# PLANKTON DYNAMICS IN THE SALINE LAKES OF THE VESTFOLD HILLS, EASTERN ANTARCTICA

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Thesis submitted to the University of Nottingham for the Degree of Doctor of Philosophy

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#### **DECLARATION**

I hereby declare that the work presented in this thesis is my own and has not been submitted for any other degree. All sources of information have been acknowledged by reference to the authors.

mor bell.

Elanor Margaret Bell

"It seemed we had suddenly come into an oasis, for instead of the usual mass of snow with occasional patches of rock there were large valleys of exposed rock, with scarcely any snow to be seen."

Stephenson (1938) a member of the British Graham Land Expedition of 1934-1937.

"Those who have spent an Antarctic night bear an indelible mark on their innermost self, a feeling which a person finds hard to define and explain to another."

Len Sales (1964) New Zealand Antarctic Society Bulletin, 9, pp. 414.

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# ABSTRACT

The paradigm of aquatic food webs has undergone fundamental revision over the past twenty years. Research suggests that a significant proportion of organic material and energy in aquatic ecosystems flows through a "microbial loop" before passage into the classic aquatic food web. Unique Antarctic conditions mean such "bottom-up" control can be of extreme importance during the austral winter. In order to investigate this, the microbial communities and physico-chemical properties of three saline lakes in the Vestfold Hills, eastern Antarctica, Ace, Highway and Rookery, were investigated between December 1995 and February 1997.

The lakes of the Vestfold Hills were formed approximately 8000 years ago following a period of post-glacial marine transgression, which isolated seawater in glacially scoured basins. Post-formation, the chemistry and biology of this isolated seawater underwent significant changes creating a suite of lakes and ponds with highly varied chemical and biological compositions. This study was concerned with meromictic Ace Lake in particular. Physico-chemical results demonstrated that the lake was highly stratified. An upper, nutrient-poor *mixolimnion* was separated by a *thermocline/halocline* from a lower, nutrient-replete *monimolimnion*. The monimolimnion was further stratified in terms of oxygen; an *oxycline* separated an oxic upper stratum from an anoxic lower stratum. At the oxycline, organic substrate sedimenting from above and inorganic nutrients diffusing from below were entrained. This stable stratification exerted a profound influence on the microbial dynamics of Ace Lake.

Over the course of its evolution the microbial food web of Ace Lake was truncated and a simple, low diversity community of bacteria, algae and Protozoa, with a paucity of metazoan zooplankton, now dominates. This evolutionary process was illustrated by the intermediate diversity of the protozoan community in recently formed, Rookery Lake. The most dominant micro-organisms in Ace Lake were those which were highly motile and employed versatile nutritional strategies, such as mixotrophy, to remain physiologically active during the austral winter.

Ace Lake is an oligotrophic system which receives negligible allochthonous inputs of organic carbon and inorganic nutrients. However, a simple model of the carbon flux within Ace Lake highlighted the fact that autotrophic production within the plankton was insufficient alone to maintain the level of heterotrophic activity observed. Instead, the microbial plankton were dependent on regenerative fluxes of inorganic nutrients and slow-turnover autochthonous carbon, consistent with the concept of the microbial loop.

# Chapter 1: INTRODUCTION

#### 1.1: THE PROTOZOA

The Protozoa are classified as a sub-kingdom within the Kingdom Protista (Levine *et al.*, 1980). They are generally considered to be eukaryotic, single celled organisms although there are examples of multicellularity amongst the larger protozoan species (Fenchel, 1987). Protozoa occur in the planktonic environments of lakes and seas from pole to pole, and in some extreme environments they are the sole 'zooplankters'. Within aquatic environments there are thousands of species (Austin, 1988) and although protozoan diversity is reduced in Antarctica, there have still been reports of a surprisingly large number of species from continental and maritime freshwaters (Vincent, 1988). Artificial substrata incubated in Lake Fryxell, McMurdo Dry Valleys, Antarctica, yielded 35 species of Protozoa from the water column and 55 species from the littoral zone (Cathey *et al.*, 1981), and 75 species were recorded in maritime Antarctic lakes on Signy Island (Hawthorn & Ellis-Evans, 1984).

#### 1.1.1: The flagellated Protozoa

The sub-Kingdom Protozoa is divided into the phyla Sarcomastigophora and Ciliophora, or more simply, the flagellated and ciliated Protozoa. The flagellates are an abundant and widely distributed group in aquatic environments. Taxonomically the flagellates belong in the sub-phylum Mastigophora, which is divided into two classes; the Phytomastigophora (phytoflagellates) and the Zoomastigophora (zooflagellates) (Levine et al., 1980). Despite what the class names suggest, as a rule flagellates are nutritionally versatile and both classes contain heterotrophic, mixotrophic and autotrophic species (Laybourn-Parry, 1992). The group is extremely heterogeneous, morphologically and physiologically. It is characterised by the possession of one or more flagella which function as locomotory or feeding organelles (Fenchel, 1987). Flagellates reproduce asexually by symmetrigenic binary fission and, in some orders, sexually (Laybourn-Parry, 1992). Among the most easily recognised of the flagellates are the colourless choanoflagellates with their distinctive silica loricae and the thecae covered dinoflagellates. In this study, the heterotrophic nanoflagellates (HNAN) included all colourless flagellates between 2 and 20 µm in diameter, and the autotrophic or phototrophic nanoflagellates (PNAN) included flagellates in the same size range which possessed chlorophyll (Figure 1.1).





Figure 1.1: Examples of planktonic Protozoa in the lakes of Vestfold Hills. a, b & c are flagellated Protozoa and d & e are ciliated Protozoa. (a) Cryptomonas sp., (b) Pyramimonas sp., (c) Acanthoecopsis unguiculata (d) Euplotes sp. and (e) Mesodinium rubrum. Not drawn to scale

#### 1.1.2: The ciliated Protozoa

The ciliated Protozoa, or ciliates as they are more commonly termed, belong to a large, homogeneous group encompassing over 7000 species. The group is characterised by its complex ciliated cortex where cilia, cirri and membranelles are arranged over the cell. The cilia function in locomotion and feeding (Laybourn-Parry, 1992) (Figure 1.1). Ciliates reproduce both asexually by means of transverse binary fission and sexually by conjugation. They typically have a resting cyst phase in their life cycle and this encystment plays a critical role in the annual survival of ciliates, in particular in environments where temperature and food supplies are seasonally unavailable. In most instances, cysts reach the sediments from which they are resuspended by tides, currents and wind-induced mixing. The large dormant, encysted population can rapidly excyst when favourable conditions return and recolonise the plankton. Excystment is thought to be triggered by internal biological clocks in combination with the return of favourable environmental conditions (Rengefors & Anderson, 1998). Ciliates are commonly heterotrophic, but on occasion mixotrophic or autotrophic (e.g. Smith & Barber, 1979; Lindholm, 1985; Stoecker *et al.*, 1987a).

# 1.2: THE IMPORTANCE OF PROTOZOA IN CARBON CYCLING: THE "MICROBIAL LOOP"

In the last decade there has been a significant expansion of the planktonic protozoan community database. Generally studies have concentrated on the ciliate component of aquatic ecosystems and there are instances when these play the major role in the flow of energy and cycling of carbon, phosphorus and nitrogen (Sherr & Sherr, 1987). However, where the whole community has come under scrutiny it has become increasingly apparent that heterotrophic nanoflagellates play a greater role (Landry *et al.*, 1984; Sanders *et al.*, 1989; Weisse *et al.*, 1990; Laybourn-Parry *et al.*, 1992b).

#### 1.2.1: The traditional paradigm

Conventional descriptions of trophic level systems have always been based upon a linear, vertical, hierarchical food chain in which the major pathways flow from primary producers, dependent on a pool of nutrients, to zooplankton (chiefly metazoan herbivores), to fish, with some excreted products recycled simultaneously (Stone & Weisburd, 1992) (Figure 1.2). These traditional notions tended to portray positive feedbacks as undesirable features in that they delayed the time needed for a system to recover from perturbation and resulted in explosive, unstable population growth.



Figure 1.2: The major trophic pathways in the classical vertical food chain structure of aquatic ecosystems. (Taken from Stone & Weisburd, 1992, pp. 265)



Figure 1.3: The recycling introduced at the base of the food web by the 'microbial loop'. Note that the pathway from phytoplankton via bacteria indicates that a large proportion of autotrophic production becomes available for bacterial uptake via indirect pathways such as algal exudates and 'sloppy feeding'. (Taken from Stone & Weisburd, 1992, pp. 265)

Evolutionary arguments, therefore, deemed it unlikely that organisms would develop strategies that depend upon such unstable processes. Instead, two main hypotheses were proposed as control mechanisms for linear trophic models. Firstly, 'bottom up' control, whereby competition for nutrients between phytoplankton species determined the higher trophic levels, and secondly, 'top down' control which argued that predation effects cascade down the linear chain and are responsible for ecosystem control (e.g. Pace & Cole, 1994; Pedrós-Alió, 1994).

#### 1.2.2: A new paradigm

Pomeroy's (1974) seminal paper revolutionised concepts regarding aquatic microbial food webs. Before 1974, bacteria and Protozoa were not included as significant components of food web models (Steele, 1974), thus, ecological activity, and therefore functional importance, was assumed to be in direct proportion to the size of an organism, concentrating at the upper end of the size spectrum (Stone & Weisburd, 1992). This meant that bacteria were considered to be involved in the decomposition of faecal material and nutrient mineralisation only, the majority of primary production utilised instead by herbivores and then passed upwards via the organisms comprising the "grazing food chain". However, with the development of new techniques post-1974 perceptions began to alter (Hobbie et al., 1977; Porter & Feig, 1980). Whilst the classical picture of planktonic food chains was still viewed as essentially correct, the fact that bacteria and protists were found typically to comprise approximately half of the total biomass suggested that this model was incomplete (Fenchel, 1982). Moreover, the recognition that rates of potential growth and weight specific metabolism are more or less inversely proportional to length at the lower end of the size spectrum, led observers to realise that microbial organisms are responsible for by far the largest part of the energy flow in aquatic systems (Fenchel, 1988). Initially, it was demonstrated that bacterial abundance had been underestimated (Hobbie et al., 1977). Cells smaller than 20 um maximum linear dimension were shown to account for as much as 90 % of the total phytoplankton carbon (Garrison & Gowing, 1993) and ultra-microbacteria (< 0.2 µm), up to 28 % of bacterial production (Velimirov, 1994). Subsequently, it was determined that bacterial productivity rates were so rapid as to require one third to one half of the total primary productivity in aquatic ecosystems to support them. Indeed, Stone and Weisburd (1992) determined that bacteria commonly consume 20 to 60 % of total primary production and have a carbon biomass equivalent to between 10 and 40 %of total phytoplankton standing stock. Together these new findings indicated that bacteria are in fact a "dynamic, metabolic and trophic component representing a major pathway for matter and energy flux in the food web" (Azam et al., 1983). Consequently, during the 1980's there was a major shift in the understanding of aquatic

food webs and a new paradigm developed around the concept of a "microbial loop", a pathway via which energy released as dissolved organic material (DOM) from phytoplankton is returned to the main food web by a loop of bacteria  $\rightarrow$  flagellates  $\rightarrow$  micro-zooplankton (Figure 1.3). This concept has since been incorporated into the classic planktonic food chain (Figure 1.3). The new paradigm of plankton dynamics included the recognition that firstly, phototrophic and heterotrophic micro-organisms play a substantial, sometimes dominant, role in the cycling of matter in aquatic ecosystems. Secondly, that a large fraction of total primary production is not consumed directly by herbivorous consumers but is channelled through a pool of organic matter before it becomes available, via bacterial production, to phagotrophic organisms (Fenchel, 1988). Hence, the microbial loop increases the length of aquatic food chains (Painting *et al.*, 1992) and produces multiple links to further the flow of carbon, energy and nutrients through the classic aquatic food web (Boraas *et al.*, 1992).

# 1.2.3: The importance of the "Microbial Loop" in Antarctic aquatic ecosystems

It has been suggested by Azam et al. (1991) that the microbial loop in Antarctic aquatic ecosystems performs some roles which are fundamentally different from those in oligotrophic temperate and tropical waters due to the Antarctic's unique conditions. Their suggestions commence with the theory that during winter bacterial production, at the expense of slow-turnover dissolved organic matter (DOM) from the previous summer phytoplankton blooms, could be a significant factor in the survival of overwintering populations. DOM is produced in various ways. Phytoplankton have been shown to exude up to 70 % of their fixed carbon into their surroundings (Vollenweider, 1974; Coveney, 1982; May, 1982; Wood & Van Valen, 1990; Sharp & Priscu, 1991; Wetzel & Likens, 1991; Sell & Overbeck, 1992; Tranvik, 1992; del Giorgio & Peters, 1993b; Leboulanger et al., 1998). Early models such as Steele's (1974) suggested that herbivores consumed phytoplankton without the loss of cell contents, despite Hutchinson (1961) proposing the "paradox of the plankton" whereby phytoplankton encourage bacterial colonisation by releasing extracellular carbon, colonisation by the very organisms with which they compete for limited resources. Evidence now confirms that loss of cell contents is significant, especially when fragile phytoflagellates burst during handling or "sloppy feeding" (Eppley et al., 1981; Azam & Cho, 1987). Alternatively, physico-chemical stresses and viral infections are known to cause lysis in microbial populations, producing a microzone of DOM to which bacteria are attracted (Proctor & Fuhrman, 1990; Thingstad et al., 1993; Bratbak et al., 1994; Kirchman, 1994; Suttle, 1994; Weinbauer & Peduzzi, 1995; Gobler et al., 1997). High viral to

bacterial abundance ratios have been recorded in most aquatic ecosystems. On average between 10 and 20 % of the bacterial population is lysed daily, but the percentage may be as high as 40 %, and approximately 20 % of heterotrophic bacteria are virally infected (Bratbak et al., 1994; Suttle, 1994). Bacteria have a greater risk of viral infection in dense, actively growing bacterial populations. Virus enrichment experiments suggest that bacterial productivity would be 2 % higher in the absence of viruses and overall, 2 to 3 % of productivity is lost to viruses (Suttle, 1994). Viral lysis is also important for the turnover of nitrogen (N) and phosphorus (P) in the system. Lysing cells release proteins, nucleic acids, and other organic N and P compounds, including decaying virus particles which themselves make nutrients available to bacterial and phytoplankton communities (Bratbak et al., 1994). In their investigation of nanoflagellate grazing on viruses, González and Suttle (1993) concluded that viruses may represent 0.2 to 9 % of the carbon, 0.3 to 14 % of the nitrogen and 0.6 to 28 % of the phosphorus that flagellates obtain from the ingestion of bacteria. Viruses may, therefore, be of nutritional importance to phagotrophic flagellates. Moreover, healthy phytoplankton cells have been observed to exude DOM as a viral defence mechanism, supporting bacterial biomass (Murray, 1995). Berman and Stone (1994) have gone as far as suggesting that a viral "mini-loop" consisting of, bacteria  $\rightarrow$  virus  $\rightarrow$  DOM  $\rightarrow$ bacteria, could exist which potentially increases resource availability in the microbial loop and offers another explanation for Hutchinson's "paradox of the plankton", viruses increasing the benefits that plankton receive from attracting bacterial 'competitors' (Hutchinson, 1961; Bratbak et al., 1994).

Studies have also shown that phosphate, ammonia and organic faecal pellets are major components of micro- and macro- zooplanktonic excreta (Hand & Burton, 1981; Tanimura *et al.*, 1984). Particulate organic matter (POM) can be hydrolysed by bacteria (Chróst *et al.*, 1989; Turley, 1994). The hydrolysates can be taken up by the bacteria or diffuse into the environment as DOM. Bacteria colonising faecal pellets of *Calanus pacificus* labelled with radioactive <sup>14</sup>C released two to three times as much carbon as they assimilated (Azam & Cho, 1987). The implicit assumption throughout is that the DOM pool exhibits slow turnover. There is evidence to support this assumption and suggest that longevity is increased when utilisable substrates bind with humic or fulvic material, constituting a 'nutritional buffer' (Azam *et al.*, 1991). Alternatively long lived polymers, for example complex carbohydrates, may be directly employed by organisms. Indeed, Marchant (1990) proposed that choanoflagellates can directly utilise such higher molecular weight polysaccharides in the pelagic system.

The implications of such bacteria-organic matter coupling are far reaching. Firstly, since all DOM originates from some particulate source it will be found in discrete loci with concentration gradients. Behaviour for sensing these gradients is, therefore, essential to the bacteria. Secondly, due to the fact that "sloppy feeding" and excretion are episodic, and exudation, autolysis and hydrolysis sustain DOM inputs, the bacteria must have strategies for the uptake of both long and short lived nutrient supplies (Eppley et al., 1981). Finally, it has been shown that only 5 % of the DOM produced is utilisable by the bacteria and of that utilisable portion only a tiny fraction can be used without first hydrolysing the constituent proteins, polysaccharides and nucleic acids into monomers, inferring both tight coupling between DOM inputs and utilisation, as well as specialised modes of uptake. It is logical, therefore, that the optimum bacterial feeding strategy would be to perform digestion at the cell surface, or in the periplasmic space of algal agglutinates. Hence, it follows that bacterial repellents may be secreted by algae, explaining why healthy algae are generally free of attached bacteria whereas dead algae are heavily colonised. This would constitute a "metabolic feedback" between the algae and the bacteria, but bacterial nutrient recycling must then be coupled to herbivore grazing and the conventional food web (Azam & Cho, 1987 and references therein).

In systems where metazoan micro-zooplankton components exist, their bacterivory increases the efficiency of the microbial loop. If bacterivorous Metazoa are directly consumed by upper-level consumers, the flagellated protozoan component of the food web is bypassed or short-circuited (Simek & Straskrabová, 1992). The path of bacterial organic matter through the microbial loop can then be shortened before Lindeman's law<sup>1</sup> reduces trophic efficiency to trivial levels (Turner & Tester, 1992). Such short-circuiting constitutes the second fundamental difference unique to Antarctic oligotrophic waters because cyanobacteria, which dominate total primary productivity in lower latitude oligotrophic waters, are not considered to be digestible by metazoan herbivores (Azam *et al.* 1991).

But what is it that makes this microbial loop so important to the dynamics and structure of aquatic ecosystems? Hutchinson's (1961) "paradox of the plankton" questions the potentially detrimental effects for plankton that attract bacteria by releasing extracellular organic carbon. Stone's (1990) model predicted that this seemingly detrimental behaviour was in fact highly stabilising and provided the phytoplankton with indirect

<sup>&</sup>lt;sup>1</sup>Lindeman linked trophic levels in series to form a simple trophic chain, a model which equates the energy not respired by the populations aggregated to form a trophic level to the productivity of the populations assigned to the next level (Burns, 1989).

advantages. Indeed, the paradox can be resolved by bringing bacterivorous Protozoa into the equation. Protozoa are rapid grazers and remineralisers and can alleviate the competitive effect of bacteria on the phytoplankton. Thus, indirectly, Protozoa exert a positive effect on the phytoplankton and contrary to popular belief, this positive feedback is an integral part of the system's stability (Stone, 1990). Similarly, if the phytoplankton themselves are capable of bacterivory (i.e. are mixotrophic) then they can benefit from a commensal relationship by providing "cheap" dissolved organic carbon (DOC) for the bacteria whilst grazing "expensive" bacterial nutrients, such as mineralised phosphorus and nitrogen, which may be growth limiting for the phytoplankton. The term "bootstrapping" was employed to describe this bacterial-algal coupling, the basis for the microbial loop (Hutchinson, 1961; Azam & Cho, 1987; Stone & Weisburd, 1992; Thingstad et al., 1996). Moreover, by virtue of their small size and large surface area to volume ratio the bacteria can absorb low concentrations of nutrients that might otherwise be unavailable to the phytoplankton and this enhanced nutrient cycling strengthens positive feedback links in aquatic environments (Fenchel, 1982; Stone & Weisburd, 1992). Hence, the bacteria facilitate rapid recycling and remineralisation by holding nutrients within the system and making them available for repeated use by the phytoplankton. Marine bacteria have been observed to employ both biochemical and behavioural strategies for competing with other organisms for the utility of dissolved and particulate organic matter, and these are the basis for such "bootstrapping". However, due to the scale differences, bacteria may have problems keeping up with the sinking velocity of algal cells and their major importance might, therefore, be restricted to vertically stratified waters where phytoplankton are (temporarily) entrained i.e. at a thermocline, a situation common in Antarctic, icecovered lakes. Nevertheless, this slow sinking velocity also means that bacteria and nutrients remain in the photic zone for longer than those incorporated directly into the food chain, since bacteria associated with particulate matter sink out of the mixed layer faster than those free living bacteria comprising the microbial loop, reducing the time available for utilisation of nutrients and their subsequent passage through the conventional planktonic food web (Azam et al., 1983). Moreover, an attached bacterial population is not as readily accessible to grazing flagellates because individual cells are often too firmly attached (Laybourn-Parry & Marchant, 1992b).

Spatial and temporal heterogeneity on different scales is an important aspect of community ecology. Urban *et al.* (1992) observed a temporal succession from a diatom based food chain in the winter and spring to one based on the microbial loop in the summer and autumn in stratified systems. Indeed, Cho and Azam (1988) concluded that the distribution patterns of growth and dynamics of free bacteria could strongly influence the spatial and temporal patterns of biogeochemical cycling of materials in the

oceans interior. Certainly, past studies of such species organisation have shown a dynamic structuring with depth (Burch, 1988). But vertical stratification need not exist. In the oligotrophic environment of tropical coral reefs it is thought that the communities are only able to sustain such a high standing biomass and gross primary productivity by congruous positive feedback systems (Stone & Weisburd, 1992).

The new paradigm is not without its critics. Taylor & Joint (1990) developed a model which implied that the microbial loop does not exert a large influence on the recycling of nitrogen because, although they found rates of carbon assimilation and respiration to be high within the loop itself, only a small fraction of the carbon was transferred to higher trophic levels, contradicting Azam's theory (1983). Similarly, they cited findings claiming that carbon assimilated by bacteria could not provide a significant food source for larger organisms. Such studies claimed that the basic premise of the microbial loop should be re-examined. Despite these few critics modelling attempts and field experiments are generally consistent with the fact that the microbial loop is an important, stabilising and integral part of many aquatic ecosystems. It may well be that we still have not yet realised the full importance of such microbial feedbacks especially in terms of current and future threats to environmental stability. These threats will require a full understanding of the regulatory mechanisms of the microbial loop since many aquatic environments are now subject to natural and anthropogenic addition of nutrients and/or organic carbon which threaten to uncouple the phytoplankton from the microbial loop (Hendl, 1992), and perhaps of more urgency are the implications the microbial loop has for the sequestration of carbon pertinent to relieving the current threat of global warming (Legendre & Lefevre, 1992).

#### **1.3: ANTARCTIC LACUSTRINE ECOLOGY**

#### 1.3.1: The Antarctic environment

The Antarctic continent experiences the lowest temperatures (annual mean -20 °C coupled with a mean wind speed of 5.0 ms<sup>-1</sup>), the lowest precipitation and the lowest relative humidity levels (annual mean relative humidity < 50 %) on the planet, rendering the continent the driest on Earth (Simmons *et al.*, 1993). Approximately 98 % of the Antarctic continent is covered by ice, but despite this ice-free areas do exist, primarily on the continental margin (C.I.A., 1978; Pickard, 1986; Simmons *et al.*, 1993) (Figure 1.4). These areas have been termed "oases" since, like oases in hot deserts, life is concentrated in them (Pickard, 1986). An Antarctic oasis is defined as "a substantial ice-free area separated from the ice sheet by a distinct ablation zone, and which is kept



Figure 1.4: Map of Antarctica illustrating the major ice-free areas and lake research locations (Taken from Ellis-Evans, 1996, pp. 1396)

free from snow by ablation due to low albedo and positive radiation balance" (Shumskiy, 1957). Such ice-free areas vary considerably in size and character. The McMurdo Dry Valleys of southern Victoria Land have the largest area ( $4000 \text{ km}^2$ ) and the highest in elevation (> 2000 m) (Pickard, 1986). Many ideas have been put forward to explain the existence of oases, ranging from orographic, through climate change, to proglacial ice formation. The oases vary in age; some may have been ice free for as long as 4 to 5 million years, others appear to be very young. Lakes in the Taylor Valley (McMurdo Dry Valleys) are the remnants of Glacial Lake Washburn, which existed 10 000 to 24 000 years ago (Simmons *et al.*, 1993 and references therein) by comparison, the lakes of the Vestfold Hills are young at approximately 8000 years old (Pickard, 1986; Peterson *et al.*, 1988). The climate in the ice-free, coastal areas is largely determined by the interaction of dry polar and moist oceanic winds and climatic conditions are generally less arid than those prevailing inland (Heywood, 1977 and references therein).

#### 1.3.2: Antarctic lacustrine environments

Despite its arid nature, the Antarctic continent harbours 70 to 90 % of the world's fresh water (Heywood, 1984; Pickard, 1986). Most is held in the continental ice sheet in a solid state, but some is present in its liquid state in lakes. Although lakes do exist below the continental ice sheet (Heywood, 1977), most are confined to the ice-free oases and to coastal and offshore islands. As illustrated above, the Antarctic environment is very inhospitable for colonisation by micro-organisms. Nevertheless, the biota in lacustrine environments are buffered from the low temperatures, low humidity, sand abrasion and freeze-thaw cycles and for this reason most of the Antarctic non-marine biomass is concentrated in the lakes (Wright & Burton, 1981; Simmons et al., 1993). However, because of the continent's harsh environmental conditions, many life forms and interactions normally associated with temperate lakes and streams do not exist in Antarctic aquatic environments. Riparian vegetation does not exist; higher assemblages of annelids, molluscs, arthropods, fish, and vascular plants do not exist; and, for the most part, habitats are free of anthropogenic perturbations (Simmons et al., 1993). Despite the fact the charismatic megafauna of the continent, penguins and seals, are the most readily recognised of Antarctic inhabitants, these animals are sustained entirely by the marine ecosystem. It is in fact micro-organisms which dominate the true biota of Antarctic the continent.

#### 1.3.2.1: *Ice-cover*

High latitude lakes are subject to long continuous periods of ice-cover. Only, if they are extremely saline do lakes avoid freezing, an example being Deep Lake in the Vestfold Hills, which has a salinity approximately eight times that of seawater and water temperatures as low as -17 °C (Ferris & Burton, 1988). Most ice-covered lakes thaw peripherally each summer, forming moats via which the water comes into contact with the atmosphere and running water for a few weeks each year (Simmons et al., 1993) but some lakes, chiefly those in the McMurdo Dry Valleys, are perennially ice-covered by up to 6 m of ice, although, epiglacial lakes in all oases remain perennially icecovered. Possessing an ice-cover exerts a strong influence on the lake environment. The ice eliminates turbulent wind-driven mixing, restricts gas exchange between the atmosphere and water column, reduces light penetration to the water column, restricts catchment exchange and is responsible for the distribution pattern of lake-floor sediments (Simmons et al., 1993; Ellis-Evans, 1996). Horizontal currents as a result of geostrophic forces and thermal or density induced convection have been observed in a number of lakes, but this level of mixing is far less dynamic than that which is windinduced. Thus, an ice-cover can also lead to the development of chemical stratification, hypoxia or total anoxia due to stagnation of the water column (Vincent, 1988; Ferris et al., 1991; Ellis-Evans, 1996; Tyler et al., 1998). Ice-covered lakes are generally supersaturated with gases, principally nitrogen and oxygen, since the gases are forced out of the freezing water into the liquid waters below (Wharton et al., 1986; 1987). However, ice cover can also act as a vehicle for gas removal via ablation (Simmons et al., 1993 cite Craig et al., unpublished data). Almost invariably an inverse temperature profile exists below the ice in Antarctic lakes, and this imposes some stability on the water column (Ellis-Evans, 1996). Such stable temperatures are much more common in Antarctic lakes than their Arctic counterparts (Hobbie, 1973).

The quality of the ice-cover is very important when considering its influence on the environmental parameters listed above, particularly in terms of light penetration. The continental lakes of Antarctica have fewer freeze-thaw cycles and less cracking of ice than maritime Antarctic lakes, which consequently reduces the opacity of the ice-cover (Ellis-Evans, 1996). In addition, because of the drier climate, precipitation is reduced and snow cover is not as important an environmental factor in continental Antarctic regions as in the maritime regions, since katabatic winds rapidly disperse any snow that accumulates on coastal terrain and lake surfaces (Ellis-Evans, 1996).

The most important environmental variable in any ecosystem is light, because light provides energy to heat the system, as well as the energy for photosynthesis. By virtue of the fact that most Antarctic lakes are covered with ice, light is also a major limiting factor for the development of microbial communities. In most cases 99 % of light striking the lake surface is absorbed, which means that the microbial communities living in the lakes are well adapted to low light levels and production can proceed, albeit at low levels per unit biomass (Light et al., 1981; Hawes, 1985; Priddle et al., 1986; Vincent 1988; Simmons et al., 1993). High latitude lakes are subject to low annual Photosynthetically Active Radiation (PAR) and the solar flux is generally less than 190 umol m<sup>-2</sup> s<sup>-1</sup>. Light levels are also extremely seasonal. Lakes receive continuous light during the summer but in winter endure complete darkness for up to two months. Mean daily radiation can similarly vary over several orders of magnitude (Priddle et al., 1986; Ellis-Evans, 1996). Generally, Antarctic lakes do not lose their ice-cover until after the summer PAR peak therefore light penetration into the water column is impeded. Conversely, in summer open-water situations high light levels may cause photoinhibition in the phytoplankton (Goldman et al., 1963). When combined with the effects of cloud cover, low water temperatures and ice-coverage, the low levels of PAR can render these ecosystems extremely unproductive (Vincent, 1988; Ellis-Evans, Of additional importance are the high UV levels. Each spring 1996). (October/November) an 'ozone hole' develops over Antarctica, allowing UV radiation to reach the ground and this may be detrimental to aquatic microbial communities (Schindler & Fee, 1975; Hawes, 1985; Priscu, 1995).

#### 1.3.2.3: Water chemistry and nutrient status

There is a wide disparity of water-chemistry characteristics within and between Antarctic lakes. Some lakes have very low solute concentrations, others are extremely saline (e.g. Vincent & Vincent, 1982; Ferris & Burton, 1988). This project was concerned with Antarctic saline lakes only. The dividing value between fresh and saline water lakes is generally considered to be  $3 \, \eta_{\infty}$  total dissolved salts. However, this is an arbitrary figure with no fundamental biological reality, as biological change in fresh and saline water systems follows a continuum. There are four classes of Antarctic saline lake: those produced by 1) the evaporation of freshwater, 2) ectogenic meromictic lakes, 3) sea water isolated by relative sea level change, and 4) lakes produced by volcanic activity (Burton, 1981a; 1981b). Associations with the sea, the presence of ice cover, the arid nature of the environment, and in some instances the chemistry of inflowing streams, combine to promote extreme chemical stratification in all Antarctica

saline lakes and lead to a permanent stratification/meromixis in many (Burton, 1981a; 1981b). Meromixis will be discussed further in Section 2.1.2.1. The saline lakes of the Vestfold Hills are examples of those formed as a result of isolation from the sea. In contrast, of the lakes in the McMurdo Dry Valleys, few have a marine signature (e.g. Lake Fryxell) (Green *et al.*, 1988) and their current ionic composition is thought to derive mainly from nutrient loading by inflowing streams. The salinity of Lake Vanda is influenced by calcium chloride-rich ground water from the nearby Don Juan Basin (500 Kg m<sup>-3</sup> calcium chloride brine) and the influence of the Onyx River (e.g. Torii *et al.*, 1980; Wright & Burton, 1981; Tomiyama & Kitano, 1985; Vincent, 1988).

Salinity plays a very important role in the microbial ecology of Antarctic lakes through its combined effect with temperature and chemical stratification. Salinity affects several other environmental parameters. For example, as salinity increases osmotic strength increases, the freezing point of water is depressed, ice cover is reduced and ice opacity increased (Wright & Burton, 1981). Furthermore, as the waters of a saline lake freeze, salt exudes from the ice increasing the salinity of the water below, a process termed freeze-concentration (Niedrauer & Martin, 1972; Martin, 1979; Burton, 1981a). The effects can be significant in a small lake, for example, the conductivity of Bird Pond, Ross Island, increased to almost seven times its ice-free value by this process (Spurr, 1975).

The barren nature of the recently deglaciated Antarctic lake catchments means that runoff entering a lake contains little dissolved material and essential elements such as carbon, nitrogen and phosphorus may be in short supply. As a consequence, Antarctic lakes generally range from ultra-oligotrophic to oligotrophic. The exceptions are those lakes which lie in close proximity to the coast and receive enrichment in the form of sea-spray, or those near which penguin rookeries and seal wallows and enriched by inputs of faecal material. On occasion, anthropogenic eutrophication may also occur (Hawes, 1983; 1985; Smith, 1985; Simmons et al., 1993; Ellis-Evans, 1990; Ellis-Evans, 1996; Laybourn-Parry et al., 1996). Lake Vanda, in the McMurdo Dry Valleys, is phosphorus limited but fixed nitrogen compounds are generally regarded as the most important of limiting nutrients in Antarctic Lakes (Vincent, 1981; 1988). Nevertheless, in some instances, lakes can be naturally enriched by fixed nitrogen from melting glaciers, mainly in the form of urea, and by Auroral activity (Hand & Burton, 1981). It has been demonstrated by most investigators that Antarctic lakes are rich in dissolved organic compounds in comparison with temperate-latitude lakes (Simmons et al., 1993). The kinds and amounts of compounds alter with depth and are paralleled by a known zonation of micro-organisms.

The classic view in the Antarctic marine environment is that the waters are oligotrophic in terms of primary production, despite their high residual nutrients. However, Azam *et al.* (1991) coined the term "hypoproductive" in order to distinguish them from oligotrophic waters at lower latitudes that exhibit low primary productivity due to low nutrient concentrations. The extremely low productivity of most continental Antarctic lakes is in stark contrast to the fertility of the Southern Ocean and the sea-ice encircling the continent. However, Parker *et al.* (1982) showed that some Antarctic lakes have planktonic production values that could be classified as mesotrophic or eutrophic on the basis of temperate-lake values.

#### 1.3.3: The microbial flora and fauna of Antarctic Lakes

#### 1.3.3.1: Endemism

It is generally accepted that, contrary to what one might expect, the majority of species found in Antarctica are cosmopolitan, with very few endemics (Heywood, 1977; Hawthorn & Ellis-Evans, 1984; James *et al.*, 1995). There is also little direct evidence that organisms growing in Antarctica have adapted their metabolism in some way to live there. It may be that the youth of the present ecosystems has not allowed sufficient time for adaptations to occur. Heywood (1977) considered that this youth, together with the difficulty of transport both to and within Antarctica, was the main factor limiting species diversity in lakes. He supported this contention with the observation that Antarctic lake biota is poor when compared with that of the Arctic. Environmental conditions in both environments are equally as severe, hence, the smaller number of species found on the Antarctic continent must be a reflection of the youth and isolation of the ecosystems. This may indeed be the case in most freshwater and saline lakes but in hypersaline lakes, even though inoculations are possible, it is the temperature and salinity of the lakes themselves which ultimately limit biodiversity (Wright & Burton, 1981).

#### 1.3.3.2: Protozoa

Protozoa were first recorded in Antarctic lacustrine ecosystems by Murray (1910), but despite their diversity and wide distribution in Antarctic lakes they have only recently received attention. Taxonomic groups identified include flagellates, ciliates, heliozoans and rhizopods. Antarctic Protozoa are less diverse than in Arctic and temperate lakes, but are well adapted to their lacustrine environments and show potential to readily respond to environmental changes (Ellis-Evans, 1996). Planktonic Protozoa in

Antarctic lakes are subject to the same laws governing their behaviour as those controlling plankton in aquatic environments elsewhere (Priddle *et al.*, 1986). However, the Antarctic lacustrine ecosystem presents extremes of conditions which profoundly influence the success of the plankton. To re-iterate: temperatures are consistently low and the extreme year-round variation in radiation climate is further exacerbated by winter ice-cover and a short ice-free growing season (Ellis-Evans, 1996).

There are a wide variety of inland water bodies on the Antarctic continent and protozoan plankton occur in all but the most hypersaline of these. Under ice-cover, the phytoplankton typically consist of small flagellated Protozoa such as chlorophytes and chrysophytes, while in open water conditions cryptophytes tend to dominate (Light *et al.*, 1981; Vincent, 1981). The ice-cover restricts water movements and in order to survive such stable and often stratified conditions, organisms must either be able to regulate their position in the water column or possess a buoyancy mechanism. Consequently, motile flagellated and/or ciliated Protozoa dominate the plankton in lakes, in contrast to the marine ecosystem where diatoms are predominant (Spaulding *et al.*, 1994). Truly planktonic diatoms are virtually absent in Antarctic lakes, although they are found associated with benthic algal mats. The majority of diatoms observed in the water columns of Antarctic lakes during summer open-water periods are benthic forms suspended by wind-, density- or temperature-induced mixing (Ellis-Evans, 1996). Their small size enables protozoans to feed efficiently on small eukaryotic algae and bacteria (Garrison & Gowing, 1993).

Relatively few Antarctic lakes have been subject to year round sampling (e.g. Hand & Burton, 1981; Burch, 1988; Heath, 1988; Light *et al.*, 1981; Hawes, 1983; 1985; Laybourn-Parry *et al.*, 1995; Bayliss *et al.*, 1997). In the lakes studied, phytoplankton have been shown not only to be capable of surviving long periods of near or total winter darkness, but also to be capable commencing photosynthesis and growth during the spring, despite persistent ice cover and low ambient radiation fluxes (Light *et al.*, 1981; Hawes, 1985). They are in effect shade-adapted and, thus, photosynthetically poised and ready to respond quickly to the appearance of light in spring (Heath, 1988). However, the size of these spring populations is often determined by the availability of inorganic nutrients. If annual ice-cover disappears completely from a lake, as is common in coastal locations, a second taxonomically distinct population may develop in the open-waters which has extremely high production rates. In contrast to these highly productive coastal lakes, summer phytoplankton populations in the perennially ice-covered lakes of continental Antarctica exhibit extremely low rates of photosynthetic

production. Production rates in these lakes, where nutrient cycling is thought to be slow, are consistent with extrapolated Arctic data (Priddle *et al.*, 1986).

Stratification and ice-cover exert the most influence on the regulation of plankton communities in Antarctic lakes. A distinct taxonomic distribution may exist in stratified lakes as a result of species being well adapted to the particular set of environmental conditions present in each stratum. Since the niches in each stratum are distinctly separated by strong light and nutrient gradients, competitive exclusion occurs within each and selection for organisms which are either strong swimmers or so small that they sink slowly, results (Hardin, 1960; Simmons *et al.*, 1993; Spaulding *et al.*, 1994). The majority of biomass and biological activity of plankton is found at the oxic-anoxic interface of a water column. This deep, well-defined planktonic layer is often referred to as the Deep Chlorophyll Maximum or DCM. Lake Vanda in the McMurdo Dry Valleys is an excellent example of how chemical stratification can influence planktonic distributions. The lake has three floristically distinct algal communities distributed in its water column, each specifically adapted to the temperature, light and nutrient conditions prevailing in each stratum (Vincent & Vincent, 1982; Priscu *et al.*, 1987).

#### 1.3.3.3: Bacteria

The phytoplankton of Antarctic lakes has received great attention but the role of bacteria in these lakes is becoming better known. Direct epifluorescence enumeration has been a major breakthrough (Porter & Feig, 1980) (Section 2.2.3.1). Bacterial growth and production in general, mirrors that of the phytoplankton, emphasising the nutrient cycling between the two groups. Picocyanobacteria are probably most widespread in their occurrence in Antarctic lakes and may be significant components of most lakes (Ellis-Evans, 1996; Rankin *et al.*, 1997). Extensive populations of both aerobic and anaerobic bacteria exist in Antarctic lakes depending on the relative conditions that prevail. In some instances, bacterioplankton dominate, for example, in hypersaline lakes such as Don Juan Pond, McMurdo Dry Valleys, and Deep Lake, Vestfold Hills (Cameron *et al.*, 1972; Siegel, 1973; Siegel *et al.*, 1979; Burke & Burton, 1988; Ferris *et al.*, 1988; Vincent, 1988; Ellis-Evans, 1996).

#### 1.3.3.4: Metazoa

Metazoa inhabit Antarctic lakes in limited numbers. Bayly and Eslake (1989) studied the vertical distribution of two copepods, *Paralabidocera antarctica* (I.C. Thompson), a calanoid species, and *Amphiascoides* sp., a planktonic harpacticoid, in Lake Abraxas, a meromictic lake in the Vestfold Hills. Lakes Ace and Burton in the same area, have also

been found to contain metazoan zooplankton. Burton Lake still retains contact with the sea and contains two calanoid copepods, an Anthomedusa and a cydippid ctenophore (Bayly, 1978; Bayly & Burton, 1987). Rotifers have also been reported from lakes in the Vestfold Hills (Dartnall, 1997). In contrast, the lakes of the Dry Valleys, are characterised by a virtual absence of planktonic zooplankton, only rotifers have been observed in the Dry Valley meromictic lakes (Heywood, 1972; Parker & Simmons, 1985; Vincent, 1988). Difficulties in dispersal are probably a main reason why no crustacean has colonised the truly fresh-water lakes in the McMurdo Sound area (Heywood, 1972). Heywood (1977) showed that the occurrence of zooplankton decreased with increasing latitude. The grazing component of the planktonic ecosystem may be entirely absent from some lakes or may interact with phytoplankton for only a brief period during the year, thus, the grazing pressure exerted on the protozoan and bacterial populations in Antarctic lakes is highly variable (Hawes, 1985).

#### 1.3.3.5: Microbial mats

Benthic microbial mats are probably the most striking feature of Antarctic lakes because the mats are invariably highly pigmented and extremely widespread in both terrestrial and aquatic environments. They have been observed extensively in the lakes of the maritime Antarctic, the Schirmacher Oasis, the McMurdo Dry Valleys, the Larsemann Hills, and the Vestfold Hills (Ellis-Evans, 1996). The mats are composed primarily of shade-adapted cyanobacteria, pennate diatoms and heterotrophic Protozoa. In addition, often a community of Metazoa is observed associated with the mats. Reduced grazing pressure in Antarctic systems and the ecophysiological flexibility of cyanobacteria, has allowed these microbial mats to flourish (Ellis-Evans, 1996).

#### 1.4: THE LAKES OF THE VESTFOLD HILLS

The Vestfold Hills form a low lying, ice-free area occupying approximately 411 km<sup>2</sup> on the north-eastern shore of Prydz Bay, eastern Antarctica (Pickard, 1986; Simmons *et al.*, 1993). The Vestfolds area contains hundreds of lakes and ponds each with distinctive chemical compositions and biota. The lakes originate from a period of postglacial marine transgression that followed glacial retreat approximately 8000 years ago, although this date is the subject of some controversy (Adamson & Pickard, 1986; Peterson *et al.*, 1988). Following marine transgression, isostatic uplift of the ice free terrain isolated seawater in basins formed by glacial corrasion (Heywood, 1972). Their evolution means that the lakes' chemistry derives from relic seawater. Over intervening years the chemical composition of the lakes has altered considerably due to the relative balance between evaporation, ablation, dilution by freshwater, precipitation and biological modification, which is dependent on their location in relation to the ice cap and the ocean, catchment area, topography and volume to surface area ratio (Hand & Burton, 1981; Volkman *et al.*, 1988). The lakes that exist at present vary from deep, freshwater basins (Crooked Lake; 157 m deep, salinity  $0.02 \, {}^{\prime}_{\infty}$ ) to shallow, hypersaline basins (Deep Lake; 36 m deep, salinity  $280 \, {}^{\prime}_{\infty}$ ) (Ferris *et al.*, 1988; Laybourn-Parry & Marchant, 1992b). Lakes lying in close proximity to the coast have lost their connection with the ocean relatively recently, for example Lakes Burton and Rookery. A net annual increase in terrestrial habitat and the number of lakes in the region still takes place due to the 'heat island' effect and a continued glacio-isostatic uplift of approximately 2 mm a<sup>-1</sup> (Burton, 1981a; Zhang *et al.*, 1983; Zhang & Peterson, 1984).

Post-formation, the biological communities of the Vestfold Hills lakes have also undergone significant changes. The original marine communities have evolved and simplified to the extent that many lakes now contain a scarcity of living species (Campbell, 1978). The original marine food webs have effectively been truncated leading to the existence of simple communities of bacteria, algae and Protozoa, and limited numbers of Metazoa. In these communities microbial loop dynamics dominate with energy flowing through microbial autotrophs and heterotrophs (Azam *et al.*, 1983; Laybourn-Parry *et al.*, 1992a; 1992b).

#### 1.5: THE AIMS OF THE PROJECT

There were two main aims within the project:

1) To investigate the seasonal variation in species composition, abundance, size and distribution of micro-organisms living in the saline lakes of the Vestfold Hills, in particular Ace Lake, and relate these to the physico-chemical properties of their lacustrine environments.

2) To elucidate the major pathways of carbon and microbial trophic interactions in Ace Lake with a view to modelling carbon flux within the system.

The project was conducted at the Australian Antarctic Division's Davis Station, in the Vestfold Hills (Photograph 1.1).


Photograph 1.1: Aerial photograph of the Australian Antarctic Division's Davis Station, Vestfold Hills, eastern Antarctica. Taken during the summer, hence the lack of snow

# Chapter 2: ANNUAL PLANKTON DYNAMICS IN ACE LAKE

### 2.1: INTRODUCTION

The last decade has seen a significant expansion of the database concerning the nature and dynamics of planktonic protozoan communities. Generally studies have concentrated on the ciliate component and there are instances when these have been shown to play the major role in the flow of energy and cycling of carbon, phosphorus and nitrogen (Sherr & Sherr, 1987). However, where the whole community has been investigated it has become increasingly apparent that heterotrophic nanoflagellates play a greater role (Landry *et al.*, 1984; Sanders *et al.*, 1989; Weisse *et al.*, 1990; Laybourn-Parry *et al.*, 1992a; Laybourn-Parry *et al.*, 1995). In this study, a balanced approach was adopted to investigate all components of the planktonic community, together with the physico-chemical properties of Ace Lake in the Vestfold Hills, eastern Antarctica.

### 2.1.1: Lake Stratification

#### 2.1.1.1: Meromixis - a definition

Some lakes never mix throughout their entire depth. Such lakes are termed meromictic. Meromixis is unusually common in the saline basins of the Vestfold Hills (Burton, 1981a; 1981b). Worldwide, Walker and Likens (1975) listed only 120 meromictic lakes, but at least thirty such basins, ranging from marine embayments having yearround tidal exchange with the sea to fully isolated lakes, are known in the Vestfolds (Ferris et al., 1991; Gibson & Burton, 1996); one such basin is Ace Lake. Wetzel (1983) described meromixis (in open-water situations) as the stratification occurring when a deeper stratum of water, the *monimolimnion*, is perennially isolated from an overlying *mixolimnion*, which periodically circulates. These two strata are commonly separated by a steep salinity gradient termed the chemocline. The chemocline lends such lakes "concentration stability" (Wetzel, 1983). In most lakes the salinity gradient is not sufficient to prevent wind energy from causing holomixis (circulation throughout the entire water column) but in lakes such as Ace Lake in the Vestfolds and Lake Fryxell in the Dry Valleys (Vincent & Ellis-Evans, 1989), the present meromixis is maintained ectogenically by both fresh, meltwater inputs which seasonally enhance the stability of the chemocline, and a protective ice-cover (Burke & Burton, 1988). In other instances a crenogenic or biogenic meromixis results when the salt in the monimolimnion originates from deep water springs, or minerals released during decomposition in the deep waters and sediments, respectively (Lampert & Sommer, 1997).

Approximately twenty years ago when the first geochemical surveys were carried out on Ace Lake, the chemocline was far less pronounced than at present. The lake was meromictic, but there was a relatively steady increase in conductivity with depth which rendered the terms mixolimnion and monimolimnion misleading. Therefore, limnologists preferred to divide Ace Lake into three zones based on dissolved oxygen concentration, an upper *oxylimnion* separated from a lower *anoxylimnion* by a transition zone, or *oxycline* (Burton, 1980; Hand & Burton, 1981). For the purposes of this study, however, the classic limnological terms mixolimnion and monimolimnion will be employed for the strata in Ace Lake. Nevertheless, it is recognised that these terms relate specifically to physical forces driving lake stratification. Since Ace Lake is ice-covered for the majority of each year, the major physical force responsible for mixing, wind, is restricted. Consequently, there are fundamental differences between ice-covered and ice-free lakes.

In order to define the extent of the mixolimnion and monimolimnion in Ace Lake the situation existing during an ice-free period must be considered. Throughout this study the mixolimnion extended as far as the thermocline/halocline at 8 m and the monimolimnion from 8 m downwards. Theoretically, if the lake remained ice-free and in its current water balance, over time diffusion would result in chemical uniformity in terms of nutrients and oxygen throughout the entire monimolimnion. However, the chemistry of the monimolimnion is biogenically influenced. The upper monimolimnion (from 8 m to 10 m) is aerobic, and nutrient limited, the lower monimolimnion is anaerobic and relatively nutrient rich by virtue of its micro-inhabitants. These two pseudo-strata are separated by an oxycline between 10 and 12 m (Figure 2.1). The terms oxylimnion and anoxylimnion coined by Hand and Burton (1981) define Ace Lake's chemical stratification but they are not in general usage and will be avoided where possible.

