184	4.50E+05	4.90E+05	4.70E+05	4.10E+05	3.90E+05	4.10E+05	4.10E+05	4.10E+05	4.70E+05	3.80E+05	4.40E+05	3.80E+05	3.90E+05	5.70E+05
208	7.40E+05	7.20E+05	6.80E+05	7.20E+05	6.70E+05	7.90E+05	7.90E+05	7.60E+05	5.00E+05	6.00E+05	5.30E+05	5.40E+05	5.10E+05	7.80E+05
256	6.00E+05	6.70E+05	6.70E+05	6.80E+05	6.30E+05	6.20E+05	5.70E+05	6.10E+05	4.70E+05	4.20E+05	1.80E+05	1.60E+05	1.30E+05	1.90E+05
304														
352	1.07E+06	7.96E+05	9.20E+05	9.29E+05	9.88E+05	7.81E+05	7.01E+05	7.78E+05	1.38E+05	6.12E+04	4.97E+04	4.30E+04	3.52E+04	5.10E+04
426														
496														
570	1.07E+06	1.21E+06	1.39E+06	1.42E+06	7.62E+05	8.62E+05	3.53E+05	4.88E+05						
690														
858														
1002	2.09E+05	4.20E+05	2.05E+05	1.13E+05	1.85E+04	1.22E+05	1.29E+03	2.26E+03	2.34E+02	4.13E+03	9.14E+02	2.88E+03	4.13E+03	3.50E+03
1194	1.56E+05	3.20E+05	1.02E+05	2.97E+04										

Table A5.3: Final cell numbers following 312 h exposure to different light intensities. Treatments are light intensity (μmol photons m^{-2} s^{-1}); numbers brackets are the hours per day that cells were exposed to the light intensity.

Replicate	20 (12)	20 (24)	200 (24)	5 (24)
A	5.48E+07	1.21E+08	4.20E+07	2.45E+06
B	4.50E+07	1.04E+08	3.80E+07	1.94E+06
C				1.48E+06
D				1.71E+06

Table A5.4: Histogram statistics from flow cytometric readings for the relative change in FL2 fluorescence produced by *Synechococcus* cells exposed to different light intensities.

Treatment*	Events	Peak Channel	Mean	Median	SD	CV %
20 (12)	10000	79.15	114.11	93.06	71.77	62.89
20 (24)	10000	8.06	8.00	7.04	5.01	62.71
300 (12)	10000	403.15	558.55	474.00	326.35	58.43
5 (12)	10000	63.21	97.85	79.86	57.09	58.34

*** Numbers in this column are light intensity (μmol photons m^{-2} s^{-1}). Numbers in brackets are number of hours per day that cells were exposed to the light intensity.**

Table A5.5: Histogram statistics for flow cytometric readings for the relative change in FL3 fluorescence produced by *Synechococcus* cells exposed to different light intensities.

Treatment*	Events	Peak Channel	Mean	Median	SD	CV %
20 (12)	10000	7.23	7.24	5.94	6.45	89.04
20 (24)	10000	4.00	4.99	4.18	3.07	61.42
300 (12)	10000	30.78	27.20	26.66	8.71	32.03
5 (12)	10000	11.14	13.16	10.09	9.26	70.41

*** Numbers in this column are light intensity (μmol photons m^{-2} s^{-1}). Numbers in brackets are number of hours per day that cells were exposed to the light intensity.**

Table A5.6: Salinity tolerence of the Ace Lake, Pendant Lake and Lake Abraxas *Synechococcus* strains.

Lake	Undiluted Lake Water			2/3 Lake Water			1/3 Lake Water			No Lake Water		
Ace Lake	Ace Neat			Ace 2/3			Ace 1/3			Ac MQ		
Time (h)	0	7	28	0	7	28	0	7	28	0	7	28
a	1.68E+06	3.85E+06	1.08E+07	2.01E+06	3.78E+06	1.19E+07	1.23E+06	2.80E+06	2.56E+06	1.69E+06	2.02E+06	9.11E+04
b	1.49E+06	3.75E+06	1.18E+07	1.49E+06	2.81E+06	8.57E+06	1.29E+06	2.58E+06	5.41E+06	1.86E+06	1.78E+06	4.15E+04
c	1.53E+06	3.56E+06	1.07E+07	1.68E+06	3.62E+06	8.62E+06	1.16E+06	2.58E+06	4.64E+06	2.03E+06	2.06E+06	1.03E+05
Pendant Lake	Pd Neat			Pd 2/3			Pd 1/3			Pd MQ		
Time (h)	0	7	28	0	7	28	0	7	28	0	7	28
a	3.23E+06	2.69E+07	1.54E+08	3.02E+06	4.31E+07	9.25E+07	1.70E+06	1.92E+07	4.50E+07	2.03E+06	3.30E+06	5.12E+06
b	3.56E+06	4.98E+07	1.24E+08	2.94E+06	2.56E+07	7.75E+07	2.21E+06	2.36E+07	4.04E+07	1.72E+06	4.69E+06	4.33E+06
c	3.38E+06	5.29E+07	1.47E+08	3.48E+06	3.16E+07	7.70E+07	2.48E+06	1.96E+07	3.72E+07	1.61E+06	3.58E+06	4.50E+06
Lake Abraxas	Ab Neat			Ab 1/3			Ab 2/3			Ab MQ		
Time (h)	0	7	28	0	7	28	0	7	28	0	7	28
a	6.59E+06	4.11E+07	1.20E+08	4.01E+06	2.10E+07	9.48E+07	4.60E+06	3.38E+07	2.30E+07	4.26E+06	3.00E+06	3.12E+06
b	6.45E+06	2.86E+07	1.17E+08	3.89E+06	1.78E+07	8.97E+07	3.79E+06	3.39E+07	3.39E+07	3.88E+06	4.16E+06	8.07E+06
c	8.30E+06	2.96E+07	1.17E+08	4.44E+06	2.10E+07	9.26E+07	4.39E+06	2.49E+07	2.86E+07	3.77E+06	4.24E+06	3.50E+06

Appendix 6

Raw data from Chapter 6

Table A6.1 *Synechococcus* cell numbers in samples from the nine preservation protocols. Numbers correspond to those in Table 6.1

Control	1	2	3	4	5	6	7
2.20E+06	1.89E+06	1.73E+06	2.70E+06	8.02E+05	1.59E+06	2.00E+06	1.60E+06
2.00E+06	1.80E+06	1.53E+06	2.22E+06	1.04E+06	2.16E+06	2.13E+06	2.04E+06
1.90E+06	2.70E+06	1.86E+06	1.92E+06	1.74E+06	1.96E+06	2.20E+06	2.30E+06
1.80E+06	2.25E+06	1.93E+06	1.80E+06	1.96E+06	1.79E+06	2.00E+06	1.97E+06
2.10E+06	2.02E+06	1.82E+06	1.98E+06	1.85E+06	1.78E+06	1.75E+06	1.69E+06
1.94E+06	2.05E+06	1.84E+06	1.92E+06	2.18E+06	1.92E+06	1.78E+06	1.75E+06
1.60E+06		2.10E+06			2.18E+06	1.75E+06	
2.00E+06		2.50E+06			1.99E+06	1.78E+06	
2.00E+06		2.10E+06			1.89E+06		
1.97E+06		1.95E+06			1.93E+06		
		1.91E+06			1.95E+06		
		1.88E+06			2.30E+06		
		2.03E+06			1.83E+06		
					1.79E+06		

1 = No preserving ageent; 2 = 1 % gluteraldehyde; 3 = 1 % gluteraldehyde, 10 % DMSO; 4 = 1 % gluteraldehyde, 10 % DMSO; and 10 % glycerol; 5 = 1 % formalin; 6 = 1 % formalin, 10 % DMSO; 7 = 1 % formalin, 10 % DMSO, 10 % glycerol.

