Ecophysiology of the brine dinoflagellate,

Polarella glacialis,

and

Antarctic Fast Ice Brine Communities

by

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Abstract

Extremes in salinity and temperature and high levels of incident ultraviolet radiation (UVR) characterise the brine pockets and channels of upper Antarctic fast ice. Data on the composition and distribution of the microbial community inhabiting this environment is limited. Furthermore, how this community tolerates the immoderate physical and chemical parameters of the upper ice brine is poorly understood.

The microbial community in the Davis upper fast ice consists of cryo- and halotolerant autotrophic flagellates, a few diatoms, one ciliate species and several heterotrophic species. Small autotrophic dinoflagellates and chrysophytes dominate a community containing greater flagellate diversity than previously reported. A cryptomonad and two species of *Pyramimonas* are reported for the first time.

The abundant dinoflagellate of Davis fast ice, identified using molecular taxonomy, is *Polarella glacialis* Montresor *et al.* Observations of *P. glacialis* in this study from fast and pack ice brine samples along the East Antarctic coastline, with others from the literature, indicate that this species has a circumpolar distribution. The perfect match between the large subunit ribosomal RNA sequences of the Davis and McMurdo Sound *P. glacialis* strains suggests a single Antarctica population of this species.

The Davis fast ice brine community shows a remarkable resemblance to that of McMurdo Sound. The dominant taxa of the Davis ice, *P. glacialis* and the chrysophytes, are abundant in McMurdo Sound. These similarities point to at least a disparate distribution of this community throughout Antarctic fast ice. However, the probable circumpolar occurrence of *P. glacialis* and similar taxa from other coastal regions indicate a more continuous fast ice distribution.

For the first time, *P. glacialis* is described in terms of its pigments and lipids. The extremely high polyunsaturated fatty acid composition of this species (up to 76%) most likely enhances its tolerance of the cold brine environment. The sterol profile of *P. glacialis* is atypical of dinoflagellates and is dominated by the 4-desmethylsterol,

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27-nor-24-methylcholest-5,22E-3ß-ol. This sterol is rare in other dinoflagellates and unknown in other algal classes, suggesting its potential as a biomarker for *P. glacialis* in the environment. Sediment records of this biomarker may prove useful in determining past Antarctic climate change and fast ice extent.

The upper fast ice of Davis Station is characterised by extreme and transitory salinities and temperatures over the spring-summer transition. Salinities decrease from 96 to 2.5 psu whilst temperatures increase from -4.5 to -0.3 °C. These parameters and the relatively high UVR environment of the upper ice are identified as major ecophysiological stresses in the brine channels.

The osmoregulatory and cryoprotectant role of dimethylsulphoniopropionate (DMSP) in *P. glacialis* and the Davis fast ice community are investigated in the field and the laboratory. DMSP concentrations in the brine (450 to 230 nM) decrease in linear proportion to salinity and temperature, implicating DMSP as a possible osmolyte and cryoprotectant. In laboratory studies, *P. glacialis* cultures produce up to 47 fM DMSP cell^{-1}. However, under salinity upshock, no clear relationship between DMSP production and salinity is evident. Under the nitrogen replete culture conditions used here, DMSP is not considered a primary osmolyte in *P. glacialis*.

The effects of UV radiation (280 - 400 nm) on the brine community are assessed *in situ* using spectral perturbation methods. Under the conditions of this experiment, UV-B radiation (280 - 320 nm) inhibited chl *a* production within the upper ice whilst UV-A (320 - 400nm) appeared to have little effect. All UV radiation retarded the growth of *P. glacialis* asexual cells, however it is not possible to differentiate between the effects of UV-A and UV-B. DMSP concentrations within the brine are reduced under the influence of UV-A alone. UV absorbing compounds, with absorption spectra between 310 and 335 nm, are evident in the brine. As light perturbation did not show trends in the accumulation of these compounds, it is concluded that the UV absorbing compounds present are not photoinducible.

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Chapter 1 Introduction

Antarctic sea ice extent reaches its maximum coverage of 20 million km^2 during the austral spring and its minimum of 4 million km^2 in late summer (Zwally *et al.* 1983; Cosimo and Zwally 1984). Even at its minimum the remaining ice offers a vast habitat for sea ice algae. Sea ice algae are estimated to contribute between 20 - 50% of primary productivity in the Southern Ocean during spring (Voytek 1989; Legendre *et al.* 1992; Kirst and Wiencke 1995). Positioned at the base of the food chain, they support a large and diverse zooplankton community responsible for the maintenance of higher trophic levels (Knox 1994 p.64).

Three main types of algal communities (bottom, surface and interior assemblages) develop in sea-ice (Horner 1985a). To date, most work has focused on the community producing the highest biomass, the bottom ice assemblages. These studies range from species identification, community composition and biomass, productivity investigations, photosynthetic properties, ice incorporation mechanisms, phytoplankton seeding studies (reviewed in Horner 1985b; Garrison 1991; Knox 1994; Scott *et al.* 1994; McMinn 1996; McMinn and Ashworth 1998) through to the effects of enhanced ultraviolet B radiation (UV-B) (reviewed in Weiler and Penhale 1994; McMinn 1997; McMinn *et al.* in press).

Included within the interior communities are the brine channel assemblages (hereafter called brine communities) first alluded to in sea ice adjacent to Davis Station, East Antarctica (McConville and Wetherbee 1983). Brine communities occur in the brine pockets and channels of sea ice and are distinct from the interior band communities (Horner *et al.* 1988). Whilst the brine channel communities occur throughout the ice, this thesis concentrates on those in the upper 50 cm for ease of comparison with previous studies in the Ross Sea. In comparison to the bottom assemblages, only scant information exists on the brine communities.

In land fast sea ice, American researchers (Stoecker *et al.* 1992; 1993; 1997; 1998) have provided the most detailed study of brine communities to date. Their studies in

McMurdo Sound fast ice described a cold, hypersaline environment dominated by a photosynthetic dinoflagellate and chrysophyte statocysts distinct from the diatom dominated bottom communities. The brine dinoflagellate, newly described as *Polarella glacialis* Montresor *et al.* (Montresor *et al.* 1999), produces a spinose cyst also reported from pack ice (Garrison and Buck 1989; Garrison 1991; Buck *et al.* 1992; Garrison and Close 1993) and annual fast ice in other regions around Antarctica (McMinn and Hodgson 1993; McMinn 1996). The description of the harsh brine environment, the lack of knowledge of the ecophysiology of this community and the possible circumpolar distribution of the brine dinoflagellate lead to this thesis.

The extreme and dynamic nature of the brine channels offers a unique environment for studying the ecophysiology of sea ice algae. In the brine channels, extremely high salinities (>3 times that of seawater) and extremely low temperatures during spring transit quickly to almost fresh waters and higher temperatures (Chapter 3 this thesis; Stoecker *et al.* 1992; Stoecker *et al.* 1993; Stoecker *et al.* 1997). High levels of solar radiation and damaging UV-B penetrates the ice to irradiate a community essentially entrapped during springtime ozone depletion and long summer days. In comparison to the bottom ice assemblages where salinity, temperature and light vary relatively little, the brine channel environment is a challenging habitat for microalgae.

This thesis is an ecophysiological study of the upper ice brine communities in annual fast ice adjacent to Davis Station. It identifies the abundant dinoflagellate of the upper ice, investigates its pigments and lipids for specific biomarkers and addresses its Antarctic distribution. It describes the brine channel environment and community in Davis fast ice and compares findings with those in the Ross Sea. It attempts to answer how this community overcomes the osmotic and low temperature stresses of the high salinity, low temperature environment it survives in. In this regard, DMSP (dimethylsulphoniopropionate) production in the brine and its role as a possible osmolyte in a dinoflagellate isolated from this community was investigated. DMSP acts as an osmolyte and compatible solute in some micro- and macroalgae (reviewed in Kirst 1996) and is the precursor to DMS (dimethylsulphide). DMS is the most

abundant volatile biogenic sulphur compound produced in the marine environment and is vital in balancing the sulphur budget in the global sulphur cycle (Bates *et al.* 1992). DMS flux from the oceans is believed to ultimately affect global climate by providing cloud condensation nuclei (CCN) (Turnipseed and Ravishankara 1993). Previous DMSP/DMS investigations in Antarctic sea ice have been undertaken but most have been species non-specific studies, relating DMSP/DMS levels with chlorophyll *a* concentration.

The thesis then examines the effects of ozone hole enhanced UV-B radiation on the Davis fast ice brine community. A plethora of scientific research into the effects of UV-B on the Southern Ocean ecosystem followed the discovery of the ozone hole in 1985 by Farman *et al.* (1985). In relation to microalgae, most of this research focused on the phytoplankton and bottom ice assemblages. This is the first study that examines the effect of UV-B in the higher light climate of the upper ice brine community. The effects of UV-B are investigated in terms of species composition and succession, pigment and lipid content and DMSP production. A preliminary screening of the brine community for UV absorbing compounds is carried out.

The specific aims of this thesis are to:

1. Identify the abundant brine dinoflagellate in Davis fast ice, characterise it in terms of its pigment and lipid composition and determine its Antarctic distribution;

2. Determine the auto- and heterotrophic species composition of the Davis fast ice brine communities;

3. Compare the Davis community with other studies in the literature, with particular reference to the abundant dinoflagellate;

4. Investigate DMSP production in fast ice brine;

5. Investigate the role of DMSP as an osmolyte in *Polarella glacialis*;

6. Investigate the effects of UV-B on brine communities through species
   composition and succession, lipid analyses and DMSP production; and to

7. Conduct a preliminary screening of UV absorbing compounds in the upper ice
   community.

The importance and relevance of this work is several fold. Sea ice microbial
communities are believed to influence sea ice porosity and strength and contribute to
its melting and subsequent break out through the absorption of solar radiation
(McConville and Wetherbee 1983; Maykut 1985; Eicken *et al.* 1991). In turn, sea ice
cover in the Southern Ocean influences global ocean circulation and weather and
climate patterns (Horner 1985a; Maykut 1985; Garrison and Buck 1991).
Understanding the sea ice algal communities that undermine ice strength is clearly
important. Furthermore, little is known about the circumpolar distribution and
ecophysiology of the brine communities. Knowledge of DMSP production in the
brine and by the abundant dinoflagellate will contribute to answering how these
communities survive a hypersaline environment. Understanding the effects of UV-B
in this potentially high light environment will help predict possible future changes, if
any, in the productivity of the Antarctic region.

Chapter 2 identifies the abundant dinoflagellate in Davis fast ice as *Polarella
glacialis* and for the first time reports on its lipid and pigment composition and its
circumpolar distribution. Chapter 3 describes the brine environment in Davis fast ice
in terms of temperature, salinity, brine volume and vertical distribution of the
community in the sea ice. This chapter also describes the community composition
and compares the Davis community with those in the literature. Chapter 4
investigates DMSP production in the brine and in clonal cultures of *Polarella
glacialis* and examines the possible biological roles of DMSP. Chapter 5 examines
the effects of UV-B on the brine community and reports on the production of UV
absorbing compounds. Chapter 6 concludes this thesis with a summary of the major
findings.

Chapter 2 Molecular taxonomy, pigment and lipid composition and Antarctic distribution of the brine dinoflagellate, *Polarella glacialis*

2.0   Introduction

Polarella glacialis Montresor *et al.* is a recently described gymnodinioid dinoflagellate isolated from McMurdo Sound fast ice (1991; 1996) where it can reach densities of > 10^6 cells l^{-1} of ice (Stoecker *et al.* 1997). Based on plate tabulation of the vegetative cell, Montresor *et al.* (1999) established the new genus and species, *Polarella glacialis,* and placed it within the family Suessiaceae, order Suessiales (Fensome *et al.* 1993). Phylogenetic analysis, using small subunit ribosomal RNA (SSU rRNA), confirmed this placement and a close relationship with the genus *Symbiodinium* Freudenthal, family Symbiodiniaceae, a group of symbiotic dinoflagellates in marine invertebrates. *P. glacialis* and *Symbiodinium* spp. represent the only extant members of the Suessiales (Montresor *et al.* 1999).

The excystment, growth and population dynamics of *P. glacialis* in McMurdo Sound fast ice are now well established (Stoecker *et al.* 1992; 1997; 1998). However, the Antarctic distribution of this new species and its pigment and lipid composition are unknown. A large population of unidentified, small, motile dinoflagellates have been recorded near Syowa Station (Hoshiai 1977) and a spinose resting cyst (Buck *et al.* 1992; Montresor *et al.* 1999) similar to that of *P. glacialis* has been noted near Davis Station (McMinn and Hodgson 1993; McMinn 1996). In pack ice, cysts have been reported from the Weddell and Scotia Seas (Garrison and Buck 1989; Buck *et al.* 1992).

This chapter extends the known distribution of *P. glacialis* in Antarctic fast and pack ice. The brine dinoflagellate from Davis Station fast ice is confirmed as this species through comparison of large subunit (LSU) rRNA with that of culture on which Montressor et al. (1999) based their original description. Examination of additional

fast ice samples from Mawson and Casey Stations, the Larsemann Hills, and several pack ice sites extends our understanding of this distribution.

This chapter reports for the first time the photosynthetic pigment and lipid composition of *P. glacialis*. Pigments have been used as a chemotaxonomic tool in identifying algal classes in oceans (Everitt *et al.* 1990; Williams and Claustre 1991; Mackey *et al.* 1996; Wright *et al.* 1996; Peekin 1997) and show potential in the detection of toxic dinoflagellate blooms (Millie *et al.* 1997). Dinoflagellates typically have chlorophylls *a* (chl *a*) and c_2 (chl c_2) and peridinin and diadinoxanthin as the dominant carotenoids. Other carotenoids in dinoflagellates include β,β carotene, diatoxanthin, dinoxanthin, P-457 and P-468, peridinol and pyrrhoxanthin (Jeffrey *et al.* 1997). Characterising the pigments of *P. glacialis* will be of use in future studies examining its presence in sea ice.

Lipids of microalgae have also been used as chemotaxonomic markers (biomarkers) in identifying algal classes (reviewed in Volkman *et al.* 1998) and in identifying trophic links between ice algae and pelagic zooplankton (Scott *et al.* 1999). Fatty acids and sterols are useful tools in identifying species in the water column (Hallegraeff *et al.* 1991), determining sources of organic matter in sediments (Volkman *et al.* 1990; Volkman *et al.* 1993; Volkman *et al.* 1998), and as evidence for reassigning species to a different class (Jones *et al.* 1983). Furthermore, fatty acids and sterols may be useful in identifying genera within an algal class (Withers 1987).

Dinoflagellate fatty acids are typically high in polyunsaturated fatty acids (PUFAs), with the major fatty acids being 16:0, 18:4 (n-3), 18:5 (n-3), 20:5 (n-3) and 22:6 (n-3) (Holz 1981; Nichols *et al.* 1984).

Dinoflagellate sterols also show some promise as biomarkers for this algal class. Most dinoflagellates are typified by a high composition of 4α-methyl sterols (rare in other algal classes), minor proportions of 4-desmethylsterols and a high proportion of stanols (Withers 1987; Volkman *et al.* 1998; Mansour *et al.* 1999). Dinosterol (4α,23,24-trimethyl-5α-cholest-22E-en-3ß-ol) is often the most abundant of the 4α-methyl sterols (Shimizu *et al.* 1976; Alam *et al.* 1979; Kokke *et al.* 1981; Nichols *et*

al. 1984; Withers 1987; Harvey *et al.* 1988; Piretti *et al.* 1997) and has been regarded as a biochemical marker for dinoflagellates in sediments (Boon *et al.* 1979; Robinson *et al.* 1984). The sterols of *P. glacialis* described in this chapter confirm the placement of this species within the Dinophyceae and are used to examine the occurrence of specific biomarkers for *P. glacialis* in sea ice, the water column and in sediments.

2.1 Methods and Materials

2.1.1 Isolation and culture techniques

Fresh brine samples were collected from the upper fast ice adjacent to Davis Station by collecting brine accumulated in holes drilled to 50 cm depth. Sample portions were transferred to 150 ml culture flasks, enriched with f/2 growth medium and incubated at 100 µE m^{-2} s^{-1} and 4 °C. To minimise encystment of the dinoflagellates, the mixed cultures were regularly sub-cultured into fresh, f/2 medium at 35 psu.

Isolation of single cells was carried out using the micropipette method and axenic techniques at 6 °C. Motile dinoflagellate cells were removed from the mixed cultures using micropipettes and successively isolated and washed in a series of fresh, sterile f/2 medium droplets. Solitary cells were then placed in a sterile, well petri dish in a small volume of fresh, sterile 35 psu f/2 medium. Isolated cells were incubated at 100 µE m^{-2} s^{-1} and 4 °C. After one month of incubation, the wells were examined for growth using a Zeiss inverted microscope.

Subsequent clonal cultures were then transferred using axenic techniques into 150 ml culture flasks containing 20 ml of f/2 medium. This process was continued, using progressively larger inoculation volumes, until 200 ml cultures were established. Culture conditions were maintained at those previously described and regular sub-cultures were made.

CCMP 1383, a clonal culture of *P. glacialis* established from the upper fast ice of McMurdo Sound, was obtained from Bigelow Laboratory for Ocean Sciences, West Boothbay Habor, ME, USA. On arrival, this culture was allowed to equilibrate to 4 °C over 1 day in a growth cabinet before axenic inoculation into several 150 ml

culture flasks containing fresh, sterile f/2 medium. The CCMP 1383 cultures were
then grown in a similar manner as described for the Davis dinoflagellate.

2.1.2 Large subunit rRNA extraction, PCR conditions and sequencing

LSU rRNA was extracted from CCMP 1383 and FL1B, a Davis clonal culture, at the
School of Plant Science, University of Tasmania in collaboration with Dr. Chris
Bolch.

Ten ml of late exponential phase cultures were harvested by centrifugation at 2000 g
for 5 min and the growth medium was decanted away. The cell pellet and residual
medium were transferred to a 1.5 ml plastic centrifuge vial, repelleted and excess
growth medium carefully removed. DNA extraction was carried out using the "gentle
lysis" and phenol:chloroform:isoamyl alcohol extraction method (Scholin *et al.* 1994)
modified as described by Bolch *et al.* (1999). The conserved regions and intervening
variable domains of the LSU rRNA gene were amplified with the PCR (polymerase
chain reaction) primers of Scholin *et al.*(1994) and Daugbjerg (unpublished) (D1R
and D3B, 28-860F and 28-1973R), using the PCR conditions described in Bolch *et al.*
(1999).

Completed PCR reactions were checked for successful amplification and non-specific
PCR products by electrophoresis of 10 µl of product through 1% agarose/TBE gels.
PCR buffer and unincorporated primers and deoxynucleotides (dNTPs) were
removed from the remaining product by ultrafiltration using regenerated cellulose
fibre centrifuge columns (30,000 NMWL UltraFree-® MC, Millipore, Bedford, MA,
USA) according to the manufacturers instructions. Samples were resuspended in
double distilled water and frozen until sequencing could be carried out.

Both strands of PCR product were sequenced in separate reactions using either
forward or reverse amplification primers (Bolch *et al.* 1999). Sequence data were
checked by manual inspection of the electropherograms of both forward and reverse
sequences and ambiguous calls. Base-call conflicts were resolved by comparison of
both strands using the program Sequence Navigator 1.0.1 (PE Applied Biosystems,
USA) and a consensus sequence established. Completed sequences were aligned
using the Clustal alignment option of Sequence Navigator, using the default settings

for gap inclusion and extension. Resulting alignments were checked visually and alignment errors corrected manually.

2.1.3 Photosynthetic pigments and carotenoids

FL1B and CCMP 1383 were analysed for pigment identification and composition. 10 ml of each culture (late exponential phase for FL1B; stationary phase for *P. glacialis* CCMP 1383) were filtered through 13 mm GF/F filters for pigment extraction. Another 10 ml were fixed with Lugol's Iodine and enumerated after settling 2 – 5 ml in a 10 ml settling chamber. Triplicate counts of 15 fields of view at x40 magnification were made using a Ziess Televal 31 inverted microscope. Standard error for the triplicate counts ranged between 2 – 11.9%.

Each filter was extracted within one hour of filtering in 1.8 ml of MeOH to which 100 µl of apo-8'-β-carotenal (Fluka) (internal standard pigment at 176 ng/25 µl MeOH) was added. Filters were sonicated for 1 minutes at 100 watts using a Braun Labsonic 1510 sonicator. The extract was then filtered and pigments identified through HPLC (High Performance Liquid Chromatography) using the methods of Wright *et al.* (1991; 1996) using a Waters 626 LC quaternary pump and Millenium 32 (ver. 3.05.01) software. The method of Zapata *et al.* (submitted) was used to identify the chlorophyll *c* peak and to confirm pigment identifications from the initial HPLC runs.

2.1.4 Lipid and carbon analysis

Duplicate cultures of exponential phase FL1B were analysed for lipid classes, fatty acids and sterols. Fifty ml of each culture of the Davis dinoflagellate were filtered through 13 mm GF/F filters and subsamples taken for carbon analysis at the Central Science Laboratory, University of Tasmania. Ten ml of each duplicate were fixed and enumerated as previously described, with standard errors for the counts ranging between 2 – 11.9%.

Lipids were extracted using the modified one phase $CHCl_3$-MeOH-H_2O Bligh and Dyer method (Bligh and Dyer 1959). After phase separation the extracted lipid was recovered from the lower $CHCl_3$ layer, concentrated by rotary evaporation at 40 °C to

dryness and the sample made up to 100 μl in CHCl$_3$. This total lipid extract was stored under N$_2$ at –20°C.

Lipid classes were resolved by thin layer chromatography (TLC) in conjunction with a flame ionisation detector (FID) using an Iatroscan Mk V TH-10 TLC-FID analyser (Volkman *et al.* 1989; Volkman and Nichols 1991). Peak areas of the individual classes were quantified using DAPA chromatography software. The solvent system used was hexane-diethyl ether-acetic acid (60:17:0.2 v/v/v).

The sterol and fatty acid fractions were obtained through saponification of 50% of the total lipid extract. The total lipid extract was blown down under N$_2$ gas to dryness, 3 ml of a solution of 5% KOH in methanol/water (80/20 v/v) were added and the sample heated at 80 °C for 2 hours. After cooling, sterols were separated by the addition of 1 ml of Milli Q water and 2 ml of pre-extracted 4:1 hexane (C$_6$H$_{14}$):CHCl$_3$, centrifugation and the removal of the upper layer. This process was repeated 3 times to maximise sterol recovery. Sterols were converted to trimethyl silyl (TMS) ethers by reaction with bis(trimethylsilyl)trifluoroacetamide) (BSTFA) at 50 °C overnight. The excess BSTFA was blown down under N$_2$ gas and the sterol fraction made up to 50 μl.

The fatty acid fraction was treated by acidification of the lower layer of the saponified extract to ≤ pH 2 and addition of 3 ml of 4:1 C$_6$H$_{14}$:CHCl$_3$. This fraction was reduced to dryness under N$_2$ gas and treated with MeOH-HCl-CHCl$_3$ (10:1:1) for conversion to fatty acid methyl esters (FAMES) at 80 °C for 2 hours. The FAMES were extracted after the addition of Milli Q water and 4:1 C$_6$H$_{14}$:CHCl$_3$, centrifugation and recovery of the top layer. The addition of 4:1 C$_6$H$_{14}$:CHCl$_3$ and removal of the top layer was repeated three times to maximise FAME recovery.

Gas chromatographic (GC) analysis of the FAMES was performed with a 50 m x 0.32 mm id HP1 cross-linked methyl silicone fused silica capillary column. The GC was fitted with a flame ionisation detector (FID) set at 310 °C and a purged split/splitless injector set at 290 °C. Samples were injected using a Hewlett Packard 7673A autosampler and the oven was programmed from 50 to 150 °C at 30 °C/min, 2 °C/min to 250 °C and 5 °C/min to 300 °C.

Fatty acid identifications were made through comparison of retention times of laboratory standards and using mass spectral data gathered by gas chromatography-mass spectrometry (GC-MS). GC-MS was performed using a Fisons MS800 system. The GC was set at an initial injector temperature of 280 °C. Samples were injected at 50 °C onto a 50 m x 0.2 mm HP1 column. After 1 min the oven temperature was raised to 180 °C at 45 °C/min, 250 °C at 2 °C/min and 300 °C at 5 °C/min. Mass spectrometer operating conditions were electron impact energy 70 eV, transfer line 310 °C, source temperature 250°C, 0.8 scan sec^{-1}, and mass range 40 to 650 daltons.

Sterol identifications were confirmed by comparison of retention times with those of authentic laboratory standards, by the calculation of relative retention times (RRT), and from mass spectral data. GC-MS analysis was performed using a HP 5890 GC and 5790B MSD using an open split interface with helium as the carrier gas (4 ml min^{-1}). One μl samples were injected at 260 °C by splitless injection and the detector temperature was ramped from 60 – 230 °C at 30 °C min^{-1} and to 290 °C at 5 °C min^{-1}. MSD conditions were as previously described. Stereochemistry of the sterols was not determined.

2.1.5 Antarctic distribution of *P.glacialis*

Apart from areas sampled near Davis Station, brine samples of opportunity were collected from several other fast and pack ice sites in 1996 and 1997 (Table 2.0). Additional sites in the pack ice of the East Antarctic sector and one fast ice site adjacent to the Larsemann Hills was sampled by the author. Ms. Vanessa Noble collected samples from Mawson Station fast ice in late November 1996 whilst Mr. Paul Goldsworthy collected brine samples from Casey Station fast ice in November 1997. These brine samples were collected as described in Chapter 3 and fixed with Lugol's Iodine. The samples were concentrated by settling (see Chapter 3) and examined for the presence of *P. glacialis* cysts and asexual cells using a Televal 31 inverted microscope.

Table 2.0 Samples of opportunity collected in fast and pack ice

locations in East Antarctica

Date collected	Fast ice	Pack ice
20 December 1996	Horseshoe Harbour, Mawson Station 67°36'S 62°53'E	
5 October 1997		64°51.79'S 109°37.32'E
13 October 1997		63°04.41'S 92°47.53'E
18 October 1997		62°56.87'S 92°58.00'E
23 October 1997		63°46.76'S 74°10.49'E
26 November 1997	Browns Bay, Casey Station 66°17'S 110°32'E	
16 November 1997	Nella Fjord, Larseman Hills 69°23'S 76°23'E	

2.2   Results

2.2.1 Davis dinoflagellate identification

LSU rRNA sequences from FL1B and CCMP1383 are shown in Table 2.1. Almost the entire LSU rRNA gene ($\approx$ 2000 base pairs (bp)) was successfully sequenced from FL1B. From CCMP 1383, only the 28-860F and 28-1973R regions were successfully amplified and sequenced, giving a total of 729 bp. The CCMP 1383 sequence was identical to the same region in FL1B. Faulty D1R and D3B primers appeared responsible for the poor amplification of this region.

Table 2.1 Large subunit rRNA sequence alignment between FL1B (Davis dinoflagellate) and CCMP 1383 (*Polarella glacialis*) clonal cultures

```
             10        20        30        40        50        60
FL1B  GAAGCAAGCGGAGGTAACAAGGAACTAAATAGGATTCCCTTAGTAATGGCGAACGAACAG
CCMP  ------------------------------------------------------------
             70        80        90        100       110       120
FL1B  GGATGAGCTCGGCTTGGAAACTGGGGCCTTGGCTCTGGATTGTAGCCTCGAGACATATTG
CCMP  ------------------------------------------------------------
             130       140       150       160       170       180
FL1B  CCAACGGAGGCGCAGGTGTAAGCCTCTTGGAAAAGAGCATCAACGTGGGTGAGAATCCTG
CCMP  ------------------------------------------------------------
             190       200       210       220       230       240
FL1B  TGTTTCATCTGCAGTCCACCGTGCACGGCACATGTTCTCAGAGTCGCGTTCCTCGGAATT
CCMP  ------------------------------------------------------------
             250       260       270       280       290       300
FL1B  GGAGCGTAAAGTGGGTGGTAAATTTCATCTAAAGCTAAATATAGGCTTGAGACCGATAGC
CCMP  ------------------------------------------------------------
             310       320       330       340       350       360
FL1B  AAACAAGTACCATGAGGGAAAGATGAAAAGGACTTTGAAAAGAGAGTTAAAAGTGCTTGA
CCMP  ------------------------------------------------------------
             370       380       390       400       410       420
FL1B  AATCGCTGAAAGGGAAGCGAATGGAACCAGTCCTCTGGCTGAGATTGTTGCATGCTCCTG
CCMP  ------------------------------------------------------------
             430       440       450       460       470       480
FL1B  TGATACCTGGCTGCTTCAGCGCAAGCGTTGCAGTTGGTGTTGAGCGGCTGTGTGTGGTGA
CCMP  ------------------------------------------------------------
             490       500       510       520       530       540
FL1B  CTCTCAGCTTGTTAGTCAACCGCTGTTCATACTTGAGGAAAGCCCCAGGTTCATGATTCT
CCMP  ------------------------------------------------------------
             550       560       570       580       590       600
FL1B  TCTCTGTGGAGTCGGTCTTGTGCTTGTGGGGACCCAGGTGTGTACAGTTTCCTGAGTGTC
CCMP  ------------------------------------------------------------

             610       620       630       640       650       660
FL1B  TGGTGCTAACGCCTCGTGATGCTCTGTGTGGGTGTTGCTTTTCCAAGCTTCGCTCGCTCG
CCMP  ------------------------------------------------------------
             670       680       690       700       710       720
FL1B  GGTGTCCTCGGTGATCTCCTGACATACAGTGGTGACGAAATGGTTCTCTTCGACCCGTCT
CCMP  ------------------------------------------------------------
             730       740       750       760       770       780
FL1B  TGAAACACGGACCAAGGAGTCTAGCACATGTGCGAGTTCACAGGTGGTAAGCCTGCTTGC
CCMP  ------------------------------------------------------------
             790       800       810       820       830       840
FL1B  GCAATGAAGGTGACAGCTGGGATTCTTGCACCAGCAACCGACCAATCAATTGAGAGAAGT
CCMP  ------------------------------------------------------------
             850       860       870       880       890       900
FL1B  TTGAGTATGAGCATATGTGTTAGGACCCGAAAGATGGTGAACTATGCCTGCGAAGGGCAA
CCMP  ------------------------------------------------------------
             910       920       930       940       950       960
FL1B  AGTCAGGGGAAACTCTGATGGAGGCTCGTAGCGGTACTGACGTGCAAATCGTTCGTCATA
CCMP  ------------------------------------------------------------
```

```
              970       980       990       1000      1010      1020
FL1B  CGTGGGTATAGGGGCGAAAGACTAATCGAACCATCTAGTAGCTGGTTCCCTCCGAAGTTT
CCMP  ------------------------------------------------------------
              1030      1040      1050      1060      1070      1080
FL1B  CCCTCAGGATAGCTGGAGTTGCACAGTTTTATCAGGTAAAGCGAATGATTAGAGGAATCG
CCMP  ------------------------------------------------------------
              1090      1100      1110      1120      1130      1140
FL1B  GGGACGCGTTGTCCTCGACCTATTCTCAAACTTTAAATGGGTAAGATCCAGTGGTTACTT
CCMP  ------------------------------------------------------------
              1150      1160      1170      1180      1190      1200
FL1B  CATTGAACTTCTGGGATAAATGACAACTCTAAGTGGGCCGTTTTTGGTAAGCAGAACTGG
CCMP  ------------------------------------------------------------
              1210      1220      1230      1240      1250      1260
FL1B  CGATGAGGGATGAACCTAACGTTGGGCTAAGGTGCTTAACTGCACGCTCATCAGATACCA
CCMP  ------------------------------AGGTGCTTAACTGCACGCTCATCAGATACCA
              1270      1280      1290      1300      1310      1320
FL1B  CAAAGGGTGTTGGTTCATTTTAACAGCAGGACGGTGGTCATGGAAGTCGAAATCCGCTAA
CCMP  CAAAGGGTGTTGGTTCATTTTAACAGCAGGACGGTGGTCATGGAAGTCGAAATCCGCTAA
              1330      1340      1350      1360      1370      1380
FL1B  GGAGTGTGTAACAACTCACCTGCCGAATGGACTAGCCCCGAAAATGGATGGCGCTCAAGC
CCMP  GGAGTGTGTAACAACTCACCTGCCGAATGGACTAGCCCCGAAAATGGATGGCGCTCAAGC
              1390      1400      1410      1420      1430      1440
FL1B  GGGTGACCGATGCCCAACCATTGTTGCAAATGCTGCATCAATGAGTAGGAGGGCGCGGAG
CCMP  GGGTGACCGATGCCCAACCATTGTTGCAAATGCTGCATCAATGAGTAGGAGGGCGCGGAG
              1450      1460      1470      1480      1490      1500
FL1B  GTTGTGATGAAGCTCTTGGCGACAGCCTGGGTGAAACAGCCTCTGGTGCAGATCTTGGTG
CCMP  GTTGTGATGAAGCTCTTGGCGACAGCCTGGGTGAAACAGCCTCTGGTGCAGATCTTGGTG
              1510      1520      1530      1540      1550      1560
FL1B  GTAGTAGCAAATATTCAAATGAGAACTTTGAAGGCCGAAGTGGAGAAGGGTTCCATGTGA
CCMP  GTAGTAGCAAATATTCAAATGAGAACTTTGAAGGCCGAAGTGGAGAAGGGTTCCATGTGA
              1570      1580      1590      1600      1610      1620
FL1B  ACAGCAATTGGACATGGGTTAGTCGATCCTAAGAGACAGGGATGTTCCGCACATGCAAGC
CCMP  ACAGCAATTGGACATGGGTTAGTCGATCCTAAGAGACAGGGATGTTCCGCACATGCAAGC
              1630      1640      1650      1660      1670      1680
FL1B  TTTGCTTCGTCTCTCGAAAGGGAATGGGGTTAACATCCCCCAACCAGGACGTGGATACTG
CCMP  TTTGCTTCGTCTCTCGAAAGGGAATGGGGTTAACATCCCCCAACCAGGACGTGGATACTG
              1690      1700      1710      1720      1730      1740
FL1B  TACGGCAACGTAAGAGAATTCGGCGACGCAGGGGGGGGCCCTGGGAAGCGTTGTCTTTTC
CCMP  TACGGCAACGTAAGAGAATTCGGCGACGCAGGGGGGGGCCCTGGGAAGCGTTGTCTTTTC
              1750      1760      1770      1780      1790      1800
FL1B  TTTTTAACCGCCTTTTACCCTGGAATCAGATTGCCTGGAGATAGGGTTAAAGAGCGGGTA
CCMP  TTTTTAACCGCCTTTTACCCTGGAATCAGATTGCCTGGAGATAGGGTTAAAGAGCGGGTA
              1810      1820      1830      1840      1850      1860
FL1B  AAGCACCTTGTCTTCCGAGGTGTCCGGTGCGCTCTCAACTGCCCTTGAAAAGCCGAAGGA
CCMP  AAGCACCTTGTCTTCCGAGGTGTCCGGTGCGCTCTCAACTGCCCTTGAAAAGCCGAAGGA
              1870      1880      1890      1900      1910      1920
FL1B  CAGACTTATTCACACCCCTGGTCGTACCCATAACCGCATCAGGTCTCCAAGGTTTGTAGC
CCMP  CAGACTTATTCACACCCCTGGTCGTACCCATAACCGCATCAGGTCTCCAAGGTTTGTAGC

              1930      1940      1950      1960      1970      1980
FL1B  CTCTGGTCGATAGAACAAAGTAGGTAAGGGAAGTCGGCAAAATAGATCAAGTATCAATCA
CCMP  CTCTGGTCGATAGAACAAAGTAGGTAAGGGAAGTCGGC----------------------

FL1B  AAGGTACA
CCMP  --------
```

2.2.2 Photosynthetic pigments

Photosynthetic pigments and carotenoids of both cultures were resolved as P457, peak 2 (an unknown pigment), chl c_2, peridinin, cis-peridinin, dinoxanthin, diadinoxanthin, cis-diadinoxanthin, chl a and β,β-carotene (Figure 2.0). Minor amounts of chl a breakdown products (peak a, chlorophyllide a (chlide a) and peak c, epimer) were also identified. The percent contribution of these products were combined under chl a, contributing 18.0% of the total.
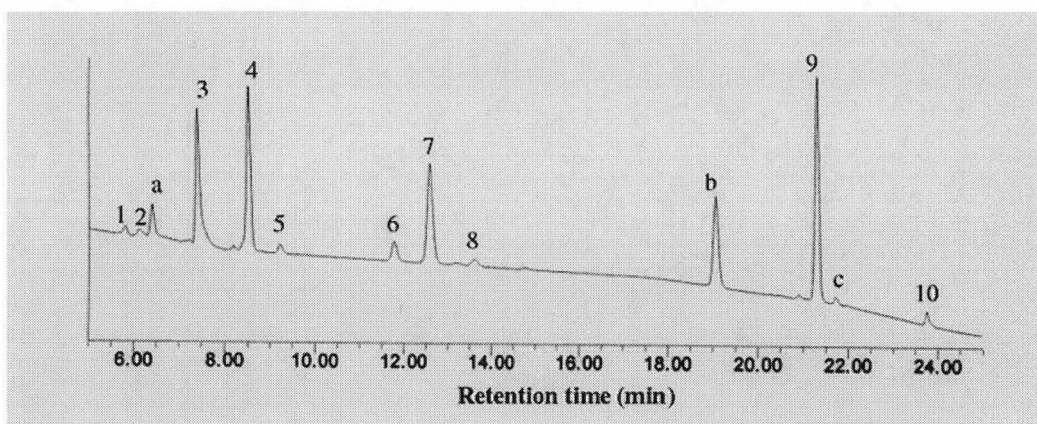
Figure 2.0 High performance liquid chromatogram of photosynthetic pigments in CCMP 1383 (*Polarella glacialis*) (peak numbers refer to pigments in Table 2.2; a = chlide *a*, b = internal standard (apo 8), c = epimer)

Both cultures showed identical pigment profiles (Table 2.2), with the major pigments being chl a, peridinin, diadinoxanthin and chl c_2. However, variation in the percent composition of chl a and peridinin occurred between the cultures. CCMP 1383 contained 51.2% and 24.4 % chl a and peridinin respectively whilst FL1B contained less chl a (44.3%) and a higher composition of peridinin (31.1%). CCMP 1383 had over twice as much chl a per cell (12.5 pg cell^{-1}) than FL1B (5.2 pg cell^{-1}) whilst both cultures showed similar chl a:chl c ratios (7.73, FL1B and 8.45, CCMP 1383). The peridinin:chl a ratios were 0.7 in FL1B and 0.48 in CCMP 1383.

At the time of harvesting, a notable difference in cell size between the cultures was evident. FL1B showed little variation in cell size, with cells ranging between 12 – 15 μm in length. CCMP 1383 showed greater variation, with approximately one third of

the cells having a larger cell length of between 15 – 20 μm. A proportion of these cells were identified as planozygotes by the presence of two longitudinal flagellae.

Table 2.2 Photosynthetic pigment content and composition (% of total pigment) of the cultures FL1B (Davis dinoflagellate) and CCMP 1383 (*Polarella glacialis*) (peak numbers refer to Figure 2.0)

		FL1B (Davis dinoflagellate)	CCMP 1383 (*Polarella glacialis*)
	Cells ml^{-1}	53580	17503
	Total chl a (μg ml^{-1})	0.28	0.22
	Chl a (pg cell^{-1})	5.2	12.5
	Chl a:chl c ratio	7.73	8.45
	Peridinin:chl a ratio	0.70	0.48
Peak no	**Pigments**	**Percent Composition**	
	Chlorophylls		
9	Chlorophyll a (chl a)	44.3	51.2
3	Chlorophyll c(chl c_2)	5.7	6.1
	Carotenoids		
1	P457	1.4	1.1
2	Unkown	0.6	0.6
4	Peridinin	31.1	24.4
5	Cis–peridinin	1.1	1.3
6	Dinoxanthin	2.4	2.1
7	Diadinoxanthin	10.9	11.0
8	Cis–diadinoxanthin	0.8	0.9
10	β-Carotene	1.6	1.3

2.2.3 Lipid composition of FL1B

The duplicate cultures varied in cell density (16084 - 32476 cells ml^{-1}) and lipid per cell (5.1 - 12.2 ng cell^{-1}, Table 2.3). Sterol content ranged from 0.3 to 0.5 ng cell^{-1} whilst carbon per cell ranged between 13.4 - 26.5 pg cell^{-1}. In these cultures, chl a content ranged between 1.3 - 2.7 pg cell^{-1}.

Lipid classes

The percent composition of polar lipids (81 - 91%), sterols (3.6 - 4.9%) and hydrocarbons (2.1 - 2.2%) showed little variation between replicate FL1B cultures (Table 2.4). Considerable variation was apparent, however, in the triglyceride (1.5 - 10.7%) and free fatty acid content (0.7 - 2.3%) of the replicates.

Table 2.3 Lipid concentration in duplicate *Polarella glacialis* cultures

Replicate	# 1	# 2
Mean cells ml^{-1} (s.d)	32476 (2529)	16084 (620)
Total lipid (μg l^{-1})	4273	5072
Total sterols (μg l^{-1})	210	183
Total chl a (μg l^{-1})	42.8	43.6
Total carbon (mg l^{-1})	20.4	20.1
Lipid (pg cell^{-1})	132	315
Sterol (pg cell^{-1})	6.5	11.4
Chl a (pg cell^{-1})	1.3	2.7
Carbon (pg cell^{-1})	628	1248

Table 2.4 Lipid class composition in duplicate cultures of *Polarella glacialis*

Lipid classes	Percent composition	
	# 1	# 2
Hydrocarbon	2.1	2.2
Triglyceride	1.5	10.7
Free fatty acid	0.7	2.3
Sterol	4.9	3.6
Polar lipid	90.8	81.2

Fatty acids

FL1B contained fatty acids ranging between carbon chain lengths of C_{14} to C_{22} (Table 2.5). Polyunsaturated fatty acids (PUFAs) were the most abundant, contributing up to 76.3% of the total fatty acids over the saturated (up to 16.6%) and monounsaturated (up to 8.6%) components.

The major fatty acids were 18:5 (n-3) (up to 49%) and 22:6 (n-3) (up to 19%), contributing almost 70% of the total fatty acids. 14:0, 16:0 and 20:5 (n-3) each

contributed approximately 8%. 18:4 (n-3) was not detected in either replicate.

Branched chain fatty acids (i15:0 and a15:0) accounted for less than 1% of the total.

Table 2.5 Fatty acid composition in duplicate FL1B cultures (fatty

acids are designated x:y (n-z) where x is the number of carbon atoms, y is the

number of double bonds and z is the position of the ultimate double bond from the

terminal methyl group; i = iso: a = anteiso).