# 2.1.1.2: Evolution of the current meromixis in Ace Lake

The meromictic state of Ace Lake has not been continuous. Burton and Barker (1978) argued that one or more episodes of meromixis have taken place in the lake. They suggested a three stage development for Ace Lake based upon its sulphur chemistry. An initial stage encompassed several cycles of mixing and meromixis during which most of the original seawater was replaced by fresh runoff and 76% of the sulphur disappeared from the system through biological reductions in the monimolimnion. This was followed by a period of climatic warming which increased evaporation from the lake surface and led to the production of more saline, denser surface waters. This, coupled



Figure 2.1: Diagrammatic representation of the stratification in Ace Lake

with a decrease in the stabilising ice-cover, destroyed the stability of the chemocline and promoted a period of holomixis. During this period the lake sulphur content was replenished. Finally, the present stage of meromixis developed when local freshwater became layered over older water remaining after the cessation of holomixis.

This thinking was reaffirmed by Bird *et al.* (1991) based on the analysis of sulphur,  $\delta^{13}$ C, and water content of the lake sediment. Bird *et al.* (1991) divided the core into three units of varying composition. The second unit was of marine origin, units one and three were not, although the origin of unit three (the oldest unit) was difficult to interpret. They also noted that aerobic and anaerobic prostrate algal mats found in units one and three, were similar to those from nearby Highway Lake and the lakes of the McMurdo Dry Valleys (Wharton *et al.*, 1983). This correlated well with hydrocarbon analyses and suggested that the lake was initially entirely oxic but moved through a period of hypoxia to reach its currently stratified state with a permanent anoxic basin, over the last 1000 years (Volkman, *et al.*, 1986). However, disparities led Bird *et al.* (1991) to hypothesise that Ace Lake may contain sediments solely of lacustrine origin, unit two representing a period when the lake was holomictic rather than influenced by marine incursions and separated by two phases of meromixis (units one and three). This argument is consistent with the sulphur-isotopic analyses performed by Burton and Barker (1978) but inconsistent with the timing of the lake's isolation event.

More recently Fulford-Smith and Sikes (1996) analysed sedimentary diatom assemblages and predicted a five stage evolution for Ace Lake. They suggested that Ace Lake was initially a marine inlet influenced by the dynamic mixing of ocean and meltwater inputs. As glacial retreat commenced, isostatic uplift led to isolation of the lake from its marine influences, thereby allowing flushing by meltwater from the retreating ice sheet. Over the course of 800 years (approximately 9200 to 8400 <sup>14</sup>C years BP) meromixis evolved as the result of a freshwater lens developing over the water of marine origin. However, the sediment diatom assemblages suggested that 6700 years ago, concurrent with the Antarctic sea level maximum, seawater flooded over the sill of the lake disturbing this freshwater meromixis. Since the sediments from this period were found to be laminated and contained elemental sulphur, Fulford-Smith and Sikes (1996) contended that the marine input must have been limited and low in energy. Marine inputs ceased approximately 5500 years ago and the lake gradually became meromictic once more, reaching a stable state over a 1700 year period. Little change to this stable state was apparent in sediments laid down during the last 4000 years.

#### 2.1.1.3: Recent small-scale changes in Ace Lake's stratification

Small scale changes in the stratification of Ace Lake are highly dependent on the water balance of the lake. Water levels in closed basin lakes such as Ace Lake are determined by the balance between melt-water inflow from nearby glaciers (e.g. in the McMurdo Dry Valleys) or snow banks (e.g. Vestfold Hills) and water loss by ablation (when ice-covered), and evaporation (Gibson & Burton, 1996).

When ice first develops on a lake it leads to the formation of a convective cell of water (a new mixolimnion or epilimnion) in the wind-mixed portion of the water column and a decrease in water temperature which leads to the production of more ice. Further ice formation leads to increased salt rejection from the ice, a process termed freezeconcentration (Niedrauer & Martin, 1972; Martin, 1979; Gibson & Burton, 1996) and this salt is essentially integrated into a smaller volume of water, causing an increase in salinity. The increasing salinity leads to a corresponding increase in density, and ultimately a deeper penetration of the mixolimnion. The process of ice formation is enhanced by the fact that increasing salinity, suppresses the freezing point of the water column, thus decreasing the temperature of the mixolimnion and stimulating ice formation. The base of the new mixolimnion is constrained by the depth of summer wind mixing initially but as density increases so does penetration of the mixolimnion into the lake.

With the cessation of ice formation three scenarios are possible: 1) if a winter were milder than the previous one, the ice covering on the lake would be thinner and the mixolimnion would penetrate less deeply. Consequently, an interval of approximately isopycnal (water of equal density) water (which has not undergone vertical mixing during the year and should therefore be considered part of the monimolimnion) would result between the current and previous mixolimnia. 2) If ice formation were approximately equal to that of the previous year the mixolimnion would penetrate to approximately the same depth. 3) If ice formation were increased, a deeper penetration of a new mixolimnion would result, entraining previously unmixed waters from the monimolimnion. Such changes are, however, rare due to the relatively constant annual pattern of ice formation on Ace Lake (Gibson & Burton, 1996).

The effects of water level changes on water column structure are similar but more marked to those resulting during ice-cover changes. The effect of a decreasing or negative water balance i.e. a decreasing water level, is analogous to that of an increasing ice-cover. Effectively, decreasing water level decreases the volume of the mixolimnion and causes an increase in salinity. Hence the maximum density within the mixolimnion increases over time, the depth of penetration of the mixolimnion increases and the annual minimum temperature within the mixolimnion decreases. Extended periods of decreasing water level result in a deep penetration of successive mixolimnia thus the elimination of structures such as pycnoclines and isopycnal zones previously present in the water column profile. It therefore follows, that a significant drop in water level could lead to a period of holomixis, thus "resetting" the lake structure (Gibson & Burton, 1996).

Conversely, periods of increasing or positive water balance are analogous with decreased ice formation. Increasing water levels, increase total lake volume and thus decrease salinity. Assuming the same volume of ice-cover, this leads to a decreased density and decreased penetration of the mixolimnion. Consequently, the base of any new mixolimnion will be constrained by summer wind mixing. Model calculations suggest that even 0.1 m (easily achieved in the summer months) water level changes can have a marked effect on the salinity of the reduced epilimnion (Gibson & Burton, 1996). Successive periods of positive water balance create a considerable depth of near isopycnal water extending from the base of existing mixolimnia to the depth of mixing in the last period of negative water balance, i.e. the water level minimum. These intervals are termed palaeoepilimnia and are indicative of prior minimum water levels and successive increases. Isopycnal intervals deeper in the lake represent older periods of minimum water level. The older the palaeoepilimnion, the more smoothed and diffuse it will appear in the lake profile. Hence, such artifacts are extremely useful when assessing lake evolution.

#### 2.1.2: The anaerobic monimolimnion of Ace Lake

The earliest studies performed on Ace Lake in the mid- to late seventies, concentrated on the biogeochemistry of the anoxylimnion, or anaerobic monimolimnion as it will be termed in this study. Burton (1980) and Hand (1980) discussed the bacterioplankton residing in this lower stratum of Ace Lake and the consequent sulphur and methane cycles taking place. The concentration ratios of major ions in Ace Lake are similar to those observed in seawater, with one exception (Burton & Barker, 1978); the successive periods of meromixis have reduced the sulphate concentration in the bottom waters of the anaerobic monimolimnion by 76 %. These unique circumstances mean that sulphate-reducers dominate the upper regions of the monimolimnion and sulphide concentrations (principally in the form of hydrogen sulphide,  $H_2S$ ) are high (Burton, 1980; Mancuso *et al.*, 1990; Gibson *et al.*, 1991). Oxidation of this sulphate by photosynthetic sulphur bacteria produces elemental sulphur and sulphate which are reduced back to sulphide by sulphate-reducing bacteria of the genus *Desulfovibrio*. Dissolved and particulate organic carbon resulting from this cyclical process supports populations of heterotrophs; aerobes in the oxycline and anaerobes in the anaerobic monimolimnion. The aerobic heterotrophs in the oxycline are subsequently responsible for the maintenance of this steep oxygen gradient as they reduce dissolved oxygen during their oxidation of organic materials (Hand, 1980).

Organic matter sedimenting from the mixolimnion is the source of carbon and energy for the bacteria in the anaerobic monimolimnion of Ace Lake (Burton, 1980). When decomposed, organic matter in such anaerobic environments is converted into increasingly smaller molecules, ultimately forming carbon dioxide (CO<sub>2</sub>) and methane  $(CH_4)$ . However, if a sufficient concentration of sulphate is present,  $CH_4$  production is usually inhibited, even if methanogenic bacteria are present. This occurs because sulphate is the preferred terminal electron acceptor and reducing sulphate to sulphide is more efficient in terms of the energy gained by the bacteria than reducing organic matter to CO<sub>2</sub> or CH<sub>4</sub>. Sulphate-reducers, therefore, have an adaptive advantage in most anaerobic environments (Burton, 1980). An early study carried out by Burton and Barker (1978) demonstrated that the bottom waters of Ace Lake are sulphate-free but that sulphate was present in the upper portion of the anoxic monimolimnion. Thus, H<sub>2</sub>S is produced in this upper layer, but in the lower portion (below 22 m) where sulphate is absent, CH<sub>4</sub> is produced (Burton, 1980). Biological sulphate-reduction in these bottom waters has completely removed sulphate, and CH<sub>4</sub> concentrations are now saturating. At these high concentrations, ebullition occurs. As bubbles of CH<sub>4</sub> rise through the lake they remove  $N_2$  from the water column, resulting in the depletion of  $N_2$  observed in this stratum. Conversely, CO<sub>2</sub> concentrations are higher than those in the aerobic strata of the lake (Burton, 1980; Mancuso, 1990).

Methanogenic bacteria are ubiquitous in most anaerobic environments, where they perform the terminal step in the mineralistaion of organic carbon (Zeikus, 1977; Mancuso *et al.*, 1990). The CH<sub>4</sub> present in the bottom waters of Ace Lake is produced by such methanogenic bacteria (Volkman *et al.*, 1986; 1988). Organic geochemical studies of organic-rich lake sediments have provided evidence for the existence of methanogens, as well as other microbes (Volkman *et al.*, 1986; 1988). The cell membranes of methanogenic archaebacteria are unique and consist of lipids formed with ether linkages and isoprenoid branching. Identification and quantification of these phospholipid-derived ether lipids provide a means by which the methanogenic bacterial component of the microbial community of Ace Lake can be estimated. Matsumoto (1989) and Mancuso *et al.* (1990) found a high abundance of isoprenoid hydrocarbons in the bottom waters and sediments of Ace Lake which were attributable to the dominant

population of methanogenic archaebacteria inhabiting these areas. The majority of known species of methanogens obtain their energy from acetate or from the reduction of carbon dioxide (CO<sub>2</sub>) by molecular hydrogen (H<sub>2</sub>) or formate, although in some environments, methanogens obtain energy for the reduction of CO<sub>2</sub> from acetate and higher fatty acids through interspecies hydrogen transfer (Franzmann *et al.*, 1991). Where sulphate is depleted, methanogenesis usually occurs via CO<sub>2</sub> reduction, since after sulphate, this is the next favourable electron acceptor remaining for the oxidation of organic matter (Franzmann *et al.*, 1991). Consequently, H<sub>2</sub> concentrations in the monimolimnion of Ace Lake are below the limit of detection (Burton, 1980). The CH<sub>4</sub> produced in the sediments and bottom waters of Ace Lake, diffuse upwards to be utilised by methane-oxidising bacteria, completing the carbon cycle (Ellis-Evans, 1985c). Consequently, no new carbon is introduced into the lake by this microbial cycling. Only the phytoplankton and benthic algal mats in the aerobic strata contribute to the carbon pool (Ellis-Evans, 1985c).

Information on the structure of the microbial community of Ace Lake provides insight into the carbon cycling of the system, but more detailed analyses are required to determine the rate of this cycling. Mancuso *et al.* (1990) employed phospholipid analyses of water column particulate and sediment samples to determine the rate of microbial degradation of organic carbon within the system. They concluded that the relatively high methanogenic biomass in the lake implied that degradation rates occurred at extremely slow rates. Further work by Franzmann *et al.* (1991), confirmed a slow rate of methanogenesis, despite high populations of methanogens, suggesting that they might be working well below their optimum temperature. No evidence exists for the presence of psychrophilic methanogenic bacteria but it is possible that Antarctic methanogens show lower optima and minima temperatures for growth compared when with their taxonomic counterparts at lower latitudes, as has been shown for halobacteria (McMeekin & Franzmann, 1988). However, although methane is produced very slowly in Ace Lake, the very stable waters of the monimolimnion entrain the gas accounting for the saturation concentrations observed (Mancuso *et al.*, 1990; Franzmann *et al.*, 1991).

#### 2.1.3: The aerobic mixolimnion and upper monimolimnion of Ace Lake

The earliest investigations of the micro-organisms dwelling in the aerobic mixolimnion and upper monimolimnion of Ace Lake were conducted for metazoan zooplankton (Bayly, 1978; Bayly & Burton, 1987; Bayly & Eslake, 1989). The first recorded incidence of the calanoid copepod *Paralabidocera antarctica* (I.C. Thompson) in a lacustrine environment was documented for Ace Lake, and it was noted that Ace Lake contained a much higher abundance of zooplankton than any other lake in the Vestfold Hills (Bayly, 1978). Populations of the copepod peaked both below the ice and just above the anaerobic monimolimnion, corresponding with peak population abundance of their flagellated protozoan and bacterial prey (Bayly & Burton, 1987). Females were found to dominate the near-surface waters and males the deeper levels (Bayly & Eslake, 1989).

Hand and Burton (1981) were the first to perform a full annual study of the lake's microbial populations, concentrating on the physico-chemistry and bacterioplankton of the entire lake. Since then, researchers have performed taxonomic studies to identify the bacterioplankton of Ace Lake (Franzmann et al., 1987; 1991a; 1991b; 1992; 1997; Volkman et al., 1988; Franzmann & Dobson, 1993; Franzmann & Rohde, 1991; 1992; Spielmeyer, 1993). Volkman et al. (1988), reported that the major species of bacterioplankton in Ace Lake were photosynthetic green sulphur bacteria, Chlorobium vibrioforme and C. limnicola Nadson. Species of purple bacteria and methanogenic and sulphate-reducing bacteria were also identified. Franzmann et al. (1991a; 1992) identified the methanogen, Methanococcoides burtonii, and the lactic acid-producing, Carnobacterium funditum and C. alterfunditum. More recently, Rankin et al. (1997) also reported that picocyanobacteria of the genus Synechococcus were also present at abundances 40 000 times greater than those recorded for species of the same genus in southern polar marine waters, therefore, contributing greatly to the autotrophic productivity of Ace Lake. Butler et al. (1988) determined that total iodine concentration in Ace Lake was closely correlated with bacterial abundance in the aerobic water column, suggesting a biological mediation of iodine distributions. They revealed that the element is assimilated by phytoplankton, which are subsequently grazed by metazoan zooplankton and iodine consequently sinks in faecal pellets and other biodetritus, to be remineralised by heterotrophic bacteria at the oxycline or in the nearbottom, sediment pore waters (Butler et al., 1988).

Burch (1988) performed the first annual study of Antarctic lake phytoplankton on Ace Lake in 1979. He found only four species of autotrophic flagellated Protozoa in Ace Lake and demonstrated a distinct, vertical, taxonomic stratification throughout the aerobic strata of the lake. He also determined that overall production of the phytoplankton is limited by the availability of inorganic nutrients in the water column above the oxycline. Van den Hoff *et al.* (1989) subsequently investigated the microstructure of cysts from the dominant phytoflagellate, *Pyramimonas gelidicola* McFadden (Chlorophyta: Prasinophyta), and made the first record of a prasinophyte with two distinctly different scale types at different stages of its cell life cycle. Laybourn-Parry and Perriss (1995) identified the autotrophic ciliated protozoan, *Mesodinium rubrum* Lohmann (Ciliophora; Haptorida), as a dominant component of Ace Lake's photosynthetic community and the first annual study of this ciliate was conducted by Gibson *et al.* (1997) between 1994 and 1995.

It is clear from the above that the biogeochemistry and bacterioplankton of Ace Lake have received far more attention than the protozoan communities, and that annual data sets are scarce. It is hoped that the work performed during this study will help to readdress the balance.

### 2.1.4: Ace Lake as a study site

Antarctic lakes in general are excellent laboratories where the limits of biological adaptation and the interaction between microbial populations can be studied without the background noise usually imposed by herbivory, predation and terrigenous inputs. Ace Lake itself represents an ideal model system in which to study the dynamics of microbial marine plankton since evolution of the relict marine communities has led to the loss of virtually all metazoan components, and hence the domination of a relatively simple, low diversity community of Protozoa, algae and bacteria. In addition, Ace Lake can be considered a closed system; insofar as is known, inflows are negligible, there are no outflows and the carbon within the system is almost entirely autochthonous, derived by the fixing of atmospheric carbon dioxide by the phytoplankton and benthic algal mats (Hand & Burton, 1981; Vincent, 1988). However, Vincent and Howard-Williams (1985) have pointed out that the low diversity of the planktonic communities does not necessarily mean that the processes that govern the flow of carbon and energy within these systems are any less complex than their low latitude counterparts.

This chapter describes the seasonal variations in species composition, abundance, size and distribution of the bacterioplankton and protozoan plankton community in Ace Lake, and relates these to the physico-chemical properties of the lacustrine environment.

#### 2.2: MATERIALS AND METHODS

#### 2.2.1: The sampling site

Ace Lake is located at 68° 28.4′S, 78° 11.1′E on Long Peninsula, the most northerly of three peninsulas comprising the Vestfold Hills area, and is flanked by Prydz Bay to the north and Long Fjord to the south (Figure 2.2). It lies 10 km from the ice cap and only 150 m from the nearest sea. The lake has a surface area of 1.8 x  $10^5$  m<sup>2</sup>, a maximum depth of 25 m and drains a catchment area of approximately 3.55 x  $10^5$  m<sup>2</sup> (Hand &



Figure 2.2: Map of the Vestfold Hills showing the location of Ace Lake, Rookery Lake and Highway Lake. Hatched area represents sea-ice. Modified from Burke and Burton (1988), pp.14

Burton, 1981; J.A.E. Gibson, personal communication). Ace Lake is a saline lake of marine origin. In general the lake is completely ice-bound from March to December. During this study Ace Lake was ice-free for a period of six weeks from late January to February each summer. In some years only a wide moat melts out (Volkman *et al.*, 1988).

#### 2.2.2: Sampling procedure

The mixolimnion (upper oxygenated waters above the halocline/thermocline), the upper aerobic monimolimnion and the oxycline of Ace Lake were sampled approximately fortnightly (weather and transport permitting) between 1995 and 1997 on: 23 December, 3 February, 13 April, 31 May, 1 & 18 July, 5 & 19 August, 13 September, 1, 10 & 25 October, 17 & 25 November, 9 & 31 December, 6 & 20 January and 5 & 19 February, from a fixed site at the southern end of the basin (Photograph 2.1). On occasion, samples were taken from the anaerobic monimolimnion below the oxycline for comparison. Logistics prevented samples being collected in January, March and June 1996. Samples were taken from a boat on three open water occasions in February 1996 and February 1997, but when the lake was ice-covered a Jiffy Ice-drill (Feldmann Engineering, Sheboyan Falls, Wisconsin, USA) was employed to gain access to the water column.

#### 2.2.2.1: Physical parameters

Detailed thermal profiles were measured using a Conductivity-Temperature-Depth recorder (CTD) (Platypus Engineering, Loyetea, Tasmania), factory calibrated and accurate to  $0.05 \,^{\circ}$ C. The CTD, set to sample every 10 s, was lowered in steps of 1 m and held for 40 s at each depth. The 0 m depth was referenced to the water surface level in the drilled ice-hole. Due to problems with the light meter employed during the study data obtained in previous studies was used when discussing the light climate of Ace Lake. The light readings cited were collected by Pitman and Rankin in 1992 using a Digital Scalar Irradiance Meter and an underwater quantum sensor, as close to the solar noon (14:00) as possible. Ice thickness was measured on each sampling occasion using a ruled pole. Three seasonal ice cores, 11 cm in diameter, were collected using a 'sipre' ice auger (Journal of Glaciolgy, 1958) with the aid of a wooden template. The cores were placed in sealed, plastic tubes for transport and subsequently stored at - 20 °C.



Photograph 2.1: Aerial photograph of Ace Lake. + marks sampling site



**Photograph 2.2:** DAPI stained autotrophic nanoflagellates (*Pyramimonas gelidicola*). Arrow indicates chlorophyll fluorescence

#### 2.2.2.2: Biological parameters

The water column was sampled at 2 m intervals from 0 m to 12 m using a 4.5 l nonmetallic Kemmerer bottle. From each depth interval 250 ml of water was immediately fixed in buffered glutaraldehyde (to a final concentration of 2 %) for determination of bacterial and nanoflagellate abundance. For the enumeration of ciliate, dinoflagellate, diatom and metazoan abundance and biomass, 1 l was fixed in Lugol's iodine (final concentration 2 %).

#### 2.2.2.3: Chemical parameters

One litre samples were taken for chlorophyll *a* and nutrient analyses, and further samples for dissolved and particulate organic carbon (DOC and POC) analyses. In addition water samples were collected in stoppered glass bottles and treated *in situ* with divalent manganese and a strong alkali in accordance with the Winkler technique. The precipitated manganous hydroxide was dispersed throughout the vessel to facilitate fixation of dissolved oxygen whilst oxidising an equivalent amount of divalent manganese to basic hydroxides for later titration (Parsons *et al.*, 1984).

All samples were transported to Davis Station in insulated boxes and analyses commenced within three hours of collection.

#### 2.2.3: Analysis of samples

#### 2.2.3.1: Bacterial abundance, mean cell volume and carbon biomass

Bacterial abundance was determined by staining 5 ml aliquots with a 0.01 % solution of the fluorochrome DAPI ((4',6-diamidino-2-phenylindole), Sigma) and filtering (< 2 mm Hg vacuum) onto black 0.2  $\mu$ m polycarbonate filters (Poretics Corporation, Livermore, USA.), placed over 2  $\mu$ m filters to distribute the vacuum (Fuhrman & Azam, 1982). The 0.2  $\mu$ m filters were placed on microscope slides with a drop of immersion oil and covered with a coverslip. The slides were then examined at x 2000 (oil immersion) using epifluorescence microscopy on a Zeiss Photo-microscope III. Bacterial cells in ten randomly selected Whipple Grid fields of view were enumerated using ultra-violet (UV) excitation (365 nm). Autotrophs were identified by their yellow fluorescence and heterotrophs by their blue fluorescence under UV excitation. Cyanobacteria were identified by their orange fluorescence under UV excitation with a blue filter. This method was adapted from that of Porter and Feig (1980).

Bacterial cell volume was determined by measuring the dimensions of a representative sample of cells. The equation below was then used to compute mean cell volume.

 $V = (\Pi/4) \times W^2 \times (L-W/3)$  (Bratbak, 1993)

where:

V = cell volume ( $\mu$ m<sup>3</sup>) L = cell length ( $\mu$ m) W = cell width ( $\mu$ m)

The formula was based on the assumption that the bacteria were straight rods with hemispherical ends, but worked equally well for cocci. Mean cell volume was subsequently converted into carbon biomass using the conversion factor 0.22 pg C  $\mu$ m<sup>3</sup> (Bratbak & Dundas, 1984).

#### 2.2.3.2: Nanoflagellate abundance, mean cell volume and carbon biomass

Heterotrophic and phototrophic nanoflagellate (HNAN and PNAN, respectively) abundance and mean cell volume were determined by staining 50 ml aliquots with DAPI and filtering onto 2.0  $\mu$ m filters placed over 5.0  $\mu$ m filters. Twenty replicate fields of view were examined, under both UV and blue excitation (450 to 490 nm) to distinguish chlorophyll autofluorescence in PNAN (Birk, 1984) (Photograph 2.2). Representative samples of HNAN and PNAN were measured to compute mean cell volume using the standard geometric equations: a) for approximately spherical nanoflagellates, b) for *Cryptomonas*-like species and c) for *Pyramimonas gelidicola* (Tikkanen, 1986):

Equation a:	$\mathbf{V} = (\Pi \mathbf{x} \mathbf{d}^3)/6$
Equation b:	$\mathbf{V} = (\Pi \mathbf{x} \mathbf{h} \mathbf{x} \mathbf{d}^2)/6$
Equation c:	$V = (\Pi x l x d^2)/12$

where:  $V = cell volume (\mu m^3)$   $d = width (\mu m)$   $h = height (\mu m)$  $l = length (\mu m)$  Biovolume was converted into carbon biomass using the conversion factor 0.22 pg C  $\mu$ m<sup>3</sup> (Børshiem & Bratbak, 1987).

# 2.2.3.3: Ciliate, dinoflagellate, diatom and metazoan abundance, mean cell volume and carbon biomass

Lugol's fixed material was concentrated by settling and counted in a Sedgewick-Rafter counting chamber, using a Zeiss Axioscop microscope at x 160. Where possible a minimum of 100 cells of each species (of ciliate, dinoflagellate or diatom) were enumerated. In addition, a minimum of 50 cells were measured and identified at x 320. Ciliate biovolume was estimated from measurements of cell diameter and length and subsequent equation with the closest geometric shape (equations as in Section 2.2.3.2). This biovolume was then converted into ciliate carbon biomass employing a value of 0.19 pg C  $\mu$ m<sup>3</sup> (Putt & Stoecker, 1989). Dinoflagellate biovolume was estimated as above and converted into carbon biomass using the equation derived by Mullin *et al.* (1966):

 $\log_{10} C = 0.76 \times \log_{10} V - 0.29$ 

where:

C = cell carbon (pg) $V = cell volume (\mu m^3)$ 

Diatom biovolume and biomass were calculated using the closest geometric shape and the conversion factor 0.11 pg C  $\mu$ m<sup>3</sup> (Strathmann, 1967).

The abundance of *Paralabidocera antarctica* (Copepoda: Calanoida), *P. antarctica* faecal pellets and *Notholca* sp.(Rotifera: Monogononta), in the whole sample were recorded. Copepodid and nauplius biomass were estimated using the equations derived by Tanskanen (1994) for *Acartia bifilosa*, assuming that prosome length was 77.2 % of total body length. Rotifer biovolume was estimated using appropriate geometric equations and a conversion factor of 0.20 pg C  $\mu$ m<sup>3</sup> (P. Bayliss, personal communication).

# 2.2.3.4: Chlorophyll a concentration

Chlorophyll *a* samples were filtered onto 47 mm Whatman GF/F glass fibre filters. The chlorophyll was extracted in methanol during a 20 h period of dark refrigeration (Jones,

1977) and the final concentrations determined spectrophotometrically at 665 nm (the main chlorophyll *a* peak) and 750 nm (background reading) using a GBC UV/VIS 916 spectrophotometer (Talling, 1974). On each sampling occasion 90 % methanol was employed to calibrate the instrument taking into account the dilution effect of the water saturated filters. The amount of chlorophyll *a* contained in each extract was calculated and converted to  $\mu g l^{-1}$  as follows:

# Chlorophyll *a* ( $\mu$ g l<sup>-1</sup>) = 13.9 x (D<sub>665</sub> - D<sub>750</sub>) x (v / (V x 4)) (Talling, 1974)

where:

 $D_{665}$  = spectrophotometer reading at 665 nm  $D_{750}$  = spectrophotometer reading at 750 nm V = volume filtered (l) v = volume of 90 % methanol (ml) 4 = pathlength of cuvette (cm)

#### 2.2.3.5: Nutrient analyses

The filtrate from the chlorophyll samples was collected for nutrient analysis. Soluble reactive phosphorus (SRP), ammonia (NH<sub>3</sub>), nitrate (NO<sub>3</sub>) and nitrite (NO<sub>2</sub>) concentrations were determined using standard wet chemical methods (Parsons *et al.*, 1984). Water samples for ammonia determination were analysed within 48 hours. Where possible, the remaining nutrients were analysed soon after but if necessary, the samples were stored frozen at -20 °C in pre-muffled, glass bottles.

#### 2.2.3.6: Dissolved and particulate organic carbon analyses

In the case of DOC and POC, three 60 ml subsamples from each depth were filtered through 25 mm GF/F glass fibre filters, pre-muffled at 450 °C for 8 h. The filtrates were stored frozen in pre-muffled Kimble bottles until they could be analysed, following acidification with sulphuric acid, using a Shimadzu TOC-5000 total organic carbon analyser. The filters were dried overnight at 60 °C and similarly stored frozen for POC mean ash dry weight analysis at a later date.

#### 2.2.3.7: Dissolved Oxygen

On return to the laboratory, dissolved oxygen was determined by the Winkler method (Parsons *et al.*, 1984). The precipitated manganous hydroxide was acidified in the presence of iodide, reverting the oxidised manganese to its divalent state and liberating

iodine equivalent to the oxygen dissolved in the original sample. Subsequently, this liberated iodine was titrated with standardised thiosulphate, using starch to determine the end point, to yield the absolute concentration of dissolved oxygen in the original sample.

### 2.2.3.8: Ice cores

The bottom 20 cm of each of three seasonal ice cores brought back from Ace Lake were melted down in, first 0.45  $\mu$ m then 0.22  $\mu$ m, filtered seawater. A 1:1 weight to volume ratio was used to ensure the correct final salinity and prevent cell lysis. Cells within the melt were subsequently concentrated onto an 0.8  $\mu$ m filter and this concentrate was fixed in either glutaraldehyde or Lugol's iodine. Three 1 ml subsamples were settled in counting chambers per ice core and examined at 40 x using a Zeiss Axiovert 135 microscope.

All seasonal data were mapped using the Surface Mapping System, Surfer Version 5.02, using the Kriging method of interpolation.

# 2.3: **RESULTS**

# 2.3.1: Physico-chemical characteristics of Ace Lake

#### 2.3.1.1: Ice cover

Complete ice-cover was maintained on Ace Lake until the end of December 1996. Maximum ice thickness was achieved in October 1996 (1.6 m). The lake was completely ice-free for only six weeks of each year between January and early March (Figure 2.3).

# 2.3.1.2: Temperature

A marked thermal stratification was evident in Ace Lake throughout the year (Figure 2.4). An inverse temperature profile exists in Ace Lake (Vincent, 1988) which Burch (1988) suggested could be divided into four thermal zones. In the mixolimnion, a near-surface zone remained at  $-1^{\circ}$ C to  $1^{\circ}$ C for most of the year, however, during the ice-free period this zone warmed to 5 °C. A thermocline region was evident between 6 and 8 m, in which the temperature increased by as much as 5 °C over a narrow depth interval. This region was seen to broaden and move upward during the summer heating period. In the monimolimnion, a zone existed throughout which temperatures increased steadily



Figure 2.3: Annual variation in ice-cover on Ace Lake

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-7		+	+		+	+	+	+	+	+	+	+ ·	+	+ +	+	+	+	+	+	F
-8		+	+		t	+	+	+	+	+	+	+	+	+ +	+	+	+	+	+	F
-9		+	+		+	+	+	+	+	+	+	+	+	+ +	+	+	+	+	+	F
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# Figure 2.4: Depth-time isotherms in Ace Lake, December 1995 - February 1997 (degrees Centigrade) + sample points; x ice thickness



9

with depth to a maximum of 9 °C before, declining steadily in the zone between 13 and 23 m.

### 2.3.1.3: Salinity

The fact that an inverse profile exists demonstrates the comparative weakness of thermally induced density gradients in Ace Lake. In common with other meromictic lakes, salinity is the major factor maintaining the long-term stratification of Ace Lake (Figure 2.5). A marked halocline was apparent in Ace Lake between 6 and 8 m, which was highly stable when compared to the thermocline. In this region, conductivity was observed to increase by up to 12 mS cm<sup>-1</sup> over a 2 m depth interval. The mixolimnion had a conductivity of 8 to 16 mS cm<sup>-1</sup>. However, in summer meltwaters decreased the conductivity of the surface waters by up to 8 mS cm<sup>-1</sup>, the minimum recorded was 10.3 mS cm<sup>-1</sup> in January 1997. Conductivity in the monimolimnion ranged between 20 and 40 mS cm<sup>-1</sup>.

This salinity stratification enhanced the stability of the water column when the lake was ice free, the period in which the meromixis was threatened by wind-mixing. Maximal vertical penetration of wind-mixing occurred in February 1996 and 1997. These results illustrate the potential annual variation. The zone of wind driven mixing appeared to be deeper in the summer of 1997 and meteorological data corroborates this. In February 1997 the Vestfold Hills region experienced 10 days of strong winds (>21 kt) and 4 days of gale force winds (>33 kt), making it the third windiest February on record.

# 2.3.1.4: Dissolved Oxygen

Ace Lake's stratification can also be described in terms of its dissolved oxygen concentration (DO) (Burton, 1980; 1981a). During this study, the mixolimnion and the upper monimolimnion (0 to 10 m) were oxygenated. Throughout DO slowly declined from a maximum of 11 mg l<sup>-1</sup> to approximately 6 mg l<sup>-1</sup>. Between 10 and 12 m an oxycline existed in which DO rapidly reduced to zero (Photograph 2.3). No oxygen was detected below 12 m (Figure 2.6). The anoxic waters of the monimolimnion extended from 12 m downwards. In the absence of ice the gradual decline in DO concentration was relatively constant, however, once an ice-cover had developed a pattern of two maxima seemed to be adopted, one lying at approximately 4 m and the other at 8 m, before a rapid decline across the oxycline ensued. These maxima corresponded closely with maxima observed in photosynthetic bacteria and nanoflagellates (Figures 2.15 & 2.17).

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# Figure 2.5: Depth-time isopleths for the salinity of Ace Lake, December 1995 - February 1997 (mS/cm) + sample points; x ice thickness



**Photograph 2.3:** Illustration of the oxycline at approximately 11.5 m in Ace Lake. The boundary between clear oxic waters and yellow anoxic waters is clearly visible in the kemmerer bottle



**Photograph 2.4:** Deep chlorophyll maximum at 12 m in Ace Lake. The sample on the far right is from Rookery Lake



#### Figure 2.6: Dissolved oxygen concentration in Ace Lake over time (mg/l) + sample points; x ice thickness

#### 2.3.1.5: Photosynthetically Active Radiation

Typical profiles of Photosynthetically Active Radiation (PAR) levels in Ace Lake are illustrated in (Figure 2.7) (T. Pitman & L.M. Rankin, personal communication). Incident photosynthetic radiation varied by over two orders of magnitude throughout the year. Surface quantum irradiation ranged from 300.5  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> in December to 8.6  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> in August. In December 1992 complete attenuation was achieved by 12 m, in August the limit of light penetration was reduced to approximately 9 m. No light was detectable below 12 m at any time in Ace Lake, due to a band of photosynthetic sulphur bacteria.

#### 2.3.1.6: Nutrients

Soluble reactive phosphorus (SRP), was the most abundant of the nutrients analysed, detectable throughout the water column in all but the uppermost regions (Figure 2.8). Concentrations in the mixolimnion ranged from undetectable to 20  $\mu$ g l<sup>-1</sup>. The highest concentrations occurred consistently in the lower, anaerobic monimolimnion (> 300  $\mu$ g l<sup>-1</sup>). A broadening of this concentration band was apparent concomitant with ice formation. By April 1996 a full ice-cover had developed and SRP levels between 10 and 12 m rose rapidly. The maximum SRP concentration of > 360  $\mu$ g l<sup>-1</sup> was attained in September 1996, one month prior to the October peak in ice thickness. Conversely, as ice thickness diminished, SRP concentrations decreased and the concentration band narrowed. In the months when Ace Lake was totally ice free, SRP levels within the monitored water column were relatively constant.

Similarly, ammonia was usually limiting in the mixolimnion, but increased markedly in the monimolimnion (Figure 2.9). Levels were observed to increase throughout the water column as winter progressed. The concentrations within the mixolimnion ranged from undetectable to 21.6  $\mu$ g l<sup>-1</sup>, whereas concentrations within the oxycline reached levels of > 700  $\mu$ g l<sup>-1</sup>. Nitrate concentrations were consistently low at all depths ranging from 0.02  $\mu$ g l<sup>-1</sup> to 1.12  $\mu$ g l<sup>-1</sup> where detectable (Figure 2.10). Little variation in concentration was observed, although the concentrations at 10 m tended to be greater than those higher in the water column. Concentrations were also greater in the summer, the maximum concentration of 1.12  $\mu$ g l<sup>-1</sup> was measured in December 1995. Nitrite remained virtually undetectable throughout the study period with a range of 0.02 to 1.19  $\mu$ g l<sup>-1</sup> when present (Figure 2.11). An anomalous peak in October 1996 was probably the result of experimental error.



Figure 2.7: Photosynthetically active radiation in Ace Lake in December (open circles) and August (closed circles). Data from 1992 (T. Pitman & L.M. Rankin, personal communication)



Figure 2.8: Soluble reactive phosphorus concentration in Ace Lake over time (ug/l) + sample points; x ice thickness



#### Figure 2.9: Ammonia concentration in Ace Lake over time (ug/l) + sample points; x ice thickness



#### Figure 2.10: Nitrate concentration in Ace Lake over time (ug/l) + sample points; x ice thickness



#### Figure 2.11: Nitrite concentration in Ace Lake over time (ug/l) + sample points; x ice thickness

#### 2.3.1.7: Dissolved and particulate organic carbon

Dissolved organic carbon (DOC) concentrations showed significant seasonal variation ranging between 4.5 mg l<sup>-1</sup> and 15.2 mg l<sup>-1</sup> (Figure 2.12). Distribution of DOC throughout the water column showed little consistent variation, however, concentrations were generally higher in the monimolimnion across the oxycline. POC ranged between 83.8 and 416.7 mg l<sup>-1</sup> throughout the year. A peak was noted in the mixolimnion in February 1996, but was not apparent in the following February (Figure 2.13).

# 2.3.2: Biological characteristics of Ace Lake

### 2.3.2.1: Chlorophyll a

The chlorophyll *a* (Chl *a*) values recorded in the mixolimnion reflected the light and nutrient limitation of the phytoplankton (Figure 2.14). Chl *a* concentrations showed surprisingly little seasonality. Throughout the winter, levels in the mixolimnion remained relatively constant within the range of 0.32 to 4.42  $\mu$ g l<sup>-1</sup>. Even during the midwinter period when solar radiation reached a minimum and the sun did not rise above the horizon for approximately seven weeks, there was no marked decline in Chl *a* concentration. A Deep Chlorophyll Maxima (DCM) was apparent throughout the year just below the thermocline/halocline and in the oxycline. In this, chlorophyll *a* concentrations reached a maximum of 167.5  $\mu$ g l<sup>-1</sup> (Photograph 2.4).

### 2.3.2.2: Bacterioplankton

A bacterial 'plate' resided at the oxylimnion. In this zone bacterial abundances were consistently maximal. Concentrations of photosynthetic bacteria ranged between 0.02 x  $10^8$  cells l<sup>-1</sup> to 8.94 x  $10^8$  cells l<sup>-1</sup>, peaking in January 1997 at 12 m (Figure 2.15). A second maxima in bacterial autotroph numbers was observed at 8 m in most instances, corresponding with favourable growth conditions at the thermocline/halocline. While some of these were picocyanobacteria contributing to photosynthesis in the mixolimnion, the majority were Proteobacteria confined to the anoxic lower waters.

Heterotrophic bacterial abundance exceeded that of photosynthetic bacteria throughout the study period, ranging from  $1.26 \times 10^8$  cells l<sup>-1</sup> to  $72.80 \times 10^8$  cells l<sup>-1</sup> (Figure 2.16).



Figure 2.12: Dissolved organic carbon concentration in Ace Lake over time (mg C/l) + sample points; x ice thickness



#### Figure 2.13: Mean ash dry weight in Ace Lake over time (mg C/l) + sample points; x ice thickness



#### Figure 2.14: Chlorophyll a concentration in Ace Lake over time (ug/l) + sample points; x ice thickness



Figure 2.15: Spatial and temporal distribution of autotrophic bacteria in Ace Lake (cells/l+E08) + sample points; x ice thickness


Figure 2.16: Spatial and temporal distribution of heterotrophic bacteria in Ace Lake (cells/1+E08) + sample points; x ice thickness

The highest densities occurred in the monimolimnion at the oxycline, and were probably sulphate reducing bacteria which produced hydrogen sulphide as a byproduct, rendering the waters of the oxycine yellow in colouration (Photograph 2.3). A peak in aerobic heterotrophic bacteria occurred in the mixolimnion during winter (June/July) when DOC was relatively high (8.00 mg  $l^{-1}$ ). A second, smaller maximum in bacterial abundance in November followed elevated DOC concentrations in October (8.00 to 9.00 mg  $l^{-1}$ ).

The mean cell volume of bacteria exhibited a marked seasonality. The abundance of photosynthetic bacteria showed the greatest variation, ranging between 1.4 x  $10^1 \ \mu m^3$  and 2.0 x  $10^3 \ \mu m^3$ . Heterotrophic bacterioplankton abundance ranged from 1.5 x  $10^1 \ \mu m^3$  to 2.0 x  $10^2 \ \mu m^3$ . Maximum biovolume was achieved by the photosynthetic and heterotrophic bacteria in July and December, respectively. Biomass ranged between 0.25 to 1.79 x  $10^6 \ \mu g \ C \ l^{-1}$  for photosynthetic bacteria and 0.19 to 1.46 x  $10^8 \ \mu g \ C \ l^{-1}$  for heterotrophic bacteria.

### 2.3.2.3: Nanoflagellates

Photosynthetic nanoflagellate (PNAN) numbers peaked during the summer months The highest densities occurred between the base of (Figure 2.17). the thermocline/halocline (8 m) and the top of the oxycline (12 m) in the water column. These PNAN maxima formed Deep Chlorophyll Maxima (DCM) which appeared to shift position seasonally, moving down from 8 m in the summer to 10 m in the autumn. Dissolved oxygen maxima occurred concomitantly as a result of photosynthesis. No diel migration of PNAN was noted. The most common PNAN species was Pyramimonas gelidicola McFadden (Chlorophyta: Prasinophyceae), with lower numbers of Cryptomonas sp. (Chlorophyta: Cryptophyceae), and a chlamydomonad sp. (Chlorophyta: Chlorophyceae) (Plate 2.1). P. gelidicola reached densities of 4.60 x 10<sup>6</sup> cells 1<sup>-1</sup> and was observed in all months except August (Figure 2.17). Interestingly, during the winter it lost its chlorophyll. Preliminary experiments using bacteria sized fluorescently labelled microspheres (FLM) revealed phagotrophic behaviour, suggesting that P. gelidicola may have been employing mixotrophy as a survival strategy. This will be discussed further in Chapter 5. Cysts of P. gelidicola were also observed (Plate 2.1). The maximum abundance of 1.16 x  $10^7$  cysts  $l^{-1}$  occurred in November 1996, preceding the active bloom in December.

Heterotrophic nanoflagellates (HNAN) occurred throughout the water column in both the mixolimnion and upper monimolimnion (Figure 2.18). Taxonomic analysis



Figure 2.17: Spatial and temporal distribution of autotrophic flagellates in Ace Lake (cells/l+E06) + sample points; x ice thickness



Figure 2.18: Spatial and temporal distribution of heterotrophic flagellates in Ace Lake (cells/l+E06) + sample points; x ice thickness

Depth (m)

- 14 - 12 - 10 - 8 - 6 - 4 - 2

20 18

16

indicated that there were two distinct communities. Community abundance in the upper monimolimnion remained fairly high throughout the year (0.92 to 40.2 x  $10^6$  cells  $1^{-1}$ ), reflecting the abundant bacterial food resources in this region of the water column. In contrast the aerobic mixolimnion community showed a late summer maximum in 1996 and an indication of the development of a late summer maximum in 1997. A small winter peak was also apparent. These abundance patterns coincided with peaks in photosynthetic and heterotrophic bacteria.

Total nanoflagellate biomass derived from cell counts varied between 1.22 and 44.41  $\mu$ g C l<sup>-1</sup>, at peak biomass HNAN accounted for approximately twice that of the PNAN.

## 2.3.2.4: Dinoflagellates

Photosynthetic dinoflagellates, chiefly *Gyrodinium* sp., *Gymnodinium* sp. and *Gonyaulax* sp. (Sarcomastigophora: Dinoflagellida) (Figure 2.19; Plate 2.2) were present at relatively low abundances throughout the winter. Their abundance increased dramatically in late spring and summer to a maximum of 2.65 x  $10^4$  cells  $1^{-1}$  in early December 1996. Subsequently a mass encystment of the community was observed in late summer. By February 1997 dinoflagellate cysts had reached an abundance of 0.29 x  $10^4$  cysts  $1^{-1}$  (Figure 2.20; Plate 2.2). Dinoflagellate biomass derived from cell counts varied between 0.04 and 3.45 µg C  $1^{-1}$ . The choanoflagellate, *Acanthoecopsis unguiculata* Thomsen (Choanoflagellida: Acanthoecideae) was also observed on occassion (Plate 2.2).

#### 2.3.2.5: Diatoms

Diatoms were present in low numbers throughout the year, with the exception of February to April 1996 (Figure 2.21). During the winter cells generally concentrated in the upper 4 m of the mixolimnion however, from August onwards abundance increased throughout the mixolimnion and upper monimolimnion. The maximum abundance was  $2.48 \times 10^5$  cells l<sup>-1</sup> in January 1996 (Plate 2.3).

#### 2.3.2.6: Ciliated Protozoa

The unique marine photosynthetic ciliate, *Mesodinium rubrum* Lohmann (Ciliophora: Haptorida), was an important component of the phytoplankton in Ace Lake and dominated the ciliate community (Figure 2.22: Plate 2.2). Although *M. rubrum* was observed throughout the year, the highest abundance was recorded in summer (maximum  $6.2 \times 10^4$  cells l<sup>-1</sup>). The cells concentrated in the upper 4 metres of the



#### Figure 2.19: Spatial and temporal distribution of dinoflagellates in Ace Lake (cells/l) + sample points; x ice thickness



# Figure 2.20: Spatial and temporal distribution of dinoflagellate cysts in Ace Lake (cells/l) + sample points; x ice thickness



#### Figure 2.21: Spatial and temporal distribution of diatoms in Ace Lake (log10 cells/l) + sample points; x ice thickness

3.0 2.5 2.0 1.5 1.0 0.5

0.0

5.0

4.5

4.0



Figure 2.22: Spatial and temporal distribution of <u>Mesodinium rubrum</u> in Ace Lake (log10 cells/l) + sample points; x ice thickness

mixolimnion. Mean cell volume was typically 9.3 x  $10^3 \ \mu m^3$  and exhibited little seasonal variation. During November a portion of the *M. rubrum* population began to encyst. By January cyst numbers had increased to 4.1 x  $10^4$  cysts l<sup>-1</sup>. Cysts were absent from the water column in winter suggesting that they had settled out (Figure 2.23).

The ciliate community (including M. rubrum) showed distinct seasonality (Figure 2.24). Cell concentrations fell to very low levels in winter (August) increasing towards a peak in mid-summer (December). The species diversity of the ciliate community was low. The only common ciliate besides M. rubrum was Euplotes sp., however its abundance was low in comparison to M. rubrum, and occurrence confined to the summer months.

## 2.3.2.7: Metazoa

Although the plankton of Ace Lake was dominated by microbes, a small number of Metazoa did occur. The endemic, euryhaline, marine calanoid copepod, *Paralabidocera antarctica* Thompson, was the only species of planktonic crustacean found to inhabit Ace Lake (Figure 2.25). A peak of copepodid stages (64 individuals  $l^{-1}$ ) was recorded in January 1997 at 12 m. Naupliar stages were observed throughout the year with the exception of February and March 1996, and exhibited cyclical maxima at roughly two monthly intervals between September 1996 and January 1997. Peak naupliar abundance (50 individuals  $l^{-1}$ ) was reached at 10 m in late November, however, similar naupliar abundances were recorded throughout the summer at this depth (Figure 2.26). The mean body length of copepodids and nauplii was 0.8 mm and 0.2 mm, respectively.

The numbers of faecal pellets varied concomitant with copepod abundance (Figure 2.27). Maximum abundance was observed in December at > 5000 pellets  $l^{-1}$  however, the average abundance was far lower at 88 pellets  $l^{-1}$ . As would be expected, in general faecal pellet maxima occurred lower in the water column than the correspondent copepod maxima. The average length of the faecal pellets was 79.3 µm and the mean volume 87.2 µm<sup>3</sup>.

A species of *Notholca* (Rotifera: Monogononta) was observed in low numbers. A maximal abundance of 6 individuals  $l^{-1}$  was recorded in January 1997 at a depth of 6 m (Figure 2.28).

## 2.3.2.8: Ice core content



Figure 2.23: Spatial and temporal distribution of <u>Mesodinium rubrum</u> cysts in Ace Lake (log10 cells/l) + sample points; x ice thickness

Depth (m)



#### Figure 2.24: Spatial and temporal distribution of ciliates in Ace Lake (log10 cells/l) + sample points; x ice thickness



#### Figure 2.25: Spatial and temporal distribution of <u>Paralabidocera antarctica</u> in Ace Lake (individuals/l) + sample points; x ice thickness



Figure 2.26: Spatial and temporal distribution of Paralabidocera antarctica nauplii in Ace Lake (individuals/l) + sample points; x ice thickness



Figure 2.27: Spatial and temporal distribution of <u>Paralabidocera antarctica</u> faecal pellets in Ace Lake (log10 numbers/l) + sample points; x ice thickness

Depth (m)

2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2

0.0

3.0 2.8 2.6



#### Figure 2.28: Spatial and temporal distribution of Notholca sp. in Ace Lake (individuals/l) + sample points; x ice thickness

The main aim of taking ice cores was to look for evidence of an ice dependent stage for the copepod, *P. antarctica* (Tanimura *et al.*, 1996). However, no evidence was found of *P. antarctica* (although a single nauplius individual was observed, perhaps trapped as the ice froze). The only organisms found consistently were diatoms, *Nitzschia longissima* in particular. These reached abundances of up to 2.07 x  $10^3$  cells l<sup>-1</sup> in the spring (Figure 2.29; Plate 2.3).

