INCORPORATION OF SEA ICE ALGAE INTO NEWLY FORMING SEA ICE, USING FIELD AND LABORATORY BASED EXPERIMENTS.

by

Paul Anthony Scott (BSc, Grad Dip ASOS Hons)

Submitted in fulfilment of the requirements for the degree of Master of Science

University of Tasmania (April, 2000)

QUOTE...

'If you fight the dragon too long, you become the dragon.' (My crazy ex-housemate)

DECLARATION...

This Thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the Thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgment is made in the text of the Thesis.

PAUL ANTHONY SCOTT

AUTHORITY OF ACCESS...

This thesis may be made available for loan and limited copying in accordance with the *Copyright Act 1968*.

PAUL ANTHONY SCOTT

ABSTRACT

Sea ice algae play a critical role as major primary producers in the Southern Ocean ecosystem. Each autumn, algae are incorporated into newly forming sea ice in vast numbers, but the mechanisms by which algal cells are incorporated into young sea ice remain unclear. Early experiments with ice-forming tanks suggested that frazil-harvesting is the most significant process leading to algal accumulation. In the present study, both field and laboratory experiments are used to investigate factors related to algal inclusion in sea ice. Field experiments were used to evaluate the natural extent of algal cell incorporation, while laboratory experiments tested specific processes.

Field samples were collected in areas of new ice formation off eastern Antarctica during the spring of 1992 and the autumns of 1993 and 1996. One to three orders of magnitude higher algal biomass was found in ice and interstitial water samples than in the underlying water column. Diatoms contributed approximately two thirds of the biomass with small unidentified flagellates contributing the remainder. Field experiments indicate some diatom species were selectively incorporated into ice which forms in different conditions (ie that which forms in rough versus calm conditions). There was evidence of size specific incorporation of algae and microspheres into the natural and laboratory-formed sea ice. This size selectivity discriminated against large microspheres and large algal cells.

Experiments using a laboratory-based ice tank and a similar tank at Casey Station Antarctica, revealed that laboratory-based ice tanks are an effective way of simulating both sea ice production and the incorporation of phytoplankton into the sea ice. Experiments were conducted to simulate water being pushed through the ice matrix (similar to a wave field agitation system). Results of ice tank experiments indicate that there are several physical processes responsible for algal enrichment in sea ice, these are, 'pumping' of water through the newly forming ice, water circulation patterns and scavenging of phytoplankton by frazil ice crystals. The ability of phytoplankton to remain suspended in the upper water column is an important biological factor which influences algal incorporation into newly forming sea ice.

Laboratory-based experiments using temperate water diatom species revealed a level of incorporation into sea ice similar to that of Antarctic species. Experiments with glass microspheres indicate that microspheres < 53 μm in diameter were incorporated in to sea ice at levels above the microsphere numbers in the underlying water. Microspheres with a greater diameter rapidly settled out of the system. Despite the spatial and temporal constraints of laboratory-based tanks, Antarctic phytoplankton species were incorporated into slurry within the ice at concentrations as great as 7 times the levels in ice and water samples.

These results imply algae are actively incorporated into very young sea ice. Incorporation microspheres into sea ice at a similar level to Antarctic taxa indicates that in an agitated system the incorporation results from a physical process. The enrichment of specific species in calm conditions indicates there are important biological processes involved in the algal incorporation. Results of this study indicate that the 'pumping' of water through an ice matrix, water circulation patterns and algal cell harvesting, are important physical mechanisms responsible for algal enrichment in sea ice. Preferential incorporation of some species occurs in calm conditions as a result of a combination of biological and physical processes.

ACKNOWLEDGMENTS

Thanks must go to my supervisors Dr Andrew McMinn and Dr Ian Allison, they kept talking to me even though at times they probably shouldn't have. Andrew was always enthusiastic and welcoming.

Thanks must go to Dr Simon Wright at the Australian Antarctic Division (AAD). He graciously provided pigment data presented in Chapter 3 of the thesis. Doctor Andrew Davidson provided Antarctic phytoplankton cultures used in Chapter 5 as well as his time, energy and enthusiasm to assist me with culturing at AAD.

During frequent times of despair, there were a number of people who spurred on the old sluggish pony. Particular Ross, together we battled our respective nemesis like two little bearded cave men with pointy sticks trying to slay some massive hairy beast. Vicky, Naomi and Thommo were also there shooting arrows at said beast. I would like to thank Gary for dragging me away from time to time, he kept me sane.

Mum took to my writing with red pen in hand. Her comments and corrections were very much appreciated. Thanks to mum and dad for being proud of me no matter what I choose to do. Thanks to Jen and Pete for just been cool. Thanks are also due to Margaret Young for her wise and impartial words.

This research was supported by The Institute of Antarctic and Southern Ocean Studies and the Antarctic Co-operative Research Centre at the University of Tasmania, and the Australian Antarctic Division in Hobart Tasmania, Australia.

TABLE of CONTENTS

LIST OF FIGURES...

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

LIST OF TABLES...

Chapter 2

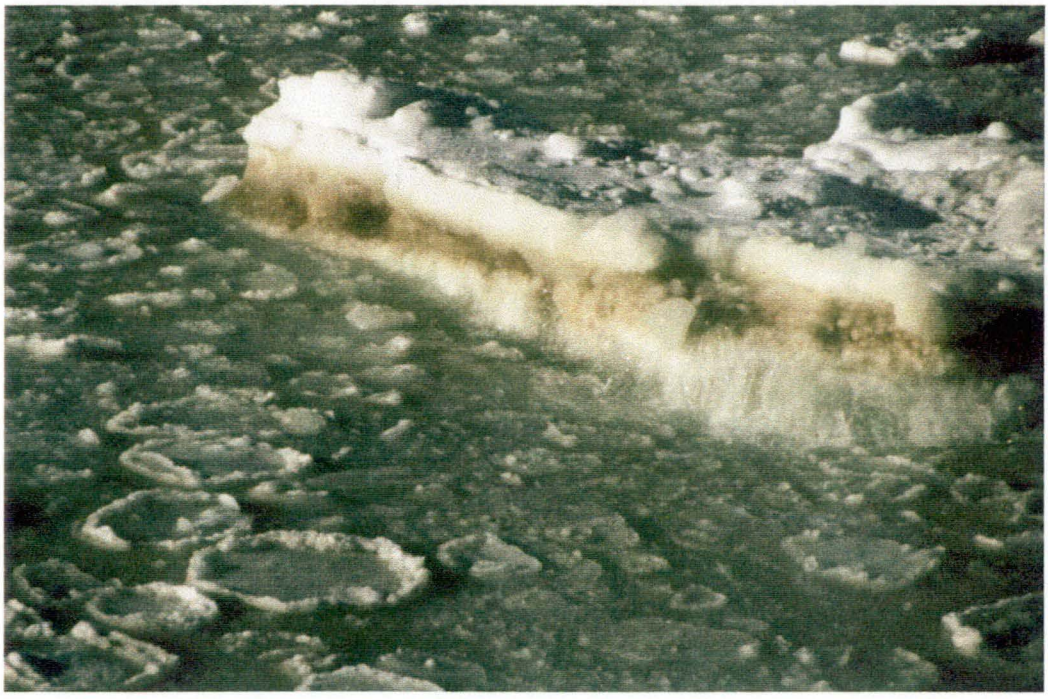
Chapter 3

Chapter 4

Chapter 5

1.0

Introduction...



1.0 INTRODUCTION

1.1 GENERAL INTRODUCTION...

1.1.1 Importance of the Study...

Annually 16 x 10^6 km^2 of sea ice form and decay around Antarctica. This newly forming expanse of ice provides a vast habitat for sea ice algae which are incorporated in huge numbers. The sea ice habitat offers a stable substrate for the algae which ensures exposure to adequate light levels (necessary for photosynthesis) and enables them to avoid settling out of the photic zone. The ice algae together with the phytoplankton then make an equal contribution to the base of the food chain in the Southern Ocean. Sea ice algae have been noticed since the early voyages of exploration in the 1800s where their presence was noted to stain the berg and pack ice. Despite over 150 years of investigation into the mechanisms which facilitate the incorporation of phytoplankton into the ice are still not well known.

Over the past 15 years, several physical and biological mechanisms have been proposed as potential processes responsible for the incorporation and enrichment of phytoplankton into sea ice. Incorporation as a result of physical mechanisms will result in enrichment of any particles which are in the upper part of the water column at the time. This process may be size specific. Incorporation as a result of biological mechanisms will favour phytoplankton that have specific adaptations which increase the likelihood of their entrapment within newly forming ice. Mechanisms described to date are: scavenging, nucleation, wave field pumping, water circulation patterns and the exudation ice active substances.

Laboratory-based facilities allow investigations into the above mechanisms without the logistical difficulties associated with working in regions of newly forming sea ice. Experimental ice tanks are limited in the extent that they are able to replicate the natural environment. However, they are still useful tools for investigating specific processes for algal incorporation into sea ice.

1.1.2 Aim of this Study...

This study was structured to investigate the incorporation of phytoplankton into sea ice. Field and laboratory-based experiments were designed and carried out to determine whether physical or biological processes were responsible for the incorporation. Thus the aim of the thesis was to determine the mechanism or mechanisms responsible for algal enrichment in sea ice.

1.1.3 Research Approach...

To achieve this aim Chapters 2 and 3 discuss the distribution and biomass of algae in newly forming sea ice, which was collected in the Eastern Antarctic pack. Chapter 4 compares samples from an experimental ice tank used in Antarctica, to samples which were concurrently collected from Newcomb Bay, Casey, Antarctica. Chapter 5 describes results from a laboratory-based experimental sea ice tank. Chapter 6 presents conclusions from the four preceding chapters. Physical and biological mechanisms which assist the incorporation of phytoplankton into sea ice are suggested.

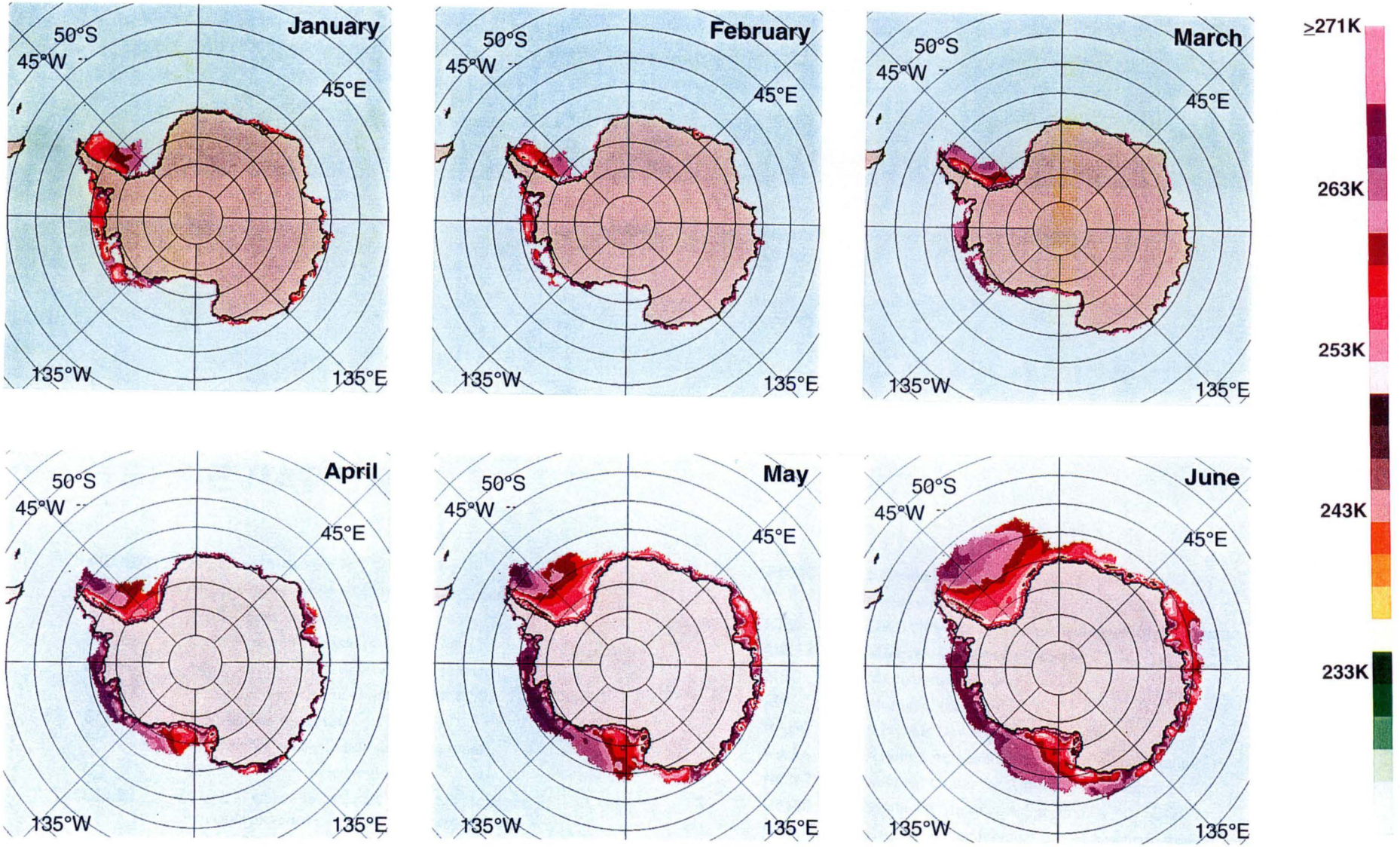
A comprehensive review of sea ice formation processes can be found in Untersteiner (1988). In the next sections, only the processes relevant to this thesis are reviewed. First the seasonal cycle and areal extent of sea ice is discussed (Section 1.2). Section 1.3 reviews ice formation processes, and section 1.4 discusses sea ice biota. Section 1.5 reviews the proposed algal incorporation mechanisms.

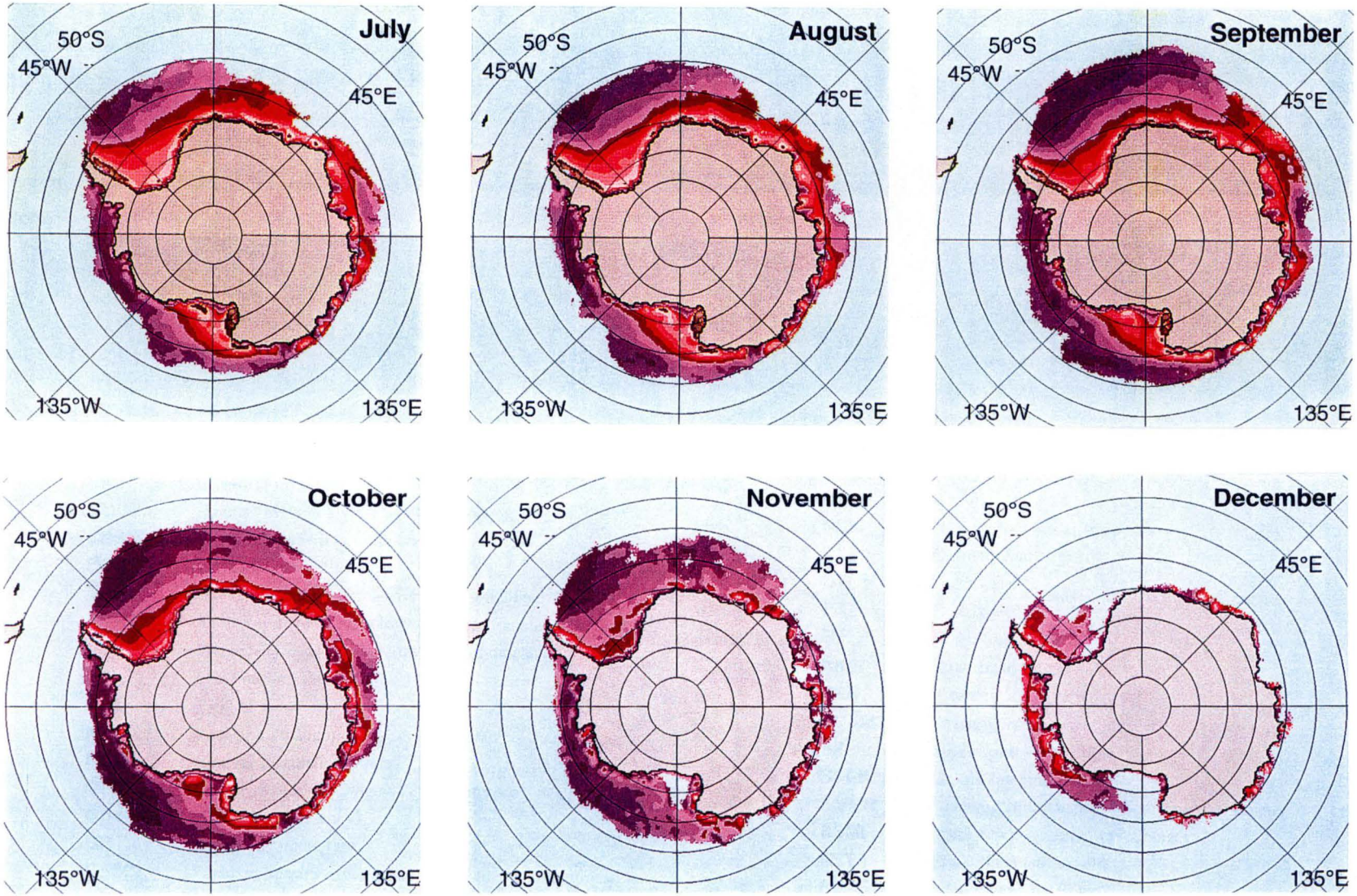
1.2 DISTRIBUTION AND EXTENT OF ANTARCTIC SEA ICE...

During winter up to 20 x 10^6 km^2 of sea ice is present around Antarctica, extending as far as 2200 km from the Antarctic continent. Annually 16 x 10^6 km^2 or more sea ice forms and decays. The monthly average sea ice extent for the period 1978 - 1987, derived from satellite passive-microwave observations, is shown in Figures 1.1 and 1.2 (Gløersen *et al* 1992). During winter, the sea ice covers an area greater than the permanent ice cover on the continent, however the volume of sea ice is several thousand times less than that of the continental ice (Zwally *et al* 1983). Eastern Antarctic pack ice (20° - 160°E) has a mean, undeformed ice thickness (including the open water fraction) which ranges from 0.31 m in December to 0.52 m in August (Worby *et al* 1998). Areas of open

Figure 1.1 Average mean monthly sea ice concentrations for January - June 1978 - 1987 (Gløersen *et al* 1992)

Figure 1.2 Average mean monthly sea ice concentrations for July - December 1978 - 1987 (Gløersen *et al* 1992)


January
February
March
April
May
June
50°S
45°W
45°E
135°W
135°E
≥271K
263K
253K
243K
233K


July
50°S
45°W
45°E
135°W
135°E
August
50°S
45°W
45°E
135°W
135°E
September
50°S
45°W
45°E
135°W
135°E
October
50°S
45°W
45°E
135°W
135°E
November
50°S
45°W
45°E
135°W
135°E
December
50°S
45°W
45°E
135°W
135°E

water exist throughout the pack ice in the form of leads or polynyas. These areas of open water may form up to 5 x 10^6 km^2 in areal extent (Gordon and Cosimo, 1988; Horner *et al* 1992). These areas are important sites for new ice formation.

The Southern Ocean can be divided into sectors, as shown in Figure 1.3 (Gløersen *et al* 1992). Data from satellite passive-microwave observations for the years of 1978 - 1987, indicates the Weddell Sea sector contains over a third of all sea ice. The Ross Sea and Indian Ocean sectors contained each 16 % of total Antarctic sea ice. The Pacific Ocean and the Bellingshausen-Amundsen Seas sectors contain 20 %. Newly formed ice described in Chapters 2 and 3, was collected in the Pacific Ocean Sector around 145° E.

Both inter-annual and annual variations occur in sea ice cover around Antarctica. The observed Southern Ocean sea ice cover for individual years can deviate significantly from the multi-year averages (Gløersen *et al* 1992). The seasonal sea ice cycle varies not only from one part of the Southern Ocean to another but also from year to year and may be linked to other global climate systems (White and Peterson, 1996).

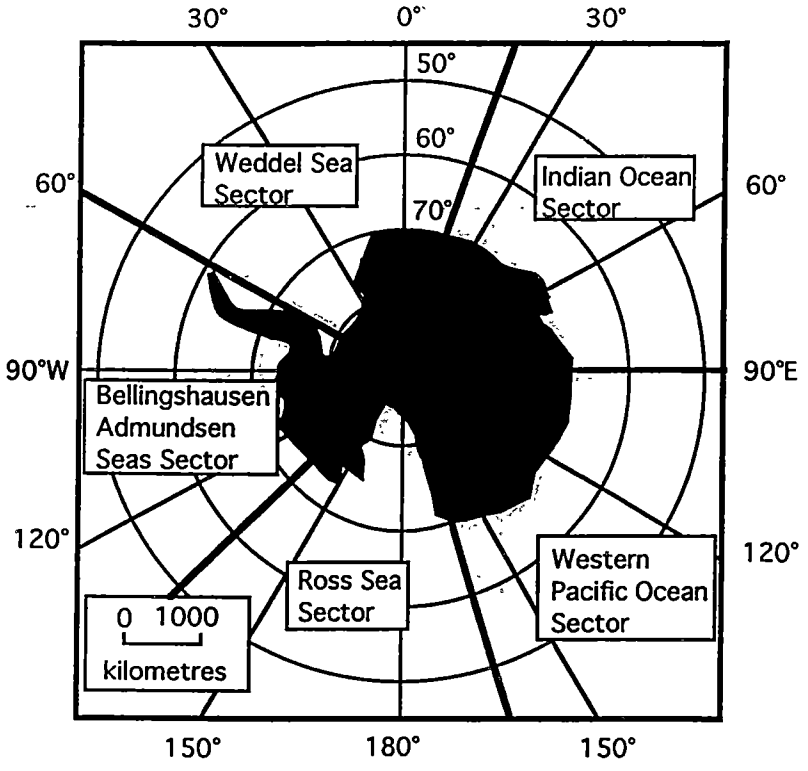
1.3 SEA ICE FORMATION AND EVOLUTION...

1.3.1 Ice Crystal Type...

Before sea ice growth can proceed, the upper layer of the water column needs to be at or slightly below its freezing point; the depth of this layer may be up to tens of metres (Stavn, 1971). Initial ice crystal formation processes are comprehensively reviewed by Weeks and Ackley (1982).

Initial sea ice formation is in the form of minute spheres of pure ice (Hobbs, 1974). Growth rapidly changes these spheres into small discs and needles called frazil ice. Production of frazil ice is most common in leads or polynyas and near glacial ice shelves and ice edges (Weeks and Ackley, 1982; Barry, 1988). Discs are typically 2-3 mm in size and grow at rates often exceeding 1 cm/hr. Discs may continue to grow into hexagonal dendritic stars (Weeks & Ackley, 1982). In calm conditions the star-like crystals grow rapidly across the surface of the water eventually freezing together to form a thin ice skin. If, however, there is wind or wave-induced turbulence during the initial phase of ice formation, extensive discoidal growth is favoured. The frazil ice crystals may be mixed down several

Figure 1.3 Antarctic sea ice sectors (Gløersen *et al* 1992).



meters or more into the water column by turbulence. The growth sequence of the initial ice cover is from spheres -> thin circular discs ->to hexagonal dendritic stars (Weeks and Ackley, 1982).

Under calm conditions, after a thin ice skin forms, growth of columnar crystals will occur. In the zone between frazil and columnar crystals, transition ice grows, (Perry and Pounder, 1958). In this zone, crystals growing in unfavourable directions are eliminated by geometric selection. Crystals growing in favourable directions are able to rapidly grow ahead of crystals with less favourable orientations. Geometric selection and ice crystal orientation is reviewed in Untestiener (1988). The transition zone extends for about 0.05 - 0.10 m below the frazil ice.

Immediately below the transition zone, congelation ice starts to dominate (Untestiener, 1988). This type of ice is comprised of columnar crystals. In calm conditions, these crystals will grow to over a metre in length (Weeks and Ackley 1982). In fast ice, congelation crystals are usually the dominant type. Congelation ice tends to reject biological material (Clarke and Ackley 1984; Scott *et al* 1994; Helme and Weyland, 1995), whereas frazil ice tends to concentrate it (Garrison *et al* 1983). Congelation ice was initially thought to compose the majority of the sea ice in the polar oceans. While this may be true in the Arctic, work in the Weddell Sea area by Gow *et al* (1982); in the Bellingshausen and Amundsen Seas by Jefferies *et al* (1997); and in the Pacific and Indian Ocean sectors by Worby *et al* (1998), indicates that up to 50% of pack ice may be comprised of frazil ice. Clarke and Ackley (1984) and Ackley *et al* (1979) reported pack ice in the Weddell Sea was comprised of up to 70 % frazil ice. Such high concentrations of frazil ice result when ice crystals form in the presence of wind-driven turbulence. Snow ice (Worby *et al* 1998) and platelet ice (Vincent, 1988; Dieckmann *et al* 1993) also occur in the Antarctic but are not spatially significant and so are not discussed further in this thesis.

1.3.2 Newly Forming Sea Ice...

Newly forming ice refers to all categories of ice up to a thickness of 0.1m (World Meteorological Organisation, 1970). Frazil ice crystals combine to form a variety of newly formed ice types.

Grease ice is a very early stage of newly forming ice. Coagulation of frazil crystals results in a soupy layer on the sea surface. Grease ice forms in both calm and rough conditions, however greater thicknesses occur when there is some

agitation. Grease ice has a dark, matt appearance (Stringer *et al* 1984). This ice formation may be accelerated when snow falls into the sea; it becomes saturated and mixes with the water forming a viscous floating mass of ice crystals. With a large amount of wind stress, accompanied by agitation induced by wave action, grease ice may collect into spongy white lumps called shuga. The lumps are typically a few centimetres in diameter.

Under turbulent conditions, the frazil ice crystals may form ice pancakes ranging from 0.30 - 3 m in diameter. During the initial stages of their formation, the pancakes are only semi-consolidated surrounded by water which is relatively free of ice. These ice-free areas act as source regions for new crystals which enlarge the pancakes. Wave action results in repeated contacts and separations between pancakes which not only acts to retain the shape but also results in the pancakes gaining raised edges (Weeks and Ackley, 1982). Pancakes may eventually collide with one another and become rafted, continued freezing may result in a solid sheet of ice. If at any time the turbulence stops, grease ice will form between the pancakes resulting in a composite ice sheet.

In calm conditions, grease ice or shuga can undergo a transition to form a consolidated cover called nilas. Nilas is a thin (< 0.1 m), elastic crust of ice with a matt appearance and is an important mechanism for ice growth within the ice edge (Allison and Worby, 1994). Newly formed open water areas, which are far enough inside the ice edge to avoid ocean swell, allow the formation of substantial quantities of nilas.

1.3.3 Distribution of Ice Types...

Consolidation of frazil crystals is initiated when the concentration of crystals exceeds about 40% by volume (Martin & Kauffman, 1981). Wind action, which mixes nuclei in the area of active freezing, can result in accumulations of frazil ice up to 1 m thick.

Analysis of pack ice cores from the Pacific and Indian Ocean Sectors indicate that while the average pack ice thickness is up to 0.59 m, the thickness of individual layers within the ice is only 0.12 m (Worby *et al* 1996). These layers are indicative of the importance of rafting to increase ice thickness. Wind or water induced divergence results in areas of open water within the Southern Ocean pack ice. Frazil ice crystals quickly form in these areas of open water during autumn and winter. In areas of fast ice formation and growth, congelation ice continues to grow (in calm conditions). Congelation ice crystals frequently account for 90% of

ice type within fast ice cores. The biological composition of the two types of ice varies significantly (Scott *et al* 1994).

1.4 REVIEW OF SEA ICE BIOTA...

Comprehensive reviews of Southern Ocean phytoplankton and sea ice algae have been undertaken by several authors [eg Horner (1985), Medlin and Priddle (1990), Garrison *et al* (1991), Ackley and Sullivan (1994) and Knox (1994)]. The following section briefly describes the ecology of phytoplankton and the factors influencing its growth. Specific information relating to phytoplankton species distribution and biomass is considered later in the relevant experimental chapters.

1.4.1 Ecology of Phytoplankton...

Distribution

Most of the primary production in the Southern Ocean is by the phytoplankton (Everson, 1988). The phytoplankton are a highly diverse assemblage of photosynthetic (autotrophic) organisms. The autotrophs, or primary producers provide, directly or indirectly, nutrition for all forms of marine life (Price, 1990). Among the phytoplankton, diatoms often dominate. Some authors (Round *et al* 1990) have suggested that they are perhaps the most widespread group of plants on earth, certainly they are the most abundant of all unicellular planktonic algae.

Diatoms are unicellular, eukaryotic micro-organisms which are pigmented and photosynthetic. Up to 60 % of the dry weight of a diatom cell is silica (Round *et al* 1990). The diatom cell wall consists of many parts; two valves are always present as are several thinner, linking structures known as girdle elements. The valves lie at each end of the cell and the girdle elements surround the region in between. An often-used analogy describing how diatom valves fit together refers to the two halves of a petri-dish. The whole structure is held together by coatings of inorganic material.

Most diatoms have a relatively simple shape, the valves being radially or bilaterally symmetrical, parallel to each other and connected by a cylindrical girdle. The symmetry is based on a series of silicate ribs which grow out from a circular or elongated centre during valve formation. The primary rib system can be organised in two main ways. In some diatoms the ribs radiate from a ring, or annulus (centric diatoms); in others they extend out from an often thicker,

principle rib, or sternum; these are the pennate diatoms. Centric diatoms commonly have round or polygonal valves while pennate diatoms have bipolar, elongated valves. There are three types of pennate diatoms: those with a simple sternum (araphid) and those with a sternum which contains two raphe slits (raphid diatoms) and those that have a raphe only on one valve. Diatoms are usually found as individuals but several species form colonies, often in chains.

Over 200 algal species have been reported on, in, or in association with the Antarctic sea ice (Garrison, 1991). Algal production, both in the ice and in the water column at the ice margin, either directly or indirectly provides an abundant source of food for all higher trophic levels (Horner, 1991). Species found within the ice are often more diverse per given volume than species found in the underlying water column (Melnikov, 1995). Bacteria are also common in sea ice [Sullivan and Palmisano (1984), Sullivan (1985b) and Kottemeir and Sullivan (1987)]. The productivity of bacteria appears to be coupled with ice algal growth and production (Grossi *et al* 1984). A significant proportion of the biomass from pack ice may be comprised of heterotrophic flagellates and ciliates (Garrison *et al* 1986 and Garrison and Buck, 1989). Amoeba and heliozoans, foraminiferans and dinoflagellates are also found in the ice (Garrison, 1991).