Fatty Acid	Percent Composition	
Replicate	#1	#2
Saturates		
14:0	6.9	7.5
i15:0	0.2	0.1
a15:0	0.2	0.2
15:0	0.4	0.3
16:0	7.8	7.5
18:0	0.8	1.0
Sum	16.3	16.6
Monounsaturates		
16:1 (n-7)	1.7	1.2
16:1 (n-5)	0.1	0.1
18:1 (n-9)	4.9	7.1
18:1 (n-7)	0.4	0.2
Sum	7.1	8.6
Polyunsaturates		
18:5 (n-3)	49.4	43.4
18:3 (n-6)	0.4	0.3
18:2 (n-6)	1.8	3.0
20:5 (n-3)	7.7	8.2
22:6 (n-3)	16.9	19.3
22:5 (n-3)	0.1	0.2
Sum	76.3	74.4
Other	0.3	0.4
Total	100.0	100

Two unknown compounds, eluting between 18:0 and 20:5 (n-3), contributed up to

0.4% of the fatty acids. Another unknown compound eluted prior to 18:0 but was not

included in the percent composition calculations. The mass spectrum of this

compound (Figure 2.2) indicated it was possibly a C_{19} or branched chain C_{20} fatty

acid but was not conclusive. If included in the percentage composition calculations,

this compound would have contributed significantly (up to 16%) to the fatty acid composition.
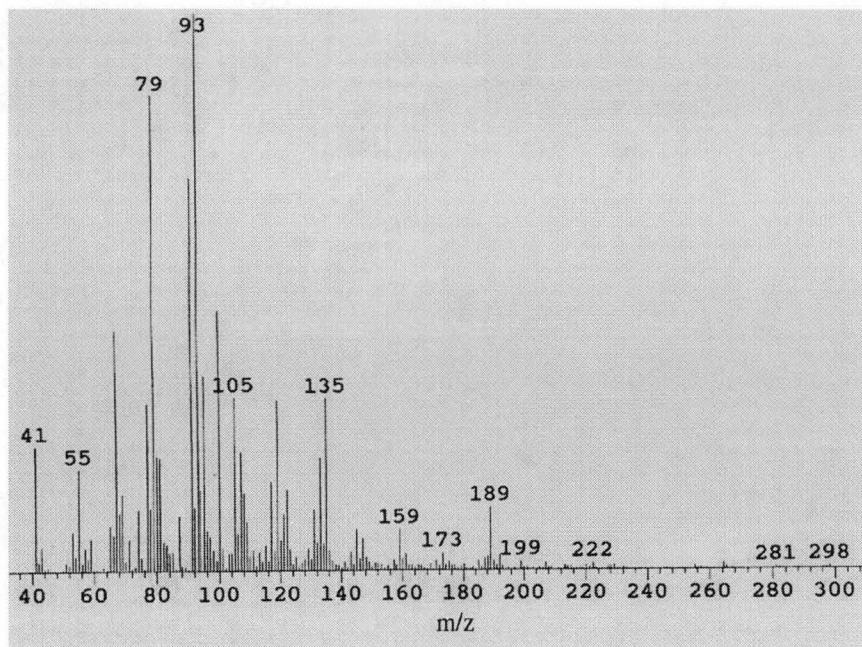
Figure 2.2 Mass spectrum of unknown compound (as a methyl ester) in Davis dinoflagellate culture FL1B

Sterols

Sterols of FL1B consisted of 4-desmethylsterols, 4α-methyl sterols and stanols (Figure 2.3, Table 2.6). 4-Desmethylsterols contributed up to 78.5% of the total sterols, with 27-nor-24-methylcholesta-5,22E-dien-3ß-ol the most abundant sterol (up to 64.2%). Other 4-desmethylsterols included 24-methylcholesta-5,22E-dien-3ß-ol (up to 7.5%, brassicasterol/crinosterol), 23,24-dimethylcholesta-5,22E-dien-3ß-ol (up to 5.7%) and 24-norcholesta-5,22E-dien-3ß-ol (2.4%). Cholest-5-en-3ß-ol (cholesterol) was present in only one of the replicates at 0.2%. The major sterol, 27-nor-24-methylcholesta-5,22E-dien-3ß-ol, was differentiated from cholesta-5,22Z-dien-3ß-ol (*cis*-22-dehydrocholesterol) on the basis that all other sterols in *P. glacialis* showed *trans* configuration. 27-Nor-24-methylcholesta-5,22E-dien-3ß-ol was chromatographically resolved from cholesta-5, 22E-dien-3ß-ol (*trans*-22-dehydrocholesterol).
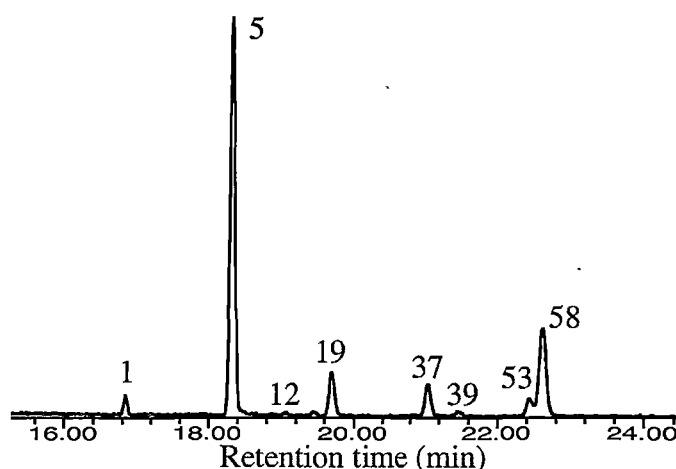
Figure 2.3 Gas chromatogram of sterols as trimethylsilyl ethers from Davis dinoflagellate culture FL1B (replicate # 2) (peak numbers identified in Table 2.6)

4α-Methyl sterols and the one stanol contributed up to 23.7% of the total sterols (Table 2.6). The major 4α-methyl sterol and second most abundant sterol in *P. glacialis* was 4,24-dimethyl-5α-cholestan-3ß-ol (4,24-dimethylcholestanol), contributing up to 19.6% of the sterol total. Also present were 4,24-dimethyl-5α-cholest-24(28)-en-3ß-ol (9.0%, also a stanol) and a sterol tentatively identified as 4,24-dimethyl-5α-cholest-22E-en-3ß-ol (up to 0.9%). Relative retention times (RRT) and mass spectral data for each sterol are given in Table 2.6.

2.2.4 Distribution of *Polarella glacialis*

Cysts and motile cells similar in size and morphology to *P. glacialis* (Montresor *et al.* 1999) were observed in fast ice adjacent to Mawson and Casey Stations, in Nella Fjord, Larsemann Hills and at one pack ice location in the Indian Ocean sector (62°56.87′S 92°58.00′E).

Table 2.6 Sterol composition in duplicate Davis dinoflagellate clonal cultures (FL1B) (a, peak numbers as identified in Jones (1994): b, relative retention times calculated as cholesterol = 1.00; 4,24-dimethyl-5α-cholestan-3ß-ol =1.69)

Peak no.[a]	Sterol	Trivial name	Percent Composition		Sub-totals		RRT[b]	M⁺	Base peak	Other major ion fragments (m/z)
	4-Desmethylsterols		#1	#2						
1	24-Norcholesta-5,22E-dien-3ß-ol		2.4	2.4			0.58	442	97	353, 313, 255, 215, 130
5	27-Nor-24-methylcholest-5,22E-dien-3ß-ol	Occelesterol	64.2	60.5			0.87	456	111	411, 366, 351, 327, 255, 129
12	Cholest-5-en-3ß-ol	cholesterol	0.0	0.2			1.00	458	129	443, 368, 253, 329, 247
19	24-Methylcholesta-5,22E-dien-3ß-ol	brassicasterol/crinosterol	6.9	7.5			1.13	470	69	455, 380, 365, 255, 129
37	23,24-Dimethylcholesta-5,22E-dien-3ß-ol		5.0	5.7	78.5	76.3	1.39	484	69	469, 394, 372, 343, 255, 139, 83
	4α-Methyl sterols and stanols									
39	4,24-Dimethyl-5α-cholest-22E-en-3ß-ol		0.6	0.9			1.47	486	69	388, 359, 271, 121
53	4,24-Dimethyl-5α-cholest-24(28)-en-3ß-ol		2.8	3.2			1.66	486	402	387, 359, 269, 75
58	4,24-Dimethyl-5α-cholestan-3ß-ol	4,24-dimethylcholestanol	18.1	19.6	21.5	23.7	1.69	488	75	473, 398, 359, 229
	Totals		100	100	100	100				

2.3   Discussion

2.3.1   Identification of the Davis dinoflagellate

The perfect match of the LSU rRNA sequences of FL1B and CCMP 1383 unambiguously identifies the Davis dinoflagellate as *P. glacialis*. In another Antarctic study, using SSU rRNA, researchers genetically differentiated between several cultures of *Phaeocystis antarctica* (Medlin *et al.* 1994). They found an intraspecific variation in the SSU rRNA molecule between 0 – 5 bases for cultures from the Weddell and Scotia Seas and McMurdo Sound, indicating discrete populations. In this dissertation, the lack of genetic differentiation between FL1B and CCMP 1383, isolated from the disparate locations of Davis Station and McMurdo Sound suggest a single Antarctic circumpolar population of *P. glacialis*.

2.3.2   Pigments in *Polarella glacialis* (FL1B and CCMP 1383)

The photosynthetic pigments of both cultures of *P. glacialis* were identical and typical of those found in peridinin-containing dinoflagellates (Johansen *et al.* 1974; Jeffrey *et al.* 1975; Jeffrey *et al.* 1997). Furthermore, from the limited number of samples analysed here, no regional variation in pigment composition was evident between the cultures. Chl $a{:}c_2$ ratios of the cultures were similar to those reported for dinoflagellates of similar size by Jeffrey *et al.* (1975) and peridinin:chl a ratios correspond to those found in *Gymnodinium catenatum* Graham (Hallegraeff *et al.* 1991).

One source of variation between the two cultures was the difference in chl *a* per cell (FL1B, 5.2 pg and CCMP 1383, 12.5 pg). When expressed as chlorophylls $a + c_2$ $\mu g/10^6$ cells, these values are 5.9 $\mu g/10^6$ cells for FL1B and 14.0 $\mu g/10^6$ cells for CCMP 1383. The value for FL1B corresponds to those found in similarly sized cells calculated by Jeffrey *et al.* (1975). In CCMP 1383, the chlorophylls $a + c_2$ concentration is more comparable to larger cell sizes. A probable explanation for this is the larger cell size found in the stationary phase of growth of CCMP 1383. Chl *a* and peridinin percent compositions between the cultures also showed variation

despite being grown under identical conditions. These differences possibly reflect the differing growth phases and life cycle stages between the two cultures. Jeffrey et al.

2.3.3 Lipids in *Polarella glacialis* (FL1B)

Lipid classes

Polar lipid was the major lipid class in *P. glacialis* and similarly high compositions are found in most microalgae (Dunstan *et al.* 1993). Polar lipids are the membrane components of the cell and include glycolipid, phospholipid and chlorophyll (Harwood and Russell 1984). The polar lipid, sterol and hydrocarbon compositions varied little between the replicates and are similar to values found in log and stationary phase *G.catenatum* (Hallegraeff *et al.* 1991).

In the replicate *P. glacialis* cultures, triglycerides and free fatty acid composition showed some variation. Triglyceride is a storage lipid produced either directly or from the conversion of polar lipid whilst free fatty acids are breakdown products that may indicate some physiological stress. The higher content of triglyceride and free fatty acid content of replicate '2' may reflect a more advanced culture phase over replicate '1', despite identical culture conditions.

Fatty acids

The individual fatty acids of *P. glacialis* are typical of dinoflagellates although the PUFA composition is the highest recorded in dinoflagellates to date. The PUFA proportion in *P. glacialis* (74 -76%) is higher than values found by Nichols *et al.*(1984) in *Gymnodinium wilczeki* Pouchet (48.4%) and *Prorocentrum cordatum* Ostenfeld (32%), in exponential and stationary phase *G. catenatum* (41.7 − 52.0%) (Hallegraeff *et al.* 1991) and in *Symbiodinium microadriaticum* Freud (52.7%) and *Gymnodinium sanguineum* Hirasaki (51.3%) (Mansour *et al.* 1999). Comparable saturated and monounsaturated compositions were also found in these species.

Proportions of lipid classes may change with differing culture conditions and growth phase, with a increase in PUFAs often observed between the exponential and stationary phases (Volkman 1989; Dunstan *et al.* 1993). It is possible therefore, that the high percentage of PUFAs in *P. glacialis* are influenced by its stationary phase of

growth. A more likely explanation, however, lies in the inverse relationship between temperature and the degree of fatty acid unsaturation (Gill and Suisted 1978). PUFA compositions in bacteria, algae and invertebrates (Gill and Suisted 1978; Henderson and MacKinlay 1989; Desvilettes *et al.* 1994) have been demonstrated to increase with decreasing temperatures, possibly as an adaptation to maintain membrane fluidity under colder conditions. It seems likely, therefore, that the high PUFA composition in *P. glacialis* may be an adaptation to tolerate the exceptionally cold brine environment (up to -14 °C (Stoecker *et al.* 1992)) of fast ice.

The major fatty acids of *P. glacialis* were typical of dinoflagellates (Holz 1981; Nichols *et al.* 1984; Mansour *et al.* 1999). 18:5 (n-3) was the major fatty acid in *P. glacialis* (up to 49%) and has previously showed potential as a biomarker for dinoflagellates. However, this fatty acid is now known to also occur in some haptophytes (Holz 1981; Volkman *et al.* 1981; Napolitano *et al.* 1988), raphidophytes (Nichols *et al.* 1987; Bell *et al.* 1997) and prasinophytes (Dunstan *et al.* 1992) and therefore some care is necessary in using this component as a signature for dinoflagellates. Of the remaining PUFAs, levels of 20:5 (n-3) and 22:6 (n-3) in *P. glacialis* (approx. 8% and 19% respectively) fall about the middle of the range found in other dinoflagellates (1.8 - 20.9% and 9.9 - 26.3% respectively). Levels of the major saturated fatty acids (16:0 and 14:0) were similar to levels in other dinoflagellates (Nichols *et al.* 1984; Hallegraeff *et al.* 1991; Mansour *et al.* 1999).

Iso- and anteiso- branched chain fatty acids (i15:0, a15:0, i15:1 and a15:1) are bacterial signature lipids (Gillan *et al.* 1983). In the *P. glacialis* cultures, these accounted for less than 1% of the fatty acids present, indicating minor bacterial contamination. Bacteria are known to contain low amounts of several other fatty acids reported here (16:0, 18:0, 16:1 (n-7) and 18:1 (n-7c)) (Perry *et al.* 1979; Kaneda 1991) and may have contributed minor amounts to the *P. glacialis* composition.

Sterols

The sterol profile of *P. glacialis* was atypical of that of the Dinophyceae. *P. glacialis* had only moderate levels of 4α-methyl sterols (up to 23.7%) whilst 4-desmethylsterols were the dominant sterol class, contributing up to 78.5% of the total

sterols. Dinosterol was not detected in cultured *P. glacialis*. However, this sterol has been noted as absent in other dinoflagellates such as *Amphidinium* (Withers 1987), *Gymnodinium sanguineum* (Mansour *et al.* 1999) or at only low concentrations in other gymnodinioids (Withers 1987). Only one stanols was present in *P. glacialis*, contributing only a low proportion of the total sterols (up to 3.2%).

The sterol profile of *P. glacialis* appears more typical of the gymnodinoid dinoflagellates. Withers (1987) suggests that a profile with 4,24-dimethylcholestanol as a major sterol and little, if any, dinosterol may be characteristic of species of *Gymnodinium*. In *P. glacialis*, 4,24-dimethylcholestanol was the second most abundant sterol (approximately 20%) whilst dinosterol was absent. However, recent research has revealed several species of *Gymnodinium* with atypical sterol profiles. Hallegraeff *et al.* (1991) demonstrated a low level of 4,24-dimethylcholestanol (2.8%) and a high level of dinosterol (26.8%) in *Gymnodinium catenatum*, whilst similar values were found in *Gymnodinium* sp. CS-389/1 (Mansour *et al.* 1999), *G. wilczeki* (Nichols *et al.* 1984) and stationary phase *Gymnodinium* sp. (Piretti *et al.* 1997).

To be more specific, *P. glacialis* appears to belong to the small subset of gymnodinioids grouped by Mansour (1999) that satisfy the view of Withers (1987). Dinoflagellates in this grouping (*G. simplex* (Goad and Withers 1982), exponential phase *Gymnodinium* sp, GyD2 (Piretti *et al.* 1997) and some strains of *Symbiodinium microadriaticum* (Mansour *et al.* 1999)) have between 23.2 - 100% of 4,24-dimethylcholestanol and between 0 - 14% dinosterol (Table 2.7). Grouping of *P. glacialis* with *S. microadriaticum* appears appropriate considering both species belong to the order Suessiales.

Table 2.7 Sterols of *P. glacialis* compared with gymnodinioid subset

suggested by Mansour *et al.* (1999)

Sterol	Percent composition			
	Polarella glacialis	*S. micro-adriactium*[1]	*Gymnodinium* sp. GyD2[2]	*G. simplex*[3]
24-Norcholesta-5,22-dien-3ß-ol	2.4			0.7
27-Nor-24-methylcholest-5,22E-dien-3ß-ol	62.3	2.8		53[4]
Cholest-5-en-3ß-ol (cholesterol)	0.1	14.2		
24-Methylcholesta-5,22E-dien-3ß-ol (brassicasterol/crinosterol)	7.2	6.3		32
24-Methylcholesta-5,24(28)-dien-3ß-ol		0.7		
24-Methylcholest-5-en-3ß-ol		30.7		
23,24-Dimethylcholesta-5,22E-dien-3ß-ol	5.4	6.1		14
4α,24-Dimethyl-5α-cholest-22E-en-3ß-ol		3.7		
4,24-Dimethyl-5α-cholest-22E-en-3ß-ol	0.7			
4,24-Dimethyl-5α-cholest-24(28)-en-3ß-ol	3.0			
4,24-Dimethyl-5α-cholestan-3ß-ol (4,24-dimethylcholestanol)	18.9	23.2	100	
4α,23,24-trimethyl-5α-cholest-22E-en-3ß-ol (dinosterol)		5.7		
Others		6.6		0.3
Total	100	100	100	

[1] (Mansour *et al.* 1999); [2] (Piretti *et al.* 1997); [3] (Goad and Withers 1982); [4] In *G.simplex*, 27-nor-24-methylcholest-5,22E-dien-3ß-ol was identified as the structurally very similar 27-nor-(24*R*)-24-methylcholesta-5,22-dien-3ß-ol.

Other interesting aspects of the *P. glacialis* sterol profile include the moderate

composition of brassicasterol/crinosterol (approximately 7%) and the occurrence of

27-nor-24-methylcholest-5,22E-dien-3ß-ol (up to 64.2%) as the major sterol.

Brassicasterol was initially presented as a biomarker characteristic of diatoms

(Patterson 1987). However, more recent studies have shown that brassicasterol does

not dominate the sterols of most diatoms (Barrett *et al.* 1995; Volkman *et al.* 1998)

and is a major component in some prymnesiophytes (Volkman 1986; Volkman *et al.*

1990; Farmer 1993; Conte *et al.* 1994) and cryptomonads (Goad *et al.* 1983; Gladu *et*

al. 1990). Furthermore, it has been recorded in the Cyanophyceae, Rhodophyceae,

Chrysophyceae and in at least one member of the Euglenophyceae, Raphidophyceae

and the Prasinophyceae (Ballantine *et al.* 1979; Volkman 1986; Patterson 1991). In

the Dinophyceae, apart from *P. glacialis*, brassicasterol has been noted as a major

sterol of *G. simplex* (32%) (Goad and Withers 1982). Clearly, from this study and

others, brassicasterol can no longer be considered a unique biomarker for diatoms (Barrett *et al.* 1995; Volkman *et al.* 1998).

The moderate presence of 24-methylcholesta-5,22E-dien-3ß-ol in *P. glacialis* and species of the Chrysophyceae has implications for sea ice algal research. The upper fast ice algal communities of McMurdo Sound are dominated by *P. glacialis* and chrysophytes. Furthermore, Stoecker *et al.* (1997; 1998) has demonstrated high concentrations of both algae towards the lower ice. Considering the relatively small size (to most diatoms) and the previously undescribed nature of *P. glacialis* (10 - 15 µm) and the chrysophytes (<10 µm), they may be easily overlooked during microscopic observations. Therefore, due to the increasingly recognised distribution of 24-methylcholesta-5,22E-dien-3ß-ol in microalgae and the recently demonstrated fast ice populations, care must be taken in interpreting the presence of this sterol in sea ice as an indicator of diatom presence.

27-Nor-24-methylcholest-5,22E-dien-3ß-ol, the major sterol in *P. glacialis*, is rare in dinoflagellates and unknown in other algae. Mansour *et al.* (1999) recorded 2.8% in *S. microadriaticum* and 0.3% in *Fragilidium* sp. whilst Teshima *et al.* (1980) detected a low percentage in *Noctiluca scintillans* (Macartney) (although it was unclear whether this phagotrophic species actually produced the sterol). It has been reported from the water column and sediments of the central Pacific and the Black Sea (Wakeham and Beier 1991; Wakeham 1995) and 27-nor-(24*R*)-24-methylcholesta-5, 22-dien-3ß-ol, structurally very similar to 27-nor-24-methylcholest-5,22E-dien-3ß-ol (with the opposite configuration at C-24), is the major sterol in *G. simplex* (Table 2.5). In McMurdo Sound fast ice, Nichols *et al.* (1989; 1990) noted 27-nor-24-methylcholest-5,22E-dien-3ß-ol as a minor component (0.2 – 1.2%) in a diatom dominated community. As this sterol has not been recorded in diatom species and considering the preponderance of *P. glacialis* in the upper ice in McMurdo Sound, this species probably was the likely source of 27-nor-24-methylcholest-5,22E-dien-3ß-ol in these studies.

The rare occurrence of 27-nor-24-methylcholest-5,22E-dien-3ß-ol in dinoflagellates and its absence in other algal species suggests this sterol as an excellent biomarker for

identifying *P. glacialis* in ice and the water column. Whilst *P. glacialis* cysts are known from pack ice (Garrison and Buck 1989; Buck *et al.* 1992; Garrison and Close 1993), this species appears to be more abundant and widely distributed in fast ice (see following section and Chapter 3; Stoecker *et al.* 1992; Stoecker *et al.* 1997; Stoecker *et al.* 1998). As such, it may provide a distinctive marker for past fast ice presence or absence in sediments and prove useful in determining past Antarctic climate variability and fast ice extent.

Palaeoenvironmental studies indicate a late Holocene climatic optimum in summer temperatures that resulted in glacial retreat and reduced sea ice cover (Denton *et al.* 1991; Leventer *et al.* 1993; Domack *et al.* 1994; Leventer *et al.* 1996; Goodwin 1998). In coastal areas (suitable for fast ice growth), decreasing sea ice coverage between approximately 2500 – 4000 B.P. has been interpreted from occupation records of Adélie penguin rookeries (Goodwin 1993; Baroni and Orombelli 1994). In areas where fast ice and *P. glacialis* populations predominate, 27-nor-24-methylcholest-5,22E-dien-3ß-ol records in marine sediments may be useful in confirming these climatic events.

Mansour *et al.* (1999) states that shifts in major sterol distributions in dinoflagellates are unusual although relative proportions may vary (as found in a comparison between exponential and stationary phase *Gymnodinium* sp. CS-380/3). Hallegraeff *et al.* (1991) found similar results between the two phases in *G.catenatum* cultures. In contrast, Piretti *et al.* (1997) noted major differences in the dominant sterols of *Gymnodinium* sp. between the two growth phase cultures. Clearly, comparative studies between growth phases of *P. glacialis*, together with other environmental factors such as temperature and salinity, are necessary to determine if variation in the sterol profile occurs .

2.3.4 Distribution of *Polarella glacialis*

If the assumption is made that the cysts and motile cells observed in this study from sites other than Davis Station are *P. glacialis*, then these sightings indicate a widespread distribution in East Antarctic fast ice. This distribution ranges from an eastern limit at Casey Station (66°17'S 110°32'E) to the western limit at Mawson

Station (67°36'S 62°53'E), including the sites of Davis Station and the Larsemann
Hills (Figure 2.6). *P. glacialis* has also been recorded in McMurdo Sound fast ice
(Stoecker *et al.* 1992; 1997; 1998) and a large population of unidentified, small
motile dinoflagellates, similar to that described from McMurdo Sound, have been
recorded near Syowa Station (Hoshiai 1977). In pack ice, this study recorded the *P.
glacialis* cyst in the Indian Ocean sector whilst others have reported cysts from the
Weddell and Scotia Seas (Garrison and Buck 1989; Buck *et al.* 1992).
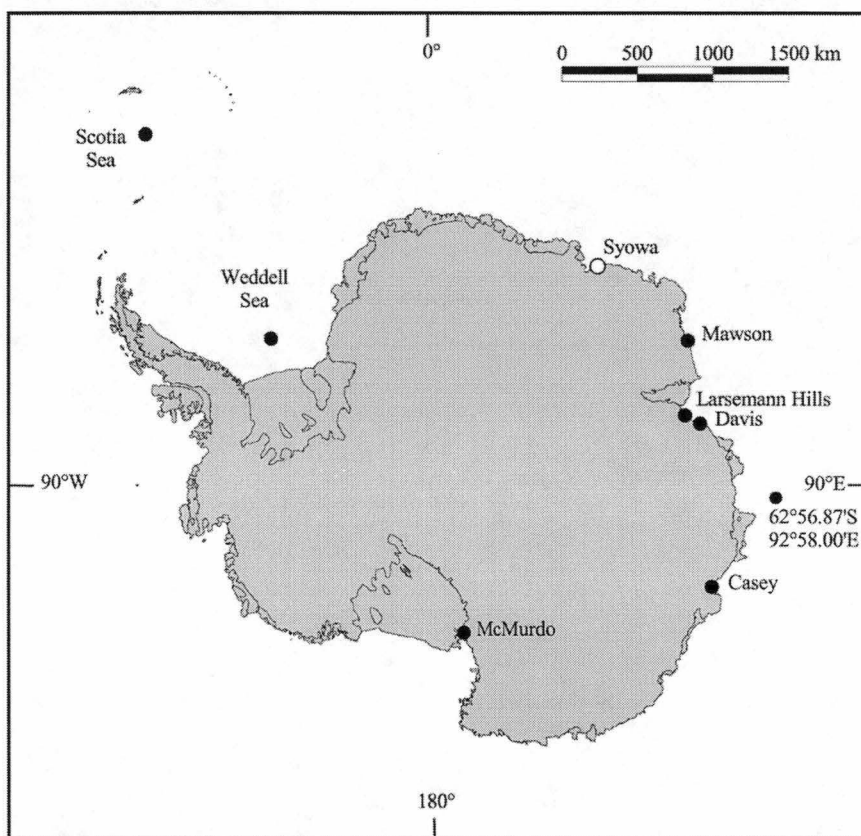
**Figure 2.6 Circumpolar distribution of *Polarella glacialis* in Antarctic
fast and pack ice (●** indicates recorded presence of *P.glacialis* or
morphologically similar motile cells and cysts; McMurdo Sound data **(Stoecker
et al. 1998; Montresor *et al.* 1999),** Weddell and Scotia Seas data
(approximate locations) **(Buck *et al.* 1989; Garrison and Buck 1989);**
Syowa Station marked as presence of photosynthetic dinoflagellate population in
fast ice **(Hoshiai 1977))**

Overall, *P. glacialis* appears to have a circumpolar distribution (Figure 2.6). Such a distribution is not surprising as a survey of polar algal species complied by Horner (1985) and other authors (Hart 1942; Baker 1954; Fryxell and Hasle 1979) reveals many Antarctic species (such as *Thalassiosira perpusilla, T.gracilis, Fragilariopsis curta, Entomoneis kjellmanii* and *Phaeocystis antarctica*) exhibit such a distribution. The Antarctic Circumpolar Current (ACC) and the inshore Antarctic Coastal Current (Knox 1994) most likely supply the force responsible for this distribution, carrying cysts and cells within broken ice floes and the water column about Antarctica.

In addition to its Antarctic circumpolar distribution, it is possible that *P. glacialis* also occurs in the Arctic. Meunier (1910), Ikävalko and Gradinger (1997) and Lovejoy (pers.comm) have all recorded similar spinose cysts from this region. Clearly, comparative molecular genetics, pigment and lipid studies between these cysts and those of Antarctic *P. glacialis* would clarify the question of its bipolar distribution.

2.4   Conclusions

A dinoflagellate isolated from Davis Station fast ice was identified as *P. glacialis* on the basis of the identical LSU rRNA sequences in comparison with CCMP 1383 (*Polarella glacialis*). The lack of genetic variability between the two cultures points to a single circumpolar population of this species. Cells and cysts morphologically indistinguishable from *P. glacialis* were also found along the East Antarctic coastline between Casey and Mawson Stations and from a pack ice location in the Indian Ocean. Combined with reports from McMurdo Sound and the Weddell and Scotia Seas, it is concluded that *P. glacialis* has a circumpolar distribution.

The photosynthetic pigments of *P. glacialis* were typical of dinoflagellates and showed no regional variation between Davis Station and McMurdo Sound. Peridinin was the major carotenoid present, followed by diadinoxanthin and dinoxanthin. The only sources of variation between the two cultures (chlorophylls $a + c_2$ concentrations per cell and chl a and peridinin percent compositions) were attributed to differences in culture and life cycle phases.

The fatty acid profile of *P. glacialis* was also typical of the dinoflagellates, with 18:5 (n-3), 16:0, 22:6 (n-3) and 20:5 (n-3) being the most abundant components. Overall, PUFAs contributed the highest percent composition of the fatty acids over saturates and monounsaturates. Furthermore, the PUFA composition of *P. glacialis* was very high in comparison to other dinoflagellates. It seems likely that the high PUFA content is an adaptation to survive the cold temperatures of the fast ice brine environment. Neither the pigment or fatty acid profiles of *P. glacialis* revealed specific biomarkers for this species. Despite this, both profiles are useful indicators of this and related dinoflagellate species.

In contrast to the pigments and fatty acids, the sterol profile of *P. glacialis* was atypical of dinoflagellates. 4-Desmethylsterols were the most abundant sterol class over the more typical 4α-methyl sterols found in dinoflagellates and dinosterol was absent. This composition is more typical of a small subset of gymnodinioid dinoflagellates, including *S.microadriaticum*, a close relative of *P. glacialis*. The moderate composition of brassicasterol/crinosterol in *P. glacialis*, once considered a biomarker of diatoms, must be considered during future sea ice studies utilising this sterol as a diatom marker.

This study identifies 27-nor-24-methylcholest-5,22E-dien-3ß-ol as a major dinoflagellate sterol for the first time. This sterol, the most abundant in *P. glacialis*, has been recorded only in low compositions in *S. microadriaticum*, *Fragilidium* sp. and possibly in *N. scintillans*. Furthermore, 27-nor-24-methylcholest-5,22E-dien-3ß-ol is unknown in other algae. The limited distribution of this sterol in microalgae, therefore, suggests its usefulness as a specific biomarker for *P. glacialis* in future sea ice studies. This biomarker could be used either alone or in combination with other components, such as fatty acids and pigments. The predominance of *P. glacialis* in fast ice, in comparison to pack ice, may also render 27-nor-24-methylcholest-5,22E-dien-3ß-ol a useful sediment biomarker for interpreting past Antarctic climate variability and fast ice extent.

Chapter 3 Davis fast ice brine environment and community composition

3.0 Introduction

Sea ice production annually dominates the polar marine environment in the Southern Ocean (Legendre *et al.* 1992) and provides an immense substrate for the colonisation and growth of sea ice algae (Horner 1985; Garrison 1991). Several types of algal communities develop in association with sea-ice (Horner 1985; Garrison 1991; Knox 1994). In pack ice, surface and interior communities are common and consist predominantly of diatoms and photosynthetic flagellates (Garrison and Buck 1985; Garrison *et al.* 1987; Garrison and Buck 1989; Garrison and Buck 1991; Fritsen *et al.* 1994). In land fast ice, bottom and sub-ice communities are dominated by diatoms (Bunt and Wood 1963; Burkholder and Mandelli 1965; McConville and Wetherbee 1983; Grossi *et al.* 1987; SooHoo *et al.* 1987; Watanabe and Satoh 1987; Watanabe 1988; McMinn 1996; McMinn 1997; McMinn and Ashworth 1998; McMinn *et al.* 1999) whilst surface communities of diatoms and flagellates are also known (Burkholder and Mandelli 1965; McConville and Wetherbee 1983; Watanabe and Satoh 1987).

Over the past decade, an interior community has been described from the brine channels and pockets of upper fast ice of McMurdo Sound (Stoecker *et al.* 1992; 1993; 1997; 1998). These studies revealed an environment characterised by extreme and transitory temperatures and salinities, ranging from $-13.8\ °C$ and 200 psu (practical salinity units) in early spring to $-0.2\ °C$ and 20 psu by late summer. This community was dominated by photosynthetic dinoflagellates (the most abundant form now identified as *Polarella glacialis*) and their hypnozygotes (resting cysts), chrysophytes and their statocysts and small photosynthetic flagellates. Diatoms, plastidic ciliates and heterotrophs were also present but at lower densities. Stoecker *et al.*(1997) demonstrated the excystment and growth of *P. glacialis* and the chrysophytes in this extreme environment, with both species reaching $>10^6$ cells l^{-1} of

ice. Stoecker *et al.*(1998) proposed life cycles for the dominant species, concluding that their resting stages are adaptations for dispersal during ice decay and, after incorporation back into the ice, overwintering survival until the following spring.

Whilst the physical environment and population dynamics of the upper fast ice of McMurdo Sound are now well described, little is known of the Antarctic distribution of this community. *Polarella glacialis* appears to have a circumpolar distribution (Chapter 2) and large populations of small dinoflagellates similar to those described from McMurdo Sound have been reported in Davis and Syowa Station fast ice (Hoshiai 1977; Archer *et al.* 1996). Additionally, Takahashi *et al.* (1986) has reported chrysophyte statocysts, some similar to those found at McMurdo Sound, also near Syowa Station. It seems likely, but unproven, that brine channel communities are widespread in Antarctic fast ice.

This chapter describes the physical and chemical parameters of the brine channel environment and its community composition in Davis Station fast ice. It compares and contrasts the Davis fast ice environment and community with that of McMurdo Sound. Furthermore, it expands on the known distribution of this community about Antarctica.

To begin this chapter, the principal study area at Davis Station, East Antarctica is described. The methods used in sampling the sea ice and subsequent analyses are detailed. Results from these analyses will include physical and chemical parameters of the brine environment, the vertical distribution of the phototrophic community, its composition, and its population dynamics. Finally, Davis and McMurdo Sound upper fast ice communities are compared and conclusions as to the distribution of this community are made.

3.0.1 Study Area

Field work was carried out during the spring summer transition of 1996 and 1997 in fast ice near Davis Station in the Vestfold Hills, East Antarctica (68° 43.2'S 77° 56.4'E) (Figure 3.0). The Vestfold Hills represent an approximate 400 km^2 area of ice free Antarctica. Its coastline is studded by numerous rocky outcrops and islands

and intersected by several fjords. In summer, this area is largely snow free and the underlying rocks and loose soils are exposed.
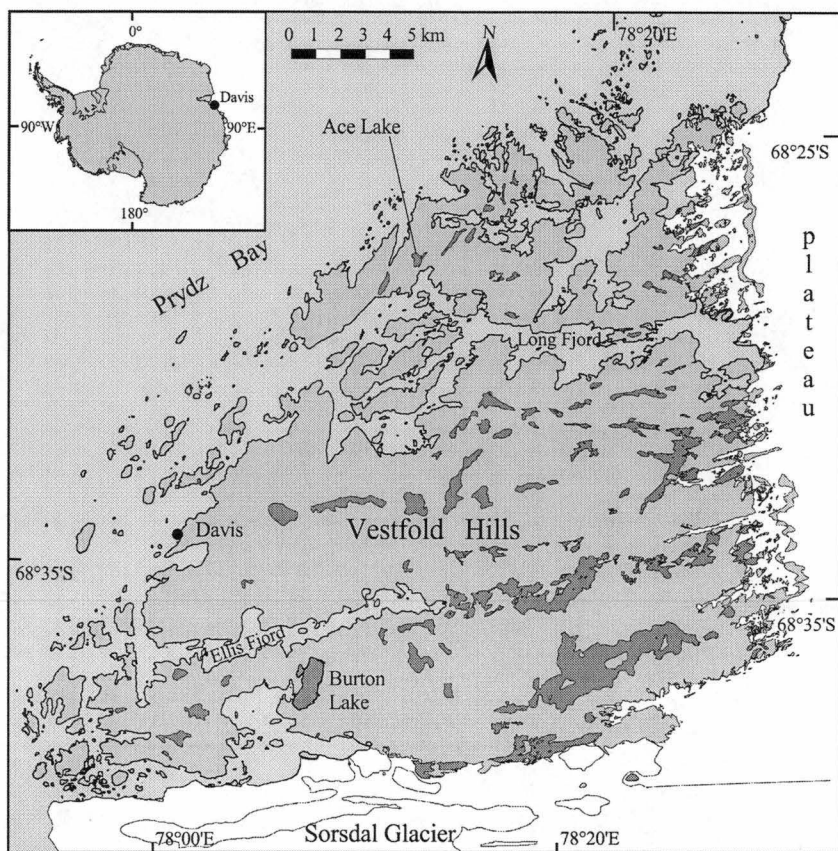
Figure 3.0 The location of Davis Station and the Vestfold Hills (includes location of Ace and Burton Lakes where samples of opportunity were taken)

Fast ice growth typically begins in March and maintains slow thermodynamic thickening until late September. Its structure is dominated by congelation ice (McConville and Wetherbee 1983; Worby *et al.* 1998) and it reaches a maximum thickness of up to 2 m and a maximum extent of about 15 km from Davis Station (Worby *et al.* 1998). During winter and early spring, a substantial snow cover may accumulate on the ice surface although snow free areas are common in late spring and summer (unpublished observations, Thomson 1996, 1997). Surface ablation, exacerbated by wind deposited dust, and bottom melt occurs from September until the ice breaks out in late December or early January (Heil *et al.* 1996). Ice break out

generally occurs sectionally due to entrapment by the numerous inshore islands.
Final ice break out is followed by a period of open water that persists until sea ice
formation commences in March. Sea ice thickness and snow cover observations by
Davis ANARE wintering staff for 1996 and 1997 are shown in Figure 3.1.
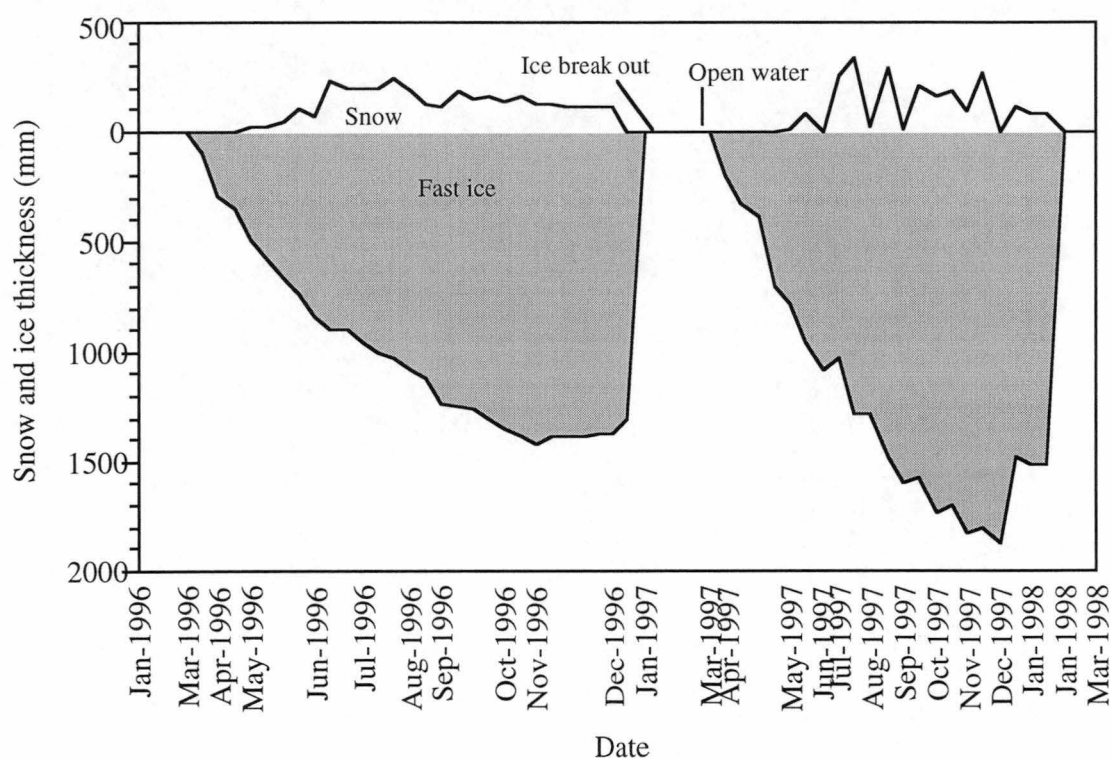
Figure 3.1 Snow and ice cover adjacent to the Vestfold Hills, East Antarctic from January 1996 – March 1998 (data from Davis ANARE wintering staff, 1996 – 1998)

3.0.2 Study sites

Sampling sites were chosen with longevity of sampling in mind. In the 1996 study,
three routine sampling sites were chosen. These were O'Gorman Rocks, Flutter
Island and an unnamed outcrop adjacent to Airport Beach (referred to as Airport
Beach). In 1997, these locations, plus points near Plough and Bluff Islands, were
sampled (Figure 3.2). Water depth beneath the fast ice sites ranged from 15 – 25 m.

During both summers, sites were lost sequentially as sections of the fast ice broke out
along the coast. In 1996, the last accessible site remaining until late December was

O'Gorman Rocks, with Flutter Island and Airport Beach lost mid December. In 1997, all sites except O'Gorman Rocks were lost by mid December. Samples of opportunity were taken throughout the Vestfold Hills, in Ellis and Long Fjords, near the Sørsdal Glacier and from Ace and Burton Lakes (Figure 3.0).
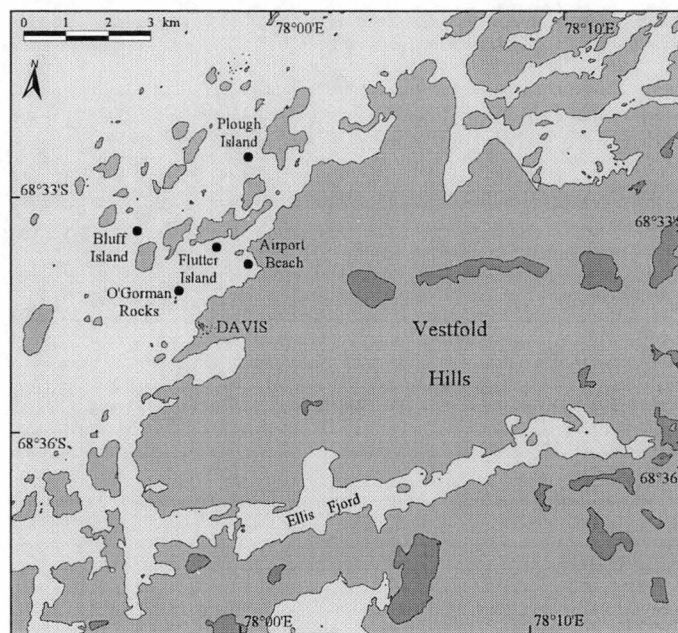
Figure 3.2 Routine sampling sites near Davis Station.

3.1 Methods and Materials

3.1.1 Brine Sampling

Brine sampling was carried out every three days at each of the primary sites when weather conditions permitted. Snow cover at each site was recorded and triplicate, 500 mm deep holes were drilled within one metre of each other by Jiffy drills 30 cm in diameter. The drill holes were allowed to flood with brine from the surrounding ice and the quantity of brine in the holes (hereafter called brine quantity, calculated from the accumulated brine depth and hole diameter) was used as an indicator of ice decay (porosity) and brine volume. Early in the season when the low brine volume would be expected, brine slowly accumulated overnight and was collected the following day. As the season progressed, brine collection was possible immediately.

Brine flooding and collection, therefore, ranged from hours to a matter of minutes. Brine volume estimations, through measurement of the ice temperature and bulk salinity (Frankenstein and Garner 1967), were not carried out.

Brine temperature and salinity was measured using a Hanna Checktemp C digital thermometer and a hand held Shibuya (Type 147) salinity refractometer directly from the hole as soon as possible after drilling. The brine was thoroughly mixed and approximately 1L samples were collected and fixed immediately with acid Lugol's Iodine. Fresh samples were taken for microscopic identification, isolation purposes, chl *a*, pigment analyses and nutrient analyses. All samples were transported back to the laboratory in an insulated container within 2 hours of collection.

3.1.2 Ice Coring

Triplicate ice cores were taken on a weekly basis at O'Gorman Rocks in both seasons, weather permitting, until ice porosity and high brine quantities rendered coring unsuitable for collection. Cores were taken using a 100 mm SIPRE ice corer through the depth of the ice. Cores were sectioned into 10 cm intervals and placed in plastic buckets for transportation back to the laboratory, thereby minimising brine loss. An equal volume (to the ice core section) of 0.45 μm GF/F filtered seawater (FSW) was added to the containers and the cores were melted at 4 °C in the dark. The addition of FSW minimised the loss of flagellates and ciliates by buffering salinity and osmotic changes during melting (Garrison and Buck 1986).

3.1.3 Species Identification

In 1997, High Performance Liquid Chromotography (HPLC) was used to identify pigments and carotenoids in the brine and to aid in taxonomic identification. Between 24 November and 9 December, one litre of fresh brine was filtered through a 47 mm GF/F filter and frozen at –80 °C for later pigment analysis. The filters were transported back to Hobart in liquid nitrogen and stored at –80 °C until they were extracted (within 12 months). Extraction and analysis of the field samples followed the methods of Wright *et al.* (1991; 1996). One quarter of each filter was extracted in

2 ml of MeOH to which 25 µl of APO-8 (standard pigment mixture) was added. The filter was sonicated for 2 minutes at 100 watts using a Braun Labsonic 1510 sonicator and the extract filtered (Milex-SR 0.5 µm). Only one quarter of the filter was extracted due to the apparent dense filtrate on the filter surface.

Pigments and carotenoid concentrations were analysed using CHEMTAX. CHEMTAX utilises factor analysis and a steepest descent algorithm to find the best fit to data based on pigment ratios of the algal classes to be determined (Mackey *et al.* 1996). HPLC and CHEMTAX analysis was carried out at the Australian Antarctic Division, Kingston, Tasmania with Dr. Simon Wright.