#### 2.3.2.9: Relative contribution of the microbial fractions to the carbon pool

Table 2.1 and Figure 2.30 summarise the relative contribution of the different microbial components of Ace Lake's food web to the microbial carbon pool. Heterotrophic bacteria dominated the biomass in the mixolimnion throughout autumn, winter and spring, and in each season in the monimolimnion. HNAN were the second highest contributors in each instance. Both the phototrophic bacteria and PNAN made limited contributions. The proportion of the carbon pool contributed by the ciliate community reached a maximum in the summer throughout the water column. *Mesodinium rubrum* dominated in the mixolimnion at this time, surpassing the individual contributions made by the other microbial components. Its contribution to the carbon pool diminished during autumn and winter. The dinoflagellate carbon contribution was relatively small and limited to spring and summer. The *Paralabidocera antarctica* copepodid contribution was marked during the summer months. In contrast, nauplii made their highest relative biomass contribution made by both the diatoms and rotifers were negligible.

#### 2.4: DISCUSSION

#### 2.4.1: Physico-chemical characteristics of Ace Lake

#### 2.4.1.1: The stratification of Ace Lake

Most Antarctic lakes are covered by ice through all but a few weeks of the year. This single feature has a pervasive influence on the physical, chemical and biological properties of Ace Lake. As is apparent from the results, lake ice greatly reduces the transfer of wind energy and momentum to the water column beneath and thus promotes physically stable conditions throughout the year. Lack of turbulence also favours motile ciliates, flagellates and cyanobacteria able to adjust their position in the water column.



Figure 2.29: Seasonal ice core content with standard error bars

Microbial component	Mixolimnion				Monimolimnion			
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
Autotrophic bacteria	0.2459	1.5062	0.2062	0.6779	0.1346	0.7918	0.2679	2.0600
Heterotrophic bacteria	15.7010	27.3563	10.4830	12.2005	15.1447	27.2450	32.2386	27.7392
PNAN	1.0886	0.3234	2.4384	4.6753	0.3184	0.2337	1.0544	13.6236
HNAN	7.6261	4.5316	3.3212	9.5805	10.6852	11.0831	9.8507	9.3295
M. rubrum	0.9767	0.0907	1.3092	22.8230	0.4066	0.0558	0.6557	4.1472
Other ciliates	0.3450	0.0935	0.0175	5.8770	0.1237	0.0056	0.1034	3.2038
Dinoflagellates	0.1884	0.1486	1.1891	3.4460	0.10863	0.0414	0.7220	2.2315
Diatoms	<0.0001	0.0031	0.0276	0.0732	0	<0.0001	0.1144	0.0869
P. antarctica copepodids	1.4770	1.3715	0.8324	<u>9.7</u> 766	1.3187	0.2637	1.2600	15.6455
P. antarctica nauplii	0.1783	0.4829	0.5994	1.0006	0	0.3343	0.8671	1.5476
Notholca sp.	0	0	0	0.0912	0	0	0	0.0912

Table 2.1: Seasonal biomass of the microbial components of Ace Lake ( $\mu g C l^{-1}$ )







Plate 2.1: The dominant nanoflagellate species in Ace Lake. (a) Pyramimonas gelidicola, (b) Pyramimonas gelidicola cyst, (c) the characteristic 'hairy' flagellae of P. gelidicola and (d) P. gelidicola scales, (e) a chlamydomonad sp. and (f) its encystment phase, (g) Cryptomonas sp. and (h) Paraphysomonas sp.



Plate 2.2: The dominant dinoflagellates, choanoflagellate and ciliate in Ace Lake.

- (a) Gymnodinium lachyna, (b) Gymnodinium sp. cyst, (c) Gonyaulax sp.,
- (d) unidentified cyst, (e) Acanthoecopsis unguiculata and (f) Mesodinium rubrum



Plate 2.3: The dominant diatom species in Ace Lake. (a) Navicula distans,
(b) Fragilariopsis centris, (c) Pinnularia sp., (d) Navicula sp., (e) Pinnularia sp., (f) a diatom chain, (g) Pinnularia sp. and (h) Nitzschia longissima



The results indicate an inherently stable stratification of Ace Lake both thermally and chemically. A thermocline/halocline lay at between 6 and 8 m in the water column throughout the sampling period (Figures 2.1, 2.4 & 2.5). The position of the thermocline/halocline in Ace Lake varies annually. Recently, Ace Lake experienced a period of positive water balance i.e. lake levels increased. However, Ace Lake water levels are now beginning to decline (J.A.E. Gibson, personal communication). Periods of negative water balance decrease total lake volume and thus increase salinity. Assuming the same annual ice-cover and thickness, this leads to an increased density and increased penetration of the mixolimnion. As a result the stratification boundaries described in 1995 to 1997 were deeper than those described in 1979 when the thermocline lay at least 2 m above their present position (Burch, 1988; Gibson & Burton, 1996).

Despite maximal solar inputs occurring at the December solstice, effective heating of the water column and destabilisation of the thermocline did not commence until late January, early February when the ice and snow cover had diminished sufficiently to allow wind-driven mixing of the mixolimnion. This contrasts with lakes such as hypersaline Deep Lake, Vestfold Hills, which are ice-free throughout the year and warm coincidentally with increasing solar radiation in the spring (Burch, 1988). As ice-cover diminished a lens of relatively fresh meltwater developed at the lake surface strengthening the salinity gradient (Figure 2.5). Conversely, during winter surface salinity increased from a summer low of 10 mS cm<sup>-1</sup> to a high of 19.2 mS cm<sup>-1</sup>, weakening the salinity stratification and deepening the halocline by approximately 1 m. This effect was attributable to freeze concentration, the exclusion of salt by the developing ice-cover (Niedrauer & Martin, 1972; Martin, 1979; Gibson & Burton, 1996). In general, ice-cover buffers thermal changes within the water column. This was illustrated within the monimolimnion where annual temperatures exhibited little variation (Figure 2.4).

In addition, Ace Lake had three well defined strata in terms of its measured dissolved oxygen content (DO) content (Figure 2.6). An oxygenated stratum lay between 0 and 10 m, an oxycline between 10 and 12 m, and an anoxic stratum extended downwards from 12 m. A DO maxima was apparent at 8 m corresponding with the thermocline/halocline and increased autotrophic nanoflagellate and bacterial abundances. In this respect, Ace Lake exhibits remarkable similarities to Lake Fryxell in the Taylor Valley, Dry Valleys which has permanent gradients of temperature, salinity and oxygen down to 9.5 m (Vincent, 1988).

Ice-cover coupled with low annual Photosynthetically Active Radiation (PAR) limits light penetration into the water column. The extent of the limitation is dependent upon albedo, the fraction of ice with dark material in it, attenuation coefficient of the ice, ice thickness and snow cover (Vincent, 1988). Unlike the lakes of the McMurdo Dry Valleys, Lake Miers and Trough Lake in particular, wind-blown sediments and moraine do not accumulate in the ice-cover of Ace Lake, thus, the associated light limitation does not occur (Vincent, 1988). In addition, due to its north-south aspect and proximity to the plateau, katabatic winds are channelled along the lake surface and remove the majority of snow from the ice surface. However, attenuation is still rapid. At the height of summer light levels below the ice in Ace Lake were 300  $\mu$ mol m<sup>-2</sup> sec<sup>-1</sup>, decreasing to < 50  $\mu$ mol m<sup>-2</sup> sec<sup>-1</sup> at 4 m (T. Pitman & L.M. Rankin, personal communication) (Figure 2.7). Thus, low light levels are likely to inhibit primary production in Ace Lake.

## 2.4.1.3: The nutrient status of Ace Lake

The stratification in Ace Lake influenced the vertical pattern of nutrients and organic matter throughout the water column. Studies of Lake Vanda, McMurdo Dry Valleys, suggested that phosphorus availability can exert an overall control on phototrophic biomass in high latitude, oligotrophic lakes (Vincent & Vincent, 1982). In Ace Lake, soluble reactive phosphorus (SRP) was observed in low concentrations throughout the mixolimnion and aerobic monimolimnion, but rose abruptly across the oxycline (Figure 2.8). The increased availability of this limiting element accounted for the population maxima of photosynthetic bacteria at 12 m. Potentially, this bacterial population severely restricted the availability of phosphorus to phototrophs living under more favourable light conditions higher in the water column (Vincent, 1988). In Lake Vanda, McMurdo Dry Valleys, communities of phototrophs dwelling above the bacterial oxycline receive some additional SRP inputs from a single inflow, the Onyx River. However, Ace lake has no such inflows and it is likely that phosphorus recycling rates were rapid at all depths in order to sustain the autotrophic demand. Azam and Cho (1987) stated that clustered bacteria create a high phosphorus microzone around an algal cell. Most of this phosphorus is released by the bacterioplankton themselves sustaining a high phosphorus concentration within the micro-environment and accounting for the close correlation between phosphate levels and bacterial maxima observed at the oxycline.

It is clear that maximal SRP concentration also corresponded with increased ammonia levels. noteably between April and November (Figures 2.8 & 2.9). The preference for nitrogen in the form of ammonia  $(NH_3)$  or ammonium  $(NH_4)$  over nitrate  $(NO_3)$  is a well known phenomenon in aquatic systems (Wheeler et al., 1982; Dortch, 1990) and is of fundamental importance in Antarctic systems. In Antarctic waters it has been shown that, 30 to 90 % of nitrogen demanded for primary production can be satisfied by regenerated ammonium despite nitrate concentrations of 15 to 30 µmol 1<sup>-1</sup> (Rönner *et* al., 1983; Koike et al., 1986). LeCome (1993) demonstrated that Gyrodinium aureolum blooms could be maintained almost exclusively by such in situ bacterial remineralisation of remnants of previous diatom blooms. Such direct use of ammonium spares the reduction potential, and hence energy, that phytoplankton would otherwise use to reduce nitrate to ammonium. This can mean a saving of as much as one third of the reducing power produced by photosynthesis essential in low light environments (Lasoda & Guerrero, 1979; Syrett, 1981). In addition, both nitrate and nitrite were present in low or undetectable concentrations throughout the study (Figures 2.10 & 2.11), whereas phosphate and ammonia concentrations increased markedly at 8 and 12 m concomitant with increasing ice-cover, decreasing light levels (Moon & Matuyama, 1998) and increasing bacterial abundance, corroborating the fact that such regenerative fluxes are taking place in Ace Lake (Figures 2.8 & 2.9).

The source of regenerated ammonia in Ace Lake was the monimolimnion, primarily due to sulphate-reduction. Much of this ammonia was free to diffuse upwards, beyond the oxycline and into the mixolimnion. The density of photosynthetic bacteria at the oxycline was a less effective trap for ammonia than for phosphate and, thus, some diffused further up. A portion of this ammonia would have been oxidised by nitrifying bacteria into nitrate, nitrite and nitrous oxide, which exhibited distinct bands in the water column at certain times of the year, noteably in December 1995 and October/November 1996 when a simultaneous decline in ammonia levels was observed. These oxidised nitrogen compounds had two possible fates: they may have been assimilated by phytoplankton and/or diffused downwards into the anoxic monimolimnion to be consumed by denitrifying bacteria.

Adequate levels of ammonia and orthophosphate were available to support production in the monimolimnion (Hand & Burton, 1981). However, although phosphate levels in the mixolimnion were in excess of the 0.076 g  $l^{-1}$  Fortner *et al.* (1976) found was not limiting in Lake Bonney, Dry Valleys it would, therefore, appear that the concentration of inorganic nitrogen potentially limits production in the mixolimnion of Ace Lake (refer to Chapter 3).

It is likely that Ace Lake receives virtually no allochthonous inputs of carbon, although this remains to be experimentally proven. Instead, as with most other Antarctic lacustrine systems, it can be assumed that the organic carbon in the lake is autochthonous carbon, fixed in situ during autotrophic production (Hand & Burton, 1981: Bayliss et al., 1997). Dissolved organic carbon (DOC) levels in Ace Lake remained relatively high throughout the year. Seasonal variations were apparent but levels never dropped below 4.5 mg  $l^{-1}$ . This minimum concentration exceeded that measured in other lakes in the Vestfold Hills, for example ultra-oligotrophic Crooked Lake  $(2 \text{ mg } l^{-1})$  and the lakes of the nearby Larsemann Hills  $(0.51 \text{ to } 2.65 \text{ mg } l^{-1})$ , and was in the upper range recorded in temperate, oligotrophic Loch Ness, Scotland (2.0 to 4.5 mg l<sup>-1</sup>) (Laybourn-Parry et al., 1994; Bayliss et al., 1997; Ellis-Evans et al., 1998). Instead. DOC concentrations in Ace Lake were in the same order of magnitude as those reported for Lake Fryxell, McMurdo Dry Valleys, and showed a similar increase with depth (McKnight et al., 1991). It is interesting to note that the peak concentration recorded was 15.2 mg l<sup>-1</sup> in the monimolimnion, and although lower, is of the same magnitude as the level recorded in humic Lake Mekkojärvi, Finland (25.1 mg  $l^{-1}$ ) (Salonen et al., 1992).

DOC may originate from several in situ biological processes, but it is ultimately derived from autotrophic production (Carlson et al., 1998). Significant amounts of newly produced DOC have been shown to accumulate during or after phytoplankton blooms supporting heterotrophic microbial populations (Salonen & Hammar, 1986; Carlson et al., 1998 and references therein). Although, in some situations dissolved organic substrate production by phytoplankton is limited (del Giorgio, 1992; del Giorgio & Peters, 1993a; 1994; Karl, 1993). In Ace Lake, dissolved organic carbon supply via phytoplankton exudation (May, 1982), 'sloppy feeding' (Azam & Cho, 1987) or viral and physio-chemical lysogeny (Thingstad et al., 1993) was probably a principle source of DOC. Such sources would have been maximal during peak PNAN and HNAN abundance but, due to their rapid utilisation by bacterioplankton and nanoplankton, a decrease rather than increase in DOC concentration was observed during the summer months (Figure 2.12). However, in late summer/autumn DOC levels rose, particularly around the thermocline, due to the progressive accumulation of dead and dying microbial matter (Figure 2.12). DOC concentrations were consistently higher in the monimolimnion and, coupled with nutrient availability, supported a relatively constant year round bacterioplankton population (the principle oxidisers of DOC) and higher abundances of HNAN at this level in the lake (Figures 2.12, 2.15, 2.16 & 2.18) (Azam & Hodson, 1977). A different scenario was apparent in Lake Fryxell, McMurdo Dry Valleys, where the sediments accounted for the majority of the DOC present in the water column. The remaining fraction derived from algal mats and the plankton. Although no data on the origin of the DOC pool is available it is possible that the sediments and algal mats in Ace Lake made a substantial contribution to the dissolved organic carbon in the lake but further work would be required to confirm this.

POC concentrations in Ace Lake were very high (83.8 to 416.7 mg l<sup>-1</sup>) (Figure 2.13). This may be an artifact of the ashing method employed since the levels observed far exceed the levels recorded in other Antarctic lakes, Loch Ness and humic, Lake Mekkojärvi (Laybourn-Parry & Marchant, 1992b; Salonen *et al.*, 1992; Laybourn-Parry *et al.*, 1994; Priscu *et al.*, 1995; Ellis-Evans *et al.*, 1998). In the lakes of the McMurdo Dry Valleys, Priscu (1995) found that concentrations of POC were closely correlated with chlorophyll *a* concentrations indicating that microbial biomass (bacterial, protozoan and phytoplankton) concentrates around an oxycline when present. Despite the presence of an oxycline in Ace Lake, no such trend was apparent. Instead, POC concentrations were maximal in the mixolimnion in the autumn following the summer plankton blooms (Figure 2.13).

Hand and Burton (1981) suggested that a considerable quantity of the POC in Ace Lake was likely to be the faecal pellets of *Paralabidocera antarctica*. Similarly, Azam (1985) found faecal pellets of *Calanus pacificus* provided a source of POC for bacterial colonisation. High numbers (up to 62 individuals  $1^{-1}$ ) of *P. antarctica* lived in the water column of Ace Lake all year, producing substantial amounts of faecal material (Figures 2.25, 2.26 & 2.27). An accumulation of POC was apparent in Ace Lake following the summer productive phase and copepod maxima, but it was not possible to determine for which proportion of this accumulation faecal material was responsible. In addition, after probable breeding episodes dead copepods colonised by bacteria were observed in the anoxic monimolimnion making a further contribution to the POC pool.

Significant variations in the proportion of total organic carbon (TOC) present in aquatic systems, attributable to either DOC or POC, exist. Carlson *et al.* (1998) investigated the dynamics of the carbon pool and the role it played in the marine carbon cycle during a spring phytoplankton bloom. Their data demonstrated clear differences in the production, biolability, and accumulation of DOC between the Ross Sea polyna and the Sargasso Sea. In the Ross Sea 89 % of the TOC that accumulated was partitioned as POC, with the remaining 11 % partitioned as DOC. In contrast, in the Sargasso Sea 86 % of TOC accumulated as DOC and as little as 14 % as POC. If the POC data are

assumed to be correct, it would appear that POC accounted for a higher proportion of the TOC than DOC in Ace Lake.

## 2.4.2: Biological characteristics of Ace Lake

The stable stratification and resultant physico-chemical conditions have led to the development of two limnetic communities in Ace Lake: an aerobic microbial community in the mixolimnion and upper monimolimnion dominated by nanoflagellates and ciliates, and an anaerobic community dominated by bacterioplankton at and below the oxycline. The aerobic plankton community observed in Ace Lake during this study was compositionally similar to that described for Ace Lake in previous years (Burch, 1988) and for many inland Antarctic waters (Vincent, 1988). The common planktonic species included *Pyramimonas gelidicola* (Chlorophyta: Prasinophyceae), *Cryptomonas* sp. (Chlorophyta: Cryptophyceae), a Chlamydomonad sp. (Chlorophyta: Chlorophyteae), dinoflagellates, in particular *Gyrodinium* sp., *Gymnodinium* sp. and *Gonyaulax* sp. (Sarcomastigophora: Dinoflagellida), and diatoms (Plates 2.1, 2.2 & 2.3).

Previous studies reported that the bacterioplankton of Ace Lake was composed primarily of cyanobacteria, sulphate-reducing and methanogenic bacteria (e.g. Burton, 1980; Hand, 1980; Hand & Burton, 1981; Burke & Burton, 1988; Franzmann *et al.*, 1991; Franzmann *et al.*, 1992; Franzmann & Rohde, 1991; 1992; Rankin & Burton, 1993; Spielmeyer *et al.*, 1993; Bowman *et al.*, 1997; Franzmann *et al.*, 1997; Rankin *et al.*, 1997). No taxonomic work was performed with bacteria during this study.

## 2.4.2.1: Photosynthetic bacteria, phototrophic nanoflagellates and Deep Chlorophyll Maxima

In marked contrast to marine ecosystems, diatoms rarely dominate the phototrophic biomass in Antarctic inland waters. Instead the dominant phototrophs are generally cyanobacteria, phytoflagellates and chlorophytes. The phytoplankton community in Ace Lake was dominated by free-living nanoflagellates. The success of the nanoflagellates is largely attributable to their ability to maintain their position in the water column. They were not subject to wind induced turbulence during ice-free periods and were able to alter their position in the water column according to their requirements. Some nanoflagellate genera appear to be particularly successful in Antarctic lacustrine environments. For example, the genera *Cryptomonas*, *Chlamydomonas* and *Pyramimonas*, have all been observed to form monospecific populations in highly stable lakes (Vincent, 1988). In Ace Lake the dominant phytoflagellate was *Pyramimonas gelidicola*.

The seasonal abundance of *P. gelidicola* during 1996 was comparable to that reported by Burch (1988) for 1979, the lowest abundance occurred in the winter and the highest from late spring to summer when greater PAR was available. However, the highest abundances of PNAN, *P. gelidicola* in particular, were consistently observed in the monimolimnion between the base of the thermocline/halocline and above the oxycline where light was limited (Figures 2.17). A seasonal downward migration of this maximum was apparent through summer and autumn (December to April) with shadeadapted cells moving through the water column in order to reside in a lower light environment as summer progressed and ice-cover diminished. A similar progressive, downward migration in spring/summer was observed in Char Lake, Canada (Kalff *et al.*, 1972). In accordance with Moll *et al.* (1984), no diel migrations were observed during this study.

The development of substantial phytoplankton biomass well below optimum light levels for photosynthesis has been reported in aquatic environments from the tropics (Lagoa Carioca, Eastern Brazil: Reynolds et al., 1983) to Antarctica (Lake Vanda, McMurdo Dry Valleys: Vincent, 1988; Spaulding et al., 1994), in oceans, large lakes, and small lakes (e.g. Pick et al., 1984; Gervais, 1998). These subsurface chlorophyll layers or Deep Chlorophyll Maxima (DCM) are usually found near a strong vertical gradient, such as a thermocline, nutricline, pycnocline or oxycline. DCM are often associated with light levels below the 1 % surface level and always below the 5 % level (Moll et al., 1984). Rodhe (1955) suggested that heterotrophic or photo-heterotrophic uptake of organic solutes by phytoplankton could contribute to survival under such low light conditions. However, later work suggested that low-light adaptation alone was sufficient (e.g. Pechlaner, 1971). Indeed, Antarctic phytoplankton have been shown to possess high photosynthetic efficiencies and be well suited to growth in dimly lit environments, where they utilise nutrients remineralised by bacterioplankton near a lake's oxycline (Vincent, 1981; Wright & Burton, 1981; Priddle et al., 1986; et al., 1996). As well as low light levels, DCM phytoflagellates situated at or near oxyclines may also be subject to dissolved oxygen concentrations lower than 1 mg l<sup>-1</sup>, a situation entirely possible in Ace Lake (Gervais, 1998).

Although DCM have been identified in many aquatic environments, the contribution of these deep-living cells to net areal autotrophic productivity remains in doubt. Estimates range from 2 % to > 90 % of the algal biomass and autotrophic production in lakes (Fee, 1976; 1978a; 1978b; Moll *et al.*, 1984; Konopka, 1989; Gasol *et al.*, 1992; Gasol *et al.*, 1993). As a consequence of this doubt several hypotheses have been developed which attribute DCM formation to passive processes rather than active growth.

However, Steele (1964) modelled the DCM in the Gulf of Mexico and concluded that considerable subsurface growth was required for its formation. Moll *et al.* (1984) also suggested that active biological processes rather than physical processes were the principle mechanism maintaining the DCM in Lake Michigan. Their results indicated that nutrient uptake and primary production by phytoplankton were more important in determining chemical and chlorophyll vertical profiles than diffusivity.

Gasol et al. (1993) summarised the explanations most commonly cited for the formation of deep chlorophyll accumulations. i) Nutrients may be available in the area of the DCM or easily obtained from this vantage point (Fee, 1976; Pick et al., 1984). ii) Nutrientlight interactions may influence the particular position of a population in the water column, as is the case with Oscillatoria rubescens (Konopka, 1982; 1989). iii) Algae use dissolved organic matter or bacteria as sources of nutrients and/or carbon and these are often more abundant in the area of the DCM (Vincent, 1988). However, Tranvik et al. (1989) showed that less than 2 % of the carbon requirements of Cryptomonas ovata were met by bacterivory, thus this may be of a lesser importance. iv) The lake mixolimnion (or epilimnion) may have comparatively harsh conditions (Gasol et al., 1993). This is particularly pertinent in Ace Lake where a more favourable conductivity and temperature regime exists in the region below the thermocline/halocline. v) Reduced respiratory losses have been proposed for DCM populations but when compared to the potential productivity gains of existing higher in the water column this strategy appears unprofitable (e.g. Pedrós-Alió et al., 1987). vi) Predation pressure may also be reduced. Gasol et al. (1992; 1993) showed that predators did not follow Cryptomonas sp. into the sulphide-rich waters of Lake Cisó when the algae migrated at night. They estimated that this was sufficient to reduce the grazing pressure on the protist by as much as 70 %. Thus, if metalimnetic algae are not efficiently used by predators production by the DCM does not end up in the zooplankton but is lost to the sediments or respired in the microbial loop (Gasol et al., 1992). This latter scenario is surprising since flagellated algae are generally considered excellent food sources for zooplankton. vii) In addition, depth regulation must be achieved by some means to maintain a DCM (Moll & Stoermer, 1982). Consistent with Fee's (1976) observations in small Canadian Shield lakes, the DCM in the mixolimnion of Ace Lake were predominantly composed of motile flagellates. In contrast, cyanobacteria maintain their vertical position in the water column by controlling their density. Low density gas vesicles counterbalance high density molecules within the cell, a process strongly influenced by light and nutrient levels (Konopka, 1984; 1989). It is likely that a combination of factors is responsible for the formation of DCM in aquatic environments (Gasol et al., 1993). Further investigations are needed to determine the precise factors involved in the formation of the DCM in Ace Lake, however, it is likely that the trade-off between being close to the nutrients entrained in the monimolimnion, in combination with a suitable light climate for shade-adapted protists, more favourable temperature regimes below the thermocline/halocline, and to a lesser extent reduced grazing pressure, all play a part in the persistence of the DCM in Ace Lake (Figure 2.14 & Photograph 2.4).

In his investigation of DCM in Lake Schlachtensee, Germany, consisting of *Cryptomonas* spp., Gervais (1998) concluded that *in situ* growth within DCM was entirely due to shade-adapted photosynthesis rather than mixotrophy. However, there is evidence to suggest that *P. gelidicola* in Ace Lake is nutritionally versatile, remaining active during the winter as a result of mixotrophy (the assimilation of carbon via both photosynthesis and bacterivory). During winter active cells were observed to lose their chlorophyll entirely, thus, amphitrophy, a complete switch from autotrophic nutrition to heterotrophic nutrition, may in fact be a better description of this behaviour (M. Laval-Peuto, personal communication). This will be discussed further in Chapter 5.

A proportion of the *P. gelidicola* population relied on other strategies, bet-hedging to ensure survival of the population in the absence of light. Part of the population encysted during winter, and presumably lay dormant in sediments in the littoral zone until a more favourable light regime developed. *P. gelidicola* cyst abundance in the plankton was observed to follow a similar trend to that of the active cells. The maximum abundance of cysts in the water column occurred in early summer (November) probably having been flushed from the littoral zone by inflowing meltwaters. These cysts subsequently excysted and led to formation of the active cells in Ace Lake were larger and stained more darkly with iodine (indicating starch reserves) in summer than in winter when cells were smaller, a phenomenon not tested during this investigation. Thus, further alternatives may include the sequestration of endogenous energy reserves during summer for use during winter.

Further to phytoflagellate DCM in the monimolimnion of Ace Lake, the highest abundance of photosynthetic bacteria was consistently recorded at the oxycline concurrent with a persistent plate of bacterial chlorophyll (between 10 and 12 m) (Figures 2.14 & 2.15). It is well known that a considerable concentration of photosynthetic bacteria generally exist at or below the oxycline in meromictic lakes (Pfenning, 1978), although concentrations are usually several orders of magnitude higher in meromictic lakes at lower latitudes than observed in Ace Lake (Hammer, 1981; Moon & Matsuyama, 1998). At the oxycline photosynthetic bacterial requirements for light and hydrogen sulphide (H<sub>2</sub>S) were met. Organic matter and nutrients were entrained and ambient temperatures (fluctuations in which were buffered

by the ice-cover), coupled with decreasing oxygen levels provided ideal conditions for bacterial production. Although these populations were photosynthetically active, Hand and Burton (1981) concluded that only phytoplankton and cyanobacterial photosynthesis in the mixolimnion and aerobic monimolimnion of Ace Lake was actually fixing atmospheric carbon into the lake and could be termed primary production. Instead, in the immediate vicinity of the oxycline phototrophic bacteria were refixing carbon dioxide ( $CO_2$ ) and small organic molecules, released by sulphatereducers and other heterotrophs during oxidative heterotrophy of organic matter falling and diffusing from above. The refixed carbon was eventually converted into methane or deposited in the sediment. The rate of methane production and the proportion of carbon turned into methane was governed by the production of hydrogen and organic molecules of low molecular weight by bacteria other than the methane-producers, which in turn was influenced by the rate of fall-out of POC from the oxylimnion (Hand & Burton, 1981).

Despite a reduction in the abundance of photosynthetic bacteria in the monimolimnion during late winter/spring, a DCM persisted at the oxycline (Figures 2.14, 2.15 & Photograph 2.4). It is possible that the salinity and reducing environment in the anoxic waters retarded chlorophyll degradation and it accumulated in a reduced state, as observed by Tominaga and Fukui (1981) in Lake Suribati, Syowa Oasis, eastern Antarctica. In contrast, chlorophyll *a* concentrations in the mixolimnion dropped to very low levels during midwinter, indicating a cessation of photosynthesis in phytoplankton residing in this stratum (Figure 2.14). The chlorophyll *a* concentrations of Ace Lake and other Vestfold Hills lakes are comparable to those recorded in many of the McMurdo Dry Valley Lakes (Heath, 1988; James *et al.*, 1998).

#### 2.4.2.2: Heterotrophic bacteria and heterotrophic nanoflagellates

The phototrophic populations in Ace Lake supported a wide range of microbial heterotrophs. As in the pelagic system the bacterial heterotrophs were an important component of Ace's lacustrine food web, as regenerators of nitrogen which sustained phytoplankton growth, and as a food source for heterotrophs at higher trophic levels in the food web. Their abundance was consistently higher in the monimolimnion where entrained nutrient and DOC/POC levels were greatest (Figures 2.12, 2.13 & 2.16). Bacteria were observed on numerous occasions to associate with dead or dying flagellates and algal cells (as well as other forms of POC) temporarily trapped at the thermocline/halocline or settling through the monimolimnion. A peak of heterotrophic bacteria was evident at midwinter, which was apparently not exploited by the heterotrophic nanoflagellates (HNAN). It is possible that these bacteria were
unpalatable or that the HNAN community was unable to effect a grazing response at low winter temperatures. In contrast the HNAN community in the upper monimolimnion, which experienced higher winter temperatures (maximum 9 °C), sustained relatively high winter populations.

Heterotrophic nanoflagellates were observed in higher numbers where their bacterial prey predominated, chiefly at the oxycline (Figure 2.18). The abundance of HNAN in Ace Lake was similar to that reported in the literature for lakes with low concentrations of organic matter (Carrais *et al.*, 1998) and summer HNAN abundances were comparable to those of other saline lakes in the Vestfold Hills and the meromictic lakes of the McMurdo Dry Valleys (Perriss & Laybourn-Parry, 1997; James *et al.*, 1998). There are no winter data for HNAN available for the Dry Valley lakes and the only other winter data collected for saline lakes in the Vestfold Hills was obtained during this study. HNAN abundances in nearby Highway Lake ranged from 3.77 x 10<sup>8</sup> cells 1<sup>-1</sup> in late summer to 8.29 x 10<sup>6</sup> cells 1<sup>-1</sup> in early winter (Section 6.3.1). However, the freshwater lakes of the Vestfold Hills showed a similar decline during winter with numbers building up in spring towards a summer maximum (Laybourn-Parry *et al.*, 1995; Bayliss & Laybourn-Parry, 1996).

# 2.4.2.3: Dinoflagellates, diatoms and cysts

Dinoflagellate cysts and diatoms appeared in the plankton in large numbers during the summer, concomitant with ice melt (Figures 2.20 & 2.21). It is possible they may have been held within, or by the ice in shallow basins but were released as the ice melted. Or, that since productivity would have increased dramatically in the zone that was ice but became water, there was a greater abundance spread throughout the lake. There are large, shallow areas at the north eastern end of Ace Lake and it is likely that productivity increased in these basins prior to the main body of the lake. Once a moat had formed at the perimeter of the lake (commencing in December), the water would have warmed relatively rapidly compared with the ice-covered waters, setting up strong convection currents which transported certain diatoms and cysts from the benthos across the lake. Once favourable conditions developed in the plankton, in particular in terms of temperature, excystment commenced and active cell blooms were observed (Rengefors & Anderson, 1998) (Figures 2.20, 2.21 & 2.23). Blooms of other species of diatom, in particular Nitzschia longissima, may have been 'seeded' by the ice (Plate 2.3). There was a clear trend of diatom abundance in the water column increasing from the surface downwards as spring progressed and ice thickness decreased, concomitant with the highest abundances of diatoms recorded in the ice (Figures 2.21 & 2.29). It is therefore possible that the bloom of N. longissima began in the ice itself as PAR increased. In general Antarctic lake-ice has no or a limited ice community in comparison to that of sea-ice (Garrison & Mathot, 1996; Gleitz *et al.*, 1996; McMinn, 1996; Gradinger & Ikävalko, 1998; Knight, 1998; Priscu *et al.*, 1998) but in common with sea-ice communities diatoms dominated the lake-ice community of Ace Lake (e.g. Gleitz *et al.*, 1996; McMinn, 1996; Gradinger & Ikävalko, 1998)

# 2.4.2.4: Ciliated Protozoa

*Mesodinium rubrum* (Ciliophora: Haptorida) is a ubiquitous member of the marine plankton occasionally responsible for the formation of 'red tides' (Lindholm, 1985; Crawford, 1989; Satoh & Watanabe, 1991). The species has adapted successfully to life in a lacustrine, meromictic environment and occurs widely in the saline and brackish lakes of the Vestfold Hills (Perriss & Laybourn-Parry, 1997). In contrast, *M. rubrum* has not been observed in the McMurdo Dry Valley lakes or the lakes of the Larsemann Hills, an ice-free oasis some 50 miles east of the Vestfold Hills (Ellis-Evans *et al.*, 1998). Both suites of lakes predate the last major glaciation (Chinn, 1993; Doran *et al.*, 1994; Burgess *et al.*, 1994) whereas the Vestfold Hills postdate it. Therefore, their age may account for the absence of the species.

As is apparent in other aquatic ecosystems, M. rubrum was the dominant component of the ciliate community in Ace Lake throughout the year (Bernard & Rassouladegan, 1994; Sanders, 1995) (Figures 2.22 & 2.30). During the winter months M. rubrum was most abundant just below the ice. In the summer active cells were observed throughout the mixolimnion and aerobic monimolimnion, and were often abundant near the oxycline where nutrient concentrations and temperatures were higher. An interannual variation in abundance is apparent from the literature. Gibson et al. (1997) observed active cell numbers an order of magnitude greater than those recorded in the present study, however the biovolume measured was lower in 1994 than 1996. Grev et al. (1997) reported maximal concentrations of M. rubrum comparable to winter populations found in Ace Lake, in Ellis Fjord, Vestfold Hills. In contrast, the Antarctic marine environment harbours population densities comparable to the summer abundances recorded during this study. For example, 2 to 3 x 10<sup>4</sup> cells 1<sup>-1</sup> under the sea ice off Syowa Station, eastern Antarctica (Satoh & Watanabe, 1991). It should be noted that M. rubrum is a fragile species (Crawford, 1989; Lindholm, 1985) and due to cell loss during sampling and storage, the numbers reported are likely to underestimate true abundance.

*M. rubrum* is an autotrophic ciliate which relies on translocated photosynthate from cryptophycean endosymbionts to meet its energy requirements. The species is highly

motile, an equatorial band of cilia enabling it to take advantage of optimal environmental conditions for photosynthesis by means of vertical migration (Plate 2.2). Moreover, fluorescent microscopy showed that the active cells in Ace Lake retained chlorophyll, and therefore their photosynthetic capacity, all year. Thus, it can be assumed that M. *rubrum* was photosynthesising albeit at a minimal rate, throughout the winter beneath the ice where the highest light levels would have been available. Adaptation of phytoplankton to very low light levels and continued year round photosynthesis has been demonstrated in other Antarctic systems (Palmisano *et al.*, 1985; Priscu *et al.*, 1987; Stoecker *et al.*, 1991). The abundance of *M. rubrum* observed in Ace Lake during the summer months rendered it a major component of the phytoplankton, able to make a considerable contribution to the lake's productivity (Table 3.2). This is apparent in other Vestfold Hills lakes (Laybourn-Parry & Perriss, 1995) and in other localities at lower latitudes. For example, Sanders (1995) reported that autotrophic ciliates such as *M. rubrum* could account for up to 23 % of community photosynthesis in the Damariscotta Estuary, Maine, USA.

*M. rubrum* cells were observed in this and aforementioned studies to possess endogenous concentrations of carbon-rich material, probably starch (Perriss *et al.*, 1995) and Smith and Barber (1979) reported evidence that *M. rubrum* may also take up dissolved organic compounds. These observations may constitute a means by which over-wintering populations supplement photosynthesis, providing carbon and nutrients in the otherwise nutrient poor waters of the mixolimnion. Moreover, low water temperatures in this stratum minimised cell metabolism, thus, conserving these valuable resources.

Previous studies have suggested that, like *Pyramimonas gelidicola*, *Mesodinium rubrum* may encyst as a means to survive light limitation and low temperatures during winter (Lindholm, 1985; Perriss *et al.*, 1993; 1995). However, during their winter investigation Gibson *et al.* (1997) found no evidence of this. The present study noted the presence of active cells throughout the winter coupled with the occurrence of cysts in early summer (Figure 2.23). It is possible encystment in such instances may be a result of photoinhibition (J.A.E. Gibson, personal communication) or a bet hedging strategy to ensure the continuation of the population. It may be that active cells encysted during 1995, these cysts accumulated in the aerobic sediments of the littoral zone (therefore were not sampled), and were released into the water column by convection currents in the summer of 1996. Thus, the population of active cells in the plankton was increased from hundreds per litre in the winter to tens of thousands per litre in the summer following excystment. In late summer 1997 cyst abundance increased once more as active *M. rubrum* cells encysted in preparation for the ensuing winter. Clearly a

prolonged study of the population dynamics of this unique ciliate is required to elucidate the dynamics of *M. rubrum* populations in Ace Lake.

The ciliate community of Ace Lake as a whole displayed low species diversity, and was dominated by *M. rubrum* (Figure 2.24). Low species diversity is a characteristic of the ciliate community of all lakes in the Vestfold Hills (Laybourn-Parry & Marchant, 1992b; Perriss & Laybourn-Parry, 1997). In contrast, the older, more complex lakes of the McMurdo Dry Valleys exhibit greater ciliate species diversity and more complex communities, which include raptorial, or ciliate predating, ciliates (Laybourn-Parry *et al.*, 1997; James *et al.*, 1998). It is possible that the species diversity reflects the different age and history of this suite of lakes.

### 2.4.2.5: Metazoa

Few of the lakes of the Vestfold Hills harbour metazoan herbivores. *Paralabidocera* antarctica occurs in three (Bayly, 1978; Bayly & Burton, 1987). In agreement with Bayly and Burton (1987) the peak in metazoan predator abundance in Ace Lake coincided with the spatial distribution of phytoplankton peaks (Figures 2.25, 2.26 & 2.28). One explanation is that *P. antarctica* is presumptively herbivorous and nourished by flagellates, including the dominant phytoflagellate *Pyramimonas gelidicola*, and/or *Mesodinium rubrum* (Tranvik & Hanson, 1997). It is highly likely that *P. antarctica* exerts a degree of "top-down" on the plankton at certain times of the year (e.g. Stoecker & Capuzzo, 1990; Kleppel, 1993; Dobson *et al.*, 1997; Tranvik & Hanson, 1997; Swadling *et al.*, 1997; Barquero *et al.*, 1998; Strom & Loukos, 1998). Spencer and Ellis (1998) determined that zooplankton did not regulate the phytoplankton community of oligotrophic, Flathead Lake (Montana, USA) unless nutrient concentrations were artificially enriched. However, in Ace Lake a decline in HNAN abundance was apparent, particularly near the oxycline, concurrent with the summer increase in copepod abundance, indicating a seasonal grazing impact (Figures 2.18 & 2.25).

*P. antarctica* were not observed to concentrate at the under surface of the ice to feed on diatoms associated with it, as observed by Hoshiai *et al.* (1987) and it is improbable that food sources such as detritus and bacterioplankton could sustain the standing crop of *P. antarctica* in the mixolimnion. Nevertheless, it is quite possible that the *P. antarctica* population residing at the oxycline could supplement their diet with bacteria (Bayly & Burton, 1987). In addition, copepods are able to withstand short periods of anoxia without ill effects (Tinson & Laybourn-Parry, 1985; Bayly & Eslake, 1989; DeMeester & Vyverman, 1997; Tiselius, 1998) and may be diving into the anaerobic monimolimnion to feed on the larger bacteria, behaviour reported previously in the

cladoceran, Daphnia longispina (Salonen & Lehtovaara, 1992; Tranvik & Hanson, 1997).

Unlike populations in the marine environment, *P. antarctica* in Ace Lake never entered the lake ice during its life cycle (Figure 2.29). In addition, the life cycle was markedly different from that observed in the marine environment. Individuals from each developmental stage (1 egg, 6 nauplii and 6 copepodid) were present throughout the year rather than restricted to a summer bloom, and successive breeding episodes were apparent in terms of nauplii abundance (Tanimura, 1996). A further discontinuity between lacustrine and marine populations existed in terms of size. The mean length of both copepodids and nauplii was less than that reported for individuals from marine environments (Bayly, 1978).

A corollary of *P. antarctica* presence in Ace Lake was the observation of faecal pellets in the water column, identified according to Tanimura *et al.* (1984) (Figure 2.27). Abundances of > 5000 pellets  $1^{-1}$  were recorded in December and their numbers varied concomitantly with copepod abundance. Pellet length and volume was far less than that recorded by Tanimura *et al.* (1984) but this is not surprising considering that the copepods in Ace Lake are smaller than their marine counterparts (Bayly, 1978). The general trend was for maxima to occur slightly lower in the water column than the copepod maxima due to settling. Interestingly, there was no concentration of faecal material apparent at the thermocline/halocline or oxycline. It is possible that, owing to their size, faecal pellet settling was not impeded by the density gradients. The faecal pellets provided a source of POC which was utilisable by bacteria and other planktonic species (Roman & Gauzens, 1997).

Dartnall (1997) has reported three species of rotifer associated with the benthic algal mats in the lake shallows, *Encentrum spatitium*, *Encentrum salinum* and *Notholca* sp. (Rotifera: Monogononta). Only one of these, *Notholca* sp., was observed in low numbers in the plankton of Ace Lake (Figure 2.28). *Notholca* sp. was absent from the plankton for the majority of the study. Like the diatoms and cysts it was probably a migrant from the benthos and littoral algal mats. Carrais *et al.* (1998) suggest that rotifers may have a greater grazing impact than copepods on nanoflagellate abundance, however, it is unlikely that the few individuals of *Notholca* sp. observed in the plankton of Ace Lake (maximum of 6 individuals  $l^{-1}$ ) would exert any "top-down" control. Carrais *et al.* (1998) also discussed the importance of ciliate predation, but once again this is unlikely to be significant by virtue of the fact that the quantitatively dominant ciliate, *M. rubrum*, is autotrophic.

As such the findings of the investigation thus far indicate that a meromictic system such as Ace Lake, that does not receive allochothonous carbon inputs from the surrounding environment, possesses a microbial plankton that is highly dependent on the flux of carbon, nutrient regeneration and positive feedbacks described by the microbial loop concept.

# Chapter 3: AUTOTROPHIC PRODUCTION

### 3.1: INTRODUCTION

#### 3.1.1: Terminology

The term "primary production" is difficult to define precisely. It is a term which is typically, but loosely, employed to describe production at the base of a food chain (Flynn, 1988). In aquatic microbial foodwebs the term "primary production" is generally considered to be limited to autotrophy or carbon (C) fixation, however, in higher plants only a small proportion of plant cells are photosynthetic. These cells release dissolved organic carbon (DOC) into the phloem, and this DOC is used by heterotrophic cells elsewhere. The biomass of the whole plant, including autotrophic and heterotrophic cells, is thus considered when assessing plant production. By strict analogy, primary productivity in aquatic microbial food webs must, therefore, include heterotrophs using DOC leaked or released by autotrophic organisms (Flynn, 1988). The term is then better defined as "...the formation of organic particulate material at the primary stages of the food chain" (UNESCO, 1973). This definition is also pertinent when considering radioactively labelled carbon dioxide  $({}^{14}CO_2)$  fixation. A more accurate estimation of potential primary production would be gained by including with autotrophic assimilation the heterotrophic use of DOC, since a significant proportion of fixed <sup>14</sup>CO<sub>2</sub> is subsequently released or leaked by algae (Vollenweider, 1974; Coveney, 1982; Wood & Van Valen, 1990; Sharp & Priscu, 1991; Wetzel & Likens, 1991; Sell & Overbeck, 1992; Tranvik, 1992; del Giorgio & Peters, 1993b; Leboulanger et al., 1998). However, there are problems with this analogy; DOC is released by activities other than photosynthesis, for example, grazing and lysis at cell death. Such "old" released DOC is difficult to distinguish from "new" DOC produced during photosynthesis. In addition, competition between heterotrophs and autotrophs for nutrients may be too great to allow their inclusion as primary producers in the same trophic level. Consequently, the concept of primary production as "borrowed" from terrestrial systems cannot be applied accurately to aquatic ecosystems (Flynn, 1988). Instead, terms such as "autotrophic production", "heterotrophic production" or "CO, fixation" are better substitutes (Flynn, 1988). The latter is particularly useful as it describes the methodology used and is not misleading since <sup>14</sup>CO<sub>2</sub> fixation methods measure photosynthesis not production (Talling, 1984). These terms will be employed in this chapter.

#### 3.1.2: Photosynthesis in aquatic environments

### 3.1.2.1: Summary of photosynthesis

The use of light as an energy source in photosynthesis is by far the most important process by which inorganic matter is converted to organic matter (Lampert & Sommer, 1997). Carbon dioxide ( $CO_2$ ) and water ( $H_2O$ ) interact with photosynthetic pigments to produce carbohydrates and oxygen, aerobically (Austin, 1988). Photosynthesis can be presented simply by the formula:

# $\mathbf{6} \ \mathbf{CO}_2 + \mathbf{12} \ \mathbf{H}_2\mathbf{0} \rightarrow \ \mathbf{C_6H_{12}O_6} + \mathbf{6} \ \mathbf{H_2O} + \mathbf{6} \ \mathbf{O_2} \uparrow$

Photosynthetic sulphur bacteria utilise  $H_2S$  in place of water as an electron and hydrogen donor for photosynthesis; accordingly sulphur is formed in the place of oxygen. This process is strictly anaerobic (Lampert & Sommer, 1997).

It is clear from the formula that photosynthesis can be measured as either release of oxygen or the use of carbon dioxide (CO<sub>2</sub>). Oxygen changes can be measured with relative ease in water, but a more sensitive measure of photosynthesis is gained by the use of radioactively labelled <sup>14</sup>C sodium bicarbonate to determine the incorporation of CO<sub>2</sub>. The latter was employed in this study.

#### 3.1.2.2: The aquatic photic environment

Almost all available energy on Earth comes from solar radiation. Solar radiation can be divided into ultraviolet (300 to 380 nm), visible radiation including (Photosynthetically Active Radiation (PAR) = 400 to 700 nm) (380 to 750 nm) and Infrared Radiation (750 to 3000 nm). When radiation reaches a body of water, some is reflected and the remainder penetrates the water where it is absorbed. The proportion reflected depends upon the angle of the sun, the wavelength and the surface conditions of the water (Kirk, 1983). Since light always comes from above a lake and is absorbed as it passes through the water, there is a vertical light gradient in every lake which has a profound affect on the production and life in the lake. As light passes through water it is attenuated. It is either scattered, absorbed as heat or transformed into other energy sources. The degree of light retention by a layer of water is termed *light extinction*, and the amount of light passing through *light transmission*. The reduction of light intensity does not decrease linearly as it passes through water but rather by a fixed proportion of the

light still remaining at each depth. Thus, light is subject to an exponential decrease described by a *vertical attenuation coefficient*. Each penetrating wavelength has a different attenuation coefficient. The higher the coefficient the more rapidly it is absorbed. Thus, red light is the most readily absorbed, typically within the first 0.5 m, and blue light the most readily transmitted (Kirk, 1983; Vincent, 1988). 65 % of red light (720 nm) is absorbed in the first metre of water compared with 0.5 % of blue light (475 nm) (Kirk, 1983).

There are many laws that govern the attenuation process and many factors that influence it. Nevertheless, essentially all the light absorption which takes place in natural waters is attributable to four components of the aquatic ecosystem; the water itself, for example its transparency, yellow pigments (gilvin) resulting from the microbial decomposition of organic matter, the photosynthetic biota (phytoplankton, and macrophytes where present), and inanimate particulate matter (tripton) (Kirk, 1983). The colour of the sediments and the geography of the lake basin, sea bed or coast may also exert an effect. The depth at which 1 % of light is still available in a water column is often used as a rough estimate of the lower boundary of the euphotic zone; the region in which there is a positive energy balance due to photosynthesis (Lampert & Sommer, 1997). However, Talling (1984) pointed out that low-light adapted cells can achieve an appreciable growth rate (0.2 divisions d<sup>-1</sup>) at a quantum flux of 1  $\mu$ E m<sup>-2</sup> s<sup>-1</sup> thus the 1 % PAR delimitation of the euphotic zone can have conspicuous exceptions.

### 3.1.2.3: Modelling photosynthesis

Non-acclimated photosynthetic rate is usually described with a modified Blackman model, where acclimation is defined as the physiological adaptation of a photosynthetic organism to a new environment or condition (Lampert & Sommer, 1997) (Figure 3.1). In this model the photosynthetic rate decreases above a certain intensity, producing what is usually referred to as a *P-I curve* (P = photosynthetic rate, I = irradiance) which describes an immediate photosynthetic reaction to light conditions. All the parameters of this curve are influenced by the species of phytoplankton in question as well as by their physiological condition. At high light intensities *light inhibition* results, caused by photochemical damage of the chloroplasts by UV radiation as well as increased photorespiration. As a result, photosynthetic rate ceases to fit the Blackman model (Schindler & Fee, 1975; Lampert & Sommer, 1997; Geider *et al.*, 1998). In terms of low light, two physiological adaptations are apparent in plants (Jørgensen, 1969); either chlorophyll content increases resulting in higher rates of photosynthesis for low-light adapted cells, or there is a restructuring of the photosynthetic apparatus which elevates the initial slope of the *P-I* curve. The ability to adapt to low light intensities means that



 $V = S \propto, \text{ for } S < S_k$   $V = V_{max}, \text{ for } S > S_k$  $\propto = V_{max} / S_k$ 

### MICHAELIS-MENTEN MODEL



 $V = V_{max} \times S / (S + k_t)$ 

where:



the long-term response to light, unlike the instantaneous response, does not show the linear increase of the Blackman model and is better described by a Michaelis-Menten model (Figure 3.1). This light-adapted model better describes the long term correlations between underwater light environment and the occurrence of different species.