Table A6.2: *Cryptomonas* sp. numbers in cycloheximide and ampicillin treatments.

Control	a	b	c	d	e	Mean	SD
0	3.96E+04	4.16E+04	5.70E+04	2.90E+04	4.30E+04	4.20E+04	1.00E+04
24	3.90E+04	3.70E+04				3.76E+04	3.29E+03
48	3.89E+04	4.01E+04				3.95E+04	8.49E+02
72	5.86E+04	5.20E+04				5.53E+04	4.67E+03
216	2.41E+05	2.03E+05				2.22E+05	2.69E+04
Cy.	**a**	**b**	**c**	**d**	**e**		
0	3.96E+04	4.16E+04	5.70E+04	2.90E+04	4.30E+04	4.20E+04	1.00E+04
24	3.10E+04	4.30E+04	4.50E+04	3.90E+04		3.95E+04	6.19E+03
48	2.87E+04	3.58E+04	5.69E+04	2.75E+04		3.72E+04	1.36E+04
72	3.50E+04	3.20E+04	3.40E+04	3.03E+04	2.50E+04	3.13E+04	3.94E+03
216	3.40E+04	3.30E+04	1.94E+04			2.88E+04	8.16E+03
Cy. + Amp.	**a**	**b**	**c**	**d**	**e**		
0	3.96E+04	4.16E+04	5.70E+04	2.90E+04	4.30E+04	4.20E+04	1.00E+04
24	3.70E+04	3.30E+04	4.20E+04			3.73E+04	4.51E+03
48	4.12E+04	4.02E+04	4.21E+04			4.12E+04	9.50E+02
72	5.40E+04	5.10E+04	6.40E+04			5.63E+04	6.81E+03
216	3.13E+05	1.98E+05	2.56E+05			2.56E+05	5.75E+04

Table A6.3: *Synechococcus* numbers in cycloheximide and ampicillin (0.1 - 5 mg l^{-1}) treatments.

Control	a	b	c	d	e	Mean	SD
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.20E+05	4.60E+05	5.00E+05	6.30E+05	5.50E+05	5.32E+05	5.71E+04
48	7.20E+05	3.04E+05	6.80E+05	5.61E+05	5.60E+05	5.65E+05	1.45E+05
72	7.89E+05	1.06E+06	7.30E+05	7.40E+05	8.48E+05	8.33E+05	1.21E+05
120		1.80E+06	2.30E+06	1.70E+06	1.60E+06	1.85E+06	2.69E+05
216	5.38E+06	4.28E+06	5.14E+06	5.08E+06	3.60E+06	4.70E+06	6.61E+05
Amp 0.1	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.05E+05	4.83E+05	5.39E+05	4.84E+05	5.10E+05	5.04E+05	2.05E+04
48	5.20E+05	5 46E+05	5.62E+05	8.10E+05	4.40E+05	5.76E+05	1.24E+05
72	4.35E+05	3.64E+05	3.66E+05	5.60E+05	6.15E+05	4.68E+05	1.02E+05
120	3.30E+05	3.20E+05	3.20E+05	3.05E+05		3.19E+05	8.93E+03
216	6.75E+04	6.28E+04	3.60E+04	4.24E+04	2.76E+04	4.73E+04	1.54E+04
Amp 0.25	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	4.69E+05	3.59E+05	3.91E+05	5.73E+05	4.25E+05	4.43E+05	7.44E+04
48	5.40E+05	3.03E+05	4 07E+05	4.50E+05	6.80E+05	4.76E+05	1.27E+05
72	3.36E+05	3.07E+05	3.10E+05	5.10E+05	4.10E+05	3.75E+05	7.72E+04
120	3.10E+05	4.05E+05	2.20E+05	2.30E+05		2.91E+05	7.44E+04
216	5.55E+04	6.37E+04	7.60E+04	3.83E+04		5.84E+04	1.37E+04

Table A6.3 cont: *Synechococcus* numbers in Cy and Amp (0.1 - 5 mg l^{-1}) treatments.

Amp 0.5	a	b	c	d	e	Mean	SD
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	4.40E+05	5.34E+05	4.84E+05	4.08E+05	4.34E+05	4.60E+05	4.43E+04
48	3.30E+05	4.47E+05	4.90E+05	3.80E+05	3.40E+05	3.97E+05	6.19E+04
72	7.70E+05	3.90E+05	2.96E+05	4.20E+05	4.27E+05	4.61E+05	1.62E+05
120	2.00E+05	4.80E+05	2.90E+05	3.40E+05	4.80E+05	3.58E+05	1.22E+05
216		1.02E+05	5.17E+05	4.89E+04	4.87E+04	1.79E+05	2.27E+05
Amp 1.0	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.48E+05	5.31E+05	4.74E+05	4.11E+05	4.31E+05	4.79E+05	5.37E+04
48	3.48E+05	5.69E+05	4.86E+05	5.45E+05	3.74E+05	4.64E+05	8.90E+04
72	3.20E+05	3.66E+05	4.10E+05	3.60E+05	6.70E+05	4.25E+05	1.26E+05
120	3.50E+05	4.80E+05	3.70E+05	1.40E+05	2.70E+05	3.22E+05	1.13E+05
216	5.90E+04	8.79E+04	6.13E+04	3.56E+04	4.46E+04	5.77E+04	1.78E+04
Amp 2.5	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	3.85E+05	4.40E+05	4.10E+05	4.92E+05	6.62E+05	4.78E+05	9.88E+04
48	5.17E+05	5.50E+05	5.00E+05	5.40E+05	4.15E+05	5.04E+05	4.80E+04
72	4.40E+05	3.30E+05	4.60E+05	5.00E+05	4.22E+05	4.30E+05	5.65E+04
120	2.96E+05	2.60E+05	3.30E+05	5.00E+05	1.30E+05	3.03E+05	1.19E+05
216	7.10E+04	5.60E+04		1.96E+05	3.65E+04	8.99E+04	6.25E+04
Amp 5	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.09E+05	5.03E+05	4.08E+05	5.36E+05	5.60E+05	5.03E+05	5.18E+04
48	4.99E+05	3.15E+05	6.40E+05	4.10E+05	4.37E+05	4.60E+05	1.08E+05
72	3.46E+05	3.03E+05	3.98E+05	5.80E+05	5.96E+05	4.45E+05	1.21E+05
120	3.25E+05	3.90E+05	3.37E+05	4.20E+05	1.20E+05	3.18E+05	1.05E+05
216	5.79E+04	6.60E+04	7.70E+04	1.06E+05		7.67E+04	1.82E+04
Cy	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	7.00E+05	5.60E+05	5.06E+05	9.80E+05	8.40E+05	7.17E+05	1.75E+05
48	5.00E+05	6.60E+05	6.75E+05	7.42E+05	7.57E+05	6.67E+05	9.14E+04
72	6.90E+05	8.50E+05	1.00E+06	1.03E+06	1.20E+06	9.54E+05	1.73E+05
120	2.40E+06	2.30E+06	2.40E+06	2.99E+06	1.98E+06	2.41E+06	3.27E+05
216	5.18E+06	4.66E+06	5.70E+06	7.67E+06	3.09E+06	5.26E+06	1.49E+06
Cy +Amp 0.1	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.80E+05	6.02E+05	6.37E+05			6.06E+05	2.35E+04
48	4.36E+05	4.61E+05	4.41E+05	3.81E+05	4.88E+05	4.41E+05	3.53E+04
72	4.10E+05	3.60E+05	3.70E+05	3.40E+05	3.66E+05	3.69E+05	2.29E+04
120	3.50E+05	3.40E+05	2.20E+05	3.40E+05	3.99E+05	3.30E+05	5.91E+04
216	7.50E+04	9.30E+04	3.89E+04	1.40E+05	1.85E+05	1.06E+05	5.10E+04
Cy + Amp 0.25	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.20E+05	4.98E+05	5.66E+05	5.55E+05	3.70E+05	5.02E+05	7.02E+04
48	4.20E+05	4.02E+05	4.05E+05	5.50E+05	3.35E+05	4.22E+05	7.02E+04
72	4.20E+05	3.10E+05	3.10E+05	3.90E+05	7.80E+05	4.42E+05	1.75E+05
120	1.08E+05	2.92E+05	1.93E+05	2.57E+05		2.13E+05	7.00E+04
216	2.99E+04	6.22E+04	3.71E+04	4.40E+04	4.37E+04	4.34E+04	1.07E+04