Microscopic identification of species was carried out using both light and electron microscopy. Where light microscopy was used, subsamples of up to 1L of fresh brine were filtered through Activon 0.8 µm cellulose acetate membrane filters until a small volume of concentrated sample remained above the filter. The cells were gently resuspended and a drop of the concentrate was placed on a microscope slide, covered, and examined using an Axioscope microscope at 400 and 1000x magnification. Cells were identified as photosynthetic though epifluorescence microscopy. The remaining solution was fixed with 5% glutaraldehyde solution made from the filtrate.

A Phillips Electroscan Environmental SEM with Environmental SEM System 2020 Version 3.2 software, was used to identify some species in the fixed samples. The glutaraldehyde and Lugol's fixed samples were prepared for the SEM by filtering 1 ml of concentrated samples through 0.8 µm membrane filters. Twice filtered Milli-Q water was used to wash the material. The filters were air dried overnight on No.1 Whatman filter paper, attached to stubs the following day and gold sputter coated.

3.1.4 Enumeration of species

The Lugol's fixed brine samples were concentrated using the inverted microscope method (Hasle 1978). The volume of the Lugol's fixed samples was measured and cells were allowed to settle for three days in the collecting containers. Following this settling period, the supernatant was carefully siphoned off to leave a volume of approximately 100 ml. The remaining volume was transferred to a 100 ml graduated

volumetric measuring cylinder and the volume was recorded. After further settling, this volume was reduced to 20 ml and the sample was transferred to glass vials. Additional Lugols' iodine was added if necessary and the samples were stored in the dark at 4 °C until counts were made.

Before counting, the concentrated 20 ml samples were gently mixed and subsamples of 5 – 10 ml (depending on cell concentration) were settled in 10 ml settling chambers (Utermöhl 1958). Counts were carried out on two inverted microscopes. In 1996, cells were counted at x32 and x40 magnification on Telaval 31 inverted microscope. In 1997, an Axiovert inverted microscope was used to enumerate species under x40 and x100 magnification. Cells within an E29 Whipple Grid were counted and fields of view (FOV) at each magnification were calculated. Fifteen replicate FOVs were counted, averaged and the concentration of cells per litre was calculated for the known volumes settled. Observations from the fresh samples aided in identification and enumeration of fragile species otherwise distorted in the Lugol's fixed samples.

3.1.5 Chlorophyll *a* analysis

Chlorophyll *a* concentrations were determined spectrophotometrically as soon as possible after collection (Parsons *et al.* 1984). Fresh brine samples of up to 1L in volume were filtered through either 13 mm or 47 mm GF/F filters under vacuum. The filters were folded, blotted dry, placed in labelled centrifuge tubes with 90% acetone and the chlorophyll extracted overnight in the dark at −18°C. The volume of acetone used to extract chlorophyll varied with the diameter of the filter used. For the 47 mm filter size, 5 mls was used while for the 13 mm filters, 3 ml of acetone was used.

The following day the filters were squeezed using forceps to remove residual chlorophyll from the filters and the extracts centrifuged at 2000 rpm for 5 minutes. The extracts were then transferred into 1 cm light path cuvettes and analysed in a GBC 916 UV visible spectrophotometer where absorbency was measured at 750,

664, 647 and 630 nm. Chlorophyll a concentrations were then calculated using the following equation;

$$\text{Chlorophyll } a = 11.85 \, E_{664} - 1.54 \, E_{647} - 0.08 \, E_{630}$$

where E is the absorbancy at different wavelengths.

Brine chl a values, expressed as $\mu g \, l^{-1}$, determined from the five sites were averaged to give a mean value for Davis fast ice. To account for dilution of chl a within the ice by increasing brine volumes, the mean chl a measurements were normalised to the initial brine salinity at the start of sampling using dilution factors. Dilution factors for each sampling date were calculated by dividing the initial brine salinity at the start of sampling by the current salinity. Both diluted and normalised chl a measurements are presented.

Where chl a was determined from melted ice core sections, the entire volume of melted section and the FSW melting medium was filtered and extracted as previously described. In this case, chl a concentrations were averaged between the triplicate cores and expressed as mg/m^3.

3.1.6 Nutrient analysis

Weekly samples were taken for nutrient analysis at O'Gorman Rocks between 17 November and 9 December, 1997. Duplicate, ice free samples were collected directly from one drill hole, frozen at $-20 \, °C$ and transported back to Hobart. Nutrient analysis was undertaken at CSIRO Marine Laboratories in Hobart with the assistance of Mr. Neale Johnston. Dissolved inorganic nitrogen (DIN) ($NO_3^- + NO_2^-$), dissolved inorganic phosphate (DIP) (PO_4^{3-}) and silica (SiO_2) (measured as reactive silicate (H_4SiO_4)) were analysed with an ALPKEM Auto Analyser. Analysis was carried out following the ALPKEM Methodology Manual (1992). To minimise disturbances in the matrix due to the varying salinities, the samples were divided into three categories of high (65 – 100 psu), medium (35 – 65 psu) and low salinity (20 – 35 psu). Autoanalyser carrier solutions and standards of 85, 45 and 30 psu respectively were

used in conjunction with these sample categories. DIN measurements were at detection limit of 0.2 μM with a precision of 0.86% full scale deflection (FSD). Ortho-phosphate measurements were accurate to 0.01 μM with a precision of 0.57 FSD. Silicate measurements were measured with a detection limit of 0.1 μM and a precision of 0.73% FSD.

3.1.7 Seawater samples

Sub-ice and open water samples were taken adjacent to O'Gorman Rocks in the 1996 season. Sub-ice samples were taken weekly when possible through a 30 cm hole drilled through the ice cover. A Kammera water sampling device was lowered through the ice and into the water column where discrete samples at 0, 5, 10, 15 and 20 m depth were collected. The approximately one litre samples were fixed with Lugol's iodine and triplicate counts, specifically of *P. glacialis* cysts, were made after settling as previously described. After ice break out, water samples were taken at approximately the same location on a weekly basis when weather conditions permitted.

3.1.8 Statistical analysis

Mean values and standard deviations for temperature, salinity and cells counts were performed in Microsoft Excel 98. Where necessary, data was log transformed and Student t-tests, ANOVAs and post hoc analyses were performed using Systat. Tukey's Test was used in calculating changes in brine temperature and salinity.

3.2 Results

Sampling began as soon as possible after arrival on station. The earliest samples were taken around 11 November (1996) and sampling ceased late December (both years) when sites were lost and sea ice travel become unsafe. From the 15 December in both years, O'Gorman Rocks was the only remaining site.

Prior to sampling in 1996, snow cover on the fast ice was light (Figure 3.1) and during the sampling period this same year, the fast ice was mostly free of snow (Table 3.0). In 1997, the ice was buried under a substantial snow cover of 200 mm.

After sampling began, high winds dispersed some of this cover, however episodic snowfalls created further coverage of up to 200 mm during the sampling period.

Table 3.0 Average snow cover, brine depth, accumulated brine quantity (1996, 97) and nutrients (1997) in Davis upper fast ice (I = immediately)

1996	11/11	14/11	17/11	21/11	24/11	2/12	4/12	8/12	10/12	16/12	21/12
Snow cover (mm)	0	20	0	55	0	0	0	0	0	0	0
Accum. brine quantity (l)	7.6	7.6	17.7	17.7	20.3	21.3	20.3	20.3	20.3	21.3	22.8
Time to fill (hr)	24	17	19	17	1.5	<1	<1	I	I	I	I
1997	12/11	14/11	17/11	21/11	24/11	27/11	1/12	9/12	12/12	15/12	22/12
Snow cover (mm)	200	50	210	60	200	40	0	0	0	0	0
Accum. brine quantity (l)	7.6	10.1	12.7	17.7	20.3	21.3	20.3	20.3	22.8	20.3	21.3
Time to fill (hr)	24	14	6.5	<1	<1	I	I	I	I	I	I
Nutrients											
$NO_3^- + NO_2^-$ (µM)			0.28	< 0.2	< 0.2	< 0.2	0.31	< 0.2			
PO_4^{3-} (µM)			4.85	1.15	1.75	1.15	0.95	0.27			
SiO_2 (µM)			54.35	32.1	48.95	41.05	38.25	8.75			

Increasing brine quantity filling the drill holes was evident from 11 November through to late December in both seasons (Table 3.0). Brine did not accumulate in the holes prior to 11 November. In early December in both years, a freeboard layer similar to that described by Stoecker *et al.* (1998), McConville and Wetherbee (1983) and Fritsen *et al.* (1994), developed in the upper layers of the ice. This layer, up to 250 mm in depth and partially filled with brine and meltwater, was capped with an icy surface crust of about 100 mm thick. Numerous meltpools, up to metres in diameter, were also evident at this time. These developments generally did not affect the sampling sites.

3.2.1 Physical and chemical parameters

Mean brine temperatures increased from early November through to late December (Figure 3.3) and a post hoc Tukey's Test indicated where these differences existed

(Table 3.1). In 1996, statistically significant warming occurred between 11
November and 18 November. Between 18 November and 8 December, brine
temperature increases were less dramatic and rose insignificantly until another
warming phase from the 10 – 16 December.
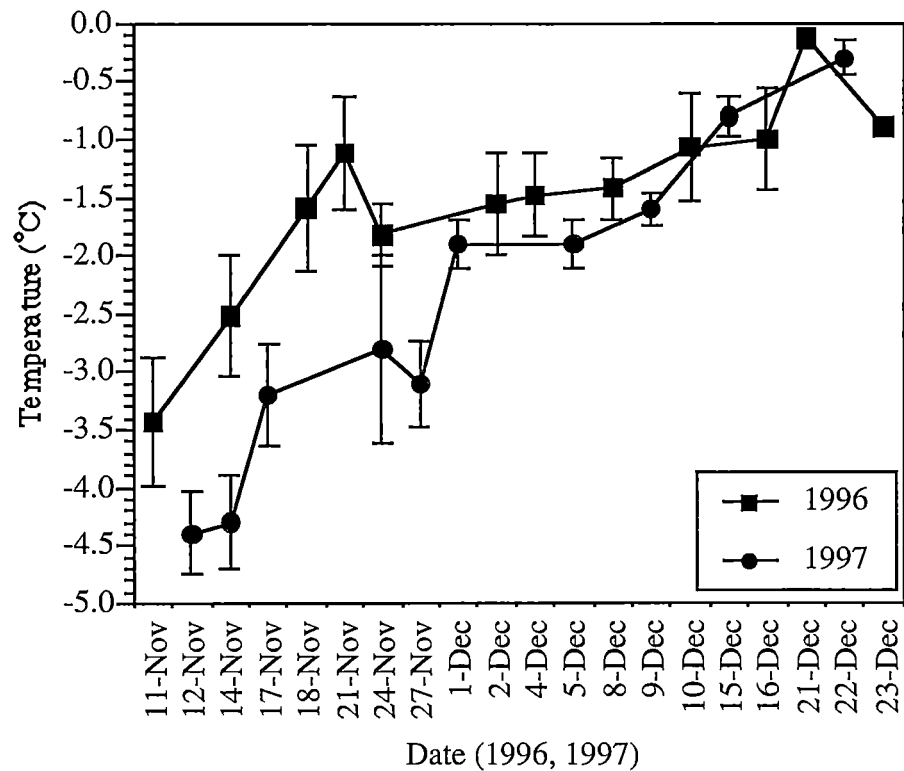
**Figure 3.3 Mean brine temperature in Davis fast ice in 1996 and 1997 (n = 3,
1996, n = 5, 1997 except for 16 December onwards where single measurements
taken only at O'Gorman Rocks. Error bars = 1 sd)**

In 1997, temperature increases were more remarkable and occurred in four steps
(Table 3.1). Similarly to 1996, the first step of significant warming occurred between
14 and 17 November and no change was recorded again until the next step between
27 November and 1 December. The third step occurred between 5 and 9 December
and the fourth after 9 December.

Table 3.1 Post hoc Tukey's test for significant difference between brine temperatures and salinities in Davis fast ice within 1996 and 1997 seasons (same letters represent no significant difference at $\alpha = 0.05$. Degree of overlap of letters indicates the gradient of the curve, ie. the more overlap, the more gradual the change)

1996 Date	11/11	14/11	18/11	21/11	24/11	2/12	4/12	8/12	10/12	16/12	21/12
Temperature (df = 20)	A	A									
		B	B		B	B	B	B			
			C	C	C	C	C	C	C	C	
Salinity (df = 20)	A	A	A								
		B	B	B	B	B					
			C	C	C	C	C	C			
								D	D	D	

1997 Date	12/11	14/11	17/11	24/11	27/11	1/12	5/12	9/12		15/12	22/12
Temperature (df = 38)	A	A									
			B	B	B						
						C	C				
							D	D			
								E		E	E
Salinity (df = 34)	A	A									
		B	B								
				C	C						
						D	D				
							E			E	E

The initial rapid warming of the brine to mid November in both years corresponded with increases in ambient air temperature (Figure 3.4). The lag in air temperature increases from mid November to early December was also reflected in the lack of significant brine warming during this time. Further air temperature increases to late December were followed by continued brine warming. Air temperature in 1997 warmed more quickly than in 1996, rising to approximately 0 °C by 18 November. At the same time in 1996, the air temperature was around –1 °C. From mid November 1997, temperatures generally remained higher than in 1996 until early December.
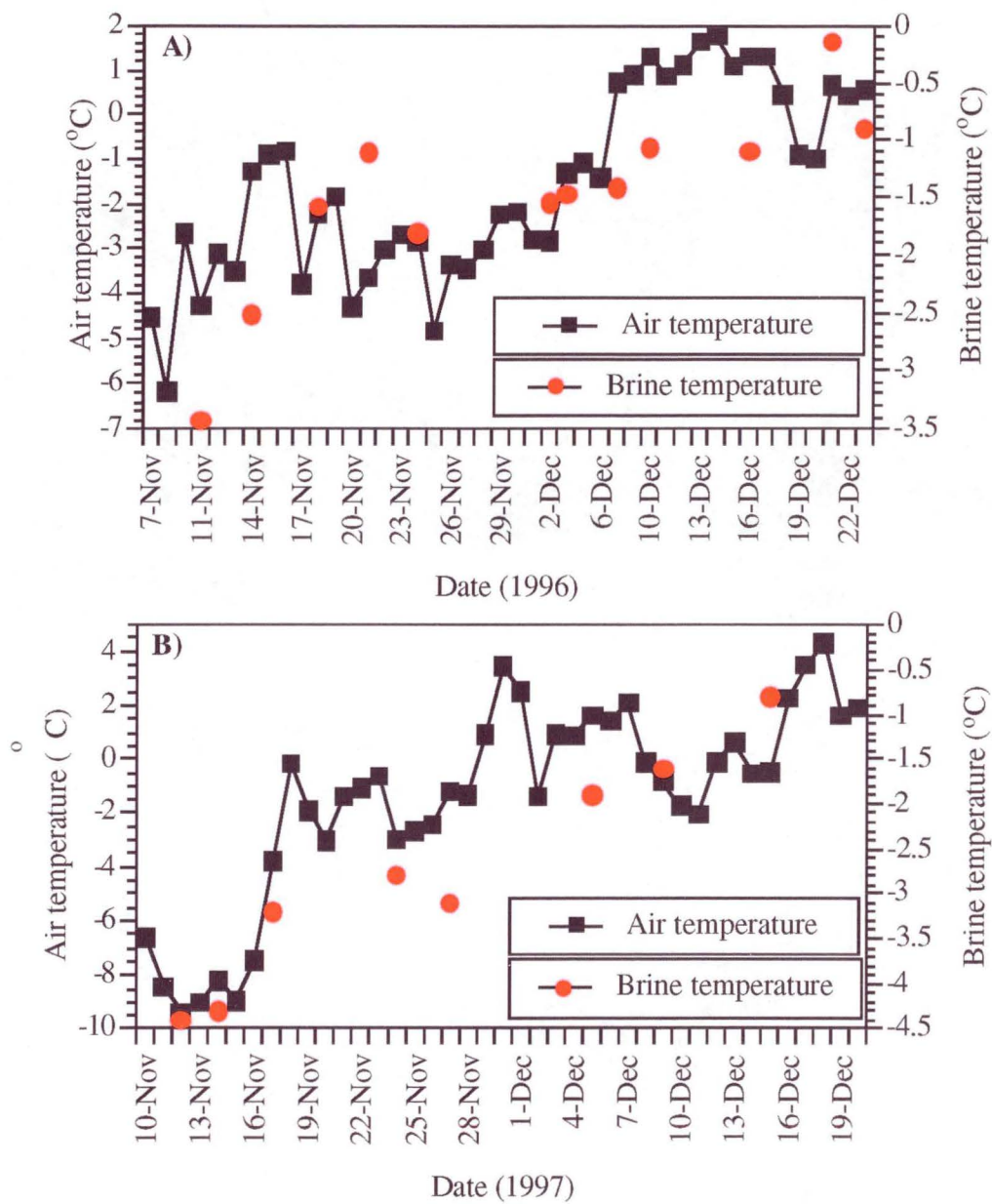
Figure 3.4 Mean air and brine temperatures at Davis Station in A) 1996 and B) 1997 (mean daily air temperatures calculated from 3 hourly measurements and supplied by the Climate and Consultancy Section, Antarctica Regional Office of the Bureau of Meteorology)

In both years, the brine was hypersaline in November and fell to extremely low values by late December (Figure 3.5). Brine salinity trends closely followed those described for temperature (Table 3.1). In both years, temperature and salinity showed a negative correlation of r = -0.86 (1996) and -0.98 (1997).
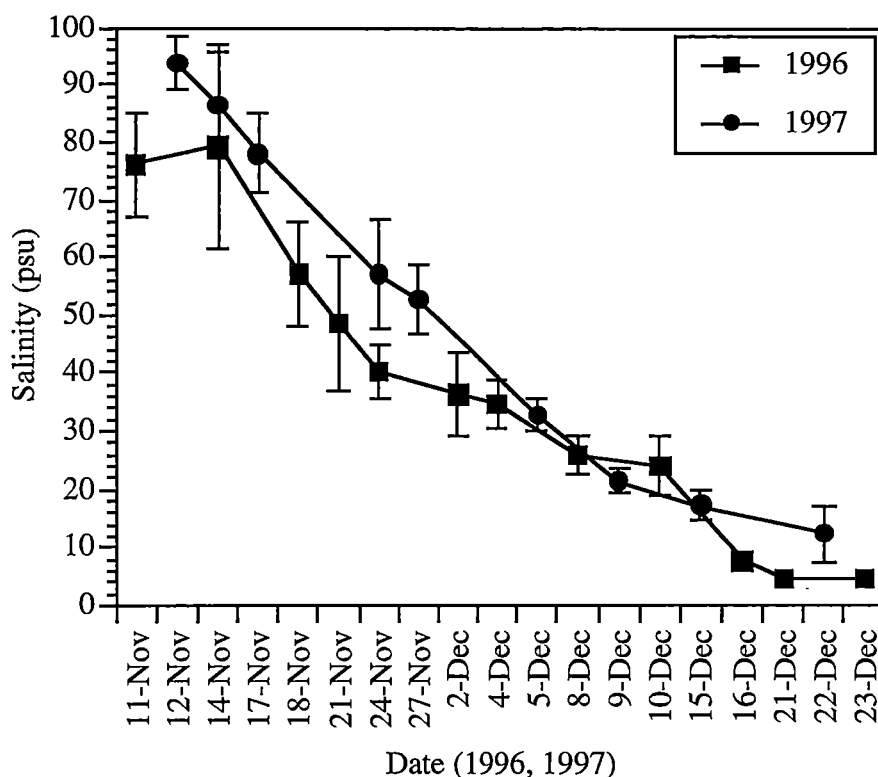
Figure 3.5 Mean brine salinity in Davis fast ice in 1996 and 1997 (n = 3, 1996, n = 5, 1997 except for 16 December onwards where measurements taken only at O'Gorman Rocks. Error bars represent 1 sd)

Nutrients within the brine generally decreased in concentration during the sampling period (Table 3.0). DIN on 17 November 1997 was low at 0.28 μmol l^{-1} and decreased to below detection levels (< 0.2 μM) on subsequent dates with the exception of 4 December. Phosphate and silicate similarly decreased as the season progressed (4.85 μM to 0.27 μM and 54.35 to 8.75 μM respectively). The low DIN concentrations precluded further analysis using these nutrient values.

3.2.2 Vertical distribution of the upper ice, autotrophic community

At the start of sampling in 1996, the phototrophic community at O'Gorman Rocks was concentrated between 40 – 50 cm depth in the ice (Figure 3.6), at a mean maximum of 4.19 mg/m^3. Less than 1 mg/m^3 of chl a was present at all other depths sampled. Chl a values increased throughout the upper ice as the season progressed, however, the greatest concentrations of chl a remained below 30 cm in the ice.
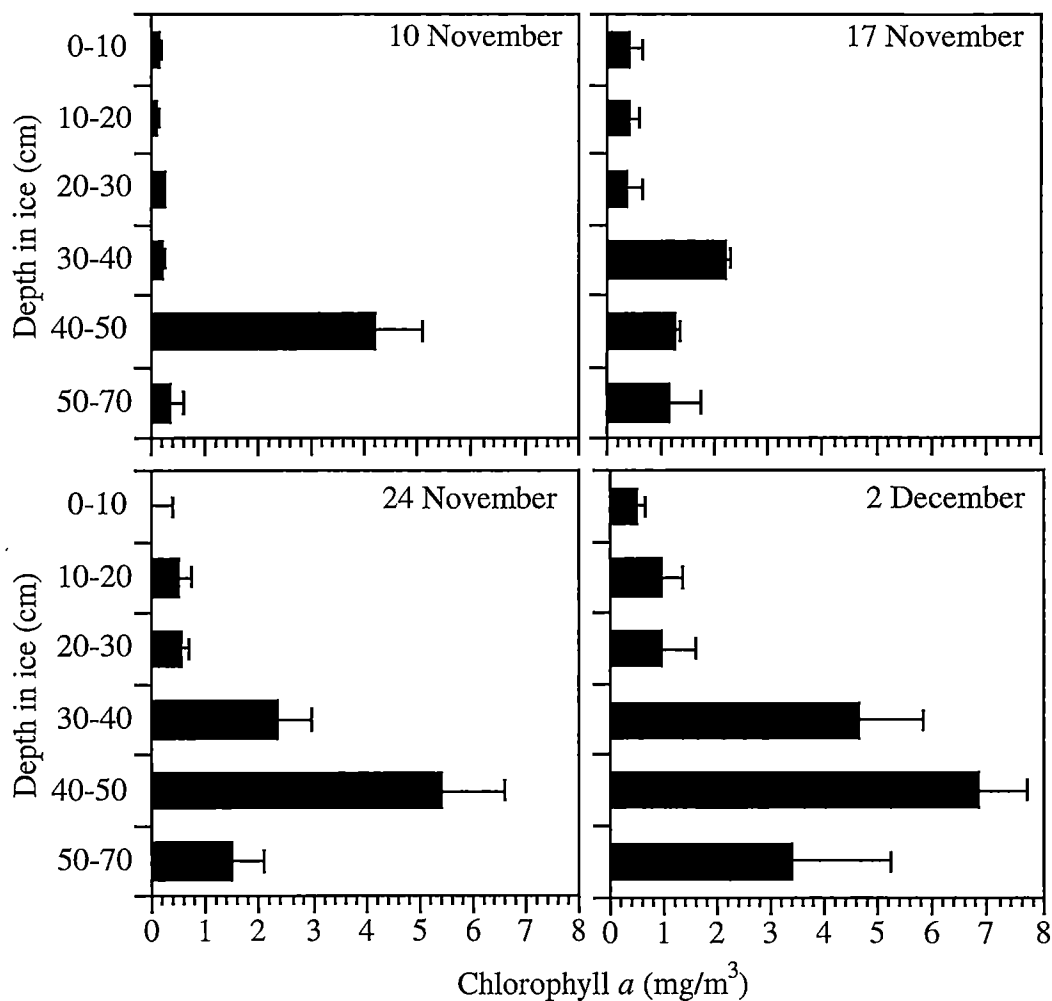
Figure 3.6 Vertical distribution of the phototrophic community in upper fast ice at O'Gorman Rocks in 1996 (as mean chlorophyll *a* values, n = 3, error bars = 1 sd)

At the beginning of the sampling in 1997, chl *a* showed the phototrophic community concentrated between 50 and 70 cm depth (Figure 3.7). By 14 November, this peak had moved to between 20 and 30 cm and the community was more evenly distributed in the upper ice. Ice cores on 21 November showed bimodal peaks at 0 - 10 cm and 50 – 70 cm. By 14 December, the population was again concentrated between 50 – 70 cm. Chl *a* in 1997 was generally lower than that recorded in 1996.
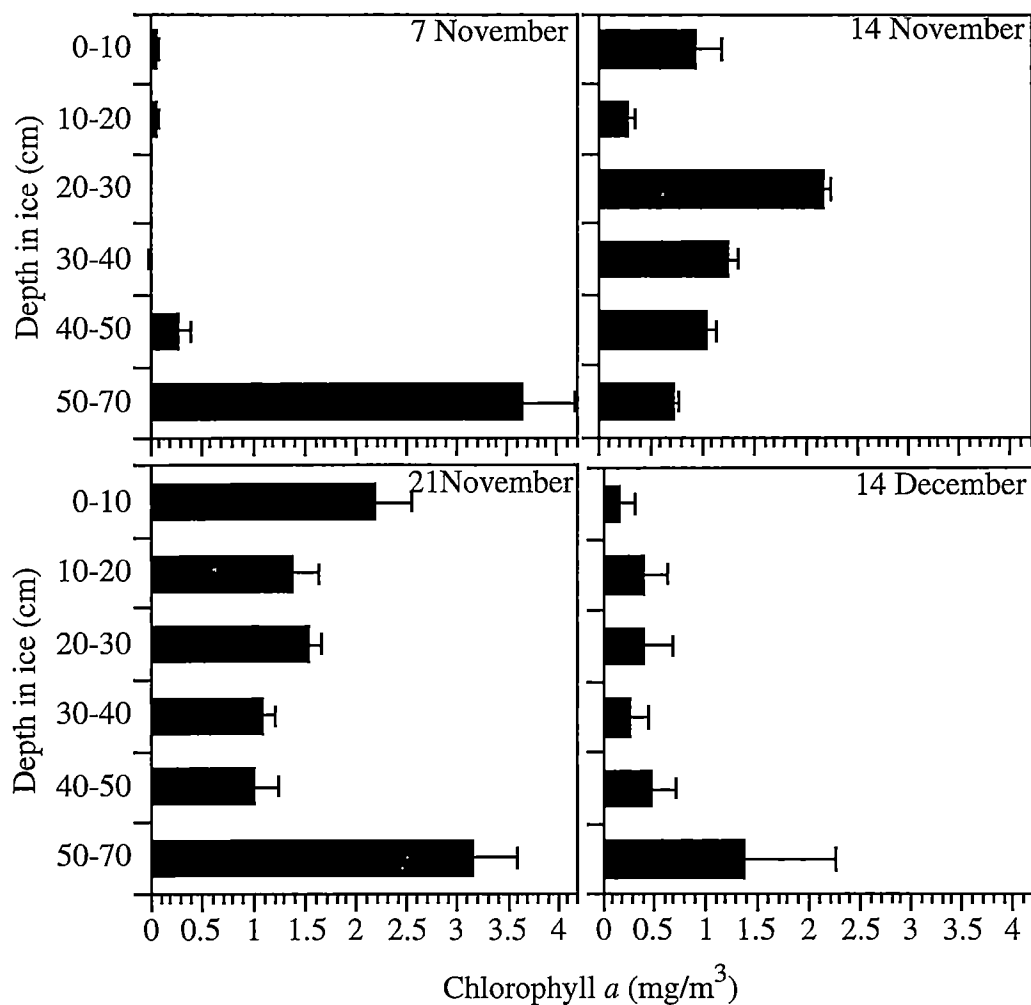
Figure 3.7 Vertical distribution of the phototrophic community in the upper fast ice at O'Gorman Rocks in 1997 (as mean chlorophyll *a* values, n = 3, error bars = 1 sd)

3.2.3 Percentage composition of ice horizons in fast ice

The upper (50 cm) of fast ice at Davis Station accounted for between 5 - 42% of chl *a* in 1996 and 1997 (Table 3.2). Where a substantial bottom ice community was present, the percentage composition of the upper 50 cm of ice ranged from 4.8 – 8.6% of the total chl *a* measured in both years. In the absence of a bottom community, the upper 50 cm accounted for up to 42.4% of total chl *a* in the ice. The 50 – 100 cm horizon generally accounted for less of the total chl *a* then the upper ice. In 1996, the percentage composition of this horizon ranged between 3.1 – 14.5%. The exception

occurred on 7 November 1997, when 92.1% of chl *a* was concentrated between 50 – 100 cm.

Table 3.2 Chlorophyll *a* content and percentage composition in three ice horizons from ice cores at O'Gorman Rocks in 1996 and 1997 (bottom ice = lower 20 cm of ice; bdl = below detection limits)

Year	1996				1997			
Depth in ice (cm)	10/11	17/11	24/11	2/12	7/11	14/11	21/11	14/12
	Chl a (mg/m³)							
0 – 50	4.82	4.99	8.79	13.78	0.4	5.64	7.17	1.75
50 – 100	3.01	2.56	5.34	9.13	4.65	1.73	4.16	2.38
Bottom ice	12.89	75.15	88.04	163.87	bdl	103.62	138.88	bdl
Total Chl *a*	20.72	82.7	102.17	186.78	5.05	110.99	150.21	4.13
Percent composition								
% 0 - 50	23.3	6	8.6	7.4	7.9	5	4.8	42.4
% 50 - 100	14.5	3.1	5.2	4.9	92.1	1.6	2.8	57.6
% Bottom ice	62.2	90.9	86.2	87.7	0	93.4	92.5	0

3.2.4 Brine chlorophyll *a* measurements

Considerable variation between chl *a* values was evident between the five sites in 1997 (Figure 3.8). On 17 November chl *a* values ranged between 0.3 μg l^{-1} at Airport Beach and almost 14 μg l^{-1} at the Plough Island site. Considering mean chl *a* values for Davis fast ice (Figure 3.8), this Plough Island value was responsible for a mid November peak that was not representative of the remaining 4 sites. Although mean values followed an increasing trend from 5 December to a maximum of 6.6 μg l^{-1} by 12 December, no significant difference was calculated between chl *a* at the beginning and end of the season.

Chl *a* data to this point does not take into account the diluting effect of the increasing brine volume, estimated here from increasing brine quantity and decreasing salinity. By the completion of sampling, brine quantity had increased by up to 3 times that measured on 12 November. Normalised chl *a* values, calculated using dilution factors based on brine salinity decreases from 12 November, show a significant

increase ($F_{0.05(1), 8}$ = 5.32) in chl *a* between 12 November and 9 December 1997 (4.32 µg l^{-1} to 27.38 µg l^{-1}) (Figure 3.9).

After extraction, some material was still apparent on the filters. It was unclear whether this material was organic in nature or a result of dust particles deposited on the ice.
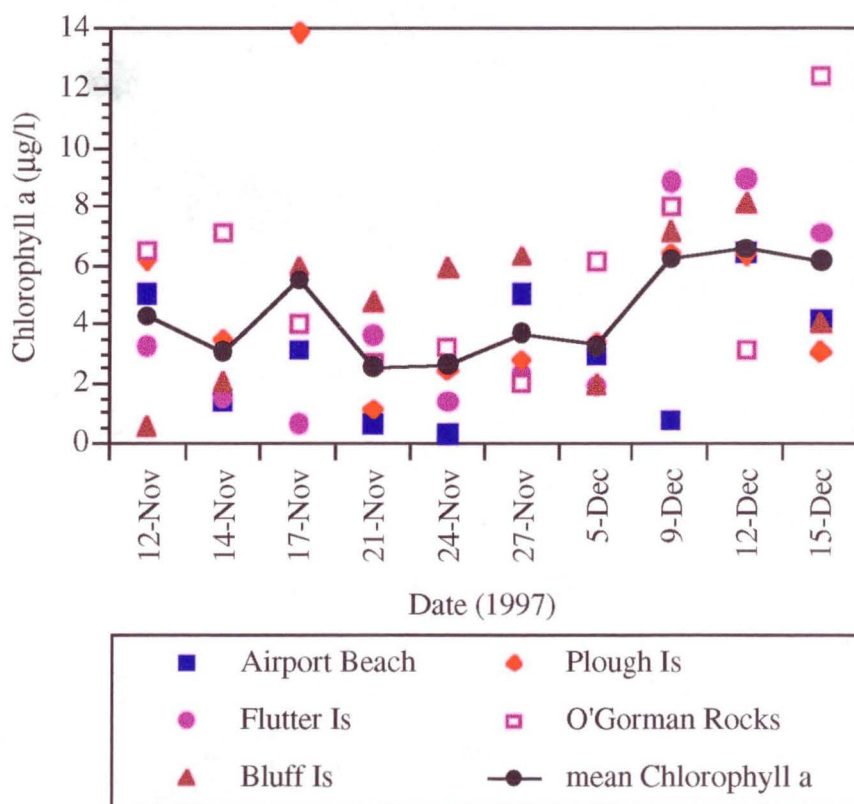
Figure 3.8 Mean and all sites chlorophyll *a* values in the brine from the upper 50 cm of Davis fast ice in 1997 (for means, n = 5)
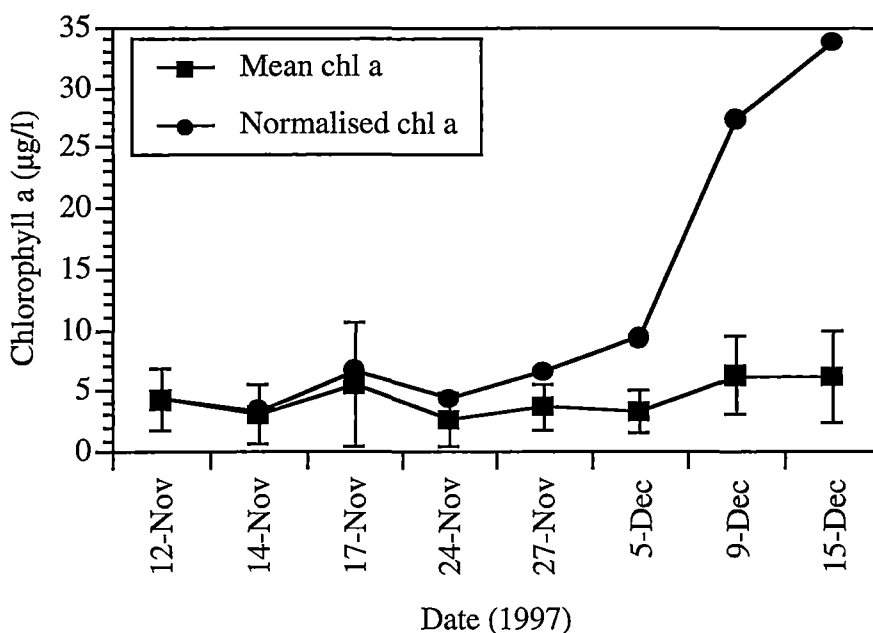
Figure 3.9 Mean and normalised chlorophyll _a_ concentrations in Davis fast ice brine in 1997 (chl _a_ normalised to salinity to account for increasing brine volume within the ice; n = 5, error bars for means = 1 sd)

3.2.5 Community composition and population dynamics

CHEMTAX failed to distinguish algal classes, other than the Dinophyceae and the Cryptophyceae, based on the number and concentration of pigments identified. Table 3.3 shows the pigments identified in the brine by HPLC. Of these, two pigments represent unambiguous markers for different algal classes. Peridinin, a major pigment specific to photosynthetic dinoflagellates, was present throughout the sampling period. Alloxanthin, a major carotenoid known only in the Cryptophyceae, indicated the presence of this class to mid November. The remaining pigments were ambiguous, being present in several algal classes.

Microscopic analysis revealed an upper ice brine community consisting of gymnodinoid dinoflagellates and their life cycle stages, autotrophic flagellates, diatoms, a ciliate and several heterotrophs (Table 3.4).

Table 3.3 Algal pigments identified in Davis fast ice in 1997 (• presence)

Pigment	21/11	24/11	1/12	5/12	9/12
Chlorophyll *a*	•	•	•	•	•
Chlorophyll *b*	•				•
Chlorophyll *c*	•	•	•	•	•
Chlorophyllide *a*					
Peridinin	•	•	•	•	•
Fucoxanthin	•	•	•	•	•
19' Hexanoyloxyfucoxanthin		•	•		
Violaxanthin	•		•		
Dinoxanthin	•		•		•
Diadinoxanthin	•	•	•	•	•
Alloxanthin	•	•			
Diatoxanthin	•	•			•
ß,ß-carotene	•				

Table 3.4 Composition of Davis upper ice brine communities during the summers of 1996 and 1997.

Phototrophic Component Genus/species/life cycle stages	Heterotrophic Component Genus/species
Dinoflagellates	**Dinoflagellates**
Polarella glacialis	*Gyrodinium* sp.1
Asexual/motile stage (10–15 μm)	*Gyrodinium lachryma* Meunier
Sexual planozygote stage (15–25 μm)	*Gymnodinium* sp 2
Cyst stage	*Protoperidinium* sp.
Gymnodinium sp.2 (25–40 μm)	**Others**
Flagellates	Euglenoid sp.
Chrysophytes	*Cryothecomonas* sp 2.
flagellated stage	*Cryothecomonas armigera*
coccoid stage	**Metazoan**
statocyst stage	Larval copepod
Cryptomonas sp.	
Mantoniella sp.	
Flagellate sp.3	
Pyramimonas sp.	
Diatoms	
Nitzschia subcurvata	
Nitzschia lecontei	
Entomoneis kjellmanii	
Fragilariopsis kerguelensis	
Navicula delicatula	
Amphora sp.	
Ciliate (plastidic)	
Strombidium sp.	

Dinoflagellates

Three size classes of gymnodinoid dinoflagellates were evident (10 – 15, 15 – 25 and 25 – 40 μm) in the brine. The smallest size class (10 – 15 μm) included a photosynthetic, gymnodinoid form and its cyst (Figure 3.10a, c). The cyst was morphologically indistinguishable from *Polarella glacialis* (Montresor *et al.* 1999) and the asexual, motile form was identified as *P. glacialis* (Chapter 2). The *P. glacialis* motile cells increased in length as the ice decayed. Early November, cell length was approximately 10 μm. By late November and early December, cell length was 15 μm. *P. glacialis* motile cells and their cysts were observed throughout the fast ice in the Vestfold Hills, being evident in Ellis and Long Fjords, in fast ice adjacent to the Sorsdal Glacier and in the ice cover of saline Ace and Burton Lakes.

The motile, asexual stage of *P. glacialis* was abundant in the brine in November and December of both years (Figure 3.11). In 1996, motile cell numbers peaked on 14 November at a mean concentration of 1.4×10^6 cells l^{-1} before declining towards late December. This contrasted with the lower concentrations in 1997 and the later population peak around 5 December at 0.9×10^6 cells l^{-1}. Despite the apparent increase in motile cell numbers between 12 November and 5 December 1997, no significant growth was calculated ($F_{44, \, 2054} = 2.037$).