# 3.1.3: Factors affecting photosynthesis

Autotrophic production in lakes is controlled by the interaction of many factors. These can be divided into three groups: 1) physical factors originating directly or indirectly from solar radiation e.g. light conditions, temperature, mixing and wind-induced turbulence, and thus, the degree of stratification; 2) the availability of nutrients and/or inorganic substrate (e.g.  $CO_2$  or  $H_2S$ ) in the euphotic zone of lakes, and 3) the interaction of the organisms present in the plankton community which may promote or hamper the production of certain species (Findenegg, 1969).

# 3.1.3.1: Light and photosynthetic pigments

As described in Section 3.1.2.2, unlike air in the terrestrial environment, water both absorbs and scatters light. In all but the shallowest waters light is, therefore, potentially limiting for primary production. Aquatic plants must compete not only with one another, as in the terrestrial system, but with other light absorbing components within the aquatic medium. For this reason, over the course of evolution, each of the major groups of algae has acquired characteristic arrays of light-harvesting pigments which are of major significance for the understanding of the adaptation of the algae to their ecological niche (Kirk, 1983).

Higher plants have the whole spectrum of incident light available to them i.e. all the colours as well as UV. They have two photosystems, Photosystem I (PSI) and Photosystem II (PSII), situated in the thylakoids and containing the accessory pigments chlorophyll a and b (Chl a, Chl b) and carotenoids (Car). Chl a absorbs maximally in the blue (430 to 470 nm) and red (620 to 700 nm) regions of the spectrum (Kirk, 1983). Absorption is very low, but not zero, in the green region and green light is therefore transmitted. However, for marine plants the red radiations are not available at depths greater than 0.5 m so Chl a can only function in the blue region of the spectrum. For this reason aquatic plants have different pigment compositions. Many different algal groups possess both Chl a and Chl b. Chl b has the effect of widening the absorption spectrum of the algae in the red and blue regions. Most algae also possess Car. These absorb maximally in the blue region but widen the algal absorption band further toward the yellow-green wavelengths. However, even when the Chl a, Chl b

and Car spectra are combined, it is apparent that there is a 'gap' in the central spectral region corresponding to the red-orange wavelengths of incident light that the algae cannot utilise. Many algal groups contain different carotenoids with slightly different absorption capabilities but with each the 'gap' still remains. It is for this reason that some algal groups; Rhodophyta, Cyanophyta and the highly specialised Cryptophyta, have evolved further accessory and auxiliary pigments that to date have been found in no other algal groups. These pigments are termed the 'phycobiliproteins' and evidence that they were active in algal photosynthesis was first obtained by Engelmann in 1883 (Kirk, 1983).

It should be noted that not all autotrophic production is photosynthetic. There are autotrophic bacteria that obtain energy for biosynthesis from exergonic chemical reactions rather than from light. These *chemolithotrophic* organisms use chemical energy sources, inorganic electron donors, and  $CO_2$  as a carbon source. Chemolithotrophic bacteria often live at the boundary between oxic and anoxic zones e.g. the oxycline, where the anoxic water provides a constant supply of reduced compounds into boundary layers (Lampert & Sommer, 1997).

NB: Although it is not entirely correct to assume that respiration is not affected by light, this assumption is adequate for most practical purposes (Reynolds, 1984).

### 3.1.3.2: Temperature

In the light-limited range of photosynthesis the photosynthetic rate is determined exclusively by the photochemical processes that are not temperature dependent. Thus the increase of the *P-I* curve (Figure 3.1) is independent of temperature. However, the magnitude of light-saturated photosynthesis is temperature dependent and evidence for temperature effects on photosynthesis have been widely reported (e.g. Talling, 1984; Tilzer *et al.*, 1986; Hawes, 1990a; Devos *et al.*, 1998; Fritsen & Priscu, 1998).

### 3.1.3.3: Nutrients

In addition to the elements C, O and H that are required in photosynthesis, many other elements are essential components of the biomass of living plants. These are divided into *macro-elements*, those making up greater than 0.1 % of the organic matter e.g. N, P, S, K, Mg, Ca, Na, Cl, and *trace elements* which are required in far smaller quantities e.g. Fe, Mn, Cu, Zn, B, Si, Mo, V, Co. Some trace elements, such as Si, are macro-elements for certain species e.g. diatoms and crysophytes (Lampert & Sommer, 1997). All of these elements must be taken up from a dissolved pool of

nutrients. Generally they are available in excess but all have the potential to become limiting, in particular, N, P and Si (Lampert & Sommer, 1997). Certain cyanobacteria can utilise molecular nitrogen  $(N_2)$  by nitrogen fixation, whereas eukaryotic algae and all higher plants are dependent on nitrate or ammonium (occasionally urea).

Availability of nutrients in aquatic ecosystems is largely dependent upon what are received from the catchment area via runoff, meltwaters (Torii *et al.*, 1988; Knox, 1990; Priscu, 1995), the atmosphere (Paerl, 1988) and those remineralised in the aquatic system itself. "New" water entering the lake may be relatively rich in phosphate and nitrate but contains small amounts of organic material. Conversely, "old" water has large amounts of phosphate and nitrate in the form of suspended or dissolved organic matter. When the nutrients of "new" water are taken up by phytoplankton no reserve is available and autotrophic production is stopped until the phytoplankton population declines and begins to decompose. In "old" water the uninterrupted replenishment of nutrients goes on by means of the mineralisation of both dissolved and particulate organic matter, which is the more effective the higher the concentration of organic material (Findenegg, 1969).

### 3.1.3.4: Dissolved inorganic carbon and pH

Dissolved inorganic carbon (DIC) occurs in three forms: carbon dioxide  $(CO_2)$ , hydrogencarbonate  $(HCO_3^{-})$  and carbonate  $(CO_3^{-2})$ . The abundance of each depends on the pH of the water. All plants can utilise CO<sub>2</sub> but when the supply of CO<sub>2</sub> has been depleted, the pH rises to 9 and only plants that produce the enzyme carbonic anhydrase can continue to photosynthesise using HCO<sub>1</sub>, essentially the only form of inorganic carbon present at this pH. When the supply of  $HCO_3^-$  is exhausted, the pH continues to rise to pH 11. Thus, if the total amount of DIC remains constant, but the pH changes, the form of DIC present alters. Consequently, photosynthesis is affected. The photosynthetic rate of HCO<sub>3</sub> using plants decreases as the pH exceeds 7 but they can photosynthesise to pH 11. Conversely, CO<sub>2</sub> users can only photosynthesise when the pH is below 9. When CO<sub>2</sub> is the only form of DIC present (at less than pH 6.3), respiration and photosynthesis have no affect on the pH, but when other forms of DIC are present pH is dependent on photosynthesis, respiration and nitrogen assimilation. Often, a vertical gradient of pH develops in stratified lakes (pH is usually higher in the epilimnion or monimolimnion) as a result of the inorganic carbon and phytoplanktonic species present (Lampert & Sommer, 1997). In addition, diurnal variations in DIC content were recorded in lakes of the Experimental Lakes Area, Canada, which severely limited autotrophic production (Schindler & Fee, 1973).

Nitrogen assimilation by aquatic organisms also can affect the pH of a lake (Lampert & Sommer, 1997). If ammonium ions  $(NH^{4+})$  are used as a source of nitrogen pH decreases, but if nitrate ions  $(NO^{3-})$  are used, pH increases. Since nitrogen assimilation is not as important as carbon assimilation, its affect on pH is most important at low pH (Lampert & Sommer, 1997).

# 3.1.3.5: Stratification

In lacustrine ecosystems, the trophogenic zone often comprises of layers of very different character, with remarkable gradients of temperature and chemical properties. Sometimes there are also layers of turbidity and different plankton communities because the thermocline may still be part of the euphotic zone (Findenegg, 1969). Therefore, in contrast to the oceans, primary production in lakes generally takes place in non-homogeneous layers and the "normal" assimilation curves of depth distribution are seldom found (Figure 3.1). The wind-protected or ice-covered situation of some lakes can lead to meromictic stratification, as described in Section 2.1.1 (e.g. Wetzel, 1983). Such pronounced stratification may lead to a mixolimnetic nutrient depletion at certain times of the year and does not, therefore, favour production. Part of the plankton biomass sinks down after death and is not mineralised before reaching the lake bottom. Consequently, essential nutrients such as SRP and nitrogen, do not readily return to trophogenic layers (Findenegg, 1969). The concentration of nutrients entrained in the monimolimnion can be high.

It is common in oligotrophic, stratified lakes for different maxima of autotrophic production to develop and considerable photosynthesis to take place below the thermocline (Findenegg, 1969; Schindler & Fee, 1975; Gasol *et al.*, 1992; 1993; Gervais, 1998). Autotrophic production in the aerobic monimolimnion is generally limited by low-light availability, but it is favoured by the relatively high content of dissolved nutrients. The reverse is true in the mixolimnion. Thus, in well stratified lakes with good light transmission, as a rule two plankton communities are present: one consisting of species tolerant of the conditions in the mixolimnion, and another composed of low-light adapted species in the region below the thermocline (the metalimnion) (Findenegg, 1969). Moreover, a plate of photosynthetic bacteria can develop below the maxima of phytoplankton in lake with an anaerobic monimolimnion and a build up of  $H_2S$ , as long as some light penetrates the  $H_2S$  zone.

According to the Michaelis-Menten model (Figure 3.1), when there is adequate surface light one might expect to find most species of phytoplankton at depths of their optimal

(i.e. saturated) light requirements. However, it is not always possible for phytoplankton to remain stratified at particular depths. A wind velocity of only 3 ms<sup>-1</sup> can destroy flagellated algal stratification (Lampert & Sommer, 1997). Thus, in an open-water situation, phytoplankton in the mixolimnion are passively exposed to an average light intensity within this strata. However, when the lake is ice-covered, clear vertical separations are maintained and growth rate studies indicate that these are close to optimal light conditions for the resident species (refer to Chapter 5).

### 3.1.3.6: Biotic interactions

Light is a consumable resource (Lampert & Sommer, 1997). Tilzer (1983) demonstrated that 70 % of light attenuation in Lake Constance, Germany, was due to phytoplankton chlorophyll. Phytoplankton shift the absorption maximum to the longer green wavelengths, and dissolved humic substances to shorter yellow wavelengths, hence, both light intensity and spectral composition alter with depth depending on the phytoplankton residing in the water column. This is of great importance to photosynthesis which functions only in the range of 400 to 700 nm. In stratified lakes in particular, there is the potential for phytoplankton maxima in upper strata to consume light (and nutrient) resources required by phytoplankton maxima in lower strata. This problem is avoided in some instances, for example, sulphur bacteria residing in the upper monimolimnion of meromictic lakes have requirements that are comparable to low-light-adapted phytoplankton but avoid competition for light because their spectral optima for photosynthesis is at wavelengths that are weakly absorbed by the phytoplankton stratified above them (700 to 760 nm for green sulphur bacteria and greater than 800 nm for purple bacteria) (Lampert & Sommer, 1997).

In meromictic lakes, where nutrients are entrained in the monimolimnion, phytoplankton residing in lower lake strata can function as an effective "trap" for the diffusive flux of nutrients, especially SRP, that would otherwise reach the mixolimnion (Vincent, 1988). This can lead to depleted levels available to phytoplankton in the mixolimnion, at certain times of the year. It is apparent, therefore, that phytoplankton species are faced with a "trade-off" between their light and nutrient requirements and, if they are not sufficiently motile to migrate, will reside at a level in a lake at which their relative needs are met.

#### 3.1.3.7: Trophic status

Autotrophic production variability increases with lake trophic status (Alvarez Cobelas & Rojo, 1994). However, the self-shading effects of phytoplankton on autotrophic

production increase dramatically with increasing trophic status (Alvarez Cobelas & Rojo, 1994 cite Kalff & Knoechel, 1978). This may help to explain the paradoxical situation whereby, on the basis of Chl *a* content, phytoplankton of hypertrophic lakes photosynthesise less than lakes of lower trophic status (Alvarez Cobelas & Rojo, 1994).

# 3.1.3.8: Latitude

Comparative investigations conducted by the International Biological Programme have shown that the magnitude of autotrophic production in productive lakes declines along a geographical gradient from the tropics to the poles (Lampert & Sommer, 1997). The gradient is less apparent in the least productive lakes in each geographical location. The potential causes of such a gradient include the fact that, 1) the amount of light energy is higher in the tropics, 2) the summer growth period is longer in the tropics and appears to be more important than the long summer day length experienced at high latitudes, 3) nutrients are remineralised more rapidly at higher temperatures and are therefore more readily available in the mixolimnion/epilimnion for autotrophic production, and 4) the polymictic mixing regime at tropical latitudes continually cancels out the spatial separation between the availability of light and nutrients (Brylinski & Mann, 1973; Platt & Sathyendranath, 1995).

# 3.1.4: Photosynthesis in Antarctic aquatic environments

Lakes and streams are a prominent feature of desert landscapes around the margins of the Antarctic continent (Vincent *et al.*, 1998). As documented in Chapter 2, such high latitude aquatic systems are subject to low annual PAR, extremes of PAR availability, random cloud cover (Schindler & Fee, 1975) and oligotrophic conditions, unless in the vicinity of penguin rookeries or seal wallows (e.g. Hawes, 1990b). In addition to these factors, ice-cover is of particular importance to photosynthesis in high latitude lakes.

Ice-cover can significantly alter the optical characteristics of a lake. Clear ice has the optical characteristics of distilled water and allows many algae to grow attached to the underside of the ice. However, air bubbles within, icicles beneath and snow on top of the ice, strongly affects light transmission (Vincent, 1988; Kaup, 1994; Neale & Priscu, 1995). A 20 cm thick snow cover can absorb and reflect 99 % of incident light, thereby seriously limiting photosynthesis under the ice. Ice also selectively transmits blue-green irradiance, with a maximum flux at 500 nm, thus limiting spectral distribution (Neale & Priscu, 1995). Therefore, phytoplankton in ice-covered lakes are exposed to a range of light variation in terms of both intensity and spectral distribution

during the austral spring and summer. However, in perennially ice-covered lakes a certain degree of constancy is maintained due to the permanency of the ice-cover and the stratification and low turbulence it promotes. For example, summer phytoplankton populations in Lake Bonney, McMurdo Dry Valleys, dwell in a stable light environment due to the continuous daylight characteristic of the Antarctic summer and the vertical stratification (Vincent, 1981).

In higher plants, modification of the structure and function of photosynthetic apparatus is an important response to a light environment and has advantages in increasing quantum yields of photosynthesis, in allocation of plant resources and in protecting against high-light stress. However, less is known about the importance of changes in the photosynthetic apparatus of phytoplankton (Neale & Priscu, 1995). Most nonphycobilin containing eukaryotic microalgae (in particular chlorophytes) have a lower photosynthetic efficiency in blue-green light compared to other wavelengths. Better utilisation of blue-green light could be achieved by, 1) synthesis of light-harvesting pigment-protein complexes which absorb in the predominant spectral bands and efficiently transfer energy to reaction centres, 2) increased numbers of light-harvesting pigment molecules (increasing antennae size), or 3) increasing density of reaction centres within the cell, thus, increasing maximum quantum yield for photosynthesis. Most phytoplankton increase antennae size (Neale & Priscu, 1995). Results suggest that photosynthetic pigments, e.g. carotenoids, in Lake Bonney phytoplankton are more important in light harvesting than photoprotection. Moreover, natural populations of Chlamydomonas subcaudata utilised blue-green light for photosynthesis more efficiently than laboratory cultured strains (Neale & Priscu, 1995).

With the depletion of the ozone layer above the continent, Antarctic communities must now contend with the additional stress of increasing levels of short-wave ultraviolet radiation (UVR) (Banerjee *et al.*, 1998; Vincent *et al.*, 1998). Results from work performed by Vincent *et al.* (1998) show that polar desert lakes are optical extremes in terms of their water-column transparency to UVR, and that their dilute, mostly autochthonous chromophoric dissolved organic carbon (CDOC) offers little protection against the ultraviolet-B (UV-B) radiation flux that is continuing to increase over polar regions. In many lakes in temperate zones, aquatic biota are protected from UV-B, and to a lesser extent UV-A, by the presence of CDOC. CDOC is composed of aromatic humic and fulvic acid brought in from vegetation and leaf litter in the surrounding catchment. Polar desert catchments tend to be largely devoid of plants, and the input of these allochthonous materials is greatly reduced relative to lower latitudes. Although DOC concentrations in polar desert lake waters can be well above the limits of analytical detection, much of it is generated autochthonously by microbial processes within lakes and streams (McKnight *et al.*, 1991).

Antarctic lakes offer a unique opportunity to examine the acclimation of natural phytoplankton populations to their irradiance environment in a non-turbulent environment (Sharp & Priscu, 1991; Neale & Priscu, 1995). In a turbulent environment phytoplankton are subject to rapidly fluctuating light, thus, a more accurate assessment of autotrophic production can be made in non-turbulent environments. Furthermore, in these ice-covered Antarctic lakes there is a virtual absence of planktonic grazers. In most pelagic environments grazing results in the significant loss of phytoplankton biomass (Sharp and Priscu, 1991), therefore, the absence of grazing is amenable to autotrophic production and growth studies

# 3.2: MATERIALS AND METHODS

### 3.2.1: Quench correction curves

Initially, quench correction curves were set up on a Beckman LS6500 scintillation counter to determine count efficiency. Water samples from discrete depths within Ace Lake were collected, spiked with a <sup>14</sup>C labelled sodium bicarbonate standard of known activity and 'quenched' with a known quantity of chloroform. The final activity of each sample was determined in the scintillation counter, plotted and the regression equation derived used to estimate count efficiency.

### 3.2.2: Photosynthetic rate

Photosynthetic rate was determined using a modified version of the protocol set out in Schindler *et al.*, (1972). Experiments were carried out successfully on five occasions: 19 August 1996, 17 November 1996, 31 December 1996, 31 January 1997 and 28 February 1997. On each occasion, lake water was collected from beneath the ice and at depths of 2 m, 6 m, 10 m and 14 m, using a blackened Kemmerer bottle to minimise potential 'light shocking'. A sample from 2 m was only taken on 19 August 1996. Manipulation of samples was performed in the darkened cab of a vehicle parked adjacent to the sampling hole, in a tent pitched on the ice (Photograph 3.1) or, during open water periods, in a lakeside field hut depending on the thickness of the lake-ice. Three replicate 7 ml aliquots from each depth sample were placed into a series of glass scintillation vials to act as background samples (BG). A further 80 ml of each depth sample was decanted into a blackened bottle and spiked with an 100  $\mu$ l aliquot of <sup>14</sup>C-



Photograph 3.1: Experimental manipulations in a tent pitched on the lake ice

sodium bicarbonate working standard (specific activity 250  $\mu$ Ci ml<sup>-1</sup>). Immediately following this addition, three replicate 7 ml aliquots of the spiked sample were placed into glass scintillation vials containing 200  $\mu$ l of 6N hydrochloric acid (HCl). These 'T0' vials were the controls for the experiment. Similarly, three replicate 7 ml aliquots of the spiked sample were added to 'IA' scintillation vials containing 200  $\mu$ l of 6N sodium hydroxide (NaOH). The NaOH trapped the inorganic CO<sub>2</sub> (including the <sup>14</sup>Clabelled fraction) yielding a precise measure of the amount of isotope added. Three replicate 10 ml spiked samples from each depth were placed in Whirl-paks and incubated *in situ* at the respective depths for five hours (including solar noon). After five hours, 7 ml of water were taken from each replicate Whirl-pak and transferred in to a series of 'T5' scintillation vials containing 200  $\mu$ l of 6N HCl. In order to determine the concentration of dissolved inorganic carbon (DIC), towards the end of the experiment at least two replicate water samples were drawn from each depth and used to fill to overflowing pre-weighed, 125 ml glass, stoppered bottles. All samples were transported back to the laboratory in darkened boxes.

In the laboratory the 'T0' and 'T5' vials were agitated on a mechanical shaker at 200 r.p.m. for three hours to drive off uncomplexed <sup>14</sup>C. After three hours, 400  $\mu$ l of 6N NaOH were added to fix all remaining <sup>14</sup>C. As soon as possible (typically within 6 hours of collection) 10 ml of scintillation cocktail (Ultima Gold XR) was added to each of the vials (BG, IA, T0 and T5), and they were left to stand in the dark for 15 min before disintegrations per minute (DPM) were determined in the scintillation counter.

# 3.2.3: Dissolved inorganic carbon analysis

Gran titrations were performed on each of the 125 ml DIC samples. The bottles were weighed to obtain a precise volume of water and initial pH was recorded using a standard pH probe. A few drops of BDH 4.5 indicator and a magnetic stirrer were placed in each bottle. The samples were then titrated with 0.5 N HCl whilst the sample was constantly stirred. Volumetric readings of each titrant addition were made in the ranges pH 7.6 to 6.6 and pH 4.4 to 3.7 (Mackereth *et al.*, 1989).

### 3.2.4: Analysis of results

#### 3.2.4.1: Photosynthetic rate

The photosynthetic rate throughout the incubation was calculated using the equation (Vollenweider, 1974):

$$P_{rate} = 1.06 \text{ x } (\text{T5 - T0}) \text{ x } \text{DIC}$$
$$\text{T x IA}$$

where:

 $P_{rate} = \text{photosynthetic rate (}^{12}\text{C assimilated) (}\mu\text{g C I}^{-1}\text{ h}^{-1}\text{)}$ IA = mean DPM count for the initial isotope addition T0 = mean DPM count at time zero T5 = mean DPM count after 5 hours DIC = Dissolved inorganic carbon concentration (}\mu\text{g C I}^{-1}\text{)} T = incubation time (5 h) 1.06 = the isotope correction factor which allowed for the fact that <sup>14</sup>C is assimilated less readily than <sup>12</sup>C

#### 3.2.4.2: Assimilation numbers

Photosynthetic rate per unit chlorophyll *a* (assimilation number) for microbial photosynthesis was determined after Vollenweider (1974):

$$A = P_{rate} / \text{Chl } a$$

where:

A = Assimilation number (µg C (µg Chl a)<sup>-1</sup> h<sup>-1</sup>)  $P_{rate}$  = photosynthetic rate

Chl a = mean concentration of Chl a

Photosynthetic efficiency was calculated after Heath (1988):

$$P_{eff} = A / I$$

where:

 $P_{eff} = \text{Photosynthetic efficiency } (\mu g \text{ C} (\mu g \text{ Chl } a)^{-1} \text{ h}^{-1} (\mu \text{mol } \text{m}^{-2} \text{ s}^{-1})^{-1})$   $A = \text{Assimilation number } (\mu g \text{ C} (\mu g \text{ Chl } a)^{-1} \text{ h}^{-1})$  $I = \text{Photon flux } \mu \text{mol } \text{m}^{-2} \text{ s}^{-1}$ 

### 3.2.4.4: Dissolved inorganic carbon

The concentration of dissolved inorganic carbon (DIC) at each depth was calculated subsequent to Gran Titration using the equations (Mackereth *et al.*, 1989):

F1 = (antilog (b - pH)) x (Vs + v) x (V<sub>2</sub> - v) F2 = (antilog (a - pH)) x (Vs + v) V1 = V<sub>1</sub> x (1000/(Vs x N)) V2 = V<sub>2</sub> x (1000/(Vs x N)) DIC = (1000/Vs) x N x (V2-V1)

where:

**F1** = function for higher pH range

F2 = function for lower pH range

 $\mathbf{a} \& \mathbf{b} = \text{convenient numbers e.g. 5 \& 8}$ 

Vs = volume of sample

**v** = titrant volume added

 $V_1$  = v-axis intercept on regression plot of F1 against v

 $V_2 = v$ -axis intercept on regression plot of F2 against v

**V1** = free  $CO_2$  acidity (meq l<sup>-1</sup>)

V2 = total carbonate alkalinity (meq l<sup>-1</sup>)

(Vs + v) = correction factor for dilution by titrant

(V2 - v) = term allows for decreasing alkalinity during the titration

N = normality of titrant

**DIC** = dissolved organic carbon ( $\mu g l^{-1}$ )

### 3.3: RESULTS

### 3.3.1: Features of the water column

### 3.3.1.1: Ice-cover and light profiles

The extent of ice-cover on Ace Lake varied throughout the year (Figure 2.3). A peripheral moat developed in late December, but the lake was not totally ice-free until February (Table 3.1). Light transmission was limited during the winter (Figure 2.7), but increased by two orders of magnitude in the summer months. During the winter virtually no light was detectable below the ice and light was never detectable below 12 m.

### 3.3.1.2: Temperature

Temperature variations are described in detail in Chapter Two (Section 2.3.1.2). The minimum temperatures were recorded beneath the ice on each experimental occasion, ranging between -2 and 2 °C (Figure 2.4). The maximum temperature recorded during autotrophic production experiments was 7.36 °C at 10 m in February 1997 (Figure 2.4).

### 3.3.1.3: Chlorophyll a

Chlorophyll *a* concentrations increased with depth in Ace Lake (Figures 3.2 & 2.14). The levels measured were consistently low in the upper 6 m of the water column but increased by an order of magnitude at 14 m on each experimental occasion. The maximum Chl *a* concentration recorded was 37.81  $\mu$ g l<sup>-1</sup> at 14 m in January 1997, the minimum 0.62  $\mu$ g l<sup>-1</sup> beneath the ice in August 1996.

#### 3.3.1.4: Nutrient concentrations

The concentrations of nitrate (NO<sub>3</sub>) (Figure 3.3), ammonia (NH<sub>3</sub>) (Figure 3.4) and soluble reactive phosphorus (SRP) (Figure 3.5) were usually low throughout the experimental period. The highest concentration of NO<sub>3</sub> recorded concomitant with autotrophic production experiments was 1.62  $\mu$ g l<sup>-1</sup> at 14 m in November 1997. Concentrations of NH<sub>3</sub> were an order of magnitude higher than NO<sub>3</sub> concentrations at all depths. Levels of NH<sub>3</sub> peaked between July and August 1996 at all depths for which data were available. On each experimental occasion NH<sub>3</sub> concentrations were highest at

Date	Depth	pН	DIC	Chl a	Ice	Irradiance	P <sub>rate</sub>	A	P <sub>eff</sub>
	(m)		(µg C l <sup>-1</sup> )	(µg l <sup>-1</sup> )	(m)	(µmol m <sup>-2</sup> s <sup>-1</sup> )	$(\mu g C l^{-1} h^{-1})$	$(\mu g \ C \ (Chl \ a)^{-1} \ h^{-1})$	$(\mu g C (Chl a)^{-1} h^{-1})$
									$(\mu mol m^{-2} s^{-1})^{-1})$
19-Aug-96	UI	7.90	$2.16 \times 10^4$	0.62	1.3	0.614*	nd	-	-
	2	8.18	1.71 x 10 <sup>4</sup>	0.79		0.365*	nd	-	-
	6	8.28	1.75 x 10 <sup>4</sup>	0.96		0.199*	nd	-	-
	10	8.26	$12.39 \times 10^{4}$	1.00		0+	nd	-	-
17-Nov-96	UI	8.41	1.38 x 10 <sup>4</sup>	1.86	1.6	-	nd	-	-
	6	8.46	$1.24 \times 10^4$	2.01		-	nd	-	-
	10	8.38	$1.63 \times 10^4$	4.60		-	nd	-	-
	14	7.89	16.89 x 10 <sup>4</sup>	34.91		-	nd	-	-
31-Dec-96	UI	8.29	$0.69 \times 10^4$	0.95	1.3	123.18+	nd	-	-
	6	8.24	1.26 x 10 <sup>4</sup>	1.38		36.17+	nd	-	-
	10	8.12	$0.78 \times 10^4$	3.49		15.24+	1.15	0.330	0.022
	14	7.60	$34.80 \times 10^4$	34.87		0+	9.25	0.265	265.211
31-Jan-97	UI	8.35	8.95 x 10 <sup>4</sup>	2.42	0.75	-	8.72	3.603	-
	6	8.27	$18.64 \times 10^4$	1.27		-	nd	-	~
	10	8.56	13.37 10 <sup>4</sup>	2.88		-	nd	-	-
	14	7.71	$37.62 \times 10^4$	37.81		-	nd	-	-
28-Feb-97	UI	8.26	$22.65 \times 10^4$	1.03	0	-	10.36	10.058	-
	6	8.07	$22.54 \times 10^4$	0.96		-	nd	-	-
	10	8.13	35.39 x 10 <sup>4</sup>	6.68		-	nd	-	-
	14	8.07	$26.93 \times 10^4$	10.16			nd	-	-

**Table 3.1:** Photosynthetic parameters for Ace Lake: pH, dissolved inorganic carbon (DIC), chlorophyll *a* concentration (Chl *a*), irradiance, photosynthetic rate ( $P_{rate}$ ), assimilation number (*A*) and photosynthetic efficiency ( $P_{eff}$ ). **nd** not detectable; + L.M. Rankin & T. Pitman, personal communication; - no data available



Figure 3.2: Mean chlorophyll *a* concentration in the water column of Ace Lake. Error bars (n = 2-3). Data from Chapter 2



Figure 3.3: Mean nitrate concentration in Ace Lake. Error bars (n = 2-3). Data from Chapter 2



Figure 3.4: Mean ammonia concentration in Ace Lake. Error bars (n = 2-3). Data from Chapter 2



Figure 3.5: Mean Soluble Reactive Phosphorus concentration in Ace Lake. Error bars (n = 2-3). Data from Chapter 2

10 and 14 m. A maximum  $NH_3$  concentration of 48.9 µg l<sup>-1</sup> was measured during the experimental period at 10 m in August. Similarly, concentrations of SRP were consistently highest at 10 and 14 m; a maximum of 269.8 µg l<sup>-1</sup> was recorded at 10 m in September.

### 3.3.1.5: Dissolved organic carbon, inorganic carbon and pH

Dissolved organic carbon concentrations (DOC) varied with depth within the range  $4.48 \times 10^3$  to  $13.47 \times 10^3 \mu g l^{-1}$  (Figures 3.6 & 2.12). The lowest concentration was measured beneath the ice in December, however, levels of DOC recorded beneath the ice were high in August and November 1996. The highest DOC concentrations were consistently observed at 14 m. DOC concentrations also varied seasonally. Generally, higher concentrations were recorded in winter, and these subsequently decreased through spring and mid-summer, increasing once more towards the end of summer (Figure 3.6). The highest concentration of dissolved inorganic carbon (DIC) in the experimental period (3.8 x  $10^5 \mu g l^{-1}$ ) was observed at 14 m in January 1997, the lowest (6.9 x  $10^3 \mu g l^{-1}$ ) beneath the ice in December 1996 (Figure 3.6). Levels were consistently highest at 14 m and DIC peaked in the summer months at all depths. pH ranged between 7.60 and 8.56 and generally decreased with depth, although the reverse was true in August 1996.

### 3.3.2: Autotrophic production

#### 3.3.2.1: Photosynthetic rate

Photosynthesis was undetectable throughout much of the year (Table 3.1). A measurable photosynthetic rate ( $P_{rate}$ ) was first recorded at 10 and 14 m in December 1996 (midsummer). Concomitant with diminishing ice-cover, photosynthesis was measured beneath the ice in January 1997 and at the surface in February 1997 (Table 3.1). Rates of autotrophic production ranged from 1.15 to 10.36 µg C l<sup>-1</sup> h<sup>-1</sup>. Maximum  $P_{rate}$  was recorded at the lake surface in February 1997.  $P_{rate}$  was closely related to the maximal abundance of autotrophs in Ace Lake (Table 3.2).  $P_{rate}$  was high at 10 and 14 m in December 1996, concomitant with the maximum abundance of autotrophic bacteria at these depths. Similarly,  $P_{rate}$  was high beneath the ice and at the lake surface in January and February 1997, respectively, coincident with the respective maximal abundance of *Mesodinium rubrum* and autotrophic dinoflagellates and diatoms (Table 3.2).



Figure 3.6: Mean concentration of dissolved organic (filled circles) and inorganic (open triangles) carbon in Ace Lake. Error bars (n = 2-3). DOC data from Chapter 2

Date	Depth (m)	Abact	PNAN abundance	Dinoflagellate abundance	Diatom abundance	<i>M. rubrum</i> abundance
	(11)	(cells $l^{-1} \times 10^8$ )	(cells $l^{-1} \times 10^6$ )	(cells $l^{-1}$ )	(cells l <sup>-1</sup> )	(cells 1 <sup>-1</sup> )
19-Aug-96	ŪI	0.30	0.09	80	0	0
	2	0.51	0.17	60	0	0
	6	0.62	0.10	46	0	0
	10	0.69	0.78	32	2	0
17-Nov-96	UI	0.23	0.73	3726	14214	420
	6	0.90	1.06	1764	26198	18
	10	0.41	1.67	130	21682	20
	14	0.04	0.58	0	0	0
31-Dec-96	UI	4.14	0.69	1800	47875	4900
	6	1.73	1.38	3900	31350	19050
	10	6.19	0.69	5750	35400	12800
	14	4.76	0.58	0	0	0
31-Jan-97	UI	1.89	1.90	19300	6000	62000
	6	0.46	1.18	7100	37700	4100
	10	6.49	2.59	5900	58900	4900
	14	5.93	0.58	0	0	0
28-Feb-97	UÍ	0.97	0.66	1200	17500	1100
	6	1.36	0.66	300	18800	500
	10	4.10	0	900	1100	300
	14	31.10	0.58	0	0	0

 Table 3.2: Mean abundance of the dominant autotrophs in Ace Lake; autotrophic bacteria (Abact), autotrophic flagellates (PNAN), dinoflagellates, diatoms and *Mesodinium rubrum*. Data from Chapter 2

#### 3.3.2.2: Assimilation number

Chlorophyll *a* specific photosynthetic rates (assimilation number; *A*) ranged from 0.265 to 10.058  $\mu$ g C (Chl *a*)<sup>-1</sup> h<sup>-1</sup> (Table 3.1). Photoinhibition was evident at midsummer since no photosynthesis was detected at the surface despite measurable photosynthesis at depth. No photosynthesis was detected at 6 m on any experimental occasion.

### 3.3.2.3: Photosynthetic efficiency

Photosynthetic efficiency  $(P_{eff})$  ranged between 0.022 and 265.211 (Table 3.1). Although limited light data are available, it would appear photosynthetic efficiency increased with depth.

### 3.4: DISCUSSION

#### 3.4.1: Photosynthetic rate

The extreme conditions of Antarctic aquatic ecosystems elicit distinct seasonal, phytoplankton production strategies (Heath, 1988). Within Ace Lake, the autotrophic components of the microbial food web varied in their response to these conditions (Table 3.2). <sup>14</sup>CO<sub>2</sub> fixation rates in autotrophic nanoflagellates (PNAN) varied from no detectable assimilation in the winter darkness, to summer peaks of production (Table 3.1) coincident with peak biomass throughout the water column (Figure 2.30). Photosynthesis beneath the ice in January was attributable to the autotrophic ciliate Mesodinium rubrum and autotrophic dinoflagellates. M. rubrum is known to form "red water" blooms with densities of over  $10^6$  cells  $l^{-1}$ , and achieve photosynthetic rates of greater than 500 µg C l<sup>-1</sup> h<sup>-1</sup> (Smith & Barber, 1979; Lindholm, 1985; Stoecker et al., 1991). The summer bloom in Ace Lake reached 6.2 x  $10^4$  cells  $l^{-1}$  (Table 3.2) with a corresponding  $P_{rate}$  of 8.72 µg C l<sup>-1</sup> h<sup>-1</sup> (Table 3.1), both values two orders of magnitude less than the aforementioned levels. In February, peak  $P_{rate}$  was observed concurrent with a diatom bloom in Ace Lake. Hardly surprising since diatoms dominate the world's net autotrophic productivity, contributing 20 to 25 % of the total estimated net autotrophic productivity (Austin, 1988).

Maximum autotrophic bacterial photosynthesis and biomass (Figure 2.30) peaked at midsummer (December) in the region of the oxycline and below, coinciding with maximum PAR levels, increasing temperatures and high ambient concentrations of nutrients remineralised during the winter months (e.g. Findenegg, 1969) (Table 3.1).

Similar, summer maxima of photosynthetic rates  $(P_{rate})$  have been observed in other Antarctic lakes, for example Crooked Lake, Vestfold Hills (Bayliss et al., 1997). However, this contrasts with lakes in temperate regions where most of the production, and much of the annual production, of phytoplankton is supported by a spring bloom (Geider et al., 1998) and other Antarctic lakes, for example, Watts Lake, Vestfold Hills (Heath, 1988), Lake Verkhneye, Schirmacher Oasis (Kaup, 1994) and Sombre Lake, Signy Island (Hawes, 1990a). Where detectable,  $P_{rate}$  in Ace Lake was generally higher than rates observed in other Antarctic lakes, instead comparing favourably with  $P_{rate}$  for temperate waters (Table 3.3). The rates measured during this study also exceeded those recorded in Ace Lake during 1979 by Hand and Burton (1981), which may be a reflection of the lower chlorophyll concentrations in the lake at that time. Surprisingly, despite the Deep Chlorophyll Maximum (DCM) apparent near the thermocline in Ace Lake (6 to 8 m) (Section 2.4.2.1), no photosynthesis was detected at 6 m. In Dry Valley lakes and other lakes of the Vestfold Hills, peak photosynthesis occurs in the DCM region. In some cases, deep living algae are responsible for most of the phytoplankton biomass and autotrophic production in lakes. Konopka (1989) determined that Aphanizomenon flos-aquae in Crooked Lake, Indiana was responsible for 88 % of the biomass and 23 to 37 % of the total areal autotrophic production. In Lake Michigan, the DCM contributed 63 % to areal autotrophic production (Moll et al., 1984). In several of the lakes in the Experimental Lakes Area, Canada, 40 % of the plankton biomass was concentrated in the metalimnion and was responsible for 2 to 20 % of the autotrophic production (Fee, 1976; 1978a; 1978b). In Lake Cisó, Spain, the metalimnetic peak of Cryptomonas phaseolus is responsible for > 60 % (often > 90 %) of total lake algal biomass and essentially all the oxygenic carbon fixation (Gasol et al., 1992; 1993). Indeed, Fee (1976) stated that a large error could be made in estimating autotrophic production and biomass estimates if the metalimnetic algal populations were to be ignored. The disparity in Ace Lake may be due to the uncoupling of growth and photosynthesis which will be discussed in Section 3.4.4. The cyanobacterial genus Synechococcus is responsible for 10 % of total marine productivity (Waterbury et al., 1979). This was the most abundant cyanobacterial genus in Ace Lake and contributes to the second DCM observed at the oxycline (Section 2.4.2.1) (Rankin et al., 1997).

# 3.4.2: Photoinhibition, low-light adaptation and photosynthetic efficiency

Photoinhibition was apparent in the phytoplankton of Ace Lake at certain times during the summer. During midsummer, no photosynthesis was detectable in the surface waters concomitant with maximum solar radiation and 24 h daylight. In contrast, photosynthesis was detected below the ice in January and increased to a maximum in February at the lake surface, as incoming solar radiation declined and the period of 24 h daylight ceased, despite the absence of a protective ice-cover. At low light intensities production is proportional to the total energy of visible light, but it has been known for some time that strong natural daylight inhibits phytoplankton photosynthesis (Goldman et al., 1963; Schindler & Fee, 1975). The chemical composition of cells changes in response to cues received from the environment. This adjustment of cellular physiology is termed acclimation (Geider et al., 1998). Typically it is assumed that acclimation serves to increase growth rate under sub-optimal conditions to above the value that would be achieved if cellular chemical composition were static. However, acclimation may also serve to limit the damage that can be incurred following exposure to adverse environmental conditions. Damage to the photosynthetic apparatus can occur at high light intensities when the excitation energy in absorbed photons exceeds the ability of cells to dissipate excess heat, or causes photochemical damage due to the formation of O<sub>2</sub> free radicals. The potential damage occurring from excess excitation energy in cells acclimatised to the extreme Antarctic conditions is exacerbated when nutrients are scarce, a situation which is readily apparent in Ace Lake (Figures 3.2, 3.3, 3.4, 3.5 & 3.6). Thus, acclimation may include a trade-off between maximising growth at low irradiance versus minimising the potential for photooxidative damage at high irradiance (Raven, 1980). As will be discussed in Chapter 5, measurable growth rates, albeit the lowest in Ace Lake, were apparent in the lake's surface waters during the summer despite photoinhibition.

One way for phytoplankton to overcome light and/or nutrient limitation in stratified waters where irradiance is high at the surface and nutrients supplied by upward diffusion, is to cycle between the high-light/low-nutrient environment of the mixolimnion and the low-light/high-nutrient environment of the monimolimnion (Geider *et al.*, 1998). Vertical migration may, therefore, be important in Ace Lake where the dominant phytoplankton, *Pyramimonas gelidicola* and *Mesodinium rubrum* (Sections 2.4.2.1 & 2.4.2.4), are both highly motile (Geider *et al.*, 1998). Likewise, buoyancy is regulated in the predominant cyanobacterial populations in the monimolimnion allowing populations to migrate over short distances (Konopka, 1984; 1989).

Glover *et al.* (1985a; 1985b) suggested that photosynthetic efficiency  $(P_{eff})$  is greater during periods of low photon flux. Conversely, Putt and Prezelin (1985) recorded peak rates of photosynthesis for cyanobacteria at midday, the period of highest photon flux. Increased phytoplankton  $P_{eff}$  in lower light intensities at depth is apparent in Ace Lake, despite limited data (Table 3.1). Such increases have been widely reported in Antarctic lakes (e.g. Heath, 1988; Lizotte *et al.*, 1996; Bayliss *et al.*, 1997). Moreover, as a result of photoinhibition,  $P_{eff}$  usually declines through the austral summer, decreasing at the surface first (Lizotte *et al.*, 1996). These trends suggest that Antarctic phytoplankton, are adapted to low-light intensities. Low-light adaptation has indeed been demonstrated in Watts Lake and Crooked Lake, Vestfold Hills (Heath, 1988; Bayliss *et al.*, 1997) and in Lake's Fryxell, Vanda and Bonney, McMurdo Dry Valleys (Vincent, 1981; Vincent & Vincent, 1982; Lizotte *et al.*, 1996).  $P_{eff}$  values for Ace Lake lie within the range reported for these lakes (Table 3.3). However, the  $P_{eff}$  of autotrophic bacteria in Ace Lake was much higher, probably due to their accessory pigments (Kirk, 1983) (Section 3.1.3.1) and/or the use of wavelengths weakly absorbed by phytoflagellates (Lampert & Sommer, 1997) (Section 3.1.3.6). Further work would be required to establish diurnal patterns in  $P_{eff}$ 

### 3.4.3: Chlorophyll a and assimilation number

Variations in the thickness of ice and snow cover over Antarctic lakes can induce abrupt changes in photosynthesis by the planktonic and benthic autotrophs beneath. Chlorophyll *a* concentrations in Ace Lake were comparable to those measured in other oligotrophic Antarctic lakes confirming Ace Lake's oligotrophic status (Table 3.3). Photosynthetic rates exhibited a strong positive response to more favourable energy supplies with increasing rates of chlorophyll *a* specific photosynthesis (assimilation number; *A*). In Ace Lake, *A* values exceeded those reported from Crooked Lake in the Vestfold Hills and lakes from the McMurdo Dry Valleys (Table 3.4). Instead, they lay within the range reported for maritime Antarctic lakes (with the exception of the exogenically enriched Wallows (Ellis-Evans, 1991)), which reportedly approach theoretical maxima for phytoplankton efficiency during summer open-water periods (Light *et al.*, 1981; Priddle *et al.*, 1986; Hawes, 1990a) (Table 3.4). Maximum *A* in Ace Lake was less than that reported for Watts Lake in the Vestfold Hills, one of the most productive Antarctic Lakes (Heath, 1988).

Typical values of A in temperate zone plankton are 3 to 6 µg C (µg Chl a)<sup>-1</sup> h<sup>-1</sup> (Parsons & Takahashi, 1973), thus, the assimilation numbers for microbial photosynthesis in various Antarctic waters are unusually low (Table 3.4). Similarly low assimilation numbers have been observed in Arctic lakes (Vincent, 1988), which have been attributed to low ambient temperatures (Rigler, 1978). However, temperature does not seem to be the primary variable governing A in Ace Lake, since no correlation was observed between temperature and  $P_{rate}$ ,  $P_{eff}$  or A (p > 0.05), or for other Antarctic Lakes. Indeed, large variations in A for lakes, all at near freezing temperatures, are apparent (Table 3.4). Conversely, in McMurdo Dry Valley lakes such as Lake Vanda, assimilation numbers remain low despite relatively warm temperatures in the lake's bottom waters (Vincent, 1988). Goldman *et al.* (1963) stated that assimilation numbers

Location	Chl a	pН	DIC	DOC	EDOC	P <sub>rate</sub>	P <sub>eff</sub>	P <sub>areal</sub>	Source
	(µ <b>g l</b> ⁻¹)		(µg C l <sup>·1</sup> )	(µg C l <sup>-1</sup> )	% of	$(\mu g C l^{-1} h^{-1})$	(μ <b>g</b> Č (μ <b>g</b>	$(mg \ C \ m^{-2} \ d^{-1})$	
					P <sub>rate</sub>		Chl $a$ ) <sup>-1</sup> h <sup>-1</sup>		
							$\mu$ mol m <sup>-2</sup> s <sup>-2</sup> )		
Crooked Lake, Vestfold Hills	< 1 <sup>1</sup>	*	*	30-1155 <sup>2</sup>	*	<b>0-0.56</b> <sup>1</sup>	0.001-0.028 <sup>1</sup>	*	<sup>1</sup> Bayliss et al., 1997
									<sup>2</sup> Laybourn-Parry et al., 1995
Watts Lake, Vestfold Hills	0.1-10	*	*	*	*	*	0.001-4.760	0-340	Heath, 1988
Deep Lake, Vestfold Hills		*	2.48 x 10 <sup>4</sup>	*	*	0.15-0.18	*	27.4	Campbell, 1978
Saline lakes, Bunger Hills	*	*	*	*	*	0.54-7.13	*	*	Kaup et al., 1993
Verkhneye, Schirmacher Oasis	0.05-0.50	*	*	*	*	*	*	1.1-10.1	Kaup, 1994
Glubokoye, Schirmacher Oasis	0.23-0.80	*	*	*	*	*	*	9.8-37.7	Kaup, 1994
Pomornik, Schirmacher Oasis	0.45-0.65	*	*	*	*	*	*	5.9-22.0	Kaup, 1994
Lake Bonney, Dry Valleys	0.03-3.80	*	*	*	*	0-0.13	0.0025-0.0049	*	Lizotte et al., 1996
Lake Fryxell, Dry Valleys	*	*	*	*	*	0.2-4.0	*	*	Vincent, 1981
Lake Vanda, Dry Valleys	*	*	*	*	*	0.003-0.019	*	*	Vincent & Vincent, 1982
Sombre Lake, Signy Is.	1.6	7.08	51.46+	*	*	*	*	*	Ellis-Evans, 1991
Heywood Lake, Signy Is.	18.9	7.45	41.50+	*	2.1	*	*	*	Ellis-Evans, 1991
The Wallows, Signy Is.	211.0	9.54	35.82+	*	17.5	*	*	*	Ellis-Evans, 1991
Elephant Is., Southern Ocean	0.7	*	*	*	*	*	*	374	Helbling et al., 1995
Atlantic Ocean, Arctic	*	*	*	*	*	0.12-2.0	*	*	Smith et al., 1985
Baltic Sea	*	*	*	*	*	0.2-3.4	*	*	Larsson & Hagström, 1982
Oslofjorden, Norway	*	*	*	*	*	0.1-30	*	*	Throndsen, 1978
Celtic Sea	*	*	*	*	*	0.7-12.4	*	*	Joint & Pomroy, 1983
Mediterranean	*	*	*	*	*	2.5-9.2	*	*	Berman et al., 1984
South China Sea	*	*	*	*	*	0.01-0.16	*	*	Saijo & Takesue, 1965
Pacific Ocean, Subtropical	*	*	*	*	*	0.75-1.5	*	*	Bienfang & Takahashi, 1983
Pacific Ocean Tropical	*	*	*	*	*	0.2-45	*	*	Li et al., 1983
Exmouth Gulf, Australia	0.2-0.3	*	*	*	*	1.04	*	*	Ayukai & Miller, 1998

**Table 3.3:** Comparative photosynthetic rate  $(P_{rate})$ , photosynthetic efficiency  $(P_{eff})$  and areal production  $(P_{areal})$  values for a global range of aquatic ecosystems. + values in  $\mu$ mol l<sup>-1</sup>
Location	Ice-cover	Temperature	A	Source
	( <b>m</b> )	(°C)	$(\mu g \ C \ (\mu g \ Chl \ a)^{-1}$	
			h''	
Crooked Lake, Vestfold Hills	2	0.2-2.5	0-1.27	Bayliss et al., 1997
Watts Lake, Vestfold Hills	0.5-1.5	0.5-3.0	17.3	Heath, 1988
Lake Verkhneye, Schirmacher Oasis	*	0.5-8.0	0.03	Kaup, 1994
Lake Vanda, Dry Valleys	3.25	4.5-19.0	0.11-0.14	Vincent & Vincent, 1982
Lake Fryxell, Dry Valleys	4.5	2.0	0.05-0.11	Vincent, 1981
Lake Joyce, Dry Valleys	5.0	2.0	0.01	Parker et al., 1982
Lake Bonney, Dry Valleys	4.0	0.5	0.02	Parker et al., 1982
			0.3	Lizotte & Priscu, 1992
Algal Lake, Ross Is.	0	<5	10.0	Goldman et al., 1972
Skua Lake, Ross Is.	00	<5	7.5	Goldman et al., 1972
Sombre Lake, Signy Is.	0-1	1.5	0.3-6.7	Hawes, 1985
		3.0	6.3	Ellis-Evans, 1991
Heywood Lake, Signy Is.	0-1	1.5	1.1-11.8	Hawes, 1985
		4.4	11.2	Ellis-Evans, 1991
The Wallows, Signy Is.	*	14.2	20.6	Ellis-Evans, 1991
Elephant Is., Southern Ocean	*	-1.8-4.5	1.02-2.92	Helbling et al., 1995

 Table 3.4: Assimilation numbers (A) for Antarctic aquatic communities

in Lake Fryxell, were higher in open water conditions, due to high summer temperatures reducing the damage caused by photoinhibition. In Ace Lake, the highest value of A observed was at the surface in February 1997, concomitant with open water conditions and high water temperatures. Nevertheless, no photosynthesis was ever detected at 6 m in Ace despite temperatures of up to 5.32 °C in the summer at this depth (Figure 2.4).