1.4.2 Sea Ice Algal Biomass...

It is clear that where phytoplankton biomass is high, in the Southern Ocean it is usually diatoms which dominate the net phytoplankton and hence make the major contribution to overall biomass (El-Sayed, 1971; Xiuren *et al* 1996; Fryxell and Kendrick, 1988). Only the colonial haptophyte, *Phaeocystis pouchetii,* has been known to form in comparable biomass in the Southern Ocean (Horner, 1991). It may be that the ice serves as only a survival platform where diatoms are able to reduce their metabolic rate, increase cell storage products and increase their heterotropic ability during the transition from summer to winter [as suggested for *Fragiliaropsis cylindrus* by Palmisano and Sullivan (1982)]. Bacterial biomass and productivity generally parallel the seasonal development of the phytoplankton (Gleitz *et al* 1994). Ice algal and phytoplankton biomass is reviewed in more detail in Sections 3.4 and 4.4.

1.4.3 Location of Algae...

Ice algae within newly formed sea ice has a relatively homogeneous vertical distribution. The newly formed sea ice discussed in the experimental chapters of this thesis does not show a distinct zonation of algal communities. However, the

algal concentration may vary widely regionally and seasonally. After the newly formed ice consolidates and subsequent increases in ice thickness occur (either by growth or deformation) distinct areas of ice algal communities occur. Ice algae may still be found throughout the ice thickness but the majority of algal cells are found in four main areas: in surface communities, interior communities, bottom ice assemblages and sub-ice habitats in the form of mat-strand or platelet communities. In the pack ice surrounding Antarctica, surface-layer and internal assemblages are characteristic ice algal assemblages, whereas bottom-layer, under-ice platelet and mat-strand assemblages usually predominate in near-shore land-fast ice (Garrison and Buck, 1991).

1.4.4 Factors Influencing Growth...

Environmental factors that influence the development, survival and growth of ice algal communities include light, nutrient concentrations, salinity and temperature. The information presented in the experimental chapters of this thesis relates to ice algal communities that are generally less than 24 hours old. The factors discussed in this section affect algal growth and survival in older ice. Despite the potential for the environmental factors to stress the algae within the older sea ice, inclusion in the newly forming sea ice offers a better alternative than sinking out of the water column over winter.

Light is usually considered to be the most important factor influencing the growth and distribution of ice algae (Horner, 1991). Experiments have shown that ice algae have adapted to live and grow successfully at light levels near 0.1% of the total surface irradiance, even though 1% light level is usually used to indicate the bottom of the euphotic zone in the ocean (Steemann and Nielsen, 1977). This may help to partially explain how diatoms are able to survive the dark winter period and how other factors such as snow cover on sea ice and internal band assemblages affect the ice algae. Dark adaptation in the phytoplankton involves (among other responses) increasing the amount of chlorophyll per cell (Lizotte and Sullivan, 1991). A recent investigation by Melnikov (1998) indicates that the extent of winter ice algal production has been previously underestimated.

The micro-organisms which inhabit sea ice experience a wide variety of salinities. During melt periods, salinities as low as 3 psu occur at the ice edge (Horner, 1977). This contrasts with salinities which may exceed 50 psu during periods of brine drainage as described for the Arctic ice by Meguro *et al* (1967) and Grant and Horner (1976). Information regarding the response of sea ice diatoms to the varying salinity regimes is limited. However, results from sampling in the

Weddell Sea by Ackley *et al* (1979), associated the microflora distribution with salinity differences. Sea ice salinities may control the distribution and abundance of algae species, particularly in older ice (Scott, 1992; Poulin *et al* 1983).

Two other influences affect the growth and behaviour of planktonic diatom populations (Round *et al* 1990). The first is the availability of nutrients. Dieckmann *et al* (1991b) investigated the nutrient availability in the Weddell Sea during winter. Results from this study indicate, phosphate, nitrate and silicate levels in the sea ice fluctuated widely. Any of these nutrients could limit the growth of diatoms, however, the first nutrient to become limiting was generally silicate. Silicate limitations may result in the succession of algal assemblages to species which are less dependant on this nutrient. Nutrient levels in older sea ice may be almost depleted compared to the underlying water column (Clarke and Ackley, 1984).

Diatom cells to sink quite rapidly as a result of the high density of their siliceous walls. While silicon is the second most abundant element on earth after oxygen, its availability is quite restricted. In fact, in the open ocean, the level of silicate is often extremely low (Round *et al* 1990). Growth of diatoms is dependent on the transport of the element across the thermocline from silicate-rich deep water.

While some pennate diatoms are motile, in the absence of a substrate most diatoms lack the ability to position themselves in the water column. Diatoms are often as dense or more dense than water. Thus, in still water they will rapidly sink out of the photic zone (Smayda, 1970; Waite *et al* 1997). When part or all of the photic zone is turbulent as a result of wind, currents and convection, diatoms remain suspended. Many of the areas where phytoplankton biomass maxima occur have a stable oceanic density profile which limits vertical mixing. One such region is the marginal ice edge zone which has a layer of fresher less dense surface water. The inability of diatoms to resist sinking (settling out of suspension) in calm conditions is of particular interest to Chapters 3, 4 and 5 of this thesis.

1.5 REVIEW OF ICE ENRICHMENT MECHANISMS...

Introduction

The number of algal cells found in sea ice is often several orders of magnitude higher than in the underlying water column (El-Sayed, 1971; Fryxell and

Kendrick, 1988). Physical or biological entrapment of particles or small, living organisms has only been a focus of scientific attention for the past 15 years. During this time a number of field and laboratory-based studies have been conducted. These studies are only starting to reveal the complex processes involved in inclusion of micro-organisms in sea ice. It appears highly likely that it is a combination of inclusion mechanisms (both physical and biological) which result in the algal enrichment.

Frazil ice crystals are the dominant crystal type found in newly forming sea ice. Many authors report on the high concentrations of algal cells (Garrison *et al* 1991; Gradinger and Ikavalko, 1998) and bacteria (Helme and Weyland, 1995) in frazil ice. Congelation ice tends to reject particulate material during ice formation, and the structural and biological characteristics of congelation ice may be very different from those of frazil ice (Clarke and Ackley, 1984; Scott *et al* 1994).

1.5.1 Natural Mechanisms...

A variety of mechanisms have been linked to algal and sediment enrichment in newly forming sea ice. The incorporation process is much more complex than first assumed and cannot be wholly explained by a single mechanism. Five mechanisms are described below. These mechanisms cannot always be classified as either physical or biological processes as degree of overlap may exist.

Scavenging

Scavenging occurs when ice crystals moving up in the water column collide with and collect algal cells from the water. The high diatom biomass often observed in the newly-formed sea ice may be achieved solely by organisms being harvested by rising frazil crystals (Garrison *et al* 1983).

Dieckmann *et al* (1986) indicate that ice crystals from great depths can play an important role in establishing biological communities, in both fast ice and pack. This research relates to the formation of ice platelets at depths of 250 m near the Filchner Ice Shelf. This process is related to coastal ice shelves and should not be confused with frazil ice scavenging which may occur in areas of newly forming pack ice. Dieckmann *et al* (1986) report, the ice platelets traverse large distances through the water column and are able to accumulate larger numbers of organisms than the frazil ice crystals which traverse a relatively shorter distance. Investigations into foraminifer incorporation in sea ice found the extent of

incorporation was equivalent to the number of cells in the water column to a depth of 100 m, a concentration factor greater than 100 fold (Dieckmann *et al* 1988; Spindler and Dieckmann, 1990; Dieckmann *et al* 1991). It seems unlikely that the high levels of algal incorporation in sea ice, particularly pack ice, can be purely attributed to this mechanism due to the much shallower depths of frazil ice crystal formation and circulation. Reimnitz *et al* (1993) and Ackermann *et al* (1993) suggest scavenging combined with wave action results in sediment enrichment in laboratory based, sea ice experiments.

Nucleation

Nucleation involves ice crystals nucleating around algal cells (Ackley 1982). For this process to occur the sea water must be at its freezing point. The ice crystals continue to grow, rising through the water column bringing the algal cell to the surface. Ackley (1982) distinguished between scavenging and nucleation, while Osterkamp and Gosink (1984) treat the two mechanisms collectively. There appears to be some controversy over the role of nucleation in concentrating particulate matter in sea ice (Reimnitz *et al* 1993). Several authors have indicated frazil ice formation and hence algal inclusion in sea ice may be assisted by nucleation-like processes (Hanley and Tsang, 1984; Kempema *et al* 1986) others indicate that 'there are no substances that will nucleate ice at the small levels of supercooling measured in natural water bodies' (Daly and Stolzenbach, 1984).

It seems unlikely that nucleation alone could account for the high concentrations of algal cells found in sea ice. Frazil ice crystals grow very rapidly as they rise. Thus, if the only cells included in sea ice were as a result of nucleation, the number of cells per volume of ice would be much lower than observed levels. Due to problems associated with examining individual ice crystals it is very difficult to ascertain whether algal cells do act as a nucleus. However, this view is supported by Ackley 1982, who reported seeing some cells within ice crystals.

Wave Field Pumping

Another possible mechanism for concentrating algal cells is by wave fields that pump water through the ice and deposit organisms. This mechanism, which operates primarily in the outer realm of the pack where the ice is still subjected to notable ocean swell, might favour the incorporation of phytoplankton and sediment (Eicken 1992). Ackley *et al* (1987) and Shen and Ackermann (1988) have also investigated this mechanism. Ackley *et al* (1987) found wave field pumping may be responsible for 10 to 1000 increase in biomass in newly formed

ice compared to the underlying water. Laboratory-based experiments conducted by Weissenberger and Grossmann (1998) showed the importance of this mechanism. These authors found wave field action to be responsible for phytoplankton enrichment factors of 9 x.

Langmuir Cells - Water Circulation Patterns

Garrison *et al*, (1989) suggest that small scale circulation features such as Langmuir cells (Stavn, 1971) collect organisms suspended in the water column and deposit them against the forming sea ice. Langmuir circulation aggregates frazil ice in converging circulation cells. Once frazil crystals are buoyant enough to avoid being down-welled, they accumulate near the surface of the down-welling region and act as a filter for suspended material in the circulation cells (Garrison *et al* 1989). The process of algal concentration through Langmuir Cell circulation has been studied in laboratory-based tests (Wiessenberger and Grossmann, 1998). Results of this work indicates that Langmuir Cells can enrich ice chlorophyll concentrations up to 53 x those of the underlying water levels.

Ice Active Substances

Diatoms release many organic compounds, mainly consisting of polysaccharides, but also including proteins and other small molecular weight substances (Fogg, 1983; Painter, 1983; McConville, 1985; and Decho, 1990). These substances have been referred to as Ice Active Substances (IAS) by Raymond *et al* (1997). These IASs roughen the surface of the ice, promoting attachment. These compounds are apparently released by polar species, there is no evidence of IASs been found in temperate water diatoms (Raymond *et al* 1997). The polysaccharides released by the diatoms act as adhesives, but their roles in repelling grazers and maintaining ionic equilibrium have also been proposed (Raymond *et al* 1997).

1.5.2 Laboratory Experiments...

Few studies have investigated biological enrichment in laboratory-grown ice. Initial investigations into algal enrichment into laboratory generated ice were carried out by Garrison *et al* (1989); and Reimnitz *et al* (1991, 1993). These authors used sea ice producing columns which while successfully producing frazil ice crystals, were limited in their similarity to natural systems. Later studies (Weissenberger and Grossmann, 1998) used large volume (2400 l) sea ice tanks. These tanks were a far better replication of the natural environment. The ice

formation mechanisms used by these researchers and their results are discussed below.

Laboratory-based studies carried out by Garrison *et al* (1989), revealed that frazil ice can enrich organisms two to four times above the levels in the underlying water. These authors used a frazil-generating chamber approximately 2 m high (Figure 1.4). Frazil crystals were generated at the bottom of the tank and then floated up to the surface, harvesting particulate-matter along the way. Incorporation of two polar diatom genera, *Fragilioropsis* species and *Chaetoceros* species was investigated by Garrison *et al* (1989). Results indicated that these diatom cells were preferentially harvested from the water column, however, the extent of harvesting was not sufficient to duplicate conditions found in nature.

More recently, Reimnitz *et al* (1993) studied the incorporation of living plankton, (foraminifers and diatoms), silt, sand and mud into laboratory produced sea ice (Figure 1.5 shows the ice columns used). Results from their study indicate that diatom and zooplankton concentrations in the frazil ice were enriched over the levels in the water column. They proposed that the mechanism facilitating this incorporation was physical in nature (scavenging). They found no evidence of nucleation or adhesion of cells to the ice crystals. Silt and clay-sized particulate matter was found to be concentrated in the frazil ice, while coarser grained material was not. Sediment particles used in these experiments ranged from 1 μm - 63 μm in diameter.

Weissenberger and Grossmann (1998) conducted studies of algal inclusion into sea ice using a 2400 l tank in a cold room. The design of their tank avoided some of the difficulties associated with the designs of Garrison *et al* (1989) and Reimnitz *et al* (1991, 1993). The large volume of water in their 3 m x 1 m tank minimised wall effects of the tank. Unlike the previous studies, the sides and bottom of the tank were insulated. They found that the area of their tank was sufficient to facilitate grease ice and pancake ice production, with growth rates being comparable to those found naturally. Crystal analysis revealed that the ice crystals were not influenced by wall effects. An internal partition in their tank (Fig 1.6) created water currents similar to those found in one half of a Langmuir cell. They found chlorophyll a in the ice to be 53 times higher than the water. They also investigated the effects of wave action during ice formation by using a wave generator. They found this enrichment mechanism to be less effective than the Langmuir cell type process, which accounted for a 9 times increase in chl a biomass in the ice compared to the water. While these mechanisms were found to

Figure 1.4 Frazil ice generating chamber (Dimension = 10.8 x 10.95 x 192.5 cm; volume cf. 25 l), (Garrison *et al* 1989).

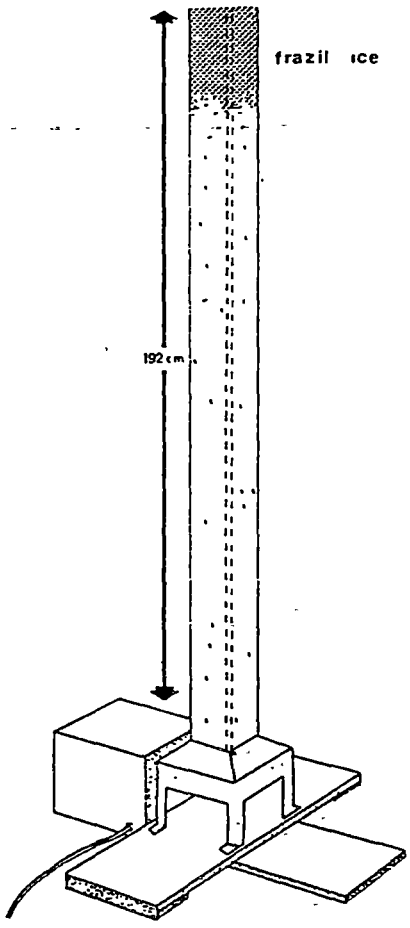


Figure 1.5 Experimental apparatus used in the study of sediment/frazil interaction: (A) simple tank using a plastic core-liner stirred with a magnet; (B) improved, rectangular tank, stirred by an electric drill and paddle, with aluminium heat exchanger to produce frazil in the base (Reimnitz *et al* 1993).

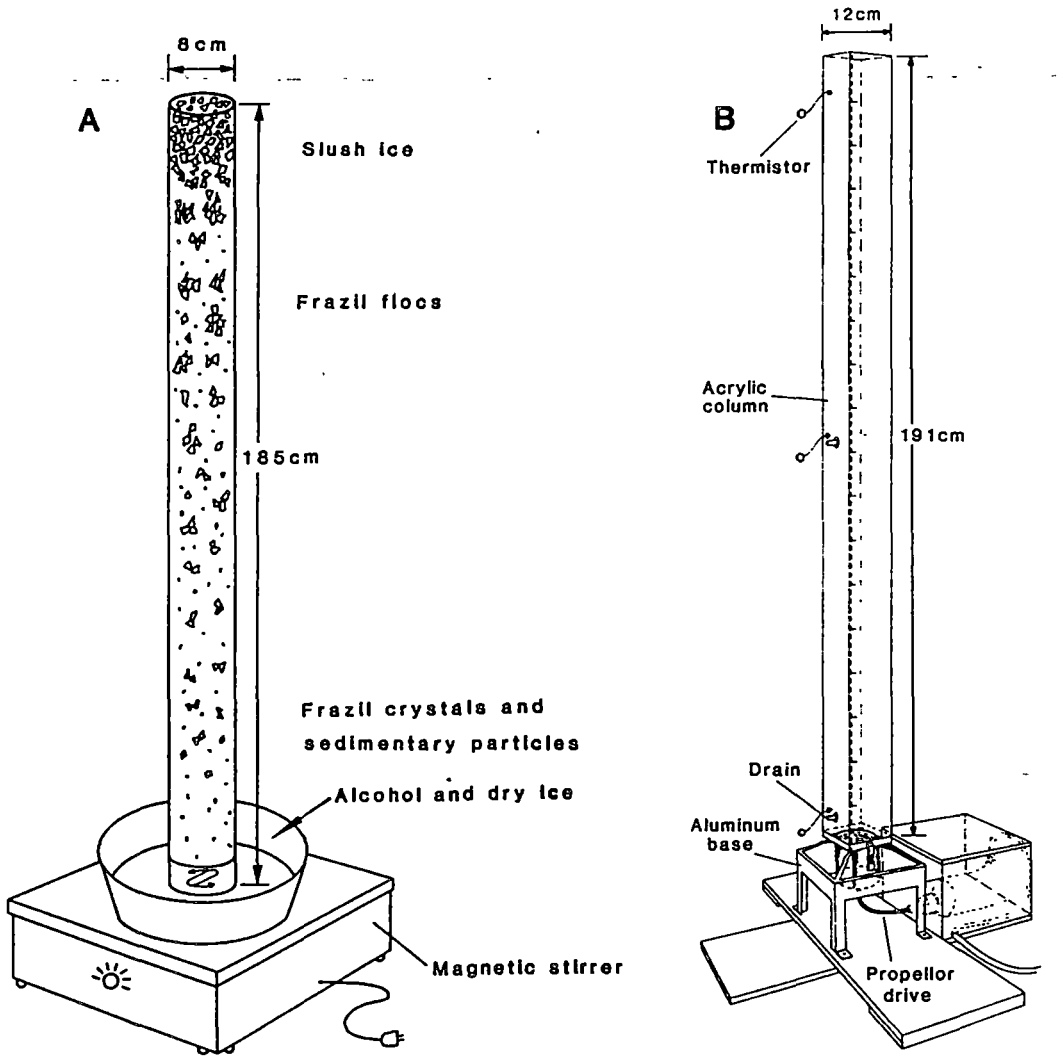
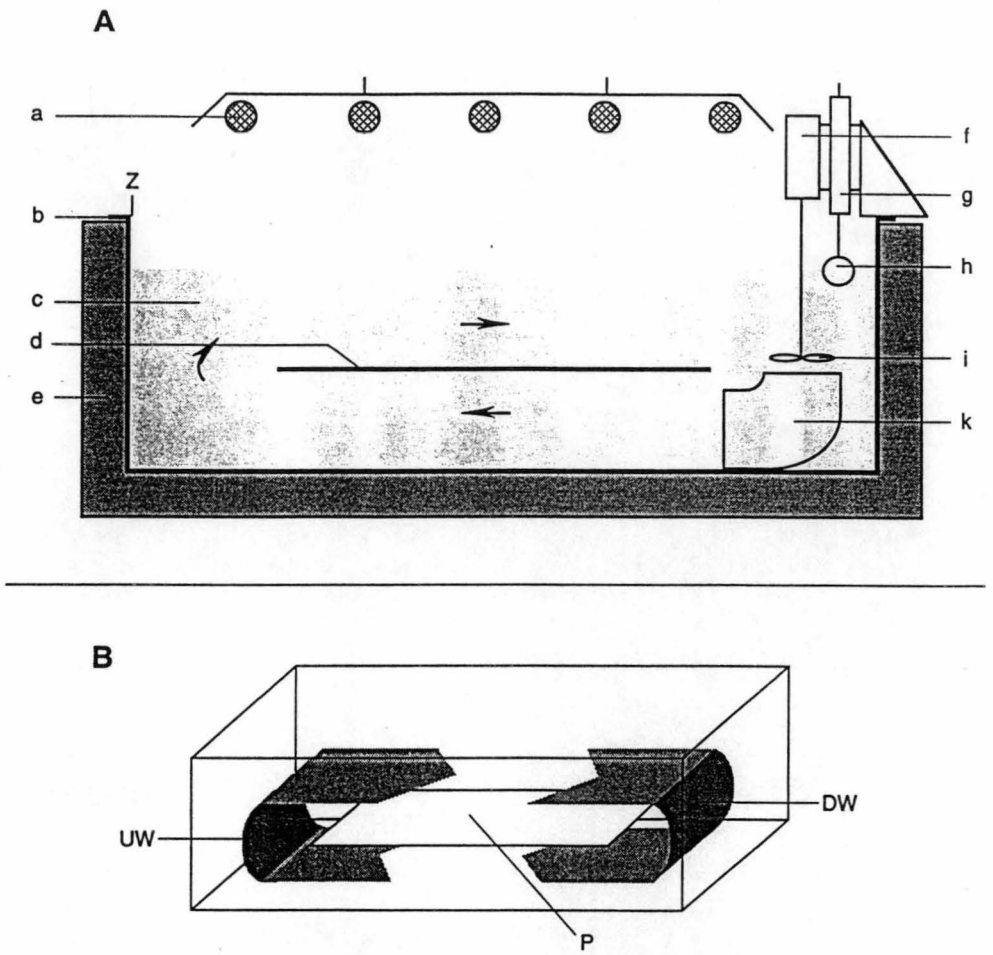


Figure 1.6 Ice tank (Weissenberger and Grossmann, 1998).



A: Schematic representation of the tank for experimental formation of sea ice. Arrows indicate water circulation generated by the flow system.

a, light tubes

b, ice/water tank (hard-polyethylene, 300 x 100 x 100 cm)

c, experimental seawater

d, plastic plate (poly carbonate, 160 x 100 0.5 cm)

e, insulation (polystyrene, 20 cm)

f, stirring motor

g, hydraulic system

h, wave generator (cylinder, 80 x 15 cm)

i, propeller

k, rectangular plastic tube (30 cm diameter)

Z = zero-point for length measurements.

B: Three dimensional diagram of the Langmuir-like circulation in the tank, generated by flow system. UW/DW, upwelling/downwelling region of the circulation cell; P, partition plate.

enrich algal cells effectively, no such enrichment was found when experiments were conducted with bacteria. Inclusion into the ice depended on bacteria cells being clumped as aggregates or being attached to algal cells (Weissenberger and Grossmann, 1998).

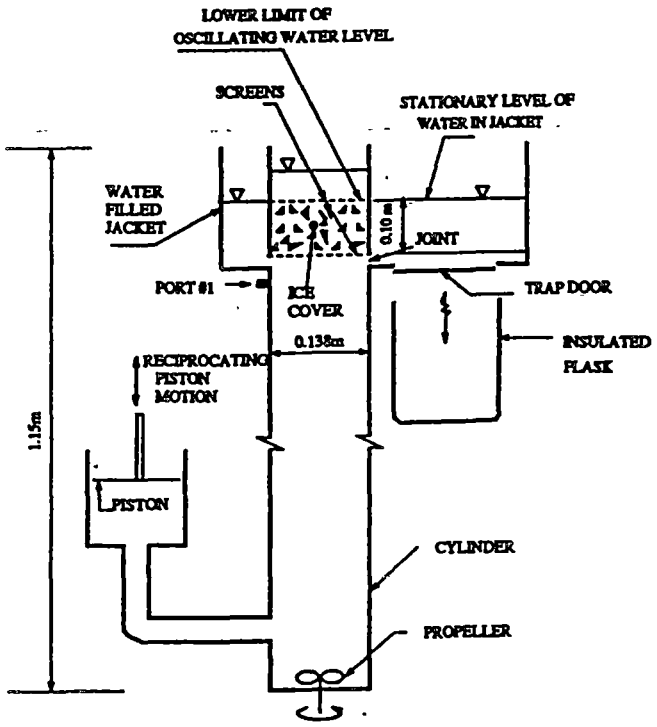
Ackermann *et al* (1990) studied wave induced enrichment and enrichment due to rising frazil (Figure 1.7). They proposed that these mechanisms were responsible for sediment enrichment in sea ice; the degree of enrichment being dependent on size of sediment grains and concentration of grains. The extent of particle enrichment was dependant on period and amplitude of the waves and duration of the experiment as well as size of the particles and water motion over and through the ice cover. Results indicated that silt particles (20 μm diameter) were enriched in the ice, however, rising frazil crystals were not buoyant enough to continue to rise when loaded with large grains of sediment (120 μm diameter). Shen and Ackermann (1988) used a 3 m long wave tank for investigating sediment enrichment in sea ice. Resultant sediment enrichment in ice samples was attributed to wave field pumping.

Problems with Ice Columns

Experimental ice columns used by Garrison *et al* (1989), Ackermann *et al* (1990) and Reimnitz *et al* (1991, 1993) proved to be a useful means for forming laboratory-based frazil crystals. However, the ice columns lacked many essential similarities to the natural environment. The sides of the columns were not insulated, thus cooling from the sides was possible. Frazil ice crystals continued to accumulate even though ice cover provided partial insulation on the top of the tank. In natural environments, this insulation would eventually result in consolidation of crystals rather than continued massing of the unconsolidated crystals. The duration of the experiments was short ($\approx$ 30 minutes) and this limits the time for contact between particles and ice crystals. The short experiment time may explain the low levels of algal inclusion (2 to 4 times) compared to the natural extent of enrichment.

Comparisons of the results of ice column experiments to natural ice formation mechanisms should be made with caution. The length of these columns is only 2 m, this contrasts with the natural depth of penetration of near freezing water which may be up to tens of meters (Stavn, 1971). This has implications for the extent and type of algal incorporation (this is explained in detail in Chapter 3). Other factors include the absence of environmental influences: wind, water

Figure 1.7 Wave column apparatus (Ackermann *et al* 1990).



circulation patterns, waves and swell. The sinking rate of sediment grains was found to be very high (Reimnitz *et al* 1993), thus the agitation mechanism used in this tank design may not have been sufficiently powerful to keep sediment in suspension. Large sediment grains also quickly settled out of suspension in the ice tank design used by Ackermann *et al* (1990).

Problems with Ice tanks

Large tanks used by Shen and Ackermann (1988), and Weissenberger and Grossmann (1998) more closely resemble natural systems. However, the simulation of natural sea ice formation in an artificial system is inevitably constrained by artefacts (Weissenberger and Grossmann, 1998). These authors attempted to limit these artefacts by insulating their large volume tank (2400 l) on the sides and bottom and by making the surface area of the tank large enough to facilitate grease and pancake ice formation. They refrigerated the tank water at 0° C for 4 weeks in a dark environment.

Reducing the water temperature to close to its freezing point and equilibrating culture stocks in the water for 2-3 days would give more representative condition for the algae. Raymond (personal communication, 1997) indicates that cultures need to be stored close to their freezing point for 2-3 days to facilitate production of potential IASs.

Summary

Many difficulties exist when attempting to produce sea ice in a laboratory. Most of these difficulties are related to replicating the process which occurs in nature. Tanks which hold approximately 30 - 2400 litres of sea water can only provide an insight into the processes occurring in the polar oceans. All of the tank experiments described above have made valuable contributions towards understanding the mechanisms behind algal incorporation into sea ice, a process we know very little about.

This thesis is the first study that field tests a laboratory-based experimental ice tank. This is done by comparing samples from a tank (which uses sea water directly from the ocean) to water, slurry and ice samples which formed naturally at the same time. Temperate water phytoplankton stocks, ten Antarctic phytoplankton cultures and sized glass microspheres are used to investigate the mechanisms associated with the inclusion and enrichment of algae into sea ice.

Results of this study indicate, both physical and biological processes are responsible for the incorporation and enrichment of algae into newly forming sea ice. In calm conditions, algal cells are scavenged by rising frazil ice crystals. This process favours algal cells which have biological adaptations which allow them to remain suspended high in the water column. In more turbulent conditions, algal incorporation and enrichment occurs in ice as a result of the filtering activity of the ice matrix. Algal species distribution and biomass is significantly different depending on the type of newly formed ice into which the algae is incorporated.

2.0

Algal incorporation into newly forming sea ice, 1993 field experiments...

2.1 EXPERIMENTAL DESIGN

Work reported in this section investigates whether diatom assemblages from different ice types have different compositions. It seeks to determine whether the level of diatom cell inclusion into the ice was influenced by the size of the diatom or the species type; and also, whether the type of newly forming ice (eg grease ice, pancake ice and nilas) affects the extent of diatom incorporation. Diatom communities in different types of newly forming ice were compared with the diatom community structures in water samples from just under the ice at 10 m and at 20 m depth.

2.1.1 SAMPLING PROGRAM FOR NEWLY FORMING ICE...

Early sea ice formation processes are shown in Figure 2.1. Ice and water samples were collected from 9 locations in the eastern Antarctic pack, near 145° E, 66° S in April and May 1993 (Fig 2.2). At each of the 9 sites up to 3 replicate samples were collected. Also included in the results, are the analyses of 3 grease ice samples collected during 1992, from 80° E, 66° S. Water samples from immediately under the ice and from depths of 5 and 10 m were also collected, although only water from immediately under the ice was collected with grease ice. New ice sample information is shown in Table 2.1. Equipment problems resulted in some of the early surface water samples not being collected. Problems with sample preservation prevented a more detailed analysis of some of the phytoplankton. These problems are discussed in Section 2.2.2. Revised sampling methods were applied to algal communities which were collected from newly forming sea ice, in a subsequent study. These results are presented in Chapter 3.