When *P. glacialis* cell numbers are normalised (by salinity dilution factors) to account for the increasing brine quantity, the peak population on 5 December 1997 was 2.5×10^6 cells l^{-1}. This represents a 12 fold increase in number from 12 November in the same year.
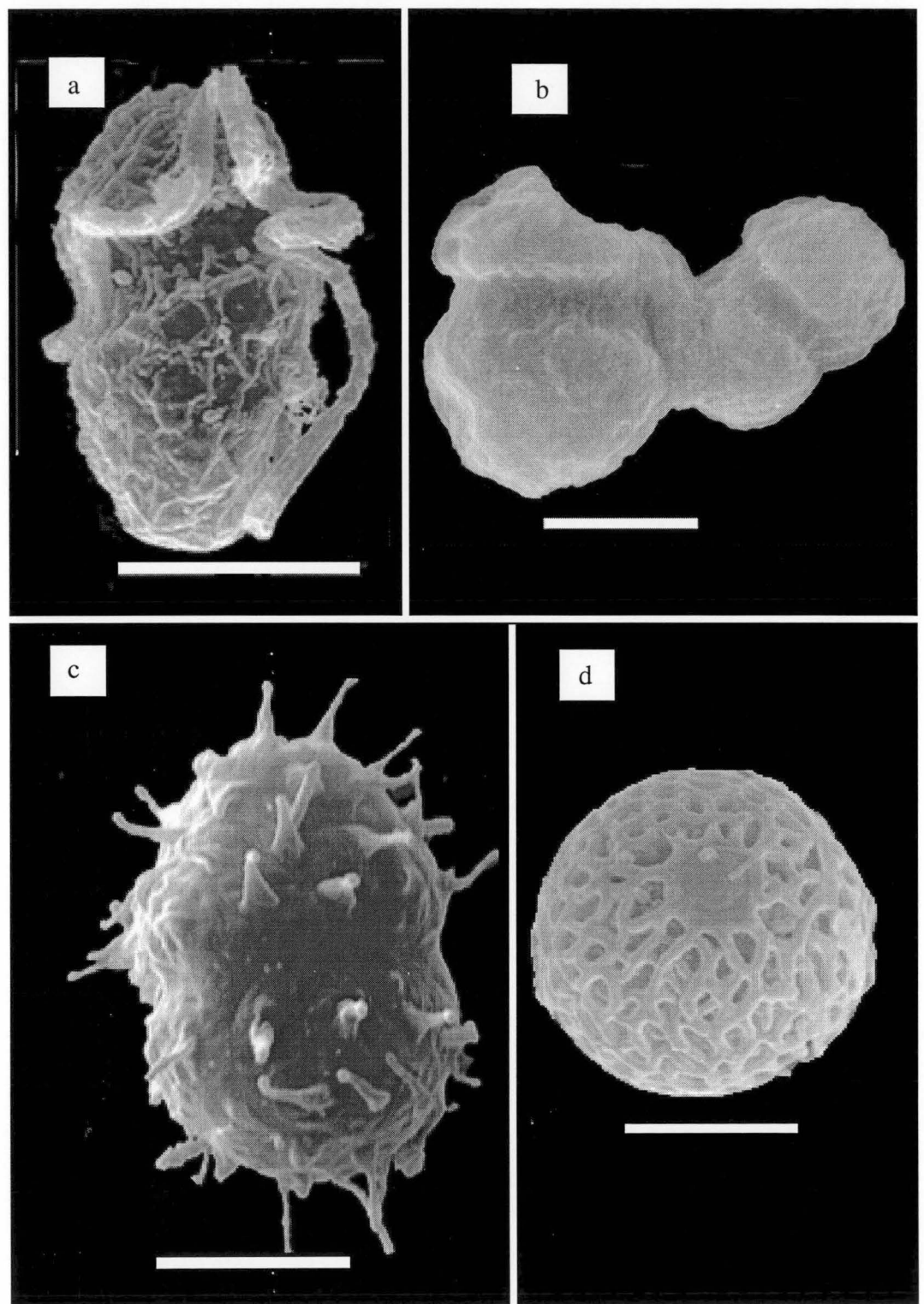
Figure 3.10 a) Lateral view of *Polarella glacialis* asexual cell (missing one flagellum), b) Lugol's preserved, fusing/dividing *P.glacialis* cells, c) *P.glacialis* cyst and d) chrysophyte statocyst similar to *Archaeomonas areolata* Deflandre (all scale bars 5 μm)
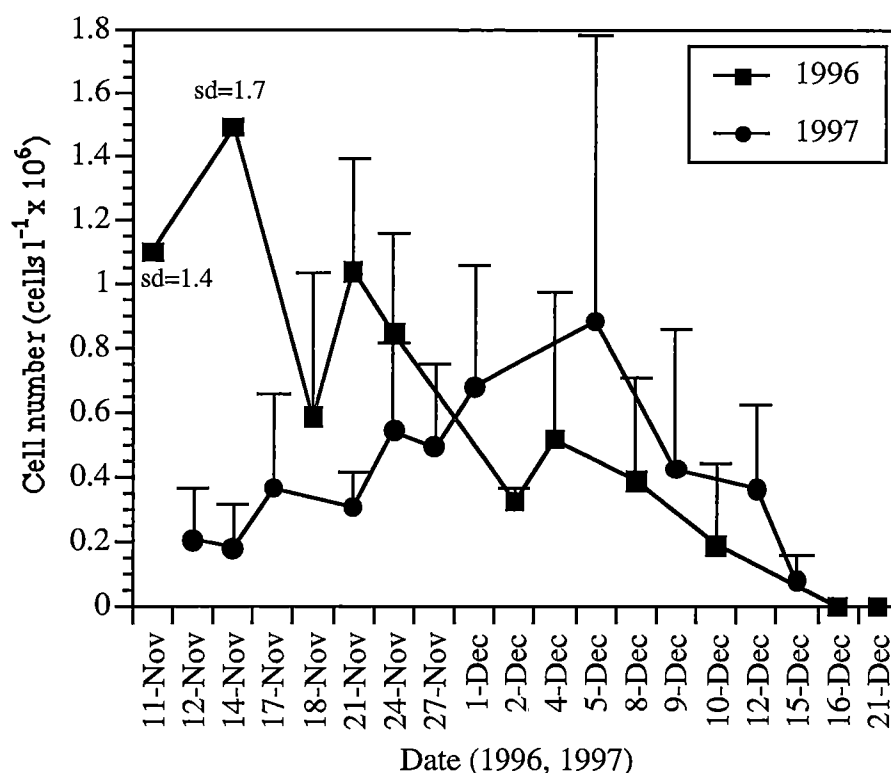
Figure 3.11 Mean motile *Polarella glacialis* cells in Davis fast ice brine in 1996 and 1997 (1996, n= 3. 1997, n = 5. Error bars represent 1 sd)

Sexuality of *P. glacialis* was evident from early November in both years when fusing/dividing cells were observed in the brine (Figure 3.10b). The second size class of dinoflagellate (15 – 25 μm), often observed with two trailing flagellae, were also evident from early November and appeared to be the planozygote of *P. glacialis*. Planozygote numbers in 1996 remained low (< 0.7 x 10^4 cells l^{-1}) until about 8 December when they increased to a maximum mean concentration of 10.4 x 10^4 cells l^{-1} (Figure 3.12). In 1997, the planozygotes increased in concentration from their first appearance on 12 November to a mean maximum of about 13.9 x 10^4 cells l^{-1} around 1 December. In both years, planozygote concentrations decreased towards the end of the sampling period.
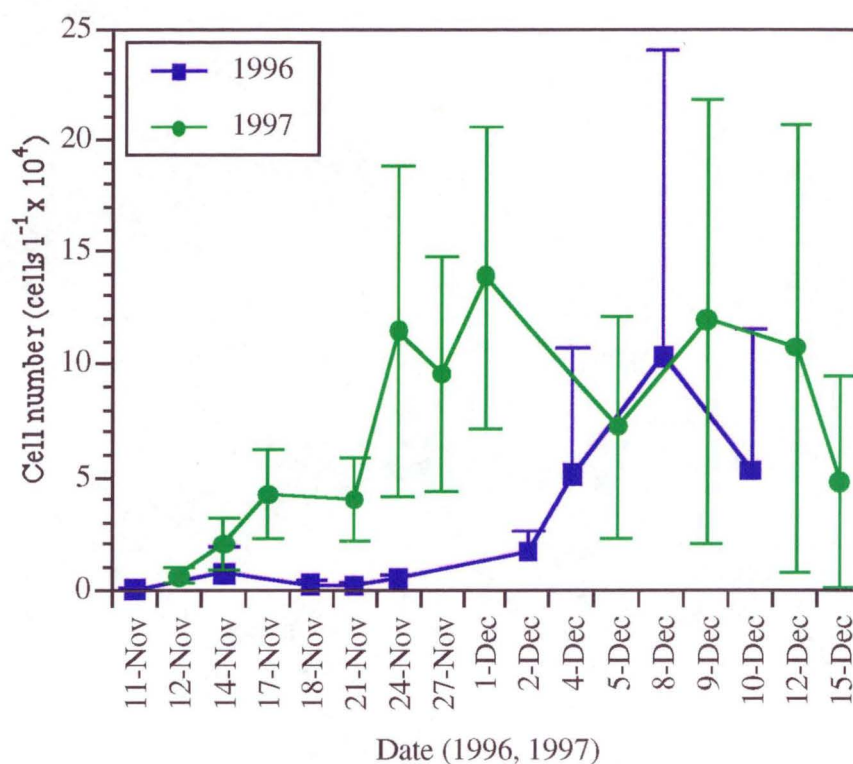
Figure 3.12 Mean *Polarella glacialis* planozygote numbers in Davis fast ice brine during 1996 and 1997 (1996, n= 3. 1997, n = 5. Error bars represent 1 sd)

In early November both years, only low numbers of *P. glacialis* cysts (< 1000 cells l^{-1}) (Figure 3.10c) were obvious in the brine (Figure 3.13). In 1996, cyst numbers began increasing from 24 November and peaked at 2.73 x 10^6 cells l^{-1} on 10 December. In 1997, fewer cysts were observed in the brine than the previous year. Cyst numbers in 1997 began increasing from 5 December and peaked at 0.43 x 10^6 cells l^{-1} (1.2 x 10^6 cells l^{-1} when normalised) on 12 December. Cyst concentrations declined rapidly after peaking in both years.
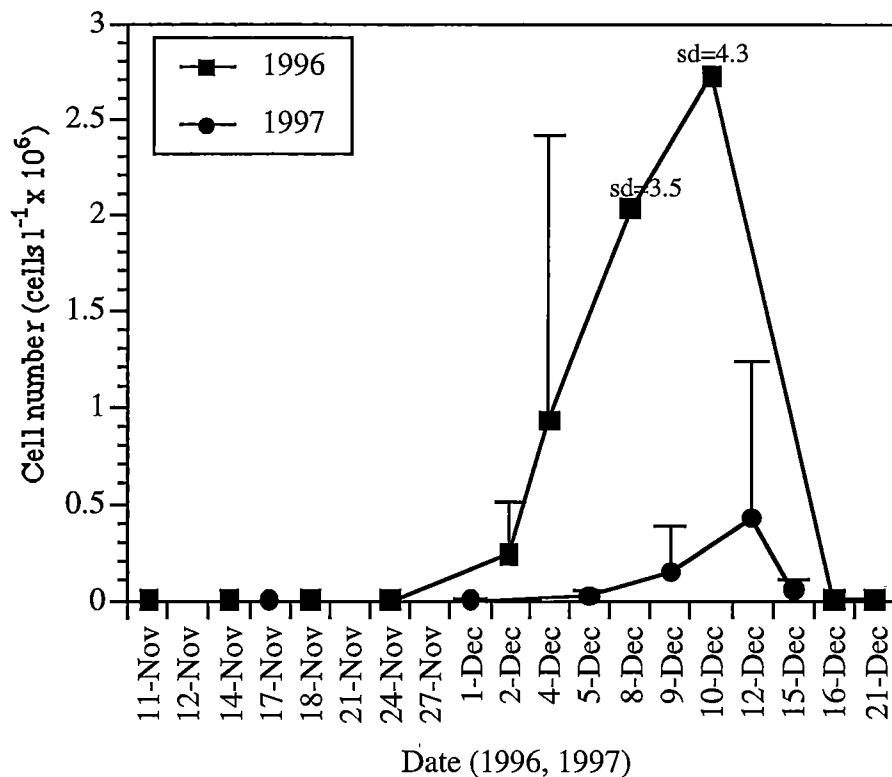
Figure 3.13 Mean *Polarella glacialis* cyst numbers in Davis fast ice brine during 1996 and 1997 (1996, n= 3. 1997, n = 5. Error bars represent 1 sd unless otherwise shown)

The final size class of dinoflagellates included a 25 – 40 μm gymnodinoid species, *Gymnodinium* sp. 2 (Figure 3.14a). In 1996, this species was present in low numbers (< 1000 cells l^{-1}) until 2 December (Figure 3.15). From this date, numbers increased to peak at 4.8 x 10^4 cells l^{-1} around 8 December. In 1997, this species was present in higher concentrations from mid November and mean maximum concentrations peaked at 3.3 x 10^4 cells l^{-1} by 5 December. Cell concentrations in the brine fell rapidly after peaking in both years. Low numbers (< 1000 cells l^{-1}) of *Gymnodinium* sp. 2 were observed fusing or dividing between 2 – 8 December in 1996.

Gymnodinium sp. 2, when observed at the lower limit of its size range, was difficult to distinguish from the *P. glacialis* planozygote. It is possible some errors in identification and counting were made when similarities in size range were encountered.
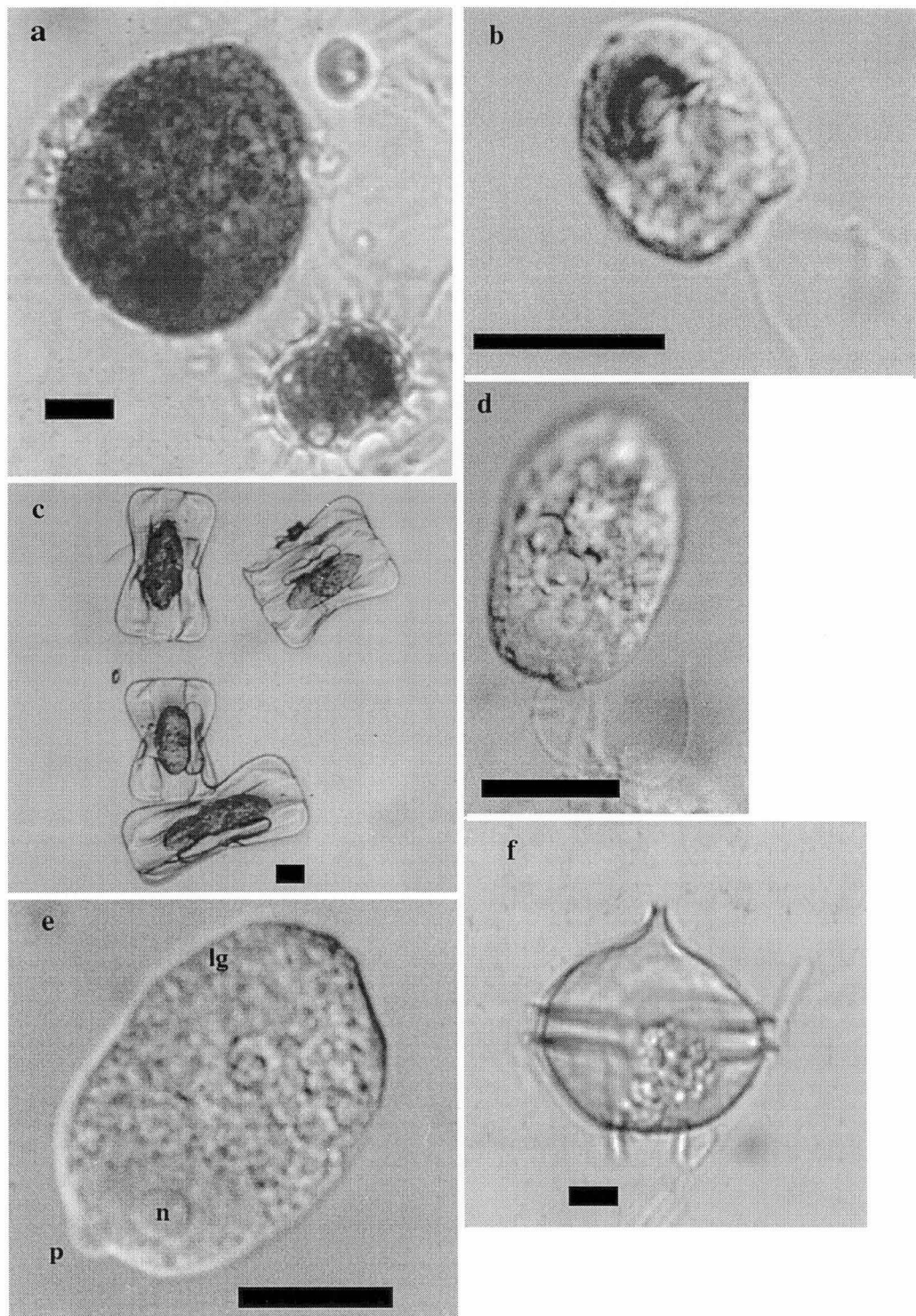
Figure 3.14 Light micrographs of auto (a – c) and heterotrophic species (d-f) in Davis fast ice brine a) 40 μm *Gymnodinium* sp. 2 cell and *P. glacialis* cyst b) *Pyramimonas* sp.2 c) late season *Entomoneis kjellmanii* cells d) *Cryothecomonas armigera* e) *C.armigera* cell showing lateral grove (lg), nucleus (n) and papilla and f) *Protoperidinium* sp. (all scale bars = 10 μm)
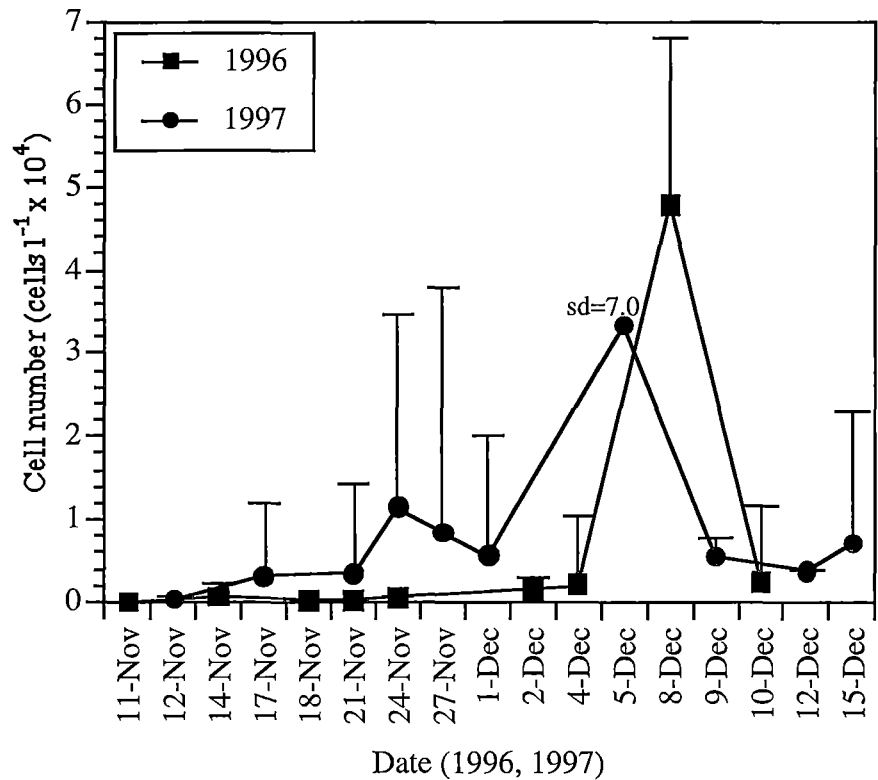
Figure 3.15 Mean *Gymnodinium* sp.2 (25 – 40 μm) numbers in Davis fast ice brine during 1996 and 1997 (1996, n = 3. 1997, n = 5. Error bars represent 1 sd unless otherwise shown)

Chrysophytes and unidentified < 10 μm flagellates

Chrysophyte statocysts were a prominent part of the brine community during early December in both years. The most numerous statocyst was a 7 – 10 μm spherical cell similar to *Archaeomonas aerolata* Deflandre (Figure 3.10d). Two other unidentified statocysts were present in mid December and were observed under LM but not under SEM. The most common of these was approximately 10 μm in diameter, appeared to have a surface ornamentation of rod-like projections and a thickened, apical collar. The remaining statocyst, observed only rarely in 1997, was 7 μm in diameter and consisted of a central, cell body surrounded by a thin sheath with long radiating spines. Positive identification of the chrysophyte vegetative and coccoid cells was only achieved in 1997.

Only low numbers of statocysts (< 100 statocysts l^{-1}) were counted on 11 November
1996 (Figure 3.16). The statocysts were not recorded again until 24 November.
From this date, statocyst numbers increased dramatically to peak at almost 2.5 x 10^6
cells l^{-1} by 8 December. After 10 December, cell numbers had declined to < 2000
cells l^{-1}.
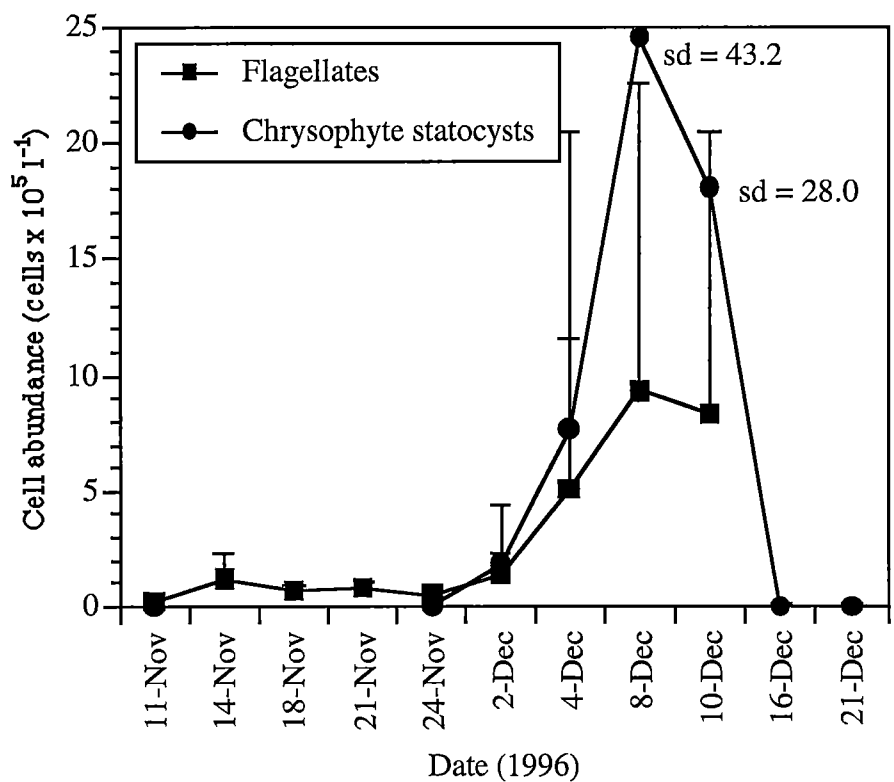
**Figure 3.16 Chrysophytes, other flagellates (< 10 μm) and chrysophyte statocysts
in Davis fast ice brine in 1996** (n = 3, error bars represent 1 sd unless otherwise shown)

In 1996, a < 10 μm class of unidentified flagellates and non motile cells (suspected to
have included chrysophyte flagellate and coccoid stages) peaked in abundance mid
December (Figure 3.16). This class increased in number (1.19 x 10^5 cells l^{-1}) to 14
November. A second peak coincided with the statocyst peak on 8 December with
cell concentrations at 9.34 x 10^5 cells l^{-1}. This class was not detected in the brine after
10 December.

In 1997, many of the <10 μm class were assigned to the Chrysophyceae. This
chrysophyte category showed bimodal peaks throughout the sampling period (Figure

3.17). The first peak occurred mid November with cell abundance at 4.40 x 10^5 cells l^{-1}. The majority of cells in this peak were attributed to 5 – 7.5 μm, biflagellated cells believed to be vegetative chrysophytes. The second peak occurred mid December at 6.12 x 10^5 cells l^{-1} and consisted predominantly of ovoid to spherical cells 7.5 – 10 μm in diameter, identified as the coccoid stage of the chrysophyte life cycle. Coccoid cells were evident in the brine until the end of sampling.

In 1997, up to 4.0 x 10^3 statocysts l^{-1} were recorded in the brine to 14 November before these cells became rare or undetected in late November (Figure 3.17). From this time, statocyst numbers increased to peak at about 1.3 x 10^6 cells l^{-1} (3.6 x 10^6 cells l^{-1} when normalised) by 12 December. Thereafter, concentrations decreased rapidly to 6.0 x 10^3 cells l^{-1} on 15 December.
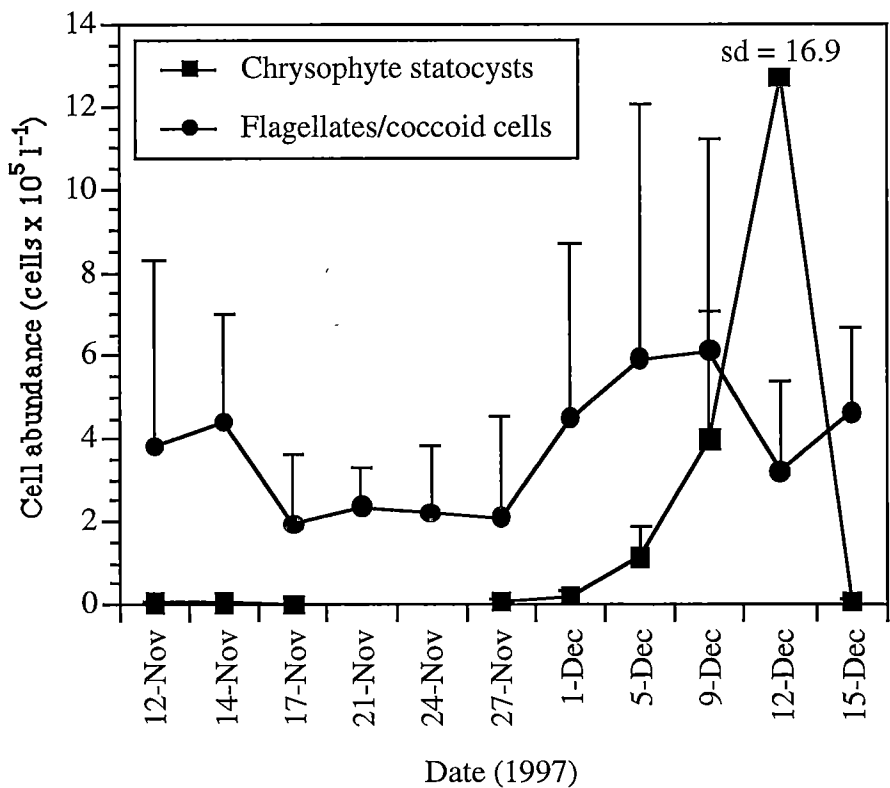
Figure 3.17 Vegetative and coccoid chrysophyte cells and chrysophyte statocysts in Davis fast ice brine in 1997 (n = 5, error bars represent 1 sd unless otherwise shown)

Other Flagellates

Other flagellates that were identified in the brine included *Cryptomonas* sp.,
Pryramimonas spp., *Mantoniella* sp. and one Euglenoid species (Table 3.5).
Cryptomonas sp., a biflagellated cell of 5 - 7 x 15 – 20 μm, was identified by a
combination of microscopic observations and by the detection of the pigment
alloxanthin. *Cryptomonas* sp. was present to mid November in both seasons. This
species peaked in abundance early in mid November at 7.32×10^4 cells l^{-1} in 1996 and
5.49×10^4 cells l^{-1} in 1997 and was not detected after this time.

**Table 3.5 Mean abundance of several flagellate species in Davis fast ice brine
during 1996 and 1997** (n = 3, 1996 except for 16 Dec onwards where n = 3 from O'Gorman
Rocks only; n = 5, 1997. 1 standard deviation shown in brackets below cell abundance)

Flagellate sp.	Mean abundance (x 10^4 l^{-1})										
1996 Date	11/11	14/11	17/11	21/11	24/11	2/12	4/12	8/12	10/12	16/12	21/12
Cryptomonas sp.	7.32 (6.74)	5.89 (1.86)	2.35 (1.54)	0.52 (0.12)							
Pyramimonas sp. 1		0.23 (0.40)	0.75 (0.14)	0.36 (0.15)	0.79 (0.34)	1.49 (1.23)	3.06 (3.56)	1.5 (1.62)	0.5 (0.64)	0.1 (0.13)	
Pyramimonas sp. 2								13.78 (22.61)	26.16 (43.65)	0.01 (0.08)	0.18 (0.35)
1997 Date	12/11	14/11	17/11	21/11	24/11	27/11	1/12	5/12	9/12	12/12	15/12
Cryptomonas sp.	4.20 (3.16)	5.49 (1.04)	0.83 (0.51)								
Pyramimonas sp.1	0.25 (0.05)	0.46 (0.45)	0.56 (0.2)	0.28 (0.17)	0.49 (0.30)	0.42 (0.18)	1.15 (0.86)	0.9 (1.4)	0.3 (0.68)		
Flagellate sp.3	0.43 (0.32)	0.65 (0.46)	1.04 (1.0)								
Mantoniella sp.				0.26 (0.20)	0.80 (0.74)	0.19 (0.51)	0.96 (0.89)	1.61 (1.37)	2.59 (1.43)	3.67 (2.72)	
Pyramimonas sp.2								3.53 (6.26)	6.28 (7.56)	27.23 (55.09)	2.29 (12.28)

Two species of *Pyramimonas* were present in the brine. *Pyramimonas* sp.1 (5 x 7.5
μm in size) was present throughout the each sampling period. This species was most
abundant early December in both years. *Pyramimonas* sp.2 (7 x 10.5 μm) (Figure
3.14b) appeared suddenly in both years from around 5 December and peaked at up to
27.23×10^4 cells l^{-1}.

Mantoniella sp., a 4 - 5 μm prasinophyte with one long trailing flagellum and one distinctly shorter one, was identified in the brine in 1997. This species was present from 21 November at approximately 0.26 x 10^4 cells l^{-1} and increased in abundance to 3.67 x 10^4 cells l^{-1} by 12 December.

A 7.5 x 12.5 μm flagellate, flagellate sp.3, was recorded in the ice between 12 and 17 November 1997, ranging from 0.43 to 1.04 x 10^4 cells l^{-1}. This species was dorso-ventrally flattened, with a weakly pronounced flagellar groove. Two flagellae, one trailing and one directed forward were present. Whilst positive identification was not made, it was suspected that this flagellate was a raphidophyte.

Diatoms

Only a few species of living diatoms were observed in the brine (Table 3.4). Of these, *Nitzschia subcurvata* was the only species reaching bloom proportions (Table 3.6). In 1996, *N. subcurvata* was present throughout the sampling period until 10 December. The Airport Beach site showed the most consistent population, although it was recorded as at least present (< 1000 cells l^{-1}) at most sites on most dates. The *N. subcurvata* population peaked at 122.33 x 10^4 cells l^{-1} on 8 December. In 1997, this species was present at much lower concentrations, reaching a mean maximum concentration of 0.61 x 10^4 cells l^{-1} by 24 November.

Navicula delicatula was evident throughout sampling in 1997 but absent in 1996 (Table 3.6). In 1997, this species reached a maximum abundance on 24 November of 2.87 x 10^4 cells l^{-1}. *Navicula lecontei*, *Entomoneis kjellmanii* (Figure 3.14c), *Amphora* sp. and *Fragilariopsis kerguelensis* occurred late in the 1996 sampling period in low numbers (< 1000 cells l^{-1}). These species were absent in 1997. Empty diatom frustules (*Cocconeis* spp., *Odontella* sp., *Chaetoceros* sp., *Corethron* sp., *Fragilariopsis curta* and *Eucampia antarctica*) were common in the brine.

Table 3.6 Diatoms in Davis fast ice brine (sd in brackets following numbers; P = present)

Diatom species	Cell numbers (x 10^4 l^{-1})										
1996	11/11	14/11	17/11	21/11	24/11	2/12	4/12	8/12	10/12	16/12	21/12
Nitzschia subcurvata	0.50 (0.68)	14.73 (25.21)	5.35 (8.54)	2.89 (4.22)	2.71 (2.44)	31.08 (50.52)	0.48 (0.83)	112.33 (185.86)	77.76 (131.05)		
Fragilariopsis kerguelensis									P	P	P
Entomoneis kjellmanii										P	P
Amphora sp.									P	P	P
1997	12/11	14/11	17/11	21/11	24/11	27/11	1/12	5/12	9/12	12/12	15/12
Nitzschia subcurvata		0.02 (0.04)	0.01 (0.02)	0.01 (0.02)	0.61 (0.07)	0.30 (0.22)	0.23 (0.30)	0.25 (0.21)	0.18 (0.40)	0.01 (0.15)	0.007 (0.014)
Navicula delicatula	0.23 (0.30)	0.87 (1.58)	0.30 (0.32)	0.47 (0.55)	2.87 (1.70)	0.55 (0.32)	1.59 (2.11)	0.49 (0.82)	0.40 (0.47)	0.54 (0.97)	0.21 (0.19)

The ciliate

Strombidium sp. was the only ciliate recorded in the brine channels. *Strombidium* sp. was present in the brine throughout the sampling period in 1996 (Figure 3.18), peaking at 2.4 x 10^3 cells l^{-1} early in December. In 1997 *Strombidium* sp. was evident from early December, with peak abundance occurring by mid December at 2.5 x 10^3 cells l^{-1}. A rapid decrease in the population occurred from this date until 21 December when they were not detected.
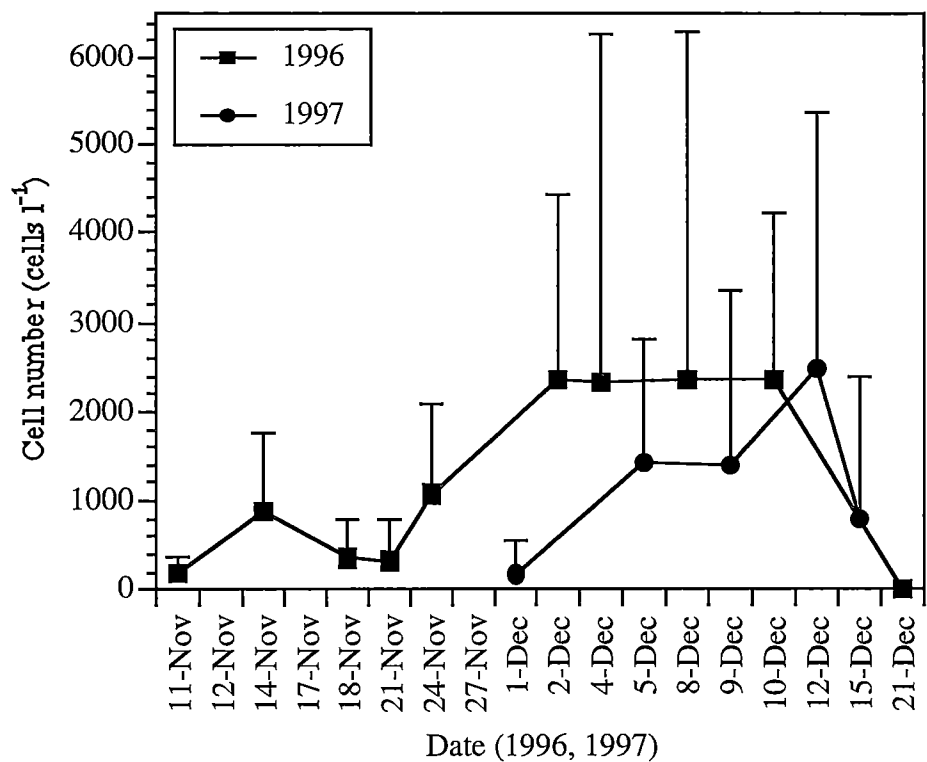
Figure 3.18 Mean *Strombidium* sp. cell numbers in Davis fast ice brine during

1996 and 1997 (1996, n = 3 except for 21 December when counts only taken at O'Gorman

Rocks. 1997, n = 5. Error bars represent 1 sd)

Heterotrophs

Several heterotrophic species were present in the brine (Table 3.4). Two species of

Cryothecomonas dominated the heterotrophic assemblage between 12 – 17

November 1997 (Figure 3.19a). *Cryothecomonas* sp.2, typically elongate (up to 20

μm x 10μm), was the more abundant and persistent species throughout the sampling

period, peaking in number on 14 November (5.49 x 10^4 cells l^{-1}) before declining

rapidly to lower concentrations. *Cryothecomonas* sp.2 remained present until early

December. The other species, *Cryothecomonas armigera* (Figure 3.14d-e), was

recorded at a mean maximum of 1.15 x 10^4 cells l^{-1} in early November before

numbers also fell quickly after 12 November 1997. This species was not detected

after this date.
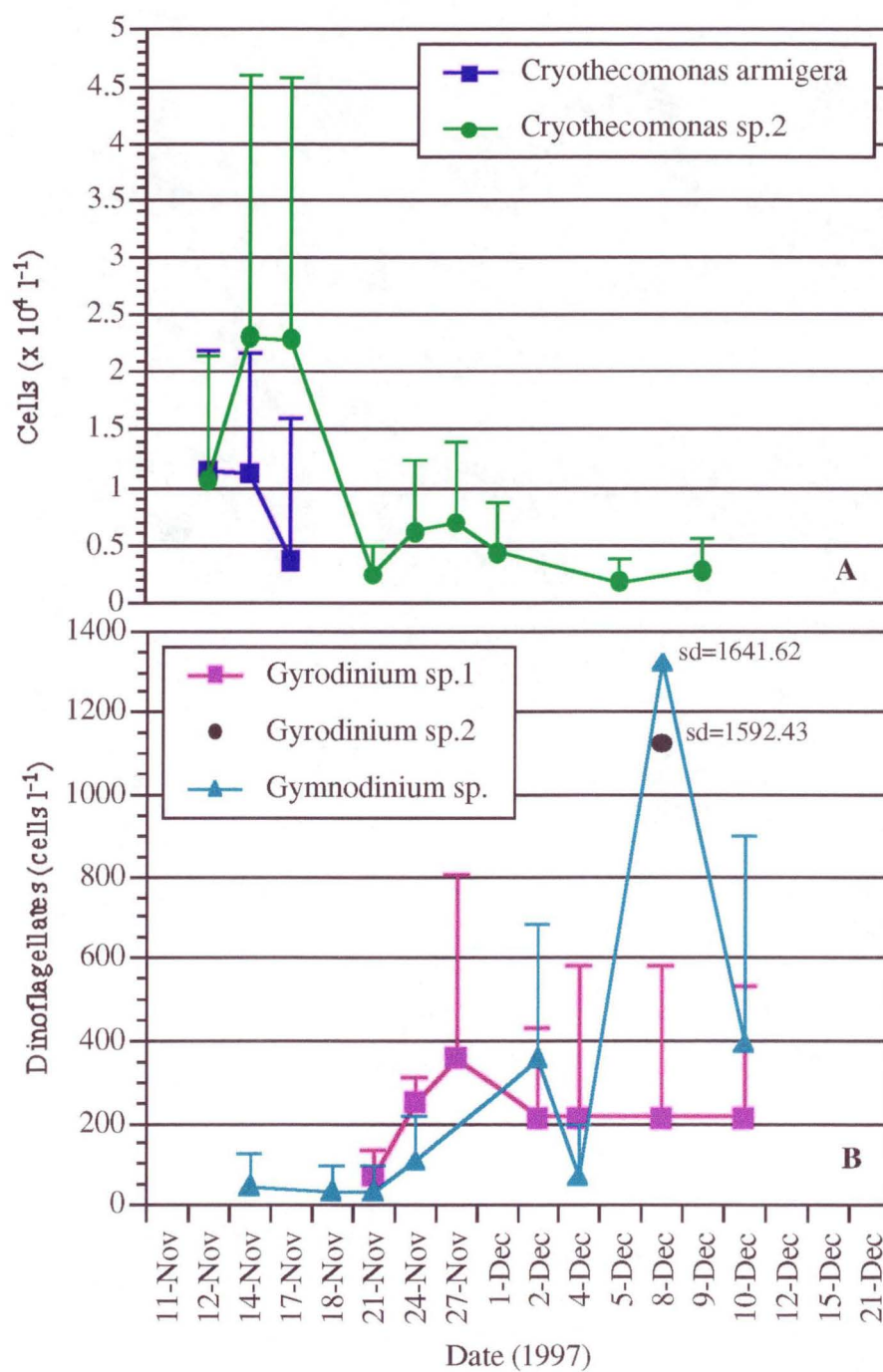
Figure 3.19 Mean *Cryothecomonas* spp. A) and heterotrophic dinoflagellate cell numbers B) in Davis fast ice brine in 1996/97 (n = 3, 1996; n = 5 1997. Error bars represent 1 sd unless otherwise shown)

Concomitant with the decline of *Cryothecomonas* spp. was the detection of a heterotrophic *Gymnodinium* sp., 17 - 20 μm in length, around 14 November (Figure 3.19b). *Gymnodinium* sp. numbers remained low (< 100 cells l^{-1}) until 24 November. Thereafter, cell numbers peaked at 1.3 x 10^3 cells l^{-1} on 27 November before dropping abruptly by 10 December.

One species of *Protoperidinium* was recorded from the brine (Figure 3.14f). *Protoperidinium* sp., 55 μm in diameter, was present in the brine in low concentrations from the beginning of sampling. Its mean abundance, however, increased rapidly from 2 December when it reached 3.2 x 10^3 cells l^{-1}. *Protoperidinium* sp. was not recorded after 10 December.

Two species of heterotrophic *Gyrodinium* were also recorded from the brine (Figure 3.19b). *Gyrodinium* sp.1, 37 μm in length, reached its mean maximum abundance of 0.4 x 10^3 cells l^{-1} by 27 November. *Gyrodinium lachryma*, 62 μm in length, was observed in the brine only on 8 December at 1.1 x 10^3 cells l^{-1}. This species was evident in the underlying seawater in November and December but did not become abundant until January.

A 30 μm Euglenoid species, similar to Euglenoid sp.2 observed by Archer *et al.* (1996) in the upper ice, was present in both years. This species was only rarely observed and at low concentrations (<150 cells l^{-1}) between 17 November and 5 December 1996 and 1997.

The metazoan

One larval Harpacticiod copepod, possibly *Drescheriella glacialis* (Dahms and Dieckmann), was observed in the brine from O'Gorman Rocks on 16 December, 1996 (Figure 3.20). *D. glacialis* is described as a sympagic copepod that enacts its life cycle principally within the bottom ice horizon of fast ice (Dahms and Dieckmann 1987). This species has previously been observed in Davis and Ellis Fjord fast ice (Kirkwood 1993; Swadling 1998) and in ice of the Weddell Sea (Dahms and Dieckmann 1987).
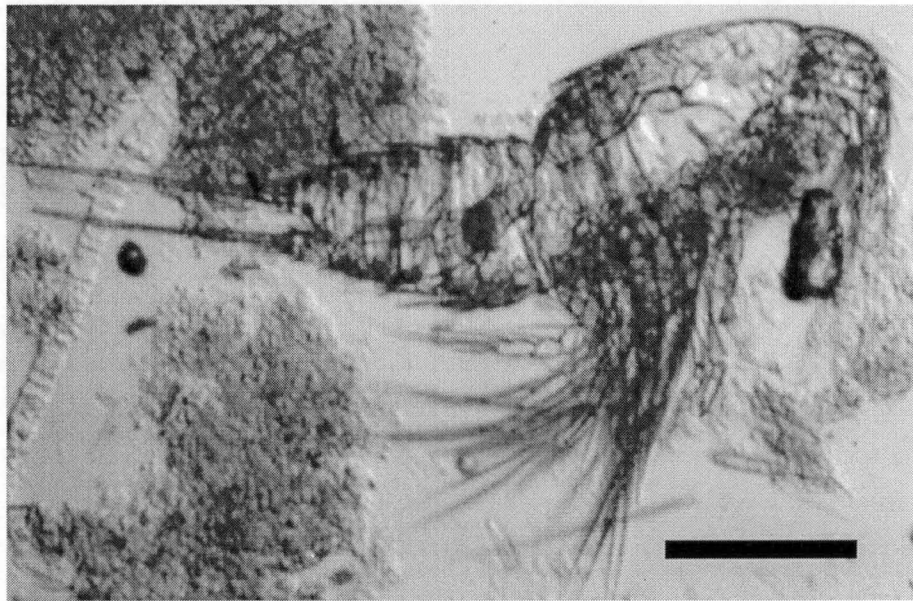
Figure 3.20 Copepod larva (possibly *Drescheriella glacialis*) observed in Davis upper ice brine mid December (scale bar = 100 μm)

3.2.6 Water samples

Seawater samples taken beneath the ice cover showed the presence or absence of *P. glacialis* cysts (Figure 3.21). In samples collected in the 1996/97 season, the cysts were not detected in the water column until 8 December. On 16 December, just prior to ice break out, a pulse of cysts of about $1.4 \times 10^4 \, l^{-1}$ was recorded in the water column between 0 and 20 metres of depth. Samples following this date were taken in the absence of ice cover and cyst numbers ranged between 0.08 and 0.2×10^4 cysts l^{-1}.
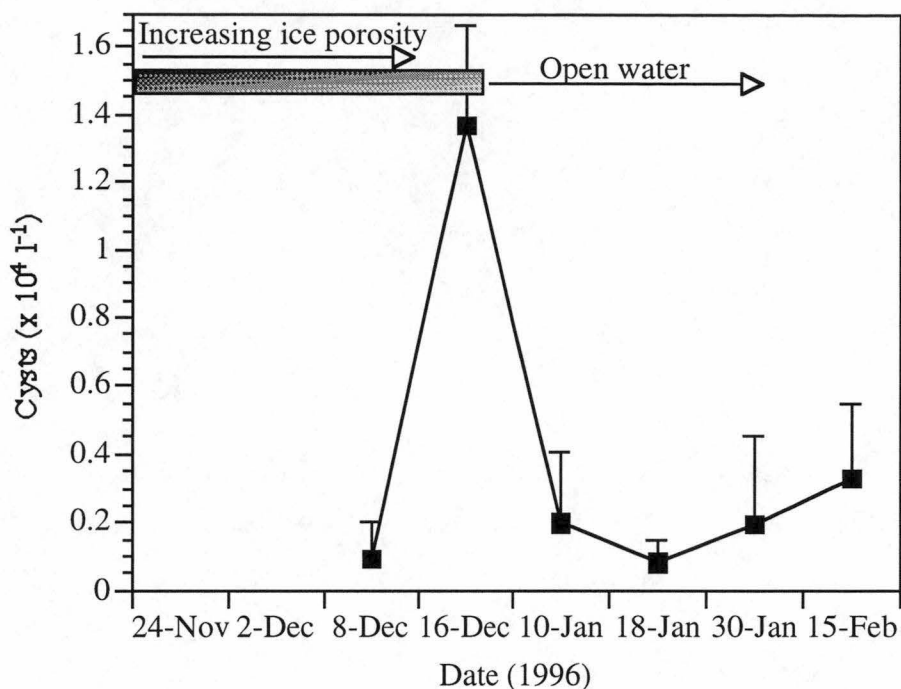
Figure 3.21 Total *P. glacialis* cysts in sub-ice and ice cover free water samples (between 0 – 20 m) taken adjacent to O'Gorman Rocks in the 1996 season (error bars indicate sd calculated from triplicate counts)

3.3 Discussion

3.3.1 Physical and chemical parameters

The upper ice brine environment was a cold habitat. The lowest mean temperature recorded in the brine was almost -4.5 °C, occurring at the beginning of sampling in November. By late December, brine temperature had risen to a maximum of about -0.2 °C.

Brine temperatures in 1996 were warmer than in 1997, particularly at the beginning of sampling where there was almost a 1 °C difference in the means. The heavy snow cover prior to the beginning of sampling in 1997 and absent in 1996 could account for this difference. Temperatures in both years continued to differ substantially until approximately 5 December when they converged. Brine warming in 1996 occurred at a faster rate than in 1997 despite the higher air temperatures that occurred in this year. Again, this was probably a result of the light snow cover in 1996. In 1997, two heavy snow falls inhibited brine warming between 17 and 27 November. Overall,

brine warming in the upper ice in both years was influenced by air temperature. Brine temperatures rose quickly with increasing air temperatures early in November and responded to the cooler air period from mid November by showing insignificant temperature increases.

The highest mean salinity recorded in both years was 94 psu, almost 3 times that of seawater. By late December, the lowest mean salinity recorded was 1/6 that of seawater, falling as low as 5 psu. Brine salinity in both years varied in thermodynamic equilibrium with brine temperature, showing a high degree of negative correlation. Both brine warming and desalination were subject to interannual variability depending on snow cover.

Seasonal fast ice decay was evident in each year. Increasing brine volume, a measure of ice decay, was identified by both the time it took for brine to flow from the ice and fill the drill holes and the quantity of brine that accumulated. From 11 November, brine accumulation times were slow (24 hrs) but by early December, the holes flooded immediately on drilling. Early in November, accumulated brine quantity in the holes was approximately 7.6 l but this quickly increased to a maximum of 23 l towards the end of December. Another indication of ice decay was the appearance of a freeboard layer in early December.

Further evidence of ice decay in 1996 is reflected in the detection of dinoflagellate cysts in the water column in mid December. *P. glacialis* cyst abundance in the upper ice decreased rapidly from about mid December when ice porosity was high and brine quantities at a maximum. Prior to 8 December, *P. glacialis* cysts were not detected in the water column. By 16 December, up to 1.4×10^4 cysts l^{-1} were recorded in the underlying water. It appears that brine drainage from the upper ice transferred the cysts to the water column at this time.

Brine drainage also explains the sudden decrease in the abundance of other cells in 1996 (statocysts, *Pyramimonas* sp.1, *N.subcurvata*) and was likely to be responsible for the decreases seen in mid December 1997. In contrast, such an event may also explain the sudden appearance of several diatom species at this time. *Entomoneis kjellmanii*, for example, was noted as the dominant species of the bottom ice

community in 1996 but was not recorded in the upper ice until mid December. It is possible that this diatom and other species, such as *Pyramimonas* sp.2, were inoculated into the upper ice by the oscillatory flow of brine and seawater during brine drainage and flushing (Weeks and Ackley 1982). The occurrence of the larval copepod in the upper ice around this time also illustrates this.