Table A6.3 cont: *Synechococcus* numbers in Cy and Amp (0.1 - 5 mg l^{-1}) treatments.

Cy + Amp 0.5	a	b	c	d	e	Mean	SD
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	6.10E+05	6.90E+05	5.91E+05	5.07E+05	6.50E+05	6.10E+05	6.16E+04
48	4.87E+05	5.01E+05	4.35E+05	5.38E+05	4.99E+05	4.92E+05	3.32E+04
72	3.40E+05	3.87E+05	2.96E+05	3.50E+05	4.40E+05	3.63E+05	4.84E+04
120	1.10E+05	2.90E+05	2.50E+05	3.40E+05	1.60E+05	2.30E+05	8.41E+04
216	3.87E+05	5.60E+05	7.62E+05	3.53E+04		4.36E+05	2.67E+05
Cy + Amp 1.0	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	6.98E+05	7.30E+05	5.80E+05	4.15E+05	4.82E+05	5.81E+05	1.21E+05
48	3.69E+05	4.65E+05	4.24E+05	4.98E+05		4.39E+05	4.82E+04
72	4.02E+05	3.66E+05	4.10E+05	3.60E+05	3.58E+05	3.79E+05	2.22E+04
120	1.20E+05	3.70E+05	3.90E+05	2.00E+05	3.40E+05	2.84E+05	1.06E+05
216	3.60E+04	4.80E+04	5.60E+04	3.10E+04	4.50E+04	4.32E+04	8.84E+03
Cy + Amp 2.5	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	5.18E+05	5.40E+05	4.66E+05	4.70E+05	5.42E+05	5.07E+05	3.31E+04
48	5.77E+05	3.96E+05	7.36E+05	3.69E+05	4.06E+05	4.97E+05	1.40E+05
72	4.50E+05	3.30E+05	4.60E+05	4.90E+05	3.10E+05	4.08E+05	7.33E+04
120	2.19E+05	3.90E+05	6.25E+05	4.70E+05		4.26E+05	1.46E+05
216	3.44E+04	6.40E+04	1.03E+05	3.67E+04	3.61E+04	5.48E+04	2.65E+04
Cy + Amp 5	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	5.70E+05	5.30E+05	4.70E+05	5.20E+05	5.20E+05	5.22E+05	3.19E+04
24	6.60E+05	5.60E+05	5.40E+05	6.80E+05	5.90E+05	6.06E+05	5.50E+04
48	5.50E+05	3.11E+05	4.20E+05	4.28E+05		4.27E+05	8.46E+04
72	4.30E+05	3.04E+05	3.98E+05	4.81E+05	5.40E+05	4.31E+05	7.95E+04
120	2.96E+05	4.20E+05		2.70E+05	1.70E+05	2.89E+05	8.91E+04
216	5.79E+04	9.20E+04	3.89E+04	4.68E+04	3.69E+04	5.45E+04	2.02E+04

Table A6.4: *Synechococcus* numbers in cycloheximide and ampicillin (0.005 - 0.1 mg l^{-1}) treatments.

Control	a	b	c	d	e	Mean	SD
0	9 40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	1.30E+06	6.50E+05	6.80E+05			8.77E+05	3.67E+05
48	8.80E+05	9.80E+05	8.85E+05			9.15E+05	5.63E+04
72	1.30E+06	1.20E+06	1.30E+06			1.27E+06	5.77E+04
120	3.00E+06	3.60E+06	4.20E+06			3.60E+06	6.00E+05
192	2.90E+06	2.70E+06	2.70E+06			2.77E+06	1.15E+05
Amp 005	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	6.90E+05	6.70E+05	6.30E+05			6.63E+05	3.06E+04
48	8.50E+05	7.60E+05	7.70E+05			7.93E+05	4.93E+04
72	1.03E+06	1.20E+06	1.10E+06			1.11E+06	8.54E+04
120	1.18E+06	9.80E+05	1.50E+06			1.22E+06	2.62E+05
192	1.60E+06	1.40E+06	1.30E+06			1.43E+06	1.53E+05

Table A6.4 continued: *Synechococcus* numbers in cycloheximide and ampicillin (0.005 - 0.1 mg l^{-1}) treatments.