Four other factors are more likely than temperature to be responsible for the low measured A values. Firstly, as mentioned previously, Antarctic phytoplankton are often shade-adapted as a result of the persistent ice and snow cover (Vincent, 1988). Such cells usually increase their light-harvesting capacity, manifested as more pigment per unit photosynthesis i.e. a lower assimilation number. For example, Signy Island lakes show a marked difference in A between summer and winter measurements (Table 3.4; the lowest values in the ranges recorded by Hawes (1985) are the winter values, the highest the summer values). Secondly, photosynthetically inactive chlorophyll may persist associated with senescing cells or chlorophyll a decomposition products, since chlorophyll a degradation is often retarded by the saline, reducing environment in stratified lakes such as Ace Lake (Section 2.4.2.1; Tominaga & Fukui, 1981). This situation is exacerbated by low temperatures since they slow rates of decomposition, and may explain the link often made between low temperatures and low assimilation numbers (Vincent & Howard-Williams, 1988). Thirdly and conversely, chlorophyll can be diluted by fresh melt water inputs (Sharp & Priscu, 1991), and this can artificially raise the observed value of A. The latter was apparent in the December assimilation 'peak' in Lake Bonney (Sharp & Priscu, 1991) and may account in part, for the rapid increase in A recorded during open-water in February in Ace Lake. Fourthly, the pigment ratio of Chl a to Chl b is known to decrease in low light indicating a decrease in Chl a concentration (Weyers et al., 1998). Since the Chl a concentration used in the calculation did not account for the relative amount of Chl a and Chl b, the measured A could have been underestimated.

#### 3.4.4: The effect of temperature on photosynthesis

Tilzer *et al.* (1986) confirmed that temperature affects photosynthetic rate  $(P_{rate})$  in limiting light conditions and Talling (1984) suggested that these temperature effects are reduced at low irradiance deep in the euphotic zone. It is likely that, as with the degradation of Chl *a*, temperature affects photosynthesis indirectly by exacerbating the affects of other factors. Hawes (1990a) investigated the influence of temperature, in combination with radiation flux, on the partitioning of photosynthetically fixed carbon into four intracellular metabolic pools for natural phytoplankton assemblages in

oligotrophic, Sombre Lake, Signy Island, Antarctica. At ambient temperatures protein synthesis was saturated at low photon flux densities. Above this flux fixed carbon was increasingly stored as lipid and polysaccharide. As a result, estimates suggested that <sup>14</sup>C incorporation into protein reflected cell growth more closely than total <sup>14</sup>C uptake.

Under adverse conditions, of low temperature and/or nutrient limitation, a small proportion of photosynthetically fixed carbon enters the protein pool (Morris et al., 1974). Therefore, even though low temperatures might be expected to result in low growth rates, <sup>14</sup>C estimates of growth have revealed rates similar or in excess of temperate and tropical lakes (Light et al., 1981), a scenario apparent in growth rates from Ace Lake, in particular at the thermocline/halocline (6 to 8 m) (Table 5.1). However, in many phytoplankton assemblages there is little relationship between the rate of carbon fixation and net cell growth (Priddle et al., 1986). Phytoplankton biomass increases are often highest under spring ice-cover, when rates of photosynthesis are low, while during the ice-free period there is little biomass increase despite high rates of photosynthesis. It is possible that this poor coupling between growth rates and photosynthesis in the permanently cold, oligotrophic waters of Ace Lake and other Antarctic lakes e.g. Lake Sombre, are due to low rates of protein synthesis at low ambient temperatures (Hawes, 1990a). Conversely, high rates of photosynthesis may represent an accumulation of reserve products (e.g. polysaccharide) rather than cell growth (Hawes, 1990a), a possibility corroborated by the fact the dominant phytoplankton species in Ace Lake, Mesodinium rubrum, has been observed to accumulate large starch reserves during the summer months presumably in order to maintain viability over prolonged winter darkness (Section 2.4.2.4) (Perriss et al., 1995). The overall patterns of photosynthesis and growth rate in Ace Lake were well suited to this contention.

The enzymes associated with the photosynthetic fixation of inorganic carbon in Antarctic phytoplankton are similar to those of temperate species. Accordingly, Jacques (1989) inferred that Antarctic phytoplankton have not developed specific mechanisms for overcoming the constraints of low temperature, a situation that Wiencke *et al.*, 1993 attributed to the short geological time spans available for physiological adaptation to low water temperatures in Antarctica. However, Devos *et al.* (1998) demonstrated that RUBISCO enzymes in *Chloromonas* spp. were adapted to low temperatures. Although, psychrophilic RUBISCO had optimum temperatures that were similar to mesophilic enzymes, they showed a greater thermosensitivity. Furthermore, the relative amount of RUBISCO sub-units was twice as high in psychrophilic algae as mesophilic algae. Such, high production of a key enzyme counter-balances its poor catalytic

efficiency at low temperature and constitutes a novel adaptive mechanism to cold environments.

#### 3.4.5: Utilisation of inorganic nutrients by phytoplankton

Nutrient availability is another crucial factor in determining the levels of autotrophic productivity that can be sustained in Antarctic lacustrine systems. In particular, phytoplankton production appears to be closely related to the *input* of nutrients. In the Experimental Lakes Area of Canada, Schindler and Fee (1975) demonstrated that increasing the input of critical nutrients such as nitrogen and phosphorus increased phytoplankton production. The catchment of Ace Lake provides a limited allochthonous supply of inorganic nutrients due to its sparse vegetation (dominated by lichens and mosses where present). Owing to its aspect, much of the snow on the lake and surrounding hills is blown away, so nutrients resulting from meltwaters are also limited and highly seasonal in their availability (Torii *et al.*, 1988; Knox, 1990; Priscu, 1995; Crittenden, 1998). Instead, nutrients (and carbon) are mainly derived from autochthonous processes, remineralisation being particularly effective in such closed systems (Findenegg, 1969).

Inorganic nitrate (NO<sub>3</sub>), ammonia (NH<sub>3</sub>) and soluble reactive phosphorus (SRP) concentrations in Ace Lake varied seasonally. Levels of these nutrients were generally higher in winter at all depths, although concentrations in the monimolimnion were an order of magnitude higher than those measured in the mixolimnion (Figures 3.3, 3.4 & 3.5). The levels measured in the mixolimnion are typically considered to limit phytoplankton communities (Vincent, 1981; Spaulding et al., 1994). Phosphorus is usually considered to be the element controlling phytoplankton growth in freshwater systems, in maritime Antarctic lakes (Hawes, 1983; Kaup, 1994), but, nitrogen has been identified as the limiting element in some continental Antarctic Lakes. Under-ice communities in Lake Fryxell, McMurdo Dry Valleys, responded to ammonium enrichment and, hence, were concluded to be nitrogen limited (Vincent, 1981). In Ace Lake, NO<sub>3</sub> and NH<sub>3</sub> concentrations were maximal in winter at all depths, when no photosynthesis was detected. Concentrations of both were extremely low in the mixolimnion during the summer which may account for the limited period in which <sup>14</sup>CO<sub>2</sub> fixation was detected. During January and February the lake-ice and surrounding snow melted, some of the nitrogen required for this brief period of production in the lake's surface waters may have derived from these meltwaters (Crittenden, 1998). Nitrogen levels were consistently higher in the monimolimnion, however, following the December maximum in  $P_{rate}$  at 10 and 14 m, both NO<sub>3</sub> and NH<sub>4</sub> concentrations rapidly decreased to undetectable levels. On the contrary, SRP was detectable at all depths, throughout most of the year. Levels were greatest in the winter and autumn months, although there was a peak in SRP availability beneath the ice in midsummer which declined concomitant with maximal  $P_{rate}$ . It is, therefore, most likely that nitrogen rather than phosphorus is the limiting element for photosynthesis in Ace Lake, a suggestion corroborated by the work of Hand and Burton (1981). Further bioassays would be required to prove this conclusively.

# 3.4.6: Relationships between inorganic nutrients and phytoplankton production

Phytoplankton communities may respond to a decreased supply of a limiting nutrient by either decreasing the maximum rate of production, or producing less efficiently at suboptimal irradiance (Schindler & Fee, 1975). For this reason, several models have been proposed to predict  $P_{rate}$  from the concentration of nutrients available (Lampert & Sommer, 1997). In the temperate regions of North America and Europe average daily autotrophic production is closely related to the concentration of total phosphorus (Smith, 1979).

# $P_{rate} = 10.4 P_{tot} - 79, r^2 = 0.94$

This model has been used to predict to a high degree of certainty autotrophic production from total phosphorus concentration. However, when applied to data collected from Ace Lake, the predicted  $P_{rate}$  was an order of magnitude greater than the observed  $P_{rate}$ (Table 3.5). This may be due to the fact that the molybdate method for phosphate analysis used in this study over-estimates phosphate in some natural waters. The discrepancy is unpredictable but may be as high as 10 to 500 times too great in oligotrophic lakes such as those found in Antarctica (Schindler & Lee, 1975). In addition, photosynthesis in Antarctic lakes is affected by a combination of aforementioned factors which have a less marked affect on photosynthesis in temperate lakes.

Other empirical models have been used to predict the biomass of phytoplankton with inorganic nutrients. Chlorophyll is often used as a surrogate parameter for biomass, the conceptual basis being that a certain amount of biomass can be formed from a certain concentration of a limiting nutrient. The best known model is the OECD model in which the relationship between biomass and phosphorus using chlorophyll a as the surrogate, is derived by the regression equation (Lampert & Sommer, 1997 cite Vollenweider & Kerekes, 1982):

Date	Depth	Actual Chl a	Predicted Chl a	Actual P <sub>rate</sub>	Predicted P <sub>rate</sub>
	( <b>m</b> )	(μ <b>g</b> l <sup>-1</sup> )	$(\mu \mathbf{g}  \mathbf{l}^{-1})$	$(\mu g C l^{1} h^{1})$	$(\mu g C l^{-1} h^{-1})$
19-Aug-96	UI	0.62	1.21	nd	nd
	2	0.79	3.70	nd	3.08
	6	0.96	19.93	nd	33.54
	10	1.00	30.89	nd	54.86
17-Nov-96	UI	1.86	4.06	nd	3.73
	6	2.01	2.75	nd	1.39
	10	4.60	22.24	nd	38.01
	14	34.91	71.89	nd	136.91
31-Dec-96	UI	0.95	10.51	nd	15.62
	6	1.38	9.83	nd	14.35
	10	3.49	18.85	1.15	30.51
	14	34.87	36.11	9.25	65.13
31-Jan-97	UI	2.42	2.53	8.72	1.00
	6	1.27	5.13	nd	5.68
	10	2.88	30.14	nd	53.39
	14	37.81	2.23	nd	0.48
28-Feb-97	UI	1.03	2.48	10.36	0.91
	6	0.96	2.51	nd	0.96
	10	6.68	30.05	nd	53.22
	14	10.16	32.10	nd	57.25

**Table 3.5:** Comparison of predicted plankton biomass using chlorophyll *a* (Chl *a*) ( $\mu$ g l<sup>-1</sup>) as a surrogate and predicted photosynthetic rate (P<sub>rate</sub>) ( $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>), using total phosphorus (P<sub>tot</sub>) ( $\mu$ g l<sup>-1</sup>), with actual values recorded in Ace Lake

## Chl $a = 0.28 P_{tot}^{0.96}, r^2 = 0.77$

This model also failed in its predictions for Ace Lake (Table 3.5). However, the predicted chlorophyll *a* concentrations were more accurate than the  $P_{rate}$  predictions from Smith's model, and a similar pattern of Chl *a* maxima and minima were apparent.

Finally, there is evidence that latitude can provide more informative, predictive information about the productivity of lakes than nutrient concentration (Brylinski & Mann, 1973; Platt & Sathyendranath, 1995). Some data suggests that nutrient input might be inversely correlated with latitude since the factors which affect nutrient availability; zooplankton, water renewal via outflow and groundwater, sedimentation and concentrations of other nutrients which affect the velocity with which phosphorus is assimilated by phytoplankton, are known to vary with latitude (Schindler & Fee, 1975).

# 3.4.7: Utilisation of dissolved organic and inorganic carbon by phytoplankton

Schindler and Fee (1975) suggested that the standing crop of phytoplankton in the lakes of the Experimental Lakes Area, Canada, was roughly a function of the amount of phosphorus added. However, the amount of carbon in the lakes governed rate at which this phosphorus governed standing crop. They subsequently made a distinction between rate-limiting and crop-limiting factors (Schindler & Fee, 1975). In Ace Lake, and others in Antarctica, evidence indicates that the majority of dissolved organic carbon (DOC) is autochthonous i.e. derived from photosynthetic activity of phytoplankton and/or cyanobacterial mats within the lake (Vincent, 1988; McKnight et al., 1991; Davey, 1993; Moorhead & Wharton Jr., 1994; Bayliss et al., 1997). Much of the organic carbon (and phosphorus) in natural lake water is bound to large, colloidsize molecules. This 'pool' of large molecules remains relatively constant throughout the year and turns over very slowly. Uptake of carbon and phosphorus from the pool is negligible. The concentrations of small molecular weight compounds are usually low, turnover extremely rapidly and are generally composed of phytoplankton excretory products of some sort (Schindler & Fee, 1975). Excretion by phytoplankton and bacteria and grazing by zooplankton all appear to be important organic carbon recycling mechanisms (e.g. Schindler et al., 1973; Coveney, 1982; Sundh & Bell, 1992). Parker et al. (1977) reported that 16 to 25 % of photoassimilated <sup>14</sup>C was excreted. This was reduced to 9 % when the samples were gravity filtered, presumably due to reduced cell breakage. This latter rate is within the range normally reported for Antarctic freshwater

phytoplankton (Heywood, 1984). DOC concentrations in Ace Lake corroborate the importance of photosynthetic excretory products in regulating DOC levels. DOC was maximal at all experimental depths in November (Figure 3.6), following a spring bloom of bacteria (Figure 2.15). In addition, levels of DOC began to increase at all experimental depths during summer peaks in phytoplankton photosynthesis. DOC variations in winter are attributable to autolysis of summer plankton blooms (Bayliss *et al.*, 1997).

Dissolved inorganic carbon (DIC) was maximal in the summer at all experimental depths (Figure 3.6). At 6 and 14 m the concentrations of DIC decreased in February, whereas concentrations beneath the ice and at 10 m continued to increase. These patterns may be due to limited allochthonous inputs via meltwaters, and an accumulation of inorganic material and respiratory products at the oxycline, respectively. pH never exceeded pH 9 indicating that there was an abundant supply of  $CO_2$  in Ace Lake. In addition, its increase with depth suggests that the nitrogen demand for photosynthesis is primarily met by  $NH_4$ , a trend highlighted in many aquatic systems (Azam *et al.*, 1991) (Section 2.4.1.3).

## 3.4.8: Summary of autotrophic production in Ace Lake

The results presented here confirm that Ace Lake is an oligotrophic system in which the productivity of the phytoplankton, and consequently the heterotrophic plankton, is severely constrained by a combination of factors, including low annual PAR, persistently low temperatures, and limited nutrient and organic substrate availability. A similar situation is known to exist in other continental Antarctic lakes, for example, Deep Lake, Vestfold Hills (Campbell, 1978). When detectable, photosynthetic rates exceeded those measured in other Antarctic lakes and aquatic systems at other latitudes. Autotrophic production was confined to the austral summer when it would appear to be uncoupled from growth. In winter no photosynthesis was detected and it is likely that the phytoplankton population remained viable throughout the winter, through a combination of mixotrophic nutrition (Chapter 5), endogenous reserves stored during the summer or by encysting.

# Chapter 4: BACTERIAL PRODUCTION

## 4.1: INTRODUCTION

#### 4.1.1: Bacterioplankton in aquatic ecosystems

#### 4.1.1.1: Roles and regulation of bacteria

For almost a century bacteria have been known to form part of the plankton in inland and marine aquatic ecosystems. Initially, research focused on the quatitative and taxonomic studies of bacteria, but recently a "process approach" has been adopted to investigate growth rates, population dynamics and production (Hobbie, 1988). Bacteria play keys roles in the functioning of natural aquatic ecosystems. Numerous studies have documented that bacteria utilise a large fraction of the carbon (C) that flows into and is produced in aquatic ecosystems. Bacteria can serve as food for protozoans and metazoans. In some systems biomass produced by bacteria is moved to a higher trophic level, in others it is consumed and respired within the microbial food web. Bacteria may also consume limiting nutrients such as nitrogen (N) and phosphorus (P), and subsequently regenerate these upon cell death (Sanders et al., 1992; Pace & Cole, 1994). Bacterial populations can be regulated in two ways. Resource or "bottom-up" regulation refers to the limitation of bacteria by carbon and nutrients derived from allochthonous inputs, primary production, and heterotrophic bacteria. Predatory or "top-down" control refers to the limitation of bacteria below levels supportable by resources alone. In most situations, bacteria appear to be subject to bottom-up control (Pace & Cole, 1994). Limiting resources for bacteria are typically labile carbon substrates, organic and inorganic N and P. These bacterial resources have multiple origins, they can be derived from allochthonous sources or from autochthonous sources; primary production, nutrient recycling, and labile carbon production via feeding, excretion and egestion by consumers (e.g. Bratbak & Thingstad, 1985; Kirchman et al., 1990; Caron 1994; Pace & Cole, 1994).

#### 4.1.1.2: Bacteria-organic matter fluxes

The major fluxes of organic matter in aquatic ecosystems occur in the microbial loop pathway: organic matter  $\rightarrow$  bacteria  $\rightarrow$  Protozoa  $\rightarrow$  Metazoa (e.g. Pomeroy, 1974; Fuhrman & Azam, 1980). The microbial loop is, therefore, a significant biological force in shaping the spatial and temporal patterns of the distribution of the bioelements carbon (C), nitrogen (N), phosphorus (P) and iron (Fe) (Azam *et al.*, 1993).

Generally, it is assumed that the microbial loop transfers organic matter equivalent to approximately one-half of the local primary production from the dissolved phase into the particulate phase, efficiently remineralises the organic matter entering it and transfers some of the carbon to higher trophic levels. However, recent work indicates that bacterially-mediated fluxes of bioelements are highly variable, both quantitatively and qualitatively, reflecting the composition and dynamics of organic and inorganic matter in the bacterium's environment (Azam *et al.*, 1993). Carbon flux into bacteria can vary from 0 to 100 % of local primary production (Pomeroy *et al.*, 1991) and bacteria can act as consumers or producers of remineralised nutrients depending on the nutrient status of their environment (Wheeler & Kirchman, 1986).

#### 4.1.1.3: The nature of the organic matter pool

Approximately 10 to 30 % of the organic matter in the ocean is readily utilisable by microbes (Morita, 1994). Aquatic systems contain a diversity of particulate and dissolved organic matter (POM & DOM). Bacteria, and other organisms, are known to attach to POM and it is thought that such attached bacteria inhabit organically rich microzones while free bacteria live in low-nutrient environments (e.g. Azam *et al.*, 1993; Pedrós-Alió, 1994; Fabiano *et al.*, 1996). Particles create heterogeneity in terms of organic matter, remineralised nutrients and microbes, and thereby influence small-scale spatial variability in rate and type of bacterial activities.

Conversion of the particulate phase regulates the flux of C, N and P into bacteria (Azam et al., 1993). The majority of organic matter flux from the particulate phase to bacteria passes through the polymer pool; particulate source  $\rightarrow$  polymers  $\rightarrow$  direct substrate  $\rightarrow$ bacteria. Thus, major controlling force of bacterial growth is not only their ability to take up utilisable DOM at low concentrations but also to hydrolyse the more complex dissolved and particulate organic matter outside the cell membrane. Extracellular enzymes hydrolyse substrates too complex to be directly transported through the cell wall and can be bound to the cell membrane or in the periplasmic space, or dispersed in the water (Turley, 1994). Dissolved polymers constitute a substantial fraction of the organic matter pool and apparently play a pivotal role in the bacteria-organic matter coupling (Chróst et al., 1989). Peptides and polysaccharides are quantitatively important potential sources of bacterial nutrients. They account for more than 50 % of the dissolved organic carbon in surface waters (Benner et al., 1992). However, despite the fact that a distinction has traditionally been made between particulate and dissolved phases within aquatic environments, it is more likely that POM and DOM blend to create a dynamic continuum of organic matter with which bacteria interact (Azam et al.,

1993). The structure and composition of the organic matter field is still poorly understood, hampering the understanding of bacteria-organic matter interactions.

## 4.1.1.4: Bacteria-organic matter coupling

Bacterial organic matter coupling depends on the utilisability of organic matter by the bacterial assemblage. Traditionally, a small, rapid turnover pool of DOM was thought to support the carbon flux to bacteria, even though 90 to 98 % of the total DOM is old, slow-to-degrade and non-utilisable. However, this view is changing as it becomes apparent that exchange between the two pools takes place (Azam et al., 1993). Recently, Rainer et al. (1996) proposed a model whereby the bioreactivity of organic matter decreased along a continuum of size (high to low molecular weight (HMW to LMW)) and diagenetic state (from new to old). This size-reactivity model suggests that the bulk of HMW DOM is more bioreactive and less diagenetically altered than the bulk of LMW DOM. Nevertheless, given time utilisable DOM transforms into slow-todegrade DOM. As a consequence, it is advantageous for bacteria to take up new DOM in tight spatial coupling with phytoplankton and particles, where they are exposed to high concentrations of new DOM and can utilise it before it is transformed. If for some reason bacterial uptake of utilisable DOM is slowed, utilisable DOM has more time to become slow-to-degrade (Hollibaugh et al., 1992; Azam et al., 1993). Subsequently, the slow-to-degrade becomes "stored" in the DOM pool. Conversely, non-utilisable DOM may become utilisable with time, for example, via breakdown by UV radiation (Mopper et al., 1991).

## 4.1.1.5: DOM storage

DOM accumulates when production processes are operating but some bacteria are unable to use it rapidly enough, either due to DOM conversion into slow-to-degrade DOM or because bacterial growth and production are inhibited by some means. Stored DOM (and POM) is subject to a downward flux (Azam *et al.*, 1993; Revsbech, 1994; Weyhenmeyer *et al.*, 1997) and such temporal export of DOM may be important in the flux of materials and energy in Antarctic waters. The productive summer, photic period generates slow-to-degrade DOM which subsequently supports bacterial production during the winter, and supplies energy to higher trophic levels (Azam *et al.*, 1991).

Recently, the traditional view that bacteria represents a "source" of inorganic nutrients for grazers and phytoplankton a nutrient "sink", has been revised (Tranvik, 1988; Caron, 1994). There is now strong evidence that heterotrophic, unicellular organisms (heterotrophic bacteria and phagotrophic protists) are responsible for the bulk of nutrient remineralisation in aquatic ecosystems via their consumption of organic matter and bacteria and phytoplankton, respectively, and subsequent excretion of inorganic nutrients and carbon (Caron, 1994; Kirchman, 1994). However, based on recent evidence it also appears certain that uptake of dissolved inorganic nutrients by natural assemblages of bacteria is much more common than believed previously and that bacteria can compete successfully with phytoplankton for these substrates (Kirchman, 1994). Thus, bacteria can also represent a "sink" for inorgnic nutrients and carbon as well as fulfilling their traditional role as a "source" for bacterivores.

## 4.1.2: Methodology

# 4.1.2.1: General principles

While the growth of photosynthetic bacteria and other components of the phytoplankton for which carbon dioxide is their principle source of carbon can be measured using <sup>14</sup>CO<sub>2</sub>, until recently there was no reliable method to determine the growth and production of heterotrophic bacterioplankton which use organic substances as a carbon source (Moriarty, 1986). Of the methods that have now been developed, the three most commonly employed are: 1) the incorporation of the purine base, nucleic acid precursor [<sup>3</sup>H]adenine into ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) by prokaryotes and unicellular algae to give total microbial production (Karl, 1982), 2) the incorporation of the nucleoside [*methyl-*<sup>3</sup>H]thymidine ([<sup>3</sup>H]-TdR) into bacterial DNA (Moriarty, 1984; 1986), and 3) the incorporation of the labelled amino acid leucine into bacterial protein (Kirchman *et al.*, 1985; 1986).

# 4.1.2.2: Thymidine incorporation into DNA

Of the two nucleic acid precursor methods, the [<sup>3</sup>H]-TdR method, though controversial, is used most frequently (Fuhrman & Azam, 1980; Moriarty, 1984; 1986; Riemann & Bell, 1990; Robarts & Zohary, 1993). Deoxythymidine (thymidine) is the deoxyribonucleoside of the pyrimidine base thymine, in which the deoxyribose is conjugated to the nucleotide thymine-2-deoxyribose; TdR (Robarts & Zohary, 1993). The theoretical basis for the use of [<sup>3</sup>H]TdR is as follows: the growth of bacteria is

marked by cell division and an increase in numbers and biomass. Synthesis of new cell material e.g. DNA, must occur before cells divide, and the culmination of DNA synthesis is cell division. Hence, DNA synthesis does not occur in non-growing cells (Robarts & Zohary, 1983 cite Brock, 1971). The method assumes that other macromolecules do not take up exogenous [<sup>3</sup>H]-TdR, therefore, bacterial growth rates can be determined from the rate of DNA synthesis or from the rate at which DNA becomes labelled with the exogenously supplied [<sup>3</sup>H]-TdR (Moriarty, 1984; 1986).

There are two principle pathways of nucleotide (deoxynucleotides form the monomers of DNA) biosynthesis which are fundamental to the understanding of the method (Figure 4.1) (Robarts & Zohary, 1993). In the *de novo* pathway, internal cellular components are combined in successive reactions to synthesise nucleotides. Nucleotides formed in this way do not pass through a pool containing free purine or pyrimidine bases or their corresponding nucleosides. However, in the salvage pathway, free bases and nucleosides arising from the breakdown of excess nucleotides or nucleic acids are converted back into nucleotide triphosphates (Figure 4.1). The [<sup>3</sup>H]-TdR assay is based on the incorporation of exogenously supplied thymidine into DNA via the salvage pathway.

Once [<sup>3</sup>H]-TdR has been transported into the cell, it must be converted to thymidine monophosphate (dTMP) by the principle salvage pathway enzyme, thymidine kinase. Thymidine monophosphate is subsequently converted into thymidine diphosphate (dTDP) and thymidine triphosphate (dTTP) by dTMP kinase and nucleoside diphosphate kinase, respectively. Only organisms that possess this transport system for thymidine and thymidine kinase are able to take up [<sup>3</sup>H]-TdR and incorporate it into DNA, thus, a further assumption of the technique is that heterotrophic bacterial populations contain organisms which possess both systems. However, not all bacteria can incorporate [<sup>3</sup>H]-TdR into DNA. Chemolithotrophic bacteria, cyanobacteria and certain aerobic heterotrophs, notably a species of Pseudomonas, do not incorporate [<sup>3</sup>H]-TdR (e.g. Fuhrman & Azam, 1982; Robarts & Wicks, 1989; Robarts & Zohary, 1993). Anaerobic bacteria, in particular sulphate-reducing bacteria, are able to take up [<sup>3</sup>H]-TdR but a smaller percentage is incorporated into DNA when compared with other macromolecules (Gilmour et al., 1990). Often, bacterial populations are treated as a homogeneous group (Fuhrman et al., 1994; Hollibaugh, 1994; Pedrós-Alió, 1994) and this can lead to errors in estimates of growth and production rates. Furthermore, according to Rivin (1986) eukaryotic phytoplankton are also able take up [<sup>3</sup>H]-TdR but only at high exogenous concentrations (75 to 150 nM) during long incubations (12 to 24 h), conditions unlikely to be employed in bacterioplankton assays.



**Figure 4.1**: Salvage (shaded), degradative (unshaded), and *de novo* pathways of thymidine nucleotide metabolism in bacterial cells. Redrawn from Robarts & Zohary (1993)

Certain problems must be overcome when employing the TdR assay. Thymidine used in ecological assays has the hydrogen of the methyl group labelled. Catabolism of thymidine to thymine can result in the loss of this methyl group and extensive labelling of non-specific macromolecules, which are subsequently incorporated into protein. Other researchers hypothesised that bacteria might use [<sup>3</sup>H]-TdR as a carbon source rather than a DNA precursor (e.g. Robarts & Wicks, 1989). However, Robarts and Zohary (1993) cite evidence that bacteria in Lake Michigan only adapted to doing this when exposed to high light. In other instances, labelled products within the cells can be released and reincorporated into other cells during [<sup>3</sup>H]-TdR incubations (Paul, 1987; 1990). Such non-specific labelling can be extensive in natural bacterial populations. Consequently, some authors stress the importance of extracting and purifying labelled DNA, though more frequently a constant proportion of DNA is assumed (e.g. Fuhrman & Azam, 1980; Wetzel & Likens, 1991; Robarts & Zohary, 1993).

Dilution of the rate of DNA labelling can also occur, both intracellularly and extracellularly (Robarts & Zohary, 1993). Internal dilution can result through dTMP produced in the *de novo* pathway becoming mixed with dTMP formed in the salvage pathway, external dilution via increased competition for uptake and transport by the presence of other nucleosides outside the cell. Moriarty (1986) concluded that the internal convergence of the two pathways was the major dilution process for [<sup>3</sup>H]-TdR. Indeed, Fuhrman and Azam (1982) suggested that the [<sup>3</sup>H]-TdR method underestimated DNA synthesis by a factor of 2.7 to 7.1 due to dilution. For this reason it is essential that the *de novo* pathway is blocked whilst labelling bacterial DNA with <sup>3</sup>H]-TdR to avoid underestimating the rate of DNA synthesis. It is generally assumed that if sufficient exogenous [<sup>3</sup>H]-TdR is supplied, *de novo* synthesis is inhibited and isotope dilution (internal and external) can be prevented (Moriarty, 1986). However, a balance must be struck; introducing the lowest concentration of exogenous [<sup>3</sup>H]-TdR possible promotes its maximum incorporation into DNA, but low concentrations of exogenous TdR may not be sufficient to block de novo synthesis (O'Donovan, 1978). Moreover, Moriarty (1986) noted that oligotrophic bacteria may not regulate DNA synthesis in the same way as bacteria of a higher trophic status and dilution might occur regardless. Performing in situ saturation experiments to determine the concentration level at which uptake of exogenously supplied [<sup>3</sup>H]-TdR helps to reduce the problem, and it is generally concluded that dilution can be prevented by using [3H]-TdR concentrations of between 10 and 20 nM. 5 nM may even be sufficient in oligotrophic systems (Fuhrman & Azam, 1982; Robarts & Zohary, 1993).

In recent years the use of radioactively-labelled leucine for the estimation of bacterial production has become increasingly popular. The method involves incorporation of the amino acid leucine into protein, providing a direct estimate of the growth rate of bacterial biomass and, thus, the flux of carbon into bacterioplankton. Kirchman et al., (1985) presented evidence that changes in leucine incorporation rate reflect changes in protein synthesis, rather than changes in the leucine content of proteins and, therefore, serve as an index of protein synthesis in aquatic systems. The regulation of amino acid uptake is also important to understand because of the role of these compounds in the dissolved nitrogen cycle and in the supply of nitrogen for bacterial growth (Kirchman et al., 1985). Furthermore, the production and concentration of exogenous amino acids versus rates of amino acid biosynthesis could affect assimilation rates of important inorganic compounds (e.g. ammonium) and organic compounds (e.g. glucose) employed in amino acid biosynthesis. In short, the regulation of amino acid uptake and biosynthesis may, to a large extent, explain changes in rates of uptake and mineralisation of selected compounds, and ultimately bacterial growth and production, in aquatic systems (Kirchman et al., 1986).

The radioactively-labelled leucine method is sensitive and reliable, leucine does not appear to be extensively catabolised during incubations, and is specific to proteins and heterotrophic bacteria (Kirchman et al., 1985; Hollibaugh, 1994). Kirchman et al. (1985) determined that more than 50 % of the bacterial population in marine environments assimilated leucine, and of that assimilated, 90 % was incorporated directly into protein rather than being used to synthesise other amino acids. The exceptions to this were oligotrophic environments where only 50 % of the leucine taken up was incorporated directly into protein. The remaining 50 % was degraded into other amino acids, which were subsequently incorporated into protein. It was hypothesised that the degree of leucine degradation was dependent upon the supply of organic carbon, rapidly regulating the relative rates of leucine degradation and biosynthesis in response to addition of organic compounds. Bacteria preferentially maximise their utilisation of extracellular leucine for protein synthesis, rather than synthesising leucine de novo or utilising leucine for the synthesis of other amino acids (Kirchman et al., 1985). Addition of nanomolar concentrations of exogenous leucine have been shown to inhibit the total incorporation of the leucine biosynthesis precursor, [<sup>14</sup>C]pyruvate, into protein, and thus, de novo amino acid biosynthesis in natural bacterial assemblages (Kirchman et al., 1985; 1986). However, rates of leucine biosynthesis still remain higher than rates of exogenous uptake and a conversion factor which relates leucine uptake rate with the rate of protein synthesis is required (Kirchman et al., 1986).

Inevitably, there are problems associated with the method. Although the rate of leucine biosynthesis decreases when leucine is exogenously supplied, it is unclear whether the leucine that is biosynthesised contributes to protein synthesis in the presence of high exogenous concentrations. If leucine biosynthesis is important then rates of exogenous leucine incorporation underestimate the rate of protein synthesis. Conversely, unlike DNA, in selected growth conditions protein can be synthesised and degraded continuously (turnover) at high rates, thus, net incorporation could be overestimated (Kirchman et al., 1986; Tranvik, 1988). In most cases (with the exception of oligotrophic environments), the rate of leucine incorporation will not overestimate the rate of protein synthesis since even at high concentrations, leucine is not transformed to other compounds which are subsequently incorporated into macromolecules. In addition, it is possible that bacterial assemblages obtain all of their carbon and nitrogen from amino acids other than leucine. Leucine (and methionine) do not appear in the dissolved amino acid pool in high concentrations, thus high rates of *de novo* synthesis may be required. However, heterotrophic bacteria have been observed to exhibit high rates of ammonium assimilation consistent with rates of amino acid biosynthesis (Kirchman et al., 1986). Since the ammonium assimilated would be used predominantly for amino acid biosynthesis and subsequent incorporation into protein, high ammonium assimilation rates by bacteria are assumed to imply that rates of amino acid biosynthesis are substantial and that bacteria can obtain carbon and nitrogen from compounds other than amino acids (Kirchman et al., 1986).

## 4.1.2.4: Dual labelling

Kirchman *et al.* (1985) suggested that rates of protein synthesis may complement measures of DNA synthesis since a large proportion of the nitrogen in bacterial cells is protein (Ingraham *et al.*, 1983), and the rate of protein synthesis is a more direct measure of the role of bacteria in utilising dissolved nitrogenous compounds. Protein is also a valuable food source for higher trophic levels and thus rates of net protein synthesis may reflect the contribution of bacteria to the nitrogen budget of bacterial grazers. Because two independent incorporation rates are measured simultaneously, the dual-label approach is more likely to separate real changes from artefacts than a single-label. Nevertheless, the dual label approach is useful as a method for measuring bacterial production only when thymidine and leucine incorporation co-vary. The proportion of primary production processed by bacteria has been estimated from rates of DNA synthesis by assuming that the uptake of organic compounds is closely coupled to the incorporation of  $[^{3}H]$ -TdR, which is the case in balanced growth, but, significant temporal and spatial differences between rates of  $[^{3}H]$ -TdR incorporation

into DNA and labelled leucine incorporation into protein occur in aquatic systems suggesting unbalanced growth (Kirchman *et al.*, 1985). Whether the bacterial assemblage is in balanced or unbalanced growth is an important question to address. Lack of co-variance between thymidine and leucine incorporation indicates periods of unbalanced growth when rates of macromolecule syntheses are uncoupled. These events reflect periods of shifting growth conditions, and their detection can be useful in the study of bacterial metabolism and its regulation by environmental conditions (Chin-Leo & Kirchman, 1988; Kirchman *et al.*, 1985).

## 4.2: MATERIALS AND METHODS

Bacterial production was estimated using a dual labelling technique. Methods for the incorporation of [*methyl-*<sup>3</sup>H]thymidine into DNA (Fuhrman & Azam, 1982; Riemann & Bell, 1990; Bell, 1993) were combined with the incorporation of [<sup>14</sup>C]leucine into protein (Kirchman *et al.*, 1985; 1986; Chin-Leo & Kirchman, 1988) in order to provide the most confidence in the results obtained. The isotopes were stored at 2 °C in an aqueous solution containing ethanol to a final concentration of 2 %, minimising the risk of autodegradation (Robarts & Zohary, 1993).

## 4.2.1: Quench correction curves

Prior to running production experiments quench correction curves were set up on a Beckman LS 6500 scintillation counter using Beckman  ${}^{14}C$  quenched standards (Section 3.2.1).

# 4.2.2: Saturation experiments

Saturation experiments were performed *in situ* using [<sup>3</sup>H]-TdR (specific activity 46 Ci mmol<sup>-1</sup>, Amersham International) and [<sup>14</sup>C]leucine (sp. act. 315 mCi mmol<sup>-1</sup>, Amersham International). These experiments were essential to determine the isotope dosage required to block *de novo* synthesis within bacterial cells via saturation with exogenous [<sup>3</sup>H]thymidine, whilst promoting maximal isotope uptake, and avoiding isotope dilution (Fuhrman & Azam, 1982; Robarts & Zohary, 1993). Saturation experiments were performed in both winter and summer to allow for potential seasonal variations in the isotope dosage required. In each, isotope concentrations ranging between 0 and 50 nM were employed. Integrated lake water samples were held in Whirl-paks (Nasco, USA) at a depth of 6 m during 60 and 90 min incubations. From the results it was determined that 25 nM of [<sup>3</sup>H]thymidine and 10 nM of [<sup>14</sup>C]leucine

should be employed in the winter and 30 nM of each isotope in the summer, during 90 min *in situ* incubations.

## 4.2.3: Bacterial production experiments

Lake water samples were collected from below the ice and at depths of 2 m, 6 m, 10 m and 14 m using a blackened Kemmerer bottle to minimise exposure to light. The samples were decanted into blackened sample bottles and from these 20 ml aliquots of water were placed in each of twenty pre-prepared Whirl-paks per isotope; three replicates and two controls per depth. Each of the replicate Whirl-paks contained a predetermined dosage of the respective isotope, and the controls, 2 ml of 10 % formalin. The Whirl-paks were incubated *in situ* at the respective depths for a period of 90 min (including solar noon). To terminate the incubation and cease production, formaldehyde was added to each Whirl-pak to a final concentration of 2 to 4 %. Further to this, at the beginning and end of the incubation period water samples were acquired from each sample depth and fixed in glutaraldehyde to a final concentration of 2 to 4 %. All samples were returned to the laboratory in cool boxes and analysed within 6 h of collection.

Once in the laboratory, 2.5 ml of 50 % ice cold tri-chloroacetic acid (TCA) was added to each of the samples before leaving them to stand on ice for fifteen minutes. The samples were then filtered through 0.2 µm cellulose acetate filters, pre-soaked in a mixture of 10 nM non-radioactive thymidine and 10 nM non-radioactive leucine for at least 2 h, a procedure which reduces label carry-over and is essential to oligotrophic samples (Robarts & Zohary, 1993). Each filter was washed three times with 3 ml of 5 % ice cold TCA, and subsequently with 5 ml of 80 % ice cold ethanol to remove the lipid fraction (Robarts & Wicks, 1989). Finally, the filters were dissolved in 1 ml of ethyl acetate in glass scintillation vials, 10 ml of Ultima Gold XR scintillation cocktail added to each, and the disintegrations per minute (DPM) for the samples counted on a Beckman LS 6500 scintillation counter. It should be noted that some criticism has been made of methods using ice-cold TCA, however, Ducklow *et al.* (1992) demonstrated that the ratio of [<sup>3</sup>H]-TdR to DNA remained unchanged throughout, and cold extraction avoided the problems of incomplete hydrolysis of DNA and destruction of deoxyribose which occur when using hot TCA (Robarts & Zohary, 1993).

Epifluorescence microscopy was employed as soon as possible after collection to characterise changes in bacterial cell abundance and mean cell volume (MCV) in the glutaraldehyde fixed lake water samples (refer to Section 2.2.3.1).

#### 4.2.4: Analysis of results

Taking into account the specific activity of the isotope added and the final concentration used, the DPM counts recorded were used to calculate the relative amount of each isotope taken up by the bacterioplankton per litre per hour (after Bell, 1993).

$$R_{thy} = (((DPM_{sample} - DPM_{control}) \times (9.79 \times 10^{-9})) / t) \times (1000 / V)$$

$$R_{leu} = (((DPM_{sample} - DPM_{control}) \times (1.43 \times 10^{-6})) / t) \times (1000 / V)$$

where:

 $R_{thy}$  = rate of [<sup>3</sup>H]thymidine incorporation (moles thymidine l<sup>-1</sup> h<sup>-1</sup>)  $R_{leu}$  = rate of [<sup>14</sup>C]leucine incorporation (moles leucine l<sup>-1</sup> h<sup>-1</sup>)  $DPM_{sample}$  = disintegrations per minute for incubated sample  $DPM_{control}$  = disintegrations per minute for incubated control 9.79 x 10<sup>-9</sup> = nmol thymidine DPM<sup>-1</sup> 1.43 x 10<sup>-6</sup> = nmol thymidine DPM<sup>-1</sup> t = length of incubation (h) V = sample volume (ml)

A conversion factor of 2 x  $10^{18}$  cells mol<sup>-1</sup> was applied to the incorporation rates of thymidine into DNA. This factor is similar to that used by Chin-Leo and Kirchman (1988), and within the range suggested as appropriate by Bell (1990; 1993), Robarts and Zohary (1993) and Cole *et al.* (1989) for oligotrophic waters. A factor of 1.42 x  $10^{17}$  cells mol<sup>-1</sup> for the incorporation of leucine was applied (Chin-Leo & Kirchman, 1988). A carbon conversion factor of 0.22 pg C  $\mu$ m<sup>3</sup> was employed to convert bacterial biomass to carbon (Bratbak & Dundas, 1984). The rate of bacterial production was calculated using the equation (Bell, 1993):

$$BP = R \times X \times C$$

where:

**BP** = rate of bacterial production ( $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>)

R = rate of isotope incorporation from above (moles l<sup>-1</sup> h<sup>-1</sup>)

X = conversion factor/cells produced per mole of thymidine incorporated (cells mol<sup>-1</sup>)

C = carbon content per cell (µg cell<sup>-1</sup>)

Natural population growth rate (k) and bacterial doubling time (d) were calculated using the equations (Laybourn-Parry *et al.*, 1995):

```
k = \ln(t_x) - \ln(t_0) / t
d = \ln(2) \ge (1 / k)
```

where:

k = specific growth rate (d<sup>-1</sup>)  $t_0$  = mean cell abundance at the start of the incubation (cells l<sup>-1</sup>)  $t_x$  = mean cell abundance at the end of the incubation (cells l<sup>-1</sup>) t = length of incubation (h) d = doubling time (d)

Finally, the percentage of bacterial production removed by grazing was calculated using the equation:

$$\% BP_{grazed} = (\frac{\text{bacterial carbon grazed daily}}{BP_{daily}}) \times 100$$

where:

 $\% BP_{grazed}$  = potential percentage of bacterial production grazed daily  $BP_{daily}$  = bacterial carbon produced daily (µg C l<sup>-1</sup> d<sup>-1</sup>) bacterial carbon grazed daily = calculated according to Sherr & Sherr (1993)

Minimum and maximum clearance rates calculated for *Pyramimonas gelidicola* during this study (Chapter 5) and clearance rates determined for HNAN in meromictic Lake Fryxell, McMurdo Dry Valleys (Roberts & Laybourn-Parry, 1999), were employed to calculate the  $\% BP_{grazed}$  by *P. gelidicola* and HNAN in Ace Lake, respectively.

#### 4.3: RESULTS

## 4.3.1: Bacterioplankton dynamics

The data presented are from the seasonal study described in Chapter 2. The data sets used are from samples collected on dates closest to those on which bacterial production experiments were performed. Heterotrophic bacterioplankton abundance and biomass varied seasonally. Numbers exhibited a winter peak (July) which declined through



Figure 4.2: Mean bacterial abundance, biomass and mean cell volume for the mixolimnion (a, b & c) and monimolimnion (d, e & f) of Ace Lake. Data from Chapter 2

Date	Depth	k	d
		(4.1)	<b>u</b> (1)
10 4.00		(a ·)	(d)
19-Aug		-	-
	2	0.37	1.87
	6	0.31	2.23
	10	-	-
17-Nov	UI	-	-
	6	-	-
	10	0.18	3.90
	14	0.57	1.21
30-Dec	UI	-	-
	6	-	-
	10	-	-
	14	-	-
31-Jan	UI	0.12	5.91
	6	-	-
	10	0.49	1.41
	14	-	-
28-Feb	UI	0.79	0.88
	6	0.32	2.14
	10	0.08	8.74
	14	0.16	4.27

**Table 4.1**: Bacterial growth rates (k) and doubling times (d) in Ace Lake. - no measurable growth. UI = under the ice

Date	Depth (m)	% BP removed by P. gelidicola daily		% BP removed by HNAN daily	
		Thymidine	Leucine	Thymidine	Leucine
19-Aug	UI	0	0	0.12-0.48	0.07-0.30
-	2	0	0	0.16-0.63	0.07-0.28
	6	1.07	0.10	0.07-0.27	0.01-0.03
	10	0.05	0.01	0.06-0.24	0.01-0.02
17-Nov	UI	0	0	1.94-7.78	0.02-0.07
	6	15.35	0.19	0.97-3.88	0.01-0.05
	10	3.03	0.03	3.53-14.11	0.03-0.12
	14	0	0	-	-
30-Dec	UI	0	0	0.44-1.77	0.14-0.55
-	6	12.07	1.93	0.76-3.05	0.12-0.49
	10	0.49	0.05	0.57-2.29	0.06-0.25
	14	0	0	-	-
31-Jan	UI	0	0	1.37-5.48	0.18-0.73
	6	35.64	1.57	2.25-9.01	0.10-0.40
	10	1.76	0.22	2.04-8.17	0.25-1.00
	14	0	0	-	
28-Feb	UI	0	0	1.01-4.02	0.05-0.19
	6	19.32	1.03	1.22-4.88	0.07-0.26
	10	2.11	0.19	2.45-9.80	0.22-0.88
	14	0	0	-	-

**Table 4.2**: Percentage of thymidine and leucine derived bacterial production (BP)grazed by Pyramimonas gelidicola and heterotrophic nanoflagellates(HNAN) in Ace Lake. - no data. UI = under the ice

spring and summer to a minimum in autumn (Figures 2.16 & 4.2a, 4.2b, 4.2d & 4.2e). The rapid decline in late summer/autumn occurred following a peak in HNAN and metazoan abundance (Figures 2.18; 2.25, 2.26 & 2.28). Heterotrophic bacterial mean cell volume (MCV) and biomass exhibited a similar winter peak (Figures 4.2c &4.2f), although the MCV maxima lagged by one month. Mean cell volume was also high in the late summer, coincident with the highest growth rates (Figures 4.2c, 4.2f & Table 4.1). Bacterial MCV ranged between 0.03 and 0.19  $\mu$ m<sup>3</sup> in the mixolimnion and 0.02 and 0.10  $\mu$ m<sup>3</sup> in the monimolimnion (mean values are presented in Figures 4.2c & 4.2f).

#### 4.3.2: Bacterial production

#### 4.3.2.1: Thymidine incorporation into DNA

Bacterial production derived from the rate of [<sup>3</sup>H]-TdR incorporation into DNA ranged from 0.05 to 6.82  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>. Bacterial production exhibited a seasonal pattern. Production was maximal in the winter (August) at all depths and decreased throughout spring and summer (Figure 4.3). Production also varied with depth in the water column. Maximum bacterial production occurred at 6 m, the top of the thermocline in Ace Lake, on all experimental occasions except December when maximal production was at 10 m (Figure 4.3). This trend suggests a temperature effect, although there was no significant correlation between temperature and bacterial production (p > 0.05). Peak thymidine incorporation coincided with maximal abundance of heterotrophic bacteria (Figure 2.16) and the highest concentrations of nutrients throughout the water column (Figures 2.8 to 2.11) but once again there was no significant correlation apparent between soluble reactive phosphorus, ammonia, nitrate or nitrite concentrations (p > 0.05). At the thermocline DOC levels also increased on each occasion and the peak in production was followed closely by a decline in DOC concentrations throughout the water column (Figure 2.12).