In 1997, nutrients in the brine decreased as the season progressed. These decreases could be attributed to a combination of factors. Algal growth would have contributed at least partially to the decrease. The earliest sample for nutrients at Davis were taken during a period of active growth of the algal community and nutrient concentrations would be expected to be somewhat depleted by this time. Furthermore, increasing brine volume during this period would have diluted nutrient concentrations. Between 1 and 9 December, at least a 3.5 fold decrease in nutrients were recorded. At this time, the ice was becoming extremely porous and it is probable that brine drainage from the ice was responsible for this decrease. Ackley and Sullivan (1994) have suggested that brine exchange with the underlying sea water when ice porosity is high is important in introducing inorganic nutrients to the ice interior. Enrichment of the upper ice with nutrients from the underlying seawater was not apparent in this study.

DIN concentrations were low throughout the sampling and did not approach the highest values of 2.4 μM recorded in fast ice in mid November by Stoecker *et al.* (1998). However, similarly low values to the Davis minimums (< 0.2 μM) were recorded in McMurdo Sound by mid December. High silicate values (54.35 − 8.75 μM) were recorded in the Davis brine and Dieckmann *et al.* (1991) have found higher concentrations of silicate in congelation ice as opposed to other ice types. The ice structure of Davis fast ice, composed primarily of congelation ice, may therefore have contributed to this high concentration. As diatom growth in the brine in 1997 was low, the decrease in silicate concentrations in the brine was most likely due to dilution by increasing brine volume and by brine drainage around mid December.

3.3.2 Vertical distribution and percentage composition of autotrophic community in the upper fast ice

In both years, the vertical distribution of the autotrophic community was initially concentrated below 40 cm depth. In 1996, the highest concentrations remained below 30 cm depth but in 1997 the autotrophic community became more evenly distributed as the season progressed. Increasing brine volume, ice porosity and therefore habitat space most likely all contributed to this spread in distribution.

The upper 50 cm horizon of Davis fast ice accounted for a significant percentage of the autotrophic community throughout the ice. In the absence of a bottom ice community, this horizon contributed up to 42% of chl a in the ice at O'Gorman Rocks.

3.3.3 Brine chlorophyll a measurements

Chl a measured directly from the brine failed to show significant growth of the autotrophic community in 1997. However, normalised values accounting for brine volume increases showed a significant increase in chl a between 12 November and 9 December.

The spatial variability of the brine communities contributed to this somewhat, with widely ranging concentrations recorded between the sites. Spatial variability in sea ice communities is not uncommon and has been previously noted (Garrison 1991). Furthermore, it was suspected that not all chlorophyll in the samples was extracted. Stoecker et al.(1997) also experienced inefficient chlorophyll extraction from McMurdo Sound upper ice communities. It is possible that extraction resistant species may have confounded chl a measurements as a biomass indicator.

3.3.4 Community Composition and population dynamics

CHEMTAX, whilst successfully used in phytoplankton (Wright et al. 1996), failed to identify algal classes in the brine other than the dinoflagellates and cryptophytes. Pigments and carotenoids identified in the samples did not produce sufficient markers to allow this. Another confounding factor was the integration within the samples of cells from 0 – 50 cm of depth and the graduation of light regimes over this depth.

When calculating pigment ratios and identifying classes, CHEMTAX does not account for varying light regimes and the subsequent effects on pigment ratios. Further work with CHEMTAX on ice samples should be carried out using sectioned ice cores and as much filtered material as possible. Despite these limitations, identified pigments were useful in confirming and identifying some species in the brine.

Microscopic and pigment analysis of the brine revealed an autotrophic flagellate dominated community consisting predominantly of dinoflagellates, chrysophytes and their life cycle stages, with a minor component of diatoms and a ciliate. Heterotrophic dinoflagellates and two species of *Cryothecomonas* were also present in the brine.

Autotrophic dinoflagellates

Polarella glacialis was the dominant dinoflagellate in the community in both years. The asexual, motile stage of *P. glacialis* was most abundant whilst the brine was cold and hypersaline, identifying the motile cell as being cryo and halophylic in nature. The increase in cell length from early to late in the season with decreasing salinities may be indicative of a response to osmotic pressure occurring at this time.

The asexual stage showed distinct, interannual variability. In 1996, the population peaked just after sampling began (14 November) whilst in 1997, the population did not peak until 8 December. Mean maximum cell concentrations also differed, with *P. glacialis* motile cells in 1996 reaching 1.4×10^6 cells l^{-1} while in 1997 this concentration was lower at 0.9×10^6 cells l^{-1}.

Interannual variability in both the timing of peak abundance and mean maximum cell numbers was attributed to the substantially colder brine recorded in 1997. Brine temperature in early November 1997 was almost 1 °C colder than the previous year. It appears that the lower temperatures, and therefore higher salinities, in 1997 retarded growth of *P. glacialis* until 17 November when the brine had warmed by about 1 °C. From this date, mean motile cell abundance increased with temperature

and decreasing salinity until 5 December (Figure 3.22). Motile cell increases in this period showed strong positive and negative correlations with temperature increases ($r^2 = 0.959$) and salinity decreases ($r^2 = -0.965$). Similar retardation of the *P. glacialis* motile cell population by colder brine temperatures were observed in McMurdo Sound by Stoecker *et al.* (1997).

Sexual reproduction in *P. glacialis* was observed in 1996 and 1997. In both seasons, low numbers of planozygotes were evident on the earliest sampling dates. However, the inducement of mass sexuality in the brine varied in time between years (Figure 3.23). In 1996, as asexual cell abundance fell towards its minimum, planozygote numbers increased rapidly from early December and peaked by 8 December. In contrast, planozygote abundance in 1997 increased in synchrony with asexual cell numbers from mid November and peaked by 1 December.

Brine temperature and salinity appeared not to be major determining factors in the inducement of sexuality (Figure 3.24). In 1996, the rapid increase in planozygotes lagged behind the initial period of warming and desalination and showed poor correlation with these factors ($r^2 = 0.362$ and -0.656 respectively). Mass sexuality in this year occurred around -1.5 °C and 36 psu. In contrast, the development of sexuality in 1997 correlated reasonably with temperature ($r^2 = 0.815$) and highly with salinity ($r^2 = -0.974$), with sexuality increasing dramatically at about -2.8 and 62 psu. The variation between years undermines any clear relationship between sexuality, temperature and salinity and it appears that some other factor or factors govern the inducement of sexuality.

Nitrogen depletion is regarded as the major factor inducing sexuality in many dinoflagellates (Pfiester and Anderson 1987; Blackburn *et al.* 1989). In 1997, DIN concentrations were mostly below detection limits and insufficient to show any pattern. Phosphate and silicate concentrations in the brine dropped markedly between 17 and 21 November, immediately prior to the mass sexuality recorded from 24 November. Due to the lack of nitrogen data and as the asexual stage was actively growing and consuming nutrients during this same period, it was not possible to correlate nutrient depletion with sexuality.
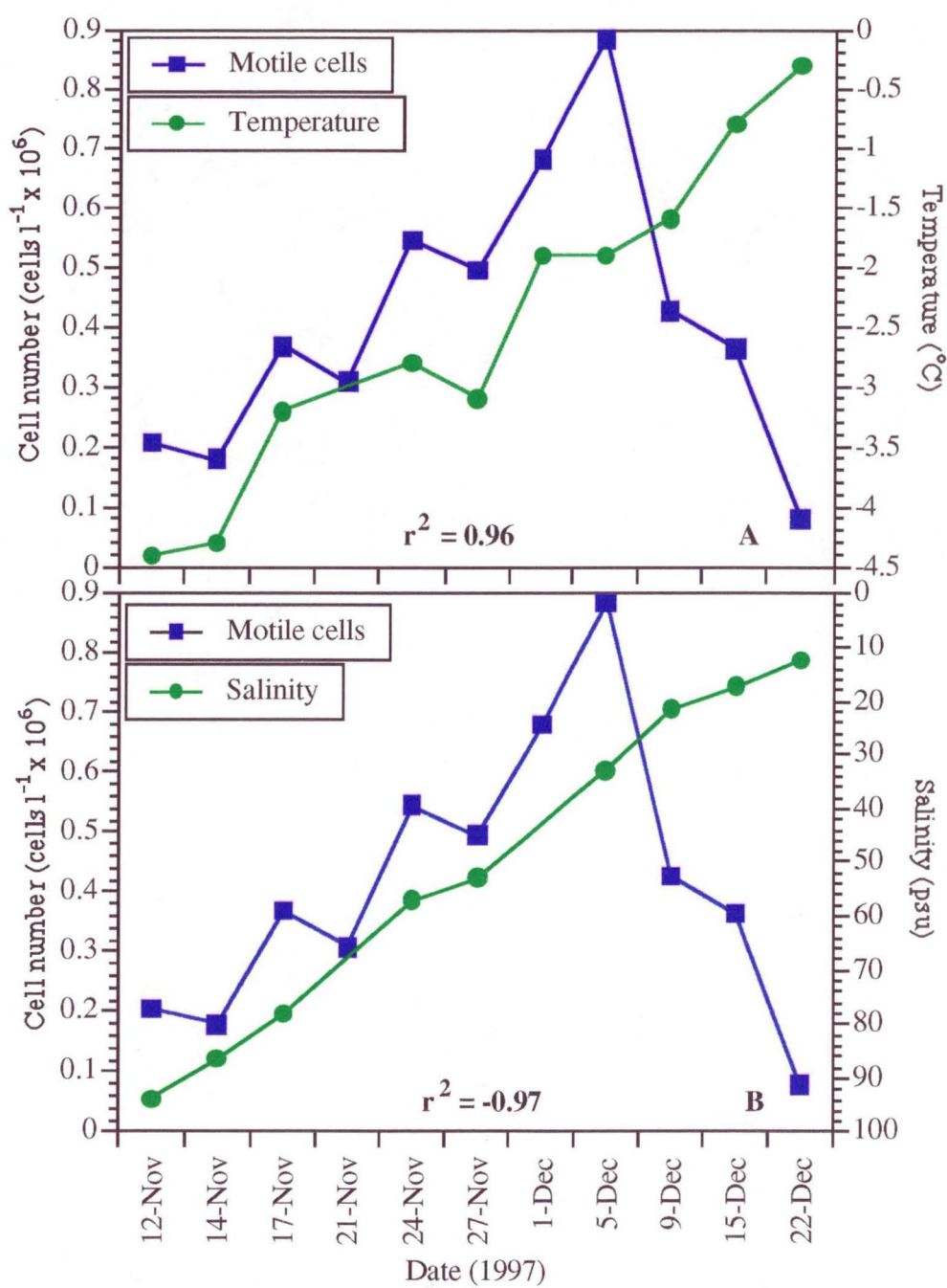
Figure 3.22 Mean *Polarella glacialis* motile cell abundance correlated with brine temperature and salinity in fast ice brine in 1997
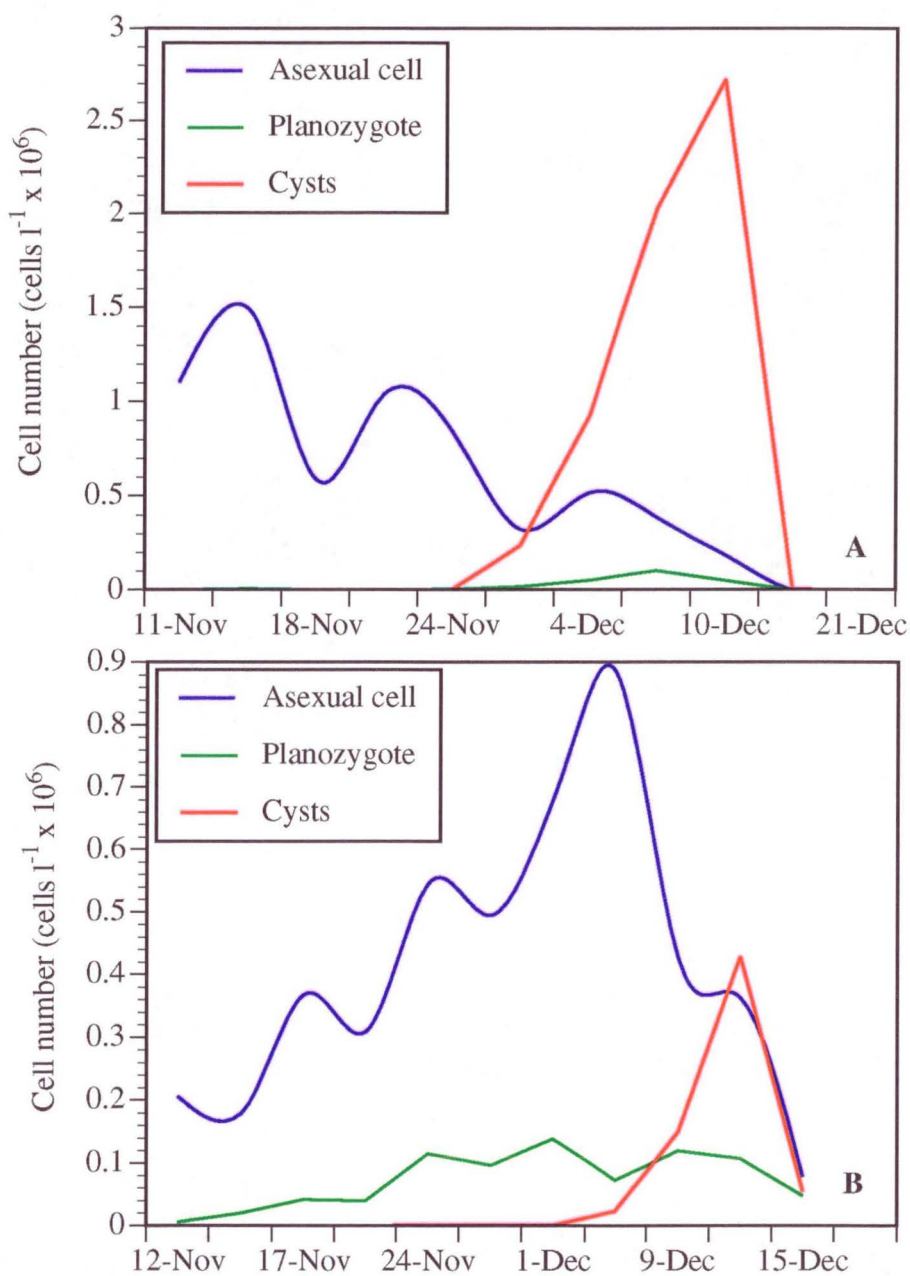
Figure 3.23 Life cycle of *Polarella glacialis* in Davis fast ice brine in A) 1996 and B) 1997 (note different scaling on y axes)
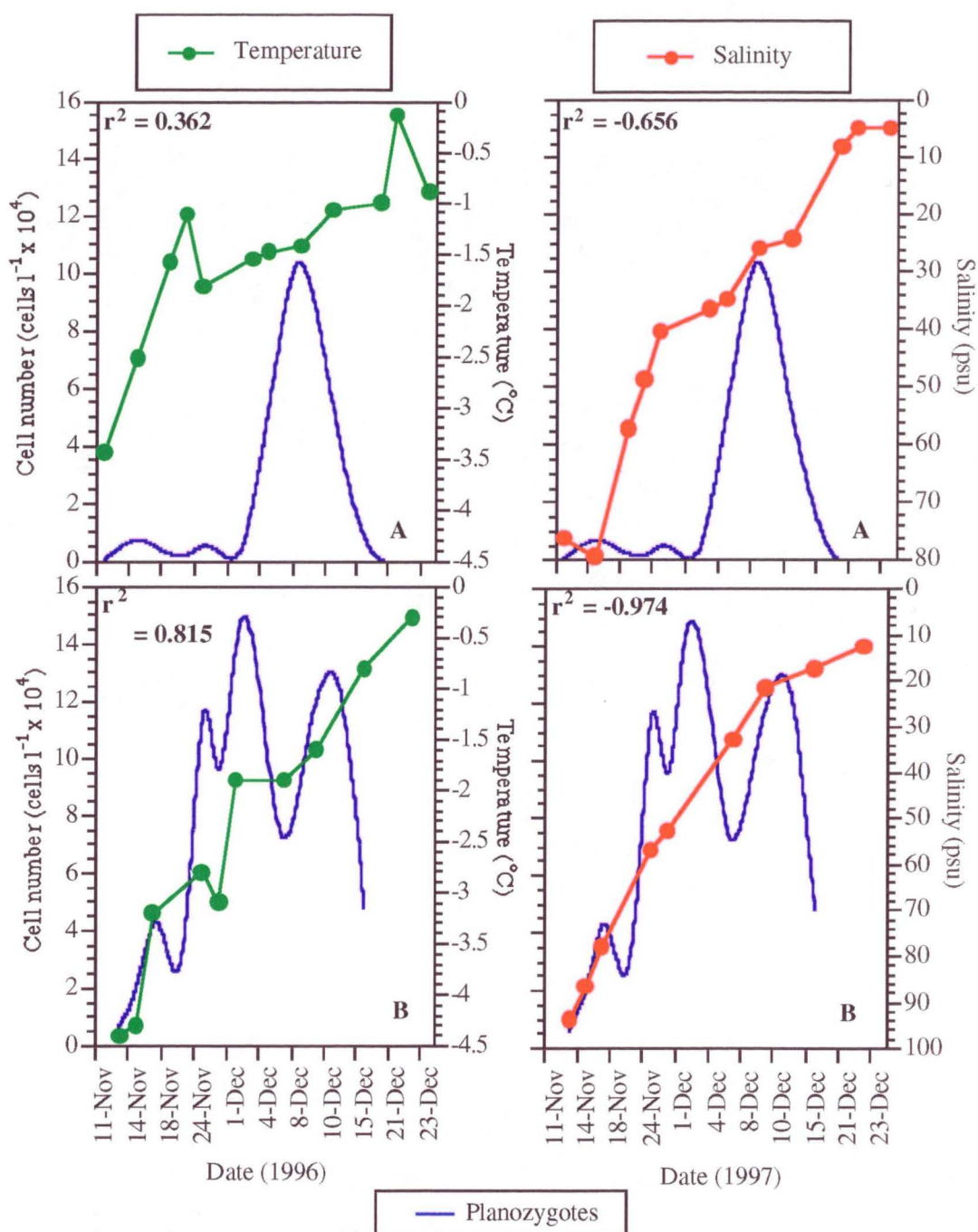
Figure 3.24 *Polarella glacialis* **planozygote production in fast ice brine correlated with brine temperature and salinity in A) 1996 and B) 1997**

Only low numbers of *P. glacialis* cysts were observed in early November both years and the mass excystment observed by Stoecker *et al*. (1998) had apparently occurred prior to sampling. This speculation would be consistent with the approximate

excystment conditions postulated by these researchers as corresponding to –6 °C and 100 psu. By early to mid December in both years, the dinoflagellate cyst became abundant in the brine as asexual cell numbers decreased and planozygote number increased. Despite the variation in the timing of asexual cell dynamics between years, peak cyst abundance occurred around the same time. Cyst abundance in 1996, elevated above asexual cells, indicate that the asexual cell abundance was underestimated in this season.

It seems likely that the pattern of cyst release and their enduring presence in the water column over summer acts to seed the reforming ice of the following year. High cyst numbers have been demonstrated in fast ice in early spring (Stoecker *et al.* 1997) so it appears that some of the cysts released to the water column are incorporated back into the ice as it forms. The morphology of the cyst may facilitate this. The recurving spines of the cyst may act to improve buoyancy in the water column as the surface ornamentation of other dinoflagellates has been postulated to do (Vesk *et al.* 1990). Furthermore, the small size of the cysts, combined with its buoyancy, may also be important in ice incorporation mechanisms such as scavenging (Ackley 1982; Garrison *et al.* 1983; Reimnitz *et al.* 1993), ice nucleation (Ackley 1982; Garrison *et al.* 1983) or the action of Langmuir cells (Garrison *et al.* 1989; Weissenberger and Grossmann 1998).

Gymnodinium sp.2 was the other autotrophic dinoflagellate present in the brine. In both years this species was most abundant from early to mid December, although its occurrence was highly variable in 1996. The size range of this species (25 – 40 μm) and the timing of its peak abundance coincides with that of the larger, photosynthetic dinoflagellate recorded in McMurdo Sound fast ice (Stoecker *et al.* 1992). However, unlike the McMurdo Sound species, mixotrophy was not observed in *Gymnodinium* sp.2.

Chrysophytes

Chrysophytes in the brine showed a similar life cycle progression to *P. glacialis*. In 1997 few statocysts were apparent when sampling began in November, although flagellated cells at this time were abundant. Apparently excystment had occurred

prior to the beginning of sampling, at a temperature < –4.5 °C and salinity > 95 psu. Like the asexual stage of *P. glacialis*, the vegetative chrysophyte stage seems well adapted to cold and hypersaline conditions. By 1 December, the coccoid chrysophyte cells became evident and the flagellated stage had declined in number. At this time, brine temperature was about –3.3 °C and the salinity was around 34 psu. Statocysts were again detected about this time and mass encystment occurred by 12 December.

Chrysophytes have been identified previously in sea ice by their characteristic statocysts or archaeomonads. Silver *et al.* (1980) described statocysts from the Weddell Sea and Takahashi *et al.* (1986) described at least 20 species from Syowa station fast ice alone, stating that their principle habitat appeared to be the brine pockets and channels of the ice. Most recently, Stoecker *et al.* (1992; 1997; 1998) found abundant statocysts in the upper fast ice at McMurdo Sound. Conditions of temperature and salinity at the time of sexuality in Davis ice closely resembled those found by Stoecker *et al.* (1998). Of the two most commonly found statocysts in McMurdo Sound, only one species (probably *A. areolata*) was positively identified in Davis fast ice by SEM studies. The presence of at least two other statocysts at Davis indicates that several species of chrysophytes inhabited the brine channels. *Paraphysomonas imperforata* Lucas has been recorded from meltpools at Davis (McConville and Wetherbee 1983). However, the fragility of the chrysophytes in the brine prevented attempts to identify them.

Other Flagellates

The remaining autotrophic flagellates showed a range of cryo and halotolerance, temperature and salinity optima, and a subsequent succession of species in the brine (Figure 3.25). Growth optima noted here do not take into account grazing pressure from the heterotrophs and are gauged from peak abundance alone. The abrupt decline in numbers of *Pyramimonas* sp. 2 and *Mantoniella* sp. late in the season is attributed to the brine drainage event previously described.
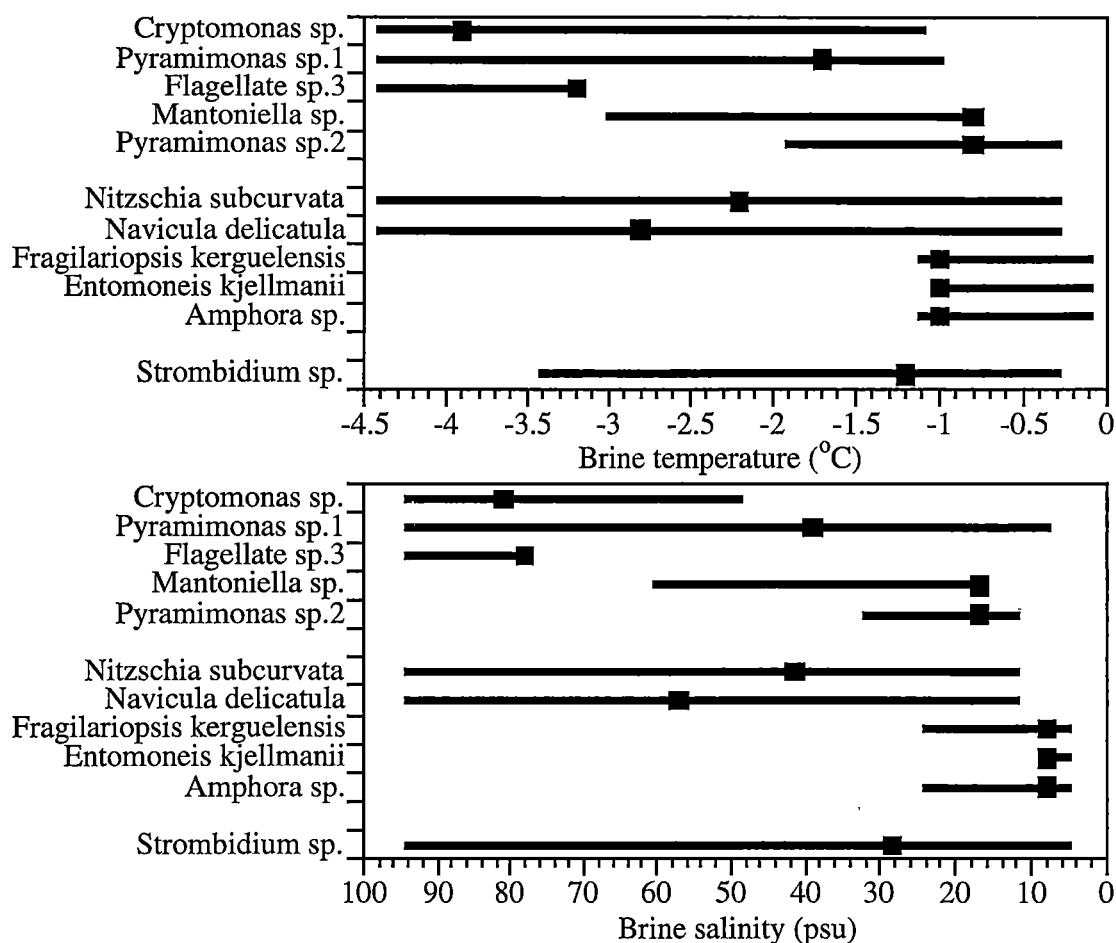
Figure 3.25 Temperature and salinity ranges and population peaks of various autotrophic flagellates and diatoms in Davis fast ice brine (■ indicates peak abundance. For species present in both seasons, ■ is calculated as mean temperature and salinity between years)

Cryptomonas sp. showed a eurythermic but more specific halophilic presence. Its temperature and salinity optima were –3.9 °C and 81 psu respectively. Several cryptomonads have been reported at Davis in the lower horizon of fast ice and surface meltpools (McConville and Wetherbee 1983; Archer *et al.* 1996) and pack ice communities (Garrison and Close 1993) prior to this study. They are also a major part of the sea water phytoplankton bloom near Davis Station (McMinn and Hodgson 1993; Gibson 1997). Considering the hypersaline preference of the species found in the brine, however, it is unlikely that this species was the same one prevalent in the phytoplankton.

Pyramimonas sp.1 was the most cryo and halotolerant of the other flagellates, being present in the brine throughout the sampling period. Its temperature and salinity optima, however, appeared to be around –1.8 and 39 psu.

The remaining flagellates showed more specific temperature and salinity requirements and less tolerance. The unidentified flagellate sp.3 was present early in November during cold and hypersaline conditions and peaked in abundance at about –3.2 °C and 78 psu. *Mantoniella* sp. became evident at about –3 °C and 60 psu but showed growth optima of -0.8 °C and 18 psu. *Pyramimonas* sp.2, present only late in the season, was the least tolerant of the flagellates and peaked in abundance under the same conditions as *Mantoniella* sp. It seems likely that *Pyramimonas* sp.2 was inoculated into the upper ice from the underlying seawater.

Diatoms and Strombidium *sp.*

Nitzschia subcurvata and *Navicula delicatula* were the only diatoms to show active growth in the brine. These diatoms showed surprising cryo and halotolerance, being present throughout the sampling period (Figure 3.25). The *N. subcurvata* population peaked at about –2.2 °C and 42 psu. The highest abundance of *Navicula delicatula* occurred at about –2.8 and 57 psu. The remaining diatoms were present only in December and were most likely transported to the upper ice at this time. *Strombidium* sp., being present throughout sampling in the 1996 sampling season, showed eurythermic and euryhaline tolerance (Figure 3.25). Its apparent temperature and salinity optima was around –1.2 °C and 29 psu.

Heterotrophs

Most heterotrophs showed eurythermal and euryhaline tolerance during the growing season and despite obvious increases in abundance, no direct evidence of grazing was observed. Only *Cryothecomonas* spp. were abundant early in the season. *Cryothecomonas armigera* presence was restricted to the cold and hypersaline conditions to mid November. The unidentified *Cryothecomonas* sp.2 was an obvious, but small, component of the brine until early December.

Cryothecomonas spp. have been recorded from sea ice in Antarctica and the Arctic (Thomsen *et al.* 1991; Garrison and Close 1993; Stoecker *et al.* 1993; Ikävalko and Gradinger 1997; Ikävalko 1998) with *C. armigera* the most frequently observed. In Davis fast ice, Archer *et al.* (1996) detected *Cryothecomonas* spp. in the bottom 10 cm of ice. *C. armigera* grazes on the 2 – 4.5 μm size class (Thomsen *et al.* 1991), a range that would include *Mantoniella* sp. Although not apparent in this study, Stoecker *et al.* (1993) observed *C. armigera* with ingested *Mantoniella* sp. cells in McMurdo Sound fast ice.

Cryothecomonas species are generally considered eurythermal and euryhaline (Thomsen *et al.* 1991) and Stoecker *et al.* (1993) found *C. armigera* present in brine to -0.2 °C and about 2 psu. The absence of *C. armigera* in Davis fast ice at more moderate temperatures and salinities may be a result of predation by the heterotrophic dinoflagellates that increased in abundance from mid November.

Gymnodinium sp. and *Protoperidium* sp. were present in the brine with the *Cryothecomonas* spp. Whilst there is no evidence from this study of predation by the dinoflagellates on either *Cryothecomonas* spp., it is likely there was some grazing impact. Both species peaked in abundance early in December, indicating successful predation on some part of the community. A similar sized species of *Gymnodinium* was recorded by Stoecker *et al.* (1993) in McMurdo Sound and *Protoperidium* sp. has been previously recorded by Archer *et al.* (1996) in O'Gorman Rocks fast ice.

Of the two species of *Gyrodinium*, only *Gyrodinium* sp.1 was evident from mid November. *Gyrodinium lachryma* was observed only on 9 December and considering its presence in the underlying water column at this time, it is likely that this species invaded the upper ice when ice porosity was high. The infrequent euglenoid has been observed with ingested *P. glacialis* cells by Archer *et al.* (1996) in Davis fast ice.

3.4 Comparison of Davis and McMurdo Sound fast ice and surface pack ice communities

In terms of transitory temperatures and salinities and seasonal ice decay, the Davis upper brine channel environment closely resembled that in McMurdo Sound fast ice (Stoecker *et al.* 1992; 1997; 1998). Generally, nutrient concentrations in Davis brine were considerably lower than those found by Stoecker *et al.* (Stoecker *et al.* 1998).

In terms of community composition, the Davis fast ice showed a greater flagellate diversity than McMurdo Sound (Table 3.7). One species of *Cryptomonas*, an unidentified flagellate (flagellate sp.3) and two species of *Pyramimonas* made the collective difference. A further difference was noted in the eurythermic and euryhaline diatom presence and abundance throughout the sampling. *N.subcurvata* was present in the brine in both years, but in 1996 it occurred in bloom proportions of millions of cells l^{-1}. *Navicula delicatula* also showed an enduring presence throughout the season, although in much lower numbers. In McMurdo Sound, diatoms were rare when brine temperatures were low and salinities high and the only recorded species was a small pennate type (Stoecker *et al.* 1998).

Several species present in McMurdo Sound were not detected in Davis fast ice. These included *Phaeocystis* sp. and a diversity of ciliates. *Phaeocystis* sp. blooms have been previously recorded in the phytoplankton of inshore Davis waters (Gibson 1997). However, these occurrences show inter-seasonal variability and in 1996 and 1997, *Phaeocystis* sp. blooms in the water column were not evident. In fact, blooms of this species have not been recorded in the water column since 1989 (pers.com, Davidson, 1999). The absence of *Phaeocystis* sp. from the water column has most likely contributed to its absence in the ice.

Stoecker *et al.* (1993) recorded a diverse ciliate assemblage at McMurdo Sound, including tinnintids, *Strobilidium* spp., *Didinium* sp., *Mesodinium* sp. and others. Oligotrichs were the most abundant, with six species of *Stombidium* recorded as opposed to the one *Stombidium* sp. detected at Davis. *Mesodinium rubrum*, and several heterotrophic ciliates, have been found at Davis but in association with the

bottom ice (Archer *et al.* 1996) and a diverse ciliate community, including several species of *Strombidium* have been recorded in the plankton (Grey *et al.* 1997).

Despite these differences, the Davis and McMurdo Sound communities closely resembled each other (Table 3.7). The numerically abundant autotrophic components of the brine, *P. glacialis* and the chrysophytes, were present at both locations. The 12 fold increase in *P. glacialis* asexual cell numbers recorded from normalised counts compares favourably with the 12.8 fold increase recorded by Stoecker *et al.* (Stoecker *et al.* 1992) and the 7 fold increase noted by Archer *et al.* (1996). Sexuality in both species at Davis occurred under similar conditions to those proposed by Stoecker *et al.*(1998) and their life cycles were enacted almost in synchrony despite the spatial and temporal differences (Figure 3.26).

The heterotrophic assemblages in both communities were also remarkably similar (Table 3.7). Of the dinoflagellates, only *Polykrikos* sp. has never been observed in Davis upper ice. Dinoflagellate sp.1 (Buck *et al.* 1990), while not observed in association with the upper ice, may be present in the lower ice (Archer *et al.* 1996). *C. armigera* was present at both locations and the smaller *Cryothecomonas* sp. at Davis was similar in size to the species observed at McMurdo Sound.

The differences between the two locations may be due to variations in ice formation, its subsequent structure and nutrient concentrations. Stoecker *et al* (1997) found that the upper 50 cm of fast ice in McMurdo Sound consisted of 10 cm of columnar/granular ice, underlaid by 20 - 30 cm of transition ice over congelation ice. Davis fast ice typically consists of a greater proportion of congelation ice (Worby *et al.* 1998). Nutrient concentrations can vary with ice texture (Dieckmann *et al.* 1991) and this alone may explain the variation in concentrations between locations.

Table 3.7 Comparison of upper fast ice communities from Davis Station and McMurdo Sound and with pack ice communities (Davis community is as observed in this study unless otherwise referenced: pack ice source is Garrison and Close (1993) unless otherwise referenced; ? indicates possible occurrence; O indicates previously recorded at Davis from other studies; a, Stoecker *et al.* (1992; 1993; 1997; 1998); b, (Buck *et al.* 1990); c, (Archer *et al.* 1996); d, (Garrison 1991))

Genera	Davis fast ice	McMurdo fast ice	Interior and upper pack ice
Autotrophs			
Polarella glacialis	●	●	● (cysts, now described)
Larger gymnodinoid sp. (25 - 40μm)	●	●	
Other Flagellates			
Chrysophytes and statocysts (*Archaeomonas areolata*)	●	●	●
Phaeocystis sp.		●	
Cryptomonas spp.	●		●
Mantoniella sp.	●	●	●
Raphidophytes	?		●
Pyramimonas spp.	●		●
Diatoms			
Nitzschia subcurvata	●		●
Nitzschia lecointei	●		●
Other diatoms	●	●	●
Ciliates			
Strombidium sp.	●	●	
Other oligotrichs		●	●
Mesodinium rubrum	O[c]	●	●
Heterotrophs			
Dinoflagellates			
Gyrodinium spp.	●	●	●
Gymnodinium spp.	●	●	●
Protoperidinium sp.	●	●	●
Polykrikos sp.		●	
Diplopeltopsis sp.	O[c]		
Dinoflagellate sp.1[b]	?[c]	●	●[b]
Other heterotrophs			
Cryothecomonas spp.	●	●	●
Cryothecomonas armigera	●	●	●
Euglenoid	●		●[d]
Didinium spp		●	●[d]
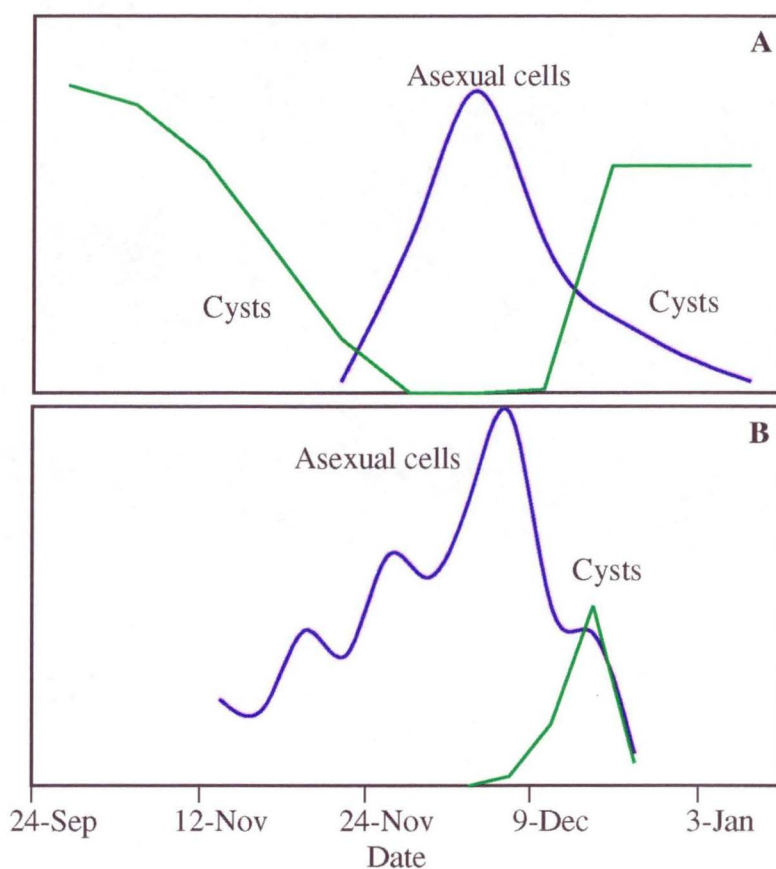
Figure 3.26 Synchronicity of the *Polarella glacialis* life cycle between A) McMurdo Sound and B) Davis Station fast ice (McMurdo Sound data compiled from Stoecker *et al.* (**1997; 1998**) where cell presence and abundance estimated from 45 cm depth in ice)

The incorporation of cells into ice as it forms will affect the diversity and number of cells in an ice cover. Ice incorporation mechanisms rely heavily on frazil ice formation in the water column (Ackley 1982; Garrison *et al.* 1983; Weissenberger and Grossmann 1998) and the thermodynamic growth of a congelation ice dominated ice sheet tends to expel brine, and entrapped cells, as it thickens (Weeks and Ackley 1982; Wakatsuchi and Kawamura 1997; Gow *et al.* 1998). Differences in the ice structure, therefore, may account for the greater diversity of genera in McMurdo Sound.

The Davis fast ice community also shared many taxa in common with pack ice surface and interior communities (Table 3.7). This commonality has been referred to

previously by Stoecker *et al.* (1992) who noted that studies in the more accessible fast ice may allow conclusions to be made on the extensive pack ice and communities contained within.

3.4.1 Known Antarctic distribution of upper fast ice communities

The similarity of the upper ice community at Davis Station and McMurdo Sound point to at least a disparate distribution around Antarctica. However, other reports of dinoflagellate and chrysophyte populations, including one similar to *Archaeomonas areolata*, indicate this community also exists near Syowa Station. It is probable that further upper ice investigations around the periphery of Antarctica will discern a circumpolar distribution of this community.

3.5 Conclusions

The upper ice brine environment adjacent to Davis Station was characterised by extreme and transitory temperatures and salinities. Low temperatures, high salinities and low brine volume were typical in mid November. By mid December, brine temperature had increased dramatically and hyposaline, almost fresh, conditions predominated. Brine warming in the upper ice was at least partially regulated by air temperature. Increasing brine quantity to mid December and the appearance of a spacious, freeboard layer was indicative of ice decay. Brine drainage events in mid December were observed in 1996 and suspected in 1997. Interannual variability in brine warming and desalination was evident. Heavier snow cover in 1997 and intermittent snow falls during the growth season appeared to be the cause of variation.

The upper ice accounted for a significant percentage of the autotrophic community in the fast ice cover. In the absence of a bottom community, the upper 50 cm of ice contributed up to 42% of chl *a*. This horizon accounted for up to 9% of total chl *a* where a bottom community was present. The vertical distribution of the autotrophic community in the upper ice varied between years. In 1996, the highest concentrations remained at or below 30 cm depth while in 1997, the community was more evenly distributed.

Cryo and halotolerant, autotrophic flagellates dominated the community over few diatoms, a ciliate and microheterotrophs. *Polarella glacialis*, chrysophytes and their life cycle stages were most abundant in the brine. *P. glacialis* asexual cells were obvious from the commencement of sampling in both years but only few cysts were observed at this time. Sexuality was induced mid November and by early December, mass encystment of *P. glacialis* was evident. It was concluded that temperature and salinity did not play a primary role in inducing sexuality and subsequent encystment. Both the timing of the asexual cell peak, their concentrations and the inducement of sexuality in *P. glacialis* varied between years. The heavy and episodic snow cover in 1997, resulting in slower brine warming, was believed responsible for this variation.

The dramatic decrease in *P. glacialis* cysts in mid December 1996 coincided with the detection of large numbers of cysts in the underlying water column. It was concluded that brine drainage, occurring when ice porosity was high, was responsible for the transfer of cysts to the water. This pattern of release most likely acts to seed the water column for incorporation of the cysts back into the ice the following year. Whilst only low cyst numbers were recorded in Davis ice early in the season, other studies have demonstrated large concentrations prior to excystment and the asexual phase of the life cycle.

The chrysophyte life cycle followed a similar pattern to that of *P. glacialis*. The unidentified, flagellate stage was recorded from mid November and was followed by an abundance of coccoid, non motile cells early December. By mid December, mass encystment resulted in high numbers of statocysts (primarily *Archaeomonas areolata*) in the upper ice. Brine drainage resulted in a rapid decrease in statocysts in the upper ice and appears to function as a mechanism for seeding the water column in a similar manner to the *P. glacialis* cysts.

Other autotrophic flagellates in the brine included *Cryptomonas* sp., two species of *Pyramimonas*, an unidentified species and *Mantoniella* sp., all of which showed a range of cryo and halotolerance. Of these flagellates, only *Pyramimonas* sp.2 appeared not to be a true member of the community. This species became evident early December and was most abundant when ice porosity was high. It seems likely

that *Pyramimonas* sp.2 invaded the upper ice from the underlying water column or was carried up the brine channels by oscillatory flow associated with brine drainage.

Of these diatoms, only *Nitzschia subcurvata* and *Navicula delicatula* were present throughout the sampling period, with *Nitzschia subcurvata* reaching bloom proportions in 1996. The remaining diatoms recorded late in the season were most likely introduced into the upper ice from the water column.

Several species of heterotrophs were present in the brine. Two species of *Cryothecomonas* were evident early in the sampling but both decreased in number with the increasing abundance of the larger heterotrophic dinoflagellates and Euglenoid. This suggests grazing on *Cryothecomonas* spp. by the larger heterotrophs, however, no evidence of predation was recorded on this or any other species.

Davis fast ice showed a greater diversity of autotrophic species, based on the detection of three flagellate species and two diatoms not previously recorded from the brine channels. A further difference was the lack of ciliate diversity recorded at Davis.

Despite these differences, however, the Davis brine channel environment resembled that of McMurdo Sound in terms of the extreme and transitory temperatures and salinities. There was also a remarkable similarity in the presence of the major taxa (*P. glacialis* and the chysophyte statocyst, *Archaeomonas areolata*) and in the synchronicity of life cycle events of *P. glacialis* in both locations. The heterotrophic component of Davis and McMurdo Sound ice were also similar. Differences between the two locations were attributed to ice formation mechanisms and subsequent ice structure, with Davis ice consisting of a greater proportion of congelation ice.

These similarities indicate at least an irregular distribution of the upper ice community around Antarctica. If the distinctive *P. glacialis* cyst and chrysophyte statocysts can be used as indicator species for this community, it is probable that further fast ice investigations around Antarctica will reveal a circumpolar distribution of the upper ice community described here.

Chapter 4 DMSP as an osmolyte in *Polarella*

glacialis and fast ice brine communities

4.0 Introduction

Dimethylsulphoniopropionate (DMSP) is a sulphur compound synthesised predominantly by photosynthetic organisms with the highest concentrations found in micro and macroalgae (Karsten *et al.* 1996). DMSP is the precursor to dimethylsulphide (DMS) (Challenger and Simpson 1948; Catoni and Anderson 1956) and is cleaved to form DMS and acrylic acid on a one for one basis (Steinke *et al.* 1996). DMS has attracted much attention as the dominant, natural sulphur emission from oceans (Bates *et al.* 1992). The atmospheric oxidation products of DMS provide the major source of aerosol particles in remote areas such as Antarctica. These aerosols may influence global climate by scattering and absorbing solar radiation and by acting as cloud condensation nuclei (CCN), forming cloud cover and subsequently increased cloud albedo (Charlson *et al.* 1987). Consequently, it has been postulated that the connection between DMSP producing algae and cloud cover represents a climate regulating, feedback mechanism (Charlson *et al.* 1987).