Figure 2.1 Diagram of early sea ice formation mechanisms

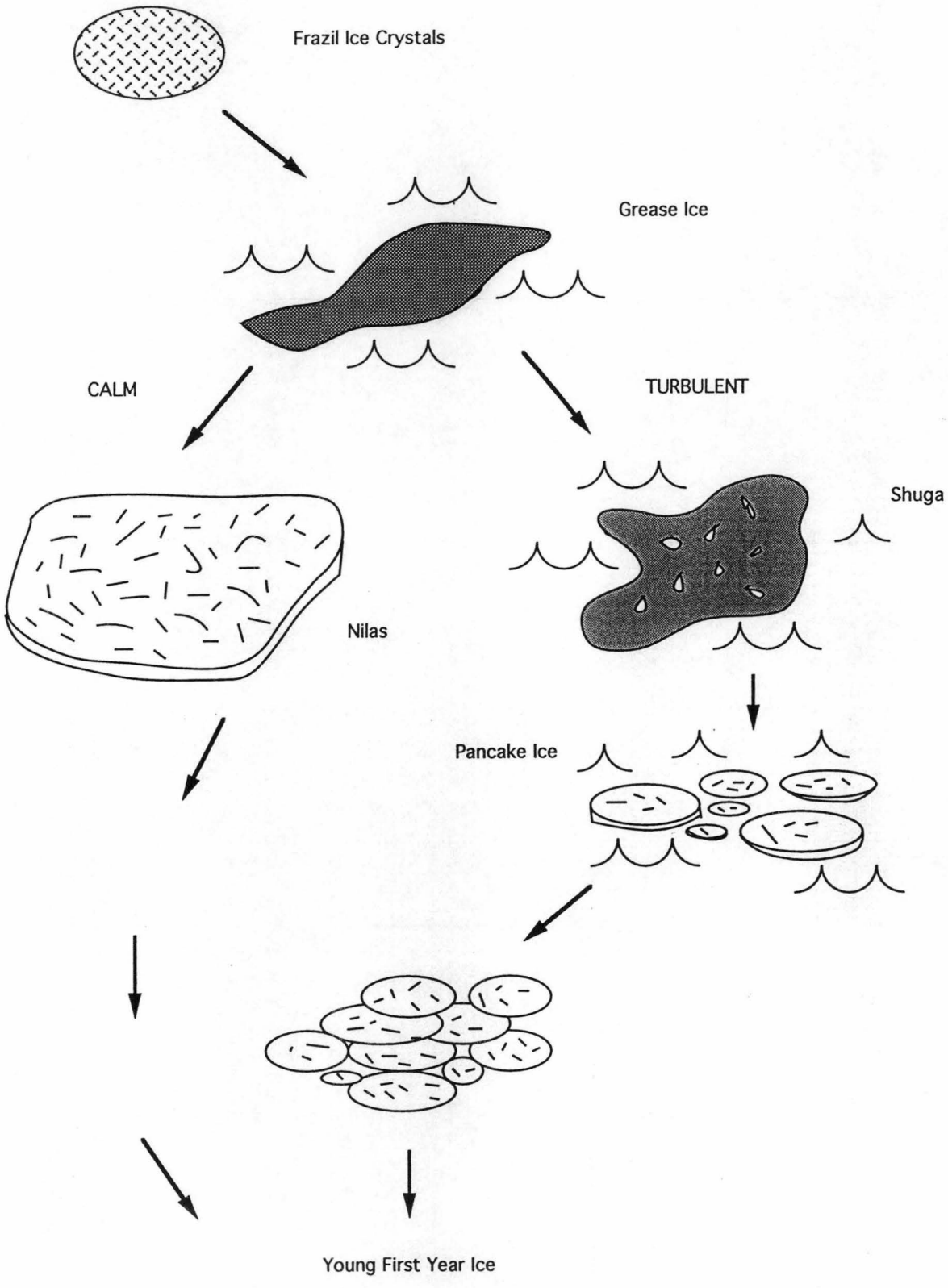


Figure 2.2 Cruise Track of *RSV Aurora australis*, V9 March - May 1993, sample areas are enlarged. 'Letters' represent sample sites.

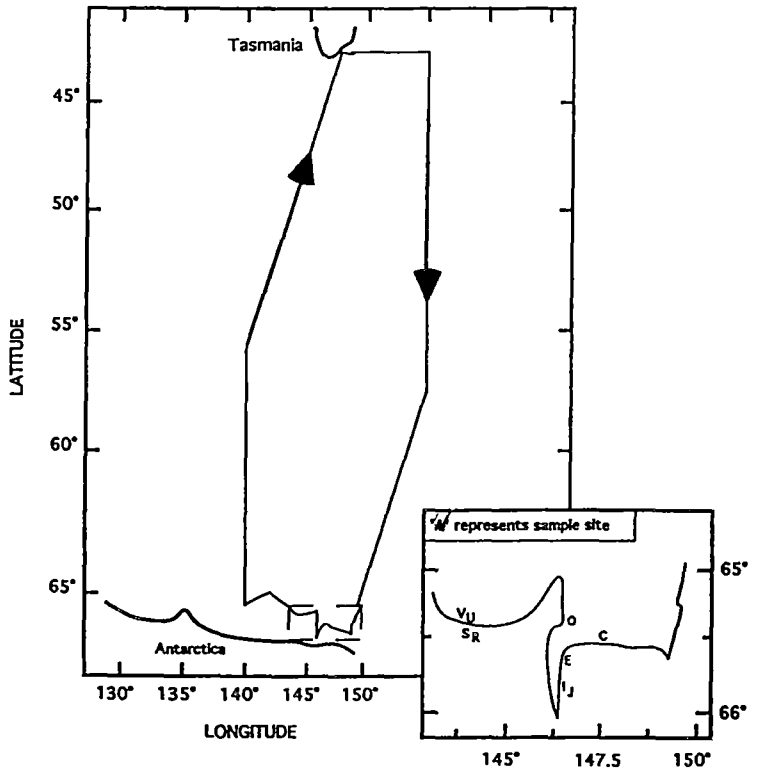


Table 2.1 Summary of New Ice samples (Salinity data from Worby and Massom, 1995)

Sample	Lat/Long	Distance to Ice Edge (km)	Date (GMT)	Ice Type	Ice Thickness (m)	Ice Salinity 1/1000	Air Temp°C	Wind Speed m/s
C1	65°35'S 145°51'E	91	28-Apr-93	Nilas	Unknown	Unknown	-9.1	5
E1	65°39'S 145°30'E	68	29-Apr-93	Nilas	Unknown	Unknown	-9.3	6
I1	65°50'S 145°30'E	106	29-Apr-93	Nilas	0.04	Unknown	Unknown	Unknown
J1	65°53'S 145°30'E	106	30-Apr-93	Nilas	0.03	Unknown	Unknown	Unknown
O1	65°24'S 145°25'E	Unknown	1-May-93	Nilas	0.03	Unknown	Unknown	Unknown
R1	65°45'S 144°51'E	59	3-May-93	Pancakes	0.03	24.4	-13.9	7
R2				Pancakes	0.03	26.9		
R3				Pancakes	0.03	26.2		
S1	65°42'S 144°48'E	57	3-May-93	Pancakes	0.03	20.5	-11.8	5
S2				Pancakes	0.03	18		
U1	65°35'S 144°47'E	57	3-May-93	Nilas	0.03	22.3	Unknown	Unknown
U2				Nilas	0.02	15.5		
U3				Nilas	0.025	20		
V1	65°34'S 144°46'E	57	3-May-93	Pancake	0.03	24.8	-18	16
V2				Pancake	0.03	19.1		
V3				Pancake	0.03	21.1		
W1	66°S 80°E	Unknown	Spring 1992	Grease	No Data	No Data	No Data	No Data
W2				Grease	Collected	Collected	Collected	Collected
W3				Grease				

2.2 METHODS

2.2.1 COLLECTION OF SAMPLES OF NEWLY FORMING ICE (1993)...

Two different sampling methods were employed to obtain ice and water samples from areas of eastern Antarctic pack ice. The first method involved a person being lowered over the side of the ship in a basket, which was suspended from the ship's crane. The basket was lowered until it was hanging less than 0.5 m above the surface of the new ice. From this position, samples of new ice could be scooped up with a spade. Water samples immediately under the ice were collected with a long tube connected to a syringe. Water samples from 5 and 10 m were collected using Niskin bottles lowered from the basket on a steel cable. The second method of sampling was carried out from inflatable rubber boats. The boats were capable of passing through unconsolidated sea ice < 0.15 m in thickness. Pancake ice samples were collected by hand and quickly transferred to buckets to minimise brine drainage. Water immediately below the sea ice was sampled using small bottles. Water at 5 and 10 m was sampled as described above.

Sea ice samples were allowed to melt in the dark at 10° C. Samples were fixed with Lugols solution and stored in the dark at room temperature for return to Hobart. Upon return samples were stored in the dark at 4° C for no more than 4 weeks, before microscopy was conducted.

2.2.2 SAMPLE PRESERVATION...

Samples were preserved using Lugols Solution (Phytoplankton Manual, 1978). This type of sample preservation is usually adequate for diatom and armoured dinoflagellate fixation, but it is not an ideal way of preserving the more fragile phytoplankton. These types of algae are likely to be under-represented. Consequently, only the incorporation of diatoms into samples of newly forming sea ice is investigated. Diatoms comprise the

majority of algal cells found in the Antarctic waters during April and May (Fryxell and Kendrick, 1988 and Xiuren *et al*, 1996). To investigate the abundance of other algal taxa cell counts were conducted on living material and biomass was determined using HPLC techniques during a later study (refer Chapter 3).

Sea ice was not melted in filtered sea water as recommended by Horner *et al* (1992). This may lead to cell lysis, which has the potential to bias the assessment of algal community composition. This destruction of cells affects fragile flagellates more than the relatively robust diatoms. The effect of cell lysis on the diatom composition is limited. Sea ice samples collected in 1996 were melted in filtered sea water before analysis. A comparison of the 1993 and 1996 samples provides an insight into the differences in preservation and melting techniques (refer 2.3.3 and 3.3.2).

2.2.3 MICROSCOPY ON SAMPLES OF NEWLY FORMING ICE...

A Zeiss Telaval 31 inverted microscope was used for microscopy. A known volume of sample was settled in a settling chamber and analysed using phase contrast under 400 x magnification. Either the contents of the whole slide or half the slide were counted (a minimum of 400 algal cells was counted).

Algal cells were categorised into size classes using a graduated eye piece. The system shown in Table 2.2, which differs slightly from that of other authors, was used to classify sizes. This system more closely represents the species seen in this study. In particular *Chaetoceros dichaeta* cells were small and had very small spines (which were ignored when applying a size class). This size classification can only be an approximate estimate.

Table 2.2 Size classification system for algal cells

Class	Long Axis	Examples
Very Small	<10 μm	*Fragiliaropsis cylindrus, C. dichaeta*
Small	<20 μm	*F. curta*
Medium	<40 μm	*N. lecointei*
Large	>40 μm	*Proboscia alata*

Samples for further taxonomic study were cleaned by soaking in 30 % H_2O_2 for 3 days (Hasle and Fryxell, 1970). Diatom species identification was made on cleaned material using a 100 x oil objective on a Zeiss Standard 20 microscope.

2.2.4 STATISTICAL ANALYSIS OF SAMPLES OF NEWLY FORMING ICE...

Student's t-Test Analysis

Algal cell concentrations in nilas, pancake and grease ice samples were compared using the Student's t-Test. P values for the Student's t-Test were generated using the Analysis Toolpack in Microsoft Excel version 7.0. P values define the probability of observing an outcome as or more extreme than that actually arising from a particular experiment. A 0.05 confidence level was used to test significance. If a comparison of 2 samples generated a P value of ≤ 0.05 then the samples are considered to be significantly different, (i.e. samples were considered to have a significantly different number of diatom cells per litre).

Cluster Analysis

Cluster analysis using a Bray Curtis matrix was undertaken on the entire sample set. This type of analysis groups samples with a similar species distribution. The Bray-Curtis algorithm is particularly useful as it does not group cells on absence of species. This is explained in greater detail in Section 2.4.

Multi-dimensional Scaling (MDS) Analysis

Multi-dimensional Scaling analysis was performed using the program Biostat. Results of this analysis presents data in a two-dimensional format, grouping similar samples together. It is possible to overlay groups generated in cluster analysis on MDS outputs, (see for example Figure 2.14).

2.3.1 CELL ABUNDANCE IN DIFFERENT ICE TYPES...

Diatom abundances in samples collected from sites where nilas, pancake and grease ice were forming are presented in the following figures. Mean diatom abundances are presented for ice samples, surface water samples and water from 5 and 10 metres. Error bars representing the standard deviations are also shown. Information relating to eight sets of samples (ice and water from the surface, 5 and 10 metres), which were collected from the nilas sites is shown in Figure 2.3. Table 2.3 summarises the mean number of cells and standard deviation for samples collected from nilas sites. Similarly Figure 2.4 and Table 2.5 relate to samples collected at pancake sites; and Figure 2.5 and Table 2.7 relate to samples collected from grease ice samples.

Student's t-Test analysis was carried out to examine whether ice and water samples had significantly different diatom abundances. Results of t-Test analysis for samples from the nilas, pancake and grease sites are shown in Tables 2.4, 2.6 and 2.8 respectively.

Nilas Samples

The mean abundance of diatom cells in nilas is a order of magnitude higher than the mean abundances in the underlying surface and water samples, (Figure 2.3). At sites 'E' and 'J', diatom abundances in nilas samples are two orders of magnitude higher than in the underlying water (Table 2.3). There is a high standard deviation amongst the ice samples from the nilas site. Consequently error bars are not shown for nilas samples on Figure 2.3. The three lowest diatom concentrations at nilas sites are from samples collected at a later date in a different area (U1, U2, U3) than the nilas samples collected from sites C, E, I, J and O, (Figure 2.1). While all the samples are nilas, there are clear variations in cell abundances within the ice type. Similarly, the number of diatoms/litre in all samples from site C is clearly lower than in the other samples from this ice type.

The results of the Student's t-Tests on cell abundance data from nilas sites are presented in Table 2.4. A 'P value' ≤ 0.05 was considered significant. Reference to Table 2.4 indicates that no samples were considered significantly different from one another on the basis of cell abundance. However, ice samples from the nilas site had P values of 0.09 when compared with surface and deeper water samples (Table 2.5). This value, while not significant, shows evidence of a greater concentration in the ice than in the water column.

Figure 2.3 Mean diatom abundance at nilas sampling sites. Error bars indicate standard deviation. Due to the large standard deviation for nilas samples, error bars are not shown (refer Table 2.3). Nilas = ice samples (ice and slurry/brine), Surface = water from immediately under the new ice cover, '5 m' water from 5 m, '10 m' water from 10 m. The Y- axis has a log scale.

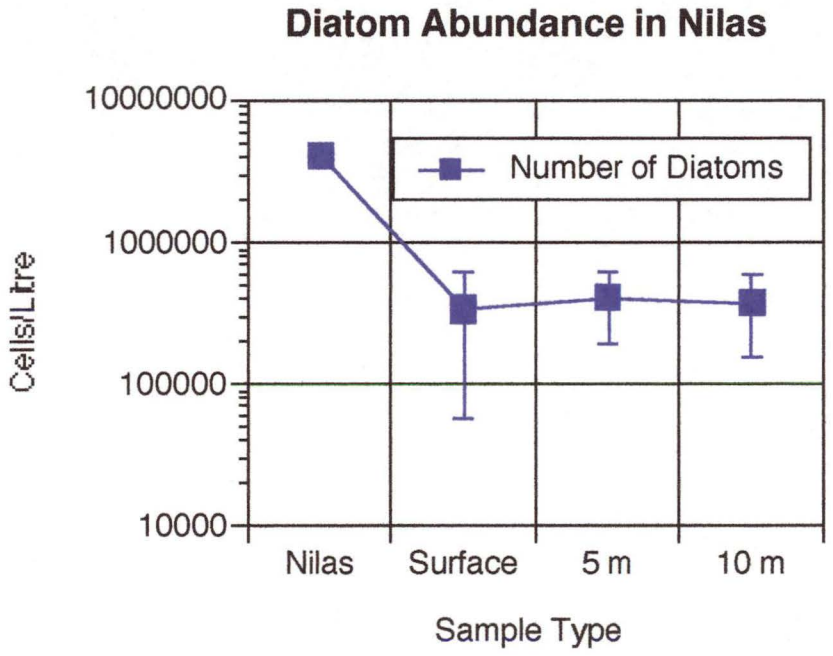


Table 2.3 Diatom abundances (cells l^{-1}) at nilas sites.

Sample	Nilas	Surface	5m	10m
C	4.52E+05	2.60E+04	8.05E+04	5.15E+04
E	1.31E+07	8.00E+04	2.30E+05	2.34E+05
I	2.41E+06	3.04E+05	6.61E+05	2.84E+05
J	1.21E+07	1.60E+05	2.53E+05	2.74E+05
O	3.86E+06	4.68E+05	3.72E+05	3.11E+05
U1	2.63E+05	7.92E+05	6.89E+05	7.25E+05
U2	3.10E+05	6.97E+05	5.48E+05	5.29E+05
U3	3.61E+05	2.48E+05	4.19E+05	5.79E+05
Mean	4.11E+06	3.40E+05	4.10E+05	3.73E+05
SD	5.40E+06	2.82E+05	2.16E+05	2.18E+05

Table 2.4 Student's t-Test analysis of all nilas sample sites C, E, I, J, O, U1, U2 and U3. $P \leq 0.05$ was considered significant.

All	Nilas	Surface Water	5 m	10 m
Nilas		0.09	0.09	0.09
Surface Water			0.64	0.84
5 m				0.77
10 m				

Pancake Samples

Diatom abundance in pancake ice samples is highly variable, being up to an order of magnitude higher or lower than in the underlying surface and water samples. Mean diatom abundances for ice and surface samples are very similar; as are the abundances of water samples from 5 m and 10 m (Table 2.4). Student's t-Tests of cell abundance data from nilas sites are presented in Table 2.6. A 'P value' ≤ 0.05 was considered significant. Analysis of data from pancake ice samples revealed deeper water samples to have a significantly lower number of cells when compared to the pancake ice and surface water samples. Comparisons of water from 5 m and 10 m depth to surface water yielded low P values; $P = 0.002$ and $P = 0.003$ respectively. Pancake ice and surface water samples comparisons were not significant (Figure 2.6).

Figure 2.4 Mean diatom abundance at pancake ice sampling sites. Error bars indicate standard deviation. Pancakes = ice samples (ice and slurry/brine), Surface = water from immediately under the new ice cover, '5 m' water from 5 m, '10 m' water from 10 m. The Y- axis has a log scale.

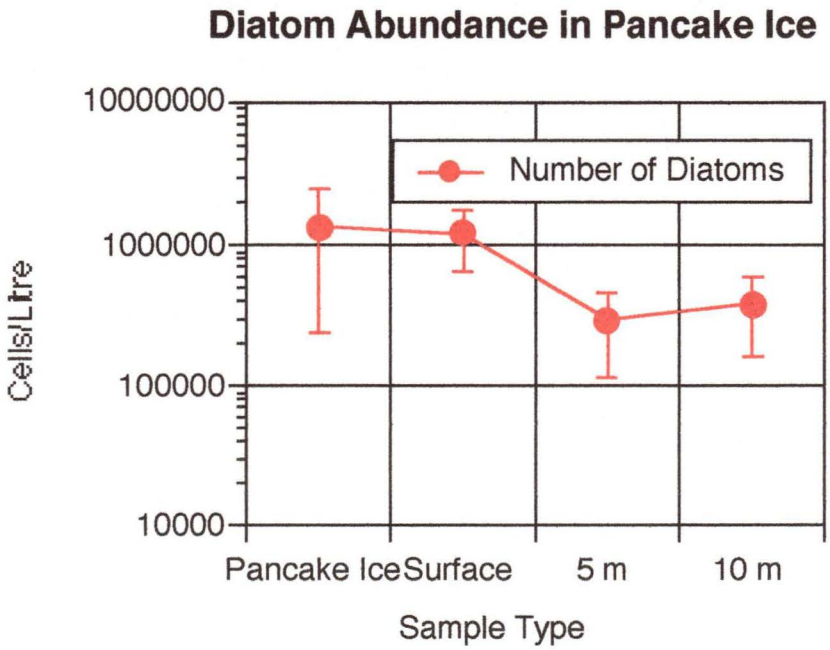


Table 2.5 Diatom abundances (cells l^{-1}) for pancake ice sites.

Sample	Pancake Ice	Surface	5m	10m
R1	2.02E+06	1.20E+06	1.08E+05	2.62E+05
R2	3.46E+06	1.31E+06	1.29E+05	3.28E+05
R3	5.88E+05	2.32E+06	1.79E+05	2.48E+05
S1	1.96E+06	1.65E+06	3.32E+05	1.74E+05
S2	1.51E+06	1.12E+06	2.81E+05	6.18E+05
V1	7.07E+05	8.24E+05	5.26E+05	7.60E+05
V2	3.88E+05	7.42E+05	5.78E+05	4.63E+05
V3	1.50E+05	5.77E+05	2.03E+05	1.86E+05
Mean	1.35E+06	1.22E+06	2.92E+05	3.80E+05
SD	1.11E+06	5.62E+05	1.77E+05	2.14E+05

Table 2.6 Student's t-Test analysis of samples from pancake sites. Significant comparisons (ie $P \leq 0.05$) are shown in red.

	Pancake Ice	Surface Water	5 m	10 m
Pancake Ice		0.77	0.03	0.04
Surface Water			0.002	0.003
5 m				0.38
10 m				

Grease Samples

Grease ice samples uniformly showed a two order of magnitude increase of diatom cells in the ice compared to water immediately under the ice, (Figure 2.5 and Table 2.7). Water samples from 5 m and 10 m were not collected.

Student's t-Test of data from grease ice sites revealed grease ice and surface water comparisons were not significant (Table 2.8).

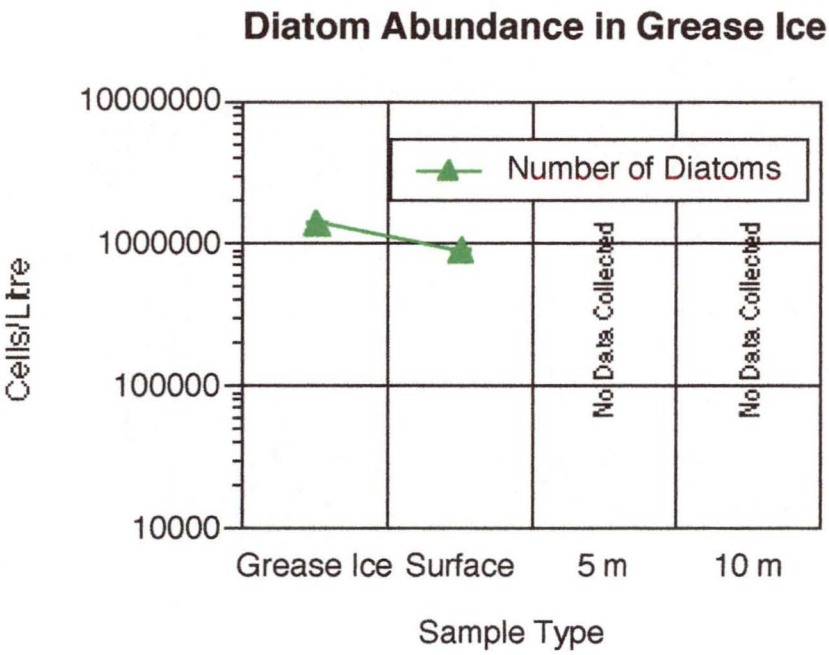
Table 2.7 Diatom abundances (cells l^{-1}) for grease ice sites. No samples were collected from 5 and 10 metres.

Sample	Grease Ice	Surface
W1	2.42E+06	6.28E+04
W2	7.32E+05	1.68E+04
W3	1.07E+06	9.78E+03
Mean	1.40E+06	2.98E+04
SD	8.93E+05	2.88E+04

Table 2.8 Students t-Test analysis of samples from grease sites.

	Surface Water
Grease Ice	0.12

Figure 2.5 Mean diatom abundance at nilas sampling sites. Error bars indicate standard deviation. Grease Ice = ice samples (ice and slurry/brine), Surface = water from immediately under the new ice cover. No samples were collected from 5 and 10 metres. The Y- axis has a log scale.

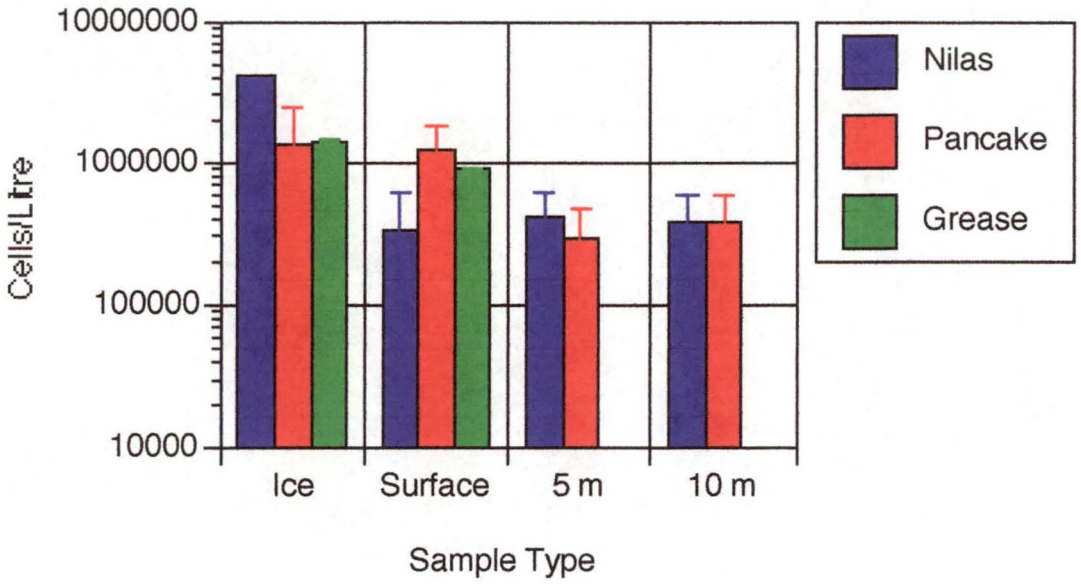


Comparative Plots

A comparison of the diatom abundances at all sites for the three ice types is shown in Figure 2.6. As they were collected at the same time of year in the same general area, it is possible to compare nilas and pancake samples. Direct comparison with grease ice samples, however, is not possible as sampling was in a different area and at a different time of year. Grease ice samples are still included for interest and general information.

Cell abundances in nilas (4.56 E+06 cells l^{-1}), pancake ice (1.80 E+06 cells l^{-1}) and grease ice (1.40 E+06 cells l^{-1}) are of a similar magnitude. However diatom abundances in water samples from the surface are lower at the nilas site (1.00 E+06 cells l^{-1}) and much lower at the grease site (2.98 E+04 cells l^{-1}) compared to the pancake site (1.87 E+06 cells l^{-1}) . Cell abundances in water samples were similar at nilas and pancake sampling sites.

Figure 2.6 Combination plot, showing average number of diatom cells l^{-1}. Nilas and pancake profiles are averaged from eight individual sets of samples. Grease ice profiles are averaged from three sets of samples. error bars represent standard deviation.



2.3.2 SIZE DISTRIBUTION OF DIATOMS...

There are difficulties associated with attempting to classify irregular shapes into specific size classes. Pennate diatoms can be categorised by relating size to the long axis of the algal cells, although, the width of these cells can vary substantially. Some centric diatoms are also very difficult to classify, as they are adorned with large spines and they can vary in size during different stages in the life cycle of the cells. However, the most abundant species were relatively easy to categorise; this is discussed further in Section 2.4.

Cell size was recorded during microscopic analysis of the samples. Overall, 60% of diatoms present were either: *Chaetoceros dichaeta, Fragiliaropsis cylindrus* (both 10 μm or smaller) or *Fragiliaropsis curta* (in the 10 μm - 20 μm size range). The cell size distribution for the various ice types is shown in Figures 2.7 to 2.9. Ice samples, surface water samples and water from 5 and 10 m, are also compared. The size range classification was discussed in Section 2.2.2. Figures 2.7 to 2.9 show the average size distribution of diatom cells from eight nilas (Figure 2.7), eight pancake (Figure 2.8) and three grease ice sample sites (Figure 2.9).

Nilas Samples

There is a decrease in the relative abundance of very small cells in nilas ice samples compared with the deep water samples, (Figure 2.7). However, a reduction in the number of very small cells is also evident at 5 m, compared with the 10 m water sample. More small cells are present in ice and 5 m water samples, compared with surface water and water from 10 m. Abundance of medium sized cells and large cells is relatively uniform over the entire data set; medium and large cells only comprised 30 % of the population.

Pancake Samples

Size distribution of diatoms in samples from pancake sites is relatively uniform (Figure 2.8). Medium and large diatoms contribute up to 30 % of the cells in the pancake ice samples. This value steadily increased with depth to reach a maximum of 40 % in the water samples from 10 metres. Very small cells contribute between 32 and 40 % of cells in all sample types. Small cells contribute between 25 and 34 % of the cells.

Figure 2.7 Size distribution of diatoms at nilas sites. The plot below is based on the average of eight separate samples per sample type. 'Ice' refers to nilas; 'surface' refers to the water immediately under the ice; '5 m' and '10 m' refers to water from those depths. The Y-axis shows percentage abundance. Size classes discussed are: very small < 10 μm, small < 20 μm, medium < 40 μm and large > 40 μm.