#### 4.3.2.2: Leucine incorporation into protein

Bacterial production based on the rate of leucine incorporation into protein ranged from 0.24 to 13.11  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>. The seasonal trend was opposite to that observed for [<sup>3</sup>H]-TdR incorporation into DNA. Bacterial production derived from leucine incorporation into protein was highest in the summer (December) (Figure 4.3). While ice was present on the lake, leucine incorporation into protein was maximal beneath it, but during the February open-water period production was maximal at 10 m (Figure 4.3). Leucine incorporation seemed to be loosely linked to the rate of autotrophic production. Peak



Figure 4.3: Histograms of bacterial production (BP) in Ace lake at various experimental depths. Based on leucine incorporation into protein (yellow) and thymidine incorporation into DNA (red)

incorporation occurred coincident with maximal autotrophic production at depth. As summer progressed and autotrophic production increased higher in the water column, bacterial production increased at depth, possibly due to dissolved and particulate organic carbon (DOC & POC) produced by the phytoplankton and sedimented downwards.

#### 4.3.2.3: Dual label variance

In most instances, the two labels did not co-vary suggesting unbalanced growth (e.g. Chin-Leo & Kirchman, 1988). However, at such low temperatures and bacterial abundance relative to lower latitude systems, the methods were stretched to their detection limits.

## 4.3.3: Bacterial growth

A measurable growth rate was not detectable on all experimental occasions. Where detectable it ranged between 0.08 and 0.79 d<sup>-1</sup>, corresponding to doubling times of 8.74 and 0.88 d, respectively (Table 4.1). It was apparent that two different growth strategies were adopted in the bacterial populations of the mixolimnion and monimolimnion. In the mixolimnion, two growth maxima were observed. The first in winter coincident with high ambient SRP and NH<sub>3</sub> concentrations, and the second in the summer coincident with DOC produced by the summer phytoplankton blooms (Table 4.1 & Figures 2.8, 2.9 & 2.17). In the monimolimnion the growth maxima followed those in the mixolimnion at a lag, suggesting that different bacterial populations were exploiting different resources and potentially, bacteria in the monimolimnion were making use of organic material settling through the water column following the mixolimnion blooms. Growth rate did not correlate significantly with temperature, heterotrophic bacterial abundance, nutrients or dissolved organic carbon (p > 0.05).

## 4.3.4: Removal of bacterial production

The percentage of bacterial production grazed daily was low to moderate. The percentage of bacterial production removed daily by HNAN during the year ranged between 0.06 to 14.11 % and 0.01 to 1.00 % for thymidine and leucine derived bacterial production, respectively (Table 4.2). The grazing impact of the mixotroph *Pyramimonas gelidicola* was higher, ranging from 0 to 35.64 % and 1.93 % for thymidine and leucine, respectively (Table 4.2). The percentage of bacterial production derived from both thymidine and leucine, removed by *P. geldicola* was maximal in the

summer at 10 and 6 m, respectively, concomitant with the summer PNAN bloom. Peak removal of thymidine and leucine derived bacterial production by HNAN occurred at 10 m in the spring and summer, respectively; the minima in winter and spring.

# 4.4: **DISCUSSION**

# 4.4.1: Bacterial population dynamics

Heterotrophic bacterial abundance in Ace Lake ranged between  $1.26 \times 10^8$  and  $7.28 \times 10^9$  cells l<sup>-1</sup> and was generally low compared to lower-latitude oligotrophic lakes, where maximum concentrations range between 0.85 x 10<sup>9</sup> and 8.0 x 10<sup>9</sup> cells l<sup>-1</sup> (Pick & Caron, 1987; Vaqué & Pace, 1992; Laybourn-Parry *et al.*, 1994). However, heterotrophic bacterial abundance exhibited a peak in the winter (August) in which abundance was comparable with these lakes (Figures 4.2a, 4.2d & 2.16). A peak in biomass and MCV were also observed during the winter (Figures 4.2 b-f). Mean cell volume reached a maximum in September, one month after peak bacterial abundance, probably due to the abundance of food and nutrients available stimulating growth in August and sustaining it through September. Heterotrophic bacterial MCV was highly seasonal in Ace Lake. Similar seasonal variations have been reported in Crooked Lake, an ultra-oligotrophic lake in the Vestfold Hills and in Lake Michigan, USA (Scavia *et al.*, 1986; Laybourn-Parry *et al.*, 1995). The winter maxima in abundance, biomass and MCV were tightly coupled with the abundance of nutrients in the lake, in particular soluble reactive phosphorus (SRP) and ammonia (NH<sub>3</sub>) (Figures 2.8 & 2.9).

# 4.4.2: Bacterial production

In Ace Lake, heterotrophic bacterial production ranged between 0.05 and 13.11  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup> based on both thymidine and leucine incorporation rates. Data on the ecophysiology of Antarctic freshwater planktonic bacteria is scarce and where present, indicate low productivity and growth in Antarctic systems. In an unenriched maritime Antarctic lake, the bacteria achieved a production rate of only 0.05  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>, whereas lakes enriched by seal faeces achieved production rates of 1.83  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>, based on the incorporation of [<sup>3</sup>H]-TdR (Ellis-Evans, 1991). The latter value is similar to rates of production reported for a Danish eutrophic lake during winter (Riemann *et al.*, 1982). Maximum production based on [<sup>3</sup>H]-TdR incorporation (6.82  $\mu$ g C l<sup>-1</sup> h<sup>-1</sup>) occurred near the thermocline in Ace Lake and greatly exceeded these values. This winter peak in production based on thymidine incorporation into DNA, occurred at all depths concomitant with peak bacterial abundance and biomass, and was followed by a peak in

MCV. It also coincided with relatively high concentrations of DOC and the maximum concentrations of SRP and  $NH_3$ . A similar winter bacterial production peak was observed in Crooked Lake, Vestfold Hills (Laybourn-Parry *et al.*, 1995). Conversely, production based on leucine incorporation into protein was maximal in the summer months at all depths. The leucine derived production rates in Ace Lake also exceeded those recorded in other lakes. For example, bacterial production based on leucine incorporation and Michigan ranged between 0.10 and 0.16 µg C l<sup>-1</sup> h<sup>-1</sup> (Pace & Cole, 1994).

It is interesting that appreciable thymidine incorporation rates were noted at all depths during the winter and yet growth was only detectable at 2 and 6 m. It is possible that the experiments were performed during the bloom when bacterial abundance was relatively stable due to rapid turnover, or that the experimental period encompassed the latter part of the July peak. It is also possible that a high proportion of the bacterial carbon produced was employed by individual bacteria to increase their MCV. Indeed, seasonal variations in MCV were closely associated with production. It has been noted in previous studies that as bacterial size decreases, protein concentration increases. Thus, as MCV decreased following the winter maximum in Ace Lake, leucine incorporation into protein exceeded thymidine incorporation into DNA (Simon & Azam, 1989; Laybourn-Parry *et al.*, 1995). The highest rate of leucine incorporation into protein at a summer corresponded with the lowest MCV recorded during the experimental period.

Incorporation of [<sup>3</sup>H]-TdR and leucine did not co-vary, suggesting unbalanced growth. Similarly, McDonough et al. (1986) found that although leucine incorporation co-varied with thymidine incorporation into protein, it did not co-vary with [<sup>3</sup>H]-TdR incorporation into DNA in a lake with an anoxic hypolimnion. Why bacterial growth should have become uncoupled is difficult to determine. Incorporation of [<sup>3</sup>H]-TdR into DNA is indicative of cell division and active population growth. This was corroborated by a population increase during the winter months concurrent with the highest rates of [<sup>3</sup>H]-TdR incorporation. However, leucine incorporation into protein was highest in the summer whilst MCV was low. Subsequent to maximal leucine incorporation rate, an increase in mean cell volume, and a smaller increase in abundance relative to the winter bloom, was apparent. This was indicative of bacterial utilisation of dissolved nitrogen compounds (Kirchman et al., 1985). It is possible that the bacteria were utilising either DOC or DON released by summer phytoplankton blooms and/or nutrients stored intracellularly following luxury uptake during the winter (Revsbech, 1994; Sbiyyaa et al., 1998). Indeed, peak leucine incorporation also followed an increase in DOC by approximately one month. Interestingly, the highest detectable peaks in nitrate and

nitrite occurred at this time (Figures 2.10 & 2.11), so it may be that the bacteria were reducing nitrate to nitrite once ammonia supplies had diminished (Kirchman, 1994).

# 4.4.3: Regulation of bacterial growth and production

## 4.4.3.1: Temperature

It had been suggested that temperature limits bacterial growth and production. Winter growth rates in Lake Pääjärvi, Finland were temperature limited (Tulonen et al., 1994) and in temperate aquatic environments relationships between temperature and bacterial growth are common (Felip et al., 1996). A number of studies indicate that there is a linear relationship between bacterial growth and temperature up to a threshold, beyond which growth is temperature independent (Scavia & Laird, 1987; Tulonen et al., 1994). Although, Felip et al. (1996) inferred that temperature limits bacterial growth uniformly over a specific range and that there is no linear relationship, rather a simple threshold below which bacterial growth is temperature limited. However, the notion that temperature alone limits bacterial growth is not supported by all field studies. In coastal Antarctic waters growth can be rapid at temperatures of less than 2 °C (Fuhrman & Azam, 1980). In Ace Lake, heterotrophic bacterial growth rates recorded in winter were comparable to those in the summer, concurrent with high [<sup>3</sup>H]-TdR incorporation rates, in particular those derived from thymidine incorporation into DNA. Indeed, no significant correlation was found between bacterial production and temperature (p > p)0.05).

It has been suggested that temperature limitations might be eleviated by high substrate concentrations (Coveney, 1982; Suttle et al., 1990; Wood & Van Valen, 1990; Pomeroy et al., 1991; Sell & Overbeck, 1992; Azam et al., 1993; Pedrós-Alió, 1994; Felip et al., 1996). Felip et al. (1996) examined the potential limitation of bacterial growth by temperature and nutrients in eutrophic Upton Lake, USA. Growth rates were ascertained using both leucine and thymidine. They concluded that temperature has little effect on growth in resource-rich environments, but a strong effect in resource-poor environments. The majority of Antarctic lakes, including Ace Lake, are oligotrophic and it would seem that the suggested interplay between temperature and nutrient effects holds true. According to Felip et al. (1996), if bacterial growth were limited by the extreme low temperatures during winter, degradable organic matter and nutrients would accumulate and affect proceeding plankton dynamics and biogeochemical cycles. Inorganic nutrients did accumulate in Ace Lake during autumn and early winter prior to peak heterotrophic bacterial incorporation of thymidine. Although this production maximum could reflect the availability of nutrients alone, a temperature effect is inferred by the fact that the highest rates of thymidine incorporation occurred near the thermocline where temperatures were higher than those recorded in the upper mixolimnion (Figure 2.4). It would appear that temperature limited heterotrophic bacterial growth and production when nutrient resources were low, but once sufficient substrate had accumulated bacterial growth and production were stimulated in the mixolimnion despite low water temperatures. This suggestion that temperature and nutrients in combination limit bacterial growth and production is further corroborated by the fact that the highest growth and leucine incorporation derived production rates were observed during the summer (February) when temperatures throughout the water column were maximal and nutrients available from the phytoplankton.

It should be noted that low temperatures can also depress the activity of bacterivores (e.g. Marasse *et al.*, 1992; Chapter 5). Nevertheless, even at low temperatures Antarctic marine heterotrophic nanoflagellates can consume up to 25 bacteria-sized particles cell<sup>-1</sup> h<sup>-1</sup> (Marchant and Scott, 1993), well within the range reported for lower latitude species. Certainly, in Ace Lake the percentage of bacterial production removed by grazers increased concomitant with increasing temperature (Table 4.2). However, it is likely that any temperature effect was indirect given that the abundance of bacterial HNAN, ciliate and metazoan predators increased with the onset of summer (and higher temperatures), and by corollary more bacterial production was grazed.

## 4.4.3.2: Inorganic nutrients

It would seem from the above that the availability of inorganic nutrients limits heterotrophic bacterial abundance, growth and production to a greater extent than temperature in Ace Lake. In the mixolimnion, bacterial abundance followed the seasonal availability of nutrients diffusing from the monimolimnion closely. In contrast, in the monimolimnion near the oxycline bacterial abundance was comparatively stable reflecting the constant availability of entrained nutrients (Figures 2.8, 2.9 & 2.16). However, care must be taken when interpreting the bacterial production results from the monimolimnion due to the anaerobic bacterioplankton and anoxic conditions prevailing below 12 m (Gilmour *et al.*, 1990; Robarts & Zohary, 1993) (Section 4.1.2.2). The idea that nutrient limitation may be of primary importance for bacterial growth and production is not new. Bacterial growth requires high-quality organic carbon (e.g. amino acids and lipids) and nutrients (e.g. inorganic and organic nitrogen and phosphorus). Studies have determined that bacterial growth is limited by the labile pool of dissolved organic matter (making no distinction between carbon, nitrogen and phosphorus) (Felip *et al.*, 1996).

Pomeroy (1970) was one of the first researchers to point out that "aerobic bacteria tend to take up all available phosphorus". Likewise, Coveney and Wetzel (1988; 1992) proposed that bacterial growth is often limited by inorganic phosphorus, noting that inorganic P additions affected thymidine incorporation and stimulated bacterial growth rates in an oligotrophic lake. P addition also caused significant increases in bacterial activity, abundance and chlorophyll a in the eastern Mediterranean Sea (Zohary & Robarts, 1998). Moreover, phosphorus is thought to be the best predictor of bacterial abundance, a relationship that is certainly likely in Ace Lake (Currie, 1990). Bacterial cells have a naturally high P content. Most of the P in bacteria is in membranes (phospholipids) and nucleic acids, therefore, it is intuitively obvious that with high surface area to volume ratios and a higher relative DNA content, bacteria contain more P than N than phytoplankton (Suttle et al., 1990; Kirchman, 1994). All the same, Kirchman (1994) cites several studies which state that because phytoplankton biomass is greater than bacterial biomass in most lakes, phytoplankton have a greater "capacity" to assimilate P over time (> 1 h). In Ace lake this is not the case; heterotrophic bacteria dominate the microbial biomass throughout the year (Figure 2.30). Other researchers have argued that nitrogen limits bacterial growth (Goldman et al., 1987; Kirchman, 1990). Although P uptake is higher than NH<sub>4</sub> uptake, heterotrophic bacteria can be responsible for up to 78 % of ammonium uptake  $(NH_4)$  (Wheeler & Kirchman, 1986). It is possible that, as with the phytoplankton, N in the form of  $NH_4$  is taken up preferentially by bacteria inhibiting the use of nitrate NO<sub>3</sub> (Pomeroy, 1970; Kirchman, 1994; Yin et al., 1998). Nevertheless, heterotrophic bacteria are capable of assimilating NO<sub>3</sub> and urea albeit at low levels (Kirchman, 1990).

The meromictic nature of Ace Lake may influence bacterial productivity. Cho and Azam (1988) noted that bacteria in deeper waters generally show a lower percentage of  $[^{3}H]$ -TdR incorporation into DNA than those in surface waters. They suggested that this was due to nutrient limitation, in particular nitrogen (Cho & Azam, 1988; Hollibaugh, 1994). Furthermore, vertical stratification is thought to induce strong inorganic nutrient uptake (Wheeler & Kirchman, 1986; Suttle *et al.*, 1990; Caron, 1994).

#### 4.4.3.3: Dissolved organic carbon

The traditional view was that bacteria mineralise organic matter and release N and P for primary producers, but it is clear from the evidence cited above that heterotrophic bacteria can account for a large proportion of the uptake of both N and P in aquatic environments. Less clear, are the factors which control this uptake and the

consequences of nutrient uptake for the plankton community and carbon flux within the food web.

Inorganic nutrient uptake by bacteria is highly variable within and between aquatic ecosystems. In some instances, uptake of dissolved inorganic nitrogen (DIN) far exceeds bacterial production. Kirchman (1994) argued that high uptake of dissolved organic carbon is necessary to balance the measured DIN uptake and Kirchman *et al.* (1990) demonstrated that  $NH_4$  uptake by heterotrophic bacteria in the sub-arctic Pacific was C limited. Phytoplankton also had a greater capacity for P uptake than bacteria in Third Sister Lake, USA, leading Cotner and Wetzel (1992) to conclude that bacterial utilisation of P may also be limited by availability of C or other nutrients. Moreover, bacterial biomass has been positively correlated with the humic content of lakes (Tranvik, 1988).

It is possible that bacteria switch among different DOC compounds and inorganic nutrients with little effect on growth rates but some experimental evidence suggests that bacterial growth and production is limited by labile DOC, corroborated by a correlation between bacterial and autotrophic production (Cole et al., 1988; Button, 1994; Kirchman, 1994). In Ace Lake this does not appear to be the case. Firstly, there was no correlation between detectable rates of autotrophic production and heterotrophic bacterial production or abundance. Bacterial growth was highest concomitant with maximal autotrophic production in February (Tables 4.1 & 3.1) and, consistent with other findings, leucine incorporation increased during the summer beneath the ice whilst autotrophic production was increasing (Chróst & Faust, 1983; Kuparinen, 1988; Tulonen et al., 1994). However, it is impossible with the data available to discern whether DOC or nutrients released during the phytoplankton bloom were responsible. Secondly, DOC within the water column of Ace Lake was relatively high throughout the year. Concentrations ranged between 4.5 and 15.2 mg  $l^{-1}$  (Figure 2.12), higher than levels recorded in other Vestfold Hills lakes, the nearby Larsemann Hills lakes and those in oligotrophic Loch Ness, Scotland, and were unlikely to have been limiting for bacterial growth and production (Laybourn-Parry et al., 1994; Bayliss et al., 1997; Ellis-Evans et al., 1998) (Section 2.4.1.4).

Kirchman (1990; 1994) and Tulonen (1994) suggested that the quality of dissolved organic material (DOM), i.e. sugars versus organic acids, has a large impact on bacterial growth and DOM mineralisation. Just as dissolved free amino acids (DFAA) and  $NH_4$  appear to account for a large fraction of the N supporting bacterial production, glucose is an important C compound in the equatorial Pacific and coastal waters of Oregon (Kirchman, 1994). Uptake of DFAA, sugars and  $NH_4$  varied along a trophic

gradient from nutrient-rich estuaries to oligotrophic open-oceans. Kirchman (1994) demonstrated that  $NH_4$  and sugar uptake rates were highest in oligotrophic waters but that DFAA uptake increased with trophic status. This pattern may be linked with grazer efficiency since the digestion of grazers is known to be more efficient in oligotrophic waters. The result being that  $NH_4$  and sugars, in contrast to DFAA, are excreted and available for bacterioplankton use (Jumars *et al.*, 1989). No investigation of grazer efficiency was made in Ace Lake, however, the general importance of  $NH_4$  is apparent during the winter in Ace Lake, bacterial abundance following  $NH_3$  concentrations almost exactly (Figures 2.9 & 2.16). Carbon would appear to be of lesser importance as a limiting factor to heterotrophic bacterial dynamics than inorganic nutrients.

# 4.4.4: The consequences of bacterial uptake of inorganic nutrients

If, as it now appears, bacterial growth can be N or P limited, intuitively bacteria can compete with phytoplankton for these nutrients. Partitioning of inorganic nutrients between bacteria and phytoplankton can, therefore, control carbon flux into bacteria, as well as rate of primary production, and in turn autotrophic production can control the carbon flux into bacteria (Azam et al., 1993). This is of particular significance in oligotrophic aquatic systems such as Ace Lake where bacteria (by virtue of their large surface area to volume ratio) are superior competitors for nutrients than phytoplankton (Bratbak & Thingstad, 1985; Suttle et al. 1990). However, if P use by bacterial assemblages were limited by DOC, Currie (1990) suggested that at steady-state "algalbacterial competition of P is replaced by mutualism". Contrary to this, Revsbech (1994) concluded that competition is most important under non-steady state conditions and Kirchman (1994) proposed that the uptake of NH₄ and P by bacteria could in fact have profound effects on the size and species composition of the phytoplankton. Nutrient limitation results in the production and release of organic material with high C:N and C:P ratios, thus, competition for and removal of inorganic nutrients by bacteria maintains phytoplankton in a physiological state where a significant fraction of total autotrophic production might be channelled into nutrient-deplete compounds. When subsequently used by bacteria, the organic compounds create intracellular N and P deficiencies and stimulate further bacterial uptake of dissolved N and P. If the bacteria then compete successfully for the growth limiting nutrient, in essence, much of the primary production is funnelled directly into the microbial loop (Caron, 1994).

Contrary to the traditional view of bacteria in facilitating the remineralisation and representing nutrient "sources" in oligotrophic environments such as Ace Lake, it is the presence of bacterivorous Protozoa which may in fact be pivotal in nutrient cycling in these ecosystems, in determining the partitioning of N and P between bacteria and

phytoplankton and the fate of bacterial production (Pace, 1988; Caron, 1994). Phagotrophic protists should be viewed as a dominant biotic control of both bacteria and of phytoplankton in aquatic ecosystems (Sherr & Sherr, 1994). They are a shunt of carbon and energy from the main phytoplankton-based food web and release phytoplankton from competition with the bacteria for limiting inorganic nutrients (Bratbak & Thingstad, 1985; Caron et al., 1988; Caron, 1994). The weight of evidence seems to favour small protists as important agents in the remineralisation of N and P in the plankton via their grazing activities (Caron, 1994). Paradoxically, the feeding activities of Protozoa can also release DOM and POM which is available to bacteria (Andersson et al., 1986), an effect not confined solely to the Protozoa. A significant bacterial biomass increase was observed in the eutrophic Lake Frederiksborg Slotssø, in Denmark due to the grazing activities of in situ meso-zooplankters (Hygum et al., 1997). Thus, in the microbial loop, the carbon released from phytoplankton as dissolved organic carbon is assimilated by bacteria and channelled back to the grazing food web via the bacterivorous flagellates. In addition to this mainstream flow of carbon, bacteria and flagellates may release DOC. However, the picture has been complicated by the discovery that some organisms can function on more than one trophic level, for example phagotrophic autotrophs and photosynthetic heterotrophs (refer to Chapter 5 for more detail). In addition, some Protozoa can utilise DOC and high molecular weight polysaccharides, thus, functioning on the same trophic level as bacteria (e.g. Smith & Barber, 1979; Berman & Stone, 1994). Alternatively, bacterivores may selectively graze the larger, growing and dividing cells within the bacterioplankton assemblages leaving valuable nutrients and carbon locked away in ungrazed bacterial populations (e.g. Marchant, 1990; Monger & Landry, 1991; Hanson, 1992).

## 4.4.5: Summary of bacterial production in Ace Lake

It has been suggested that bottom-up control is important in oligotrophic environments and Ace Lake is no exception to this (Sanders *et al.*, 1992; Pace & Cole, 1994; Pedrós-Alió, 1994). The availability of inorganic nutrients in the lake was of primary importance in the regulation of bacterial production, growth and abundance in the lake when compared with the effect of temperature and DOC concentration.

# Chapter 5: GROWTH AND GRAZING

## 5.1: INTRODUCTION

#### 5.1.1: Growth

Protozoa are important components of all aquatic ecosystems. These organisms are the dominant trophic link through which the picoplankton and nanoplankton production observed in many ecosystems is transferred to higher trophic levels. Like most unicellular organisms, Protozoa are characterised by high population growth rates and the efficiency with which they convert food biomass into protozoan biomass, under optimal conditions (Sherr & Sherr, 1984). However, little is known about the dynamics of naturally occurring protozoan populations and relatively few estimates of protozoan growth rate have been made (Carrick et al., 1992). Of the existing growth rate estimates for Protozoa, most are species-specific, yielded in laboratory observations of single species or small numbers of species under idealised conditions. Similarly, most studies of protozoan growth in situ focus on one component of the community and natural variation in these estimates is largely unknown. Others have been performed by measuring changes in *in situ* population abundance and thus, measure *net* growth rates. This problem can be overcome by pre-screening water samples to eliminate grazers, allowing an estimate of growth rate to be obtained in the absence of grazing mortality (McManus, 1993). However, questions still exist as to whether methods involving confinement of any kind provide a reasonable estimation of in situ growth rate. The aim of this investigation was to provide preliminary estimates of growth rate for the dominant components of the protozoan community in Ace Lake and elucidate possible environmental determinants.

## 5.1.2: Grazing

## 5.1.2.1: Grazing by heterotrophs

The changing paradigm of aquatic food webs has altered our understanding of the importance of grazing by heterotrophs. It now appears that the major part of primary and secondary production, and respiration can be ascribed to the smallest size fractions of the plankton (Sherr & Sherr, 1984). Azam and Cho (1987) cited evidence that initial estimates of bacterial abundance in aquatic ecosystems were gross underestimates and other investigators (e.g. Malone, 1980; Gasol *et al.*, 1995), have demonstrated that nanophytoplankton (< 20  $\mu$ m) account for the majority of the phytoplankton in the sea.

Prior to this Pomeroy (1974) had postulated that ubiquitous and abundant small producers in the ocean must support active communities of small consumers. Subsequent experimental work has demonstrated that, due to their abundance and their high size-specific predation rates, protozoans are indeed major grazers of pico- and nano-plankton in many ocean regions (as summarised by Sherr & Sherr, 1984), in some instances consuming the entire bacterial production (Stockner & Porter, 1988). As such Protozoa can influence the productivity and population dynamics of bacteria and nanophytoplankton via their grazing and the release of inorganic nutrients (e.g. Fenchel, 1988). Protozoan herbivores are capable of ingesting cells ranging from photosynthetic prokaryotes with diameters less than 1  $\mu$ m to large chain diatoms (Strom & Morello, 1998). Furthermore, protozoan population growth rates can be high enough to keep pace with increases in prey biomass (Banse, 1992). Thus protozoan grazers are important regulators of algal and bacterial biomass and species composition (Strom & Morello, 1998).

Heterotrophic Protozoa represent a significant pathway for the transfer of organic carbon from the microbial community to larger consumers, as well as shunting part of the primary and secondary production out of the food web as carbon dioxide (Sherr & Sherr, 1984). Furthermore, although it has been suggested that bacteria, rather than heterotrophic Protozoa, are responsible for much of the rapid nutrient regeneration which occurs in aquatic systems (e.g. Azam *et al.*, 1991), there is evidence to suggest that bacterial nutrient recycling itself is stimulated by the grazing activities of Protozoa (Azam & Cho, 1987; Thingstad *et al.*, 1996) and that bacterial biomass (Azam & Cho, 1987). These processes are particularly important in oligotrophic lacustrine systems such as those found in Antarctica.

This chapter does not include grazing data for the heterotrophic components of the microbial community in Ace Lake due to logistic and time constraints. Instead, the serendipitous observation of an alternative strategy, mixotrophy, is detailed. A review of the literature has been used to highlight the ecological implications of grazing by heterotrophs in Ace Lake and grazing rates derived by other authors have been employed in calculations.

## 5.1.2.2: Mixotrophy: an alternative nutritional strategy

The Protista have traditionally been divided into the plant-like algae and the animal-like Protozoa according to the presence or absence of chloroplasts. However, it has become increasingly obvious that such divisions are arbitrary (Lee *et al.*, 1985; Sanders, 1991).
It is now widely accepted that some phytoflagellates with photosynthetic capabilities exhibit "animal-like" characteristics, ingesting food particles (phagotrophy) or taking up and growing on dissolved organic matter (osmotrophy) in the dark, but photosynthesising whilst in the light (e.g. Sanders & Porter, 1988; Lampert & Sommer, 1997). Conversely, certain phagotrophic ciliates, dinoflagellates and amoebae retain photosynthetic symbionts or isolated chloroplasts following phagocytosis and benefit from translocated photosynthates (e.g. Fields & Rhodes, 1991). This mixture of autotrophic and heterotrophic nutrition is referred to as *mixotrophy*.

### 5.1.2.3: The evolution of mixotrophy

The existence of mixotrophs has been recognised for at least a century. Pfeffer (1897) was the first to define mixotrophy as "...heterotrophy by an organism bearing chloroplasts". Latterly, the definition has been expanded to include "...any organism capable of forming body substance from autotrophy or heterotrophy" (Lampert & Sommer, 1997).

The Serial Endosymbiosis Theory (Margulis, 1970; Taylor, 1974) assists explanation of how mixotrophy evolved. The theory proposes three rounds of endosymbiosis for the evolution of eukaryotes from prokaryotes. Firstly, photosynthetic prokaryotes, such as cyanobacteria, that were originally ingested as food by heterotrophic prokaryotes, were retained as photosynthetic endosymbionts. The genes needed for the independent existence of the cyanobacteria were lost and the majority of genes required for the photosynthetic functioning of the cyanobacterium were transferred to the host nucleus. This first round accounts for the plastids in Chlorophyta (higher plants) and Rhodophyta. Secondly, other eukaryotes acquired their plastids via a second round of endosymbiosis when plastid containing uni-cellular members of the Chlorophyta or Rhodophyta (from the first round) were ingested by heterotrophs. In this instance genes were transferred to the nucleus of the 'new' host rendering them incapable of independent survival. Evidence of this is found in the photosynthetic capacity of the Heterkonta (diatoms, chrysophytes and brown algae), Cryptophyta, Haptophyta, Dinophyta, Euglenophyta, and Chlorarachniophyta (Raven, 1997). In the third instance, endosymbioses developed where there was no transfer of the genes to the host nucleus that precluded the independent existence of the endosymbionts. Indeed, often endosymbionts were freshly obtained from the environment by phagotrophy or transovarian transmission in each generation. Examples include various associations between dinoflagellates and marine protists or Metazoa. In such cases the dinoflagellates possess plastids that are almost always from the second round of endosymbiosis (Fields & Rhodes, 1991). Further to the three endosymbiotic rounds, kleptoplasty (host retention of photosynthetically functional plastids from ingested algal food material) can also lead to the acquisition of a photosynthetic capacity by a host (e.g. Stoecker *et al.*, 1987a).

It is clear that phagotrophy played a universal role in the evolution of photosynthetic eukaryotes and, therefore, phagotrophy should be considered a primitive character and its absence a derived character in micro-organisms (Raven, 1997). As a consequence, it is likely that the first ancestors descending from strict heterotrophs on their way towards strict autotrophy were mixotrophs. In the first instance, organisms would have been able to switch between heterotrophic and autotrophic nutrition in response to environmental changes, for example in food availability and light, a primordial behaviour termed amphitrophy or facultative mixotrophy. Subsequently, a more constrained, obligate mixotrophy evolved in which both photosynthesis and phagotrophy were essential to cell survival (M. Laval-Peuto, personal communication). The non-phagotrophic ciliate, Mesodinium rubrum (Ciliophora: Haptorida), represents the final evolutionary step; it was once mixotrophic, by virtue of an ancient association with a cryptophycean endosymbiont, but is now considered entirely autotrophic following evidence of significant and unusual ultrastructural modifications resulting from the longstanding, permanent symbiotic association between ciliate host and algal partner (Smith & Barber, 1979; Lindholm, 1985).

Mixotrophy, therefore, represents a fortuitous ecophysiological adaptation effected in the past when environmental influences were variable, and retained by natural selection (M. Laval-Peuto, personal communication). It can be considered an intermediate stage in evolutionary terms developed from an ancestrally heterotrophic organism which kept its heterotrophy (phagotrophy or osmotrophy) whilst acquiring phototrophy by one of the following means:

- Acquisition of definitive plastids e.g. phagotrophic phytoflagellates (e.g. Tranvik *et al.*, 1989; Bockstahler & Coats, 1993; Jacobson & Andersen, 1994; Jacobson & Anderson, 1996; Li *et al.*, 1996; Tillmann, 1998).
- Associating more or less permanently with strictly photosynthetic ecto- or endosymbionts. e.g. Protozoa and invertebrates with zooxanthellae (e.g. Smith & Barber, 1979; Lindholm, 1985; Fabricius & Klumpp, 1995).
- 3) Retention and maintenance of temporary or "enslaved" plastids following phagotrophic nutrition e.g. Protozoa and invertebrates maintaining various plastids (e.g. Stoecker *et al.*, 1987a; 1987b; Stoecker *et al.*, 1988; 1989; Farmer & Roberts, 1990; Fields & Rhodes, 1991; Sanders, 1991; Jonsson, 1994).

### 5.1.2.4: The occurrence of mixotrophy

The uptake of particles has been observed in several groups of flagellates, dinoflagellates, cryptophytes, chrysophytes and flagellated green algae. Some of the best known examples are the chrysophytes *Dinobryon*, *Ochromonas* and *Chromulina*, the Dinophyceans *Gymnodinium*, *Peridinopsis*, *Heterocapsa* (Legrand *et al.*, 1998), *Prorocentrum* (Stoecker *et al.*, 1997) and *Fragilidium* (Skovgaard, 1996; Hansen & Nielsen, 1997; Jeong *et al.*, 1997), and the Cryptophycean *Cryptomonas*. It is evident that mixotrophy occurs in orders that also contain representatives that are unpigmented and purely heterotrophic.

A reciprocal type of mixotrophy is the sequestration and utilisation of ingested chloroplasts by organisms generally considered heterotrophic. Plastid retention has been described in gastropods (Smith & Douglas, 1987) and two sarcodine groups; freshwater heliozoa and marine benthic Foraminifera (Lopez, 1979; Patterson & Dürrschmidt, 1987; Lee *et al.*, 1988; Stoecker *et al.*, 1996). However, mixotrophy in heliozoa was concluded to be unstable and non-obligate because the same species were found without plastids and retained plastids were readily lost in a matter of days (Patterson & Dürrschmidt, 1987). Similarly, chloroplasts were only retained for a short period in *Elphidium* spp. although in that time they remained photosynthetically active (Lopez, 1979).

Oligotrich ciliates with "enslaved" chloroplasts have also been described in both marine and freshwater environments (Stoecker *et al.*, 1989). Individual ciliates were found to contain a variety of chloroplasts that originated from several species of algae including diatoms, Dinoflagellida, Prasinomonadina and Chrysomonadina; others were more limited (Sanders, 1991). Continued photosynthetic function was implied by the fact that chloroplasts were sequestered by the ciliates at their periphery away from central digestive vacuoles, either free in the ciliate cytoplasm or confined within host membranes, and ciliate mitochondria were frequently found in close association with them (Stoecker *et al.*, 1988).

Mixotrophy is not solely confined to the Protista. Macroalgae were generally thought to depend upon absorption of nutrients from the water column because they lacked root systems to exploit nutrients in the substratum. However, the giant marine coenocyte, *Caulerpa taxifolia*, possesses a mixotrophic 'root' system containing endocellular bacteria which can take up inorganic phosphorus and organic nitrogen from substrata and translocate nutrient products to the photoassimilatory organs (Chisholm *et al.*, 1996).

### 5.1.2.5: The physiological significance of mixotrophy

It is apparent that mixotrophy is a widely recognised phenomenon, however its importance to the physiology and ecology of protists and their relative abundance has only recently received attention. The fact that mixotrophy has been shown to be widespread across protistan taxa and its nutritional benefits have been demonstrated experimentally in several protists, strongly suggests that it is a functional adaptation. It is intuitively obvious that potential advantages arise when combining photosynthetic and phagotrophic modes of nutrition. Photosynthetic fixation of carbon and utilisation of particulate food as a source of major nutrients (nitrogen, phosphorus and iron) (Lampert & Sommer, 1997; Raven, 1997) and growth factors (e.g. vitamins, essential amino acids and essential fatty acids) could enhance growth, especially in impoverished environments (Sanders, 1991). Where light is limited phagotrophy could be important for the acquisition of carbon and conversely, photosynthesis could maintain a cell during periods when particulate food is scarce.

The relative contribution of photosynthesis and phagotrophy to growth in microorganisms varies, indeed a "mixotrophic gradient" (Sanders et al, 1990) is apparent. Since mixotrophy does not appear to be related to taxonomic grouping, a four group classification of mixotrophic protists was proposed by Jones (1997) based on their mixotrophic behaviour and position along this gradient. The first group, Group A, consists of protists whose primary mode of nutrition is heterotrophy (phagotrophy/osmotrophy/saprotrophy) and phototrophy is employed secondarily when prey concentrations limit heterotrophic growth. For example, prey density was found to determine chlorophyll concentration, and therefore phototrophic growth, in the flagellate Poterioochromonas malhamensis (Chrysophyceae). This species depends primarily on the saprotrophic uptake of dissolved organic carbon (DOC) (Lewitus & Caron, 1991a) and phagotrophy (Caron et al., 1990: Sanders et al., 1990; Zhan et al., 1996). Phototrophy only became the primary mode of nutrition when prey density fell below approximately 10<sup>6</sup> cells ml<sup>-1</sup>. Light intensity had no effect on ingestion of prey. Similarly, Ochromonas sp. (Chrysophyceae) isolated from the Baltic Sea exhibited zero growth when cultured axenically in the light, illustrating its heterotrophic dependence (Andersson et al., 1989; Keller et al., 1994).

Group B consists of protists for whom phototrophy is the dominant mode of nutrition, phagotrophy only supplementing growth when light is limiting. In such instances, ingestion of prey is inversely proportional to light intensity. For example, the obligate phototroph *Chrysochromulina brevifilum* (Haptophyceae), was unable to survive in the dark even in the presence of a flagellate prey source and exhibited an ingestion rate

which was inversely proportional to light intensity (Jones *et al.*, 1993). However, its growth rate was enhanced by the presence of the prey source when light limited growth. It was concluded that the prey was supplementing limiting nutrients. Similarly, grazing on bacteria by the algal flagellates, *Prymnesium parvum* (Prymnesiophyceae) and *Chrysochromulina polylepis*, occurred as an important means of acquiring nutrients, in particular phosphate, during periods of inorganic nutrient limitation (Nygaard & Tobiesen, 1993). The freshwater flagellate *Dinobryon divergens* (Chrysophyceae), *Ochromonas* sp. and the dinoflagellate *Amphidinium cryophilum* also fall into this category (Wilcox & Wedemayer, 1991; Jones & Rees, 1994; Keller *et al.*, 1994; Jones, 1997).

Group C consists of obligate mixotrophs for whom phototrophy is the primary mode of nutrition but phagotrophy provides essential substances for growth and ingestion is proportional to light intensity. This has been demonstrated in *Uroglena americana* (Chrysophyceae) which has a specific requirement for phospholipids obtained through the digestion of prey and proved extremely difficult to grow axenically (Kimura & Ishida, 1985). Similarly, *Dinobryon sertularia* (Chrysophyceae) and *Dinobryon cylindricum* (Jones & Rees, 1994; Caron *et al.*, 1993) and the ciliate *Laboea strobila* (Oligotrichida) (McManus & Fuhrman, 1986; Stoecker *et al.*, 1988) required the presence of both bacteria and light for growth. Bird & Kalff (1986) concluded that phagotrophy must therefore contribute a substantial proportion of the carbon budget of these species. They demonstrated that *Dinobryon* spp. can obtain greater than or equal to 50 % of their carbon from bacterivory and the remainder from photosynthesis, in light limited conditions.

Finally, in Group D phototrophy is also the primary mode of nutrition but the protists exhibit very low ingestion rates, ingesting prey or dissolved organic carbon for cell maintenance during prolonged periods of darkness. They are facultative mixotrophs or amphitrophs at the phototrophic extreme of the mixotrophic gradient. They have little impact on their prey and from an ecological standpoint can be considered primary Examples include, Cryptomonas ovata and Cryptomonas producers. erosa (Cryptophyceae) which exhibit very low ingestion rates when presented with fluorescently labelled microspheres. It was estimated that these species received less than 2 % of their carbon per day through bacterivory, thus heterotrophic nutrition was unimportant (Tranvik et al., 1989; E.C. Roberts, personal communication). However, it has been suggested bacterivory may supplement the algae with essential organic or inorganic nutrients which would place them in Group C. Further examples include Ulva lactuca (Chlorophyceae) which was shown to benefit from the uptake of glucose and acetate and, thus, survival during prolonged periods of darkness or low light intensity

was enhanced if there was a supply of dissolved organic carbon (DOC) (Markager & Sand-Jensen, 1990). *Pyrenomonas salina* (Cryptophyceae) also grew faster when provided with a source of DOC (Lewitus & Caron, 1991b).

Of additional importance to the physiology of mixotrophs is the extent to which the partners in the symbiosis have the potential to transmit their genes to future generations. Analyses performed by Raven (1993) suggested that symbiosis can increase the fitness of the phototrophic partner, perhaps even when compared with non-grazed, free-living phototrophs in similar environments.

# 5.1.2.6: The physiological costs of mixotrophy

The inherent physiological advantages of mixotrophy are so great that one could question why all protists have not evolved to employ this strategy. The answer lies with the energy required to synthesise both photosynthetic and phagotrophic apparatus. The cost of synthesising both sets of apparatus will obviously be greater than that of synthesising one set alone (Raven, 1997). Raven (1995) estimated that photosynthetic apparatus can account for 50 % of the energy, carbon, nitrogen, phosphorus and iron costs of cell synthesis whereas phagotrophic apparatus accounts for less than 10 %. Thus, a normally phagotrophic organism possessing phototrophic apparatus imposes a far higher restriction on maximum growth rate when compared with that of a normally phototrophic cell possessing phagotrophic apparatus. A further cost of phagotrophy in phagotrophs with intracellular digestion, is that volume regulation of the cells must be by active solute fluxes at the plasmalemma (in seawater) or active water efflux (in freshwater) rather than by cell walls. Although, Raven (1997) argued that phagotrophy in unicells is usually combined with motility or production of feeding currents by cilia or flagellae, which are inconsistent with turgor-resisting cell walls anyway.

### 5.1.2.7: The ecological significance of mixotrophy

The ecological significance of mixotrophy has only recently been recognised, partly due to the development of new techniques for the enumeration of microbes (e.g. Porter & Feig, 1980) and partly due to the realisation that microbes play an important role in energy and nutrient flow in aquatic ecosystems (e.g. Pomeroy, 1974; Azam *et al.*, 1983). In addition, the use of fluorescently labelled particles as tracers for ingestion has demonstrated the extent of algal phagotrophy in nature (e.g. Bird & Kalff, 1986; Sherr & Sherr, 1993).

Current estimates suggest that nanomixotrophs can be relatively abundant  $(10^2 \text{ to} > 10^3 \text{ cells ml}^{-1})$  in marine and freshwater plankton (Bennett *et al.*, 1990) and, thus, have a potentially major impact on picoplankton populations (e.g. Bird & Kalff, 1986). Of these, phytoflagellates are usually the most abundant. Grazing by phytoflagellates has some affects on the biotic community which are similar to that of completely heterotrophic organisms. These include changes in the population dynamics of the prey, and possible competition for prey with other grazers. In addition, if grazing causes prey clumping as Aaronson (1973) suggested, then the size-selective feeding of other organisms may also be affected.

The fact that mixotrophy can have a significant affect on the population dynamics of bacteria has been demonstrated on several occasions. Bennett et al. (1990) measured the abundance of heterotrophic, autotrophic and mixotrophic flagellates in Lake Oglethorpe, Georgia. Up to 38 % of the pigmented flagellates ingested particles and strong seasonal differences in the relative grazing impact of pigmented and non-pigmented flagellates were apparent. A similar situation exists in a wide range of oligotrophic and eutrophic environments (Bird & Kalff, 1986, Sanders et al., 1990). In such abundance mixotrophs are potentially responsible for more than half of the bacterivory within a community. It has been demonstrated that a single flagellate can ingest up to 70 bacteria h<sup>-1</sup>, which when bacterial abundance is taken into account, can result in uptake rates of 5 x  $10^3$  to 10 x  $10^4$  bacteria ml<sup>-1</sup> h<sup>-1</sup> (Lampert & Sommer, 1997). Phagotrophic phytoflagellates such as Dinobryon spp. (Chrysophyceae), can therefore exert up to 69 % of the total grazing pressure on bacterioplankton, so much so that Dinobryon spp. was determined to have removed more bacteria from the water column of Lake Memphremagog, USA, than crustaceans, rotifers and ciliates combined (Bird & Kalff, 1986).

Other phytoflagellates, for example, those placed in Group D (Section 5.1.2.5) may exert a limited grazing pressure. The *in situ* population of *Cryptomonas* spp. in the mesotrophic, Barber Pond, Rhode Island incorporated 0.7 to 1.7 bacteria cell<sup>-1</sup> h<sup>-1</sup>, thereby ingesting 0.3 % to 2.0 % of the total bacterial population present in the water per day, and receiving less than 2 % of its carbon per day through bacterivory. Assuming a realistic growth rate of the *Cryptomonas* population, not slower than one doubling per week, bacterivory was quantitatively unimportant to the carbon budget of the *Cryptomonas* cells (Sanders & Porter, 1988) and bacterioplankton dynamics remained relatively unaffected. Bacterivory also has the potential to influence the spatial structure of a lakes microbial population. It has been demonstrated that Deep Chlorophyll Maxima (DCM) composed of algae with phagotrophic abilities probably subsist by ingesting bacteria as discussed in Chapter 2 (Section 2.4.2.1) (Bird & Kalff, 1986; Gervais, 1997). Obviously, mixotrophs themselves are subject to grazing, although Perez *et al.* (1997) concluded that mixotrophs were less subject to copepod grazing than heterotrophs in the Ligurian Sea, northwest Mediterranean.

Diverse factors influence the contribution made by mixotrophy to community photosynthesis; from light intensity (Jones et al., 1993), through temperature (Jones & Rees 1994), to the availability of particulate food (Sanders et al., 1990), nutrients or vitamins (Lewitus & Caron, 1991a; 1991b). It is therefore difficult to draw general conclusions regarding the contribution of mixotrophy to community photosynthesis, but its role is potentially great. Indeed, the abundance and photosynthetic rates of chloroplast-retaining ciliates suggest that they can make a significant contribution to primary production in the microplankton, and occasionally total primary production (Stoecker et al., 1989; 1991; Laybourn-Parry & Perriss, 1995; Sanders, 1995). In continental shelf waters in the northwest Atlantic oligotrichs with sequestered chloroplasts reached abundance of > cells 3000  $l^{-1}$ , 18 to 47 % of the total ciliates present (Stoecker et al., 1989). In addition, the autotrophic ciliate Mesodinium rubrum comprised 1 to 59 % of the total and has been shown to dominate photosynthetic plankton populations in Antarctic lacustrine systems (Palmisano et al., 1985; Priscu et al., 1987; Stoecker et al., 1991; Perriss et al., 1995), including those in this study. In contrast, the contribution of mixotrophic phytoflagellates to primary productivity is largely unknown.

Aquatic bacteria are rich in nitrogen and phosphorus (Fagerbakke et al., 1996). Coupled with their abundance, bacteria are, therefore, an important pool of nutrients. Güde (1991) estimated that in the epilimnion of Lake Constance, the bacterial size fraction (< 1  $\mu$ m) accounted for approximately 50 % of the particulate phosphorus during the summer, stratified season. Thus, bacterial mortality was responsible for a significant mobilisation and re-allotment of nutrients. Besides viruses, bacteriovorous flagellates are usually seen as the main contributors to bacterial mortality. Thus mixotrophy, in particular phagotrophy by phototrophs, has a potential significance in biogeochemical cycling, often out of proportion with the fraction of phototroph species that perform phagotrophy (Raven, 1997). Stoichiometric considerations suggest that the role of bacteriovorous mixotrophs depends on the relative importance of photosynthetic carbon fixation. Bacterial nutrients are retained and possibly complemented by the uptake of soluble nutrients, when photosynthesis constitutes a significant part of carbon assimilation. Conversely, bacterial nutrients are released when phagotrophic nutrition prevails. For example, the mixotroph *Ochromonas* sp. grew primarily phototrophically in P-limited situations taking up and retaining nutrients for subsequent phototrophic growth and limiting the growth of other phytoplankton. However, when phagotrophic nutrition prevailed (in the dark or at high bacterial densities in the light) *Ochromonas* sp. released soluble reactive phosphorus (SRP) and ammonium, stimulating the growth of other P-limited algae. This behaviour mirrors that of exclusive phagotrophs such as *Spumella* sp. (Rothhaupt, 1997).

Bratbak and Thingstad (1985), highlighted seemingly paradoxical behaviour by algae which aggravated their own situation during nutrient limitation by producing increased levels of DOC which attracted bacteria. Bacteria are competitively advantaged in terms of nutrient uptake across membranes, thus this behaviour increased competition and subsequently decreased nutrient availability. Bjørnsen (1988) contradicted the idea suggesting instead that increased DOC production was proportional to cell biomass, irrespective of nutrient status. Nevertheless, if Bratbak and Thingstad (1985) were correct, mixotrophs able to graze the attracted bacteria and utilise bacterial nutrients, possess a compensatory mechanism to overcome the competitive disadvantage (Havskum & Hansen, 1997; Rothhaupt, 1997). Afterall, "...consumption of the competitive dominant reverses the outcome of competition" (Borass et al., 1988). In effect mixotrophs "garden" bacteria; providing the bacteria with "cheap" DOC and then harvesting "expensive" bacterial nutrients in a conveniently "pelleted" form (Thingstad et al., 1996). Such competitive reversal suggests that mixotrophy may be favoured in oligotrophic systems, such as those found in Antarctica, where nutrients for photosynthesis, as well as particulate food, may be limiting. Indeed, the relative abundance of chrysophytes, the phytoplankton group in which mixotrophy is most widespread, often increases in increasingly oligotrophic situations (Sieracki et al., 1993; Sommer et al., 1993). However, the occurrence of mixotrophy in eutrophic aquatic ecosystems (e.g. Kimura & Ishida, 1989) indicates that mixotrophy is not solely an adaptation to oligotrophic conditions but may be ecologically advantageous at any point along the trophic gradient.