Because of the importance of DMS in the biogeochemical sulphur cycle and its possible climate regulating effect, research has focused onto DMSP production by microalgae and its biological roles. Keller *et al.* (1989), in a survey of 123 microalgal species representing 12 classes, identified dinoflagellates and prymnesiophytes as high DMSP producers. They also showed a strong correlation between chromophytes (those algae with chlorophylls *a* and *c*) and significant DMSP production. Of these, diatoms are generally not significant producers of DMSP although Keller *et al.* (1989) state that in bloom proportions some species may be important. In the Antarctic and the Arctic, diatoms are the dominant members of most ice algal communities (Horner 1985; Garrison 1991) and recent reports indicate that they may be greater contributors to DMSP and DMS production then previously thought (Dieckmann *et al.* 1991; Levasseur *et al.* 1994). Chlorophytes (those possessing chlorophylls *a* and *b*) are less significant producers of DMSP (Keller *et al.* 1989).

Research to date indicates DMSP plays an important role as an osmolyte, with salinity being one of the major influences on DMSP accumulation in algae. Osmoregulation in algae is generally achieved by a combination of inorganic and organic osmotica (Kirst 1996). Under salinity upshock (increasing salinity), inorganic ions such as potassium (K^+), sodium (Na^+) and chloride (Cl^-) are initially accumulated to coarsely restore turgor pressure and cell volume (Dickson and Kirst 1986; 1987b; 1987a).

After a lag phase of approximately 3 hours, DMSP and other organic osmolytes such as glycine betaine, homarine (N-methyl picolinic acid) and glycerol are synthesised and accumulation of these organic osmotica continues in response to further upshocks (Vairavamurthy *et al.* 1985; Dickson and Kirst 1986; 1987b; 1987a; Karsten *et al.* 1992). Where the inorganic ions acted as primary osmotica, DMSP, glycine betaine and homarine appear to act as secondary osmotica, making the fine adjustments necessary to restore cell volume (Dickson and Kirst 1986). In contrast, decreasing salinities (or downshocks) cause the almost instantaneous release of DMSP, glycine betaine and homarine from the cell (1986; Dickson and Kirst 1987a; 1987b). DMSP and the other organic compounds are considered to be of greater importance in long term hyperosmotic stress (Kirst 1996).

Most organic osmolytes are also compatible solutes. Unlike inorganic ions, compatible solutes accumulated in the cytoplasm do not inhibit cellular metabolic processes and enzyme activity. They are believed to stabilise proteins under conditions of low water potential, preventing inactivation, inhibition and denaturation (Kirst 1996).

DMSP does not appear as suitable as the betaines, proline or glycerol as a compatible solute (Kirst 1996). While it is has a reasonably good solubility, it is substantially less soluble than proline and its rates of synthesis are less rapid than other organic osmotica. Nevertheless, Gröne and Kirst (1991) have shown the apparent effectiveness of DMSP as a compatible solute on enzymes of *Tetraselmis subcordiformis* (Chlorophyceae) at low temperatures.

While there is clearly a relationship between DMSP production and increasing salinity, temperature and light have also been shown to affect DMSP accumulation in algae. Karsten *et al.* (1992) showed that low water temperatures positively influenced DMSP accumulation in 4 species of macroalgae. A later study demonstrated the action of DMSP as a cryoprotectant where it stabilised and enhanced enzyme activity during freeze/thaw cycles (Karsten *et al.* 1996).

Increasing light intensity appears to stimulate DMSP production (Vairavamurthy *et al.* 1985; Karsten *et al.* 1992). Photoperiod is also important, with increasing and decreasing day lengths elevating or reducing concentrations respectively (Karsten *et al.* 1990). Nutrient availability also appears to have some influence, with nitrogen limitation enhancing DMSP accumulation in some microalgae (Dacey *et al.* 1987; Gröne and Kirst 1992; Keller and Korjeff-Bellows 1996).

To date, few studies have measured DMSP production in Antarctic sea ice and no investigations have attempted a time series study of DMSP production at one location. Kirst *et al.* (1991) investigated DMSP production and its possible biological roles in ice algal and phytoplankton populations of the Weddell Sea. Turner *et al.* (1995) measured DMS and DMSP production in surface waters and sea ice between the Drake Passage and the Bellingshausen Sea. DiTullio *et al.* (1998) determined DMSP in pack and fast ice of the Ross Sea. In East Antarctica, Curran and Jones (1998) measured DMSP in Prydz Bay pack ice and in surface waters of the Southern Ocean whilst Gibson *et al.* (1990) measured DMS in a coastal location adjacent to Davis Station.

This chapter reports for the first time on a series of temporal DMSP measurements from one location in upper fast ice brine at Davis Station. DMSP production in the brine will be discussed in relation to the extreme and transitory salinities and temperatures of fast ice during its annual spring/summer decay. Additionally, DMSP accumulation in the brine dinoflagellate, *Polarella glacialis*, was investigated. *P. glacialis* cultures, initially isolated from Davis Station fast ice, were subjected to salinity upshock and downshock treatments to investigate the role of DMSP as an osmolyte in this species.

4.1 Methods and Materials

4.1.1 Fast ice DMSP sample collection and analysis

Duplicate 20 ml samples of upper fast ice brine for the analysis of total DMSP
(DMSPt) were collected weekly from 50 cm drill holes at O'Gorman Rocks, Davis
Station, between 12 November and 9 December 1997. The samples were
acidified to pH 1 using HCl (hydrochloric acid), stored in the dark at 4 °C and
transported to James Cook University, Australia for DMSP analysis by Ms. Anne
Trevena.

DMSP analysis was based on the method of Curran and Jones (1998) using a
Varian 3400 Gas Chromatograph with a flame photometric detector. Sample
aliquots (200 - 2000 µl) were injected into a sealed purge chamber, containing 4
ml 10 M NaOH, where DMSP was quantitatively cleaved to produce DMS in 1:1
stoichiometry under the base conditions. DMS was purged from the chamber
using helium gas at 50 ml/min for 15 min and concentrated by cryogenic trapping
in a teflon sample loop immersed in liquid nitrogen. The sample loop was placed
in a room temperature water bath where the DMS was volatilised and transferred
to the GC column by the carrier gas and the use of a pneumatic valve assembly.
A polydimethylsiloxane capillary column at 130 °C was used with DMS eluting at
around 0.8 min. Quantification was by external calibration using DMSP standard
(0.47 ng S/100 µl) injections. Accuracy for DMSP analysis was ± 10%.

4.1.2 DMSP osmolyte experiment

The experiment consisted of three treatments, each with triplicate cultures
inoculated axenically from an early exponential phase *Polarella glacialis* culture
in a 10 l carboy. The inoculum was grown in 35 psu, f/2 growth medium at 4 °C
under 60 µE m^{-2} sec^{-1} light and a 16:8 hr light:dark cycle. Nine, 1 l round flasks
containing 210 ml of fresh, filter sterilised, 35 psu f/2 growth medium were
inoculated with 300 ml of starter culture. After gentle mixing 10 ml of each
culture were taken to determine initial chl *a* concentration by fluorometry, leaving
a remaining culture volume of 500 ml. Light measurements were made by a
Biospherical Instruments Inc. QSL-100 light meter and cultures were grown in a
Phasefale culture cabinet.

The experiment began after a 3 day equilibration period. Triplicate cultures were assigned to the control, upshock (increasing) and downshock (decreasing) salinity treatments. All replicates were cultured at 4 °C at 70 μE m^{-2} sec^{-1} light with a 16:8 hr light:dark cycle.

The control treatments were maintained at approximately 35.5 psu throughout the duration of the experiment. The initial salinity changes in the experimental treatments were planned to be 5 psu per day with sampling every 2 days. However, the frequent handling of the cultures and the daily salinity adjustments appeared to retard culture growth. Subsequent salinity adjustments (following the initial 5 psu) were approximately 2.5 psu every 2 days with sampling every 4 days.

At each sampling, salinity was measured using a WTW LF 320 Microprocessor Conductivity Meter (with a Tetracon 325 conductivity cell). After gentle mixing, 50 ml of each replicate culture was axenically removed using sterile, 50 ml volumetric pipettes attached to sterile, plastic syringes. Each sample was subdivided into the following volumes for later analysis:

- 20 ml for DMSPt (total).
- 10 ml for DMSPd (dissolved).
- 5 ml for chlorophyll *a* analysis by *in vivo* fluorometry.
- 5 ml of fresh culture for microscopic examination.
- 10 ml preserved in Lugol's iodine for enumeration.

50 ml of an appropriate solution was added to the replicates after sampling to restore culture volume to 500 ml. To the control treatments, maintained at approximately 35.5 psu, 50 ml of sterile f/2 medium was added. Salinity upshock was by the addition of 50 ml of 6% NaCl (5.5g NaCl/100ml) in nutrient enriched (f/2) Milli-Q water. Downshock was by the addition of 50 ml of 0.82% NaCl (0.82g NaCl/100ml) in f/2 enriched Milli-Q water. Nutrient enriched Milli-Q was added to ensure an excess supply of nutrients to the cultures over the duration of the experiment.

Chlorophyll a measurements

In vivo determination of chlorophyll *a* was carried out immediately after sampling using a Turner Designs 10-AU Digital Fluorometer. Two ml of each sub-sample

was placed directly into 5 ml cuvettes and the fluorescence reading taken. Sterile filtered seawater was used as a blank. Correction factors were calculated and applied to chl *a* measurements to overcome errors introduced by addition of medium at each sampling.

Microscopy

Fresh sub-samples were examined using a Zeiss Telaval 31 inverted microscope. Note was taken of cell health (if they were swimming vigorously or immobile) and of the life cycle stages present. Cell volume changes over the course of the experiment were assessed by measuring length and diameter of 20 cells from each replicate. Cell volumes were calculated using the geometric formula recommended for a prolate spheroid dinoflagellate (Hillebrand *et al.* 1999) and used to calculate DMSP per cell volume. Total cell volumes were calculated for ease of comparison with the study of Keller *et al.* (1989). As the cell volume estimate does not take into account non-osmotic volume of the cell, DMSP per cell volume concentrations are likely to be underestimates.

Triplicate cell counts from each treatment were made using the same inverted microscope. Two ml of Lugol's preserved sample were allowed to settle for 4 hours in a 10 ml settling chamber onto a 28 mm (25 mm counting area) glass cover slip. Motile cells and cysts were counted over 20 FOV at x400 magnification and cells per litre were calculated. As the planozygote stage was difficult to distinguish from the motile cells in this fixative and at x40 magnification, possible planozygotes were included in the motile cell counts. Correction factors were calculated to overcome the diluting effect on cell concentration of new medium added at each sampling.

Growth rates for each treatment were calculated using the triplicate cell counts and the following equations;

$$K_e = \frac{\ln(N_1 / N_0)}{t_1 - t_0}$$

Where K_e is the growth constant;

N is the mean cell number and the subscripts denote initial and final concentrations;

t is time and ln indicates the natural (base e) logarithm.

k was calculated as follows:

$k = Ke/\ln2 = Ke/0.6931$

DMSP analysis

The treatments were sampled for DMSPt and DMSPd (extracellular DMSP) at day 0 (beginning of the experiment), after 2 days and then every 4 days. The DMSPd fraction was filtered through 0.2 µm Supor ® Acrodsc ® 25 Gelman Sciences filters. The particulate DMSP (intracellular or DMSPp) fraction was derived from the difference between DMSPt and DMSPd. These samples were acidified to pH 1, stored in alfoil wrapped scintillation vials and transported to James Cook University where Ms. Anne Trevena carried out the analyses as described above. Headspace in the cultures was not analysed for DMS. As some dissolved or extracellular DMSP cleaved within the cultures may have been lost as DMS across the medium/air interface, DMSPd concentrations may be underestimates.

Statistical analyses

One tailed Student t-tests, assuming unequal variances, were used to test for significant growth in each of the treatments. Two tailed t-tests were used to test for significant changes in *P.glacialis* cyst numbers with DMSP concentrations. Correlation analysis was used to test for relationships between cell counts and chl *a* concentration and DMSP concentrations and salinity increases and decreases. Student's *t* tests and correlation were performed using the data analysis pack within Microsoft Excel 98.

4.2 Results

4.2.1 DMSP production in fast ice brine

Total DMSP measured in the brine ranged between 451 nM on 17 November to 239 nM by 9 December (Figure 4.0). A gradual decrease was evident from 17 November and by 1 December, a significant decrease ($P < 0.05$) in DMSPt had occurred.

DMSPt and chl *a* showed similar decreasing trends to early December (Figure 4.1) and a degree of correlation ($r^2 = -0.42$). DMSPt:chl *a* ratios in November

were up to 5.4 times higher than the final DMSPt:chl a ratio recorded on 9

December. DMSPt:chl a ratios were 60 (12 Nov), 111 (17 Nov), 162 (1 Dec) and

30 nmol/µg (9 Dec).
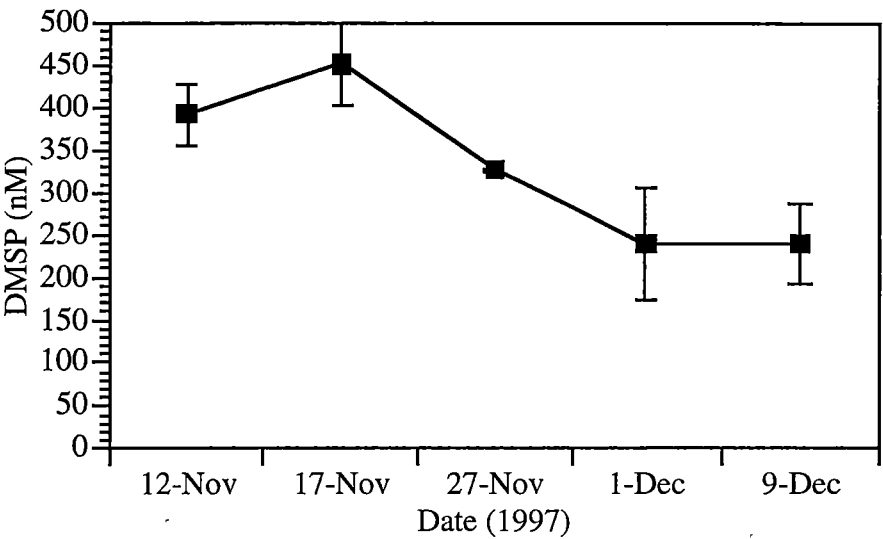
Figure 4.0 Mean, total DMSP estimates in upper fast ice brine, O'Gorman Rocks, Davis Station. (Error bars represent 1 s.d)
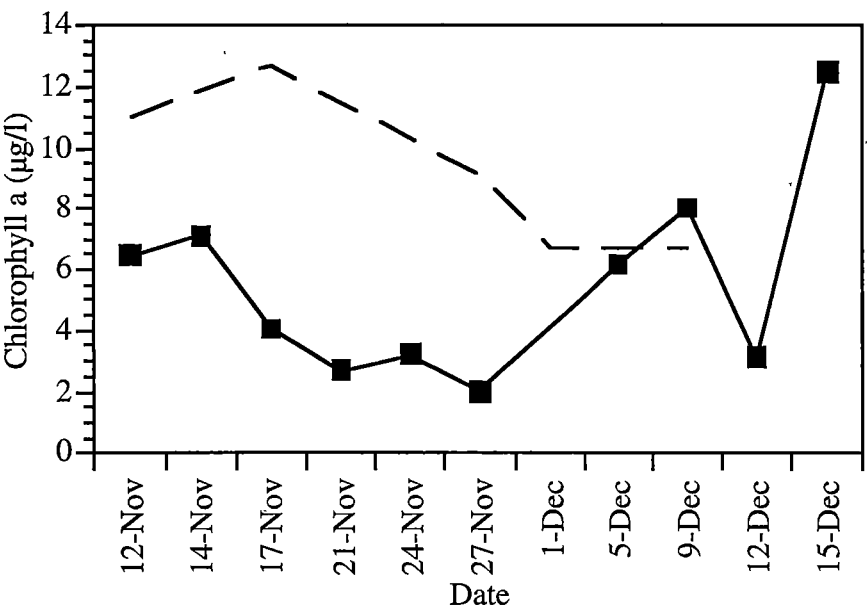
Figure 4.1 Chlorophyll a (■) and DMSPt (-) in O'Gorman Rocks brine at Davis Station, 1997

The Davis brine community was dominated by dinoflagellates (predominately *Polarella glacialis*), chrysophytes and their life cycle forms (see Chapter 3). Also present were cryptomonads, prasinophytes and diatoms. *P.glacialis* and

chrysophyte vegetative cells were the most abundant species in November when DMSPt values were high (Figure 4.2a). Vegetative cell number peaked by 5 December when the dinoflagellates and chrysophytes became sexual and began encysting. In contrast, the cryptomonads, prasinophytes and diatoms were more obvious from early December (Figure 4.2b).
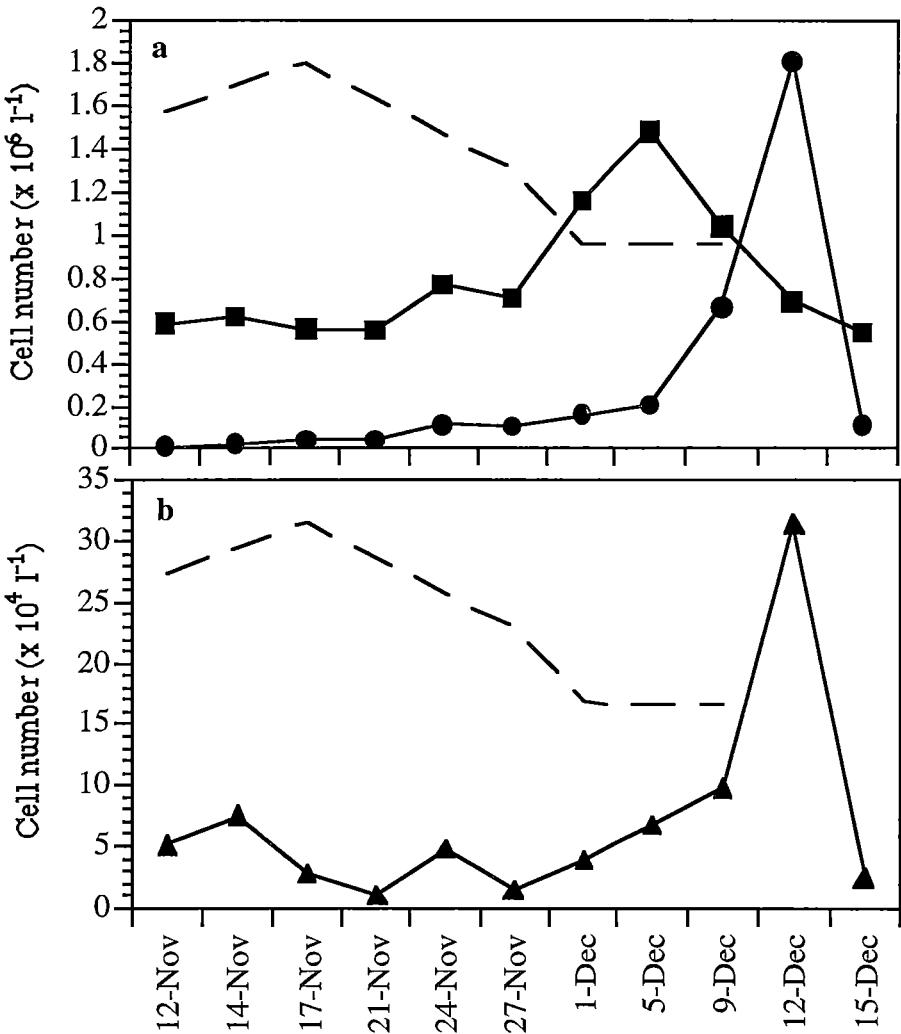
Figure 4.2 Brine microalgal species in Davis fast ice grouped as a) dinoflagellate and chrysophyte vegetative cells (■) and their sexual forms (●) and b) other species (▲) including cryptomonads, prasinophytes and diatoms (Dashed line represents DMSPt measured in brine).

The brine was hypersaline (95 - 52 psu) and cold (-4.5 to -2.5 °C) in November but was less saline (22 psu) and warmer (-1.6 °C) by 9 December (salinity and temperature data from Chapter 3) (Figure 4.3a, b). Brine salinity and DMSPt in the ice showed a high degree of correlation ($r^2 = 0.89$) as both deceased with time.

Brine temperature and DMSPt also correlated well (r^2 = -0.74) with DMSPt concentrations falling with increasing brine temperature.
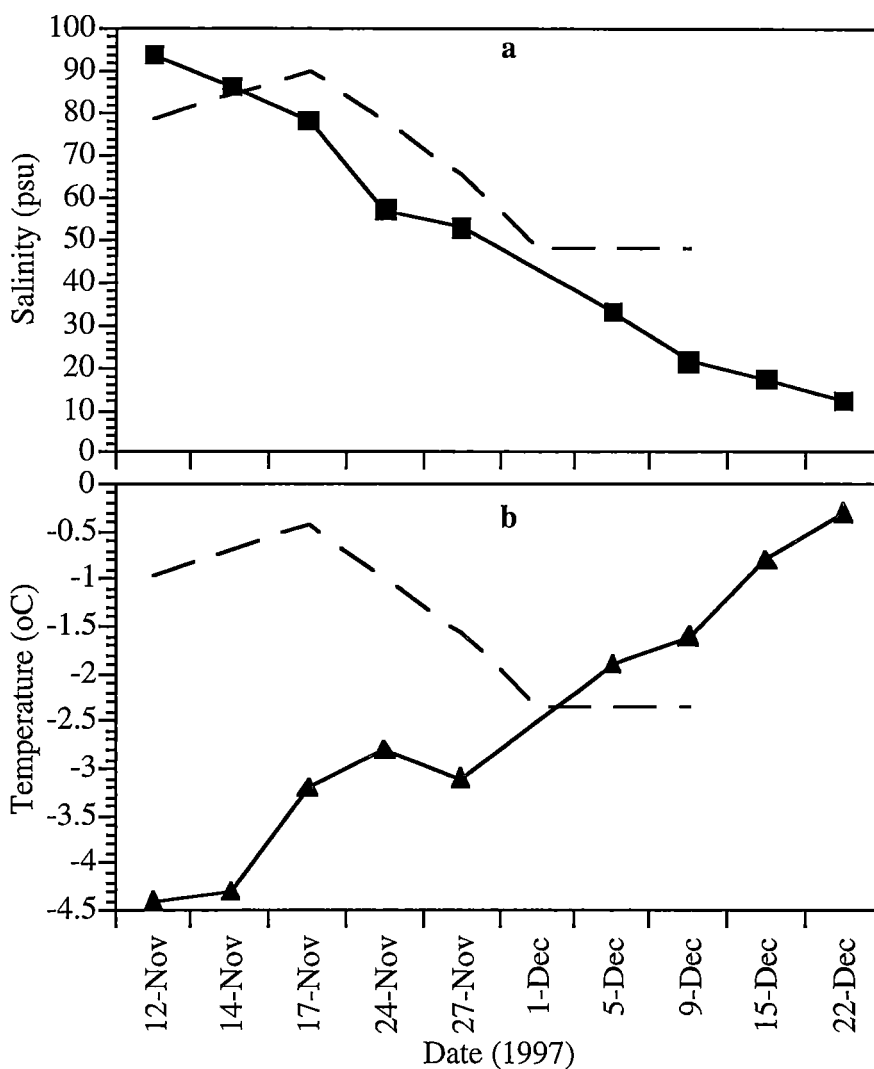
Figure 4.3 DMSPt (-) with a) brine salinity (■) and b) brine temperature (▲) in fast ice brine at Davis Station

4.2.2 DMSP osmolyte experiment

Culture conditions and growth

Salinity adjustments reached a maximum of 51.7 psu in the upshock treatment and a minimum of 21.5 psu in the downshock treatment (Figure 4.4). The control treatment was maintained at the initial starting salinity of 35.5 psu.
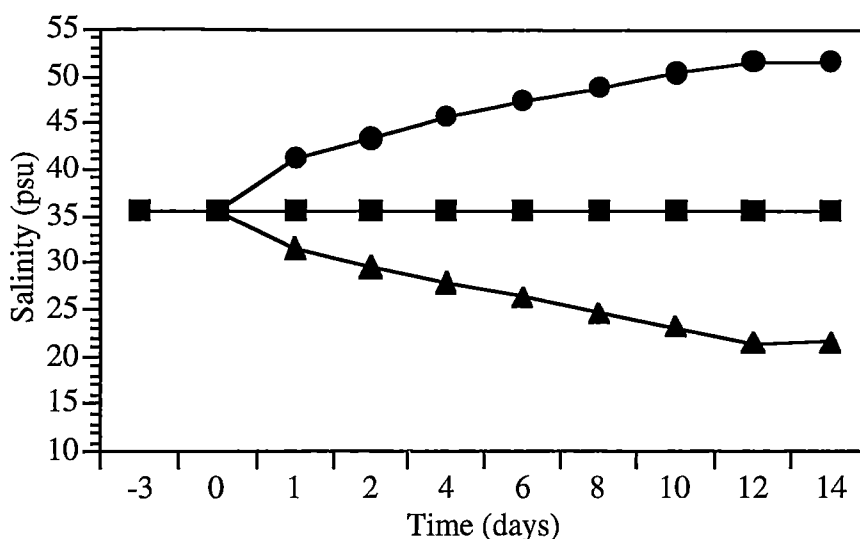
Figure 4.4 Mean salinity adjustments in the control, upshock and downshock treatments (■ control; ● upshock; ▲ downshock; n = 3; initial salinity adjustments from day 0 – day 1 were by 5 psu, followed by 2.5 psu adjustments until day 12).

Microscopic observations of fresh samples of the control and upshock treatments revealed healthy, vigorously swimming cells throughout the experiment. Significant growth was observed in the control ($P < 0.05$) and upshock treatments ($P < 0.005$), with both showing similar growth rates (k) of 0.06 divisions day^{-1} (Figure 4.5a, b). Fresh samples of the downshock treatments became progressively less healthy with salinity decreases and by day 14, many of the vegetative cells were either swimming extremely poorly or immotile. No significant change in cell numbers was recorded in the downshock treatment by day 14 (Figure 4.5c). Mean chl a measurements correlated well with cell counts in the upshock ($r^2 = 0.92$) treatment but poorly within the control ($r^2 = 0.59$) (Figure 4.5a, b, c). Mean chl a reflected the lack of growth in the downshock treatment.

Significant change in cyst numbers occurred in both the control and upshock treatments (Figure 4.5a, b). In the control, cyst numbers increased significantly ($P < 0.05$) from a mean of 1.93 to 4.63 x 10^6 cells l^{-1} by day 14. In the upshock treatment, cyst number decreased ($P < 0.05$) from 3.42 to 2.03 x 10^6 cells l^{-1}. This decrease correlated highly with the increasing salinity ($r^2 = -0.93$). However, cyst counts were variable between the replicates and empty cysts were not observed in

the upshock treatments. No significant change in cyst concentration was recorded under decreasing salinity (Figure 4.5c).
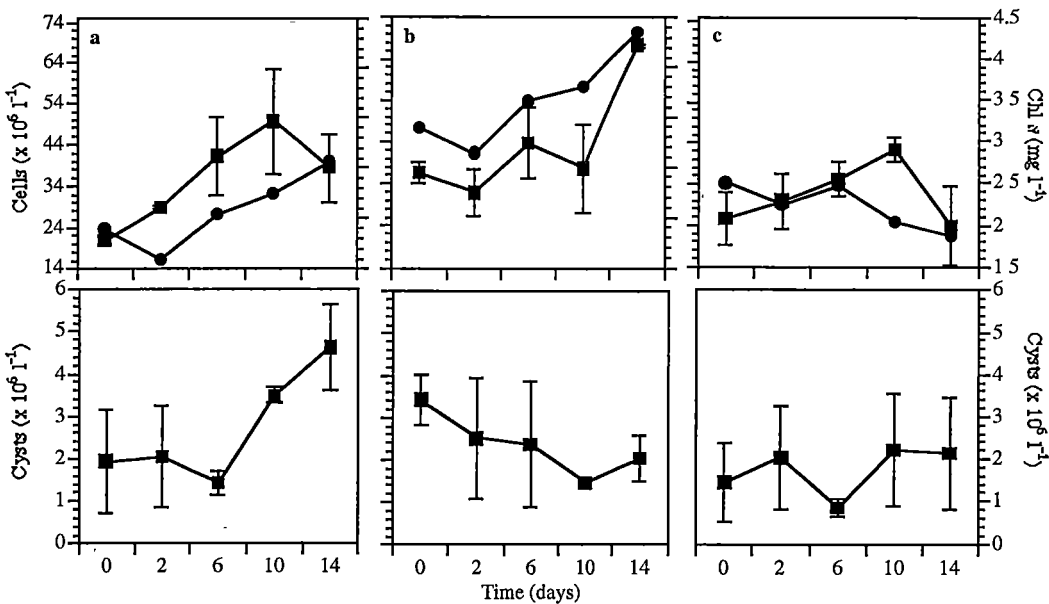
Figure 4.5 Mean cell and cyst counts (■) and chlorophyll *a* (●) in the a) control, b) upshock and c) downshock treatments (n = 3, error bars represent 1 s.d)

Significant changes in cell length and volume occurred only in the control treatment (Table 4.0). Mean cell dimensions at the beginning of the experiment was 10.9 x 6.9 μm with a mean volume of 279 μm^3. By day 14, mean cell length and volume in the control had increased significantly to 13 μm and 416 μm^3 respectively. Little change was observed in cell width. Mean cell length in the upshock treatment had decreased by day 14, although not significantly. Small increases in cell volume in the upshock and downshock treatments were apparent (Table 4.0), however the changes were not significant.

DMSP production

A significant increase in intracellular DMSP (DMSPp) was recorded only in the downshock treatment from day 6 onwards ($P < 0.005$) where DMSPp increased from 22 to 47 fM cell^{-1} (Figure 4.6a). The control treatment showed a similar increase during this period however, large variations between the triplicate cultures negated its significance. In the upshock treatment, DMSPp did not increase and remained relatively steady, ranging between 12 to 25 fM cell^{-1}.

Table 4.0 *Polarella glacialis* cell length, diameter and volume in the control, upshock and downshock treatments (mean values are followed by range in brackets)

	Day 0	Day 14
Inoculum/Control		
Cell length (μm)	10.9 (9.8 - 12.5)	12.5 (10.3 - 17.5)
Cell diameter (μm)	6.9 (5.0 - 7.5)	7.9 (7.3 - 10.0)
Cell volume (μm^3)	279.6 (114.5 - 392.9)	416.0 (301.7 - 915.8)
Upshock treatment		
Cell length (μm)		9.8 (7.5 - 11.3)
Cell diameter (μm)		7.7 (5.8 - 10.0)
Cell volume (μm^3)		298.5 (173.0 - 392.5)
Downshock treatment		
Cell length (μm)		10.9 (8.8 - 12.5)
Cell diameter (μm)		7.1 (5.3 - 10.0)
Cell volume (μm^3)		319.9 (126.2 - 654.2)

The only significant increase in extracellular DMSP (DMSPd) was recorded from the upshock treatment ($P < 0.005$) (Figure 4.6b) where DMSPd rose from 175 to 500 nM between day 6 and 10. This increase did not correspond with a decrease in intracellular DMSPp during the same period although a decrease was recorded between days 2 and 6. DMSPd in the control varied little throughout the experiment, ranging between 160 to 234 nM (Figure 4.6b). Downshock DMSPd showed a large pulse between day 6 and 10, corresponding with a decrease in upshock DMSPp from day 6. However, no significant difference in downshock DMSPd was recorded throughout the experiment.
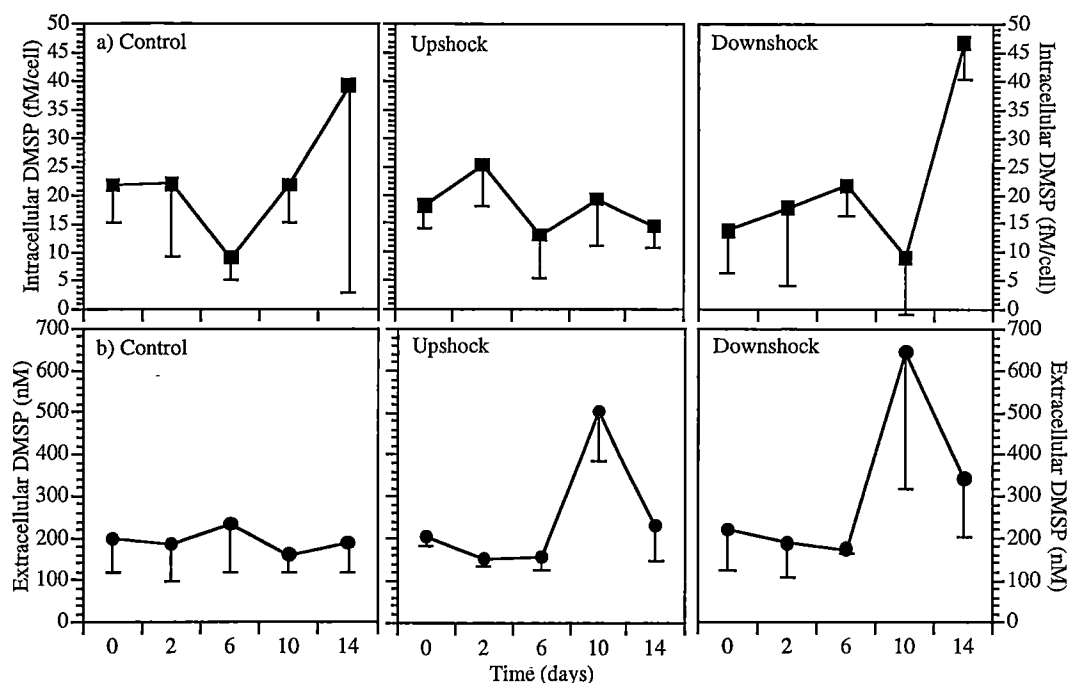
Figure 4.6 a) Intracellular and b) extracellular DMSP production in the control, upshock and downshock treatments (n = 3, error bars represent 1 s.d.)

4.3 Discussion

4.3.1 DMSP production in fast ice

The concentration of DMSPt (DMSPp + DMSPd) in Davis fast ice brine ranged from 451 nM mid November through to 239 nM by early December. Although samples were taken over a period of ice decay when brine volume was increasing as the summer progressed (see Chapter 3), the DMSPt measurements presented here are not adjusted for this diluting effect. It is argued that adjusting DMSP concentrations for the increasing brine volume is not appropriate in this circumstance. This adjustment would overcome the influences of salinity and temperature on DMSP accumulation in microalgae (see introduction) and confound interpretation of the data. Furthermore, DMSPt concentrations are a balance between DMSP production and consumption within the brine. As DMSP is produced as well as consumed by biological systems, it does not necessarily accumulate with time.

Davis fast ice DMSPt values are over 7 times higher than those found by Kirst *et al.* (1991) in pack ice brine (62 nM DMSPp; Table 4.0). The Davis estimates are closer to the infiltration, interior and brown ice values (687, 237 and 303 nM

respectively) in the Weddell Sea (Kirst *et al.* 1991). They are also comparable to fast ice from the Ross Sea (133 nM) (DiTullio *et al.* 1998), ice core sections from the Bellingshausen Sea (200 nM) (Turner *et al.* 1995) and Prydz Bay (144 nM), Eastern Antarctica (Curran and Jones 1998) (Table 4.0). The Davis brine values are up to 4 times higher than seawater DMSP values (up to 100 nM) recorded by Gibson *et al.* (1990) in coastal waters adjacent to Davis Station.

Table 4.1 DMSP and chlorophyll *a* in Antarctic pack and fast ice (modified from Kirst *et al.* (1991), Turner *et al.* (1995), Curran and Jones (1998) and DiTullio et al. (1998))

Source/date for Davis samples	Ice sample type	Chl *a* (μg a l⁻¹) Mean	Range[4]	DMSP (nM) Mean	Range	DMSP:chl *a* ratio (nmol/(μg)
[1]Kirst *et al.* (1991)	Brine	9.9	0.2-22	62	8-204	6 3
	Interior ice	26 2	7.2-156	237	4-854	9.0
	Infiltration	9.3	3.4-38	687	82-1664	73.7
	Brown ice	39.1	10.8-56	303	144-725	7.7
[1]Turner *et al.* (1995)	Ice sections	4.6	0.2-24	200	17-546	43.5
[1]Curran and Jones (1998)	Ice sections			144	8-725	
[2]DiTullio *et al.* (1998)	Ice sections	99.5	38.8-231.8	133	81-219	1.3
[2]This work	Brine					
12 November		6.5		393	367-413	60.6
17 November		4.1		451	417-485	111.29
27 November		2.0		327	326-327	161.5
1 December				240	193-287	
9 December		8.0		239	205-273	29.8

[1]pack ice, [2]fast ice. For Kirst *et al.* (1991) and DiTullio *et al.* (1998), DMSP represents particulate fraction; for Turner *et al.* (1995), DMSP represents DMSPt + DMS; for Curran and Jones (1998) and for this study, DMSP represents total DMSP.

Possible DMSP producers

Polarella glacialis and chrysophyte vegetative cells dominated the brine community over other flagellate and diatom species when DMSPt values were highest in November. Considering that dinoflagellates have been identified as high DMSP producers and some chrysophytes as significant producers (Keller *et al.* 1989), it seems likely that these taxa were responsible for the majority of DMSP production within the brine. If this assumption is made and if all the DMSP present is assumed to be intracellular, then the dinoflagellates and chrysophytes combined contained up to 108 pg DMSP/cell on 17 November. As

some of the DMSP present would have been extracellular and other species probably contributed at least partially to the DMSP total, this value is undoubtable an overestimate. However, it compares well with the DMSP content of gymnodinoid dinoflagellates (24 - 244 pg DMSP/cell) surveyed by Keller *et al.* (1989) but is much higher than the range in chrysophytes (0.24 - 2.68 pg DMSP/cell).

The higher DMSPt:chl *a* ratios in November support the hypothesis that *P.glacialis* and the chrysophytes may have been responsible for majority of DMSP produced during this period. High DMSP:chl *a* ratios have been reported to be indicative of the presence of high producers. Kirst *et al.* (1991) attributed the high ratio of 74 nmol/µg in the infiltration layer of pack ice (Table 4.0) to dinoflagellates and *Phaeocystis* (Prymnesiophyceae), despite diatoms being the prevailing algae in the samples. Similarly, Turner *et al.* (1995) found an increased biomass of prymnesiophytes at higher DMSPt:chl *a* ratios. In this study, the highest ratios were evident to early December (60, 111 and 161 nmol/µg) and coincided with the domination of the community by *P.glacialis* and chrysophyte vegetative cells.

If the dinoflagellates and chrysophytes were the main DMSP producers, DMSP production was not consistent with their population dynamics. In particular, *P.glacialis* vegetative numbers continued to increase until early December when sexuality was induced and planozygotes and cysts became apparent. However, the increasing vegetative numbers and sexual stages were not accompanied by a corresponding rise in DMSP. Conversely, a fall in brine DMSP coincided with the increasing populations and sexual stages. It appears therefore, that DMSP production is independent of density and life cycle stages of the possible high producers, particularly *P.glacialis*.

DMSP concentrations showed a poor relationship with chl *a* from early December. From this time, chl *a* increased with the *P.glacialis* cells and was enhanced by an rise in number of the so called low DMSP producing species, such as prasinophytes and diatoms. Whilst chl *a* was increasing, DMSPt concentrations fell to the lowest values measured in brine and the DMSPt:chl *a*

ratio dropped accordingly to 30 nmol/µg by 9 December. Ice decay from early December may also have contributed to the poor correlation between DMSPt and chl *a*, with meltwater from the decaying ice diluting chl *a* and DMSP concentrations.

Environmental influences on DMSP production

The highest DMSPt concentrations occurred during mid November when the brine was hypersaline (94 - 75 psu). As the brine salinity fell, a corresponding decrease in DMSPt concentration was evident and the time series measurements of these two parameters showed a good degree of correlation ($r^2 = 0.89$).

The higher DMSPt values during the hypersaline conditions in November and the correlation between falling DMSPt concentrations and brine salinity supports the role of DMSP in cellular osmoregulation. Dickson and Kirst (1986; 1987b; 1987a) have demonstrated the role of DMSP as an osmolyte in prasinophytes, diatoms and haptophytes through salinity upshock treatments. Vairavamurthy *et al.* (1985) also found a correlation between increasing salinity and intracellaular DMSP with a coccolithophorid, whilst Karsten *et al.* (1992) demonstrated the action of DMSP as an osmolyte in polar and temperate macroalgae. Considering this evidence, it is possible that DMSP was produced by at least some brine algal species to overcome the osmotic dilemma presented by the hypersaline brine.

DSMP concentrations at Davis may have been enhanced by the low nitrogen concentrations in the brine. Dissolved inorganic nitrogen levels in the brine were low (up to 0.31 µM, Chapter 3) throughout the sampling period and several authors have found that nitrogen availability effects DMSP production. Keller and Korjeff-Bellows (1996) grew four species of phytoplankton in high and low nitrate media and found that in three of the four species, increases in DMSP content occurred when nitrogen became limited. Increases with nitrogen limitation have also been demonstrated by Gröne and Kirst (1992) and Dacey *et al.* (1987). A possible explanation of the nitrogen deficiency/DMSP relationship relates to glycine betaine, another known osmolyte. Glycine betaine is the nitrogen analog of DMSP and under nitrogen replete conditions, this compound may be preferentially synthesised over DMSP. When nitrogen becomes depleted,

DMSP may be accumulated in place of glycine betaine (Keller and Korjeff-Bellows 1996). Therefore it is possible that DMSP was synthesised by the brine algal species in preference to glycine betaine under the influence of nitrogen deficiency.

Whilst DMSPt concentrations were at their highest in November, cold temperatures ranging from -4.5 to -3.0 °C were evident in the brine. As DMSPt concentrations decreased, brine temperatures were rising and the two variables showed reasonable correlation (r^2 = -0.74). Temperature, like salinity, has been found to influence DMSP production in laboratory experiments. Karsten *et al.*(1992) found that low water temperatures influenced DMSP accumulation in 4 species of macroalgae. In a later study, Karsten *et al.* (1996) identified DMSP as a cryoprotectant in macroalgae, stabilising and enhancing enzyme activity during a freeze/thaw process. Considering the higher DMSPt values when the brine was at its coldest and the correlation between the DMSPt and temperature profiles, it is possible that that DMSP was accumulated by some brine species to act at least partially as a cryoprotectant.

Other factors apart from salinity, nutrient availability and temperature appear to influence DMSP accumulation in algae. Some studies have shown that increasing light intensity stimulates DMSP production (Vairavamurthy *et al.* 1985; Karsten *et al.* 1992). Photoperiod is also important, with increasing and decreasing daylengths elevating or reducing concentrations respectively (Karsten *et al.* 1990). During sampling for DMSP in Davis brine, both light intensity (due to the increasing solar zenith angle and subsequent angle of incident radiation on the surface of the ice) and daylength were increasing as the polar summer progressed. However, considering the decrease in DMSP production in the brine with sampling as light intensity and daylength increased, these factors appeared to have little effect.

It appears that DMSP production was less necessary by 9 December when the lowest levels of DMSP were recorded in the brine. At this time, the brine was hyposaline (20 psu) and relatively warm (-0.3 °C). In contrast, the greatest DMSP production and DMSPt:chl *a* ratios occurred in November when brine salinity ranged between 94 - 50 psu and temperature was between -4.5 and -3.2 °C.

Combined with the favourable correlations between DMSPt with salinity and temperature and laboratory evidence for the action of DMSP as an osmolyte and cryoprotectant, it seems likely that DMSP was used by *P.glacialis*, the chrysophytes and other algal species in one or both of these roles.

4.3.2 DMSP as an osmolyte

Culture growth

P.glacialis cells in the control treatment were healthy and vigorously swimming throughout the experiment. Consequently, these cultures showed significant growth over the duration of the experiment. The growth rate was low (0.06 divisions day^{-1}, however, and by day 14 significant encystment was apparent. Some encystment within two weeks of subculturing of *P.glacialis* has been observed previously (unpubl. results, Stoecker pers. comm.) and was not unexpected. The significant increase in cell length and volume seen in the control was most likely a result of sexuality and the subsequent appearance of the larger planozygote cells.

In the salinity upshock treatment, *P.glacialis* cells appeared similar in health to those in the control. Cell numbers increased significantly with the rising salinity and the growth rate was identical to that of the control treatment.

In Davis brine samples (see Chapter 3), *P.glacialis* cell length increased as brine salinity decreased. Early November, when brine salinity was high (95 psu), cell length was approximately 10 μm. By early December, cell length had increased to around 15 μm. Stoecker *et al.* (1998) have also noted a similar change in *P.glacialis* cell length with decreasing salinity in McMurdo Sound fast ice. The increase in cell length in field samples with falling salinities may be a result of increased turgor pressure causing cell elongation. Subsequently, the elasticity of the cell allowing this change could be one method used by *P.glacialis* in dealing with the transitory salinities of the brine. Whilst mean cell length in the upshock treatment failed to increase significantly to 52 psu, a slight change in mean length was apparent (from 10.9 to 9.8 μm) by day 14. Whilst the elasticity theory remains unproven, the shift in mean cell length could possibly have increased with further salinity upshocks.