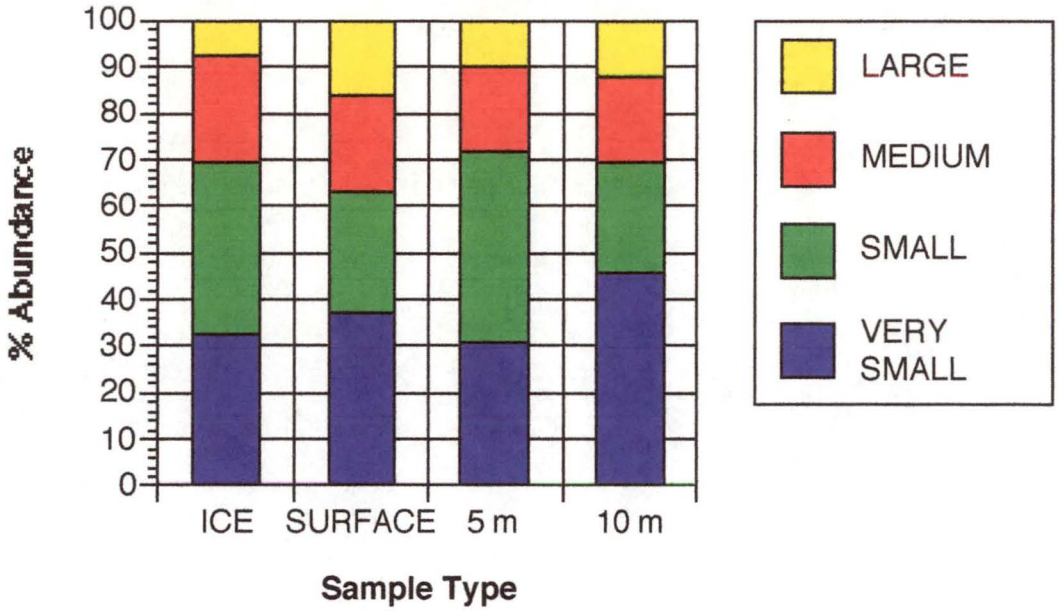
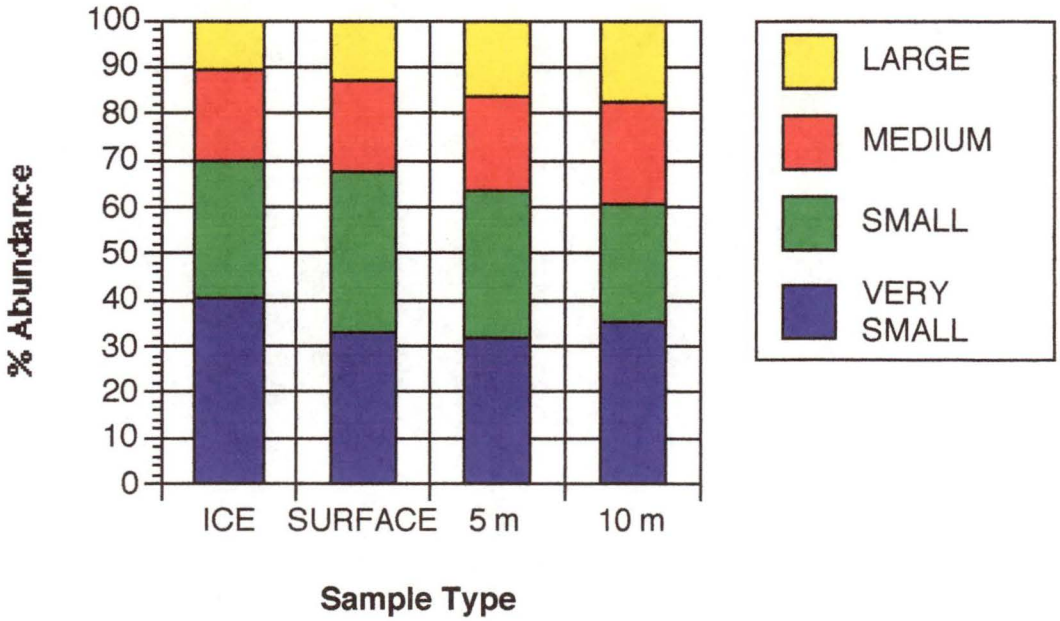


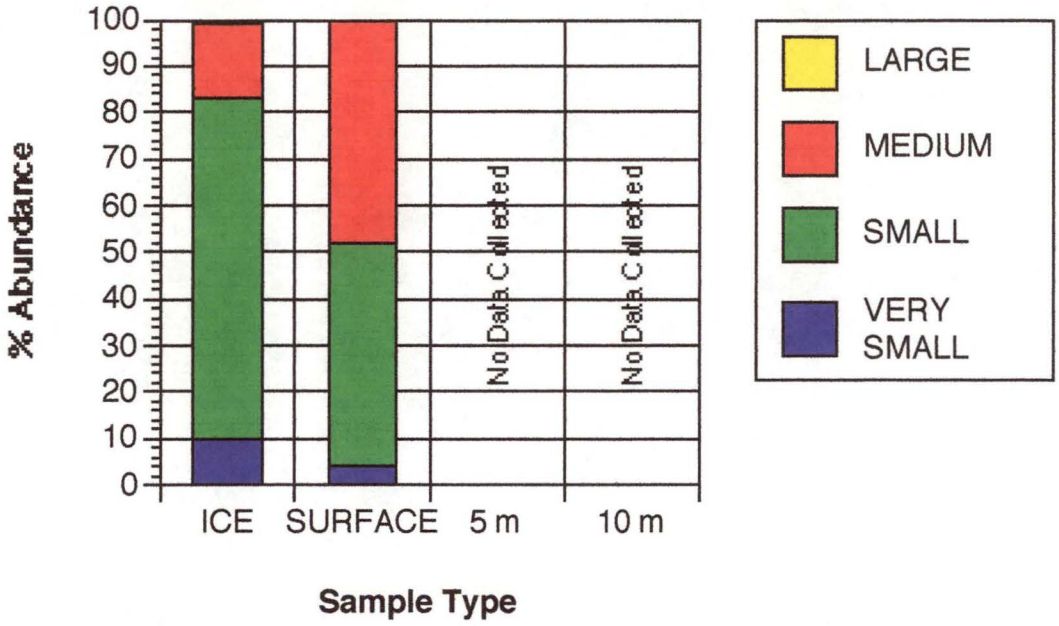
Figure 2.8 Size distribution of diatoms at pancake sites. The plot below is based on the average of eight separate samples per sample type.



Grease Samples

Grease ice samples are dominated by small diatoms (75 %). Small and medium diatoms each comprise 45 % of the slurry samples, (Figure 2.9).

Figure 2.9 Size distribution of diatoms at grease ice sites. The plot below is based on the average of three separate samples per sample type.



Summary

The distribution of size classes in nilas and pancake ice suggests that there is no incorporation of specific size classes of diatoms into newly-forming ice. Instead the size distribution of cells is homogenous, regardless of sample or ice type. There is evidence of incorporation of small diatoms into grease ice samples at levels above those in the underlying surface water. Both small and medium cells form 45 % of cell abundances in surface waters at the grease ice site. Small cells contribute to 75 % of the abundance in grease ice, whereas, medium cells only contribute 15 % of diatom abundance. Small cells were primarily *Fragiliaropsis curta*, (refer Section 2.3.3).

2.3.3 SPECIES DISTRIBUTION...

Chaetoceros dichaeta, Dactyliosolen antarcticus, Fragiliariopsis curta, F. cylindrus, F. kerguelensis, F. ritscheri, Nitzschia angulata and *Pseudonitzschia turgiduloides* were ubiquitous in distribution. Other species which occurred at an abundance level > 5% in some samples, included: *Navicula glaciei, Nitzschia lecointei, F. lineata, N. stellata, Porosira* species, *Thalassiosira gravida* and *T. tumida.* A full list of the species referred to in the text along with author citation, is shown in Appendix 8.1.

The species distribution at the nilas, pancake and grease ice sample sites is given in Figures 2.10 to 2.12. In all of the samples, *C. dichaeta, F. curta* and *F. cylindrus* combine to form between 50 and 60 % of the population. *P. turgiduloides* is common in all samples.

Nilas Samples

Fragiliaropsis curta is twice as abundant in nilas ice as in the underlying surface water. The percentage abundance increases again at 5 m depth and then decreases at 10 m. *Chaetoceros dichaeta* has a reduced abundance in the ice over levels in the underlying surface water. Its abundance decreases in the water sample from 5 m and then increases in the deeper water sample (10 m). This contrasts with data obtained in Section 3.0 (refer Section 3.3.2). *Fragiliaropsis cylindrus* is present at abundance levels of around 5 % in all samples. Around 40-45 % of total abundance is contributed by other cells, mostly those mentioned above (refer Appendix 8.2 for raw species abundance values). A summary of species distribution at nilas sites is shown in Figure 2.10.

Pancake Samples

Fragiliaropsis curta, F. cylindrus and *C. dichaeta* have a ubiquitous distribution in samples from the pancake ice sites (refer Figure 2.11). *Fragiliaropsis curta* is only half as abundant in the water sample from 10 m as in the pancake ice sample. There is an increase in the abundance of cells, other than species mentioned, in the water samples.*Pseudonitzschia turgiduloides* is common in all water samples, reaching 39 % abundance in pancake samples from site R. In addition, *Dactyliosolen antarcticus* is common in the water samples at the pancake sites (forming up to 20 % of the community), but appears only rarely in the ice (Appendix 8.2).

Figure 2.10 Species abundance at nilas sampling sites. Profiles are mean values obtained from eight different sets of samples from nilas ice formation areas. X - axis describes sample type: Surface = water immediately under ice cover, 5 m and 10 m = water from those depths. Y-axis is percentage abundance.

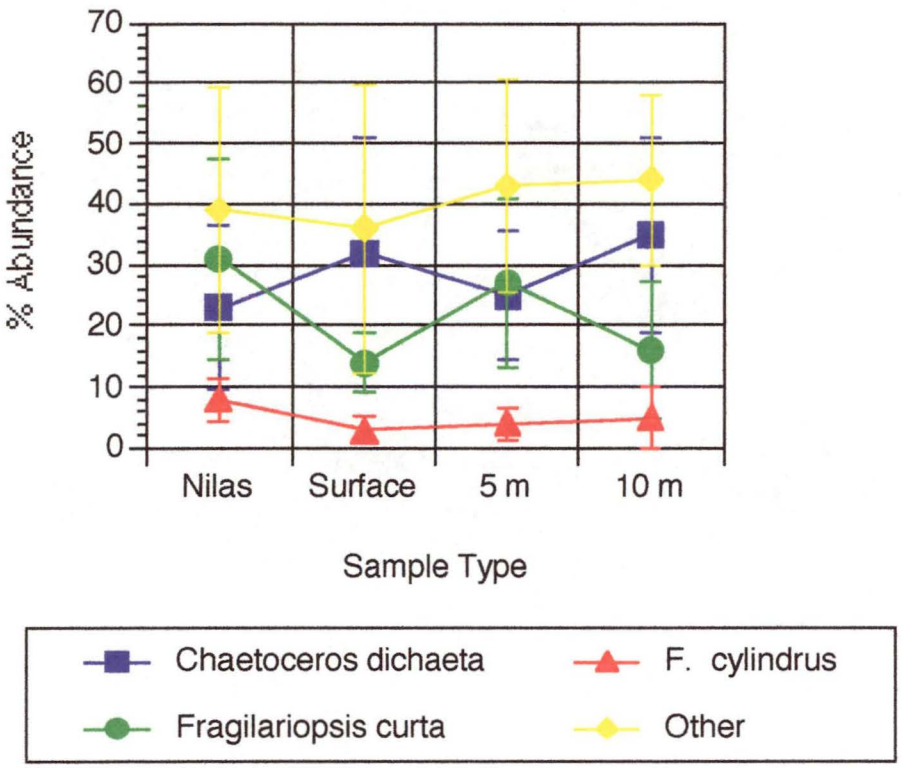


Figure 2.11 Species abundance at pancake sampling sites. Profiles are mean values obtained from 8 sets of samples from pancake ice formation areas.

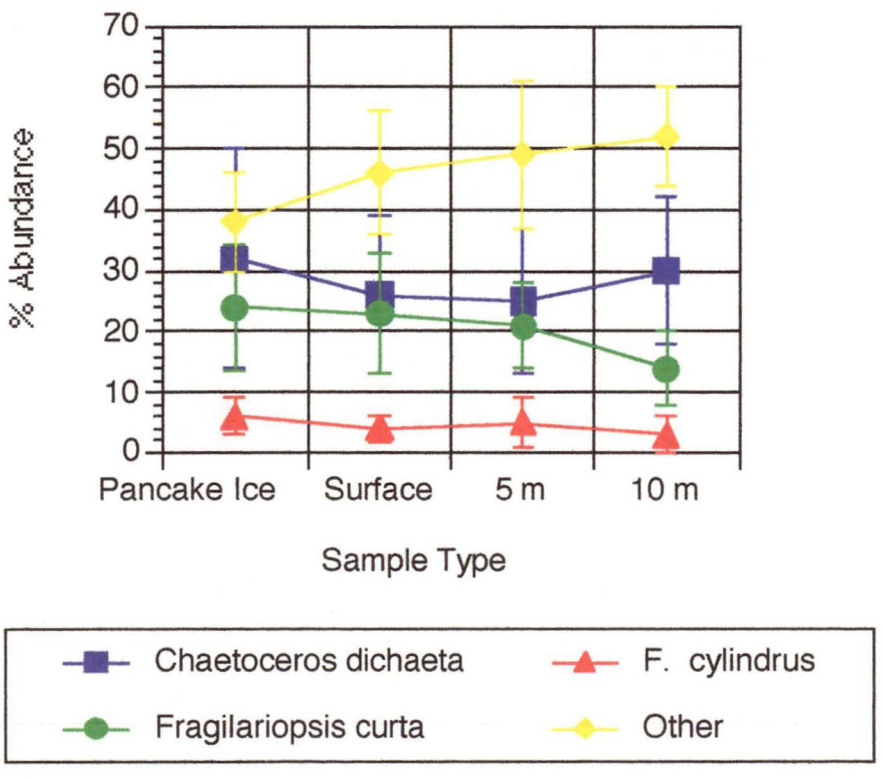
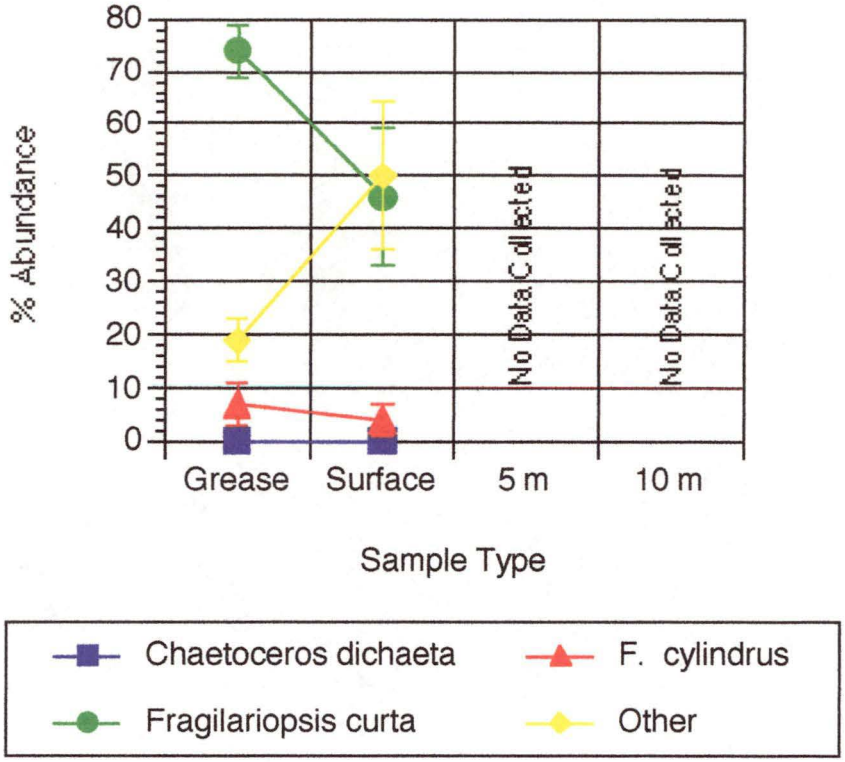


Figure 2.12 Species abundance at grease sampling sites. Profiles are mean values obtained from three different sets of samples from grease ice formation areas.



Grease Samples

Fragiliariopsis curta dominates all the grease ice samples. Its abundance is higher in the ice than the water, (Figure 2.12). This reflects closely the abundance of *F. curta* in grease ice samples studied in Section 3.0. *Thallasiosira tumida* and *T. gravida* are also relatively abundant throughout (refer Appendix 8.2). In addition, *Navicula glaciei* and *N. stellata* comprise up to 17 % of the cell numbers in the surface water samples.

Cluster Analysis

Cluster analysis using Bray Curtis matrix on relative abundance of species was conducted. Results of this analysis are shown in Figure 2.13. Sample information is related to the numbers shown on the cluster diagram in Table 2.9. A similarity of 57 % was considered to be significant. This value was chosen because it separated five distinct groups. The similarity level is considered only an approximate way of determining how similar one sample is to the next.

Group 1 was comprised of 8 samples, 7 of which were nilas samples. The second group was very large (38 samples) and comprised primarily pancake samples. Several nilas samples were also present. Group three comprised 3 samples, all of

which were from pancake sites. Group 4 contained 8 samples five of which were nilas. Group 5 contained 6 samples, which had a similarity of only 30 % when compared with the rest of the samples. All samples in group 5 were from grease ice sites.

Multi Dimensional Scaling (MDS)

Multi Dimensional Scaling (MDS) analysis reflected the results of the cluster analysis, (Figure 2.14). Some of the nilas samples are clearly segregated from other samples (Group 1) as are the grease ice samples (Group 5). Other groups (generated in the cluster analysis) are also shown on Figure 2.14.

Summary

Chaetoceros dichaeta, Fragiliaropsis curta, F. cylindrus and *Pseudonitzschia turgiduloides* combine to account for between 50 and 60 % of the algal cell abundance for all samples. The following results refer to the average species distribution for eight sets of nilas and pancake samples, and three sets of grease samples. *Fragiliaropsis curta* is twice as abundant in nilas compared to the underlying water. *Chaetoceros dichaeta* has a low abundance in nilas. *Fragiliaropsis curta* is present at twice the abundance in pancake ice compared to the underlying surface water sample and the water sample from 10 m depth. *Fragiliaropsis curta* dominates the grease ice sample with 74 % abundance. Cluster analysis and MDS analysis indicates some nilas, pancake and grease samples are distinctly different.

Figure 2.13 Dendrogram portraying results of cluster analysis of entire data set. N = samples from nilas sites; P = samples from pancakes; G = samples from grease sites. Numbers relate samples numbers presented in Appendix 8.2. Five groups become evident using 57 % similarity level.

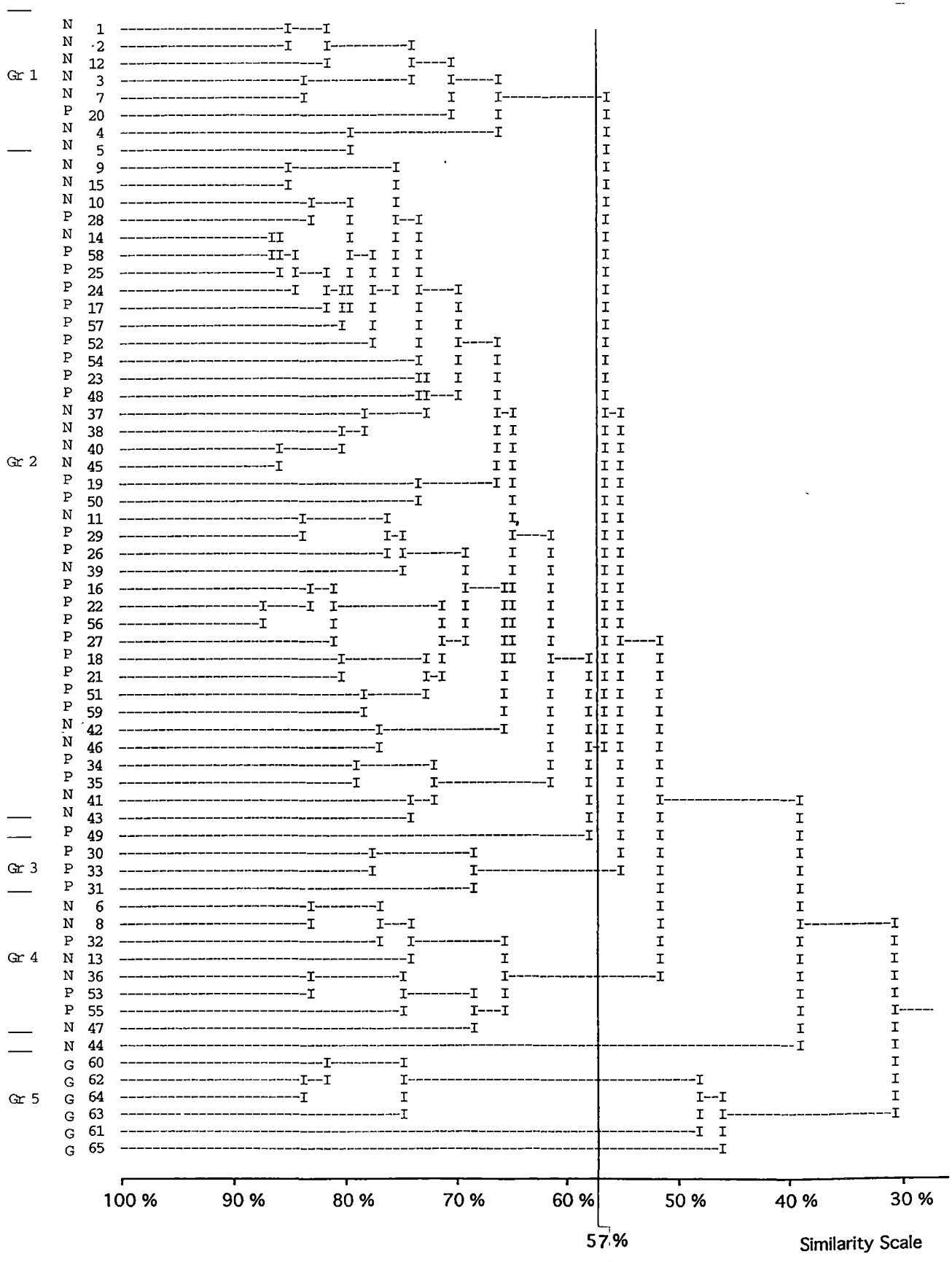
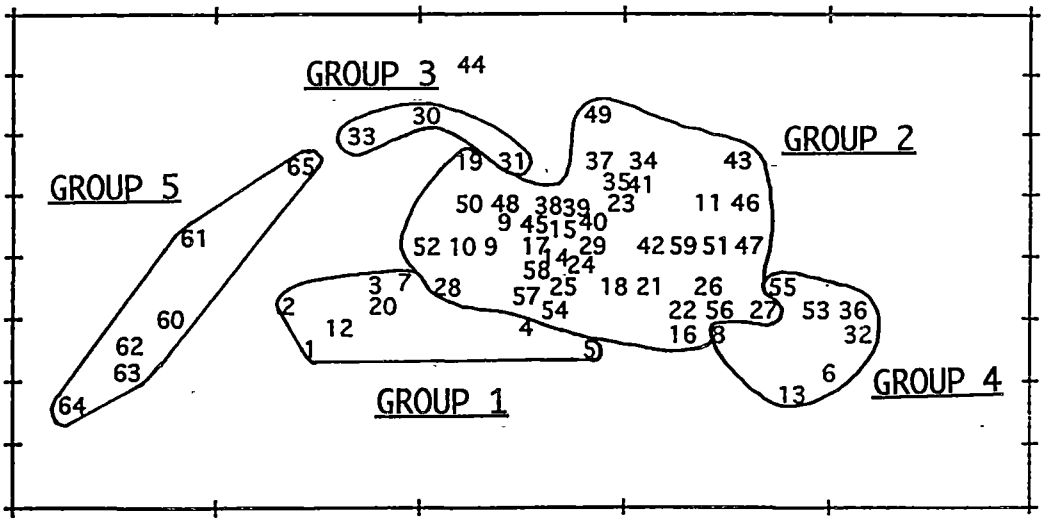


Table 2.9 Sample description for cluster analysis presented in Figure 2.13.

NUMBER	SAMPLE TYPE	ICE TYPE	SITE
Gr 1			
1	Ice	Nilas	C
2	5 m	Nilas	C
12	Ice	Nilas	O
3	10 m	Nilas	C
7	Surface	Nilas	I
20	10 m	Pancake	S2
4	Ice	Nilas	E
5	10 m	Nilas	E
Gr 2			
9	Ice	Nilas	J
15	10 m	Nilas	O
10	5 m	Nilas	J
28	Ice	Pancake	R2
14	5 m	Nilas	O
58	5 m	Pancake	V3
25	Surface	Pancake	R1
24	Ice	Pancake	R1
17	Surface	Pancake	S1
57	Surface	Pancake	V3
52	Ice	Pancake	V2
54	5 m	Pancake	V2
23	10 m	Pancake	S2
48	Ice	Pancake	V1
37	Surface	Nilas	U1
38	5 m	Nilas	U1
40	Ice	Nilas	U2
45	Surface	Nilas	U3
19	10 m	Pancake	S1
50	5 m	Pancake	V1
11	10 m	Nilas	J
29	Surface	Pancake	R2
26	5 m	Pancake	R1
39	10 m	Nilas	U1
16	Ice	Pancake	R2
22	5 m	Pancake	S1
56	Ice	Pancake	V3
27	10 m	Pancake	R1
18	5 m	Pancake	S1
21	Surface	Pancake	S2
51	10 m	Pancake	V1
59	10 m	Pancake	V3
42	5 m	Nilas	U2
46	5 m	Nilas	U3
34	5 m	Pancake	R3
35	10 m	Pancake	R3
41	Surface	Nilas	U2
43	10 m	Nilas	U2
49	Surface	Pancake	V1
Gr 3			
30	5 m	Pancake	R2
33	Surface	Pancake	R3
31	10 m	Pancake	R2
Gr 4			
6	10 m	Nilas	E
8	10 m	Nilas	I
32	Ice	Pancake	R3
13	Surface	Nilas	O
36	Ice	Nilas	U1
53	Surface	Pancake	V2
55	10 m	Pancake	V2
47	10 m	Nilas	U3
44	Ice	Nilas	U3
Gr 5			
60	Ice	Grease	W1
62	Ice	Grease	W2
64	Ice	Grease	W3
63	Surface	Grease	W2
61	Surface	Grease	W1
65	Surface	Grease	W3

Figure 2.14 Multi-dimensional scaling (MDS) results for data set presented in Table 2.8. Groups generated in cluster analysis (Figure 2.13) are superimposed on output. X and Y-axes are arbitrary.



2.4 DISCUSSION

Information obtained from analysis of newly forming sea ice and water samples was used to determine whether algal assemblages from different ice types have different compositions. Data which assisted this investigation included: diatom abundance, size and species information. Different ice types were compared using Student's t-Test analysis, cluster and MDS analysis. Cluster and MDS analysis was also used to investigate the extent of variation within the one sample type (i.e. samples collected from nilas, pancake or grease sites). The results of this study assist in discerning whether biological or physical processes are responsible for algal incorporation into newly forming sea ice.

Background...

There are few investigations into the importance of new sea ice type to algal community structure. Garrison and Close (1993) investigated grease, nilas and pancake ice, in addition to older ice sampled from the Weddell Sea in winter 1988. They found that the biomass in new sea ice was much lower than in older ice types. All new ice forms they studied, were found to have higher biomass than the water column samples. However, unlike this investigation, their study revealed pancake ice to have a higher number of algal cells than nilas ice. Algal composition in frazil and pancakes from the South Shetland Islands has been reviewed by Ligowski *et al* (1987). Results of that study indicated that air temperature may be an important factor influencing the development of diatoms in sea ice; warmer air temperatures leading to greater concentrations of cells in the sea ice. Other authors have touched on the significance of frazil ice crystals in structuring algal populations in sea ice (eg. Clarke and Ackley 1984; Horner *et al*, 1992; Scott *et al*, 1994); however extension of these results to an analysis of early ice formation has not occurred.

Much of the work on ice algal and phytoplankton in the Southern Ocean has been carried out in summer (McConville and Wetherbee 1983; Clarke and Ackley 1984; Kawamura and Ichikawa 1984; Garrison *et al* 1987; Kang and Fryxell 1991). Some authors have made comprehensive studies of the spring populations (Syvertsen and Kristian 1988; Garrison and Buck 1991) and winter populations (Ligowski *et al*

1987; Garrison and Close 1993). Watanabe and Satoh (1987), and Garrison and Buck (1989), have investigated changes sea ice algal populations over the period of a year. Early work near Syowa, by Hoshiai (1977), is one of the few studies available which analyses autumn sea ice populations. The current investigation is the first concerning algal populations in newly forming eastern Antarctic sea ice.

2.4.1 ALGAL CELL ABUNDANCE...

The abundance of algal cells in sea ice samples depends on many factors. The number of diatom cells per litre in nilas and grease ice samples reaches up to two orders of magnitude higher than underlying water samples (Figures 2.3 to 2.5). Several investigations have revealed a similar enrichment of diatoms in sea ice (eg Garrison *et al*, 1987). However, most of these investigations have been based on an analysis of sea ice cores rather than newly formed sea ice. The abundance of algal cells in pancake samples did not reach this level. It is thus apparent that ice type may be important in the degree of algal enrichment and thus the process of algal inclusion in sea ice.

On the other hand, closer inspection of diatom abundance in nilas data indicates that a more intricate process is occurring (Figure 2.3). Despite the relatively small differences in temporal and spatial factors affecting the various nilas samples (refer Table 2.1), a high variability between samples is evident. The number of diatom cells per litre in nilas samples ranges over two orders of magnitude. Surface water and water from 5 and 10 m have a relatively constant number of algal cells per litre. Nilas ice sampled from sample site U has a lower diatom abundance than surface water and deeper water samples.

The diatom cell abundance in samples from pancake ice sites is shown in Figure 2.4 and Table 2.3. Unlike the nilas sites, a large increase in the number of cells in pancake ice samples compared to water samples is not evident. Pancake ice samples and surface water samples contain roughly only 4 times the number of cells compared with water from 5 and 10 m.

Increased wind stress and wave driven agitation may contribute to a depletion in the number of diatom cells seen at 5 m depth. The lowest average number of cells is at this depth. Reference to samples collected from site R shows this trend clearly. Weather conditions at site R during sampling (30 knot winds) resulted in a considerable degree of agitation of the water column. Consequently, numbers of diatom cells were maximised in the ice and surface water samples but depleted in the

water sample from 5 m. Results from statistical analysis clearly indicate that on the basis of the number of cells per litre, pancake ice and surface water samples are significantly different to those of the underlying deeper water. A conceptual model relating wind-driven water currents to algal enrichment mechanisms is discussed in Section 3.4, (Figure 3.10).

The generally rough conditions at pancake ice formation sites, result in a greater number of diatoms in the top metres of the water column and a depletion with depth. This is not evident in samples from nilas ice sites where the minimum number of cells is found in the surface water (Figure 2.6). The average algal cell abundance from 5 m and 10 m depth, from both nilas and pancake sites, were very similar (Figure 2.6). This highlights the existence of different degrees (and methods) of enrichment in different ice types.

Results from the study of McConville and Wetherbee (1983) indicate that the abundance of cells in autumn sea ice is of the same order of magnitude as the values for the water column. Similarly, Garrison and Buck (1991) estimated a similar biomass, for three types of new ice as contained in the water column. Both these results suggest that the low number of diatom cells in pancake samples can be attributed not only to the time of year the samples are taken but also to the fact that the samples are of sea ice in its earliest stage of formation. Paradoxically, the autumn results from Ligowski (1987) indicate a concentration of diatoms in newly forming sea ice of up to 2500 times more than in the water column.

Results of this study indicate algal abundance in assemblages from different ice types have different compositions. Many factors may contribute to these differences, such as: wind-driven and wave-driven agitation, spatial and temporal factors.

The wind-driven turbulence described at sample site R, demonstrates the importance of physical processes in shaping algal populations in sea ice. While unlikely, it is possible biological processes may well have contributed to the variations in algal population seen in the nilas samples. The importance of the physical enrichment mechanisms is discussed further in Section 3.4.