Of overall concern is the fact that mixotrophy may increase the efficiency of the microbial loop (Sanders, 1991). Trophic efficiency reflects the amount of ingested food used for growth relative to losses by respiration and excretion (Fenchel, 1987). If photosynthesis provides a portion of the energy required for cell metabolism then more ingested food is available for growth. This assumes that the cost of chloroplast maintenance is low, when as we have seen in Section 5.1.2.6, these costs can be high. However, even small increases in efficiency can increase the amount of biomass supported at higher trophic levels (e.g. Michaels & Silver, 1988). Since both ciliates and mixotrophic flagellates are potentially grazed by zooplankton (Stoecker & Capuzzo, 1990; Kleppel, 1993; Dobson *et al.*, 1997; Swadling *et al.*, 1997; Barquero *et al.*,

1998; Strom & Loukos, 1998), mixotrophy may strengthen the link between the microbial food web and the classic aquatic food chain (Sanders, 1991).

The existence of mixotrophs raises questions regarding the utility of trophic-level definitions, especially in food web models. Contemporary models may be too simple (Jones *et al.*, 1994) and/or inaccurate (Wetzel, 1994; Raven, 1997) if they do not account for photosynthesising heterotrophs and phagotrophic autotrophs. But, no matter how sophisticated the model, a mixotroph's role within the microbial food web is likely to remain difficult to define since mixotrophic behaviour varies constantly with species, physiological state and environmental parameters (Jones *et al.*, 1993). Hence, the aim of this investigation was to provide a preliminary estimate of the influence of mixotrophy on the community dynamics of Ace Lake, for subsequent inclusion in a model of carbon flow.

# 5.2: MATERIALS AND METHODS

# 5.2.1: Growth Experiments

#### 5.2.1.1: Experimental procedure

Growth experiments were performed over a six day interval between 14 January and 20 January 1997. Two samples sets were incubated simultaneously *in situ*; one for flagellates, one for ciliates and dinoflagellates. Water samples were taken from three discrete, representative depths, 1.3 m (beneath the ice), 6 m and 10 m. Flagellate samples were reverse gravity filtered through 21  $\mu$ m bolting silk to remove ciliate grazers prior to incubation in three replicate Whirl-paks (Nasco, USA) for each depth. 100 ml of water was placed in each Whirl-pak. Ciliate samples were determined using unfiltered water similarly incubated in Whirl-paks. At 24 h intervals (± 0.5 h depending on helicopter logistics) 3 ml sub-samples were taken from each replicate Whirl-pak and fixed in glutaraldehyde (for flagellates) and Lugol's iodine (for ciliates and dinoflagellates). Unfortunately, on day five it was impossible to reach the lake incubation site due to inclement weather.

### 5.2.1.2: Analysis of samples

The fixed samples were analysed microscopically according to the methods described in Chapter 2. Taxon-specific mean cell abundance was plotted against time and growth curves fitted to the data points. Subsequent calculation of specific growth rate (k) and

doubling time (d) was achieved using the linear trajectory of plotted growth curves (identified according to McManus, 1993) and the equations (Laybourn-Parry *et al.*, 1995):

 $k = \ln(t_x) - \ln(t_0) / t$  $d = \ln (2) \times (1 / k)$ 

where:

k = specific growth rate  $t_0$  = mean cell abundance at the start of the linear growth phase  $t_x$  = mean cell abundance at the end of the linear growth phase t = total time of linear growth phase d = doubling time

### 5.2.2: Grazing Experiments

#### 5.2.2.1: Experiments performed at Davis Station

Initial grazing experiments were performed in January 1997 with yellow-green 0.512 µm fluorescently labelled microspheres (FLM) (Polysciences, Inc., Warrington, PA.) that had been pre-soaked at a 1:1 ratio in bovine serum albumin (Sigma) for 24 h, to improve palatability. This microsphere size was chosen as it best represented the natural population of bacteria in Ace Lake at the time. Three grazing experiments were carried out in the laboratory using representative lake depths (0.75 m (beneath the ice), 6 m and 10 m). For each experiment, 10 l of lake water from the appropriate depth were filtered through 47 mm diameter, 21 µm GF/F glass fibre filters (Whatman) to remove ciliate and copepod predators, prior to concentration by filtration onto 47 mm diameter, 0.8 µm polycarbonate filters (Millipore). 4 ml of the concentrated sample were placed in a glass bottle and incubated in the dark at ambient field temperatures (as described in Section 2.3.1.2). To the sample, 8.4 µl of pre-sonicated stock microsphere suspension was added. 8.4 µl represented 50 % of the natural bacterial abundance in Ace Lake. This was higher than the 30 % suggested by Sherr and Sherr (1993) but compensated for the fact that Ace Lake is oligotrophic. Immediately following addition, at t(ime) 0, a 200  $\mu$ l sub-sample was removed and fixed with 10  $\mu$ l ice-cold 25 % glutaradehyde in an Eppendorf tube. Subsequent sub-samples were taken at the following intervals (time in minutes): 5, 10, 15, 30, 60, 120, 180, 240, 300, 360. At the end of each experiment the sub-samples were post-stained with the fluorochrome DAPI (4',6-diamidino-2phenylindole, Sigma), filtered and enumerated according to the method described in Chapter 2. A minimum of one hundred nanoflagellate cells were counted where possible and were recorded as having ingested 0, 1, 2, 3, 4, 5 or > 5 FLM. Unfortunately, it was not possible to distinguish individual FLM and determine the exact number taken up once > 5 had been ingested.

# 5.2.2.2: Experiments performed employing cultured algae

Cultures of *Pyramimonas gelidicola*, collected and concentrated (as described in Section 5.2.2.1) from 0.75 m (beneath the ice), 6 m and 10 m in Ace Lake, were grown at 2  $^{\circ}$ C under light regimes ranging from 0 to 100 % light. F2 media was employed for the cultures (Guillard & Ryther, 1962).

4 ml sub-samples were incubated under the same culture conditions throughout the course of the experiments. The experiments were performed and results assessed according to the protocol set out in section 5.2.1.1 with the exception of the sub-sampling intervals. Sub-samples were fixed after 0, 5, 10, 15, 30, 60 and 120 minutes. In addition, further experiments were carried out using cultured bacteria from discrete depths in Ace Lake. The bacteria were heat-killed and fluorescently labelled with the yellow-green fluorescing dye, 5-(4,6-dichlorotriazin-2-yl) aminofluorescein (DTAF, Sigma) which binds protein, producing fluorescently labelled bacteria (FLB) (Sherr & Sherr, 1993). The FLB were stored frozen at - 20 °C and defrosted as required.

### 5.2.2.3: Analysis of results

Community grazing rates were determined using the calculations prescribed by Sherr and Sherr (1993), with the assumption that predators take up fluorescently labelled prey (FLM or FLB) at the same rate as they ingest non-labelled prey. For the purposes of the calculations if a cell was found to have ingested > 5 FLM the number ingested in the equations was ascribed as 6. Regression and correlation analyses were performed for grazing rates with depth, temperature, light climate, autotrophic and heterotrophic bacterial mean cell volume (MCV) and abundance, and the MCV of the species in question. In addition, correlation analyses for dinoflagellate and ciliate growth rates (with the exception of the autotroph *Mesodinium rubrum*) were performed with PNAN and HNAN abundance.

## 5.3: RESULTS

### 5.3.1: Nanoflagellate growth

Both PNAN and HNAN were combined in the flagellate growth rates cited due to the age of the samples and loss of chlorophyll autofluorescence at the time of enumeration (Figure 5.1). The growth rates of flagellates taken from beneath the ice (1.3), 6 and 10 m in Ace Lake (January 1997) were 0.078, 0.424 and 0.077 d<sup>-1</sup>, respectively. These rates corresponded to doubling times of 8.89, 1.64 and 8.95 d, respectively (Table 5.1). The results of the regression and correlation analyses performed are illustrated in Table 5.2. Flagellate growth rates did not vary significantly with depth and correlations with other environmental parameters were not significant.

#### 5.3.2: Dinoflagellate growth

The linear growth rates of dinoflagellates from 1.3, 6 and 10 m were 0.136, 0.519 and 0.354 d<sup>-1</sup>, respectively (Figure 5.2; Table 5.1). These rates corresponded to doubling times of 5.01, 1.33 and 1.96 d, respectively (Table 5.1). Dinoflagellate growth rates did not vary significantly with depth and correlations with other environmental parameters were not significant (Table 5.2).

#### 5.3.3: Ciliate growth

The linear growth rate of *Mesodinium rubrum* taken from beneath the ice (1.3 m) was 0.180 d<sup>-1</sup> (Table 5.1). This rate corresponded to a doubling time of 3.85 d. Cell survival but no growth was observed in samples from 6 and 10 m (Figure 5.3 & Table 5.1). The results of the regression and correlation analyses suggest that *M. rubrum* growth rate varied significantly with depth ( $r^2 = 0.84$ ) and was, therefore, significantly correlated with photosynthetically active radiation (PAR) (p < 0.05) (Table 5.2). The growth rates and doubling times of *Euplotes* sp. at 1.3 and 6 m were 0.462 and 0.693 d<sup>-1</sup>, 1.50 and 1.00 d, respectively (Figure 5.4 & Table 5.1). There was no cell survival in samples incubated at 10 m. *Euplotes* sp. growth rate increases correlated significantly with increasing autotrophic bacterial MCV (p < 0.05). Growth rates did not vary significantly with depth and were not significantly correlated with any other environmental parameters (Table 5.2). The growth of the other ciliate species present beneath the ice, at 6 and 10 m were 0.174, 0.080 and 3.607 d<sup>-1</sup> equating to doubling times of 3.98, 8.70 and 0.19 d, respectively (Figure 5.5 & Table 5.1). The maximum growth rate was measured for ciliates at 10 m. Growth rate varied markedly with depth







Figure 5.1: Flagellate growth curves, linear growth trajectories and growth rate equations







Figure 5.2: Dinoflagellate growth curves, linear growth trajectories and growth rate equations







Figure 5.3: *Mesodinium rubrum* growth curves, linear growth trajectories and growth rate equations







**Figure 5.4**: *Euplotes* sp. growth curves, linear growth trajectories and growth rate equations. Where no curve is plotted, no cell survival was observed







Figure 5.5: Other ciliate growth curves, linear growth trajectories and growth rate equations

Depth (m)	Under Ice (1.3)		6	<u> </u>	1	10	
Taxon	$k (\mathbf{d}^{\cdot 1})$	<i>d</i> (d)	$k (\mathbf{d}^{\cdot 1})$	<i>d</i> (d)	$k (d^{-1})$	<i>d</i> (d)	
Nanoflagellates (HNAN & PNAN)	0.078	8.89	0.424	1.64	0.077	8.95	
	$r^2 = 0.55$		$r^2 = 0.99$		$r^2 = 0.74$		
Dinoflagellates	0.136	5.01	0.519	1.33	0.354	1.96	
	$r^2 = 0.09$		$r^2 = 0.99$		$r^2 = 0.84$		
Mesodinium rubrum	0.180	3.85	-	-	-	-	
	$r^2 = 0.62$						
Euplotes sp.	0.462	1.50	0.693	1.00	0	0	
	$r^2 = 0.57$		$r^2 = 0.75$				
Other ciliates	0.174	3.98	0.080	8.70	3.607	0.19	
	$r^2 = 0.96$		$r^2 = 0.96$		$r^2 = 1.00$		

k = intrinsic linear growth rate (d<sup>-1</sup>) d = doubling time (d) o = no survival

- = survival, no growth

Table 5.1: Comparative taxon specific growth rates (k) and doubling times (d) in relation to depth for Ace Lake

Parameter	Depth	Temp.	PAR	Species	ABact.	HBact.	ABact.	HBact.	PNAN	HNAN
Taxon				MCV	nos.	nos.	MCV	MCV	nos.	nos.
Nanoflagellates	$r^2 = 0.01$	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	-	-
Dinoflagellates	$r^2 = 0.43$	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05
M. rubrum	$r^2 = 0.84$	<i>p</i> >0.05	<i>p</i> < 0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	-	
Euplotes sp.	$r^2 = 0.32$	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> < 0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05
Other ciliates	$r^2 = 0.62$	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> < 0.1	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> >0.05	<i>p</i> < 0.05

**Table 5.2:** Regression and correlation analyses of taxon specific growth rates with various environmental parameters

 $(r^2 = 0.62)$ , correlated significantly with autotrophic bacterial abundance (p < 0.1) and HNAN abundance (p < 0.05) (Table 5.2).

#### 5.3.4: Pyramimonas gelidicola grazing

The prasinophyte, *Pyramimonas gelidicola* McFadden (Chlorophyta: Prasinophyceae), previously described as autotrophic was observed to ingest fluorescently labelled microspheres (FLM). Maximum FLM ingestion occurred in samples collected from 6 m in Ace Lake. Up to 47 % of the cells present in these samples ingested 0.512  $\mu$ m FLM (Figure 5.6). Peak ingestion of FLM was observed in samples from beneath the ice (0.75 m), 6 m and 10 m after 300, 15 and 30 min, respectively (Figure 5.7).

 Table 5.3: Potential grazing rates of Pyramimonas gelidicola on bacteria in Ace Lake (January 1997) as estimated from FLM ingestion rate.

Depth (m)	Grazing rate	Cell	Community	% of	Carbon
	(FLM cell <sup>-1</sup> h <sup>-1</sup> )	clearance rate (µ1 cell <sup>-1</sup> h <sup>-1</sup> )	ingestion rate (FLM I <sup>-1</sup> d <sup>-1</sup> )	bacterial population grazed daily	biomass grazed daily (µg C I <sup>-1</sup> d <sup>-1</sup> )
				Bruzen anny	
0.75	-0.576	*	*	*	*
6	6.948	1.32 x 10 <sup>-5</sup>	1.62 x 10 <sup>8</sup>	15.83	203.91
10	0.976	1.86 x 10 <sup>-6</sup>	1.41 x 10'	0.86	25.52

The community ingestion rate of *P. gelidicola* (collected from Ace Lake in January 1997) was calculated using linear uptake rates measured during the first fifteen minutes of the grazing experiments (i.e. the first four points on the graphs in Figure 5.8). The clearance rates for samples from beneath the ice (0.75), 6 and 10 m were -0.576, 6.948 and 0.976  $\mu$ l cell<sup>-1</sup> h<sup>-1</sup>, equivalent to rates of 0, 1.62 x 10<sup>8</sup> and 1.41 x 10<sup>7</sup> FLM l<sup>-1</sup> d<sup>-1</sup>. Such rates imply the percentage daily grazing of the bacterial standing stock and bacterial carbon biomass presented in Table 5.3. *P. gelidicola* grazing rates did not vary significantly with depth and were not significantly correlated with the environmental parameters cited in Table 5.4. The strongest correlation was observed with autotrophic bacterial abundance ( $r^2 = 0.76$ ).



**Figure 5.6:** *Pyramimonas gelidicola* grazing in Ace Lake (January 1997) Percentage of the total *P. gelidicola* population observed to ingest FLM over time









Figure 5.8: Pyramimonas gelidicola grazing rates in Ace Lake

 Table 5.4: Regression and correlation analyses of Pyramimonas gelidicola grazing rates with various environmental parameters.

Parameter	Depth	Temp.	PAR	Species	ABact.	HBact.	ABact.	HBact.
				MCV	nos.	nos.	MCV	MCV
P.	$r^2 = 0.06$	<i>p</i> >0.05						
gelidicola								

It was not possible to induce grazing of FLM or fluorescently labelled bacteria (FLB) in the laboratory employing algal cultures, therefore no empirical results were produced for inclusion in this section.

#### 5.4: DISCUSSION

### 5.4.1: Growth rates and doubling times

#### 5.4.1.1: Nanoflagellate growth

The growth rates and doubling times of nanoflagellates residing beneath the ice and at 10 m in Ace Lake (k = 0.078 and 0.077 d<sup>-1</sup>, d = 8.89 and 8.95 d, respectively) were higher than those reported for freshwater Crooked Lake (Vestfold Hills, Antarctica) (k  $= -0.02 d^{-1}$ ) and Antarctic sea ice offshore from the Vestfold Hills (Archer *et al.*, 1996) but lower than those reported for the maritime Antarctic Lakes, Sombre and Heywood  $(k = 0.312 \text{ and } 0.288 \text{ d}^{-1}, \text{ respectively})$  (Laybourn-Parry *et al.*, 1996) and lakes from lower latitudes (Sherr et al., 1983; Carrick et al., 1992; 1994) (Table 5.5). In contrast, specific growth rates for nanoflagellates residing in Ace Lake at 6 m ( $k = 0.424 \text{ d}^{-1}$ ) were in the same order as those observed in flagellates from Lake Constance, Germany  $(k = 0.576 \text{ to } 0.600 \text{ d}^{-1})$  (Weisse *et al.*, 1990; Sherr *et al.*, 1983) (Table 5.5). However, the growth rate of nanoflagellates at 6 m was much lower than growth rates, and hence doubling rates, of nanoflagellates recorded for warmer latitudes where specific growth rates ranged from 1.2 to 4.8 d<sup>-1</sup> (Sherr et al., 1983; Goldman & Caron, 1985). Low temperatures are often responsible for depressing growth rates. In Crooked Lake, Vestfold Hills, Antarctica, specific growth rate and doubling time was severely depressed by a combination of low temperature and energy limitation (Laybourn-Parry et al., 1995). As is the case in Sombre and Heywood Lakes, it is unlikely that energy (carbon) limitation restricts growth in Ace Lake (Laybourn-Parry et al., 1996), but

Taxon	Location	k (d-1)	<i>d</i> (d)	Source
Nanoflagellates				
Heterotrophic	Sombre Lake, Antarctica	0.312	55	Laybourn-Parry et al., 1996
-	Heywood Lake, Antarctica	0.288	55	Laybourn-Parry et al., 1996
	Crooked Lake, Antarctica	0.0168-0.0185	37.3-41.1	Laybourn-Parry et al., 1995
	Antarctic sea ice	0.05	*	Archer et al., 1996a
	Loch Ness	1.0-2.2	7.4-16.1	Laybourn-Parry & Walton, 1998
	Lake Kinneret	1.2-4.8	*	Sherr et al., 1983
	Lake Constance	0.6	*	Sherr et al., 1983
	Lake Constance	0.576	*	Weisse et al., 1990
	In vitro (Lake Michigan)	0.04-0.6	*	Carrick et al., 1992
	In vitro (marine)	3.48	*	Goldman & Caron, 1985
Phototrophic	In vitro (Lake Michigan)	0-0.69 (size dependent)	*	Carrick et al., 1994
-	Gulf of St Lawrence	0.94-1.58	*	Tamigneaux et al., 1997
Dinoflagellates	Ace Lake, Antarctica	0.574-0.923	*	Perriss, unpublished data
-	Ekho Lake, Antarctica	0.176	*	Perriss, unpublished data
	Highway Lake, Antarctica	0.233	*	Perriss, unpublished data
	In vitro (marine)	0.41-0.48	*	Strom & Morello, 1998
Protoperidinium divergens	In vitro (Californian coast)	0.246-0.484	*	Jeong & Latz, 1994
Protoperidinium crassipes	In vitro (Californian coast)	0.107-0.308	*	Jeong & Latz, 1994
Ciliates	Sombre Lake, Antarctica	0.672	38	Laybourn-Parry et al., 1996
	Heywood Lake, Antarctica	0.240	69	Laybourn-Parry et al., 1996
	Plymouth Sound, UK	0.41-0.68	*	Leakey et al., 1994
	In vitro (marine)	0.77-1.01	*	Strom & Morello, 1998
Euplotes octocarinatus	In vitro (freshwater)	0.2-0.6	*	Kusch, 1998
Strombidinopsis chesiri	British Columbian coast	0.99	*	Montagnes et al., 1996
Halteria grandinella	Rimov Reservoir, Bohemia	*	1.21	Simek et al., 1996
unidentified oligotrich	Rimov Reservoir, Bohemia	*	1.79	Simek et al., 1996
Strobilidium spp.	In vitro (marine)	0.3-2.2	*	Montagnes, 1996
Strombidium spp.	In vitro (marine)	0.3-2.2	*	Montagnes, 1996

**Table 5.5:** Comparative taxon specific growth rates (k) and doubling times (d)

temperature is likely to play a role in physiologically constraining the nanoflagellates, curtailing their maximum growth rate. However, a temporal variation in growth rate and doubling time was apparent within the water column of Ace Lake, which did not appear to be correlated with temperature (p > 0.05) or indeed the other parameters tested. The data suggests that a combination of factors were responsible for the nanoflagellate growth rates measured in Ace Lake and that 6 m might be an optimum depth for cell survival and growth. Indeed, Section 2.4.2.1 describes in detail the presence of a Deep Chlorophyll Maximum (DCM) in Ace Lake at the thermo/halocline between 6 and 8 m where flagellates seemingly thrive in this relatively warm, nutrient replete, oxygenated zone receiving PAR inputs sufficient for shade-adapted growth, which would account for their relatively rapid growth rate and doubling time at this depth.

### 5.4.1.2: Dinoflagellate growth

Dinoflagellate growth rates in Ace Lake at 6 m and 10 m (0.519 and 0.354  $d^{-1}$ , respectively) were of a comparable magnitude to those at the lower end of the range recorded in Ace Lake in 1994 (S.J. Perriss, unpublished data) (Table 5.5). They also compared well with rates measured for cultured dinoflagellates from low latitude marine environments (Jeong & Latz, 1994; Strom & Morello, 1998). However, Ace Lake growth rates were higher than those measured in nearby Ekho and Highway Lakes. Vestfold Hills. As was the case for nanoflagellates, dinoflagellate growth was maximal at 6 m reflecting the suitable growing conditions at this depth. The lowest dinoflagellate growth rate of 0.136 d<sup>-1</sup> was recorded beneath the ice. This may reflect the physiological constraints imposed by low temperatures since the lowest temperatures were consistently recorded beneath the ice in Ace Lake (Section 2.3.1.2), but there was no significant correlation between growth rate and temperature (p > 0.05). Hansen (1992) described a linear relationship between maximum growth rate and mean cell volume (MCV) in Gyrodinium spirale which was supported by studies carried out with the larger, Protoperidinium spp., by Jeong and Latz (1994). However, no significant correlation was found between growth rate and the MCV of Ace Lake dinoflagellates or their bacterial prey. It is likely that once again a combination of environmental factors influenced dinoflagellate growth rate and doubling time.

### 5.4.1.3: Ciliate growth

Strom and Morello (1998) found that, for a given size, ciliates consistently exhibited higher growth rates than dinoflagellates *in vitro* using cultured marine protists, a finding confirmed by other authors (e.g. Banse, 1982; Hansen, 1992). This would also appear to be the case in Ace Lake. Trends in ciliate growth rates and doubling times in Ace

Lake were taxon specific. *Mesodinium rubrum*, the dominant ciliate in Ace Lake, only grew in incubations performed below the ice ( $k = 0.180 \text{ d}^{-1}$ , d = 3.85 d). In the incubations at 6 and 10 m it appeared to survive but encyst. This is not surprising, since M. rubrum is a highly motile, autotrophic ciliate that has been shown to be most abundant in the upper mixolimnion of Ace Lake where PAR levels are maximal (Section 2.4.2.4). Indeed, due to the attenuation of PAR with depth, growth rate was both significantly correlated with PAR and strongly related to depth ( $r^2 = 0.84$ ). The ciliate adopts various strategies to survive unfavourable conditions one of which is encystment. It is likely that populations of *M. rubrum* sampled from and incubated at 6 m and 10 m were captured during migrations into the relatively nutrient replete waters of the lower mixolimnion and upper monimolimnion, respectively, but had they not been confined to these depths during the experiment, would have returned to the upper mixolimnion to optimise photosynthesis and growth. By incubating the active cells at unfavourable depths they were forced to encyst prematurely in order to survive. The specific growth rates recorded for M. rubrum were low compared to those of ciliates recorded elsewhere (Table 5.5) which may reflect the low temperatures in the upper mixolimnion of Ace Lake. It also highlights the fact that bet-hedging strategy that M. rubrum employs to survive in Ace Lake is entirely responsible for its success in Ace Lake. Encystment ensures the long term survival of the population in sub-optimal conditions and the presence of active cells throughout the winter months (possibly surviving on endogenous energy supplies (Laybourn-Parry & Perriss, 1995)) suggests that they can optimise the short summer season by being photosynthetically poised and ready to commence growth, albeit at a reduced rate, as soon as suitable conditions return, without the initial energy outlay required for excystment (Hashimoto, 1963; Grimes, 1973).

The measured growth rate and doubling time of *Euplotes* sp. was maximal at 6 m ( $k = 0.693 d^{-1}$ , d = 1.00 d). This appears to be the optimum depth for cell survival and growth, slower growth being apparent at 1.3 m ( $k = 0.462 d^{-1}$ , d = 1.50 d) and no cell survival at 10 m. Growth rate was significantly correlated with increasing autotrophic bacterial MCV (p < 0.05), which may reflect a prey size preference. A protozoan preference for larger prey has been demonstrated experimentally in flagellates (Chrzanowski & Simek, 1990; Monger & Landry, 1991), dinoflagellates (Strom & Loukos, 1998) and ciliates (Christaki *et al.*, 1998; Strom & Loukos, 1998). It is possible that *Euplotes* sp. may also be exploiting nanoflagellates in the DCM but growth rate was not significantly correlated with either PNAN or HNAN abundance (p > 0.05). The growth rate of *Euplotes* sp. compares well with ciliate growth rates measured in the maritime Antarctic Sombre Lake, Signy Island (Laybourn-Parry *et al.*, 1996) and with laboratory rates for the freshwater *Euplotes octocarinatus* (Kusch,

1998). In addition, it lies in the middle of the range of growth rates determined for various marine ciliate species cited in Table 5.5.

A zonation of ciliate species in Ace Lake was apparent from the growth rate results for the other species of ciliate. The growth rate of these ciliates was higher beneath the ice than 6 m, increasing once more at 10 m suggesting that these different depths offer niches for different taxa. The maximum growth rate for all taxa investigated in Ace Lake was recorded for ciliates at 10 m at the top of the oxycline. Here nutrients were relatively abundant, temperatures greater and potential prey abundances maximal due to the existence of a second DCM (Section 2.4.2.1). Indeed, growth rate was significantly correlated with HNAN abundance (p < 0.05) and autotrophic bacterial abundance (p < 0.1) suggesting that prey abundance exerted the strongest influence on the growth rate of ciliates in Ace Lake.

The higher maximum growth rate of ciliates compared with heterotrophic dinoflagellates and nanoflagellates has implications for the whole planktonic community of Ace Lake. The growth rate disparity means that ciliate populations have the potential to increase more rapidly in response to environmental parameters such as increasing PAR, explaining their marked contribution to the microbial community biomass in the summer months, in particular the autotroph *Mesodinium rubrum* (Section 2.3.2.9).

### 5.4.2: Grazing rates

Mixotrophy in *P. gelidicola* is a hitherto unobserved phenomenon. No known reference has been made to mixotrophy in the genus *Pyramimonas* or for the species *P. gelidicola* prior to this study and ultrastructural investigations do not describe phagotrophic apparatus (e.g. Norris & Pienaar, 1978; McFadden & Wetherbee, 1982; 1985; McFadden *et al.*, 1982; 1986; van den Hoff *et al.*, 1989; Chrétiennot-Dinet *et al.*, 1993). McFadden *et al.* (1986) refer to trichocysts within *Pyramimonas* spp. from south-eastern Australia which stained heavily with the fluorochrome DAPI and, therefore, have the potential to be mistakenly identified as fluorescently labelled prey. However, since the FLM employed in this study fluoresced an entirely different colour this possibility has been ruled out.

47 % of the *P. gelidicola* population in samples taken from 6 m behaved phagotrophically, ingesting 0.512  $\mu$ m FLM. This compares well with percentage of mixotrophy in phytoflagellates from other aquatic systems. For example, 38 % in Lake Oglethorpe, USA (Bennett *et al.*, 1990), 30 to 39 % in a dystrophic lake (Salonen & Jokinen, 1988) and 65 % in the North Atlantic (Estep *et al.*, 1984).

Grazing data for lacustrine protists at low temperature is limited; most data originate from marine systems. Many marine protists have higher grazing rates than their lacustrine counterparts (Fenchel, 1982; Simek & Straskrabová, 1992). The grazing rates measured in Ace Lake at 10 m are greater than HNAN grazing rates reported in oligotrophic Crooked Lake, Vestfold Hills (Laybourn-Parry et al., 1995) and in the same order of magnitude as HNAN grazing rates from the maritime Antarctic in Lakes Sombre and Heywood (Laybourn-Parry et al., 1996). However, rates at 6 m were an order of magnitude greater and showed a greater similarity to the range of HNAN grazing rates recorded in mesotrophic Rimov Reservoir, Bohemia (Simek & Straskrabová, 1992) and eutrophic Lake Oglethorpe, USA (Bennett et al., 1990), though they were less than rates recorded for oligotrophic Loch Ness, Scotland (Table 5.6). Comparison with other taxa suggests that P. gelidicola grazing rates in Ace Lake exceeded those of heterotrophic dinoflagellates (Jeong & Latz, 1994; Archer et al., 1996b) but were generally far lower than those of heterotrophic ciliates (Sherr et al., 1989; Pedrós-Alió et al., 1995; Laybourn-Parry et al., 1996; Simek et al., 1996; Christaki et al., 1998) (Table 5.6). In terms of other confirmed mixotrophs, P. *gelidicola* grazing rates in Ace Lake were higher than those recorded for phytoflagellates in Crooked Lake, Vestfold Hills (Laybourn-Parry et al., 1996), Cryptomonas spp. (Sanders & Porter, 1988; Tranvik et al., 1989; Green, 1991; Nygaard & Tobiesen, 1993; Jones et al., 1994), various mixotrophic dinoflagellates (Sanders, 1991; Legrand 1998) and Antarctic mixotrophic ciliates (E.C. Roberts, personal et al.. communication). They compared more favourably with rates measured for Dinobryon cylindricum and Ochromonas sp. in Lake Oglethorpe (Bird & Kalff, 1986; Sanders & Porter, 1988), Prymnesium parvum (Nygaard & Tobiesen, 1993) and the mid-range of rates for Chrysochromulina spp. (Green, 1991; Nygaard & Tobiesen, 1993; Jones et al., 1993; 1994) (Table 5.7). The majority of tabulated rates were determined using fluorescently labelled food sources, which consistently yield lower rates than those determined by dilution, inhibition and filtration protocols (Vaqué et al., 1994). Antarctic phytoflagellates are known to actively discriminate against FLM (J. Laybourn-Parry, personal communication). Therefore, it is also likely that the observed laboratory grazing rates for Ace Lake are an underestimate of the potential in situ grazing rates. However, the method employed was chosen because it involved minimal manipulation of the sample and allowed relatively rapid determination of preliminary grazing rates for specific protistan components (Sherr & Sherr, 1993).

The grazing impact of *P. gelidicola* in Ace Lake was relatively low. They removed a maximum of 15.83 % of bacterial biomass daily which was equivalent to 203.91  $\mu$ g of bacterial carbon per day. In the continental Antarctic Lakes Crooked and Fryxell, a

Taxon	Location	Grazing rate	Clearance rate	% prey	Carbon biomass	Source
		(prey indiv. <sup>-</sup> n <sup>-</sup> )	$(\mu \mathbf{I} \ \mathbf{Ind} \mathbf{v} \cdot \mathbf{n})$	grazed daily	$(\mu g C l^{-1} d^{-1})$	
HNAN	Sombre Lake, Antarctica	0.51	*	100	407	Laybourn-Parry et al., 1996
	Heywood Lake, Antarctica	0.83	*	6	*	Laybourn-Parry et al., 1996
	Crooked Lake, Antarctica	0.016-0.200	*	9.7	*	Laybourn-Parry et al., 1995
	Lake Fryxell, Antarctic	*	*	4	*	Laybourn-Parry et al., submit
	Loch Ness, Scotland	10.3-24.5	*	23	*	Laybourn-Parry & Walton, 1998
	Rimov Reservoir, Bohemia	4-19	*	10-23	*	Simek & Straskrabová, 1992
	Lake Gooimer, Netherlands	3.8-64.2	*	*	*	Starink et al., submitted
	Lake Vechten, Netherlands	7	*	*	*	Bloem et al., 1989
	Lake Oglethorpe, USA	1-17	*	*	*	Bennett et al., 1990
	Lake Tuesday, USA	39-73	*	*	*	Vaqué & Pace, 1992
	Lake Paul, USA	13-30	*	*	*	Vaqué & Pace, 1992
	Red Sea	0.010-0.108	*	*	*	Weisse, 1989
Dinoflagellates	Prydz Bay, Antarctica	*	0.028-0.318	*	*	Archer et al., 1996b
Protoperidinium divergens	Californian coast	0.2	0.67	*	*	Jeong & Latz, 1994
Protoperidinium crassipes	Californian coast	0.08	0.47	*	*	Jeong & Latz, 1994
Choanoflagellates	Saroma Ko Lagoon, Japan	*	0.0007	*	*	Marchant, 1990
Ciliates	Sombre Lake, Antarctica	70.6	*	*	*	Laybourn-Parry et al., 1996
	Heywood Lake, Antarctica	119.3	*	*	*	Laybourn-Parry et al., 1996
	Rimov Reservoir, Bohemia	8-4200	*	*	*	Simek et al., 1996
	Villefranche Bay, France	*	0.025-0.175	*	*	Christaki et al., 1998
	Villefranche Bay, France	*	0.014-0.308	*	*	Sherr et al., 1989
	Lake Cisó, Spain	0.07-0.64	*	*	*	Pedrós-Alió et al., 1995

 Table 5.6: Comparative heterotroph grazing rates

Taxon	Location	Grazing rate (prey indiv. <sup>-1</sup> h <sup>-1</sup> )	Clearance rate $(\mu l \text{ indiv.}^{\cdot 1} h^{\cdot 1})$	% prey population	Carbon biomass grazed daily	Source
				grazed daily	$(\mu g C l^{-1} d^{-1})$	
Phytoflagellates	Crooked Lake, Antarctica	0.042-0.084	*	*	*	Laybourn-Parry, unpublished
Cryptomonas sp.	Lake Fryxell, Antarctica	<1-89	*	13	*	Laybourn-Parry et al., submit
Cryptomonas spp.	*	*	0.0025-0.0084	*	*	Sanders & Porter, 1988
Cryptomonas spp.	Barber Pond, USA	0.7-1.7	*	0.3-2.0	*	Tranvik et al., 1989
Uroglena sp.	In vitro	1.320	*	*	*	Bird & Kalff, 1986
Dinobryon sp.	Lake	*	0.006	*	*	Bird & Kalff, 1986
Dinobryon cylindricum	Memphremagog,USA	1.8-8.3	0.0035	*	*	Caron et al., 1993
Dinobryon sertularia	Lake Oglethorpe, USA	*	0.001-0.002	*	*	Jones & Rees, 1994
Dinobryon bavaricum	In vitro	24	0.0084	*	*	Sanders & Porter, 1988
Ochromonas sp.	Lake Oglethorpe, USA	7	0.0025	*	*	Sanders & Porter, 1988
Ochromonas danica	Lake Oglethorpe, USA	1.0	0.00013	*	*	Sanders & Porter, 1988
Ochromonas minuta	In vitro	0.8	0.00016	*	*	Sanders & Porter, 1988
Poterioochromonas	In vitro	1.5	0.00040	*	*	Sanders & Porter, 1988
malhamensis						
Chrysostephanosphaera sp.	In vitro	77	0.0260	*	*	Sanders & Porter, 1988
Haptophytes						
Prymnesium parvum	Lake Oglethorpe, USA	5.8	*	*	*	Nygaard & Tobiesen, 1993
Chrysochromulina sp.	In vitro	0.15	*	*	*	Green, 1991
C. ericina	In vitro	18	*	*	*	Nygaard & Tobiesen, 1993
C. brevifilum	In vitro	0.8	*	*	*	Green, 1991
C. brevifilum	In vitro	0.3	*	*	*	Jones et al., 1993
C. hirta	In vitro	8.3	*	*	*	Jones et al., 1994
Dinoflagellates						
Heterocapsa triauetra	In vitro	0.06-0.4	*	*	*	Legrand et al., 1998
Gymnodinium sanguinum	*	≤0.05	*	*	*	Sanders, 1991
Ciliates						
Holophrya sp.	Lake Fryxell, Antarctica	0.13-0.19	*	*	*	Laybourn-Parry et al submit
Laboea strobila	*	97-139	*	*	*	Stoecker <i>et al.</i> , 1988

maximum of 9.7 % and 13 % of bacterial biomass may be grazed daily, respectively (Laybourn-Parry *et al.*, 1995; Roberts & Laybourn-Parry, 1999), which based on bacterial production studies carried out by Takacs and Priscu (1998) equates to 100 % of bacterial production grazed daily. By comparison, 10 to 23 % of bacterial production may be removed per day in low latitude mesotrophic systems (Simek & Straskrabová, 1992) and between 55 and 100 % in eutrophic systems (Stockner & Porter, 1988; Bloem & Bär-Gilissen, 1989).

Peters (1994) reviewed protozoan grazing rates and demonstrated that they follow an exponential curve in relation to temperature. From Peters' curve the predicted ingestion rate of Ace Lake P. gelidicola populations should be approximately 4 to 10, 6 to 11, 7 to 12 bacteria indiv<sup>-1</sup> h<sup>-1</sup> at 0.75 m, 6 m and 10 m, respectively. Measured grazing rates were lower than predicted at 0.75 and 10 m which may be due to the use of FLM rather than bacteria, and the fact that once > 5 FLM had been ingested enumeration was difficult and an arbitrary figure of 6 was employed in the calculations. The grazing rates of maritime Antarctic lake protists were depressed by temperature (Laybourn-Parry et al., 1996) and it is possible that Ace Lake grazing rates could have been similarly depressed, although, grazing rate was not found to correlate significantly with temperature (p > 0.05). However, temperature is not the only factor controlling grazing rate; prey and predator concentrations are also important. A higher prey concentration often results in higher ingestion rates providing all other variables remain constant (Peters, 1994; Christou & Moraitou-Apostolopoulou, 1995). Whilst Crooked Lake Protozoa were subject to prey limitation (Laybourn-Parry et al., 1995) the Protozoa in this investigation were not; grazing rate was not significantly correlated with bacterial MCV or abundance (p > 0.05). Turbulence has also been shown to influence grazing rate, however, this is not likely to play a role in the endorheic (closed) basin of Ace Lake (Peters & Gross, 1994; Shimeta et al., 1995). It is likely that a combination of factors influenced the observed grazing behaviour of P. gelidicola. Beneath the ice no grazing occurred and the cells contained autofluorescent chlorophyll a, suggesting that photosynthesis was the dominant mode of nutrition. At 6 m the population abundance was maximal, linked with the position of the DCM. Thus, it can be assumed that optimal conditions prevailed for growth, supplemented by phagotrophy. At 10 m, although one might expect the highest grazing rates associated with the high bacterial abundance near the oxycline and low PAR, cells present may simply have migrated down the water column to make use of the relatively abundant nutrients and were physiologically constrained from 'normal' nutritional behaviour when incubated at this depth. Further experimentation would be required to rule out the possibility of anomalous results due to experimental error in this instance.

By virtue of the fact that P. gelidicola individuals were observed to lose their chlorophyll a during the winter (refer to Section 2.4.2.1) the species' mixotrophic behaviour might be more precisely defined as amphitrophy; a complete switch from autotrophy to heterotrophy in light limiting situations (M. Laval-Peuto, personal communication). This definition would place Pyramimonas gelidicola in Group D of Jones' (1997) classification: amongst facultative mixotrophs whose dominant mode of nutrition is photosynthesis but which exhibit low ingestion rates for cell maintenance during prolonged periods of darkness. The inherent advantages in such a versatile nutritional strategy are clear. The ability to take full advantage of the short austral summer despite potentially temperature- and prey-limited growth rates, necessitates an ability to sustain a year round active population of phytoflagellates that is photosynthetically poised and ready to bloom as soon as ambient PAR levels increase in spring. In addition, the maintenance of an active population, counter balances the energy expensive bet-hedging strategy of encystment and excystment (Hashimoto, 1963; Grimes, 1973), not least because there may be a significant loss of cysts to anoxic waters and sediments in a meromictic system such as Ace Lake. Moreover, the potential of amphitrophy/mixotrophy for supplementing limiting resources such as nitrogen, phosphorus, iron, vitamins, amino acids and fatty acids (e.g. Sanders, 1991; Lampert & Sommer, 1997; Raven, 1997) may have maintained populations that would not otherwise have survived the oligotrophic conditions prevailing in Ace Lake since their isolation from the marine system circa 5000 to 8000 years BP.

The ecological implications of mixotrophic behaviour in the phytoflagellates of Ace Lake are far reaching. As introduced in Section 5.1.2.7, mixotrophy contributes to the re-allotment and mobilisation of nutrients. In a meromictic system such as Ace Lake where nutrients are effectively 'locked' away in the monimolimnion, the movement of bacteriovorous protists both heterotrophic and mixotrophic strengthens the trophic link between the mixolimnion and monimolimnion of Ace Lake. Mixotrophy also increases the efficiency of the microbial loop itself, providing an additional route for the transfer of organic matter as well as strengthening the link between the microbial food web and the classic aquatic food chain (Sanders, 1991). In addition, mixotrophy shifts the competitive balance between bacteria and protists. In an oligotrophic environment where bacteria are competitively dominant, "eating ones competitor" reduces the competition and allows a mixotroph to survive as an autotroph despite its low competitive ability (Thingstad et al., 1996). Alternatively, if mixotrophic protists are important consumers of bacteria, zooplankton grazing may indirectly favour bacteria by eliminating their predators (Vaqué & Pace, 1992). In Ace Lake this is restricted to the summer when zooplankton, predominantly Paralabidocera antarctica (Calanoida:

Copepoda), make their maximum contribution to the microbial biomass and exert the highest grazing pressure (Section 2.3.2.9; Chapter 7).

In evolutionary terms the prasinophyte flagellates are the most primitive of the green algal flagellates. Phylogenetically they are also the most important class leading to the evolution of algal groups such as Chlorophyceae (Norris, 1980). Coupled with the aforementioned conclusion that phagotrophy is a primitive character (Raven, 1997) (Section 5.1.2.3), these factors hint towards the supposition that mixotrophy in prasinophytes may not be uncommon. Populations of Pyramimonas spp. in the offshore Antarctic marine environment have not been reported as mixotrophic to date, though this may be due to lack of investigation. Since these were undoubtedly the ancestors of the Ace Lake population two evolutionary scenarios are possible: firstly, that divergent evolution led to loss of phagotrophic capabilities in *Pyramimonas* spp. in the marine population whilst in Ace Lake this primitive character was retained, perhaps due to the inherent advantages of phagotrophy in such an extreme system. And secondly, that phagotrophic capabilities were in fact re-acquired by Pyramimonas gelidicola in Ace Lake as an effective survival strategy. Further work is required to determine which, if either, is most likely and the extent of mixotrophy within the prasinophytes, in particular the genus Pyramimonas.

### 5.4.3: Summary of growth and grazing

In each instance growth and grazing rates were highest at what would appear to have been the optimum environment in terms of cell growth and survival, chiefly near the thermo/halocline, represented here at 6 m. Growth rates were often comparable to those observed at lower latitudes, reflecting the fact that although the low ambient temperatures and stressful environment undoubtedly constrain Antarctic protists physiologically, they have developed strategies to allow them to take full advantage of the relatively short growing season. Relatively rapid growth rates at optimum depths in Ace Lake allow them to complete their life cycles during the short summer period. Adopting a mixotrophic nutritional strategy contributes to their survival, precluding a reliance on energetically expensive, encystment/excystment, or the need to build up photosynthetically derived reserves prior to growth and reproduction. Thus, although the results presented are those of preliminary investigations and are, therefore, subject to experimental and statistical constraints, it would appear that, contrary to the long held assumption of a physiological winter 'shut down', Antarctic organisms employ strategies that allow them to remain physiologically active, growing and grazing at rates often comparable to those reported for lower latitude aquatic systems of a higher trophic status.

# Chapter 6: A COMPARATIVE STUDY OF TWO OTHER SALINE LAKES IN THE VESTFOLD HILLS

# 6.1: INTRODUCTION

As interest in the Antarctic increases and the influence of human activities extends beyond the immediate vicinity of the scientific research stations, the likelihood of perturbation of the, as yet, largely pristine Antarctic environment also increases. Evaluating and predicting such new environmental pressures is dependent upon our appreciation of natural change within Antarctic lacustrine systems, ecosystems which are amongst those most at risk (Ellis-Evans, 1990). Unless Antarctic saline and freshwater lakes are enriched by seal or penguin faeces or are subject to human impact, they range from oligotrophic to ultra-oligotrophic. By virtue of their varied origins they also range in salinity, and across this salinity spectrum exists a gradient of species diversity and abundance (Perriss & Laybourn-Parry, 1997). In those lakes of marine ancestry, such as those in the Vestfold Hills, only a small fraction of the original marine protozoan community survived post-formation and adapted to their evolving, lacustrine environments. Nutrients became progressively limited, salinity increased, and in certain instances meromixis presented specific environmental challenges that these micro-organisms had to overcome. The evolution of the communities from complex marine to simple lacustrine systems is poorly researched, but lakes which are recently formed in geological terms, can provide clues as to the successional elimination of species. Similarly, of the lakes undergoing natural eutrophication due to nearby penguin rookeries and seal wallows, few have been investigated. Investigations carried out in the maritime Antarctic suggest that eutrophication can have profound effects on productivity and species composition (Light et al., 1981; Hawes, 1983; Ellis-Evans & Sanders, 1988; Izaguirre et al., 1993; Laybourn-Parry et al., 1996; Mataloni et al., 1998). Others indicate the importance of anaerobic processes in lacustrine systems where an anoxic sump develops under winter ice-cover (Ellis-Evans, 1982; 1984; 1985a; 1985b; Ellis-Evans & Sanders, 1988). This chapter describes the plankton dynamics in oligotrophic Highway Lake and in recently formed Rookery Lake which is undergoing natural eutrophication, and compares these two systems with Ace Lake.
#### 6.2: MATERIALS AND METHODS

#### 6.2.1: The sampling sites

Two saline lakes within the Vestfold Hills, Highway and Rookery, were sampled during the field season. Access to these lakes was highly dependent upon logistics and weather conditions, thus, both were studied for a shorter duration than Ace Lake (refer to Chapter 2). Highway Lake is a small, oligotrophic, brackish lake lying at 68° S, 78° E on Long Peninsula at the northern edge of the Vestfold Hills, within 0.2 km of the sea (Figure 2.2). It has an area of approximately 0.5 km<sup>2</sup> and a maximum depth of 15 m. Highway Lake is ice-covered for most of the year, the ice-free period lasting only 4 to 6 weeks.

Rookery Lake is situated at  $68^{\circ}$  S,  $74^{\circ}$  E on a low lying area at the tip of Long Peninsula within 0.2 to 0.5 km of the sea (Figure 2.2). It is surrounded by an Adélie penguin (*Pygoscelis adéliae*) rookery and is subject to carbon and nutrient enrichment by guano and feathers (Morgan & Westbury, 1988). The lake has an area in the region of 0.177 km<sup>2</sup> and a maximum depth of approximately 2 m. Rookery Lake was probably isolated from the sea between 1000 and 2000 years ago and is, therefore, one of the youngest among the lakes of the Vestfold Hills. The lake is subject to marine incursions during high tides and stormy weather when lake- and sea-ice are absent. Isostatic uplift is still in progress at a rate of approximately 2 mm per annum, continuously increasing the lake's isolation (Peterson *et al.*, 1988).

#### 6.2.2: Sampling procedure and analyses

Highway Lake was sampled between December 1995 and May 1996 on 23 December 3 February, 13 April and 17 May. Samples were collected at two metre intervals between 0 and 12 m, with a Kemmerer bottle from a fixed point in the centre of the lake. Sampling was carried out from a boat during open-water periods and through a hole drilled in the ice when an ice-cover prevailed. Rookery Lake was sampled between October 1996 and February 1997 on the following dates: 1 and 25 October, 25 November, 9 December, 6 and 20 January, 5 and 19 February. Due to the shallow nature of the lake, integrated depth samples were collected. During the open water phase samples were taken from the edge of the lake and during the ice-covered period the water column was accessed through a hole drilled in the ice In both instances, 1 l of water was collected for chlorophyll a and nutrient analyses and 200 ml for dissolved and particulate organic carbon analyses (DOC & POC). A further 500 ml of water was fixed in glutaradehyde for bacterioplankton and flagellate enumeration, and one litre in Lugol's iodine for ciliate, dinoflagellate, diatom and metazoan counts. On each sampling occasion dissolved oxygen concentrations were determined by the Winkler technique (Parsons *et al.*, 1984). In addition, conductivity and temperature were measured at Lakes Highway and Rookery with a conductivity, temperature, depth data logger and a hand held refractometer, respectively. All sample analyses were conducted in the laboratory using the procedures outlined in Section 2.2.3.