Amp 01	a	b	c	d	e	Mean	SD
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	6.75E+05	6.68E+05	6.87E+05			6.77E+05	9.61E+03
48	6.90E+05	7.60E+05	7.40E+05			7.30E+05	3.61E+04
72	1.10E+06	9.60E+05	9.80E+05			1.01E+06	7.57E+04
120	1.25E+06		1.10E+06			1.18E+06	1.06E+05
192	9.10E+05	1.10E+06	8.10E+05			9.40E+05	1.47E+05
Amp 05	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	7.20E+05	6.05E+05	6.57E+05			6.61E+05	5.76E+04
48	5.47E+05	5.46E+05	5.40E+05			5.44E+05	3.79E+03
72	6.80E+05	5.90E+05	5.90E+05			6.20E+05	5.20E+04
120	2.80E+05	3.10E+05	3.40E+05			3.10E+05	3.00E+04
192	1.10E+05	1.20E+05	1.10E+05			1.13E+05	5.77E+03
Amp 1	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	6.50E+05	5.40E+05	6.03E+05			5.98E+05	5.52E+04
48	4.90E+05	4.60E+05	5.05E+05			4.85E+05	2.29E+04
72	4.80E+05	5.10E+05	4.10E+05			4.67E+05	5.13E+04
120	1.70E+05	1.03E+05	1.30E+05			1.34E+05	3.37E+04
192	7.80E+04	7.00E+04	5.30E+04			6.70E+04	1.28E+04
Cy Control	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	9.60E+05	9.20E+05				9.40E+05	2.83E+04
48	7.90E+05	9.50E+05	8.35E+05			8.58E+05	8.25E+04
72	1.33E+06	1.20E+06	1.60E+06			1.38E+06	2.04E+05
120	3.15E+06	3.89E+06	3.40E+06			3.48E+06	3.76E+05
192	4.20E+06	2.70E+06	3.90E+06			3.60E+06	7.94E+05
CyAmp 005	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	7.60E+05	7.50E+05	7.40E+05			7.50E+05	1.00E+04
48	8.50E+05	7.60E+05	7.70E+05			7.93E+05	4.93E+04
72	1.20E+06	1.10E+06	1.30E+06			1.20E+06	1.00E+05
120	1.70E+06	1.50E+06	3.10E+06			2.10E+06	8.72E+05
192	1.70E+06	1.70E+06	1.60E+06			1.67E+06	5.77E+04
CyAmp 01	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	7.76E+05	8.50E+05	7.80E+05			8.02E+05	4.16E+04
48	7.10E+05	8.50E+05				7.80E+05	9.90E+04
72	9.10E+05	1.00E+06	7.50E+05			8.87E+05	1.27E+05
120	1.53E+06	1.30E+06	1.10E+06			1.31E+06	2.15E+05
192	1.20E+06	1.00E+06	9.20E+05			1.04E+06	1.44E+05

Table A6.4 continued: *Synechococcus* numbers in cycloheximide and ampicillin (0.005 - 0.1 mg l^{-1}) treatments.

CyAmp 05	a	b	c	d	e	Mean	SD
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24	6.95E+05	9.10E+05	7.60E+05			7.88E+05	1.10E+05
48	5.40E+05	5.50E+05	5.40E+05			5.43E+05	5.77E+03
72	8.60E+05	9.90E+05	8.40E+05			8.97E+05	8.14E+04
120	4.50E+05	3.60E+05	5.00E+05			4.37E+05	7.09E+04
192	1.90E+05	1.90E+05	2.10E+05			1.97E+05	1.15E+04
CyAmp 1	**a**	**b**	**c**	**d**	**e**	**Mean**	**SD**
0	9.40E+05	9.00E+05	6.60E+05	8.50E+05	8.10E+05	8.32E+05	1.08E+05
24							
48	6.50E+05	4.80E+05	6.60E+05			5.97E+05	1.01E+05
72	4.90E+05	7.30E+05	7.00E+05			6.40E+05	1.31E+05
120	1.50E+05	2.40E+05	2.00E+05			1.97E+05	4.51E+04
192	8.60E+04	5.80E+04	8.00E+04			7.47E+04	1.47E+04

Table A6.5: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* experiments to determine controls of distribution and abundance in Ace Lake. OBS = observation number, Depth = depth (m) at which the treatment was suspended in Ace Lake, Antibiot = antibiotic treatment, Nuts = nutrient treatment (WN = without nutrients, N = nutrient addition), Salt = salinity treatment (WS = without salt addition).

OBS	DEPTH	REP	ANTIBIOT	NUTS	SALT	NUTSALT	LIGHT	CELL No. 0h	CELL No. 48h	RATE 48_0
1	6	A	WA	WN	WS	WNS	10	3.70E+03	8.17E+03	1.65E-02
2	6	B	WA	WN	WS	WNS	10	4.09E+03	3.94E+03	-7.94E-04
3	6	C	WA	WN	WS	WNS	10	4.87E+03	3.98E+03	-4.23E-03
4	6	A	C	WN	WS	WNS	10	6.00E+03	3.70E+03	-1.01E-02
5	6	B	C	WN	WS	WNS	10	4.21E+03	3.84E+03	-1.92E-03
6	6	C	C	WN	WS	WNS	10	3.97E+03	4.27E+03	1.53E-03
7	6	A	A	WN	WS	WNS	10	4.03E+03	7.33E+03	1.25E-02
8	6	B	A	WN	WS	WNS	10	5.30E+03	3.91E+03	-6.33E-03
9	6	C	A	WN	WS	WNS	10	5.13E+03	4.52E+03	-2.64E-03
10	6	A	CA	WN	WS	WNS	10	3.70E+03	5.97E+03	9.95E-03
11	6	B	CA	WN	WS	WNS	10	5.30E+03	5.51E+03	8.12E-04
12	6	C	CA	WN	WS	WNS	10	4.53E+03	3.78E+03	-3.79E-03
13	6	A	WA	N	WS	WNS	10	5.17E+03	5.82E+03	2.47E-03
14	6	B	WA	N	WS	WNS	10	5.01E+03	7.03E+03	7.08E-03
15	6	C	WA	N	WS	WNS	10	4.79E+03	4.77E+03	-1.05E-04
16	6	A	C	N	WS	WNS	10	4.03E+03	5.33E+03	5.82E-03
17	6	B	C	N	WS	WNS	10	4.98E+03	3.70E+03	-6.18E-03
18	6	C	C	N	WS	WNS	10	5.21E+03	3.98E+03	-5.63E-03
19	6	A	A	N	WS	WNS	10	3.80E+03	6.28E+03	1.05E-02
20	6	B	A	N	WS	WNS	10	4.57E+03	3.62E+03	-4.88E-03
21	6	C	A	N	WS	WNS	10	4.92E+03	3.57E+03	-6.68E-03
22	6	A	CA	N	WS	WNS	10	3.52E+03	6.17E+03	1.17E-02
23	6	B	CA	N	WS	WNS	10	6.19E+03	3.81E+03	-1.01E-02
24	6	C	CA	N	WS	WNS	10	5.22E+03	4.55E+03	-2.88E-03

Table A6.5 continued: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* experiments to determine controls of distribution and abundance in Ace Lake.