In the downshock treatment, decreasing salinity appeared to adversely effect cell health and by the end of the experiment, most cells were swimming poorly or were immotile. Subsequently, no significant increase in cell numbers was recorded in the cultures. Despite the apparent poor health of these cells, the lack of a significant increase in cyst numbers indicated that there was no encystment under decreasing salinity from 35 psu. There was no significant change in cell dimensions in the downshock treatment by day 14.

DMSP production

Under the conditions of this experiment, DMSP did not appear to act as the primary osmolyte in *P.glacialis*. No significant increase in intracellular DMSP was recorded in the salinity upshock treatment, despite salinity rising from 35.5 to 51.7 psu. This result contrasts with those of other authors who have found rising DMSPp values with increasing salinity. Cultured prasinophytes, haptophytes (specifically in a coccolithophorid) and diatoms have all shown DMSP increases with increasing salinity (Vairavamurthy *et al.* 1985; Dickson and Kirst 1986; 1987a; 1987b).

A possible explanation for the lack of DMSP increase with salinity here was the availability of nutrients. In this experiment, culture media were enriched with f/2 after each sampling to restore culture volume, to adjust salinity and to enhance growth of the replicates over the experimental period. As previously mentioned, nitrogen availability can effect DMSP production in microalgae. Under nitrogen replete conditions, glycine betaine may be preferentially accumulated to DMSP. It is possible, therefore, that under the nitrogen replete conditions of this experiment, other osmolytes (such as glycine betaine) were synthesised in preference to DMSP.

In contrast, intracellular DMSP increased significantly in the salinity downshock treatment from day 10 when the medium mean salinity was decreasing from 23.1 to 21.5 psu. A similar, but insignificant, increase occurred in the control where DMSPp peaked at the end of the experiment. The reason for this pattern of DMSPp increase after 10 days in the downshock and control treatments is unclear. With the exception of media salinity, culture conditions for each of the treatments

were identical in time, light intensity, photoperiod and an excess of nutrients. In the downshock treatments, there was no significant change in cell or cyst numbers. In the control, cell growth and increases in cyst numbers were significant. Neither treatment appears to share any commonality that may relate to increases in DMSPp with time. Kirst et al. (1991) suggests that concentrations may be dependant on culture density. Cell and chl *a* concentrations in both the control and downshock treatments were lower than those in the upshock treatment. Therefore, the lower culture densities may have influenced the elevated DMSP concentrations in the two treatments over the upshock treatment.

DMSP is released from cells during senescence, cell rupture, predation and salinity downshock (Vairavamurthy *et al.* 1985; Dacey and Wakeman 1986; Gröne and Kirst 1992; Daly and DiTullio 1996). In the control, dissolved or extracellular DMSP concentrations changed little throughout the experiment. Under both increasing and decreasing salinity, pulses of extracellular DMSP were evident in the medium (significant only in the upshock treatment) between days 6 and 10. Under increasing salinity, there is no apparent reason for the increase in DMSPd and this increase was not accompanied by a corresponding decrease in intracellular DMSP. This indicates that this increase may have been an artefact of sampling, possibly caused by cell rupture during filtering.

The pulse of extracellular DMSP in the downshock treatment (despite being insignificant) was accompanied by a decrease in intracellular DMSP. Extracellular DMSP in the downshock treatment would be more easily explained if it had increased steadily with the decreasing salinity as found in a coccolithophorid by Vairavamurthy *et al.* (1985). However, in the case of *P.glacialis*, extracellular DMSP appeared as a pulse rather than a gradual accumulation. Nevertheless, the *P.glacialis* cells were unhealthy under the decreasing salinity and senescence may have contributed to this sudden increase.

DMSP and Polarella glacialis

Keller *et al.* (1989) identified dinoflagellates as one of the major producers of DMSP (although there were large variations between species). Within the dinoflagellates, DMSP concentrations ranged from 0.72 - 384 pg DMSP/cell and

0.01 - 2201.5 μM DMSP/cm^3 of cell volume. *Symbiodinium microadriaticum*
(clone HIPP), the closest known relative of *P.glacialis* (Montresor *et al.* 1999),
contained 24.2 pg DMSP/cell and 344.8 μM DMSP/cm^3 of cell volume. During
this study, the highest concentration of DMSP in *P.glacialis* was 4.9 pg
DMSP/cell by day 14 in the control treatment, or 87.4 μM DMSP/cm^3 of cell
volume. The *P.glacialis* DMSP concentrations are comparable to the values
found for other dinoflagellates by Keller *et al.* (1989) although they fall within the
lower end of the range.

P.glacialis in Davis brine was estimated to produce up to 108 pg DMSP/cell.
Under the nutrient replete culture conditions applied here, *P.glacialis* accumulated
up to 4.9 pg DMSP/cell. Taking into account that the brine estimate is considered
an overestimate and that DMSP in the cultured cells may be low due to nutrient
availability, then the field estimate of 108 pg DMSP/cell may not be grossly
overestimated. Culturing *P.glacialis* under similar low nitrogen conditions to that
of the brine may give a better estimate of its DMSP production.

4.4 Conclusions

Significant DMSP concentrations occurred in the brine channels of the upper 50
cm of Davis fast ice. Maximum DMSP in the Davis brine was more than 7 times
higher than that found in pack ice brine by Kirst *et al.* (1991) and was more
comparable to pack ice and fast ice infiltration, interior and brown ice estimates.

When DMSP estimates were highest, *P.glacialis* and chrysophytes were the most
abundant cells in the brine over cryptomonads, prasinophytes and diatoms. At
this time, high DMSPt:chl *a* ratios were also evident, indicating the presence of
high DMSP producers. Considering some dinoflagellates have been identified as
high DMSP producers and some chrysophytes recorded as significant producers,
it seems likely that one or both taxa were the main producers in the Davis fast ice.

DMSP concentration decreased with increasing vegetative and sexual cell
numbers of the *P.glacialis* population. If the dinoflagellate was a main DMSP
producer, apparently its production was independent of cell number and life cycle
stage and related to external factors.

DMSP concentration in the fast ice was highest early to mid November when the brine was hypersaline and at its coldest. Furthermore, falling DMSP concentrations over the sampling period correlated well with decreasing salinity and increasing brine temperatures. These favourable correlations, the apparent independence of DMSP production to the population dynamics of the possible high producers and laboratory evidence on the role of DMSP indicate that brine species may produce this compound as either an osmolyte, a cryoprotectant or both. Other factors such as increasing light intensity and photoperiod appeared not to be major influences on DMSP production in Davis fast ice. However, DMSP production at this location may have been enhanced as a result of low nitrogen availability within the brine.

In the laboratory experiment, *P.glacialis* in culture produced up to 4.9 pg DMSP/cell or 87.4 μM DMSP/cm^3 of cell volume. While these values are comparable to the DMSP content of other dinoflagellates, they fall towards the lower end of the dinoflagellate range. When compared to the field estimate of DMSP/cell of *P.glacialis* (108 pg) and considering nutrient availability in the cultures and the DMSP content of *S. microadriaticum*, the field estimate may not be overly exaggerated.

Under the conditions of this experiment, DMSP did not appear to act as the primary osmolyte. Clonal cultures of *P.glacialis* failed to show a significant increase in DMSP with increasing salinity. This contrasts with results from Davis fast ice where it seemed likely that DMSP was used as an osmolyte in counteracting the hypersaline brine. Furthermore, this result conflicts with previous experiments on other algal species where intracellular DMSP has been demonstrated to increase with increasing salinity.

The most likely reason for the failure of salinity upshock to induce DMSP production was the higher availability of nutrients in the experiment as opposed to availability in Davis brine. Nutrients in the experiment were replenished after each sampling period to enhance growth whilst field measurements in the ice indicated low nutrient availability. Glycine betaine, another organic osmolyte, is preferentially accumulated to DMSP under nitrogen replete conditions.

Therefore, glycine betaine was probably synthesised over DMSP under these conditions and was responsible, with other osmotica, in overcoming the osmotic dilemma of salinity upshock. Further research, using nitrogen deplete media, is required to fully assess the role of DMSP as an osmolyte in *P.glacialis*.

Chapter 5   Effects of UV radiation on fast ice brine communities

5.1 Introduction

Stratospheric ozone depletion resulting in the "ozone hole" was first reported by Farman *et al.* (1985) and is evident from Halley Bay ozone measurements (76°S 27°W) from the mid 1970s. Stolarski *et al.* (1986) confirmed these land based measurements with satellite observations. Since 1985, the ozone hole has become an annual feature of the Antarctic stratosphere (Farman *et al.* 1985; Solomon 1990), with its depth and areal extent increasing to the present day. Decreases of up to 70% of the ozone have been measured based on pre 1978 levels of 300 Dobson Units (DU: thickness of ozone when compressed at standard temperature and pressure). In 1994 and 1998, the lowest ozone values of 88 and 92 DU respectively were measured between 60 – 90° south. The 1998 ozone hole was the largest recorded at 26 million km^2 (Kerr 1998), approximately 3 times the area of Australia. As the ozone layer is the Earths foremost barrier against biologically damaging, high energy UV-B (ultraviolet B, 280-320nm) radiation, this discovery has sparked an explosion of scientific research into the effects of UVR (280-400 nm) on terrestrial and marine ecosystems.

There is no doubt that high energy UV-B radiation is biologically damaging to marine organisms (Häder *et al.* 1989; Worrest and Häder 1989; Häder and Worrest 1991; Häder *et al.* 1991). UV-B is absorbed by DNA, RNA, nucleic acids and proteins including enzymes, histones, hormones and cell membrane components (Häder and Worrest 1991; Karentz *et al.* 1991a). Subsequent effects include imperfect RNA transcription and DNA synthesis and reduced biosynthesis of amino acids, proteins, polyunsaturated fatty acids (PUFAs) and ATP. Nitrogenase activity and nitrogen metabolism may also be adversely effected (Döhler 1984; Döhler 1985; Karentz *et al.* 1991a; Döhler 1992; Goes *et al.* 1994; Arts and Rai 1997; Döhler 1998; Skerratt *et al.* 1998; Wängberg *et al.* 1999). These reduced capacities can translate to changes in membrane fluidity and permeability and a reduction in the efficiency of nutrient

acquisition and metabolism (Claustre *et al.* 1989; Döhler 1992; Goes *et al.* 1994; Döhler 1998).

Microalgae are not totally defenceless against UV-B however and may use defence strategies such as avoidance, UV absorbing compounds, UV quenching and DNA repair mechanisms for protection (Vincent and Roy 1993; Karentz 1994). Avoidance strategies include vertical migration (Vincent and Roy 1993) although no flagellated species has yet been found to exhibit phototaxis based on UV exposure alone (Häder and Worrest 1991). UV absorbing compounds have been reported in many Antarctic marine organisms such as sponges, polychaetes, molluscs and crustaceans (Karentz *et al.* 1991b). In microalgae, these compounds have been identified in green algae, diatoms, dinoflagellates and prymnesiophytes (Karentz *et al.* 1991b; Marchant *et al.* 1991; Karentz 1994).

UV radiation also has a pervasive influence through the population, community, and ecosystem levels (Vincent and Roy 1993). At the cellular and population level, UV-B exposure can have a multitude of effects. Cell size, motility, phototaxis, survival, cell division, growth and life cycle (Davidson and Marchant 1994; Nilawati *et al.* 1997) (Jokiel and York Jr. 1984; Ekelund 1990; Smith *et al.* 1992; Sebastrian *et al.* 1994) and photosynthetic capacity may all suffer (Lorenzen 1979; El-Sayed *et al.* 1990; Lesser 1996; Sundbäck *et al.* 1997; Neale *et al.* 1998; Wängberg *et al.* 1999; McMinn *et al.* in press).

At the community level, flow through effects may include reduced primary productivity (Ryan 1992; Smith *et al.* 1992; Davidson and Marchant 1994; Sundbäck *et al.* 1997) and an altered community composition and succession favouring species more tolerant to UV-B (El-Sayed 1988; Voytek 1989; Karentz *et al.* 1991a; Bothwell *et al.* 1993; Davidson and Marchant 1994; Villafañe *et al.* 1995; Davidson *et al.* 1996; McMinn 1997). Higher trophic levels may suffer from algal community changes if nutritionally undesirable or unpalatable species succeed over the preferred, but UV-B intolerant, diet species (Voytek 1989; Karentz *et al.* 1991a; Goes *et al.* 1994; Skerratt *et al.* 1998).

Some authors have speculated on global climate effects if phytoplankton and ice algal species fixing CO_2 and producing DMSP, and subsequently DMS, are effected. A reduced ocean sink capacity for CO_2 could result in higher levels of CO_2 and a greater rate of development of the greenhouse effect and global climate change (Voytek 1989; Häder 1997; Worrest and Häder 1997). One laboratory study has shown that DMSP production in *Phaeocystis antarctica* is inhibited by UV-B (Hefu and Kirst 1997). Reduced DMSP and subsequent DMS flux to the atmosphere could conceivably affect the atmospheric radiation budget though the role of DMS as cloud condensation nuclei (Worrest and Häder 1997).

Despite these studies, the effects of enhanced UV-B on the Antarctic ecosystem remain unclear. Estimated impacts range from insignificant to catastrophic (Roberts 1989) and many difficulties frustrate efforts to extrapolate laboratory findings to natural communities. These include the natural variability of incident UV-B radiation at the sea and ice surface, mediated by physical processes (cloud cover and solar zenith angle) and attenuation of UV-B through water, ice and snow (Smith and Baker 1989). UV-B may penetrate to ocean depths greater than 50 m (Smith *et al.* 1992) and is attenuated by particulate and dissolved organic compounds and the seston itself (Vincent and Roy 1993). Furthermore, vertical mixing will expose phytoplankton to a range of light climates and UV-B exposure. Sea ice is relatively transparent to UV-B during spring ozone depletion (Trodahl and Buckley 1989). However, the high albedo of snow and ice and absorption of the communities within, results in variable transmissions of UV-B through the ice and into the water column (Karentz 1991).

Biological factors also frustrate attempts to quantify the effects of UV-B in natural environments. These include the wide range of species present, their natural variability in development stages, pigment absorptive characteristics, and sensitivities and tolerances to UV radiation (Smith and Baker 1989). Furthermore, antarctic algae have now been subjected to at least 25 years of enhanced UV-B irradiance through the ozone hole. Combined with the lack of baseline data, present studies can only document responses of organisms already predisposed to higher levels of UV-B (Karentz 1994).

In Antarctic marine ecosystems, the bottom ice algal communities and phytoplankton have been the focus of much research due to their importance at the base of the food chain (Legendre *et al.* 1992) and the coincidence of the spring blooms with the formation of the ozone hole. Specific UV sea ice studies have focused on the effects of UV-B on productivity and biomass of bottom ice communities (Ryan 1992; Ryan and Beaglehole 1994; McMinn *et al.* in press; McMinn *et al.* submitted). However, in comparison to the upper ice environment, these communities are somewhat protected from UVR by the absorbing and reflecting properties of their habitats and, in the case of phytoplankton, avoidance by vertical mixing.

To date, there have been no studies on the effects of UVR on upper ice brine communities. This environment should provide a unique opportunity to study the effects of enhanced UV-B. This is presumably a higher light environment than the underside of the ice and the refraction of UV-B within the ice and the light piping effects of the brine channels should further enhance irradiation of the community (Trodahl and Buckley 1989). The brine channels and pockets of the upper ice physically entrap the community during spring ozone depletion, reducing the variation in UV-B exposure experienced by phytoplankton through vertical mixing.

This chapter examines the effects of UVR *in situ* on the upper fast ice brine community at Davis Station through a light spectral perturbation experiment. UV induced stress is assessed by changes in biomass, lipid class composition, pigments, cell counts and variations in species life cycle. The effect of UVR on DMSP production in the upper ice is also investigated. A preliminary study of UV defence mechanisms such as UV absorbing compounds is also attempted.

5.2 Methods and Materials

5.2.1 Experimental design

The light perturbation site was situated in fast ice near O'Gorman Rocks, 1 km from Davis Station. The site differed from the routine O'Gorman Rocks sampling site (Chapters 3 and 4) by the heavy snow cover (300 - 500 mm) that persisted to mid

November. The site was chosen for its snow cover and it was assumed this cover had minimised pre-exposure of the underlying brine community to UV radiation.

Three treatments of four, replicate 1.5 m^2 plots were cleared of snow and plastic screens of polypropylene, polycarbonate and mylar were attached over the clear ice plots. The control plots were covered with polypropylene sheeting that did not absorb light in any part of the spectrum (Figure 5.0) and reduced light intensity to similar levels to that of the other screens. The polycarbonate screens removed most UVR but transmitted Photosynthetically Active Radiation (PAR, 400 - 750 nm, Figure 5.0) (hereafter called PAR only). Mylar sheeting allowed the transmission of PAR and UV-A radiation whilst screening out most UV-B (Figure 5.0) (hereafter referred to as PAR + UV-A).

The dimensions of the plots reflected the minimum size necessary to overcome the influence of horizontal diffusion of unfiltered light and unscreened brine from the screen edge. Buckley and Trodahl (1987) concluded that light diffuses out to a horizontal width comparable to the thickness of the ice. As the fast ice brine was only sampled to a depth of 500 mm, this left an area of 1 m^2 under the centre of the screens unaffected by horizontal diffusion. The light 'buffer' area was assumed to also minimise the chance of sampling unscreened brine from adjacent ice. This is a reasonable assumption considering the strong vertical orientation of brine channels (Weissenberger et al. 1992). All samples were collected within the 1 m^2 area to avoid the influence of diffusing horizontal radiation and unscreened brine.

5.2.2 UVR measurements

UV radiation measurements were to be taken using a SPEX Spectramate 1680 spectroradiometer stationed at Davis Station. However, equipment failure over the duration of the experiment resulted in this data being unavailable. Consequentially, data on the effects of UVR presented here is compared on a relative basis between the perturbation treatments.

5.2.3 Brine sampling

Brine sampling was carried out by removing a section of the screen, drilling an 8"
Jiffy drill hole to a depth of 500 mm into the fast ice and collecting the brine that
accumulated in the holes. The screens were then patched with new material.
Samples were taken every 3 - 4 days where possible.
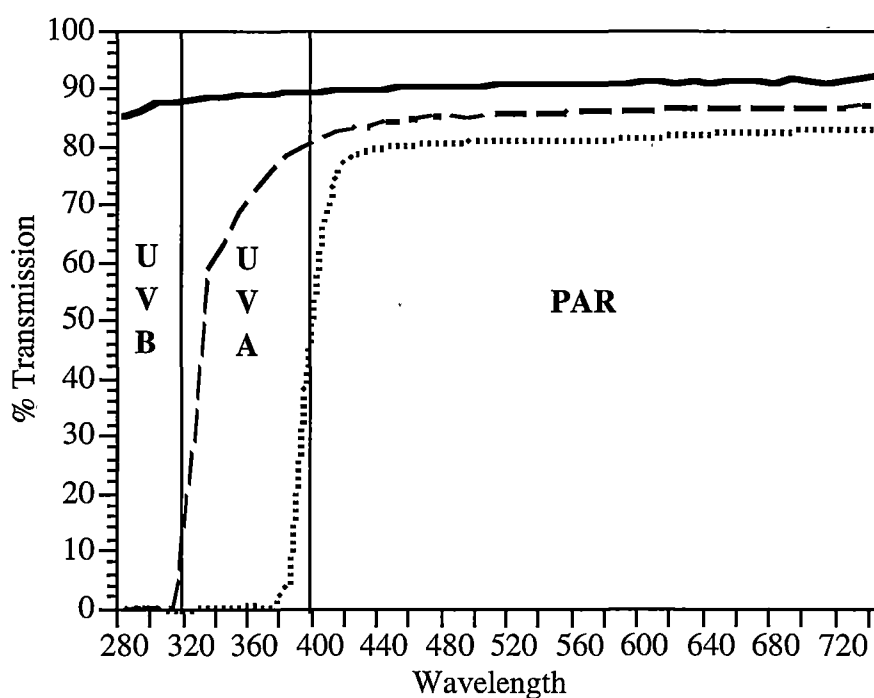
**Figure 5.0 Light transmission of the polypropylene (control, unbroken line),
mylar (PAR + UV-A, dashed line), and polycarbonate (PAR only, dotted line)
sheeting**

One plot from each treatment (Control 1 (C1), Mylar 1 (My1) and Polycarbonate 3
(PC3)) was sampled for pigment, lipid, DMSP and UV absorbing compounds.
Samples for brine temperature, salinity and cell counts were taken from each of the
four replicates. Brine temperature and salinity measurements were taken as described
in Chapter 3.

Pigment analysis

Chlorophyll *a* analysis was carried out on all replicates from each treatment. Up to one litre of each sample was filtered onto GF/F filters and extracted in 90% acetone in the dark overnight. Chl *a* concentrations were determined spectrophotometrically as described in Chapter 3.

One litre of brine from the specified replicates within each treatment (C1, My1, PC3) was also filtered for the detailed analysis of pigments by HPLC and CHEMTAX (see Chapter 3 for methods). Samples for detailed pigment analysis were not replicated.

Lipid analysis

On each sampling date, up to 3 l of fresh brine was taken from the designated plots of each treatment for lipid analysis. Samples were filtered onto muffle furnaced 47 mm GF/F filters and stored at –80°C. The filters were transported back to Australia under liquid nitrogen where they were stored again at –80°C until extraction. Lipid extraction and identification of lipid classes and sterols was as described in Chapter 2.

DMSP analysis

Duplicate samples for DMSP analysis were collected at each sampling period from the designated plots. The duplicate samples were acidified to pH 1 using concentrated HCl and stored in glass vials in the dark at 4 °C. These samples were returned to Australia for analysis by Ms.Anne Trevenna (described in Chapter 4).

Brine communities

Species identification and cell counts were made from subsamples from each replicate preserved in Lugol's iodine. One litre subsamples of Lugol's iodine preserved brine were allowed to settle and cells were counted using the method described in Chapter 3. Only species preserving well in Lugol's iodine were enumerated and fragile flagellate species (such as chrysophyte flagellated stages) may have been lost using this preservative.

UV absorbing compounds

One litre brine samples were taken for a preliminary screening of UV absorbing compounds throughout the light perturbation experiment. The samples were filtered through GF/F filters and the pigments extracted by homogenisation in 10 ml of 100% methanol. The mix was centrifuged at 4000 rpm for 10 min, transferred to cuvettes and the absorbance measured between 250 and 800 nm in a GBC 916 UV visible spectrophotometer.

5.2.4 Normalising for the effect of increasing brine volume

The increasing brine volume of Davis fast ice was described in Chapter 3 and is characteristic of the effect of the spring/summer transition on fast ice (Stoecker *et al.* 1992; 1997; 1998). Brine volume increases as the ice warms and melts. Brine pockets and channels within the ice (restricted in size whilst the ice is cold) expand, flood with fresh melt water and brine salinity decreases. The effect of the increasing brine volume as the ice warms is to dilute algal populations and to complicate the analysis of biomass measurements, cell numbers and DMSP concentrations.

To overcome the diluting effect of increasing volume over the duration of this experiment, biomass measurements such as chl *a* and total lipid were normalised to the initial brine salinity (100 psu) at the start of the experiment. As brine salinity decreases with increasing brine volume, salinity can be used as a conservative factor in estimating brine volume. Brine salinity changes were used as a correcting factor in normalising for the diluting effect of the increasing brine volume.

DMSP concentrations and UV absorbing compounds were normalised to the HPLC measured chl *a* (normalised to salinity) from the designated plots sampled for these parameters.

5.2.5 Statistical analysis

ANOVA was used to distinguish differences in chl *a*, cell and DMSP concentrations within and between treatments. Significant differences ($\propto = 0.05$) were identified using the Tukey test. Statistics were performed using the data analysis pack in Excel 98 and Systat.

5.3 Results

Under the experimental plots, brine salinity dropped from >100 psu on 21 November to 8.5 (PAR only and PAR + UV-A) and 22 psu (control) by 9 December. Brine temperature increased in dynamic equilibrium with salinity, rising from -5.3 °C to 0 °C by 9 December (Figure 5.1A,B). Whilst significant differences in mean brine salinity and temperature were not recorded between the treatments, some variability was apparent. On 1 December, both salinity and temperature in the PAR only treatment (40 psu and -2.1 °C) differed from the remaining treatments (approximately 58 psu and -3.2 °C). A further insignificant difference in temperature was apparent between the control treatment (22 psu) and the remaining treatments (approximately 8 psu) on 9 December.

Brine salinity and temperature were more hypersaline and colder in the experimental plots than in the routine sampling sites in Davis fast ice (Figure 5.1A, B). On 21 November, brine salinity under the experimental plots was > 100 psu and temperature was -5.4 °C. Mean salinity and temperature values from routine sampling in areas of lower snow cover, were approximately 68 psu and -3 °C on the same date. Following the removal of snow cover, brine salinity and temperature under the experimental plots changed a faster rate then found at the routine fast ice sites.

5.3.1 Biomass measurements

Chlorophyll a

Significant changes in normalised chl *a* were apparent in each of the treatments by the end of the experiment. Under PAR only (Figure 5.2A), spectrophotometric mean chl *a* (hereafter referred to as mean chl *a*) on 21 November (2.1 μg l^{-1}) was approximately twice that found in the other treatments. Chl *a* under this treatment increased significantly (9.3 μg l^{-1}, $P < 0.00005$) between 24 November and 1 December and again to 9 December, reaching 23.1 μg l^{-1} by 1 December.

Under PAR + UV-A (Figure 5.2B), mean chl *a* increased significantly ($P < 0.00005$) from 5 December to a maximum concentration of 28.3 μg l^{-1}. Under the full light spectrum (Figure 5.2C), chl *a* concentrations were lower than the other treatments,

ranging between 0.6 - 2.0 µg l^{-1} until 5 December. Similarly to the PAR + UV-A

treatment, a significant increase ($P < 0.05$) was only recorded from 5 December when

chl a reached 4.4 µg l^{-1}.

Between treatments, chl a concentrations under the UV screens (PAR only and PAR

+ UV-A) were in agreement from 5 December. A significant difference, however,

was recorded between these treatments and the control from 1 December ($P < 0.02$

for 5 December and $P < 0.005$ for 9 December).
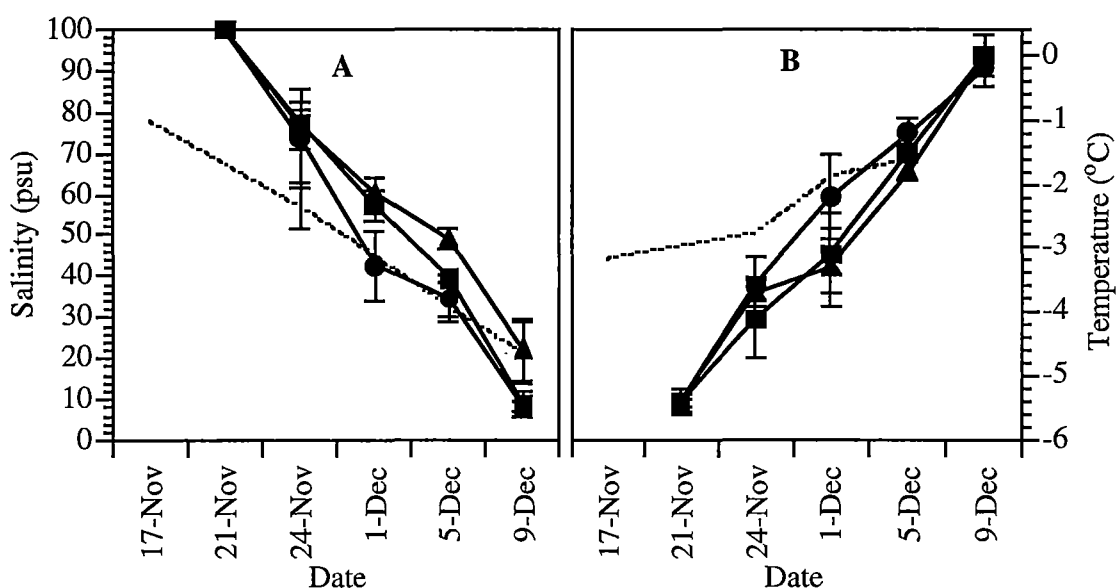
Figure 5.1 Mean brine salinity A) and temperature B) under the control (▲),
PAR only (●) and PAR + UV-A (■) treatments. Dotted line represents mean
measurements from routine fast ice sampling (Chapter 3) (n = 4, error bars represent
1 s.d)

The unreplicated HPLC chl a concentrations showed some difference to mean chl a

trends by being almost consistently lower in concentration (Figure 5.2A-C). Under

the control treatment, the HPLC chl a were in agreement with the mean

spectrophotometric measurements but failed to increase from 5 December onwards.

Similar differences are also apparent in the remaining treatments, particularly

between 5 and 9 December when HPLC chl a decreased in concentration against

steady or increasing mean chl a.
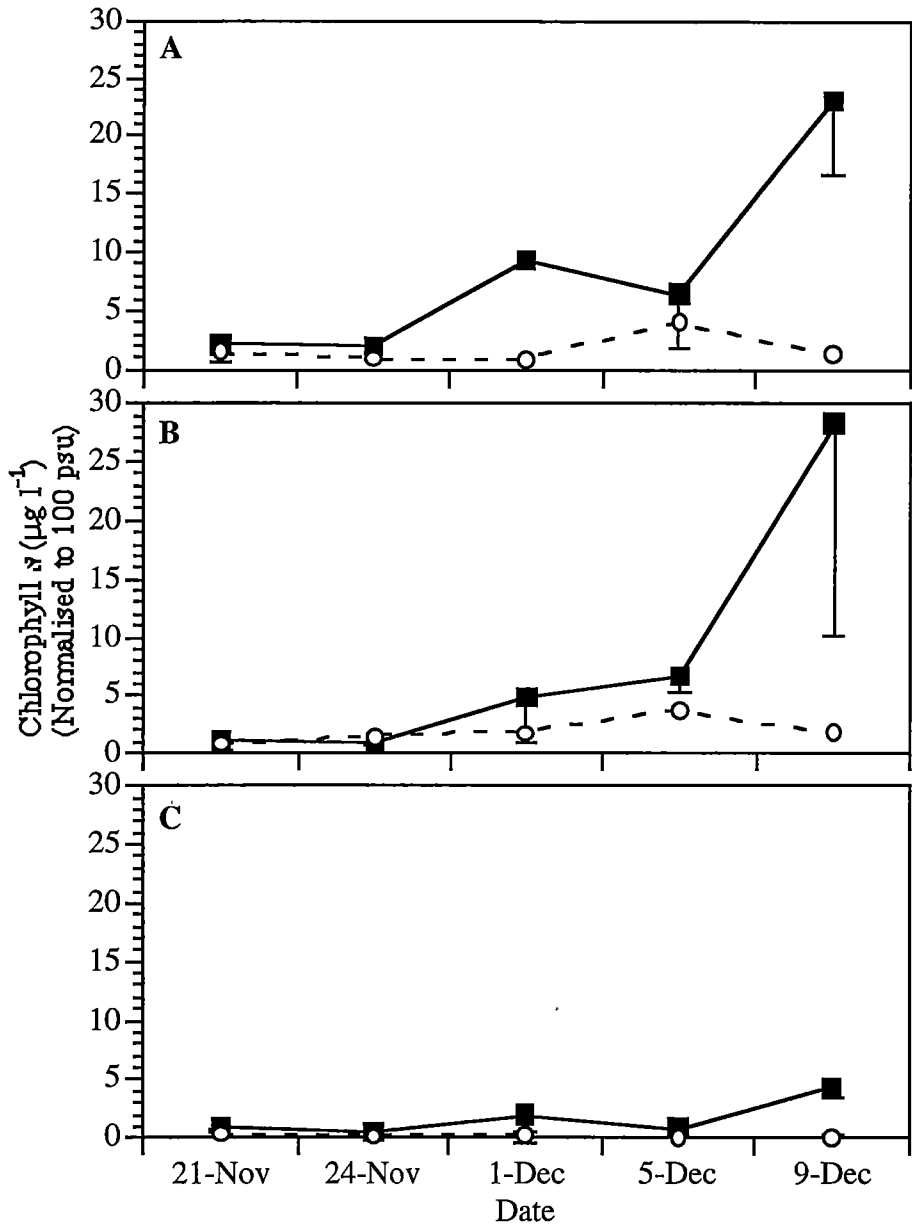
**Figure 5.2 Normalised chl _a_ (■), HPLC chl _a_ (○) in the A) PAR only, B) PAR +
UV-A and C) control treatments (normalised to 100 psu, n = 3, error bars
represent 1 s.d)**

Total Lipid

Under PAR only, normalised total lipid ranged between 1.3 - 4.1 ng l^{-1} before

increasing from 1 December to 8.3 ng l^{-1} (Figure 5.3A). A similar pattern was

evident under the PAR + UV-A treatment (Figure 5.3B). In contrast, total lipid

remained relatively steady under the control treatment, ranging between 1.5 - 4.7 ng l^{-}

[1] throughout the experiment (Figure 5.3C). General agreement between the lipid and HPLC chl *a* estimates was apparent within the treatments.
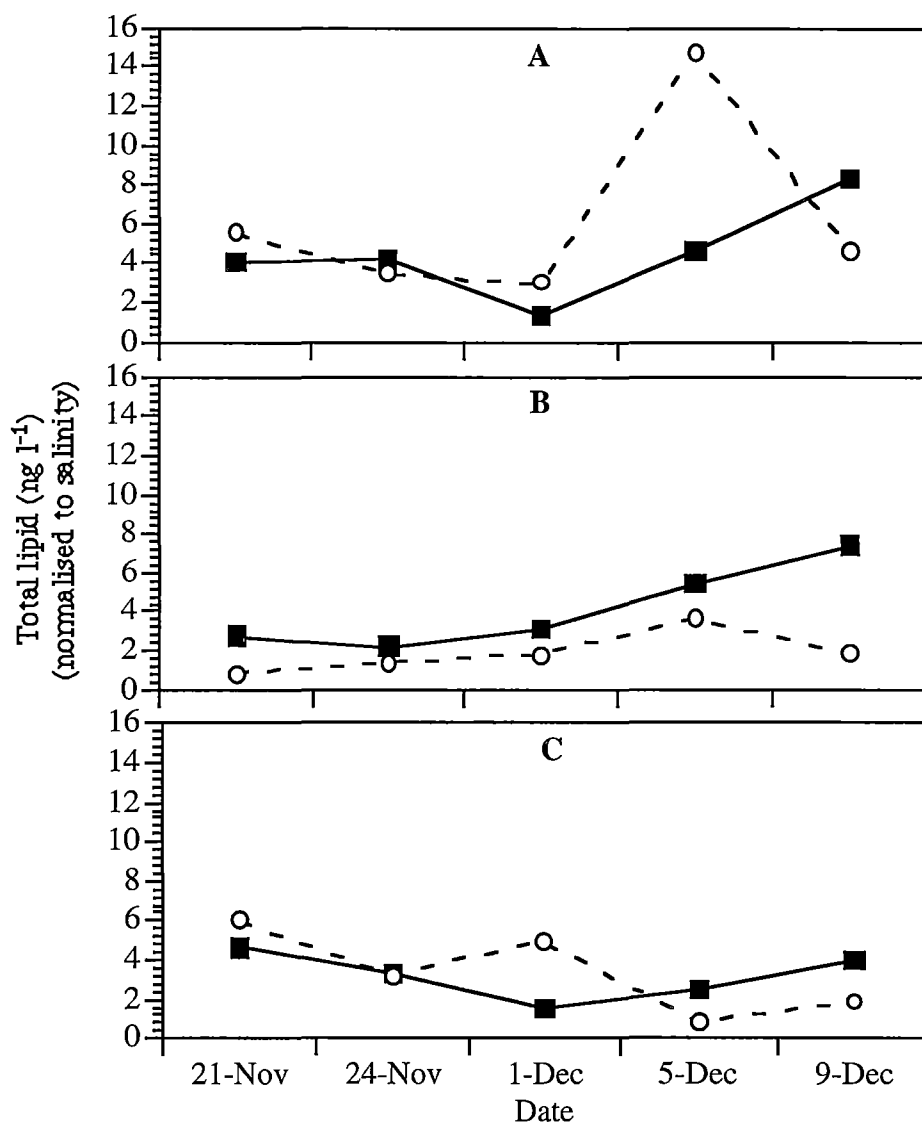
Figure 5.3 Normalised lipid (■) and HPLC chl *a* (○) as biomass indicators in the A) PAR only, B) PAR + UV-A and C) control treatments (lipid measurements unreplicated)

5.3.2 Photosynthetic pigments

Of the pigments present, peridinin, diadinoxanthin and chl *c* were the most consistently abundant. Peridinin is indicative of the presence of dinoflagellates (Figure 5.4A) whilst alloxanthin unambiguously identified cryptophytes within the

ice. CHEMTAX, therefore, distinguished only dinoflagellate and cryptophyte algal classes in the brine.
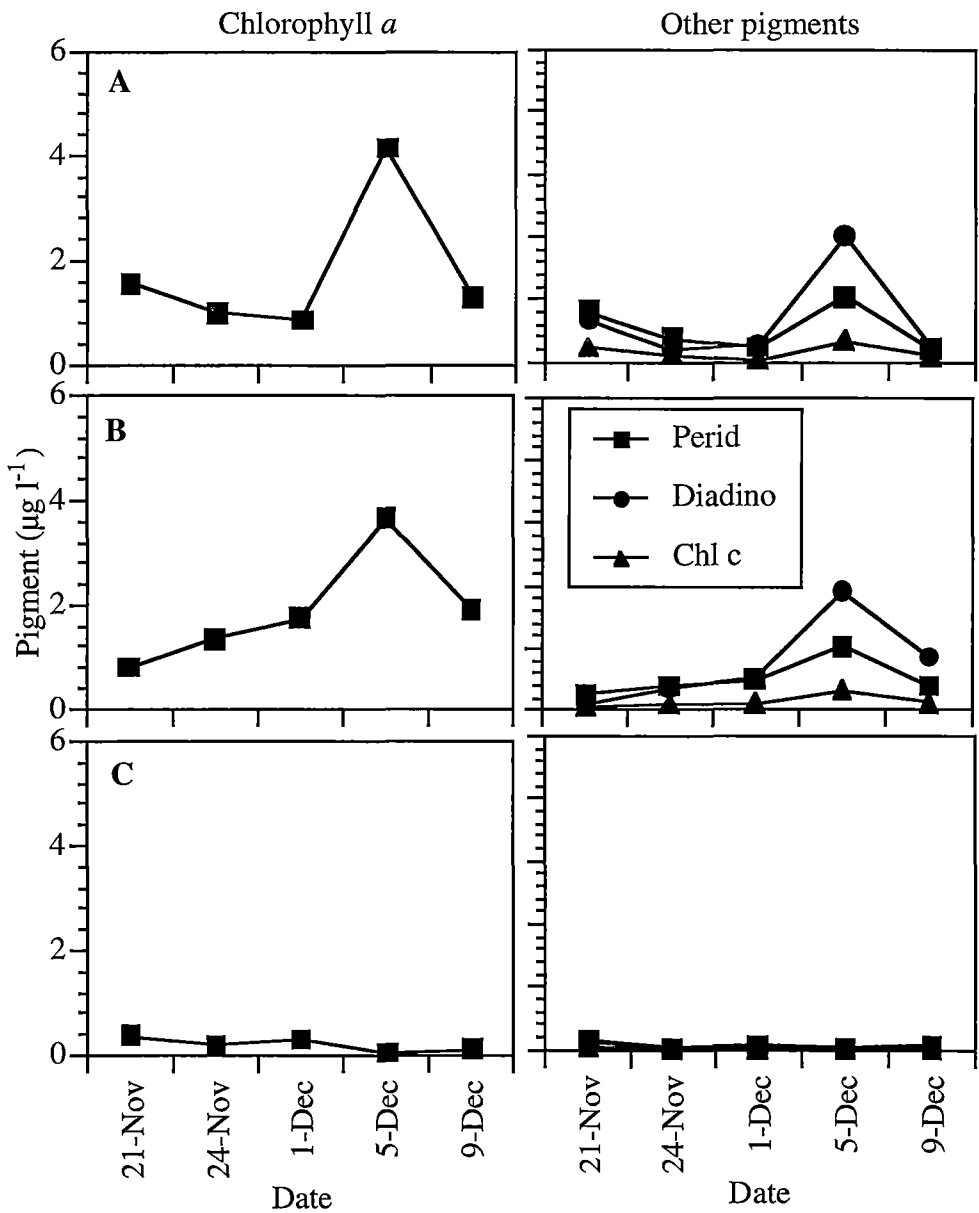
Figure 5.4 Normalised HPLC chl *a*, peridinin (perid), diadinoxanthin (diadino) and chl *c* in the A) PAR only, B) PAR + UV-A and C) control treatments (pigments normalised to 100 psu)

Under PAR only and PAR + UV-A, chl *a*, peridinin, diadinoxanthin and chl *c* concentrations all peaked in concentration on 5 December (Figure 5.4A,B).

Substantially lower concentrations of these accessory pigments were evident in the control treatment (Figure 5.4C).

Of the remaining pigments, only fucoxanthin was consistently present in all samples on all dates (Table 5.0). Chl *b*, ß,ß carotene, violaxanthin, diatoxanthin, dinoxanthin, 19'-hexanoyloxyfucoxanthin were present in low and variable concentrations in the PAR only and PAR + UV-A treatments but were often undetected under the control treatment. Alloxanthin, generally present under the UVR screens, was evident in the control at 0.02 µg l^{-1} to 24 November but was not detected after this date.

5.3.3 Lipid classes

Polar lipid was the dominant lipid class under each treatment, reaching 86% under PAR only treatment (Figure 5.5A), up to 87% in the absence of UV-B (Figure 5.5B) and up to 82% of the total lipid in the control (Figure 5.5C). Polar lipid under the control appeared to increase slightly to 1 December, with a concomitant decrease in hydrocarbons, free fatty acids and sterols.

Triglyceride was the next most abundant lipid class. Under PAR only, triglyceride showed a general increase in percent composition, rising initially from 4.5% on 21 November to vary between 7 and 11% of the total lipid. When UV-A was added to PAR, triglyceride composition increased to 1 December before falling on subsequent dates. Triglyceride in the control treatment generally increased to 15% on 5 December, mostly at the expense of free fatty acids and sterols, thereafter decreasing to 7.2%.

Free fatty acids showed a similar composition and trend between each of the treatments, each decreasing in composition to early December before rising again by 9 December. Minimal fatty acid composition in the control (0.9%) was reached by 5 December whilst in the PAR + UV-A and PAR only treatments, the lowest values (1.32 and 0.5% respectively) occurred by 1 December. Hydrocarbon and sterol composition showed similar profiles in each of the treatments. However, a higher composition of hydrocarbon (12.8%) was evident at the start of the experiment in the control treatment, as opposed to the PAR + UV-A (8.3%) and PAR only (7.9%) treatments.