### 6.3: RESULTS

#### 6.3.1: Highway Lake

A partial ice-cover persisted on Highway lake during December 1995 but by February 1996 its surface was completely ice free. An ice-cover re-developed soon after and remained throughout the rest of the sampling period. Lake water temperatures ranged from -0.32 to 2.72 °C, the highest temperatures occurred throughout the water column during midsummer (December) (Figure 6.1a). Although the lowest temperatures were consistently recorded at the air-water interface, no thermal stratification was apparent within the water column. The lake waters had a salinity that ranged between 3.3 and 11.3 mS cm<sup>-1</sup> with an average of 4.6 mS cm<sup>-1</sup> (Figure 6.1b). There was a marked increase in salinity beneath the ice during April and May due to freeze concentration (Niedrauer & Martin, 1972; Martin, 1979; Gibson & Burton, 1996). The lake was fully oxygenated to a maximum dissolved oxygen concentration of 10.76 mg l<sup>-1</sup>, however, during the winter ice covered period this concentration dropped to 2.53 mg 1<sup>-1</sup> near the sediment-water interface. Dissolved oxygen concentrations remained high throughout the rest of the water column (Figure 6.1c). Chlorophyll a concentrations ranged from 0.09 to 3.56 µg l<sup>-1</sup>, reaching a maximum in April coincident with increasing nutrient levels. (Figure 6.1d). Soluble reactive phosphate (SRP) concentrations ranged between 0 and 13.86  $\mu$ g l<sup>-1</sup> and exhibited an increase throughout the water column as autumn progressed (Figure 6.2a). Similarly, ammonia (NH<sub>3</sub>) concentrations increased during the autumn, ranging from 0 to 36.90  $\mu$ g l<sup>-1</sup>. NH, levels were maximal during the ice-covered, late autumn period near the sediment-water interface (Figure 6.2b). Nitrate and nitrite were undetectable throughout the study period. Dissolved organic carbon (DOC) concentrations increased with the developing ice-cover, reaching a maximum of 8.69 mg  $l^{-1}$  in late



Figure 6.1: Spatial and temporal distribution of (a) temperature (degrees Centigrade), (b) salinity (mS/cm), (c) dissolved oxygen (mg/l) and (d) chlorophyll a (ug/l) in Highway Lake



Figure 6.2: Spatial and temporal distribution of soluble reactive phosphate (ug/l), ammonia (ug/l), dissolved organic carbon (mg/l) and particulate organic carbon (mg/l) in Highway Lake

autumn (Figure 6.2c). The average concentration was 6.50 mg  $1^{-1}$ . In contrast, particulate organic carbon concentrations exhibited little seasonality. Levels were undetectable throughout much of the water column throughout the sampling period but the highest concentrations detected were consistently measured at the bottom of the water column (Figure 6.2d). The maximum recorded concentration was 18.30 mg  $1^{-1}$ .

The maximum abundance of autotrophic and heterotrophic bacterioplankton was recorded during the autumn (Figures 6.3a & 6.3b), following a summer peak in autotrophic (PNAN) and heterotrophic (HNAN) flagellate abundance (Figures 6.3c & (6.3d). Autotrophic and heterotrophic bacterial abundance ranged from 0.02 to 0.67 x  $10^8$  cells l<sup>-1</sup> and 4.16 to 11.00 x 10<sup>8</sup> cells l<sup>-1</sup>, respectively (Figures 6.3a & 6.3b). PNAN and HNAN abundance ranged from 0.18 to  $60.70 \times 10^6$  cells l<sup>-1</sup> and 12.5 to 2260 x  $10^6$ cells 1<sup>-1</sup>. Ciliate concentrations (excluding *Mesodinium rubrum* (Ciliophora: Haptorida)) were highest during late summer, reaching a peak of 1610 cells  $l^{-1}$  in February (Figure 6.4a). Mesodinium rubrum was the most dominant ciliate species in the lake and it too exhibited a summer peak, primarily in the surface waters (Figure 6.4b). The abundance of *M. rubrum* reached a maximum of 2292 cells  $l^{-1}$  at 0 m in February, Conversely, dinoflagellate numbers ranged from 170 to 3650 cells 1<sup>-1</sup> during the study period, increasing in the autumn concurrent with decreasing flagellate and ciliate numbers (Figure 6.4c). Metazoans in the lake included Daphniopsis studeri (Crustacea: Cladocera) and Notholca sp. (Rotifera: Monogonata) but appreciable numbers were not recorded during the study period (Laybourn-Parry & Marchant, 1992a; Bayliss & Laybourn-Parry, 1995; Dartnall, 1997). No diatoms were observed.

#### 6.3.2: Rookery Lake

Complete ice-cover persisted on Rookery Lake until late October. By late November peripheral moating had developed and the lake was totally ice-free by midsummer (December). The lake waters had a salinity of approximately 15 % $_{0}$  (approximately half that of seawater) during the open water period. However, the salinity of Rookery Lake is known to increase to at least four times that of seawater during the ice-covered period causing the precipitation mirabilite and iron sulphides (Hand & Burton, 1981). Prior to moating in November the water column was completely anoxic. Interactions between the moat waters and the atmosphere led to a gradual oxygenation of the water column and by mid-December the lake waters were fully saturated with oxygen (Figure 6.5a). Chl *a* concentrations were high, in early summer (November) levels peaked at 102  $\mu$ g l<sup>-1</sup> (Figure 6.5b; Photograph 2.4). During the anoxic phase SRP levels were also high (maximum 373.45  $\mu$ g l<sup>-1</sup>) (Figure 6.5c),



Figure 6.3: Spatial and temporal distribution of (a) autotrophic & (b) heterotrophic bacteria (cells/l+E08) and (c) autotrophic & (d) heterotrophic flagellates (cells/l+E06) in Highway Lake



Figure 6.4: Spatial and temporal distribution of (a) ciliates, (b) Mesodinium rubrum and (c) dinoflagellates (cells/l) in Highway Lake



**Figure 6.5**: Temporal patterns of (a) dissolved oxygen (DO), (b) chlorophyll *a* (Chl *a*), (c) soluble reactive phosphorus (SRP) & ammonia (NH<sub>3</sub>) and (d) dissolved (DOC) & particulate (POC) organic carbon concentrations in Rookery Lake

however, levels dropped rapidly as oxygenation of the water column took place and the microbial plankton developed (Figures 6.5c & 6.6). NH<sub>3</sub> concentrations were generally low but reached a maximum of 13.32  $\mu$ g l<sup>-1</sup> in November, thereafter dropping close to the minimum level of detection (Figure 6.5c). Levels of nitrate and nitrite were always below the level of detection. DOC concentrations ranged from 62.62 to 459.42 mg l<sup>-1</sup> (Figure 6.5d). Concentrations decreased dramatically during the summer. In contrast, POC concentrations increased in relation to increased biological activity and inputs from the penguin rookery (Figure 6.5d). Concentrations ranged between 189.17 and 270.83 mg l<sup>-1</sup>.

While some autotrophic bacteria can function under anoxic conditions, the main growth of this functional group occurred following re-oxygenation of the water column (Figures 6.5a & 6.6). Autotrophic bacterial abundance ranged from 0.71 to 49.50 x  $10^7$  cells 1<sup>-1</sup>. Contrary to this, a well developed community of heterotrophic bacteria existed during the ice-covered and anoxic period (Figure 6.6), although their numbers declined during the summer concurrent with the development of an heterotrophic protozoan community of HNAN and ciliates, and a resultant increase in grazing pressure (Figure 6.6). Heterotrophic bacterial abundance ranged between 1.00 and  $3.92 \times 10^9$  cells l<sup>-1</sup>. Although bacterial production was not measured, it is likely that the heterotrophic bacterial community maintained high productivity during the summer, resulting in the observed depression of DOC concentrations (Figures 6.5d & 6.6). An increase in phytoplankton abundance occurred as the water column became oxygenated. PNAN abundance increased rapidly in November to 5.47 cells l<sup>-1</sup> and maintained relatively high numbers throughout the summer, despite potential grazing pressure from ciliates (Figure 6.6). The maximum abundance recorded was 5.85 cells  $1^{-1}$  in February corresponding with increasing concentrations of SRP, NH<sub>3</sub>, Chl a and DOC (Figures 6.5 & 6.6). The PNAN community was dominated by Pyramimonas gelidicola (Chlorophyta: Prasinophyceae). The diatom community developed more slowly, probably as a result of longer life cycles, and exhibited a peak of  $4.18 \times 10^5$ cells  $1^{-1}$  in early January (Figure 6.6).

The ciliate community of Rookery Lake comprised marine species found commonly in the inshore marine ecosystem of Prydz Bay (J. Grey, unpublished data). The common genera included *Strombidium*, *Tontonia*, *Lohmanniella*, *Tiarina*, *Laboea*, *Euplotes* and *Codenellopsis*, and several scuticociliate species. These ciliates would have grazed primarily on PNAN, algae and HNAN, with the exception of the latter bacterivores. Dinoflagellates made only a brief appearance in the water column in mid-January, when an abundance of 20 000 cells 1<sup>-1</sup> was recorded. Large numbers of protozoan cysts were recorded throughout the summer. A maximum abundance of 1.5



Figure 6.6: Temporal patterns of autotrophic bacteria (ABact), heterotrophic bacteria (HBact), autotrophic flagellate (PNAN), heterotrophic flagellate (HNAN), ciliate, dinoflagellate, cyst and diatom abundance in Rookery Lake

x  $10^6$  cysts l<sup>-1</sup> was measured in mid-February. Metazoa were sparse in Rookery Lake; during the study period only a few nauplii of the calanoid copepod, *Paralabidocera antarctica* Thompson (Copepoda: Calanoida), were noted. Adults were never observed in the samples collected.

#### 6.4: DISCUSSION

#### 6.4.1: Highway Lake

Highway Lake is an holomictic lake which mixes throughout its entire depth during the summer months when the lake is ice-free. As such it is distinctly different from meromictic Ace Lake, and although cannot be directly compared, emphasises the influence of stratification on plankton dynamics. Highway lake was only slightly brackish with an average salinity of 4.6 mS cm<sup>-1</sup>, between half to one quarter of the salinity in the mixolimnion of Ace Lake (Figures 6.1b & 2.5). The temperature of the water column was similar to that recorded in the mixolimnion of Ace Lake, but by virtue of its lower salinity Highway Lake developed an ice-cover at higher temperatures than Ace Lake (Figures 6.1a & 2.4). Highway Lake was fully oxygenated throughout the year, although a degree of hypoxia developed near the sediment-water interface, presumably due to the settling of organic matter, the resultant microbial activity and the protective ice-cover (Figure 6.1c).

Nutrient concentrations in Highway Lake were low. Nevertheless, in common with other lakes of the Vestfold Hills, nutrient levels increased during the winter months (Figures 6.2 a & b & 2.8 to 2.11; e.g. Laybourn-Parry et al., 1992b; Bayliss et al., 1997; Bell & Laybourn-Parry, 1999). SRP levels were comparable to those recorded in the mixolimnion of Ace Lake, however, NH<sub>3</sub> levels exceeded those recorded in Ace Lake. The latter may be attributable to three factors: firstly, the catchment area around Highway Lake entrains far more snow than that surrounding Ace Lake contributing to the nitrogen levels in the lake during the summer melt period; secondly, Highway Lake lies relatively close to a penguin rookery and may receive some wind-blown guano inputs at certain times of the year; and thirdly, due to its proximity to the sea, the lake periodically receives inputs of sea-spray (Laybourn-Parry & Marchant, 1992; Perriss et al., 1993; Crittenden, 1998). The low nutrient levels are reflected in the low chlorophyll a concentrations observed during this and other studies (Figure 6.1d) (Laybourn-Parry & Marchant, 1992b; Perriss et al., 1993). Between 1995 and 1996 the maximum reported Chl a concentration was roughly half that recorded in the summer of 1991 to 1992 (Perriss et al., 1993). Dissolved organic

carbon levels were lower than those recorded in Ace lake, though higher than those noted for ultra-oligotrophic Crooked Lake, Vestfold Hills, and the lakes of the Larsemann Hills (Laybourn-Parry *et al.*, 1995; Ellis-Evans *et al.*, 1998). Levels were noticeably lower in the summer months when DOC was being used by the summer plankton blooms. POC concentrations were at least one order of magnitude lower than the maxima recorded in the monimolimnion of Ace Lake. Measured concentrations did not exhibit a marked seasonality and were consistently higher near the sediments due to the settling out of organic matter (Figures 6.2c & d).

Bacterial numbers were lower than those reported in Ace Lake. In contrast, the abundance of PNAN, HNAN and dinoflagellates was higher. The salinity of Highway Lake is at the lower limit of the reported tolerance for Mesodinium rubrum (Lindholm, 1985), however, this species still dominated the ciliate community in a similar manner to that reported in nearby Ace Lake, albeit at a lower overall abundance (Figures 6.4b & 2.22) (Perriss et al., 1993; Bell & Laybourn-Parry, 1999). Unlike other members of the ciliate community, M. rubrum exhibited a relatively constant abundance throughout the water column during the autumn sampling period reflecting its supreme adaptation to the oligotrophic conditions in the lake, potential endogenous food reserves and use of DOC (Smith & Barber, 1979; Perriss et al., 1995). Only in the summer months was a bloom noted in the lake's surface waters, taking advantage of the high light levels available (Figure 6.4b). Although the metazoan, Daphniopsis studeri, has been reported to "thrive" in Highway Lake, few individuals were recorded during this study (Laybourn-Parry & Marchant, 1992a). This reduced abundance of ciliates and Metazoa is likely to account for the higher abundance of PNAN, HNAN and dinoflagellates observed in Highway Lake when compared with Ace Lake and highlights the seasonal importance of grazing in Antarctic Lakes (Section 2.4.2.5).

#### 6.4.2: Rookery Lake

Pronounced seasonality is common in many aspects of polar aquatic ecology, although the misconception that Antarctic systems 'shut down' in the winter is far from true. In Rookery Lake such seasonality was enhanced by the annual development of anoxia and enrichment from the penguin rookery. Moreover, winter anoxia is known to result in a greater mobility of reduced compounds, such as ammonium and phosphate, which migrate up into the water column from the sediments, further enhancing the seasonal patterns (Gallagher, 1985). Relatively high levels of heterotrophic activity below the ice-cover, and the shallow nature of Rookery Lake, probably account for the development of anoxia. The formation of a

moat in November and progressive ice-loss, stimulated the development of high microbial biomass of bacteria, Protozoa and algae. The high concentrations of chlorophyll a and SRP recorded in Rookery Lake when compared with Lakes Ace and Highway, were the result of nutrient inputs from the surrounding Adélie penguin rookery (Figures 6.5b & c, 6.2a, 2.8 & 2.14; Photograph 2.4). In the nearby marine environment, chlorophyll a values were typically below 20  $\mu$ g l<sup>-1</sup> during summer, with correspondingly low levels of nutrients (J. Grey, unpublished data). Natural eutrophication is a phenomenon noted in several of the freshwater lakes in the maritime Antarctic (Signy Island, South Orkneys). There, elephant (Mirounga leonina) and fur seal (Arctocephalus gazella) faecal inputs have led to significant changes in the productivity and species diversity of lacustrine systems (Ellis-Evans & Sanders, 1988; Laybourn-Parry et al., 1996). Like Rookery Lake, Amos Lake on Signy Island is a shallow system which becomes totally anoxic beneath its ice-cover (Ellis-Evans, 1982; Ellis-Evans & Sanders, 1988). Chlorophyll a concentrations in Amos Lake ranged between 37 and 109  $\mu$ g l<sup>-1</sup> annually, values similar to those determined in the present summer study of Rookery Lake. SRP concentrations in Amos Lake were also similar to those in Rookery Lake, although, concentrations of ammonia and nitrate remained extremely high throughout the year. In contrast, Lake Otero, a shallow saline lake on the Antarctic Peninsula, had comparable levels of ammonia to those recorded during the current investigation (Mataloni et al., 1998). Hawes (1983) suggested that in enriched freshwater lakes on Signy Island phosphate inputs permitted extensive phytoplankton development, but that the developing phytoplankton become progressively nitrate limited. This may have been the case in Rookery Lake where Chl a concentrations mirrored changes in NH<sub>3</sub> concentration (Figures 6.5b & c). However, the abundance of both autotrophic bacteria and PNAN was still high despite the drop in Chl a levels, and this trend may instead reflect the high incidence of photoinhibition and self-shading imminent in such a shallow lake during the ice-free period (Figure 6.6) (Ellis-Evans, 1990). Further work would be required to clarify this.

When compared with shallow, unenriched, continental saline systems in the Bunger Hills, which are typically more productive than neighbouring freshwater lakes, chlorophyll *a* concentrations in Rookery Lake were extremely high. In a number of shallow saline Bunger Hills lakes, Chl *a* levels ranged between 0.26 and 1.93  $\mu$ g l<sup>-1</sup> and SRP between 3 and 10  $\mu$ g l<sup>-1</sup> (Kaup *et al.*, 1993). Similarly, in the neighbouring Larsemann Hills brackish shallow lakes have comparably low Chl *a* and nutrient concentrations (Ellis-Evans *et al.*, 1998). There are no data for other shallow systems in the Vestfold Hills, but in deeper unenriched saline lakes in the area Chl *a* concentrations ranged typically between 0.2 and 33.2  $\mu$ g l<sup>-1</sup> (Perriss & Laybourn-

Parry, 1997; Bell & Laybourn-Parry, 1999). The high concentrations of Chl *a* in Rookery Lake (4.3 to 102.9  $\mu$ g l<sup>-1</sup>) render it an eutrophic Antarctic lake.

Levels of POC in Rookery Lake were of the same order of magnitude observed in Ace Lake (Figures 6.5d & 2.13) despite the increased particulate load one might expect from the penguin rookery. This is likely to be due to the entrainment of particulate matter at the stratification boundaries in Ace Lake, maintaining high concentrations, when by comparison particulate matter in Rookery Lake quickly settles into the sediments. This is corroborated by the fact that DOC levels in Rookery Lake were 14 to 30 times higher than those recorded in Ace Lake, reflecting the dissolved carbon entering the lake from the rookery as runoff and the decomposition of POC in the sediments (Figures 6.5d & 2.12).

The consequences of this natural eutrophication were consistently higher concentrations of bacteria in Rookery Lake than those reported from deeper, nonenriched, saline lakes in the Vestfold Hills and the Larsemann Hills, where typical abundances ranged from 1.40 x  $10^7$  to 1.58 x  $10^{10}$  cells l<sup>-1</sup> (Figure 6.6) (Izaguirre et al., 1993; Perriss & Laybourn-Parry, 1997; Ellis-Evans et al., 1998; Bell & Laybourn-Parry, 1999). The abundance of both PNAN and HNAN was correspondingly higher than those recorded from unenriched, saline and brackish systems in both oases but within the same range reported for other naturally enriched Antarctic lakes (Figure 6.6) (Perriss & Laybourn-Parry, 1997; Ellis-Evans et al., 1998; Mataloni et al., 1998). Nevertheless, despite high numbers of PNAN there was no detectable photosynthesis. Once again, this can be attributed to a high degree of self-shading coupled with photoinhibition in this shallow lake during open water periods, corroborated by the midsummer decrease in Chl a concentration despite the high abundance of PNAN and peak abundance of autotrophic bacteria (Ellis-Evans, 1990). The PNAN in Rookery Lake were dominated by Pyramimonas gelidicola in the same manner as Ace Lake, but contrary to eutrophic lakes on the Antarctic peninsula which are dominated by *Chlamydomonas* spp. (Hawes, 1990b; Mataloni et al., 1998).

The seasonal anoxia suffered in Rookery Lake had a marked impact on its productivity. Production was curtailed post-ice formation as hypoxia progressing to anoxia developed. Deeper lakes in the Vestfold Hills have been investigated over an annual cycle, and in both freshwater and saline systems aerobic heterotrophic productivity continued during the winter (Chapter 4; Laybourn-Parry *et al.*, 1995; Bayliss *et al.*, 1997; Bell & Laybourn-Parry, 1999). In Rookery Lake the productivity was profoundly seasonal, related to loss of ice-cover and re-oxygenation of the water

column. It is likely that the Protozoa withstood this anoxic, winter phase by encysting. Large numbers of cysts (up to  $1.5 \times 10^6 \ l^{-1}$ ) were noted throughout the study, supporting this contention (Figure 6.6). The cysts appeared in the water column during the open water phase following probable resuspension from the sediments during wind-induced mixing. In contrast, in lakes with water columns that remain partially or fully oygenated beneath winter ice-covers, Protozoa can 'hedge their bets', some encysting and others remaining active or poised until the return of favourable environmental conditions in the spring and summer. This strategy renders the seasonal patterns observed, less pronounced.

The oldest lakes in the Vestfold Hills are probably no more than 10 000 years old, but at between 1000 and 2000 years of age Rookery Lake is extremely young (Adamson & Pickard, 1986; Peterson et al., 1988). Its recent emergence from, and hence close proximity to, the sea still allows occasional marine incursions during spring tides and stormy weather when sea and lake ice are absent (Burton, 1981; Peterson et al., 1988). In effect the lake is still evolving a lacustrine plankton typical of the older lakes in the Vestfold Hills area. Typically, Antarctic lakes lack a significant metazoan component and are dominated by microbial plankton (Laybourn-Parry, 1997). Indeed, Rookery Lake has already lost most of the Metazoa found in the marine environment. Only low numbers of the calanoid copepod Paralabidocera antarctica remain, of which only naupliar stages were observed. However, this is more likely to be due to the copepod's inability to withstand the complete winter anoxia than an evolutionary inevitability, since older Lakes Ace and Highway both sustain large, successful populations of Metazoa. Of particular note is the species composition of the ciliate community, which differs from that observed in the older, more evolved saline lakes of the Vestfold Hills. Despite its isolation and reduced summer salinity, Rookery Lake supports a ciliate community similar to that in the offshore marine environment of Prydz Bay, albeit of reduced diversity (J. Grey, unpublished data; Davidson & Marchant, 1990). This reduced species diversity is comparable with that observed in the six major basins of Ellis Fjord, an aquatic system in the Vestfold Hills which is still connected to the marine environment but receives freshwater inputs from a glacial stream at its head (Grey et al., 1997). In contrast, many of the older saline lakes in the Vestfold Hills are dominated by the autotrophic ciliate Mesodinium rubrum, which seems particularly well adapted to oligotrophic conditions (Figures 2.22, 6.4b & Section 2.4.2.4). Other species include members of the genera Strombidium and Euplotes (Laybourn-Parry & Perriss, 1995; Perriss & Laybourn-Parry, 1997; Bell & Laybourn-Parry, 1999). Although M. rubrum was observed in Rookery Lake it was not common, highlighting the fact that as the lakes of the Vestfold Hills have evolved and become more oligotrophic relative to the marine

system from which they originated, species diversity has been severely reduced leaving a few highly adapted species within the ciliate community. Rookery Lake represents an intermediate system which still possesses a typical marine microbial plankton but one that is undergoing a reduction in diversity. The first ciliate group that seems to disappear during the course of lacustrine evolution is the tintinnids. Only one species was observed in Rookery Lake, whereas in the nearby marine system 3 to 4 species occurred regularly within the annual cycle (J. Grey, unpublished data; Davidson & Marchant, 1990). Tintinnids are common in lower latitude lakes of the northern hemisphere but have not previously been recorded in freshwater or saline Antarctic lakes (Laybourn-Parry, 1992; 1997). Ciliate numbers peaked in late summer (February) following the peak abundance of PNAN and HNAN (Figure 6.6). This period may also represent an optimum in terms of water temperature, inducing an excystment episode (Rengefors & Anderson, 1998).

### 6.4.3: Synthesis

In combination the investigations carried out on Ace, Highway and Rookery provide useful insight into the evolution of Antarctic saline lakes and the dynamics of their microbial flora and fauna. The three lacustrine ecosystems emphasise a number of issues already raised in this study. The first is the importance of physical and chemical stratification in influencing the microbial dynamics of lakes such as Ace Lake. Highway Lake is holomictic and exhibits no chemical stratification, thus, in contrast with meromictic Ace Lake, no distinct spatial zonation was apparent in the planktonic composition of the lake. Similarly, once the ice-cover disappeared and its waters were re-oxygenated, the microbial plankton of Rookery Lake was not spatially defined.

Secondly, the dramatic effect of natural enrichment exhibited by Rookery Lake serves to highlight the potential influence of perturbations, be they natural or anthropogenic, on naturally oligotrophic Antarctic lakes. This is particularly pertinent as human presence in Antarctica increases. Enrichment of No Worries Lake in the Larsemann Hills, has occurred due to its presence within the small catchment of the Chinese research station, Zhongshan. Examination of the lake prior to the establishment of the station indicated that it was a clear, slightly brackish, oligotrophic lake with a low diversity of plankton species (Gillieson *et al.*, 1990). Subsequent, circulation of water from the lake via a pipeline to cool generators on the station and runoff from the catchment where vehicles and kitchen wastes are present has led to increased temperature and turbidity, year round anoxic sediments, a high suspended organic sediment load and an active microbial population (Ellis-Evans *et al.*, 1997). Hence, understanding the dynamics of both natural and anthropogenic enrichment events may assist the long-term conservation of the Antarctic environment.

Thirdly, although the future evolution of the plankton community of Rookery Lake may be complicated by its eutrophic nature and seasonal hypoxia, it nevertheless provides an interesting insight into how the saline lake communities in the Vestfold Hills developed from marine communities. Their isolation from the sea has led to the simplification and truncation of their microbial food webs, and the dominance of robust, flexible species adapted to cope with the extreme nature of Antarctic lacustrine environments.

# Chapter 7: A TENTATIVE MODEL OF CARBON FLUX IN ACE LAKE

#### 7.1: INTRODUCTION

To date, most models produced for aquatic microbial communities have been based upon marine systems (Jumars *et al.*, 1989; Stone, 1990; Sakshaug *et al.*, 1991; Steele & Henderson, 1992a; 1992b; Riegman & Kuipers, 1994; Cloern *et al.*, 1995; Henderson & Steele, 1995; Wood & Horwood, 1995; Blackburn *et al.*, 1997). Lacustrine models have in general been restricted to temperate systems, for example, Lake Constance, Germany (Weisse *et al.*, 1990; Gaedke & Straile, 1994; Gaedke, 1995). Few workers have considered bringing together data to develop models of community structure and interactions, carbon or nutrient budgets, for Antarctic lacustrine environments. This is unfortunate since, despite the numerous assumptions that must necessarily be made, such mathematical studies often throw new light on the processes occurring in aquatic ecosystems. Moreover, the opportunities offered by Antarctic lacustrine ecosystems to contribute to general ecological theory and the development of predictive tools for microbial systems, are yet to be realised (Ellis-Evans, 1996).

The first attempt to produce whole lake budgets for Antarctic lacustrine systems was undertaken by Ellis-Evans (1982) for oligotrophic Moss Lake and enriched Heywood Lake on Signy Island in the maritime Antarctic. This study indicated that 85 % of the autochthonous carbon in Moss lake was turned over in the water column, whereas a substantial amount of allochthonous carbon in Heywood lake was incorporated into the sediments because the lake's capacity to metabolise it was low. Ellis-Evans (1996) also produced a generalised flow diagram for the community structure of maritime Antarctic Lakes on Signy Island. Subsequently, Moorhead and Davis (1997) produced a model which simulated the carbon dynamics of microbial mats in the lakes of the McMurdo Dry Valleys and Laybourn-Parry (1997) created a simple carbon flux model for Crooked Lake, Vestfold Hills. No carbon budgets have been produced for saline lakes in the Vestfold Hills prior to this study.

The aim of this exercise was to produce a model that could generate compartmentalised flow diagrams for both the mixolimnion and the upper monimolimnion of Ace lake similar to those produced by Ellis-Evans (1982; 1996), Stone *et al.* (1993), Riegman and Kuipers (1994) and Laybourn-Parry (1997), as opposed to the empirical models produced by Stone (1990), Sakshaug *et al.* (1991), Steele and Henderson (1992a;

1992b), Cloern *et al.* (1995), Henderson and Steele (1995), Ietswaart and Flynn (1995), Wood and Horwood (1995) and Spencer and Ellis (1998), and illustrate the seasonal variations in the carbon flux within these strata.

#### 7.2: MATERIALS AND METHODS

Almost all of the data employed to produce the model was derived from the seasonal study conducted on Ace Lake between December 1995 and February 1997 (Chapters 2 to 5). When it was necessary to look to the literature for further information, data from Antarctic lacustrine systems as similar to Ace Lake as possible were introduced. This was principally required for heterotrophic ciliate, heterotrophic nanoflagellate (HNAN) and copepod clearance rates. Heterotrophic ciliate clearance rates were obtained from work carried out on Crooked Lake in the Vestfold Hills (J. Laybourn-Parry, personal communication), HNAN clearance rates from meromictic Lake Fryxell, McMurdo Dry Valleys (Roberts & Laybourn Parry, 1999), and the copepod clearance rates employed were derived for the offshore marine system from which they originated (Swadling *et al.*, 1997, pp. 43). The rotifers observed in the plankton of Ace Lake have not been included in the model since they derived exclusively from littoral, benthic algal mats and were present in such low numbers that their influence on the planktonic carbon flux was likely to be negligible.

#### 7.3: THE MODEL

Presented in Figures 7.1 to 7.8 are seasonal, compartmentalised flow diagrams for both the mixolimnion and the upper monimolimnion of Ace Lake. The average biomass ( $\mu g C l^{-1}$ ) of each microbial component, with daily production and consumption fluxes ( $\mu g C l^{-1} d^{-1}$ ) are included. Each diagram represents a 'snap-shot' in time, although the model from which these conceptual diagrams originate will ultimately be used to generate a dynamic illustration of microbial plankton carbon fluxes in Ace Lake.

## 7.4: DISCUSSION

It has become increasingly evident that complex communities of microbial organisms in aquatic ecosystems play a key role in processing and transferring carbon and inorganic nutrients from primary producers to metazoan plankton and higher trophic levels (Pomeroy, 1974; Azam *et al.*, 1983). However, the extent to which carbon fixed in microbial biomass passes to other trophic levels has rarely been quantified. Where evidence is available, its seems that protozoans can be important food and nutrient



Figure 7.1: Carbon flux in the mixolimnion during the autumn. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$ nd = not detectable



**Figure 7.2**: Carbon flux in the mixolimnion during the winter. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$ nd = not detectable



Figure 7.3: Carbon flux in the mixolimnion during the spring. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$ nd = not detectable



**Figure 7.4**: Carbon flux in the mixolimnion during the summer. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$ nd = not detectable



**Figure 7.5**: Carbon flux in the monimolimnion during the autumn. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$  nd = not detectable



**Figure 7.6**: Carbon flux in the monimolimnion during the winter. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$ nd = not detectable



**Figure 7.7**: Carbon flux in the monimolimnion during the spring. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$  nd = not detectable



**Figure 7.8**: Carbon flux in the monimolimnion during the summer. Boxes = biomass  $(\mu g l^{-1})$ , P = production  $(\mu g l^{-1} d^{-1})$ , ellipses = consumption  $(\mu g l^{-1} d^{-1})$  nd = not detectable

sources for metazoan zooplankton and the importance of the microbial loop has been emphasised (Pomeroy, 1974; Azam *et al.*, 1983; Wiadnyana & Rassoulzadegan, 1989; Carrick *et al.*, 1991; Stone *et al.*, 1993).

What is immediately apparent is that the summed carbon demand of consumers greatly outweighs the summed carbon assimilation by autotrophic producers. Three possible explanations exist: 1) The measurement of autotrophic production proved problematic and photosynthesis was not detectable on all occasions, therefore, it may be that there are errors in the autotrophic production rates employed. 2) Allochthonous carbon sources are more important than first assumed, although this is highly unlikely to account for the wide disparities observed due to the barren nature of the lake's catchment. 3) Efficient recycling of organic carbon within the microbial loop is taking place (Strayer, 1988). The latter is the most likely explanation for the disparities. Similarly, Priddle et al. (1986) suggested that autotrophic production in Signy Island lakes could not be accounted for by the measured carbon and nutrient supply, suggesting rapid recycling of carbon and nutrients within the microbial loop. More recently, Bayliss et al. (1997) demonstrated that the pool of carbon in Crooked Lake, the Vestfold Hills, was continuously recycled, albeit at a slow rate. Moreover, in temperate Lake Michigan, USA, such recycling is important; Scavia (1988) demonstrated that bacterial secondary production was also high relative to autotrophic primary production.

In addition, the models confirm the contention made in Section 2.3.2.9 that bacterial biomass (primarily heterotrophic) dominates in both the mixolimnion and monimolimnion of Ace Lake. The only exception to this was when *Mesodinium rubrum* bloomed in the mixolimnion during the summer (Figure 7.4).

What is also highlighted by the flow diagrams is that copepod biomass increased by more than 7 times in the mixolimnion and 28 times in the monimolimnion between winter and summer, and as a result the microbial carbon grazed by the copepods increased by three orders of magnitude. Previously, it has been proposed that Antarctic lacustrine ecosystems are entirely regulated by "bottom-up" control (Vincent, 1988; Laybourn-Parry *et al.*, 1995; Ellis-Evans, 1996; Bayliss *et al.*, 1997), but it is apparent in Ace Lake, that although "bottom-up" control is the dominant regulatory process throughout most of the year, "top-down" control becomes increasingly important in the summer months. A similar situation was evident in the maritime Antarctic lakes on Signy Island; copepod grazing exerted substantial "top-down" control towards the end of spring which diminished throughout the remainder of the year (Priddle *et al.*, 1986; Laybourn-Parry *et al.*, 1996).

In Ace Lake HNAN consumed the most bacteria in the winter in both strata (Figures 7.2 & 7.6). It appears that the mixotrophic phototrophic nanoflagellates (PNAN) exhibited a limited carbon consumption relative to these obligately heterotrophic organisms. However, the carbon consumed by mixotrophic PNAN is likely to be higher than that modelled during the winter when no light was available for photosynthesis, since the data employed was obtained during the summer months when both phagotrophy and photosynthesis were possible and the use of fluorescently labelled latex microspheres (FLM) may have underestimated the potential grazing impact of *Pyramimonas gelidicola* (Section 5.4.2). As would be expected, obligately autotrophic PNAN biomass was highest in the summer in both strata, when light was available for photosynthesis and high rates of photosynthesis detectable.

Given the low relative biomass of the heterotrophic ciliate component of the food web, the grazing pressure exerted on the bacterioplankton in particular was extremely high. This is most likely an artefact of the literature values employed, which give the maximum potential clearance rates for heterotrophic ciliates of all sizes and do not take into account differing conditions and species in Ace Lake.

Riemann & Sondergaard (1986) stated that, "Generalising diagrams sometimes have a tendency to be viewed in a very rigid and definitive manner as if they contain the whole and final story. This is never the case, as a diagram presents a "frozen" picture of a complex series of dynamic events. Furthermore, such diagrams are always biased by present knowledge." Flow charts such as those portrayed here should not, therefore, be regarded as definitive, ultimate statements of actual carbon fluxes in Ace Lake, rather considered as working hypotheses that need to be subjected to further testing and research but that point the way to refinements of current experimentation and theory (Stone *et al.*, 1993).

These initial carbon flux models do, however, emphasise that despite the simplicity of the trophic structure in Antarctic lake communities when compared with temperate systems, and even those of the Arctic, the evidence emerging regarding community interactions suggests a complexity of interaction that almost certainly matches that of temperate systems (Ellis-Evans, 1996). At the moment, most of the data emerging from polar lacustrine studies is descriptive. What is urgently needed are more long term investigations of community structure, temporal and spatial dynamics which include an ecophysiological component, on the diverse range of lakes on the Antarctic continent. We need to know much more about the functional dynamics of these extreme, pristine

ecosystems, in relation to the size and sources of carbon pools and nutrient dynamics (Laybourn-Parry, 1997).

## 7.5: CONTINUED EFFORTS

Work is still proceeding in collaboration with modellers at the Texas Tech University, USA, to produce a dynamic flux model which will be ready for publication in the spring of 1999.

## Chapter 8: GENERAL DISCUSSION

#### 8.1: SUMMARY OF FINDINGS

Nowhere have the changes in our understanding of ecological processes been more dramatic than in the discovery of the vast diversity of both prokaryotic and eukaryotic micro-organisms present in nature and the multitude of roles that they play in aquatic ecosystems (Wiebe *et al.*, 1994). By the early 1970's it was apparent that the simple, single, role of bacteria as nutrient mineralisers was unrealistically narrow. More recently, ecological modelling has also helped to define the roles of micro-organisms and to suggest the types of controls that could maintain the population densities observed. Although not without its critics, the new paradigm regarding aquatic microbial food webs and the concept of a "microbial loop" are now widely accepted to be of fundamental importance in aquatic systems. The paradigm itself has not changed since the early 1980's but it has advanced from a conceptual and qualitative description of microbial food webs, to a quantitative concept that incorporates rate and biomass calculations (Hobbie, 1994).

Antarctic ecosystems are extreme environments, especially in terms of the severity of their climate, their seasonality and biogeochemical isolation. These, unique Antarctic conditions suggest that the microbial loop can be of great importance in maintaining aquatic ecosystems throughout the austral winter (Azam *et al.*, 1991). Fortuitously, Antarctic lakes make excellent laboratories in which to further the understanding of microbial food webs without the background noise imposed by herbivory, predation and terrigenous inputs at lower latitudes (Ellis-Evans, 1996). This study was concerned with the saline lakes of the Vestfold Hills, Ace Lake in particular.

It is well known that extreme conditions in any environment lead to the domination by prokaryotes and lower eukaryotes. Consistent with this, evolutionary processes in all Antarctic lakes have led to the domination of simple communities of bacteria, algae and Protozoa, with a paucity of Metazoa and no known ichthyoplankton. The relic marine food web of Ace Lake has effectively been truncated and microbial loop dynamics dominate. This evolutionary process was successfully highlighted by the simultaneous study carried out on Rookery Lake, one of the youngest lakes in the Vestfold Hills (Pickard, 1986; Peterson *et al.*, 1988) (Chapter 6). Although inputs of carbon and nutrients from a nearby penguin rookery have enriched Rookery Lake when compared with other brackish and saline lakes in the Vestfold Hills, the lakes intermediate evolutionary status was clear. The diversity of the ciliate community in Rookery Lake

lay between the low diversity observed in older saline lakes within the Vestfold Hills and the high diversity apparent in the nearby marine community. Nevertheless, despite their trophic simplicity Antarctic microbial food webs exhibit a considerable complexity of interactions between trophic levels which almost certainly matches that of temperate systems (Vincent & Howard-Williams, 1985; Ellis-Evans, 1996).

Due to their complex evolution, a high proportion of the saline lakes in the Vestfold Hills are meromictic, or permanently stratified. Ace Lake is one such meromictic system. Chapter 2 illustrated the stable stratification existing in Ace Lake, a stratification which was both chemically and biogenically maintained. As a result of a salinity gradient between the freshwater of the mixolimnion and the relic seawater of the monimolimnion, a thermocline/halocline lay between 6 and 8 m. The biogenically driven stratification was apparent in terms of the lake's dissolved oxygen content; an oxygenated mixolimnion and upper monimolimnion lay between 0 and 10 m, an oxycline between 10 and 12 m, and a lower anoxic monimolimnion extended from 12 m to the lake bed. At the oxycline organic matter sedimenting from above and nutrients diffusing from below were entrained. Coupled with the relatively high ambient temperatures and optimal wavelengths of Photosynthetically Active Radiation (PAR), this zone provided increasingly ideal conditions for bacterial production. As a consequence, a bacterial 'plate' formed in the oxycline producing a marked Deep Chlorophyll Maximum, consuming the dissolved oxygen present and preventing light penetration below this depth.

It is clear from the results presented in Chapter 2 that the stratification of Ace Lake had a profound influence on the vertical pattern of dissolved oxygen, nutrients and organic matter throughout the water column, and consequently, the occurrence of bacteria and their flagellate and protozoan predators. Comparison between Ace Lake and Lakes Highway and Rookery emphasised the importance of this stratification to Ace Lake's plankton dynamics. No chemical or biological stratification was evident in holomictic Lakes Highway or Rookery, therefore, the plankton was distributed relatively evenly throughout the water column. However, two distinct limnetic populations existed in Ace Lake; an upper aerobic population dominated by algae and Protozoa and a lower anaerobic population dominated by bacterioplankton. Each lake stratum represented a distinct environment in terms of the ambient conditions that existed within it. In the mixolimnion there were relatively high levels of PAR and dissolved oxygen available, but temperatures were low and residual nutrients in short supply. Conversely, in the monimolimnion light and oxygen levels were low, but temperatures higher and inorganic nutrients and organic carbon relatively replete.

Competitive exclusion has led to the domination of species that employ strategies which allow them to compete successfully for the resources available in each stratum (Hardin, 1960). For the dominant phytoflagellate residing in the mixolimnion, Pyramimonas gelidicola, mixotrophy represented an adaptive nutritional strategy which promoted survival through the austral winter. The phytoflagellate photosynthesised during the summer months and then switched to phagotrophy during the winter darkness, precluding the energy expensive strategy of encystment/excystment (Hashimoto, 1963; Grimes, 1973), or the need to build up photosynthetically derived resources prior to growth and reproduction in the spring/summer. By maintaining an active winter population in this manner, P. gelidicola was photosynthetically poised and ready to take full advantage of the short Antarctic growing season as soon as favourable conditions for photosynthesis returned in the spring and summer. In contrast, Mesodinium rubrum, the dominant, autotrophic ciliate species in the mixolimnion, was successful by virtue of its reduced cryptophycean endosymbiont, which allowed it to build up endogenous food reserves during the summer to sustain active individuals throughout the winter. In addition, the population 'hedged its bets', a proportion of the population encysted and lay dormant during the winter months, ensuring the survival of the population in case these endogenous reserves were insufficient to maintain active cells until the resumption of the next growing season.

Due to the ice-cover on the lake wind-induced mixing was inhibited, therefore, any organism without the means to move or maintain their position in the water column would quickly sink into the sediments. In a system such as Ace Lake with an anoxic basin, this would result in cell death, thus, evolution has selected for motile micro-organisms. Both dominant autotrophs, *P. gelidicola* and *M. rubrum*, were highly motile and capable of migrating throughout the water column of Ace lake, exploiting the lake's varied conditions. Cells were able to move up to the surface waters to photosynthesise as well as make excursions into the monimolimnion to take advantage of the entrained nutrients.

In the bacterioplankton populations of the monimolimnion, cyanobacteria were extremely successful. These shade-adapted prokaryotes were able to photosynthesise efficiently at wavelengths not utilised by the phytoplankton residing in upper lake strata. They, therefore, competed successfully for PAR at a level in the lake where they also had full access to the high residual nutrients in the monimolimnion (Lampert & Sommer, 1997). In addition, cyanobacteria are known to possess buoyancy regulatory mechanisms which allowed them to maintain an optimum position within the water

column, albeit less dynamically than the dominant protozoan species in Ace lake (Konopka, 1984; 1989).

From the evidence it is clear that, contrary to popular belief, the microbial populations of Ace Lake, or indeed other Antarctic Lakes, are not required to 'shut-down' in order to survive during the austral winter. Instead they remain viable by virtue of their versatile stratagems. Indeed, the evidence presented in Chapters 3, 4 and 5 suggests that the micro-organisms in Ace Lake not only survive successfully throughout the winter, but in many instances sustain normal physiological and metabolic functions. Growth, grazing and production rates throughout the study were highest at what would appear to have been the optimum depths for cell growth and survival for each taxon, in terms of environmental conditions prevailing, reiterating the profound influence exerted by the lake's stratification. Phytoflagellate and ciliate abundance maxima were apparent at the thermocline/halocline, and bacterioplankton abundance maxima at the oxycline. In these zones, the micro-organisms could meet their requirements for light, nutrients and organic substrates, whilst benefiting from warmer temperatures and an abundance of their prey species. The measured growth, grazing and production rates of the microbial components of Ace Lake were often comparable to temperate systems in these optimal zones. Maintenance of relatively rapid winter growth rates and active populations at the optimum depths in Ace Lake also allowed the micro-organisms to take full advantage of the short summer growth period as soon as conditions within the lake improved.

Ace Lake is an oligotrophic system and, unlike Rookery Lake, it receives negligible allochthonous inputs of carbon and inorganic nutrients from its barren catchment. Thus, all of the carbon in Ace Lake is that derived autochthonously by the autotrophic production of the planktonic phytoplankton and benthic algal mats. Autotrophic production in Ace Lake was maximal during the summer months when PAR was high. The photosynthetic rates and efficiencies recorded were comparable to those documented for temperate systems in the optimal zones within the lake. Nevertheless, autotrophic production was constrained by the availability of inorganic nutrients and organic substrate, coupled with persistently low temperatures relative to lower latitude lacustrine systems. Photoinhibition also reduced the level of photosynthesis detected during open-water periods. The phenomenon was particularly noticeable in shallow, Rookery Lake (Chapter 6). When coupled with self-shading by the abundant populations of phytoflagellates, a midsummer decline in chlorophyll a was detected (Ellis-Evans, 1990).

However, the model of carbon flux presented in Chapter 7 illustrates the fact that, despite the abundance of autotrophic micro-organisms such as P. gelidicola and M.

rubrum, and the high photosynthetic rates and efficiencies, the level of autotrophic production taking place in Ace Lake was not sufficient alone to support the heterotrophic activity observed. Instead, heterotrophic production in Ace Lake was dependent upon a regenerative flux of nutrients and the slow turnover of autochthonous carbon, consistent with the concept of the microbial loop (Azam et al., 1991). This contention was corroborated by the high rate of bacterial production recorded in the oxycline during the winter when the inorganic nutrient concentrations were highest. Once again these production rates exceeded those published for other Antarctic lakes and temperate lacustrine ecosystems. These findings also highlighted the importance of resource, or "bottom-up", regulation for the microbial community. In all instances the availability of inorganic nutrients was of primary importance in the regulation of bacterial production, growth and abundance, as well as autotrophic production, and therefore, the regulation of higher trophic levels. The model of carbon flux (Chapter 7) showed clearly that "top-down" control was only important during the summer when the population of the calanoid copepod, Paralabidocera antarctica, reached its maximum abundance, in a similar manner as that described in lakes of the maritime Antarctic (Priddle et al., 1986; Laybourn-Parry et al., 1996).

#### 8.2: CONCLUSION

The project successfully achieved its twofold aims. Firstly, it collated a broad winter data set documenting the species composition, abundance, size and distribution patterns of the microbial components of Ace Lake, related these to the physico-chemical conditions persisting in the lake and highlighted the profound influence that the stratification of Ace Lake had on its micro-inhabitants.

Secondly, the project revealed that a meromictic system such as Ace Lake, which does not receive allochthonous carbon and nutrient inputs from its catchment, possesses a microbial plankton highly dependent on regenerative fluxes of inorganic nutrients and slow-turnover autochthonous carbon as described by the microbial loop concept. This was emphasised by the production of a simple model of carbon flux, the first of its kind for the water column of an Antarctic saline lake. The study also defined the trophic interactions occurring within Ace Lake, in particular an hitherto undocumented instance of mixotrophy in the phytoflagellate, *Pyramimonas gelidicola*. Although endemism does exist in Antarctic lacustrine ecosystems, it is relatively unimportant in most communities. Instead, Antarctic micro-organisms are generally robust, flexible survivors which employ versatile strategies that allow them to survive as well in the

winter darkness of Antarctic lakes as they do at lower latitudes, and precludes the previously assumed need for a physiological 'shut-down'.

### 8.3: FUTURE PERSPECTIVES

Despite recent advances in techniques and understanding there are numerous questions that remain unanswered and, with each revelation, new questions being posed. What are still required for all Antarctic lacustrine ecosystems are annual studies which take an holistic approach, considering all of the microbial components of these aquatic systems, both from a descriptive and ecophysiological perspective. This study has highlighted the need for further work in a number of specific areas:

1) The population dynamics of the unique, autotrophic ciliate, *Mesodinium rubrum* warrants further study. The two winter studies and summer work already carried out have revealed significant inter-annual variations in the number of cysts produced and the use of endogenous energy reserves to sustain active populations during the austral winter (Laybourn-Parry & Perriss, 1995; Perriss *et al.*, 1995; Gibson *et al.*, 1997). It would be extremely interesting to elucidate the behaviour of this ubiquitous species, especially in view of its known potential to form "red tides" in the oceans of the world (Lindholm, 1985; Crawford, 1989; Satoh & Watanabe, 1991).

2) In light of the highly pronounced influence that the stratification of Ace Lake exerts on the microbial dynamics of Ace Lake, and the physiological advantages bestowed on organisms able to dominate in the optimum zones of the lake, detailed studies of the planktonic organisms dwelling in thermocline/halocline and oxycline of Ace Lake are required to elucidate the precise variables to which they owe their success.

3) In the same manner, the work performed during this study only allowed for speculation as to the factors limiting cell growth and production in Antarctic lakes such as Ace Lake. Bioassays are required to say conclusively which of the environmental variables in Ace Lake limit autotrophic and heterotrophic production, in terms of inorganic nutrients in particular.

4) Photoinhibition and self-shading are known to limit autotrophic production during the summer, particularly in the shallow lacustrine systems such as Rookery Lake. Increasingly prolonged periods of ozone depletion over the Antarctic continent, and the increased the penetration of potentially harmful radiation, demands that the mechanism of this inhibitory process be investigated further. 5) Continued ecophysiological experimentation is required for the heterotrophic Protozoa and copepods in Ace lake to determine *in situ* growth and grazing rates and preclude the need to use literature values in the future.

6) Perhaps more importantly, the phenomenon of mixotrophy warrants further study. The observation of mixotrophy in *Pyramimonas gelidicola* was entirely serendipitous and one which requires elucidation and expansion. No other members of the genus *Pyramimonas* have been documented as mixotrophic until now, although numerous other Antarctic phytoflagellates are now known to be mixotrophic (Tranvik *et al.*, 1989; Roberts & Laybourn-Parry, 1999). Thus, there is the possibility that mixotrophy represents an adaptive strategy by which phytoflagellates survive in extreme aquatic habitats. Trans-latitude studies are required to determine the extent of mixotrophy in aquatic ecosystems and the importance of this behaviour, none the least because the existence of mixotrophy challenges the utility of current aquatic microbial food web models that do not account for mixotrophic behaviour.

7) Despite numerous palaeontological sediment studies in Ace Lake (Bird *et al.*, 1991; Fulford-Smith & Sikes, 1996; Volkman *et al.*, 1996), no taxonomic or process based work has been carried out on the meiofauna or microbial mats in the benthos. Such studies are essential to complete our knowledge of the carbon and nutrient fluxes taking place in Ace Lake, especially in view of the fact the autotrophic production taking place in the water column is insufficient alone to support the level of heterotrophic activity taking place within the lake.

8) No one has produced a complete taxonomic list of the species inhabiting Ace Lake and such knowledge is fundamental to furthering our understanding.

9) In terms of Antarctic lakes in general and the fragility of this pristine ecosystem, annual investigations of natural and human perturbations, such as the limited study presented for Rookery Lake, are of fundamental importance our understanding and the future preservation of one of the last true wildernesses on Earth.

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