Students t-Test analysis indicated that the cell abundance values of the nilas samples were not significantly different from one another (Table 2.4). The variation between the ice samples contributed to the lack of significance, despite some ice samples having a 2 orders of magnitude higher number of diatom cells compared with the water samples. Pancake ice and surface water samples were significantly different to deeper water samples (Table 2.6). The number of diatom cells in pancake ice and

surface water samples were consistently within a narrow range. Differences in cell abundances in grease ice samples were not significant when compared to underlying water samples (Table 2.8).

2.4.2 SIZE DISTRIBUTION OF DIATOMS...

The difficulties of classifying diatoms into specific size classes has been discussed in Section 2.2.2. Fryxell and Kendrick (1988) chose to classify *Chaetoceros dichaeta* in a 20 μm bracket. This study places the same diatom in a < 10 μm class. *Chaetoceros dichaeta* seen in this study had very small spines; in addition, its greatest diameter did not exceed 10 μm. Most diatom cells from the water and ice samples cells were ≤ 20 μm in size. This size range is similar to those reported by Kang and Fryxell (1991) who found that the phytoplankton in the outer part of Prydz Bay was dominated by small, ≤ 20 μm diatoms.

The majority of ≤ 10μm cells were small centric diatoms (specifically *Chaetoceros dichaeta*). Very small diatom cells (≤ 10 μm) were most abundant in the 10 m water sample from nilas sites (Figure 2.7) and pancake ice samples (Figure 2.8). There was a slight increase in the abundance of medium and large diatom cells in deeper water from pancake sites. Small cells clearly dominated grease ice samples (Figure 2.9), showing evidence of incorporation of small cells over medium cells into grease ice.

2.4.3 SPECIES DISTRIBUTION OF DIATOMS...

All samples had low species diversity with one to three species dominating. This appears typical of sea ice algal communities (Clarke and Ackley 1984). *Chaetoceros dichaeta* was found in all of the autumn samples. It comprised up to 67% of some samples and was regularly present in samples at abundance levels > 20%. These findings are consistent with those reported by Hasle (1969) who postulated *C. dichaeta* was important in the northern waters of the Antarctic Divergence. *Chaetoceros dichaeta* has often been reported in ice and water samples at concentrations similar to those found in this study (Clarke and Ackley 1984; Kang and Fryxell 1991; Garrison and Close 1993). Similarly, an abundance of *Dactyliosolen antarcticus, Fragiliaropsis curta, F. cylindrus, F. ritscheri, Navicula glaciei, Nitzschia stellata, N. lecointei* and *Pseudonitzschia turgiduloides*, has also been reported as common in Antarctic sea ice (Clarke and Ackley, 1984; Garrison *et al*, 1987; Ligowski *et al*, 1987; Garrison and Buck, 1991).

Figures 2.10 to 2.12 present species distribution plots for nilas, pancake and grease ice sampling sites. Raw species abundance data is presented in Appendix 8.2. *Fragiliaropsis curta* was present in nilas ice (Figure 2.10) and grease ice (Figure 2.12) at much higher abundances than the surface waters. This was not evident in pancake ice (Figure 2.11). *Fragiliaropsis cylindrus* and *Chaetoceros dichaeta* did not show evidence of preferential incorporation in, or conspicuous absence from, ice samples. In samples from all sites diatom species, other than those mentioned above, usually comprised the bulk of the community. Reference to Appendix 8.2 shows which other species were important in forming communities. Usually these other species were not present individually at abundances greater than 20 %.

The cluster analysis used the Bray-Curtis index with unweighted pairs group averaging (UPGMA) linkage (Bray and Curtis, 1957). Cluster analysis generated a similarity matrix by comparing all the samples within the data set and grouping those with similar variables. Cluster analysis of the data revealed nilas and grease ice samples were significantly different to other samples on a basis of their algal community structure (Figure 2.13). Group 1 (Figure 2.13) is comprised primarily of nilas samples from the earlier sampling sites (C, E, I, J, and O). Thus a temporal variation as well as a spatial variation exists when comparing samples to later samples (U1, U2 and U3). In addition, different formation mechanisms (resulting in different consolidation and porosity) may contribute to the separation of nilas samples seen in group 1. Grease ice samples are clearly separated on the cluster output (group 5). The results of the cluster analysis are supported by Multi-dimensional Scaling (MDS) analysis (Figure 2.14). Both group 1 and group 5 are well separated from the remaining bulk of samples in groups 2, 3 and 4.

2.5 SUMMARY

Few sea ice algal studies have investigated sea ice in its earliest forms. While it is clear that different physical factors are responsible for the formation of nilas and pancake ice; it is unclear what effect these physical parameters have on the resultant algal communities.

Results of this study indicate different diatom assemblages result from different types of ice formation. Diatom numbers in some nilas and grease ice samples reached up to two orders of magnitude higher than the underlying water. Diatom numbers in pancake ice samples only reached four times the number of cells in the underlying water. The number of diatom cells per litre in ice samples from the nilas sites was highly variable and consequently not significantly higher than cell numbers in underlying water. This was not the case with pancake ice samples and surface water samples which had significantly higher number of cells compared to deeper water samples. Also grease ice samples did not contain significantly more diatoms than underlying surface water samples. Cluster analysis and MDS analysis separated grease and some nilas samples from the remaining samples on the basis of diatom species distribution.

Most diatom cells from the water and ice samples were ≤ 20 μm in size. Small cells clearly dominated grease ice samples. There is evidence of incorporation of small cells over medium cells in grease ice.

Analysis of samples on a basis of species distribution revealed that samples typically had low species diversity (with one to three species dominating). *Chaetoceros dichaeta* and *Fragiliaropsis curta* were found in nearly all samples at abundances exceeding 20 %. Similarly, *Dactyliosolen antarcticus, Fragiliaropsis cylindrus, F. ritscheri, Navicula glaciei, Nitzschia stellata, N. lecointei* and *Pseudonitzschia turgiduloides*, were also regularly found. *Fragiliaropsis curta* showed evidence of incorporation in nilas and grease ice samples over cell concentrations in the underlying water. Enrichment of this diatom into grease ice is discussed in detail in Section 3.4.

While it is easy to note that different types of early sea ice formation result in different diatom assemblages, it is harder to attribute a definitive cause. Spatial and temporal scales were quite small (refer Figure 2.1 and Table 2.1); however water samples showed considerable variation in species composition (refer Table 2.9, note widespread distribution of water samples from 5 m and 10 m). This could in part contribute to differences seen in incorporation of diatoms into the samples of newly forming ice .

The average number of cells per litre in water from 5 m and 10 m, from both nilas and pancake sites, was very similar. This highlights the existence of different degrees (and methods) of enrichment in different ice types. Wave-driven and wind-driven turbulence at pancake sites may contribute to an increase in cells in surface water and a depletion at 5 m depth. The wave-driven and wind-driven turbulence described above demonstrates the importance of physical processes in shaping diatom populations in sea ice.

The processes involved in forming new ice diatom communities are complex. Statistical results unravel some of the complexities but not all. It is clear that there is evidence of spatial and temporal factors influencing newly forming ice diatom communities. Cluster and MDS analysis indicated different diatom communities occurred in ice which had undergone different formation processes. Student's t-Test results clearly indicate that diatoms are present in pancake ice and surface water at significantly higher levels than in underlying water. Thus the processes behind pancake formation (wind and wave-driven turbulence) are important in shaping resultant communities, this is discussed in greater detail in Section 3.4.

3.0

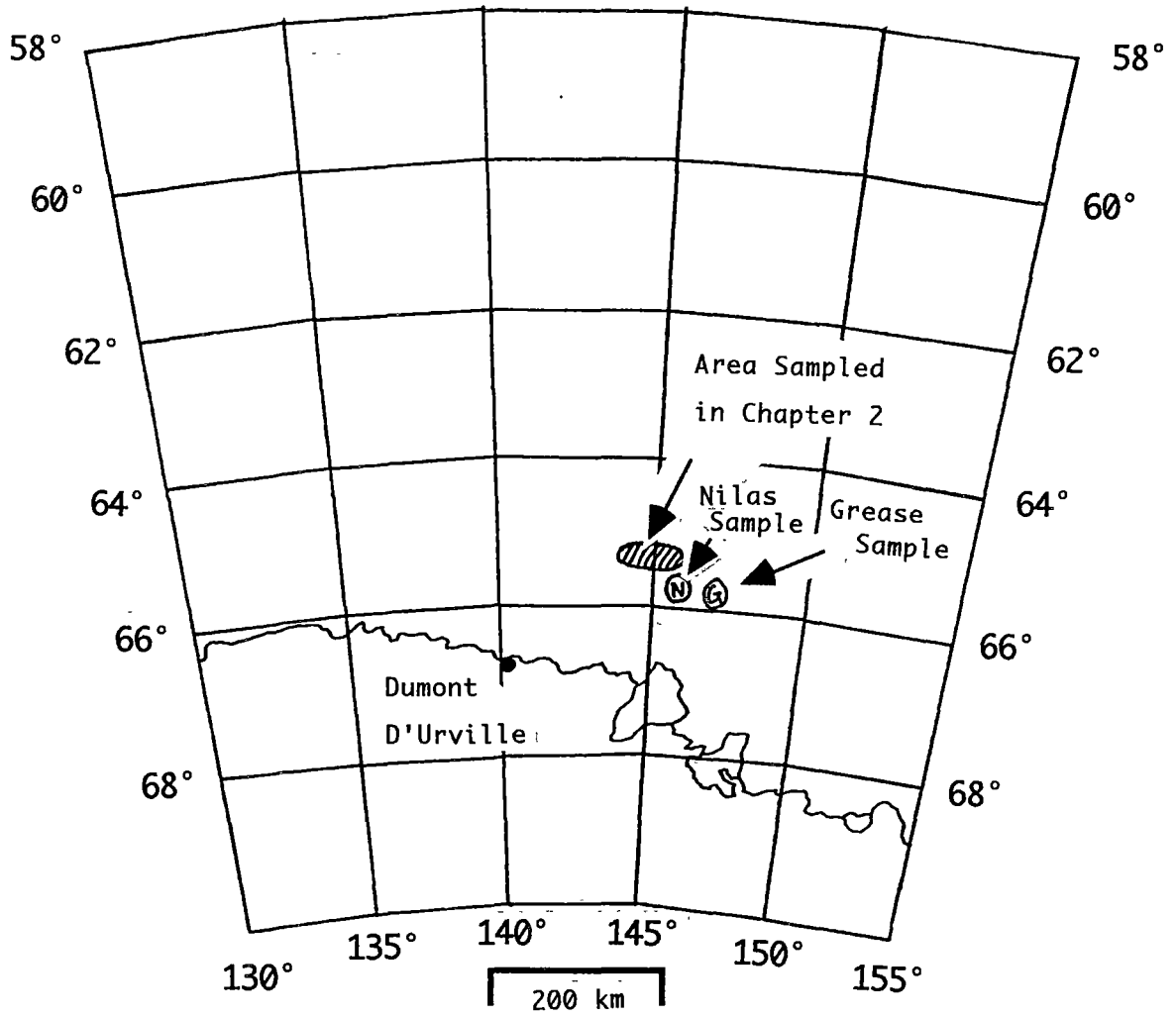
Algal incorporation into newly forming sea ice, 1996 field experiments...

3.1 EXPERIMENTAL DESIGN

3.1.1 DESCRIPTION OF THE STUDY...

The purpose of this investigation was to study the uptake of algae by newly forming sea ice. It sought to clarify further whether algal assemblages from different ice types have different compositions. Ice, slurry and water samples were collected from two sites. At one site, nilas was forming and at the other, grease ice predominated with some early pancake formation. Information presented in this chapter supplements data collected in 1993 and presented in Chapter 2. Both the 1993 and 1996 studies investigated algae in sea ice in its earliest stages of formation and the underlying water column. The 1996 study was a comprehensive analysis of two sets of samples, whereas the 1993 study was less detailed but involved many more samples. The study sites were both situated off the Terre Adelie coast and sampling was conducted in early autumn in each case. During the autumn of 1996, samples were collected from an area similar to that used for samples of newly forming ice described in Chapter 2, (Figure 3.1).

Figure 3.1 Map showing location where samples of newly forming ice and water were collected in the 1996 study. Map also shows general location of data collected during the 1993 study. 'N' refers to the site where nilas and underlying water samples were collected. 'G' refers to site where grease ice and underlying water samples were collected. Hashed area represents approximate region where data presented in Chapter 2, was collected.



3.2 MATERIALS AND METHODS

3.2.1 COLLECTION OF SAMPLES...

Niskin bottles were used to collect water samples. Each bottle had a 10 l capacity. Water was collected: just below the surface (≈ 5 m), 10, 20, 35, 50, 65, 80, 95, 110 and 125 m. At each of the depths 5 l of water was used for live plankton analysis by microscopy and bacterial counts. The remaining 5 l was used for chlorophyll a measurements using HPLC. An additional 20 l of water was collected from the top three depths for polysaccharide and protein analysis, in search of an ice active substance. However, due to the vibration and constant motion of the ship, ship-board settling proved to be unsuitable and this investigation was not continued further.

All water samples were sieved to separate out any ice crystals which had formed. No ice crystals were found at or below 5 m despite the penetration of - 1.8° C water to 350 m (from CTD profiling).

Ice samples were collected using a bucket on a rope. As the ice sampled was sea ice in its earliest stages of formation, it was easily collected in this manner. Ice was sieved immediately after collection to separate ice crystals from water. Crystals are referred to as 'ice' and drain water as 'slurry'. Slurry samples should not be confused with surface water samples from Chapter 2. Similarly ice from Chapter 2 was a combination of ice and slurry, whereas in this study slurry was allowed to drain from the ice. Ice samples were melted in seawater which had been filtered through 0.2 μm membrane filters to prevent damage to algal cells through osmotic shock, (Garrison and Buck, 1986).

The availability of shipboard microscopes and bacterial analysis facilities in 1996 allowed a more comprehensive investigation into sea ice biota than in 1993. Analysis of living material in water samples enabled fragile species and groups to be documented. The 1993 investigation, presented in Chapter 2, was based on preserved material which prevented the recognition of more fragile taxa. Chlorophyll a biomass measurements were determined for the samples from the 1996 study using the High

Performance Liquid Chromatography (HPLC) techniques of Wright *et al* (1991). Bacterial concentrations were also determined. Methods are described in the Section 3.2.

Water temperature at the sampling depths was determined using the instruments on a CTD. The approximate depth of the mixed layer was also recorded. This information was not available for the 1993 study and is an additional aspect investigated.

3.2.2 ANALYSIS OF SAMPLES...

Speciation

Between 0.5 l and 2 l of water was filtered through 2 μm membrane filters. Phytoplankton cells were gently resuspended off the filters and a drop of the concentrate was placed onto a microscope slide. Identification and determination of the relative abundance of the species was determined using a microscope in a cold room (approximately 0° C). A Ziess Axioscope was used to count a minimum of 100 cells per sample. Ice algae and phytoplankton were described to species level when possible.

Bacteria

Bacteria counts were carried out on melted ice samples, slurry samples and water from < 5, 10 and 20 m. Bacteria counts were conducted on approximately 5 ml of sample. One or two drops of Acridine Orange was added to the water samples. The water was then filtered through 0.45 μm membrane filters. These were then placed on a slide and analysed under a 100 x oil objective on a Ziess Axioscope microscope, using fluorescence microscopy. A minimum of 10 fields of view were counted for each sample, (minimum 150 bacteria cells). The number of bacteria cells per litre was determined using the following equation:

$N_{ml} = 6045 \times Cn / V$ $\qquad$ $N_l = N_{ml} \times 1000$

Area of filter glass ($\pi \times 7775^{2)}$ / Area of field of view ($\pi \times 100^2$) = 6045.

Where: C_n is the average number of cells per 10 fields of view
V is the volume of sample counted (5 ml)
N_{ml} is the number of cells per ml
N_l is the number of cells per litre

Cont....

7775 is the radius of the filter glass in μm
100 is the radius of the field of view in μm

Biomass

Between 0.5 and 2 l of water was filtered through Whatman GF/F glass-fibre-filters (47 mm diameter.) using a vacuum of less than 0.5 atm. These were then blotted dry and frozen in cryo-tubes in liquid nitrogen for return to Hobart. Analysis was conducted using the HPLC technique of Wright *et al* (1991).

Oceanographic data

A CTD was used to measure water temperatures and salinities to >1000 m. Biological sampling at sites of newly forming ice was conducted in conjunction with deeper CTD casts for oceanographic purposes. Mixed layer depths are shown in Section 3.3.1.

3.3.1 SUMMARY INFORMATION...

Sample locations and basic characteristics are shown in Figure 3.1 and Table 3.1. The physical characteristics of the ice, weather and oceanographic conditions are shown in Table 3.2.

Nilas was collected at site 1. At this site, 70 % of ice was pancake ice. Ocean swell had reduced to the extent that subsequent ice formed as nilas. Newly-forming nilas was sampled from between pancakes.

At site 2, grease ice was collected. The majority of ice in the area of site 2 was grease with some evidence of pancake ice in early stages of formation. Wind speed was high and ocean turbulence was evident. Subsequent production and growth of ice in this area would most likely result in further pancake development.

Table 3.1 Summary of sample site information for nilas and grease ice samples.

Variable	Nilas	Grease
Date	23-03-96	25-03-96
Latitude	65°55.89'	65°54.31'
Longitude	145°41.29'	146°56.88'
Ice cover	10/10	4/10
Ice thickness (m)	0.10 - 0.15	0.02

Table 3.2 Conditions at nilas and grease sample sites.

Variable	Nilas	Grease
Air temp (° C)	- 6	- 5
Wind speed (kts)	12.2	23.0
Depth of mixed layer (m)	350	350
Water state, Ice cover	10/10	4/10, slight swell
Ice thickness (m)	0.10 - 0.15	0.02
Water temp (° C) at 5 m	- 1.78	- 1.859
Water temp (° C) at 125 m	- 1.81	- 1.819
Salinity (ppt) at 5 m	33.858	33.908
Salinity (ppt) at 125 m	34.392	34.353

3.3.2 DISTRIBUTION OF MAJOR ALGAL GROUPS...

Diatoms were the most abundant of the algae found at the sampling sites. *Chaetoceros dichaeta, Fragiliaropsis curta* and *F. cylindrus* together combined to account regularly for over 80 % of the ice algal and planktonic communities in the samples. In the deeper water samples at the grease ice site, protozooplankton and small heterotrophic flagellates comprised up to 30 % of the planktonic community. Dinoflagellates comprised the majority of the protozooplankton.

Chaetoceros dichaeta was very abundant in the ice and slurry samples from the nilas site. Similarly, it formed 50 % of the cells in the samples from 5 metres and 20 metres at the nilas site. Conversely, *C. dichaeta* was found in low abundances in the ice and high in the water column, at the grease site. Its abundance increased to up to 60 % at 80 m depth (Figure 3.2). Seventy-three percent of the ice algal community at the grease ice site was comprised of *Fragiliaropsis curta.* This species was also abundant high in the water column, however, it only comprised < 10 % of the community between the depths of 35 m and 95 metres (Figure 3.3).

Fragiliaropsis cylindrus comprised up to 40 % of the algal community at 110 metres depth, at the nilas site. It was conspicuously absent in the ice, slurry and high water column samples from both sites.

Further information on the composition of the communities is available in Appendix 8.3.

Figure 3.2 *Chaetoceros dichaeta* distribution at Nilas and Grease ice sample sites. X-axis is sample type (ice, slurry and depth in metres). Y-axis is abundance of *Chaetoceros dichaeta* in percent.

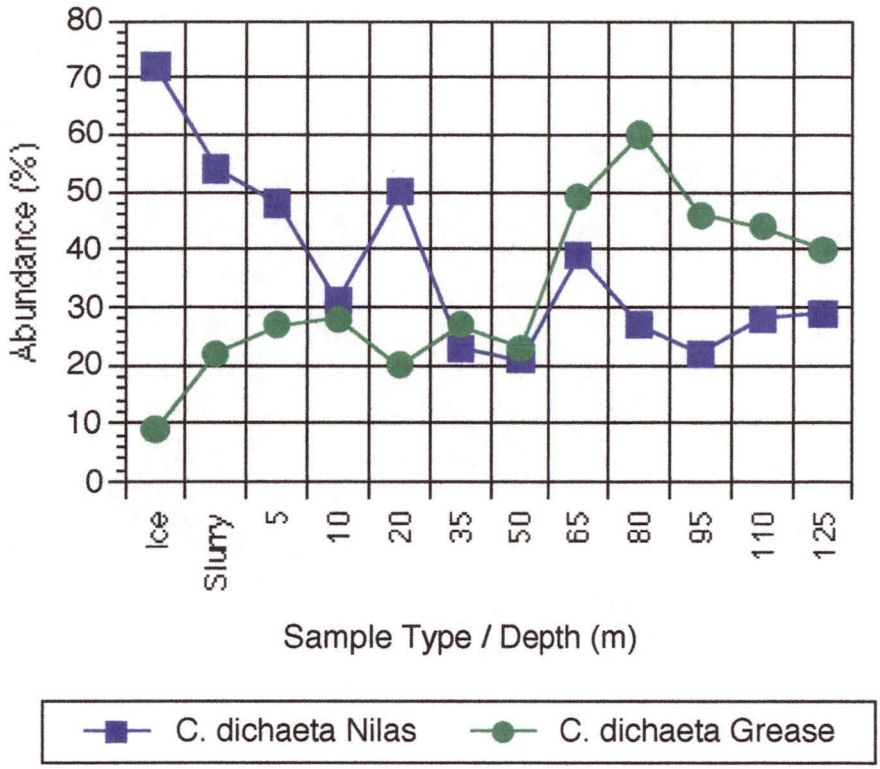


Figure 3.3 *Fragiliaropsis curta* distribution at Nilas and Grease ice sample sites. X-axis is sample type (ice, slurry and depth in metres). Y-axis is abundance of *Fragiliaropsis curta* in percent.

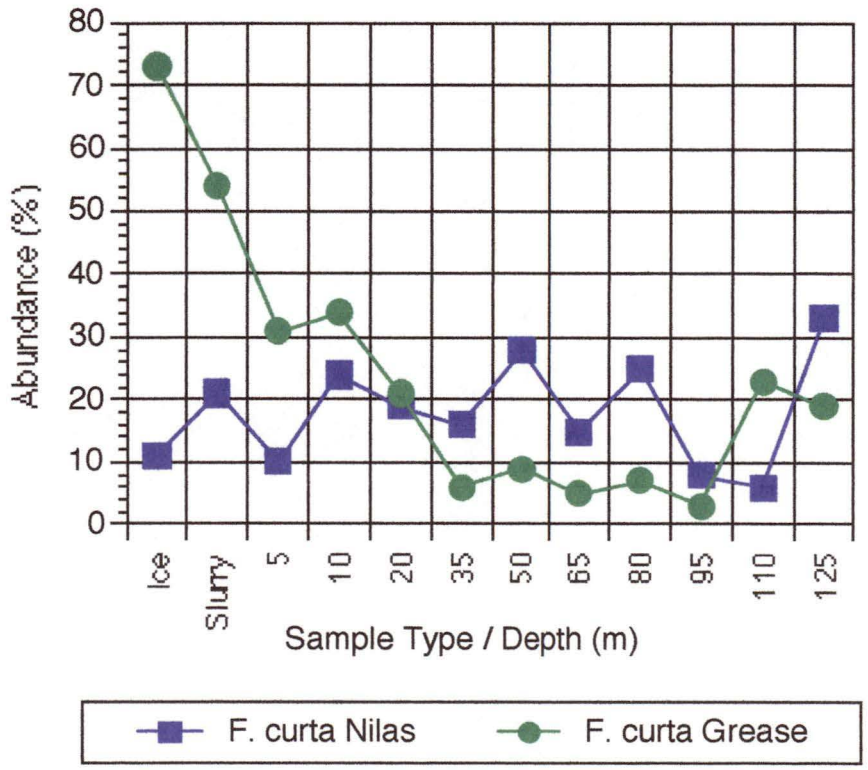


Figure 3.4 *Fragiliaropsis cylindrus* distribution at Nilas and Grease ice sample sites. X-axis is sample type (ice, slurry and depth in metres). Y-axis is abundance of *Fragiliaropsis cylindrus* in percent.

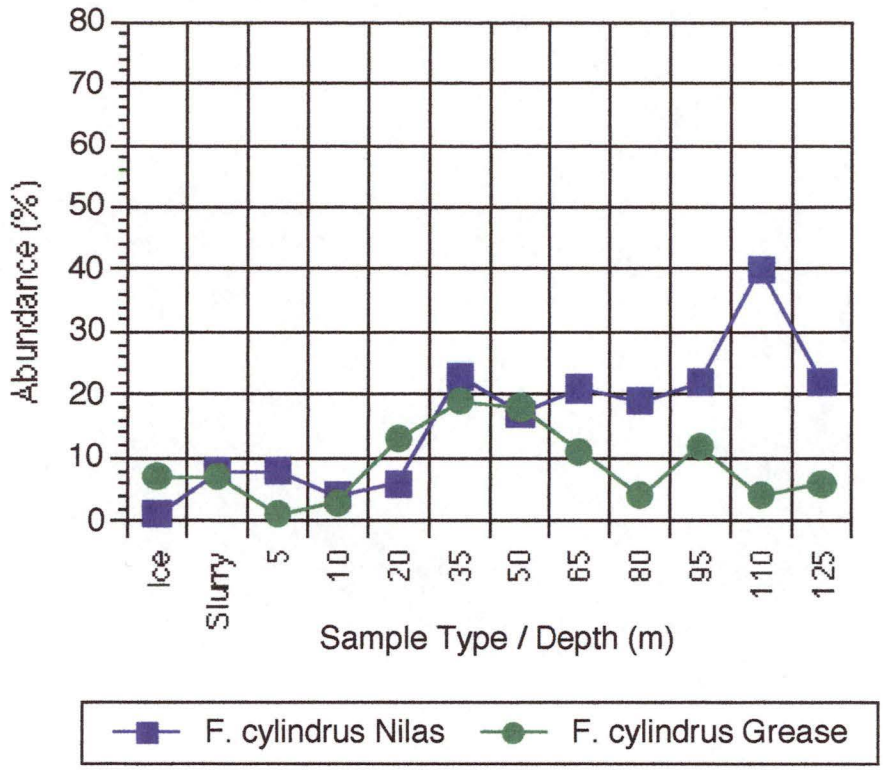


Figure 3.5 Small flagellates distribution at Nilas and Grease ice sample sites. X-axis is sample type (ice, slurry and depth in metres). Y-axis is abundance of Small flagellates in percent.

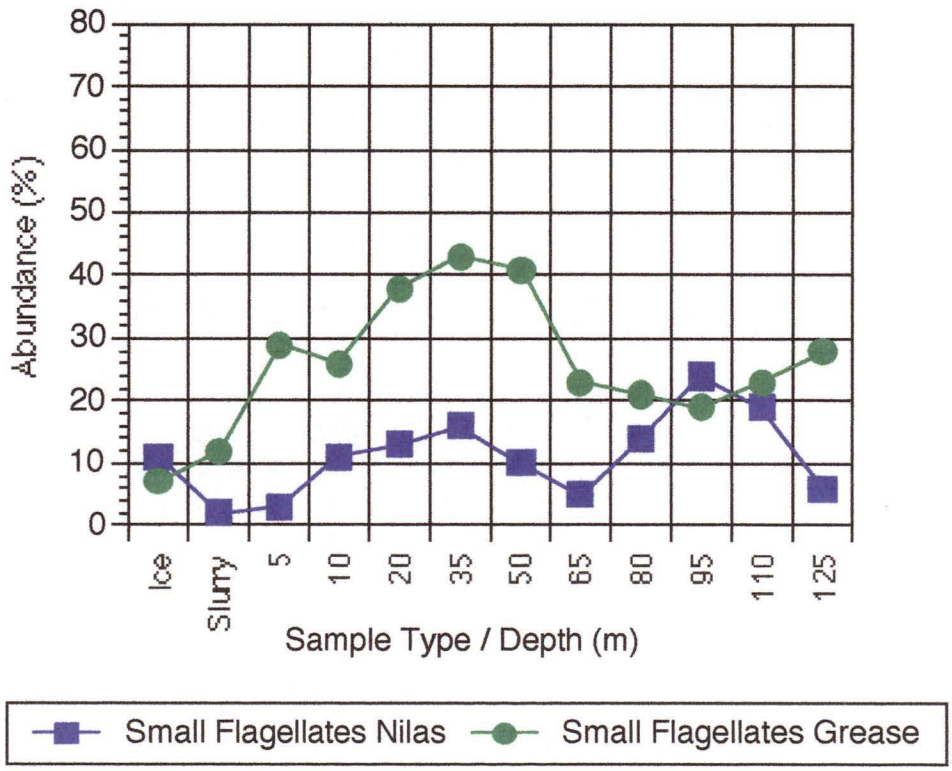
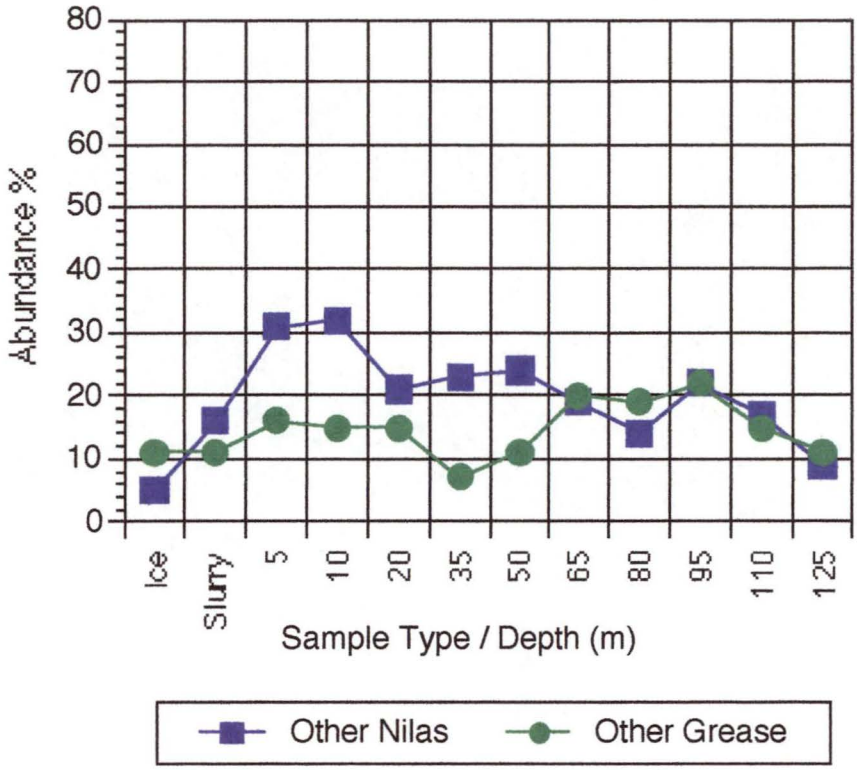


Figure 3.6 Distribution of other species at Nilas and Grease ice sample sites. 'Other' refers to all species not represented in the previous 4 diagrams. X-axis is sample type (ice, slurry or depth in metres). Y-axis is abundance of other in percent.