Table 5.0 Normalised photosynthetic pigments in the control, PAR+UV- A and PAR only treatments

(normalised to temperature, [1]- indicates pigments not detected, [2]19'-Hexanoyloxyfucoxanthin)

Treatment /date	[1]Photosynthetic pigment ($\mu g \ l^{-1}$)							
	Alloxanthin	Chlorophyll b	ß,ß Carotene	Violaxanthin	Diatoxanthin	Dinoxanthin	[2]19'-Hex	Fucoxanthin
PAR only								
21/11	0.05	-	0.28	0.11	0.05	0.05	0.04	0.3
24/11	0.04	-	0.08	0.09	0.14	0.02	0.07	0.72
1/12	0.04	-	0.23	0.04	0.03	0.03	0.03	0.13
5/12	0.27	-	0.86	0.22	0.60	0.28	0.08	0.39
9/12	0.10	-	-	-	0.21	0.04	0.14	0.27
PAR+UV-A								
21/11	0.04	0.08	0.19	0.05	0.01	0.01	0.04	0.2
24/11	-	-	0.09	0.06	0.19	0.03	0.04	0.24
1/12	0.03	0.11	0.09	0.02	0.02	0.04	-	0.09
5/12	0.18	0.07	0.67	0.10	0.32	0.17	0.08	0.17
9/12	0.27	0.18	0.12	0.03	0.67	0.13	-	0.27
Control								
21/11	0.01	0.05	0.1	0.02	0.01	0.01	-	0.04
24/11	0.02	-	-	-	0.03	-	0.3	0.26
1/12	-	-	-	0.01	-	0.01	0.02	0.12
5/12	-	-	-	-	-	-	-	0.02
9/12	-	0.03	-	-	0.02	0.01	-	0.03
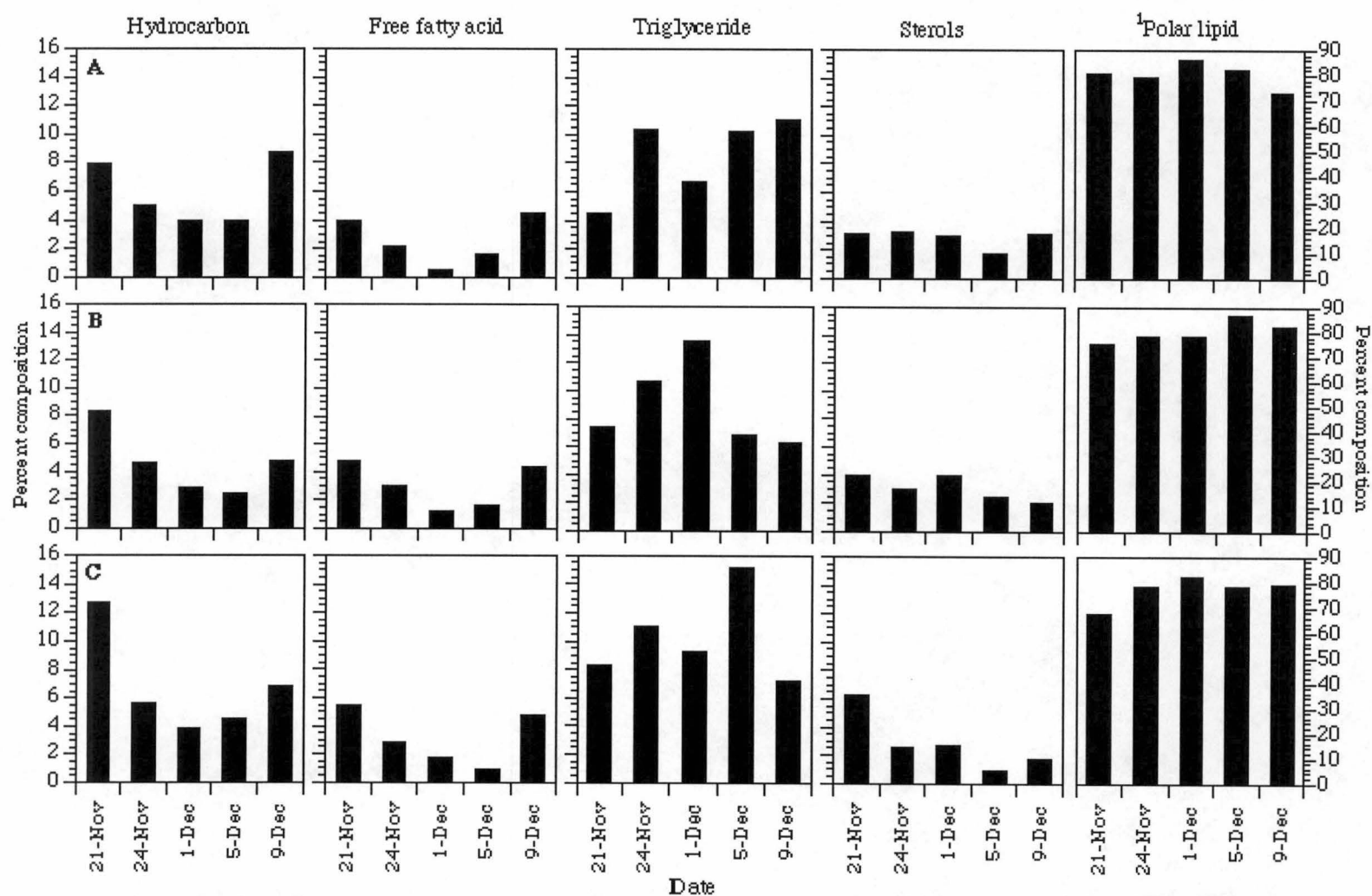
Figure 5.5 Percent composition of lipid classes in the A) PAR only, PAR + UV-A and C) control treatments ([1] note different scale for polar lipids)

5.3.4 DMSP

Under PAR only (Figure 5.6A), total DMSP ranged between 43.9 - 161 nM

DMSP/μg chl a l^{-1}. DMSP under this treatment showed an immediate significant

increase ($P < 0.005$), rising from 43.9 to 161 nM DMSP/μg chl a l^{-1} between 21 and

24 November. DMSP concentrations remained high until decreasing to around 45.1

nM DMSP/μg chl a l^{-1} from 5 December.
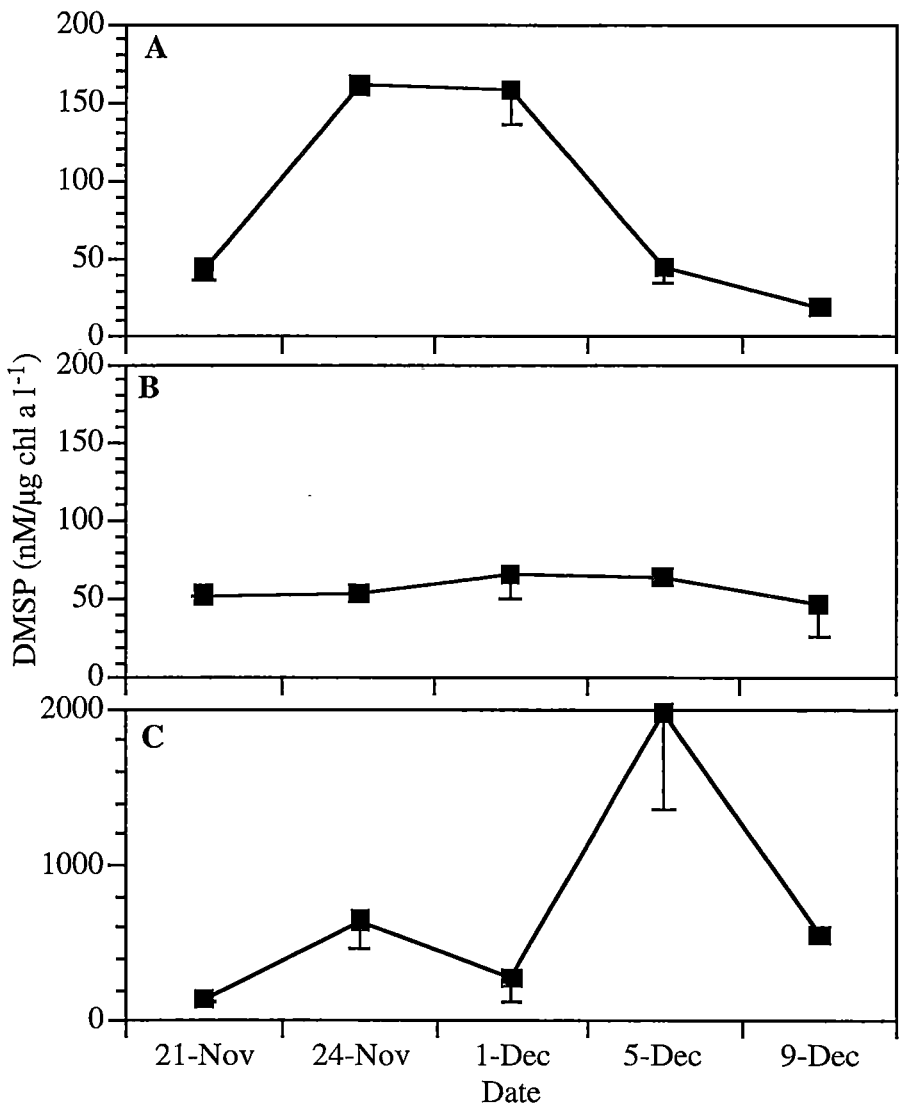
**Figure 5.6 Total DMSP per unit of HPLC chl a in fast ice brine in the A) PAR
only, B) PAR + UV-A and C) control treatments** (n = 2, error bars represent s.d unless
otherwise shown).

In the PAR + UV-A treatment (Figure 5.6B), DMSP concentrations varied little, ranging between 52.8 and 65.4 nM DMSP/µg chl a l^{-1}. These values were lower than in the PAR only treatment and no significant increase in DMSP was recorded over the course of the experiment.

Under the control treatment (Figure 5.6C), total DMSP concentrations were consistently higher than reported from under the UV screens, ranging between 148.7 and 1979.5 nM DMSP/µg chl a l^{-1}. DMSP did not increase significantly in concentration until 1 December ($P < 0.05$), rising from 278.8 to 1979.5 nM DMSP/µg chl a l^{-1}).

Between treatments, the control DMSP total concentrations were significantly different from the remaining treatments throughout the experiment. PAR only DMSP differed from the PAR + UV-A concentrations in the initial increase evident under PAR only from 21 November.

5.3.5 Community composition

Polarella glacialis, chrysophyte statocysts, *Mantoniella* sp. *Cryptomonas* sp. and flagellate sp.3 were identified in the Lugol's preserved brine samples. Two species of *Cryothecomonas*, (heterotrophs identified in Chapter 3) were also present in the UV brine samples. Notably absent from the brine at the UV experimental site were *Pyramimonas*, live diatoms, *Strombidium* sp. and the heterotrophic dinoflagellates identified in the routine sample sites in Chapter 3. Flagellated and coccoid chrysophyte cells, numerous in the routine brine samples (Chapter 3), were highly variably in occurrence and difficult to identify in the Lugol's fixed samples. Time constraints restricted the use of fresh and glutaraldehyde fixed samples necessary to enumerate these chrysophyte cells, explaining their lack of appearance in this study.

Polarella glacialis

Polarella glacialis and its sexual stages (counts all normalised to salinity) were well preserved in the samples and evident in each treatment. *Polarella glacialis* vegetative or asexual cells under PAR only increased significantly to 1 December ($P < 0.05$), peaking at 16.6 x 10^4 cells l^{-1} (Figure 5.7A). Planozygote concentrations also

peaked on this date, showing a highly significant increase ($P < 0.00005$) to 15.4 x 10^4 cells l^{-1} on 5 December. Mean cyst numbers increased in concentration, peaking at 24.8 x 10^4 cells l^{-1} by 9 December. No significant difference in cyst numbers, however, was recorded over the course of the experiment. Cell counts from the replicate PC3 closely agreed with the mean asexual and planozygote cell counts. However, cysts were not recorded from this replicate.

Under the PAR + UV-A treatment (Figure 5.7B), vegetative *P. glacialis* concentrations showed no significant change in concentration throughout the experiment. In comparison to the PAR only treatment, vegetative cell numbers were low, ranging between 1.3 - 5.6 x 10^4 cells l^{-1}. Low numbers of planozygotes and cysts were observed from 21 November, with both peaking in abundance (24.4 x 10^4 cells l^{-1} and 16.0 x 10^4 cells l^{-1} respectively) from 5 December. A significant increase was not recorded in either the planozygotes or the cysts. Vegetative and planozygote counts in My1, the individual PAR + UV-A plot, closely agreed with the mean counts. Cysts were recorded in My1 only on 9 December.

Mean *P. glacialis* vegetative cells under the control (Figure 5.7C) were comparable in concentration to those under PAR + UV-A and also failed to show any significant change in cell number. Planozygotes were apparent from 1 December, reaching a mean concentration of 23.2 x 10^4 cells l^{-1} by 9 December. This mean increase, however, was not significant. Encystment under the control treatment was variable, with cysts noted only on 1 and 5 December (0.4 and 0.3 x 10^4 cysts l^{-1} respectively).

Polarella glacialis asexual cell counts under the control plot C1 (Figure 5.7C) showed the same pattern of decrease as the mean counts but lower concentrations of cells from 24 November. Sexuality was less evident than seen in the remainder of the control replicates, with fewer planozygotes and no cysts observed on any date.

Apart from the early presence of planozygotes under PAR + UV-A, there was no significant difference in the concentrations of these cells between the treatments. Similarly, cyst numbers between treatments did not differ significantly.
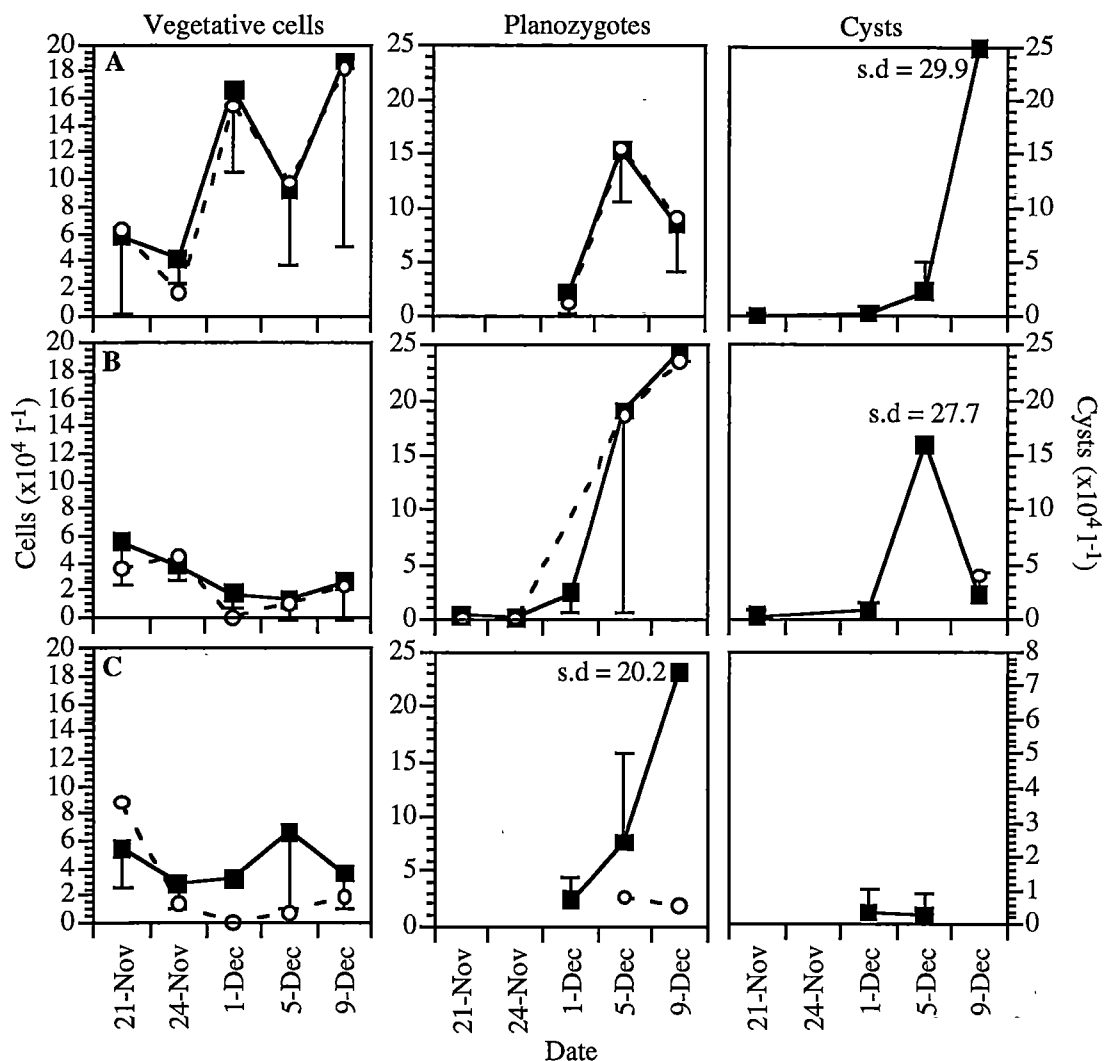
Figure 5.7 Mean *Polarella glacialis* vegetative cells, planozygotes and cysts normalised to salinity in the A) PAR only, B) PAR + UV-A and C) control treatments (note different scale on cyst chart in control treatment. Dashed line indicates counts from individual plots (C1, MY1 and PC3) within treatments, error bars represent 1 s.d unless otherwise shown)

Other algal species

The remaining microalgal species identified were relatively low in concentration and variable in occurrence within and between the treatments, with some species not detected in several of the replicates (Appendix A). *Mantoniella* sp., identified in the routine brine samples (Chapter 3), showed a general increase in cell concentration under the PAR only and control treatments to 9 December, reaching up to 16.3×10^3

cells l^{-1}. Despite these apparent trends, variation in cell number was high, with standard deviations up to 186% of mean values. In the PAR + UV-A treatment, *Mantoniella* peaked in abundance at 13.6 x 10^3 cells l^{-1} on 24 November, thereafter decreasing in number to 9 December. However, the standard deviation was 134% of the mean value on 21 November, indicating high variability between the replicates. Similar variation, evident in the *Cryptomonas* and the possible raphidophyte counts, frustrated attempts to discern trends and effects and these data were not analysed further.

5.3.6 UV absorbing compounds

The methanol extracted UV absorbing compounds in the upper ice absorbed strongly between 310 and 340 nm. The absorbance maxima under each of the screens (normalised to chl *a*) over the course of the experiment varied little, with the PAR only and full light spectrum ranges falling between 326 to 335 nm (Figure 5.8A and C respectively). In the PAR + UV-A treatment (Figure 5.8B), maximal absorbance fell between 325 to 330 nm. There appeared to be no discernible trend in the accumulation of UV absorbing compounds under any treatment.
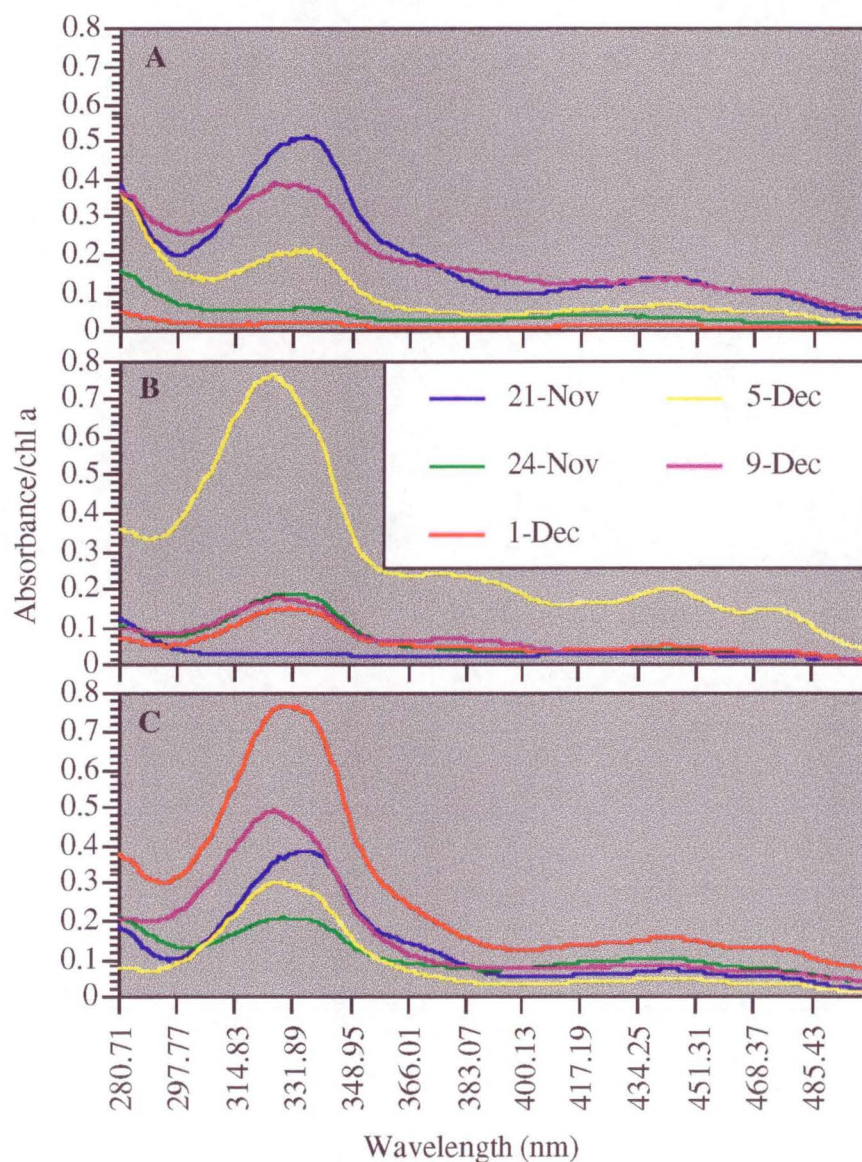
Figure 5.8 UV absorbing compounds under the A) PAR only, B) PAR + UV-A and C) control treatments (normalised to salinity adjusted chl *a*)

5.4 Discussion

Whilst significant differences in mean brine salinity and temperature were not recorded between the treatments, some variation was apparent. Disparity in salinity and temperature under PAR only on 1 December and in temperature in the control treatment on 9 December from the remaining treatments were noted. Although not significant, these differences may have resulted in sufficient variation in growth of

the brine community to add a confounding effect to the data. Therefore, subsequent conclusions based on this data must be treated with caution.

In terms of normalised chl a, UV-B radiation appeared to significantly affect biomass within the upper ice. Whilst chl a concentrations under the UV screens showed little variation, there was a substantial difference between values from the PAR only and control treatments from 24 November. From this date, biomass increased significantly in the absence of UVR (2.0 - 9.3 µg l^{-1}). Under the full light spectrum, chl a concentrations were substantially lower (0.6 - 2.0 µg l^{-1}) and did not increase until 5 December. The similarities between the PAR only and PAR + UV-A treatments and the response under the full light spectrum indicate that this effect is caused by UV-B.

HPLC chl a trends differed from those of mean chl a on several dates under each of the treatments. These variations and deviations around the mean chl a concentrations reflect the variability within the ice between and within the replicates. The lower concentrations of the HPLC chl a estimates in comparison to the mean spectrophotometric measurements was attributed to natural variation between the replicates and absorbance differences between the two methods. Spectrophotometry may overestimate chl a by up to 48% through the absorbance of pheophorbides, cholophyllide a and chl a epimers and allomers. In comparison, the HPLC method is more selective for chl a, separating this pigment and its breakdown products (Mantoura *et al.* 1997). Under each of the treatments, total lipid biomass reflected the trends in HPLC chl a.

5.4.1 Community composition

Based on the number and concentration of photosynthetic pigments identified in the brine, CHEMTAX failed to distinguish algal classes other than the Dinophyceae and the Cryptophyceae. The poor performance of CHEMTAX was due to the few pigments identified, their low concentrations and probably the integration of pigments sampled from a graduated light regime between 0 - 50 cm depth in the ice (Chapter 3). However, combined with microscopic identifications and counts, several of the pigments confirmed the presence of dinoflagellates and a species of

Cryptomonas and were useful in analysing their dynamics under the experimental plots. Microscopic identification confirmed the presence of *Gymnodinium* sp. 2, *Mantoniella*, the tentatively identified raphidophyte and two heterotrophic species of *Cryothecomonas*.

UVR appeared to adversely affect the growth of asexual *P. glacialis* cells. Under PAR only, *P. glacialis* vegetative cells increased in concentration to peak in number by 1 December and persisted at this higher concentration to 9 December. In contrast, the addition of UV-A and UV-B to the light spectrum resulted in no change in cell concentrations throughout the experiment.

UVR may also effect sexuality in *P. glacialis*. Significant increases in planozygote numbers occurred only in the absence of UVR. However, increasing concentrations were evident in the PAR + UV-A and control treatments and it is possible that the difference recorded here is due to variability within the ice.

Encystment in the presence of UV-B was substantially lower than in the other treatments. Up to 0.5 x 10^4 cysts l^{-1} were counted under the control as compared to 29.8 and 28.2 x 10^4 cysts l^{-1} in the absence of all UVR and UV-B respectively. However, cyst numbers were highly variably between the treatments so it is not possible to assign this apparent effect to UV-B with confidence.

Pigment analyses confirmed the *P. glacialis* population dynamics within the experimental plots. Peridinin, diadinoxanthin and chl *c* were the pigments most consistently evident in the samples. Peridinin is a major accessory pigment specific to dinoflagellates and in *P. glacialis* it contributed up 31% of the total pigment (Chapter 2). Diadinoxanthin (11%) and chl *c* (approximately 6%) are present in more moderate quantities in this dinoflagellate. Whilst these pigments are known in other dinoflagellates, *Gymnodinium* sp.2 was the only other dinoflagellate identified in the samples. *Gymnodinium* sp.2 was present only in low numbers and observed to early December in the PAR only treatment, only on 21 November in the PAR + UV-A treatment and to 24 November in the control treatment. It is assumed therefore that all the peridinin in the samples is contributed by *P. glacialis* unless a specific reference is made to *Gymnodinium* sp.2.

Under the influence of PAR only, peridinin, diadinoxanthin and chl c (and chl a) peaked in concentration by 5 December, coinciding with the maximum number of *P. glacialis* planozygotes under this treatment. These pigments failed to mark the vegetative cell peak on 1 December and did not reflect the persisting high numbers of these cells to 9 December. *P. glacialis* planozygotes are larger than the vegetative cells (15 - 25 μm compared to 12 - 15 μm of the asexual cells) and the larger sexual cells appear to contain greater pigment concentrations (Chapter 2). This size difference may partially explain the peak in pigments coinciding with the planozygotes, despite the higher asexual cell concentrations.

Similar peaks of peridinin, diadinoxanthin, chl c and chl a on 5 December under the remaining treatments reflected the increases in planozygote and cyst numbers recorded on this date.

Other species

Low and highly variable counts of the remaining species under each of the treatments frustrated attempts to identify effects of the modified light regimes. The pigments identified were correspondingly low in concentration and were also generally unhelpful. Alloxanthin concentrations under each treatment confirmed the presence and dynamics of the *Cryptomonas* sp. under each of the treatments.

5.4.2 Lipid classes

Lipid classes were determined from each treatment in an effort to identify physiological stress within the brine community. Laboratory studies have shown that the lipid class composition of algae may be influenced by temperature, light intensity, nutrient availability and growth phase (Mortensen *et al.* 1988; Thompson *et al.* 1990; McMinn *et al.* 1999).

Polar lipid, triglyceride and free fatty acids are useful indicators of physiological stress in algal communities and have been previously used in this manner in bottom ice algal communities (Nichols *et al.* 1989; Skerratt *et al.* 1998; McMinn *et al.* 1999). Polar lipids contain the membrane components of the cell, including glycolipid, phospholipid and chlorophyll (Harwood and Russell 1984) and high levels of polar

lipid are associated with actively growing cells (Kattner *et al.* 1983; Parrish 1987). Triglycerides are storage products generally assumed to accumulate in algae under nutrient stress during the stationary phase of growth (Parrish 1988). As such, they are likely to be the major lipid class in stressed cells (Parrish and Wangersky 1987). However, recent culture and field studies using sea ice algae have shown that increasing triglyceride composition during the log growth phase appears to be a characteristic of sea ice algae (Nichols *et al.* 1988; Smith *et al.* 1993; Henderson *et al.* 1998). Free fatty acids are degradation products and are indicative of stressed or senescent cells.

In this experiment, polar lipid was the major lipid class in each of the treatments, contributing between 82 to 87% of the total lipids. Triglycerides were the next most abundant class (up to 15 % in the control treatment) and showed a general increasing trend to early December. In contrast, free fatty acids were low in composition (between 4 and 6% on 21 November and 9 December), decreasing to minimal compositions between 0.5 and 1.3% by early December.

The high compositions of polar lipids alone indicate that the community as a whole under each treatment was healthy, incorporating most lipid into cell membrane components as opposed to storage or breakdown products. The polar lipid composition of the brine community compares favourably to studies of a diatom dominated bottom ice community at Wohlschlag Bay, McMurdo Sound where polar lipid contributed 81% of the total lipids (Nichols *et al.* 1989).

If triglyceride accumulation during log phase growth is taken as characteristic of sea ice algae then the increasing triglyceride compositions of the community under each of the treatments (up to 15 %) to early December also points to a healthy, actively growing community. Alternatively, these increasing compositions may indicate some stress within the community, increasing in effect to early December. In either case, triglyceride accumulation was low and varied little between the treatments. Other studies have showed more significant compositions of this lipid class, with neutral lipids totalling up to 94% in bottom ice algae of the Barents Sea (Henderson *et al.*

1998) and between 4 and 68% in McMurdo Sound (Nichols *et al.* 1989; McMinn *et al.* 1999).

The decrease in free fatty acids to early December supports the hypothesis that the community was under little stress to this time and is in agreement with the same indication by the polar lipid content of the community. This would also indicate that the coincidental accumulation of triglyceride is indeed characteristic of sea ice algae and does not necessarily indicate stressed cells. The free fatty acid composition under the treatments varied little and was low in comparison to other studies. Free fatty acid in bottom ice algal communities have been measured between 2 and 52% in McMurdo Sound (Nichols *et al.* 1989; McMinn *et al.* 1999). Overall, lipid class composition between the treatments varied little under the different light regimes, exhibiting little sign of physiological stress on the part of the brine community.

Using lipid classes to assess the physiological status of algal communities may not be appropriate in the upper ice brine communities. In polar regions, this technique has been used primarily on bottom ice communities dominated by diatoms that display the typical culture dynamics including the logarithmic and stationary growth phases. Furthermore, these diatom dominated communities do not show the same complexity of life cycle as seen in dinoflagellates and chrysophytes.

In contrast to the bottom ice communities, *P. glacialis*, chrysophytes and their life cycle stages dominate fast ice brine communities at Davis and McMurdo Sound. The asexual cells, planozygotes and cysts of *P. glacialis* successively peak in the brine as the life cycle progresses. During this succession, high compositions of polar lipids would be expected throughout as these lipids are necessary in the cell membranes of the succeeding life cycle stages. Furthermore, the decrease in cell numbers (asexual and sexual cells) is mostly attributed to sexuality and encystment rather than senescence as in the case of diatoms. Therefore, the accumulation of storage products such as triglyceride and an increase in breakdown products such as fatty acids seen in senescent diatom cultures (Dunstan *et al.* 1993) and communities would not be expected in a community dominated by species with a life cycle.

This argument is supported by a similar lack of change in lipid class in the temperate and planktonic dinoflagellate, *Gymnodinium catenatum*, between exponential and stationary phases of growth in culture (Hallegraeff *et al.* 1991). This probably explains the high composition of polar lipid found here and the lack of discernible change in the other lipid classes throughout the experiment. Using lipids classes to assess physiological stress in communities dominated by life cycle events, therefore, appears to be inappropriate.

5.4.3 UV effects on total DMSP

UV-A appeared to adversely affect total DMSP concentrations per unit of chl *a*. Total DMSP increased immediately from 21 November to 5 December when the community was screened from all UVR. A similar response was evident under the control treatment on the addition of UV-B. In contrast, when UV-A was added to the spectrum, increases in DMSP were not observed.

Hefu and Kirst (1997) have shown UVR effects DMSP concentrations in cultures of *Phaeocystis Phaeocystis* showed high DMSP production when screened from UVR. In contrast, the full light spectrum resulted in a significant decrease in DMSP. Whilst all UVR adversely effected DMSP content of the *Phaeocystis* cultures, UV-B (between 305 and 320 nm) was strongest in this effect. In the UV brine experiment, UV-A was the most detrimental of UV wavelength bands.

It was concluded in Chapter 4 that DMSP may play an osmoregulatory and cryoprotectant role in brine species. In the routine brine samples, DMSP concentrations decreased with decreasing salinities and increasing temperatures. In this experiment, DMSP in each treatment showed at least some increase against the trends of salinity and temperature. Whilst this occurrence is difficult to explain in terms of salinity and temperature, the role of light in regulating DMSP production may at least partially explain the initial increases seen here.

Light intensity and photoperiod (with increasing and decreasing day lengths elevating or reducing DMSP concentrations respectively) have been demonstrated as factors influencing DMSP production (Vairavamurthy *et al.* 1985; Karsten *et al.* 1990; Karsten *et al.* 1992). Furthermore, DMSP production is mainly restricted to

photosynthetic organisms (Karsten *et al.* 1996), indicating that DMSP synthesis is somehow light related. This is supported by experiments where DMSP was degraded by species kept in the dark and where, under hypersaline treatment, DMSP was only accumulated as an osmolyte by species in the light (Kirst 1989; Karsten *et al.* 1992). In this experiment, the sudden, higher light levels in the upper ice after the removal of the snow cover may have stimulated DMSP production in the brine species present, resulting in the initial increases recorded.

5.4.4 UV absorbing compounds

UV absorbing compounds were present in the brine species, with the maximal absorption spectra occurring between 310 and 335 nm. Compounds absorbing UVR over these wavelengths provide protection against the upper range of UV-B (310- 320 nm) and the lower range of UV-A (320 - 340). Absorption found here compares well to a survey for UV absorbing compounds in Antarctic marine organisms, where the absorption spectra of these organisms mostly peaked between 315 – 335 nm (Karentz *et al.* 1991b). The shoulders of the absorption spectra in the brine species would provide further protection against UV wavelengths in the lower UV-B and upper UV-A regions.

The UV absorbing compounds in the brine species did not accumulate with either time or light perturbation. This indicates that these compounds were not photoinducible and are probably inherent in at least some of the species present.

5.5 Conclusions

In terms of normalised chl *a*, UV-B appeared to adversely affect biomass production within the upper, fast ice community at Davis Station. Under PAR only and PAR + UV-A, chl *a* showed similar increasing trends with time. However, on the addition of UV-B to the light spectrum, chl *a* concentrations remained low to late in the experiment, increasing only between 5 and 9 December.

CHEMTAX failed to distinguish between specific algal classes (other than the Dinophyceae and the Cryptophyceae) sampled under the screens. At the species level, high variability and low or undetectable numbers within the treatments of most

flagellates, reflected in the pigment concentrations, frustrated attempts to identify effects on individual species. Only *P. glacialis* showed promise in this regard.

UVR radiation exhibited a detrimental effect on *P. glacialis* asexual cell numbers. Under PAR only, the asexual cells increased in number to peak by 5 December. However, UV-A and UV-B inhibited similar growth, resulting in no growth in population size over the experimental period. It was not possible to differentiate the effects of the two spectra of UV radiation based on these results.

UV radiation may have effected sexuality and encystment within *P. glacialis*. Planozygote concentrations increased significantly only in the absence of UVR. UV-B possibly reduced encystment as only very low cyst numbers were evident under this treatment. However, variable planozgygote and cysts numbers between treatments frustrated attempts to confirm these observations.

Pigment analysis using peridinin, diadinoxanthin and chl *c* (all known pigments of *P. glacialis*) confirmed the population dynamics of *P. glacialis* leading to these conclusions.

UV-A appeared to adversely effect the accumulation of total DMSP in the brine. Under PAR only and on the addition of UV-B, increases in DMSP concentrations were immediate and significant. In contrast, when UV-A was added to the light spectrum, DMSP increases were more gradual and failed to become significant until 5 December.

Lipid class analysis failed to show any indication of physiological stress under the varying light treatments. There was little evidence of variation between the composition of polar lipids, triglycerides or free fatty acids within or between the treatments. In fact, the compositions of these lipid classes indicated a healthy community. Overall, however, it was concluded that the use of lipid classes as indicators of physiological stress in brine communities was not appropriate. This conclusion was based on the markedly different redistribution of lipid classes that would be expected in a community dominated by life cycles as opposed to the culture like growth dynamics of diatom dominated communities.

The brine community was not totally defenceless against UV irradiation and contained UV absorbing compounds with absorption spectra between 310 and 335 nm. It was concluded that these compounds were not photoinducible due to the lack of discernible trends in accumulation recorded here. The extent of protection afforded by these compounds is unknown.

Chapter 6 Conclusions

The abundant dinoflagellate in the upper fast ice adjacent to Davis Station is identified as *Polarella glacialis*. The lack of genetic variability between isolates of this species from Davis Station and McMurdo Sound indicates this species has a single Antarctic circumpolar population. Observations of morphologically indistinguishable cysts to *P. glacialis* from other locations around Antarctica support this likely distribution.

The photosynthetic pigments and lipids of *P. glacialis* are described for the first time. The pigments of this species are typical of dinoflagellates. Peridinin is the major carotenoid present (24 - 31%) over moderate proportions of diadinoxanthin (11%) and chl *c* (6%). From the limited number of samples analysed, there appears to be no regional variation in pigmentation between the Davis and McMurdo Sound isolates of *P. glacialis*.

The fatty acid profile of *P. glacialis* is also characteristic of most dinoflagellates, although the PUFA composition of this species (up to 76%) is the highest recorded in dinoflagellates to date. The high proportion of PUFAs in *P. glacialis* is likely to be an adaptation to the cold brine environment. The major fatty acids of this species are 18:5 (n-3) (43 - 49%), 22:6 (n-3) (17 - 19%) and 16:0 (8%).

The sterol profile of *P. glacialis* is atypical of dinoflagellates, dominated by 4-desmethylsterols (76 - 79%) over the more characteristic 4α-methyl sterols (22 - 24%). Dinosterol is absent. This composition is more characteristic of a small subset of gymnodinoid dinoflagellates, including *Symbiodinium microadriaticum*, a close relative of *P. glacialis*.

Significantly, this study identifies 27-nor-24-methylcholest-5,22E-dien-3ß-ol as the major sterol of *P. glacialis* (61 - 64%). 27-Nor-24-methylcholest-5,22E-dien-3ß-ol is rare in other dinoflagellates and unknown in other classes of microalgae. This sterol, therefore, may prove useful as a specific biomarker for *P. glacialis* in Antarctic field studies, either alone or in combination with its fatty acids and pigments. Furthermore, 27-nor-24-methylcholest-5,22E-dien-3ß-ol may be useful as a

biomarker in sediments for interpreting past Antarctic climate change and fast ice extent.

The brine community at Davis Station consists of cryo- and halotolerant, autotrophic flagellates, few diatoms, a ciliate species and several microheterotrophs. *Polarella glacialis*, chrysophytes and their life cycle stages are the most abundant species in the brine.

Although excystment of *P. glacialis* was not observed in either year, asexual motile cells are obvious in the brine in the earliest samples (early November). Sexuality occurs from mid November, followed by mass encystment of this dinoflagellate from early December. Brine temperature and salinity, varying between years, did not appear to be the major factors inducing sexuality and encystment in *P. glacialis*. The sudden decrease in cyst numbers by mid December, coinciding with the appearance of cysts in the water column, indicates that brine drainage probably provides a mechanism for seeding the water column with cysts.

The chrysophyte life cycle progressed in a similar manner to *P. glacialis*. The flagellate stage is present from early November and is followed by an abundance of non motile coccoid cells by early December. By mid December, mass encystment results in large numbers of statocysts within the ice. Statocyst numbers decrease sharply thereafter, most likely as a result of brine drainage.

Other autotrophic species in the upper ice included a species of *Cryptomonas*, two species of *Pyramimonas* and *Mantoniella* sp. *Nitzschia subcurvata* and *Navicula delicatula* are the only diatom species of numerical importance in the upper ice. *Nitzschia subcurvata* reached bloom proportions in 1996 but was less abundant in 1997.

Several heterotrophic species are present in the brine. These include two species of *Cryothecomonas* (*C. armigera* and *Cryothecomonas* sp.2), several dinoflagellates (*Gyrodinium* sp., *Gymnodinium* sp. and *Protoperidinium* sp.) and a euglenoid.

In comparison to the upper fast ice community at McMurdo Sound, Davis fast ice is more diverse in flagellate and diatoms species. Ciliates in Davis ice, however, appear

restricted to one species of *Strombidium* as compared to several genera in McMurdo Sound.

Overall, however, the Davis ice environment and community is remarkably similar to that of McMurdo Sound. Both habitats exhibit extreme and transitory temperatures and salinities and, with the progression of ice decay, brine drainage events. Similarities in the major autotrophic and heterotrophic taxa are also evident. Furthermore, there is a remarkable synchronicity between the life cycle events of *P. glacialis* in both locations.

The similarities between the Davis and McMurdo Sound communities confirm at least a disparate distribution of this upper ice community around Antarctica. Combined with the likely distribution of *P. glacialis*, observations of a population of small dinoflagellates and chrysophytes cysts (particularly *Archaeomonas areolata*) at Syowa Station, it seems probable that this community has a circumpolar distribution.

DMSP production and the possible roles of this sulfur compound were investigated in fast ice communities and in cultures of *P. glacialis*. Significant concentrations of DMSP are produced in Davis ice and are highest when the brine is hypersaline and cold. As decreasing DMSP concentrations correlated with decreasing salinity and rising temperature, it appears that DMSP acts either as an osmolyte, cryoprotectant or both in the brine species.

P. glacialis in culture produces up to 4.9 pg DMSP/cell or 87.4 μM DMSP/cm^3 of cell volume. However, under salinity upshock, this species fails to show significant increases in intracellular DMSP. It is concluded that, under the nitrogen replete conditions of this experiment, DMSP did not act as a primary osmolyte in *P. glacialis*.

The effects of UV radiation on the upper ice community were assessed *in situ* using spectral perturbation methods. Under the conditions of this experiment, UV-B radiation inhibited chl *a* production in the brine community whilst UV-A appeared to have little effect. The growth of *P. glacialis* asexual cells is retarded by all UVR but it is not possible to separate the effect of UV-A from UV-B. UV-A alone appeared to reduce DMSP production in the upper ice.

The brine community is not defenceless against UVR and a preliminary screening of the brine community revealed compounds absorbing UV radiation between 310 to 335 nm. It was determined that these compounds were not photoinducible and appeared inherent to the brine species present.

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Appendix A Auto- and heterotrophic cell concentrations

Cell concentrations of autotrophic and heterotrophic species under the
A) control, B) PAR + UV-A and C) PAR only treatments (s.d in brackets
following mean cell counts, n = 3. nd = not detected)

	Algal species (x 10^3 cells l^{-1})				Heterotrophic species (x 10^3 cells l^{-1})	
Treatment/ date/replicate	Gymnodinium sp. 2	Cryptomonas sp.	Mantoniella sp.	Raphidophyte	C. armigera	Cryothecomonas sp.2
PAR only						
21/11	2.27 (2.64)	0.48 (0.27)	7.12 (9.56)	5.05 (3.62)	3.29 (1.85)	12.54 (3.20)
PC3 [a]	0.12	0.26	21.38	1.67	1.09	13.82
24/11	nd	0.89 (0.37)	4.12 (1.24)	1.23 (1.26)	5.70 (3.10)	2.41 (0.86)
PC3		0.58	2.61	nd	7.82	2.17
1/12	4.12 (7.11)	1.18 (0.89)	14.87 (2.96)	8.18 (4.21)	5.04 (3.59)	13.33 (8.75)
PC3	nd	nd	18.57	3.98	nd	5.3
5/12	nd	2.21 (1.72)	16.25 (3.21)	nd	2.46 (0.52)	14.27 (8.38)
PC3		nd	19.96		2.94	23.5
9/12	nd	0.18 (0.22)	13.98 (0.95)	nd	1.31 (1.37)	2.15 (0.58)
PC3		nd	15.24		3.32	2.91
PAR+UV-A						
21/11	2.74 (1.86)	0.79 (0.58)	5.92 (6.71)	11.24 (2.35)	12.72	10.77 (4.19)
MY1 [a]	4.07	nd	8.58	8.58	(11.90)	16.04
24/11	nd	0.78 (0.58)	13.63 (7.25)	4.63 (2.18)	6.24	6.90 (4.75)
MY1		1.61	17.25	5.22	9.76 (7.54)	5.62
1/12	nd	10.54 (6.44)	1.42 (0.85)	0.51 (0.61)	6.02	10.85 (9.20)
MY1		6.43	1.02	0.49	4.03 (1.10)	0.46
5/12	nd	10.22 (12.95)	0.22 (0.38)	0.65 (1.13)	nd	25.65 (22.53)
MY1		nd	0.35	0.46	10.22	3.91
9/12	nd	nd	0.22 (0.41)	nd	(9.68)	4.78 (2.57)
MY1		nd	0.25	nd	1.96	5.92
					1.49 (0.93)	
					0.42	
Control						
21/11	0.34 (0.25)	0.60 (0.38)	2.86 (2.22)	1.32 (0.36)	3.52 (0.73)	10.20 (4.03)
PP1 [a]	1.24	0.44	4.3	0.86	4.17	13.69
24/11	0.50 (1.01)	3.31 (2.56)	8.85 (8.26)	3.66 (2.30)	28.83	8.4 (6.79)
PP1	nd	1.37	1.37	0.92	(13.28)	2.75
1/12	nd	11.36 (12.12)	10.70 (9.91)	1.54 (0.79)	10.98	10.34 (7.42)
PP1		nd	nd	1.16	6.17 (0.39)	nd
5/12	nd	0.95 (0.44)	13.32 (10.5)	1.1 (0.52)	nd	4.64 (3.74)
PP1		0.81	2.59	1.13	0.66 (0.57)	2.97
9/12	nd	1.03 (0.85)	14.84	0.54 (0.51)	0.99	17.96 (11.69)
PP1		0.76	(10.36)	0.25	2.50 (2.20)	3.8
			2.89		nd	

[a]PP1, MY1 and PC3 are individual replicates within each treatment