OBS	DEPTH	REP	ANTIBIOT	NUTS	SALT	NUTSALT	LIGHT	CELL No. 0h	CELL No. 48h	RATE 48_0
25	6	A	WA	WN	S	WNS	10	4.80E+03	8.69E+03	1.24E-02
26	6	B	WA	WN	S	WNS	10	6.69E+03	4.79E+03	-6.96E-03
27	6	C	WA	WN	S	WNS	10	6.34E+03	4.17E+03	-8.75E-03
28	6	A	C	WN	S	WNS	10	5.26E+03	4.32E+03	-4.08E-03
29	6	B	C	WN	S	WNS	10	6.20E+03	4.30E+03	-7.62E-03
30	6	C	C	WN	S	WNS	10	6.29E+03	5.78E+03	-1.75E-03
31	6	A	A	WN	S	WNS	10	6.04E+03	8.56E+03	7.27E-03
32	6	B	A	WN	S	WNS	10	7.45E+03	4.19E+03	-1.20E-02
33	6	C	A	WN	S	WNS	10	5.54E+03	5.52E+03	-8.60E-05
34	6	A	CA	WN	S	WNS	10	6.71E+03	8.84E+03	5.73E-03
35	6	B	CA	WN	S	WNS	10	4.63E+03	3.60E+03	-5.27E-03
36	6	C	CA	WN	S	WNS	10	6.04E+03	6.10E+03	2.05E-04
37	6	A	WA	N	S	NS	10	5.24E+03	7.82E+03	8.33E-03
38	6	B	WA	N	S	NS	10	8.35E+03	5.28E+03	-9.55E-03
39	6	C	WA	N	S	NS	10	5.51E+03	4.51E+03	-4.17E-03
40	6	A	C	N	S	NS	10	5.85E+03	1.53E+04	2.01E-02
41	6	B	C	N	S	NS	10	6.16E+03	1.40E+04	1.71E-02
42	6	C	C	N	S	NS	10	6.59E+03	4.81E+03	-6.57E-03
43	6	A	A	N	S	NS	10	4.06E+03	9.98E+03	1.88E-02
44	6	B	A	N	S	NS	10	8.17E+03	3.84E+03	-1.57E-02
45	6	C	A	N	S	NS	10	3.22E+03	4.64E+03	7.61E-03
46	6	A	CA	N	S	NS	10	7.50E+03	8.09E+03	1.57E-03
47	6	B	CA	N	S	NS	10	7.78E+03	5.63E+03	-6.72E-03
48	6	C	CA	N	S	NS	10	6.98E+03	4.85E+03	-7.59E-03
49	6	A	WA	WN	WS	WNS	11	3.26E+03	4.96E+03	8.73E-03
50	6	B	WA	WN	WS	WNS	11	5.13E+03	3.19E+03	-9.91E-03
51	6	C	WA	WN	WS	WNS	11	4.87E+03	3.98E+03	-4.23E-03
52	6	A	C	WN	WS	WNS	11	3.22E+03	5.74E+03	1.21E-02
53	6	B	C	WN	WS	WNS	11	7.94E+03	2.93E+03	-2.08E-02
54	6	C	C	WN	WS	WNS	11	3.97E+03	4.27E+03	1.53E-03
55	6	A	A	WN	WS	WNS	11	3.33E+03	3.80E+03	2.73E-03
56	6	B	A	WN	WS	WNS	11	4.99E+03	2.99E+03	-1.07E-02
57	6	C	A	WN	WS	WNS	11	5.13E+03	4.52E+03	-2.64E-03
58	6	A	CA	WN	WS	WNS	11	3.04E+03	7.80E+03	1.96E-02
59	6	B	CA	WN	WS	WNS	11	5.52E+03	3.15E+03	-1.17E-02
60	6	C	CA	WN	WS	WNS	11	4.53E+03	3.78E+03	-3.79E-03
61	6	A	WA	N	WS	WNS	11	3.00E+03	4.40E+03	7.96E-03
62	6	B	WA	N	WS	WNS	11	6.32E+03	3.46E+03	-1.25E-02
63	6	C	WA	N	WS	WNS	11	4.79E+03	4.77E+03	-1.05E-04
64	6	A	C	N	WS	WNS	11	3.06E+03	3.58E+03	3.26E-03
65	6	B	C	N	WS	WNS	11	5.50E+03	3.62E+03	-8.71E-03
66	6	C	C	N	WS	WNS	11	5.21E+03	3.98E+03	-5.63E-03
67	6	A	A	N	WS	WNS	11	3.99E+03	3.82E+03	-9.03E-04
68	6	B	A	N	WS	WNS	11	6.29E+03	4.09E+03	-8.99E-03
69	6	C	A	N	WS	WNS	11	4.92E+03	3.57E+03	-6.68E-03
70	6	A	CA	N	WS	WNS	11	2.98E+03	4.35E+03	7.91E-03
71	6	B	CA	N	WS	WNS	11	5.92E+03	3.56E+03	-1.06E-02
72	6	C	CA	N	WS	WNS	11	5.22E+03	4.55E+03	-2.88E-03

Table A6.5 continued: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* experiments to determine controls of distribution and abundance in Ace Lake.

OBS	DEPTH	REP	ANTIBIOT	NUTS	SALT	NUTSALT	LIGHT	CELL No. 0h	CELL No. 48h	RATE 48_0
73	6	A	WA	WN	S	WNS	11	3.54E+03	5.56E+03	9.40E-03
74	6	B	WA	WN	S	WNS	11	7.07E+03	4.69E+03	-8.54E-03
75	6	C	WA	WN	S	WNS	11	6.34E+03	4.17E+03	-8.75E-03
76	6	A	C	WN	S	WNS	11	4.20E+03	7.64E+03	1.25E-02
77	6	B	C	WN	S	WNS	11	9.56E+03	4.27E+03	-1.68E-02
78	6	C	C	WN	S	WNS	11	6.29E+03	5.78E+03	-1.75E-03
79	6	A	A	WN	S	WNS	11	3.97E+03	7.05E+03	1.20E-02
80	6	B	A	WN	S	WNS	11	.	4.41E+03	.
81	6	C	A	WN	S	WNS	11	5.54E+03	5.52E+03	-8.60E-05
82	6	A	CA	WN	S	WNS	11	3.90E+03	6.44E+03	1.04E-02
83	6	B	CA	WN	S	WNS	11	.	4.07E+03	.
84	6	C	CA	WN	S	WNS	11	6.04E+03	6.10E+03	2.05E-04
85	6	A	WA	N	S	NS	11	4.64E+03	5.37E+03	3.01E-03
86	6	B	WA	N	S	NS	11	8.42E+03	4.26E+03	-1.42E-02
87	6	C	WA	N	S	NS	11	5.51E+03	4.51E+03	-4.17E-03
88	6	A	C	N	S	NS	11	3.88E+03	7.65E+03	1.41E-02
89	6	B	C	N	S	NS	11	7.55E+03	4.46E+03	-1.10E-02
90	6	C	C	N	S	NS	11	6.59E+03	4.81E+03	-6.57E-03
91	6	A	A	N	S	NS	11	3.08E+03	4.39E+03	7.34E-03
92	6	B	A	N	S	NS	11	8.65E+03	5.13E+03	-1.09E-02
93	6	C	A	N	S	NS	11	3.22E+03	4.64E+03	7.61E-03
94	6	A	CA	N	S	NS	11	3.72E+03	6.09E+03	1.03E-02
95	6	B	CA	N	S	NS	11	6.94E+03	6.19E+03	-2.37E-03
96	6	C	CA	N	S	NS	11	6.98E+03	4.85E+03	-7.59E-03
97	10	A	WA	WN	WS	WNS	10	2.66E+03	5.84E+03	1.64E-02
98	10	B	WA	WN	WS	WNS	10	3.51E+03	3.92E+03	2.27E-03
99	10	C	WA	WN	WS	WNS	10	3.45E+03	1.10E+04	2.41E-02
100	10	A	C	WN	WS	WNS	10	3.06E+03	6.15E+03	1.46E-02
101	10	B	C	WN	WS	WNS	10	3.58E+03	3.74E+03	9.13E-04
102	10	C	C	WN	WS	WNS	10	3.49E+03	6.38E+03	1.26E-02
103	10	A	A	WN	WS	WNS	10	3.24E+03	5.72E+03	1.18E-02
104	10	B	A	WN	WS	WNS	10	3.29E+03	7.00E+03	1.57E-02
105	10	C	A	WN	WS	WNS	10	3.32E+03	1.10E+04	2.50E-02
106	10	A	CA	WN	WS	WNS	10	3.29E+03	6.53E+03	1.43E-02
107	10	B	CA	WN	WS	WNS	10	5.05E+03	5.11E+03	2.27E-04
108	10	C	CA	WN	WS	WNS	10	4.05E+03	6.63E+03	1.03E-02
109	10	A	WA	N	WS	WNS	10	3.31E+03	5.50E+03	1.06E-02
110	10	B	WA	N	WS	WNS	10	6.56E+03	4.23E+03	-9.16E-03
111	10	C	WA	N	WS	WNS	10	3.42E+03	1.16E+04	2.55E-02
112	10	A	C	N	WS	WNS	10	4.41E+03	6.95E+03	9.47E-03
113	10	B	C	N	WS	WNS	10	4.75E+03	3.58E+03	-5.91E-03
114	10	C	C	N	WS	WNS	10	3.78E+03	6.89E+03	1.25E-02
115	10	A	A	N	WS	WNS	10	4.54E+03	5.50E+03	3.99E-03
116	10	B	A	N	WS	WNS	10	5.40E+03	4.02E+03	-6.15E-03
117	10	C	A	N	WS	WNS	10	3.53E+03	1.11E+04	2.38E-02
118	10	A	CA	N	WS	WNS	10	3.08E+03	6.08E+03	1.42E-02
119	10	B	CA	N	WS	WNS	10	5.93E+03	4.17E+03	-7.31E-03
120	10	C	CA	N	WS	WNS	10	4.06E+03	9.98E+03	1.87E-02