3.3.3 CHLOROPHYLL a BIOMASS...

Biomass (μg chl a l^{-1}) for the nilas sampling site is shown in Figure 3.7 and for the grease ice site in Figure 3.8. Biomass peaks in chlorophyll a, were found in the slurry sample from nilas site (3 μg chl a l^{-1}) and in the ice from the grease ice site (11 μg chl a l^{-1}). Biomass for the nilas sample was not measured. Increased chlorophyll a was found in the upper 20 m of the water column at the nilas site. Apart from a slight increase in biomass in the slurry sample, this observation was not reflected in samples from the grease ice site.

Figure 3.7 Biomass at nilas sample site. The x - axis represents sample type (ice, slurry and water [depth in metres]). The y - axis represents biomass in ug chl a l^{-1}. The ice sample at this site was not measured.

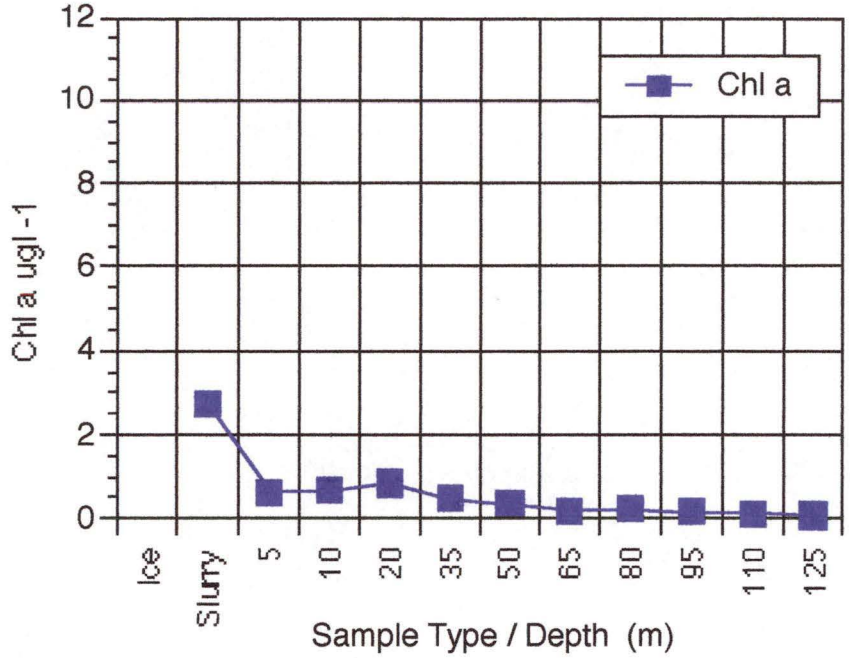
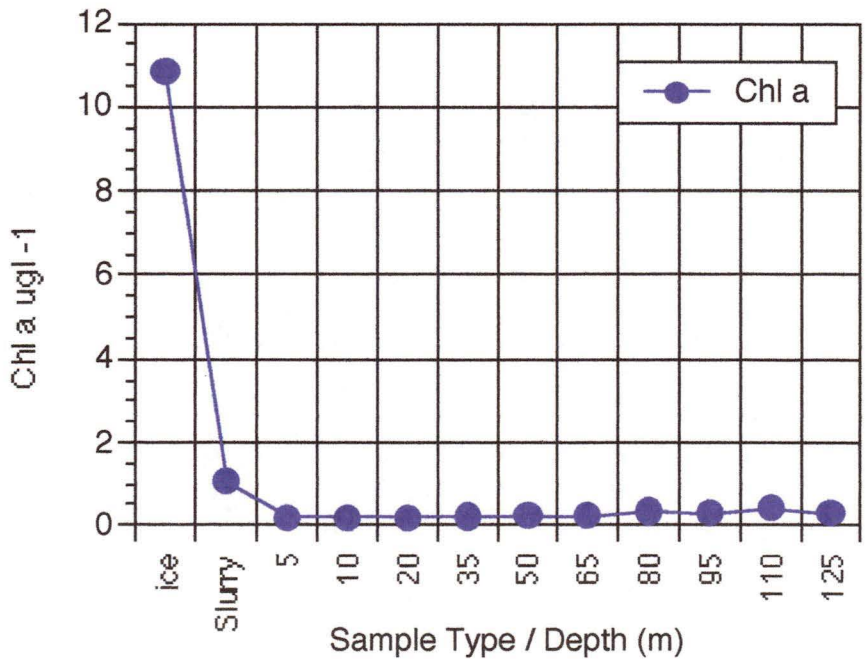


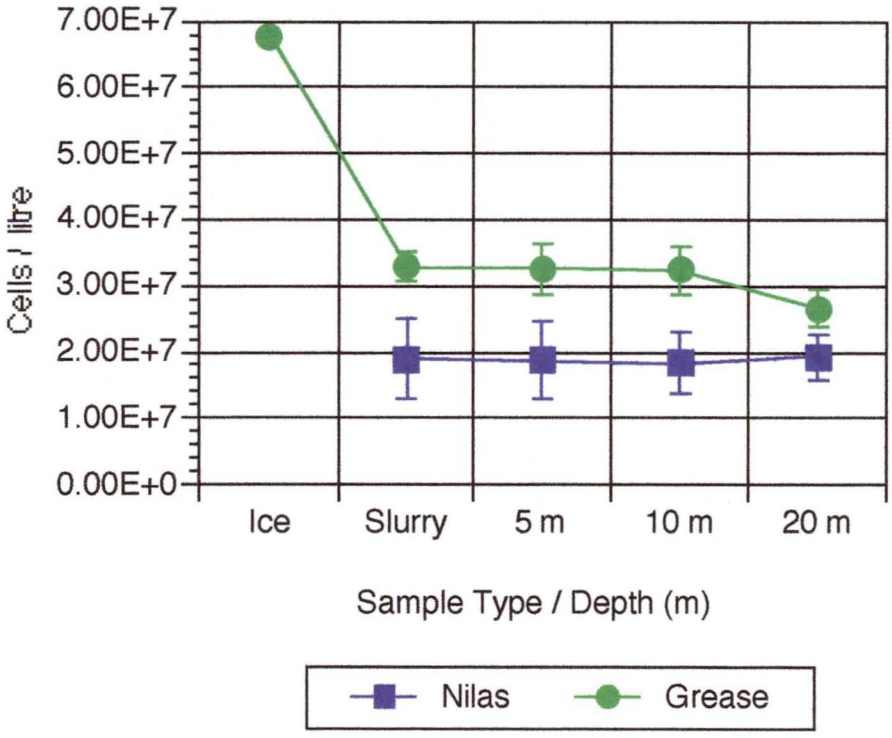
Figure 3.8 Biomass at grease sample site. The x - axis represents sample type (ice, slurry and water [depth in metres]). The y - axis represents biomass in ug chl a l^{-1}.



3.3.4 DISTRIBUTION of BACTERIA...

The distribution of bacteria cells in samples from the nilas and grease sites is presented in Figure 3.9. At the grease ice site, twice the number of bacteria cells were present in the grease ice sample than in the slurry and surface water samples. Bacteria distributions in the slurry from the grease site and water samples from 5, 10 and 20 m were also very similar. The bacteria concentration in the nilas sample was not measured. Bacteria concentrations in the slurry and water from 5, 10 and 20 m, from the nilas site, were very similar.

Fig 3.9 Distribution of bacteria cells from nilas and grease ice sites. The ice sample for the nilas site was not determined. The x - axis represents sample type (ice, slurry depth [in metres]). The y - axis is number of bacteria cells per litre.



3.4 DISCUSSION

This study was designed to determine whether algal assemblages from different ice types have different compositions. It investigates the extent of flagellate and bacterial incorporation into newly-forming sea ice. Biomass measurements are used to describe the degree of the algal incorporation. The importance of physically or biologically-driven incorporation processes will be discussed.

Background...

Diatom incorporation into sea ice in its earliest stages of formation has been reviewed in Chapter 2. High bacterial concentrations have been reported in Antarctic sea ice. Sullivan (1985a) reviewed available literature on bacteria in sea ice. Studies indicated bacterial biomass accounted for between 0.7 μgC l^{-1} and 700 μgC l^{-1}. More recently, enhanced bacterial incorporation in newly-forming sea ice has been linked to high algal concentrations (Weissenberger and Grossmann, 1998). These authors propose that bacterial cells are not scavenged by the ice crystals but that enrichment and sustainment of bacterial biomass within newly-formed ice is dependant on their attachment to algal cells or aggregates of algae. Grossmann and Gleitz (1993) also link bacterial and algal incorporation in sea ice. They propose that ice incorporated bacterial populations experience a strong metabolic inhibition, indicating bacterial cell increases in sea ice are probably not linked to in-situ growth.

The phytoplankton of high latitude Antarctic waters is dominated by diatoms and other forms of nanoplanktonic and picoplanktonic algae, (Wright *et al* 1996). Davidson and Marchant (1992) indicate the importance of autotrophic haptophytes in high latitude Antarctic waters. While many authors have focused primarily on diatom incorporation into sea ice, incorporation of other nanoplanktonic and picoplanktonic forms occurs. Garrison and Close (1993) described a wide variety of heterotrophic and autotrophic taxa found in winter sea ice in the Weddell and Scotia Seas.

3.4.1 SAMPLE SITE INFORMATION...

Results of a comparison between ice and water samples collected 50 km apart were presented in Section 3.3. Two days separated sampling at the grease and nilas sites. Despite these temporal and spatial variations, key species, biomass, bacterial abundance and oceanographic data were quite closely related. The depth of the mixing layer at both sites was 350 m. Penetration of near freezing and freezing water has been reported at depths of ≈ 250 m by Dieckmann *et al* (1986a). However these observations related to near freezing water associated with an ice shelf. Mixing layer depths of 350 m indicate scavenging processes by rising ice crystals could result in considerable enrichment of algae in sea ice. However, in reality ice crystals have not been reported at depths greater than the top few metres of the water column. In this study no ice crystals were found in water samples from any depth ≥ 5 m. Thus scavenging of phytoplankton from considerable depths cannot explain the enrichment of algae in sea ice.

While penetration of near-freezing water may have reached 350 m at both sites, local agitation due to wind and wave-driven turbulence was unequal at the two sites. At the nilas site, there was 10/10 ice cover during sampling. Ice thickness had reached 0.10 - 0.15 m and ocean swell was absent. Wind and wave-driven turbulence at this site would have been considerably less (if not completely absent) compared with the grease ice site. At the grease ice site ice thickness was 0.02 m, ice cover was 4/10 and ocean swell was evident. Increased wind and wave-driven turbulence may account for high biomass present in grease ice samples (refer Section 3.4.3).

It is possible that early ice formation at the nilas site may have formed as a result of a similar ice formation process to that which occurred at the grease ice site. Approximately 70 % of ice at the nilas site was pancake ice. This indicates that initial ice formation at this location occurred in the presence of wind and water turbulence (Weeks and Ackley 1982). As pancakes continued to form, effects of wind-driven turbulence and ocean swell would have been lessened and eventually completely dampened. Nilas would then begin to form in between the pancakes. Initial algal incorporation processes at the two sites may have been similar, due to sea ice formation in the presence of turbulence.

Sampling sites used in this part of the study were geographically close to the autumn sampling sites discussed in Chapter 2 (Figure 3.1). The 1993 sampling sites were between 50 and 100 km away from the 1996 sites. Data was collected in April and May of 1993 and late March of 1996. Both sets of sample sites had similar dominant algal species (*Chaetoceros dichaeta, Fragiliaropsis curta* and *F. cylindrus*). Both the

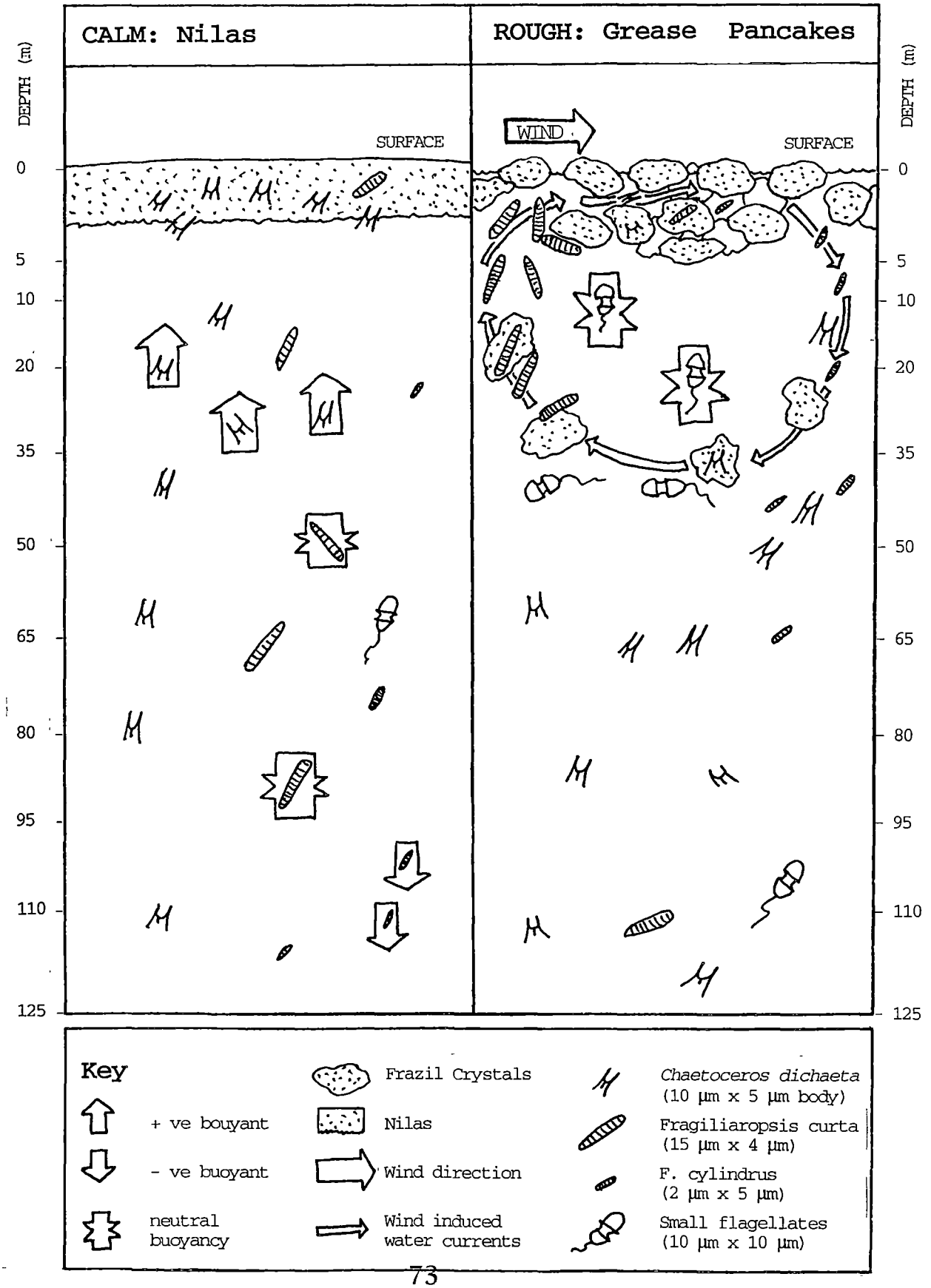
1993 and 1996 experiments sampled newly forming sea ice in its earliest stages of formation.

3.4.2 DISTRIBUTION OF ALGAL GROUPS...

There was low species diversity at both grease and nilas sites. One to four species typically combined to account for > 80 % of abundance. This reflects results discussed in Section 2.4 and the findings of other researchers (eg Clarke and Ackley, 1984; Laubscher *et al,* 1993; Ahn *et al,* 1997). Dominant species in this study have often been reported from ice and water samples. *Chaetoceros dichaeta* has been reported as a common planktonic algal species by Clarke and Ackley (1984), Kang and Fryxell, (1991) and Garrison and Close (1993). Similarly, *Fragiliaropsis curta* and F. *cylindrus* have been reported as common in Antarctic sea ice (Clarke and Ackley 1984; Garrison *et al* 1987; Ligowski *et al* 1987; Garrison and Buck 1991).

Chaetoceros dichaeta comprised 72 % of the algal cell abundance in the ice sample from the nilas site (Figure 3.2). Similarly, at the nilas site *C. dichaeta* was the dominate species in the top 5 m of the water column. *Chaetoceros dichaeta* only formed 9 % of species abundance in the grease ice sample. *Fragiliaropsis curta* was only present at 11 % abundance level in the ice sample from the nilas site. However, it accounted for 73 % of species abundance in the grease ice site and was the dominant species in the slurry which drained from the grease ice upon collection (Figure 3.3). Wind and water-driven turbulence may explain this mirror-like reflection in the dominant species in the ice and upper water column. Total ice cover (10/10) at the nilas site resulted in much reduced wind and wave-driven agitation. *Chaetoceros dichaeta* has a less silicated frustule than *Fragiliaropsis curta* and in addition *C. dichaeta* has spines (although these were quite small in the *C. dichaeta* sampled in this study); both these factors could cause an increase in the relative buoyancy of *C. dichaeta.* Thus in calm conditions, which result in nilas formation, *C. dichaeta* is more likely to be in the upper water column and incorporated into the sea ice. *Fragiliaropsis curta* is a denser, more compact diatom with a smaller surface area than a similarly-weighted *Chaetoceros dichaeta* cell. Greater agitation would lead to relative increased suspension of this cell in the upper water column. Wave fields and water circulation patterns which were able to penetrate the (still unconsolidated) grease ice, could deposit *F. curta* cells in high numbers. Once the ice consolidated, the extent and the effectiveness of the wave field pumping or water circulation patterns would be very much reduced. Subsequent algal incorporation would then result from scavenging or nucleation. The concept discussed above is shown in Figure 3.10.

Figure 3.10 Conceptual model of algal enrichment in sea ice. Enrichment of algae in Nilas is shown, as is enrichment in Grease ice (and potentially pancake ice). Enrichment of positively buoyant *Chaetoceros dichaeta* is indicated in calm conditions. Enrichment of *Fragiliaropsis curta* resulting from wind driven water circulation patterns is shown for rough conditions. This is discussed in greater detail in the text.



Fragiliaropsis cylindrus is often reported in high abundance in Antarctic sea ice (Garrison *et al* 1987; Ligowski *et al* 1987). Ice and slurry samples from both the grease and nilas sites have a very low abundance of *F. cylindrus* (Figure 3.4). This algal species is present at roughly 20 % abundance levels below 35 m at the nilas site and is less abundant deep in the water at the grease site. These results suggest, that *F. cylindrus* is less efficiently incorporated than *Chaetoceros dichaeta* in calm conditions and *F. curta* in rough conditions. The low abundance of *F. cylindrus* in the nilas and surface waters may be explained by its ratio of surface area to its overall weight. This value would be higher than that of a typical *C. dichaeta* cell seen in this study. This would reduce its buoyancy (even making it negatively buoyant) and decrease the efficiency of scavenging mechanisms, (Figure 3.10).

The majority of non-diatom algal cells were picoplanktonic flagellates (Appendix 8.3). Epifluorescence microscopy indicated the most of the flagellates (and all of the small flagellates [10 μm x 10 μm]) were autotrophic organisms. Other nanoplanktonic forms are identified by species or taxa (Appendix 8.3). The low abundance of flagellates in ice and slurry samples at both the nilas and grease sites (despite high water column abundance) indicates flagellates are less effectively incorporated into the ice than *F. curta* and *C. dichaeta*, (Figure 3.5). There was a low abundance of motile cells high in the water column at the nilas site. The majority of flagellates at this site were found at a deeper level in the water column. This may suggest some wave or wind-driven turbulence is required to maintain high abundance of flagellates high in the water. The motile nature of flagellates cells affords them some freedom over their position in the water column. It is possible flagellates cells are actively avoiding entrapment in the newly-forming sea ice.

There is a high abundance of flagellates (cf. 45 %) at the grease site between 20 and 50 m depth, (Figure 3.5). If the effect of the wind-driven turbulence was to produce Langmuir cells of water penetrating to 35 m, then this would be a sensible grazing depth for heterotrophic organisms. Maximum numbers of flagellates were found between 20 and 50 m at the grease site (Figure 3.5). By maintaining this depth flagellates have exposure to food and avoid entrapment in sea ice. The low abundance of flagellates in the grease ice and slurry sample may result from the small cells actively moving out of the unconsolidated ice or being flushed through (as discussed with *F. cylindrus*). This is supported by the greater abundance of flagellates at 5 m and 10 m depth compared to the ice and slurry samples at the grease site.

The abundance of 'other' diatom cells is shown in Figure 3.6. This classification is a summation of all other species not shown in the preceding 4 diagrams. Only two species were regularly present at abundances above 5 %; these were *Leptocylindrus*

mediteraneus and *Nitzschia lecointei*. Diatom cells in this classification from nilas sites, were poorly represented in the ice and slurry compared to abundances of 30 % at 5 m and 10 m. The abundance of other diatom cells in the ice and slurry sample at the grease ice site were more representative of the upper part of the water column, (Figure 3.6). Both *L. mediteraneus* and *N. lecointei are* less siliceous and therefore potentially more buoyant than *F. curta*, as a result these cells are found at slightly increased numbers high in the water column and *F. curta* is found to be more abundant at depth. Both *L. mediteraneus* and *N. lecointei* are larger than the similarly positively buoyant *C. dichaeta* cells; the later are therefore preferentially incorporated.

3.4.3 BIOMASS...

Chlorophyll a biomass averaged 0.38 μg l^{-1} for the water column at the nilas site and 0.26 μg l^{-1} at the grease ice site. Wright *et al* (1996) found surface chlorophyll a values of 0.20 μg l^{-1} during summer/autumn in eastern Antarctic waters of the same latitude. Chlorophyll a values of 0.12 μg l^{-1} in the Weddell-Scotia Seas were found by Cota *et al* (1992). Everitt and Thomas (1986), investigated phytoplankton biomass in inshore waters near Davis Station, Antarctica during winter and spring. Results of this study indicate biomass ranged from 0.10 μg chl a l^{-1} to 5.89 μg chl a l^{-1} in summer.

Garrison and Close (1993) conducted one of the few studies which compares biomass in sea ice in its earliest stages of formation. This study was also conducted in the Weddell-Scotia Sea during winter. Results indicated average chlorophyll a values in grease ice were 0.89 μg l^{-1}, in pancake ice were 0.87 μg l^{-1} and in nilas ice were 0.49 μg l^{-1}. These biomass values are substantially lower than the chlorophyll a biomass found in slurry samples from nilas (2.72 μg l^{-1}) and grease sites (1.05 μg l^{-1}) and in the grease ice sample (10.65 μg l^{-1}). Grease ice biomass values in this study were closer to values reported for 'first year ice and older ice ' (< 1.0 m), from Garrison and Close (1993).

Wind-driven turbulence at the grease ice site may account for the high chlorophyll a biomass (10.65 μg l^{-1}) found in the ice sample and the much lower biomass in the underlying water column (0.26 μg chl a l^{-1}). The conceptual model discussed in Section 3.4.2, demonstrates the importance of wind-driven water circulation patterns in algal enrichment in sea ice, (Figure 3.10).

3.4.4 BACTERIAL CONTENT...

Bacterial concentrations in ice samples have been reported to vary from 10^7 to 10^{10} bacteria cells l^{-1} (Sullivan, 1985a). These values encompass the bacteria concentrations found in ice and water samples from the grease and nilas sites, (Figure 3.9). Maximum concentration of bacterial cells was found in the sea ice sample from the grease site. Bacteria may be associated with ice nucleation (as described by Ackley, 1982), Sullivan (1985a). If bacteria acted as ice nuclei, high bacterial numbers in sea ice could be explained. However, more recently high bacterial numbers in sea ice have been linked to high algal concentration, (Weissenberger and Grossmann, 1998). These authors noted the relative absence of bacterial and algal cells in sea ice which formed in calm (non-agitated) conditions. They suggest bacterial enrichment in sea ice depends on the attachment of bacterial cells to algal cells or aggregates of algal cells which are then incorporated into the ice. Algal biomass and bacterial numbers are both highest in the ice from the grease ice site. However, biomass increases in slurry samples are not reflected in the number of bacterial cells from the slurry. Bacterial numbers in these samples are very similar to bacterial numbers in water from 5, 10 and 20 metres.

3.5 SUMMARY

Data which suggests different diatom assemblages occur in different ice types was presented in Section 2. This section stressed the importance of wind or wave-driven agitation in the process of algal enrichment in sea ice. Results from Chapter 2 indicate that sea ice and water samples were dominated by one to three key species, primarily *Chaetoceros dichaeta, Fragiliaropsis curta* and *Fragiliaropsis cylindrus*. *Fragiliaropsis curta* was found to be preferentially incorporated in nilas and grease ice samples. Physical enrichment processes were considered to be responsible for resultant algal assemblages in different types of sea ice samples.

Results from Chapter 3 support the findings of Chapter 2. In addition Chapter 3 provided information pertaining to flagellates, bacteria and biomass of algae. There was low species diversity at both grease and nilas sites. One to four species typically combined to account for > 80 % of abundance. Dominant species in this study were: *Chaetoceros dichaeta, Fragiliaropsis curta* and *F. cylindrus*. *Chaetoceros dichaeta* comprised 72 % of the algal cell abundance in the ice sample from the nilas site, however it only formed 9 % of species abundance in the grease ice sample. *Fragiliaropsis curta* was only present at 11 % abundance level in the ice sample from the nilas site, but it accounted for 73 % of species abundance in the grease ice. This relationship can be explained largely by physical processes.

Fragiliaropsis curta was found in high abundance in nilas samples discussed in Chapter 2. The difference between this finding and the results presented in Chapter 3 can be explained by variation in ice formation processes and phytoplankton seed stocks available. In calm conditions (conditions under which nilas would form), *Chaetoceros dichaeta* cells are more likely to be near the surface of the water column due to their probable relatively greater buoyancy. *Fragiliaropsis curta* is probably a denser diatom which is more likely to be found high in the water column in an agitated system. Thus *C. dichaeta* cells are found to have a high abundance in nilas and *F. curta* to have a high abundance in grease ice. Buoyancy of algal species most likely plays an important role as a biological enrichment mechanism. A conceptual model explaining this was developed and is shown in Figure 3.10. Wind and wave-driven turbulence would result in wave-field pumping and water-circulation patterns which

enrich algae in grease ice through both physical and biological means. Once the grease ice consolidates (to pancake ice or nilas), the extent and the effectiveness of these mechanisms would be very much reduced. Subsequent algal incorporation would then result from scavenging processes; or with reduced efficiency, as a result of wind and wave-driven turbulence. No ice crystals were found in water samples from any depth ≥ 5 metres.

The majority of non-diatom algal cells were picoplanktonic autotrophic flagellates. The low abundance of flagellates in ice and slurry samples at both the nilas and grease sites (despite high water column abundance, up to 40 %) indicates flagellates are less effectively incorporated into the ice than *F. curta* and *C. dichaeta.* This may be a result of the flagellates small size or more likely their motile nature.

Chlorophyll a biomass averaged 0.38 $\mu g\ l^{-1}$ for the water column at the nilas site and 0.26 $\mu g\ l^{-1}$ at the grease ice site. The chlorophyll a biomass found in slurry sample from the nilas site was 2.72 $\mu g\ l^{-1}$ and grease site 1.05 $\mu g\ l^{-1}$. The grease ice sample had a chlorophyll a biomass value of 10.65 $\mu g\ l^{-1}$.

Bacteria concentrations found in ice and water samples from the grease and nilas sites, were approximately 10^7 cells l^{-1}. Bacterial enrichment in sea ice may depend on the attachment of bacterial cells to algal cells or aggregates of algal cells which are then incorporated into the ice by physical means.

4.0

Field-based experimental sea ice tank, Casey Antarctica...

4.1 EXPERIMENTAL DESIGN

Naturally occurring sea ice algal populations were investigated in Chapter 2 and Chapter 3. These sections focussed on algal distribution in newly forming, nilas, grease ice and pancake ice. In addition, algal and protist distribution in the water column underneath the forming ice was studied. The experiments focussed on algal abundance, species distribution, biomass, algal size distribution and bacterial distribution.

Results from these sections indicate that different algal populations result from different types of ice formation. Enrichment of the concentration of algal and bacterial cells occurs in the sea ice, above the concentrations in the water column. Small algal cells were found to be preferentially incorporated in grease ice over medium cells. All samples (ice, slurry and water) were dominated by 1 to 3 key species. Wave and wind-driven turbulence are important physical processes responsible for shaping resultant algal populations in sea ice. The presence or absence of turbulence can be directly related to the type of algae incorporated into the newly forming sea ice. In turbulent waters, algae will be forced through the unconsolidated ice matrix, and potentially trapped. In calm conditions a combination of biological and physical processes occur resulting in the more buoyant phytoplankton species dominating the newly forming ice.

This chapter and Chapter 5 discuss the use of tanks in which sea ice was produced. Algae was then released into the tanks and the resultant algal uptake by sea ice studied. The use of the ice tanks allowed investigation into: the extent of algal uptake, the species incorporated, incorporation of microscopic glass beads, and the effects of agitation in the tank.

Experiments described in this chapter were conducted over the Austral summer of 1994/1995 at Casey Station in Antarctica. Four different experimental designs were on trail. Water was either recirculated within the tank or was allowed to flow through at a rate of 4 litres per minute. A paddle wheel agitator (refer Section 4.2.1) was used to induce 'wave-like' currents in the tank. Thus different experimental designs were: recirculating with agitation, recirculating without agitation, flow-through with

agitation and flow-through without agitation. A concurrent ice, slurry and water sampling program was conducted from Newcomb Bay, Casey, Antarctica.

An additional benefit the field-based experimental ice tank offered was that, results from the experiments could be used to test the effectiveness of using a laboratory-based ice tank to duplicate processes occurring naturally (this is discussed in Chapter 5).

4.2 MATERIALS AND METHODS

4.2.1 ICE TANK DESIGN...

The ice tank used at Casey had a 250 l capacity. The sides and the bottom of the tank were insulated with 50 mm of polyurethane. The top of the tank was left exposed. The tank was located approximately 10 m from the water's edge (Figure 4.1), in a position where its exposure to winds was similar to that of the water area (from which a concurrent sampling program was run [see Section 4.2.2]).

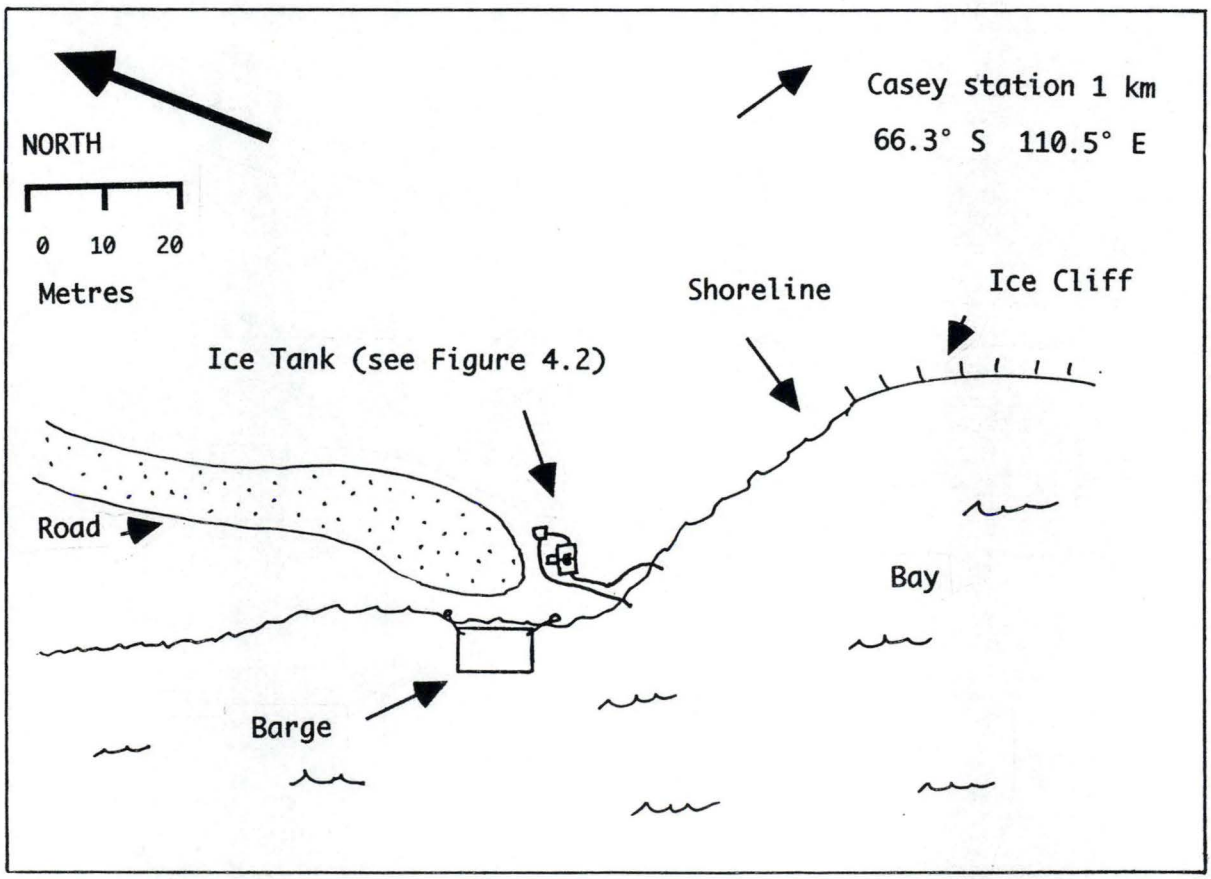


Figure 4.1 Location of ice tank at Casey. Newcomb Bay is a large bay, exposed to ocean circulation. The shoreline drops quickly reaching 10 m depth less than 20 m offshore. The tank was exposed to uninterrupted wind from all directions.

A Grunfoss, Impella-drive, self-priming, small-volume pump was used to pump water through the ice tank. Sea water was drawn along 20 mm polyurethane pipes. These pipes were insulated with foam lagging. Heat trace (10 Wm^{-1}) was used to maintain a water temperature of approximately -1.8° C. Water flow rate through the tank was 5 - 10 l min^{-1}. Agitation through water flow was not sufficient to produce frazil ice typical of the first stages of newly forming sea ice.

A paddle wheel was used to aid the production of frazil ice. The wheel had two plywood blades 350 x 150 mm which rotated at a rate of 30 turns/min. A schematic diagram of the tank set up can be seen in Figure 4.2. A photograph of the ice mechanics tank is shown in Figure 4.3. Probes were used to record water and air temperatures in and around the tank (see Section 4.3.1).

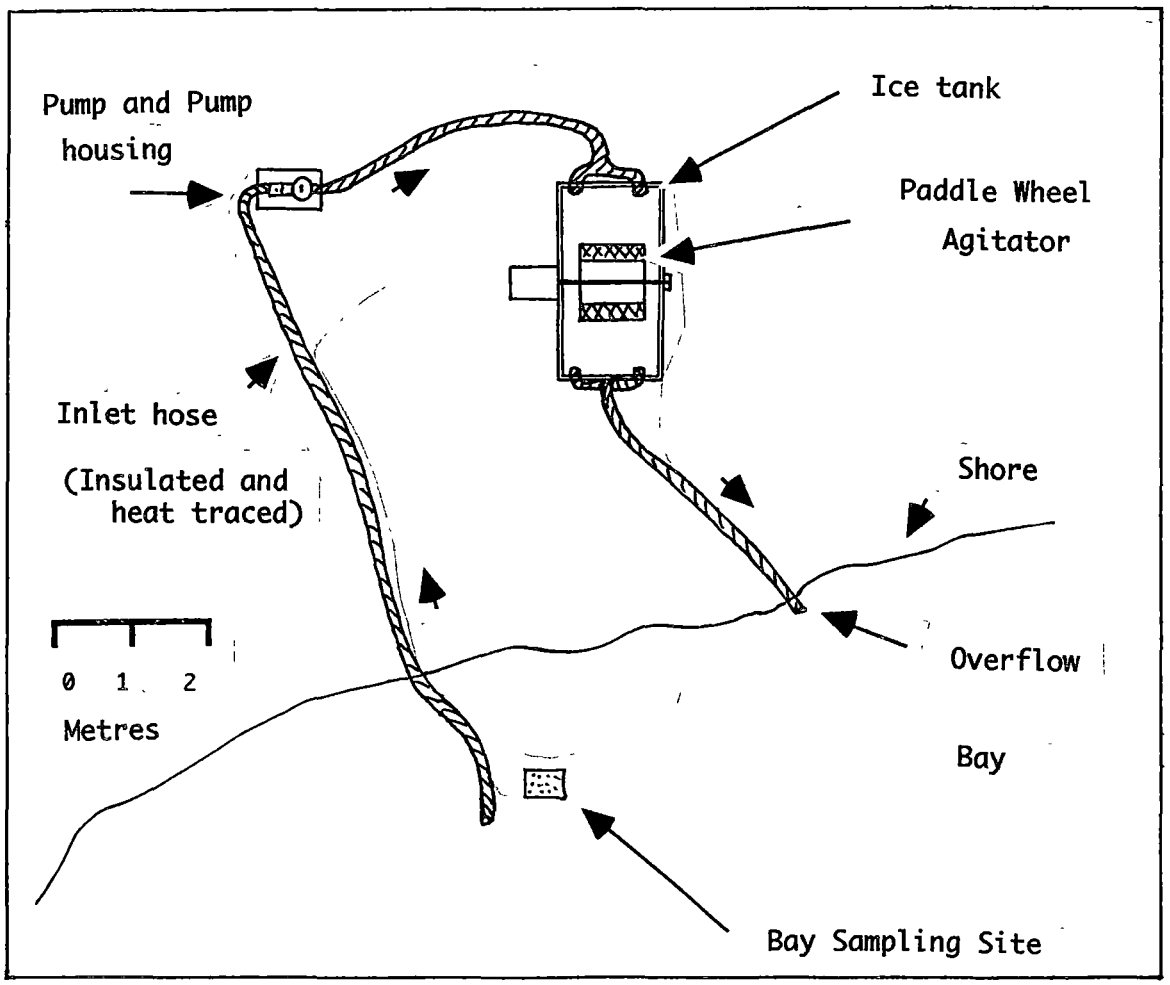


Figure 4.2 Schematic of ice mechanics tank at Casey. Note intake of water for the tank and location of bay sampling site. The tank was located approximately 0.5 m above the high water mark.

4.2.2 SAMPLING DESIGN...

Water, slurry and ice samples were concurrently sampled from the tank and the bay at 8 hourly intervals. At the time of sampling, air and water temperatures were recorded, as was a wind speed estimate. Ice conditions both in the tank were described. Ice was visually examined for crystal type (frazil or congelation), consolidation and thickness. Two litre samples were collected from the bay and the tank. Bay samples were collected from an area of Newcomb Bay which was undergoing new ice formation. Sometimes this area had to be excavated from thicker ice depending on the extent of ice in the bay at the time. Where possible bay ice and slurry were collected adjacent to the intake hose for the tank. A sieve was used to separated ice from slurry, allowing

Figure 4.3 Photograph of ice mechanics tank at Casey.

independent examination. Water samples were also collected. Part of the volume collected was used to examine species composition; the remaining volume was used for biomass analysis.

4.2.3 ANALYSIS of ICE TANK SAMPLES...

Microscopy

Only the diatom component of the phytoplankton was examined. At the time of year sampling was conducted and in such high latitude waters, diatoms comprise the majority of algal cells present. (Fryxell and Kendrick, 1988 and Xiuren *et al*, 1996). Samples for diatom analysis were prepared for microscopy by soaking in 30 % H_2O_2 for 3 days (Hasle and Fryxell, 1970). Samples were then washed and concentrated. Samples were mounted onto microscope slides using Naphrax. Analysis of ice and water samples involved microscopy using a Ziess Standard 20 microscope. A minimum of 400 cells was counted.

Biomass

Between 1 and 2 litres of sample were filtered through Whatman GFB glass fibre filters. Filters were stored in the dark at -30° C until they were analysed. Filters were extracted in 90 % acetone for 15 minutes. During this time, tubes containing the filters were sitting in an ultrasonic ice water bath. After 15 minutes in the bath, filters were left standing for 15 minutes. Four ml of each sample was transferred to a cuvette for spectrophotometric analysis using a GBC UV visible scanning spectrophotometer to determine chlorophyll a b and c values for the samples. The absorbence at 750, 664, 647 and 630 nm was determined. Each extinction was corrected for a small turbidity blank by subtracting the 750 nm from the 664, 647 and 630 nm absorptions. The amount of pigment in the original seawater sample was then determined by using the equations given below (Modified technique of Parsons *et al*, 1984):

(C*a*) Chlorophyll $a = 11.85\ E_{664} - 1.54\ E_{647} - 0.08\ E_{630}$

where E stands for the absorbence at different wavelengths obtained above (corrected by the 750 nm reading) and C*a* is the amount of chlorophyll (in μg/ml if a 1 - cm light path cuvette is used); then:

ug chlorophyll a/l $= C \times v / V \times 1000$

where v is the volume of acetone in ml, V is the volume of seawater in litres and C*a* is the chlorophyll which is substituted for C in the above equation.

Statistical Analysis

Student's t-Test analysis was conducted on recirculating experiments with agitation and recirculating experiments without agitation, using replicate data from diatom biomass measurements (chl a $\mu g\ l^{-1}$). Tank ice samples were compared to tank slurry and water; and bay ice, slurry and water samples, etc. P values for the Student's t-Test analysis were generated using Microsoft Analysis Tool Pack version 7.0. P values define the probability of observing an outcome as or more extreme than that actually arising from a particular experiment. A 0.05 confidence level was used to test significance. If a comparison of 2 samples generated a P value of ≤ 0.05 then the samples are considered to be significantly different, (i.e. samples were considered to have significantly different biomass values).

Cluster analysis was conducted on species data from the ice tank samples. Analysis was conducted on samples from individual tank experiments and on a combined data set using all the experiments. Cluster analysis was conducted on Biostat using a Bray-Curtis similarity matrix (Bray and Curtis, 1957). This type of analysis groups samples on a basis of similarity. It is particularly effective because it compares samples on the basis of the presence of species rather than the absence.

Results of the cluster analysis are presented in the form of a dendrogram. Simply described, similar samples form limbs on the same branch of the dendrogram. Dissimilar samples form different branches. It is possible to assign a similarity scale to dendrograms. Pairing sequences for the various dendrograms are shown in Appendix 8.8. Pairing sequences indicate at what level of dissimilarity samples relate to one another. This information is expressed at a decimal of 1. The closer the comparison is to 1 the more dissimilar the samples compared. This percentage can be found in the 'at distance' column of Appendix 8.8.

4.3.1 LIST of TANK EXPERIMENTS...

The types of experiments conducted are listed in Table 4.1. Recirculating experiments (RC) used a constant water mass; no water was pumped into the tank. Flow-through experiments (FT) refer to experiments which had a constant flow of water through the tank. Water was pumped into the ice tank at a rate of 5-10 l min^{-1}. Two figures are expressed under the heading '# of samples'; the first represents the number of sets of bay and tank, ice, slurry and water samples collected. These sets of samples were taken at 8 hourly intervals. The second number (in parentheses) indicates the total number of samples taken for a particular experiment. Numbers in parentheses vary as a result of the absence of bay or tank slurry or ice.

Table 4.1 List of tank experiments conducted at Casey in 1995.

Run #	Run Type	# of Samples	Date
1	RC	3 sets (14)	03-03-95
2	RC	3 sets (14)	09-03-95
3	RC	3 sets (10)	13-03-95
4	RC	3 sets (12)	19-03-95
5	RC	3 sets (14)	21-03-95
6	RC	3 sets (14)	27-03-95
7	FT	6 sets (20)	28-03-95
8	RC	3 sets (12)	03-04-95
9	FT	4 sets (18)	05-04-95
10	FT	2 sets (8)	07-04-95
11	RC	3 sets (14)	14-04-95

4.3.2 PHYSICAL PARAMETERS...

Table 4.2 provides information on the physical factors influencing the ice tank experiments. If agitation in the tank was not provided by the paddle wheel, average wind speed is shown. The tank experiments during which the paddle wheel was operational are indicated by 'PW' in the agitation category. Average air temperature is shown. Ice thickness of the ice in the tank is shown in centimetres. Thicknesses in parentheses are divided into ice type: frazil and congelation crystals are represented by 'F' and 'C' respectively.

Table 4.2 List of physical parameters for tank experiments.

Run #	Run Type	Agitation (knots)	Temperature (°C)	Ice Thickness (cm)
1	RC	PW	- 13	6 (6 F)
2	RC	PW	- 13	4 (4 F)
3	RC	20	- 3	2 (2 F)
4	RC	5	- 8	No data
5	RC	0	- 8	4 (2 F, 2 C)
6	RC	PW	- 18	10 (10 F)
7	FT	PW	- 11	1 (1 F)
8	RC	PW	- 10	12 (12 F)
9	FT	No data	- 7	7 (2 F, 4 C)
10	FT	No data	- 17	14 (2 F, 12 C)
11	RC	5	- 20	8 (2 F, 6 C)

4.3.3 SPECIES DISTRIBUTION...

Species distribution is represented in percentage abundance graphs (Figures 4.4 to 4.14). Percentage abundance data is shown in Appendix 8.6.

Percentage Abundance Graphs...

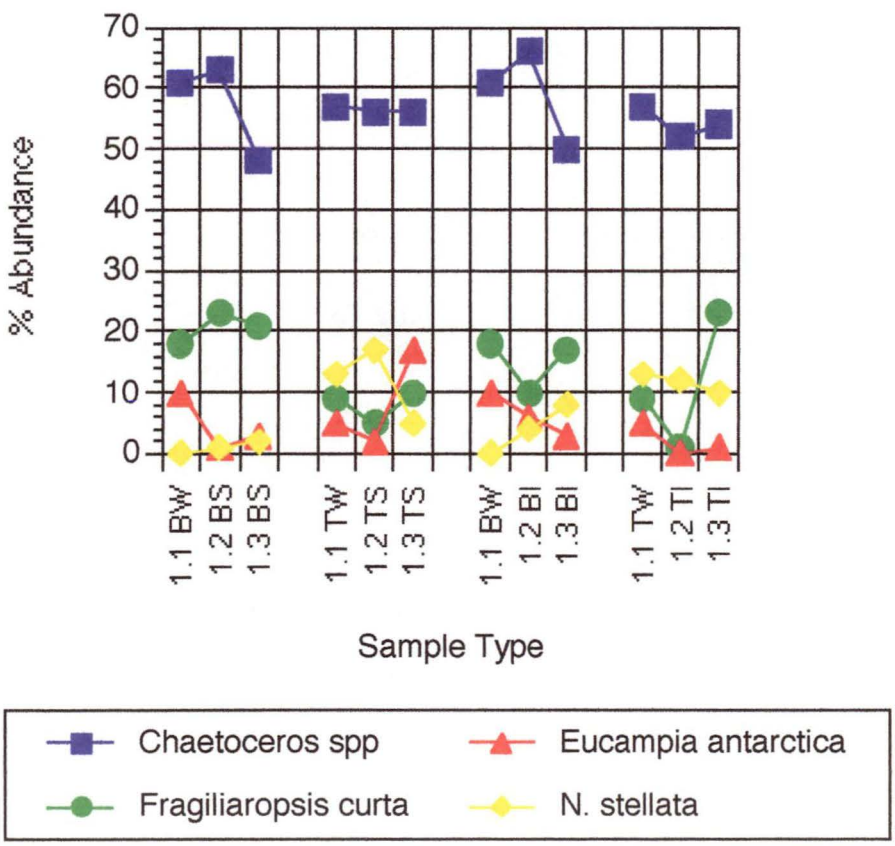
Chapters 2, 3 and 5 present detailed information regarding the incorporation of specific phytoplankton species into ice and water samples. Species data from the Casey experimental tank presents general trends in dominant species, replicate counts

were not conducted, therefor error bars are not shown and analysis of variance was not carried out.

Recirculating with Agitation

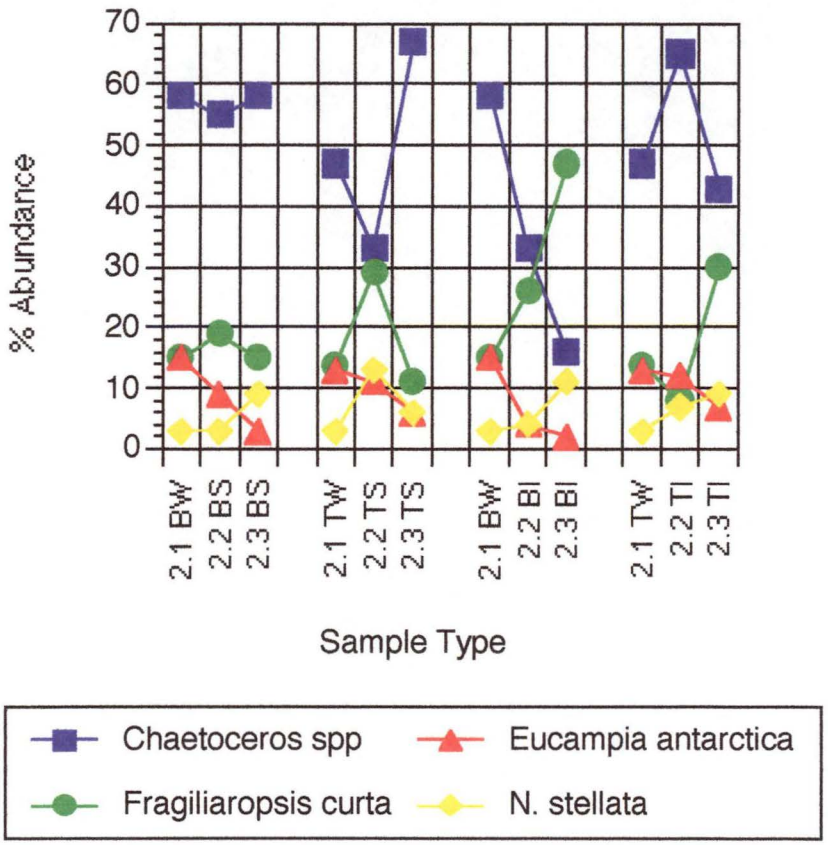
The percentage abundance of the four dominant species in each of the four recirculating experiments with agitation is shown in Figures 4.4 to 4.7. Species abundance data from the first experiment is shown in Figure 4.4. *Chaetoceros dichaeta* is uniformly the most dominant species in all samples. Bay slurry samples and bay ice samples present similar species distribution curves for the other three dominant species. This pattern is repeated for tank samples.

Figure 4.4 Percentage abundance of 4 dominant species in RC experiment with agitation (Expt #1), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



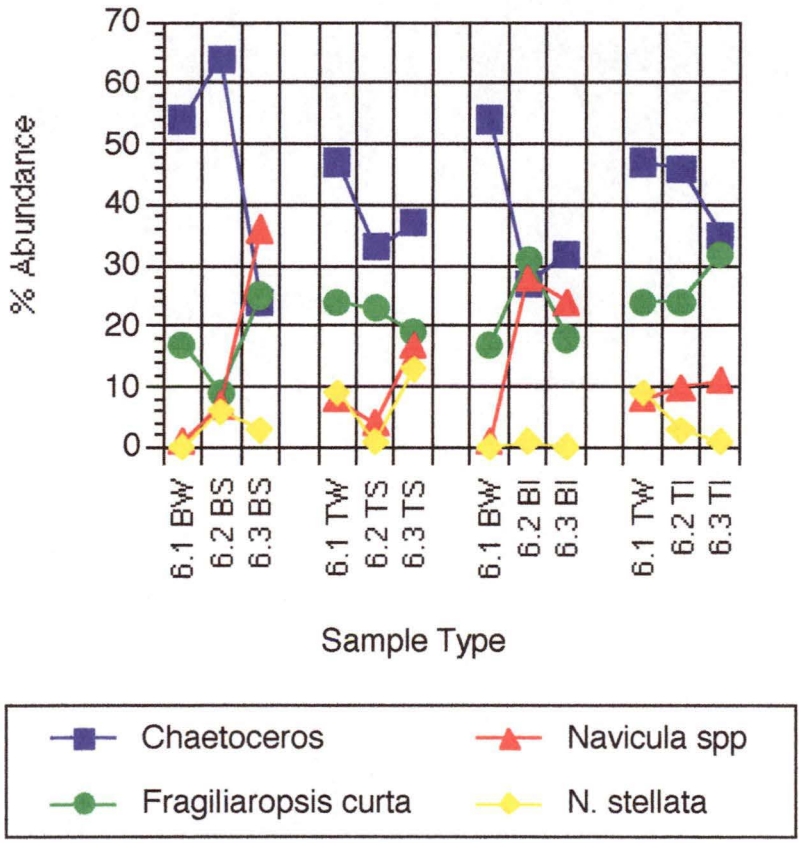
Species abundance data from the second recirculating experiment is presented in Figure 4.5. *C. dichaeta* is the most dominant species in all samples excluding the second bay ice sample (*Fragiliaropsis curta* dominates). Bay and tank profiles of *F. curta, Eucampia antarctica* and *Nitzschia stellata* show a similar shape for ice samples, with the percentage abundance of *F. curta* increasing in the later ice samples.

Figure 4.5 % abundance of 4 dominant species in RC experiment with agitation (Expt #2), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



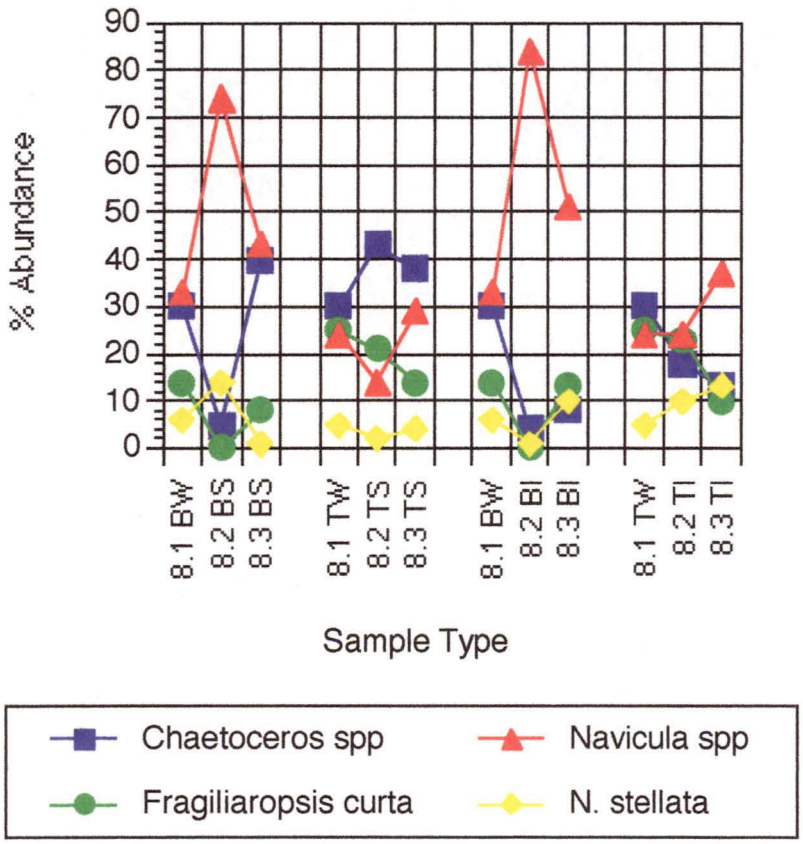
Species abundance data from the third recirculating experiment (Expt #6) are presented in Figure 4.6. *Navicula* species have replaced *E. antarctica* as one of the four dominant species in the samples. *Navicula* species are poorly represented in the water column from both the bay and tank. *Navicula* species share roughly equal distribution with *C. dichaeta* and *F. curta* in later bay and tank slurry samples and bay ice samples. *Navicula* species are not included to such an extent in tank ice samples.

Figure 4.6 % abundance of 4 dominant species in RC experiment with agitation (Expt #6), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



Species abundance data from the final recirculating tank experiment (Expt #8) is presented in Figure 4.7. Again *Navicula* species are included in the four most dominant species found in the samples. Unlike the previous experiment *Navicula* species are present at roughly the same abundances as *F. curta* and *C. dichaeta* in the initial water samples. *Navicula* species are well-represented in bay slurry and ice samples and tank ice samples.

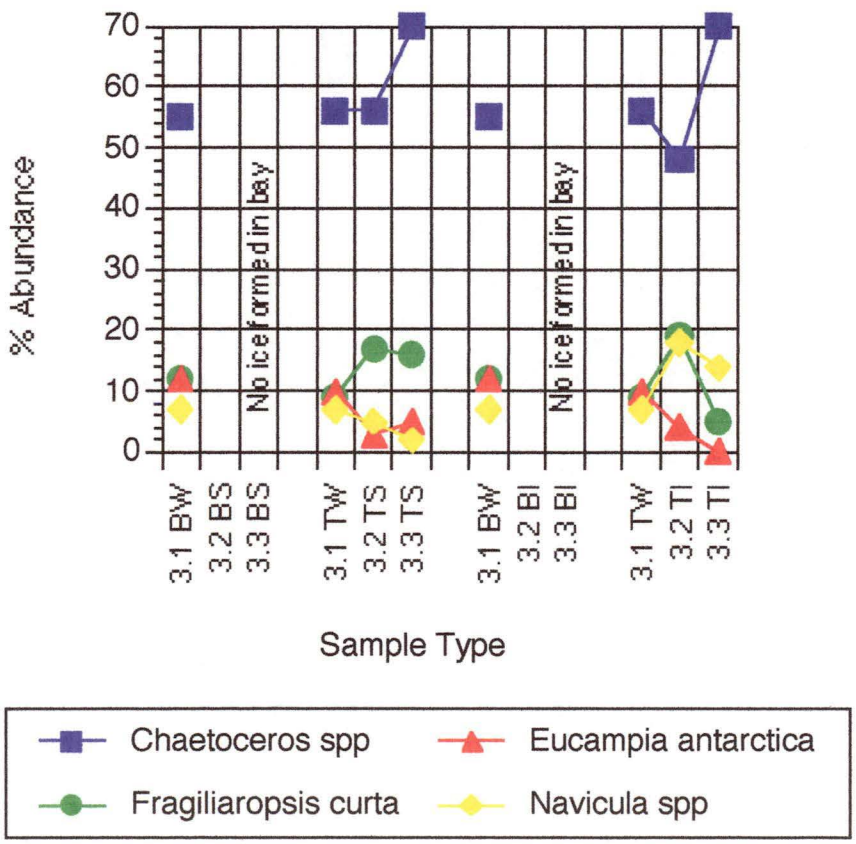
Figure 4.7 % abundance of 4 dominant species in RC experiment with agitation (Expt #8), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



Recirculating without Agitation

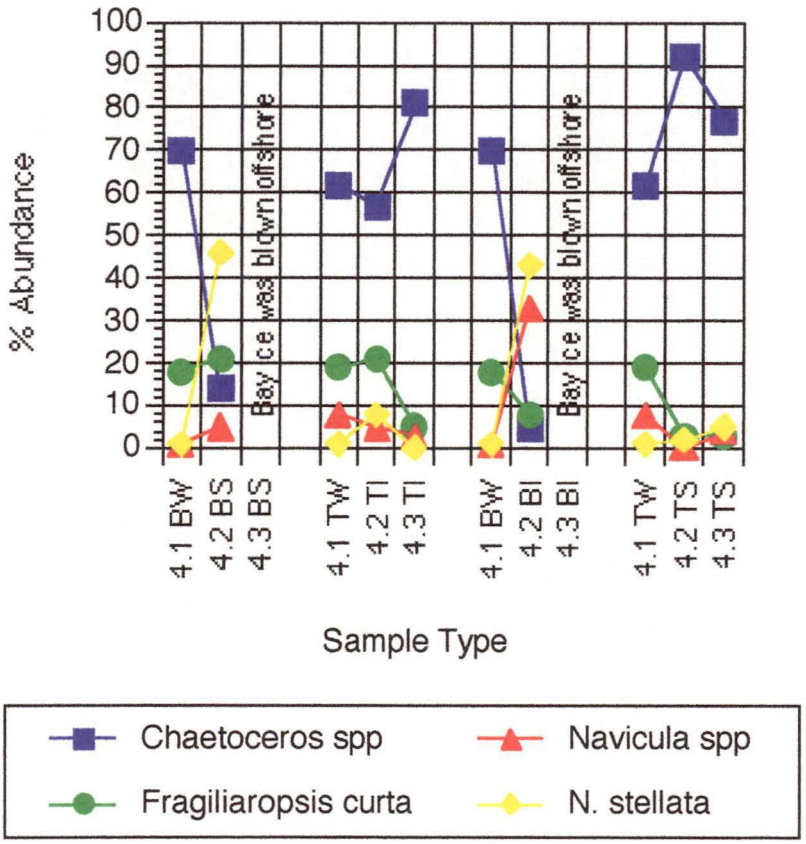
Results of recirculating tank experiments without agitation are presented in Figures 4.8 to 4.11. Results of the first experiment (Expt #3) are shown in Figure 4.8. High wind speeds (20 knots) resulted in newly-formed bay ice to be blown offshore keeping an ice free area adjacent to the shore. As a result no bay slurry or ice samples were collected. Bay water and tank water samples had very similar diatom distributions. *Chaetoceros dichaeta* was abundant in tank slurry and ice samples. Abundance of *F. curta* in the tank ice was initially increased over levels in the water sample, but decreased to below initial tank water abundances in the later ice sample.