Table A6.5 continued: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* experiments to determine controls of distribution and abundance in Ace Lake.

OBS	DEPTH	REP	ANTIBIOT	NUTS	SALT	NUTSALT	LIGHT	CELL No. 0h	CELL No. 48h	RATE 48_0
121	10	A	WA	WN	S	WNS	10	4.13E+03	1.31E+04	2.40E-02
122	10	B	WA	WN	S	WNS	10	6.49E+03	6.78E+03	9.36E-04
123	10	C	WA	WN	S	WNS	10	5.62E+03	9.33E+03	1.06E-02
124	10	A	C	WN	S	WNS	10	5.67E+03	7.12E+03	4.75E-03
125	10	B	C	WN	S	WNS	10	6.95E+03	5.17E+03	-6.18E-03
126	10	C	C	WN	S	WNS	10	3.95E+03	9.96E+03	1.93E-02
127	10	A	A	WN	S	WNS	10	3.43E+03	8.78E+03	1.96E-02
128	10	B	A	WN	S	WNS	10	5.92E+03	6.78E+03	2.84E-03
129	10	C	A	WN	S	WNS	10	3.92E+03	1.36E+04	2.59E-02
130	10	A	CA	WN	S	WNS	10	4.94E+03	7.43E+03	8.50E-03
131	10	B	CA	WN	S	WNS	10	7.00E+03	4.60E+03	-8.73E-03
132	10	C	CA	WN	S	WNS	10	5.47E+03	7.80E+03	7.39E-03
133	10	A	WA	N	S	NS	10	5.54E+03	7.78E+03	7.08E-03
134	10	B	WA	N	S	NS	10	7.45E+03	6.90E+03	-1.60E-03
135	10	C	WA	N	S	NS	10	5.43E+03	9.51E+03	1.17E-02
136	10	A	C	N	S	NS	10	4.89E+03	7.51E+03	8.95E-03
137	10	B	C	N	S	NS	10	5.62E+03	5.72E+03	3.67E-04
138	10	C	C	N	S	NS	10	4.34E+03	9.28E+03	1.58E-02
139	10	A	A	N	S	NS	10	5.42E+03	9.78E+03	1.23E-02
140	10	B	A	N	S	NS	10	6.59E+03	5.62E+03	-3.32E-03
141	10	C	A	N	S	NS	10	5.37E+03	1.73E+04	2.43E-02
142	10	A	CA	N	S	NS	10	3.72E+03	6.89E+03	1.28E-02
143	10	B	CA	N	S	NS	10	6.94E+03	4.72E+03	-8.07E-03
144	10	C	CA	N	S	NS	10	5.12E+03	1.05E+04	1.49E-02
145	10	A	WA	WN	WS	WNS	11	4.41E+03	7.74E+03	1.17E-02
146	10	B	WA	WN	WS	WNS	11	5.47E+03	4.80E+03	-2.71E-03
147	10	C	WA	WN	WS	WNS	11	3.26E+03	1.12E+04	2.57E-02
148	10	A	C	WN	WS	WNS	11	4.50E+03	7.67E+03	1.11E-02
149	10	B	C	WN	WS	WNS	11	4.96E+03	9.27E+03	1.30E-02
150	10	C	C	WN	WS	WNS	11	4.18E+03	1.01E+04	1.84E-02
151	10	A	A	WN	WS	WNS	11	3.59E+03	8.58E+03	1.82E-02
152	10	B	A	WN	WS	WNS	11	5.11E+03	7.06E+03	6.76E-03
153	10	C	A	WN	WS	WNS	11	4.13E+03	1.23E+04	2.27E-02
154	10	A	CA	WN	WS	WNS	11	2.94E+03	5.86E+03	1.43E-02
155	10	B	CA	WN	WS	WNS	11	5.05E+03	7.13E+03	7.16E-03
156	10	C	CA	WN	WS	WNS	11	3.48E+03	1.50E+04	3.04E-02
157	10	A	WA	N	WS	WNS	11	4.01E+03	8.55E+03	1.58E-02
158	10	B	WA	N	WS	WNS	11	6.71E+03	6.82E+03	3.37E-04
159	10	C	WA	N	WS	WNS	11	5.19E+03	1.43E+04	2.11E-02
160	10	A	C	N	WS	WNS	11	4.35E+03	6.67E+03	8.88E-03
161	10	B	C	N	WS	WNS	11	5.59E+03	4.97E+03	-2.44E-03
162	10	C	C	N	WS	WNS	11	3.90E+03	2.50E+04	3.87E-02
163	10	A	A	N	WS	WNS	11	2.97E+03	8.24E+03	2.13E-02
164	10	B	A	N	WS	WNS	11	5.25E+03	7.23E+03	6.69E-03
165	10	C	A	N	WS	WNS	11	3.32E+03	1.99E+04	3.73E-02
166	10	A	CA	N	WS	WNS	11	3.39E+03	6.37E+03	1.32E-02
167	10	B	CA	N	WS	WNS	11	6.41E+03	1.07E+04	1.07E-02
168	10	C	CA	N	WS	WNS	11	4.07E+03	1.50E+04	2.71E-02
169	10	A	WA	WN	S	WNS	11	3.63E+03	6.30E+03	1.15E-02
170	10	B	WA	WN	S	WNS	11	6.03E+03	7.59E+03	4.79E-03
171	10	C	WA	WN	S	WNS	11	5.08E+03	1.32E+04	1.99E-02

Table A6.5 continued: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* experiments to determine controls of distribution and abundance in Ace Lake.

OBS	DEPTH	REP	ANTIBIOT	NUTS	SALT	NUTSALT	LIGHT	CELL No. 0h	CELL No. 48h	RATE 48_0
172	10	A	C	WN	S	WNS	11	3.94E+03	1.00E+04	1.95E-02
173	10	B	C	WN	S	WNS	11	6.46E+03	7.80E+03	3.92E-03
174	10	C	C	WN	S	WNS	11	4.27E+03	1.13E+04	2.03E-02
175	10	A	A	WN	S	WNS	11	5.50E+03	1.26E+04	1.73E-02
176	10	B	A	WN	S	WNS	11	5.42E+03	5.66E+03	9.24E-04
177	10	C	A	WN	S	WNS	11	4.90E+03	2.01E+04	2.94E-02
178	10	A	CA	WN	S	WNS	11	4.31E+03	8.30E+03	1.36E-02
179	10	B	CA	WN	S	WNS	11	6.13E+03	1.01E+04	1.05E-02
180	10	C	CA	WN	S	WNS	11	4.67E+03	1.00E+04	1.59E-02
181	10	A	WA	N	S	NS	11	3.36E+03	8.73E+03	1.99E-02
182	10	B	WA	N	S	NS	11	6.45E+03	7.06E+03	1.87E-03
183	10	C	WA	N	S	NS	11	2.82E+03	1.80E+04	3.87E-02
184	10	A	C	N	S	NS	11	4.13E+03	8.80E+03	1.58E-02
185	10	B	C	N	S	NS	11	8.70E+03	7.33E+03	-3.55E-03
186	10	C	C	N	S	NS	11	6.04E+03	1.45E+04	1.82E-02
187	10	A	A	N	S	NS	11	4.95E+03	7.14E+03	7.64E-03
188	10	B	A	N	S	NS	11	6.75E+03	8.49E+03	4.78E-03
189	10	C	A	N	S	NS	11	7.12E+03	1.18E+04	1.06E-02
190	10	A	CA	N	S	NS	11	5.03E+03	1.29E+04	1.97E-02
191	10	B	CA	N	S	NS	11	5.59E+03	1.17E+04	1.53E-02
192	10	C	CA	N	S	NS	11	5.25E+03	1.12E+04	1.58E-02

Table A6.6: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* second experiments to determine controls of distribution and abundance in Ace Lake. OBS = Observation number; Depth = depth at which treatment was suspended (t1 = 6 m, t2 = 8 m and t3 = 10 m); ANTIBIOT = antibiotic treatment (NA = no antibiotics, C = cycloheximide, A = ampicillin, CA = cycloheximide and ampicillin); Cell numbers (cells ml^{-1}); Rate 48_0 = growth rate (h^{-1}).

OBS	DEPTH	ANTIBIOT	REP	LIGHT	CELL No. 0h	CELL No. 48h	RATE48_0
1	t1	NA	1	l1	1.00E+04	5.10E+03	-0.00609
2	t1	NA	2	l1	8.45E+03	6.27E+03	-0.0027
3	t1	NA	3	l1	9.54E+03	8.71E+03	-0.00082
4	t1	C	1	l1	6.25E+03	6.81E+03	0.000776
5	t1	C	2	l1	7.57E+03	5.99E+03	-0.00212
6	t1	C	3	l1	9.28E+03	7.86E+03	-0.0015
7	t1	A	1	l1	1.18E+04	6.91E+03	-0.00484
8	t1	A	2	l1	9.50E+03	5.18E+03	-0.00549
9	t1	A	3	l1	9.61E+03	6.10E+03	-0.00411
10	t1	CA	1	l1	8.18E+03	6.51E+03	-0.00207
11	t1	CA	2	l1	7.31E+03	5.96E+03	-0.00185
12	t1	CA	3	l1	3.43E+03	6.06E+03	0.00515
13	t1	NA	1	l3	.	.	
14	t1	NA	2	l3	.	.	
15	t1	NA	3	l3	5.12E+03	5.75E+03	0.00105
16	t1	C	1	l3	5.64E+03	5.56E+03	-0.00013
17	t1	C	2	l3	7.16E+03	3.99E+03	-0.00529
18	t1	C	3	l3	6.58E+03	5.63E+03	-0.00141

Table A6.6: Treatments, cell numbers at t =0 h and t = 48 h and growth rate (h^{-1}) of *Synechococcus* in *in-situ* second experiments to determine controls of distribution and abundance in Ace Lake.

OBS	DEPTH	ANTIBIOT	REP	LIGHT	CELL No. 0h	CELL No. 48h	RATE48_0
19	t1	A	1	l3	7.32E+03	7.10E+03	-0.00028
20	t1	A	2	l3	5.30E+03	.	
21	t1	A	3	l3	4.95E+03	5.95E+03	0.001665
22	t1	CA	1	l3	6.69E+03	8.97E+03	0.002653
23	t1	CA	2	l3	5.87E+03	.	
24	t1	CA	3	l3	7.50E+03	8.60E+03	0.001238
25	t2	NA	1	l2	4.41E+05	5.10E+05	0.001315
26	t2	NA	2	l2	2.62E+05	4.30E+05	0.004483
27	t2	NA	3	l2	3.36E+05	5.73E+05	0.004829
28	t2	C	1	l2	3.38E+05	3.29E+05	-0.00024
29	t2	C	2	l2	3.94E+05	3.44E+05	-0.00123
30	t2	C	3	l2	2.90E+05	4.15E+05	0.003243
31	t2	A	1	l2	4.37E+05	4.25E+05	-0.00025
32	t2	A	2	l2	3.41E+05	4.59E+05	0.002689
33	t2	A	3	l2	2.74E+05	3.49E+05	0.002189
34	t2	CA	1	l2	3.43E+05	3.24E+05	-0.00052
35	t2	CA	2	l2	3.37E+05	3.40E+05	8.02E-05
36	t2	CA	3	l2	3.56E+05	3.27E+05	-0.00077
37	t2	NA	1	l3	5.35E+05	1.19E+06	0.007233
38	t2	NA	2	l3	5.10E+05	8.91E+05	0.005048
39	t2	NA	3	l3	2.19E+05	8.89E+05	0.012676
40	t2	C	1	l3	4.97E+05	4.73E+05	-0.00045
41	t2	C	2	l3	4.38E+05	5.71E+05	0.002399
42	t2	C	3	l3	2.29E+05	5.50E+05	0.007928
43	t2	A	1	l3	4.09E+05	1.02E+06	0.008268
44	t2	A	2	l3	4.96E+05	3.78E+05	-0.00246
45	t2	A	3	l3	4.37E+05	7.39E+05	0.004753
46	t2	CA	1	l3	4.36E+05	6.25E+05	0.003258
47	t2	CA	2	l3	2.41E+05	6.12E+05	0.008432
48	t2	CA	3	l3	3.34E+05	5.26E+05	0.004109
49	t3	NA	1	l3	7.73E+05	1.66E+06	0.006915
50	t3	NA	2	l3	7.57E+05	.	
51	t3	NA	3	l3	6.87E+05	1.65E+06	0.007928
52	t3	C	1	l3	7.70E+05	1.27E+06	0.004527
53	t3	C	2	l3	7.64E+05	9.79E+05	0.002244
54	t3	C	3	l3	5.95E+05	1.29E+06	0.007002
55	t3	A	1	l3	6.12E+05	1.68E+06	0.009137
56	t3	A	2	l3	8.14E+05	2.19E+06	0.008955
57	t3	A	3	l3	8.23E+05	1.99E+06	0.007989
58	t3	CA	1	l3	8.16E+05	1.63E+06	0.00626
59	t3	CA	2	l3	7.54E+05	8.24E+05	0.000803
60	t3	CA	3	l3	6.80E+05	1.15E+06	0.004754

Table A6.7: Physcal characteristics of Ace Lake between the 13th December 1993 and the 22nd Cecember 1993.

Depth (m)	Salinity (g kg^{-1})	Temp (°C)	Depth (m)	Salinity (g kg^{-1})	Temp (°C)	Depth (m)	Salinity (g kg^{-1})	Temp (°C)	Depth (m)	Salinity (g kg^{-1})	Temp (°C)
1.5	11.22	-0.47				**1.5**					
2	17.85	1.19	**2**	10.91	-0.48	**2**	10.99	-0.46	**2**		
2.5	17.93	2.05	**2.5**	17.85	1.91	**2.5**	17.94	2.41	**2.5**	18.04	2.55
3	18.07	2.43	**3**	18.09	2.56	**3**	18.06	2.67	**3**	17.96	3.09
3.5	18.07	2.48	**3.5**	18.12	2.59	**3.5**	18.06	2.79	**3.5**	18.04	3.15
4	18.06	2.49	**4**	18.13	2.59	**4**	18.07	2.80	**4**	18.08	3.14
4.5	18.08	2.47	**4.5**	18.14	2.59	**4.5**	18.09	2.81	**4.5**	18.08	3.14
5	18.08	2.47	**5**	18.14	2.60	**5**	18.09	2.81	**5**	18.10	3.1
5.5	18.08	2.45	**5.5**	18.17	2.59	**5.5**	18.09	2.81	**5.5**	18.09	3.05
6	18.08	2.43	**6**	18.17	2.58	**6**	18.12	2.82	**6**	18.11	3.06
6.5	18.08	2.42	**6.5**	18.15	2.58	**6.5**	18.11	2.83	**6.5**	18.11	3.05
7	18.10	2.42	**7**	18.20	2.59	**7**	18.11	2.83	**7**	18.11	3.05
7.5	21.41	3.85	**7.5**	20.96	3.87	**7.5**	21.00	4.13	**7.5**	21.18	4.38
9	28.40	5.94	**9**	28.36	5.98	**9**	28.23	6.09	**9**	28.24	6.34
8.5	29.03	6.30	**8.5**	29.32	6.45	**8.5**	29.20	6.51	**8.5**	29.17	6.76
9	29.45	6.50	**9**	29.52	6.54	**9**	29.44	6.63	**9**	29.38	6.81
9.5	29.66	6.61	**9.5**	29.75	6.65	**9.5**	29.69	6.82	**9.5**	29.68	6.98
10	29.83	6.78	**10**	29.91	6.84	**10**	29.85	6.92	**10**	29.82	7.08
10.5	30.05	6.95	**10.5**	30.11	6.99	**10.5**	30.09	7.08	**10.5**	30.06	7.21
11	30.37	7.22	**11**	30.52	7.26	**11**	30.37	7.28	**11**	30.37	7.42
11.50	30.67	7.50	**11.50**	30.79	7.46	**11.50**	30.72	7.58	**11.50**	30.73	7.69
12	30.98	7.14	**12**	31.04	7.20	**12**	31.00	7.19	**12**	31.00	7.23
12.5	31.20	6.76	**12.5**	31.23	6.88	**12.5**	31.17	6.89	**12.5**	31.18	6.89
13	31.29	6.60	**13**	31.32	6.65	**13**	31.28	6.66	**13**	31.26	6.67

Table A6.8: Light intensities (PAR - $\mu m\ m^{-2}\ s^{-1}$) measured in Ace Lake between the 11th and 19th December 1993

Depth	11-Dec-93	12-Dec-93	12-Dec-93	13-Dec-93	13-Dec-93	14-Dec-93	15-Dec-93	15-Dec-93	16-Dec-93	16-Dec-93	17-Dec-93	18-Dec-93	18-Dec-93	19-Dec-93	19-Dec-93
	20:00	08:00	20:00	08:00	14:30	20:00	08:00	20:00	08:00	14:00	20:00	08:00	20:00	08:00	13:40
2	15.08	11.16	14.86	15.06	33.53	6.33	7.31	5.76	7.21	19.94	15.60	8.76	9.70	8.37	26.42
2.5	14.38	9.72	14.26	14.08	32.75	5.44	6.61	5.36	6.43	18.61	15.66	8.05	9.10	7.61	24.54
3	13.33	8.71	13.41	13.03	30.44	4.94	6.06	5.00	5.70	16.97	14.50	7.19	8.51	6.99	22.95
3.5	12.35	7.97	12.49	11.69	28.07	4.50	5.50	4.56	5.20	15.44	13.41	6.41	7.89	6.41	20.68
4	11.35	7.09	11.49	10.72	25.32	4.06	4.96	4.14	4.70	13.82	12.21	5.76	7.21	5.82	18.98
4.5	10.48	6.53	10.52	9.74	23.41	3.67	4.52	3.73	4.30	12.65	11.21	5.20	6.53	5.20	16.19
5	9.48	5.88	9.50	8.96	21.19	3.39	4.12	3.37	3.90	11.73	10.28	4.68	6.00	4.80	14.97
6	8.09	5.20	8.09	7.53	17.85	2.93	3.61	2.85	3.29	9.80	8.57	3.88	5.56	4.38	12.73
7	6.65	4.32	6.81	6.25	14.70	2.41	3.07	2.33	2.77	8.17	7.23	3.25	4.72	4.26	10.68
8	4.88	3.33	5.04	4.54	10.68	1.87	2.33	1.73	2.11	5.98	5.40	2.35	3.90	3.59	7.89
9	3.39	2.55	3.82	3.45	7.83	1.35	1.77	1.29	1.59	4.56	4.10	1.77	2.87	2.67	6.10
10	2.31	1.93	2.77	2.35	5.56	1.02	1.27	0.94	1.20	3.41	2.93	1.25	2.17	2.01	4.21
11	1.10	1.10	1.41	0.98	1.27	0.38	0.74	0.40	0.66	1.87	1.47	0.58	1.55	1.45	2.05
12	-0.08	0.24	0.16	-0.04	-0.30	0.06	0.30	-0.04	0.10	0.26	0.28	-0.12	0.84	0.88	0.16