Figure 4.8 % abundance of 4 dominant species in RC experiment without agitation (Expt #3), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours). No ice or slurry formed in the bay over the duration of the experiment.



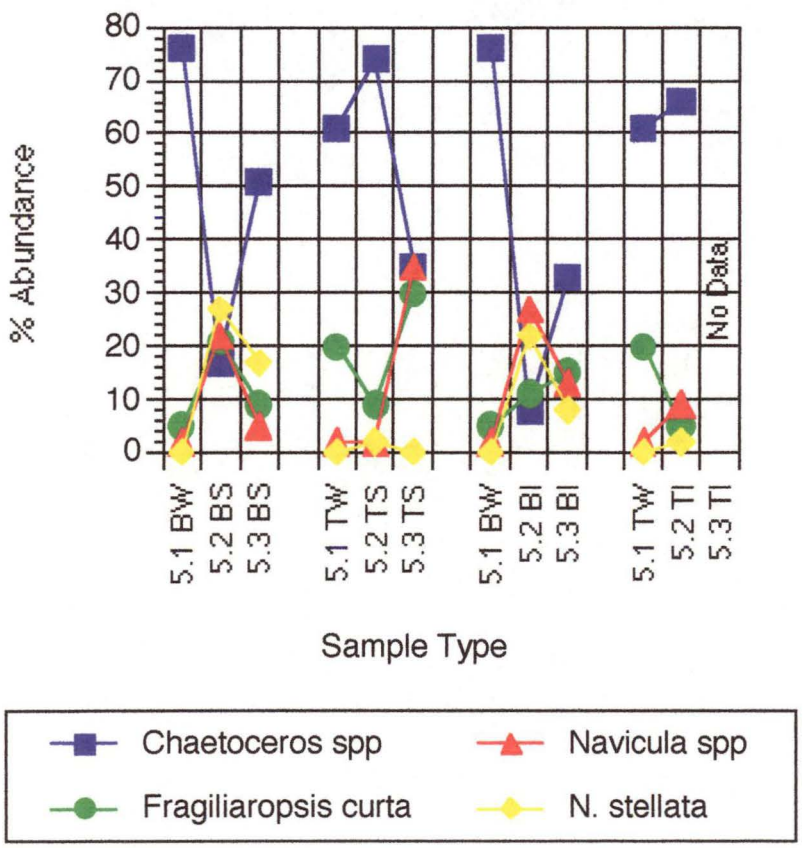
Results of the second recirculating without agitation experiment (Expt #4) are presented in in Figure 4.9. Initial bay and tank diatom populations were similar. *Chaetoceros dichaeta* was the most dominant species in tank slurry and ice samples. This was not reflected in bay samples where *Navicula* species and *Nitzschia stellata* dominated.

Figure 4.9 % abundance of 4 dominant species in RC experiment without agitation (Expt #4), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



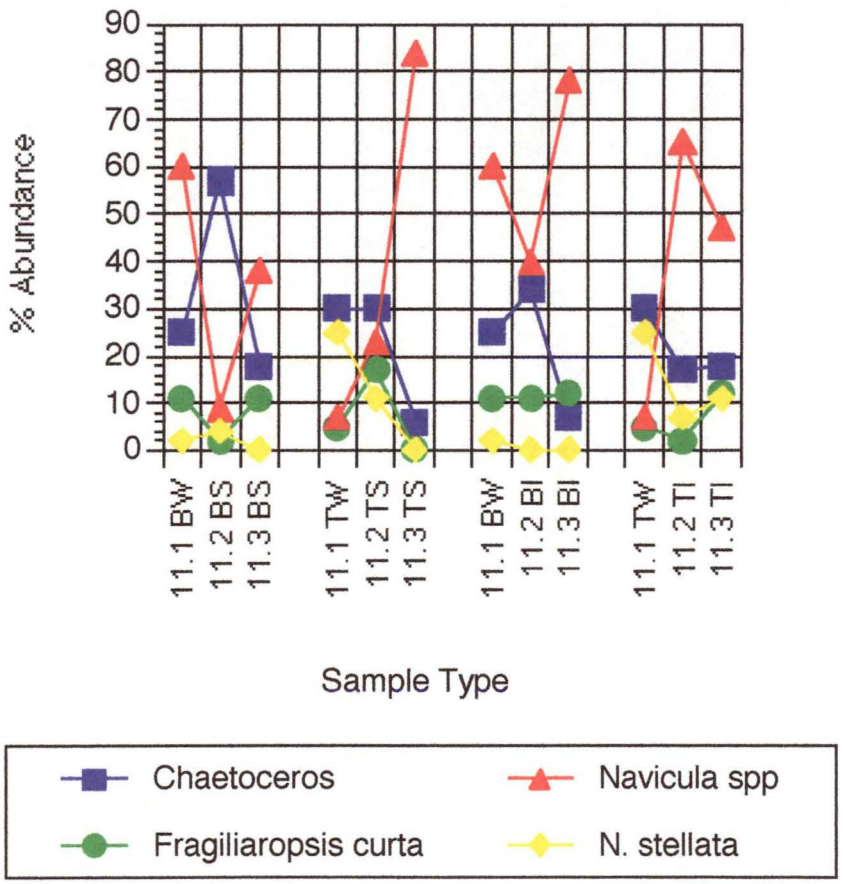
Results of the third recirculating experiment without agitation (Expt #5) are presented in Figure 4.10. *Chaetoceros dichaeta* dominates all samples with the exception of the first bay slurry and ice samples. In these samples *Navicula* species dominate despite their initial low abundance in the water column. *Navicula* species also contribute approximately 30 % of the abundance of diatoms in the second tank slurry sample, (again despite very low abundance in initial tank water). The second tank ice sample (4.3 TI) was not collected.

Figure 4.10 % abundance of 4 dominant species in RC experiment without agitation (Expt #5), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



Results of the fourth recirculating tank experiment without agitation (Expt #11) are presented in Figure 4.11. *Navicula* species dominate in the initial bay water sample and most of the bay and tank, slurry and ice samples. *Chaetoceros dichaeta* has an initial abundance of around 30 % in the bay and tank water; this abundance drops in subsequent slurry and ice samples.

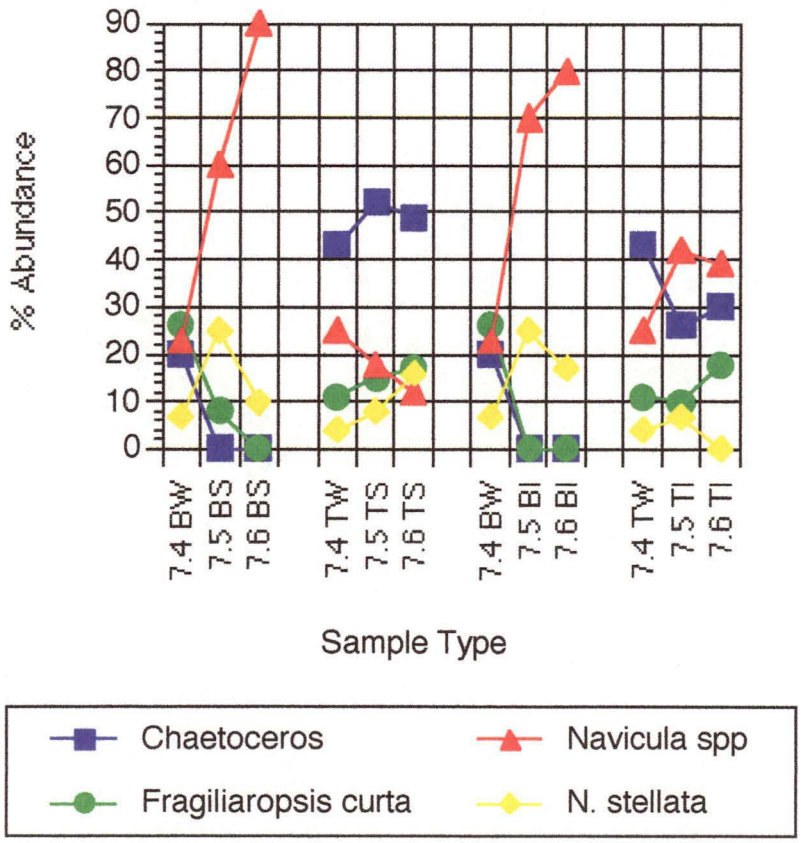
Figure 4.11 % abundance of 4 dominant species in RC experiment without agitation (Expt #11), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



Flow-through with Agitation

Results of flow-through experiment with agitation (Expt #7) are presented in Figure 4.12. Due to warm air temperature, ice formation in the bay and tank did not occur until the fifth sampling period. Hence water samples from the previous sampling period (7.4) are used for comparison. Initial water samples in the bay and tank contained different abundances for *Chaetoceros* species and *F. curta*, however *Navicula* species and *N. stellata* were of comparable levels. *Navicula* species clearly dominated bay samples, and dominated tank ice samples. *Chaetoceros dichaeta* dominated tank slurry samples.

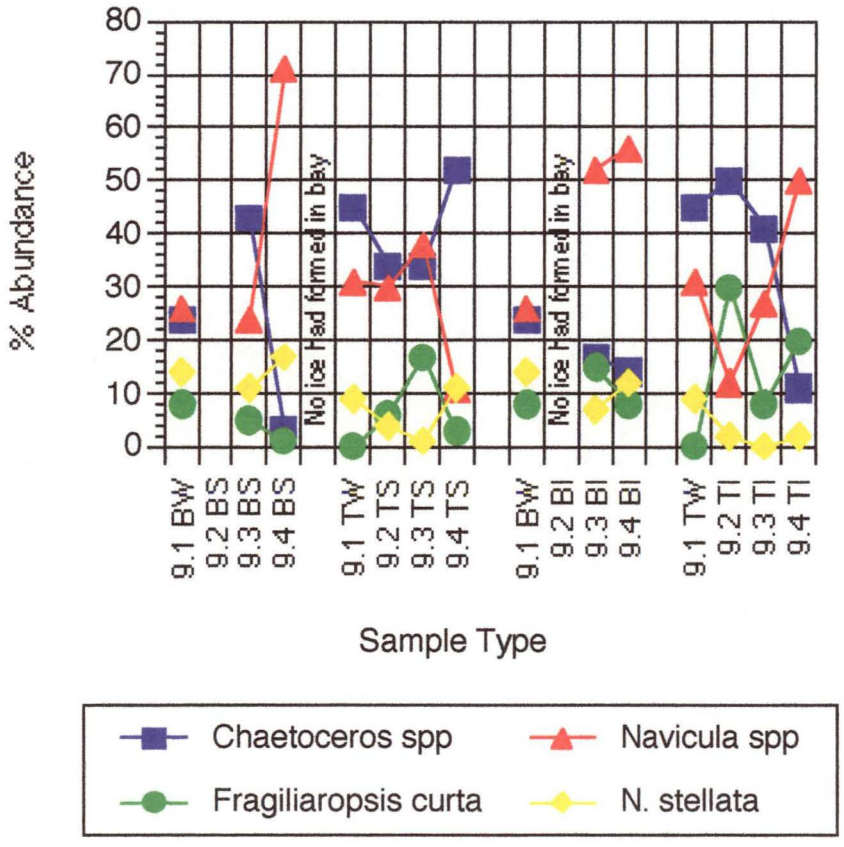
Figure 4.12 % abundance of 4 dominant species in FT experiment with agitation (Expt #7), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.4 = experiment # x, sampling time 4 (Time 24 hours), x.5 = sampling time two (32 hours), x.6 = sample time three (40 hours). Extended sampling duration was necessary as sufficient ice had not formed in the tank prior to 32 hours after the commencement of the tank experiment.



Flow-through without Agitation

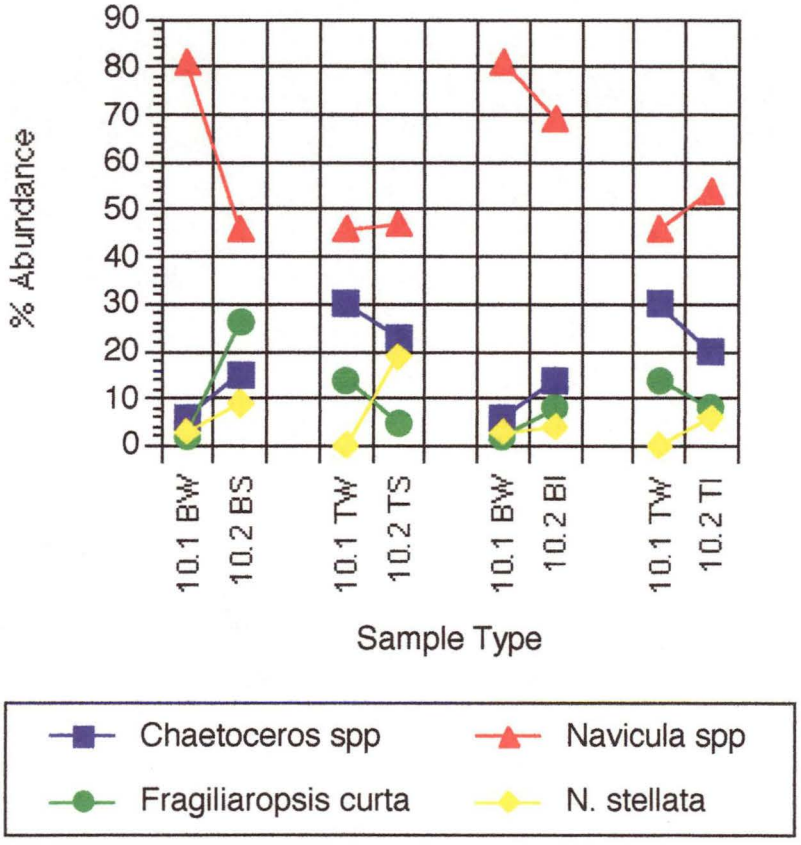
Results of flow-through experiments without agitation (Expt #9 and Expt #10) are shown in Figures 4.13 and 4.14. *Navicula* species clearly dominate bay slurry and bay ice samples (Figure 4.13). This result is not replicated in tank samples where *C. dichaeta* is the most dominant species in the majority of tank slurry and ice samples. These results are despite percentage abundance levels of the two species being similar in the initial tank and water samples (9.1 BW and 9.1 TW).

Figure 4.13 % abundance of 4 dominant species in FT experiment without agitation (Expt #9), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours), x.4 = sample time four (24 hours).



Results of the second flow-through experiment (Expt #10) without agitation are shown in Figure 4.14. *Navicula* species have a high percentage abundance in initial bay and tank water samples, and dominate slurry and ice samples. *Chaetoceros* species profiles in the tank are similar to the initial part of the corresponding profiles in Figure 4.13.

Figure 4.14 % abundance of 4 dominant species in FT experiment without agitation (Expt #10), BW = bay water , BS = bay slurry sample, BI = bay ice, TW = tank water, TS = tank slurry and TI = tank ice, x.1 = experiment # x, sampling time 1 (Time zero), x.2 = sampling time two (8 hours), x.3 = sample time three (16 hours).



4.3.4 BIOMASS MEASUREMENTS...

Biomass Summary

A summary of mean biomass measurements for experiments is presented in Table 4.3, standard deviations are shown. Note biomass values for tank slurry and tank ice samples from recirculating with agitation and flow-through with agitation experiments. Values are higher than initial water biomass measurements. This is not the case in the experiments without agitation. Analysis of bay slurry biomass values reveals higher biomass values than the underlying water samples. Bay ice samples have a lower chlorophyll a content than the bay water samples. Of the four types of experiments trailed, those with agitation, more closely represent the processes occurring in the bay. Biomass data is presented in Appendix 8.7. The results of Student's t-Test analysis on biomass data are presented later in this section and in Appendix 8.9.

Table 4.3 Summary of mean biomass of each sample type. n = 6 for recirculating with agitation (RC Ag) and for recirculating without agitation (RC x Ag), n = 1 for flow-through with agitation (FT Ag) and n = 2 for flow-through without agitation (FT x Ag). Values shown are chl a ugl^{-1} in bay and tank water (BW, TW), bay and tank slurry (BS, TS) and bay and tank ice (BI, TI). Biomass means shown in red have decreased compared to the initial value in the bay water, biomass means shown in blue have increased compared to the initial biomass in the water column.

Experiment Type	Mean/SD	BW	BS	BI	TW	TS	TI
RC Ag	Mean	1.13	0.86	0.77	1.23	1.04	1.69
	SD	0.29	0.39	0.32	0.46	0.57	1.94
RC no Ag	Mean	2.75	1.94	9.20	2.80	2.51	2.30
	SD	2.63	1.77	7.51	1.60	0.70	0.86
FT Ag	Mean	4.34	4.51	3.60	0.70	1.33	0.73
FT no Ag	Mean	1.72	0.99	1.09	1.06	0.39	0.33

The following diagrams (4.15 - 4.25) represent the percentage change in chl a biomass over the initial biomass values for the water column. The initial chl a content in the bay and tank water at time zero was expressed as 100 %. Subsequent values were expressed as a percentage increase or decrease relative to these values. The same system of labelling plots is used in the previous section (4.3.3). The biomass values of the tank and bay water at the initial time were both standardised to 100 %, despite

not being identical in value. Standardisation was carried out to make graphical comparisons easier.

Recirculating with Agitation

There was a high degree of variability amongst the recirculating with agitation experiments; however, trends are evident. Biomass maxima in both the bay and tank samples reached up to 7 times the initial biomass in the water. The biomass of the majority of slurry and ice samples from the tank exceeded the initial value for the tank water. This was not the case for bay samples. Slurry samples from the tank contained maximum biomass for two experiments; ice samples contained the maxima for the other two. Maximum biomass for the bay was found in the initial water sample (in two cases), the slurry (1 case) and an ice sample (1 case). Similarities in the biomass percentage change for bay and tank slurry samples are evident in Figure 4.15 and 4.16.

Student's t-Test Analysis...

Student's t-Test, two sample analysis, assuming unequal variances, was conducted on recirculating with agitation experiments. Comparisons were based on biomass values (chl a ug l^{-1}) and are shown in Table 4.4. None of the samples from the recirculating experiment with agitation were considered significantly different to another on the basis of chlorophyll a content. This suggests that enrichment mechanisms in the bay and tank were not significantly different. Ice and slurry samples from the tank were therefor being enriched to a similar level to the slurry and ice samples in the bay.

Table 4.4 Results of Student's t-Test analysis for biomass values from recirculating experiments with agitation. A confident level of $P \leq 0.05$ is considered significant. For simplicity, the following abbreviations will be used in this section: Bay water (BW), bay slurry (BS), bay ice (BI), tank water (TW), tank slurry (TS) and tank ice (TI).

	BS	BI	TW	TS	TI
BW	0.832	0.669	0.426		
BS		0.855		0.773	
BI					0.263
TW				0.691	0.194
TI				0.245	

Figure 4.15 Recirculating tank experiment with agitation (Expt 1).

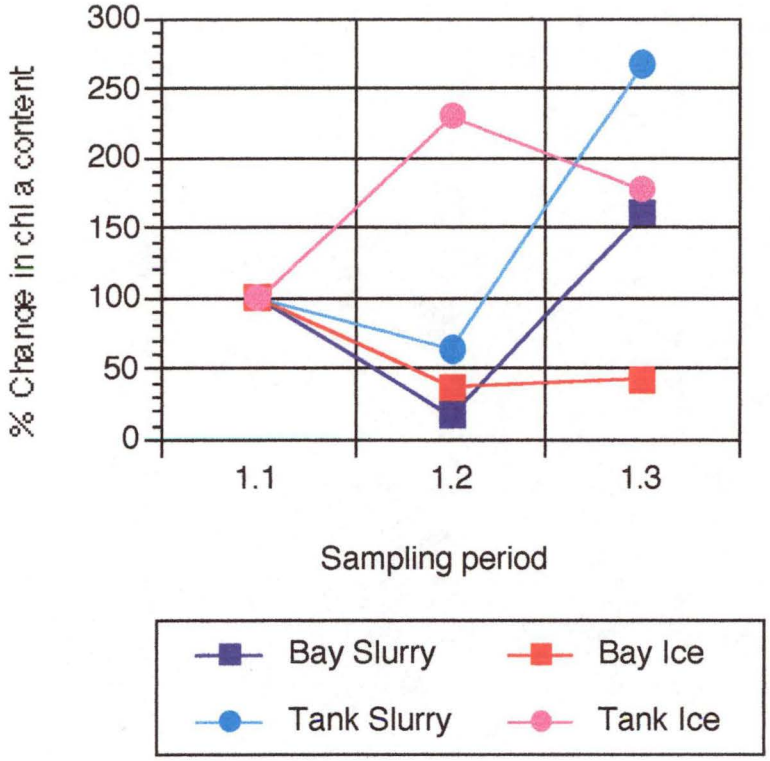


Figure 4.16 Recirculating tank experiment with agitation (Expt 2)

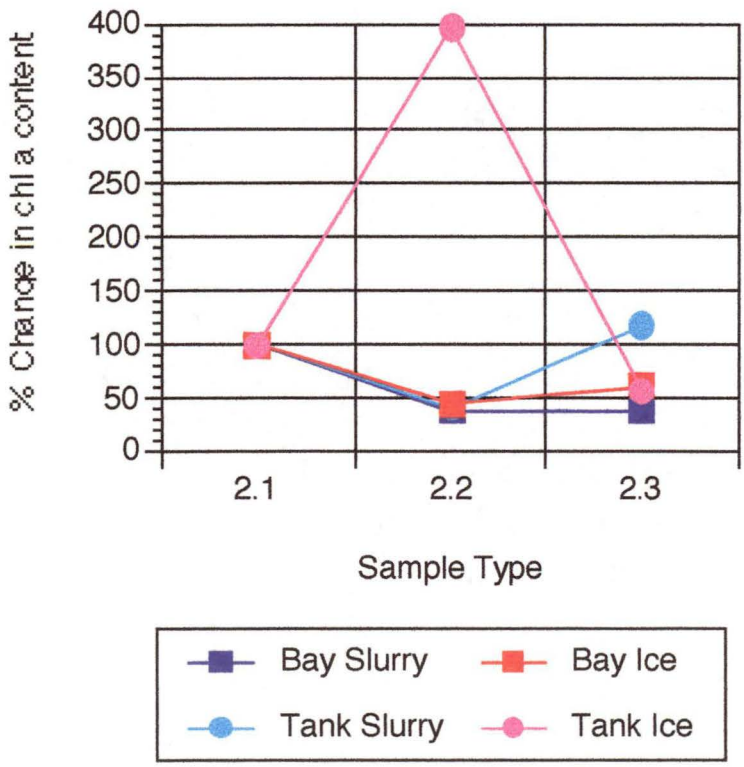


Figure 4.17 Recirculating tank experiment with agitation (Expt 6).

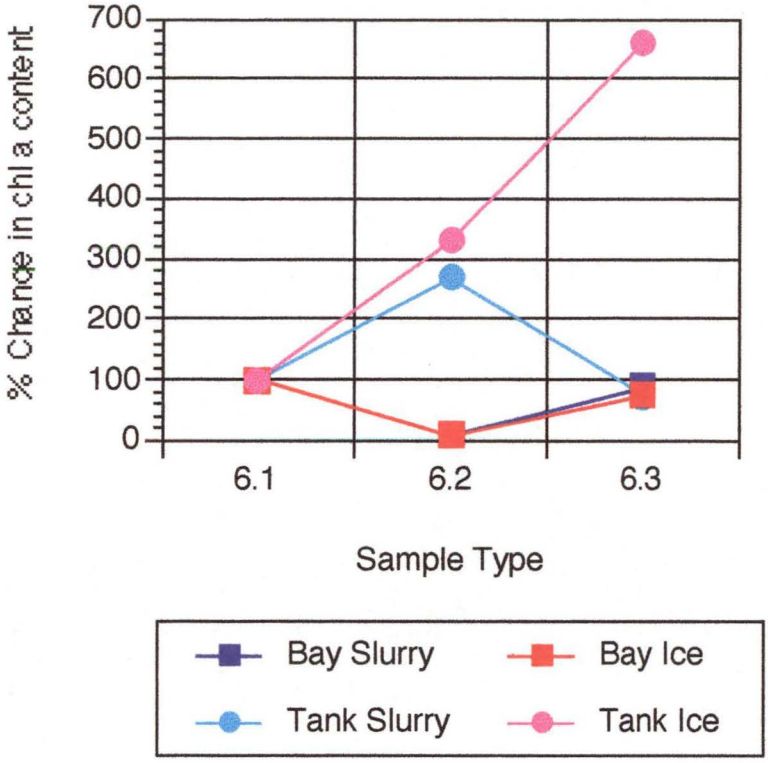
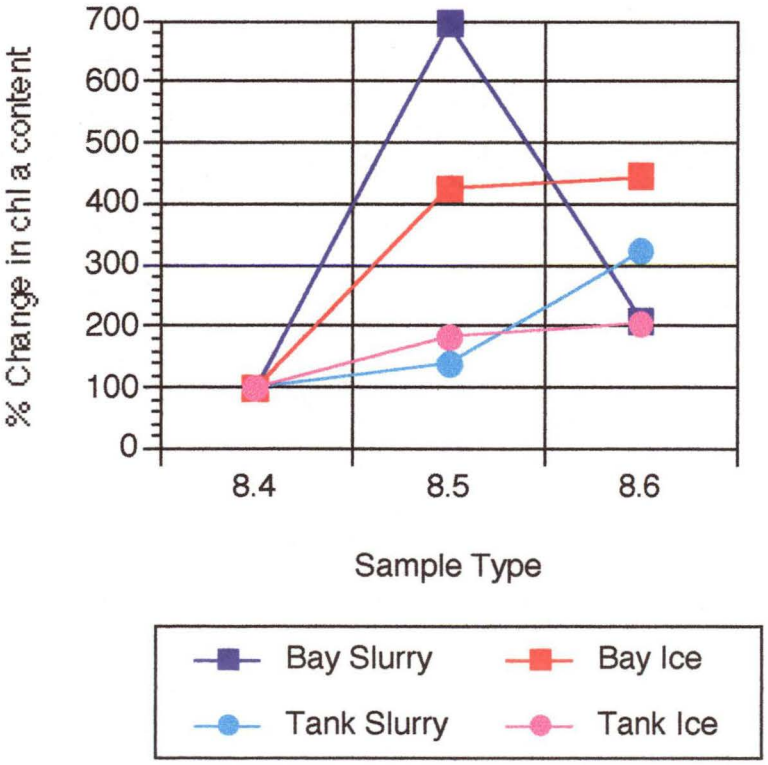


Figure 4.18 Recirculating tank experiment with agitation (Expt 8).



Recirculating without Agitation

Three of the recirculating without agitation plots show biomass in tank slurry and ice samples at reduced levels compared to the initial biomass in the water in the tank. Six to seven times the biomass is present in slurry and ice samples compared to the initial water sample in Experiment #11(Figure 4.22). Biomass maxima in Experiment # 3 (Figure 4.19) are found in the initial water sample and the slurry sample collected at 16 hours. Experiment # 11 (Figure 4.22) indicates that the maximum biomass in the tank is found in the second ice sample. The ice sample from the third sampling time (11.3 I) comparatively has a greatly reduced biomass. Biomass in bay ice samples is up to 350 times the initial value (5.1 W) in Experiment # 5 (Figure 4.21). This may be a result of accidental sampling of older bay ice instead of newer sea ice of a similar age to the tank ice. Ice did not form in the bay during Experiment # 3.

Results of Student's t-Test analysis of recirculating experiment without agitation are presented in Table 4.5. No samples were considered significantly different to one another on the basis of chlorophyll a content. However, tank ice samples produced a P value of 0.099 when compared to bay ice samples, this indicates a trend towards the bay ice and tank ice samples having a significantly different biomass. This compares with the tank ice-bay ice comparison for the recirculating experiment with agitation which generated a P value of 0.263, (Table 4.4).

Table 4.5 Student's t-Test analysis of recirculating experiments without agitation. A P value ≤ 0.05 is considered significant.

	BS	BI	TW	TS	TI
BW	0.646	0.175	0.713		
BS		0.133		0.492	
BI					0.099
TW				0.389	0.349
TI				0.805	

Figure 4.19 Recirculating tank experiment without agitation (Expt 3).

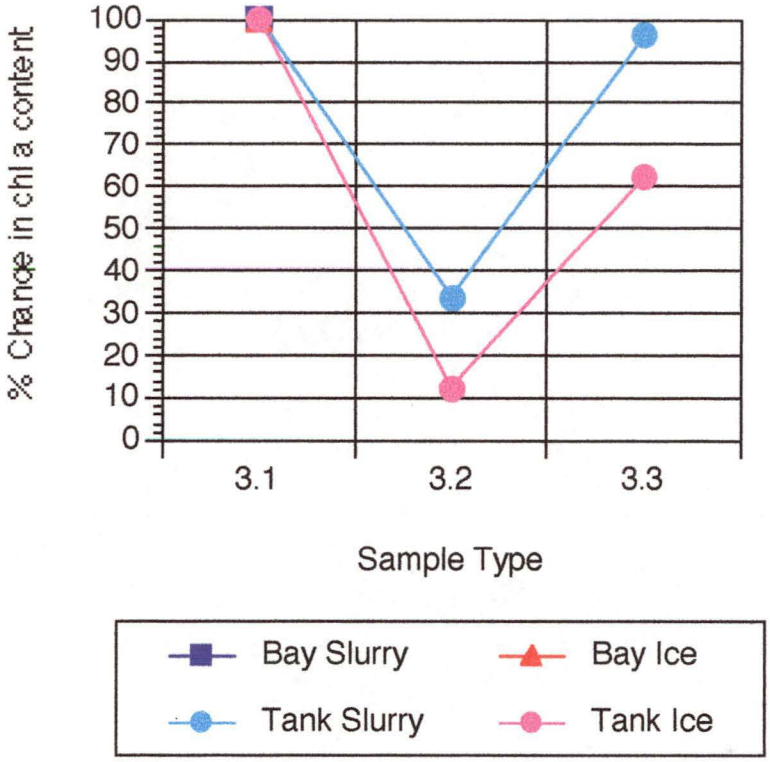


Figure 4.20 Recirculating tank experiment without agitation (Expt 4).

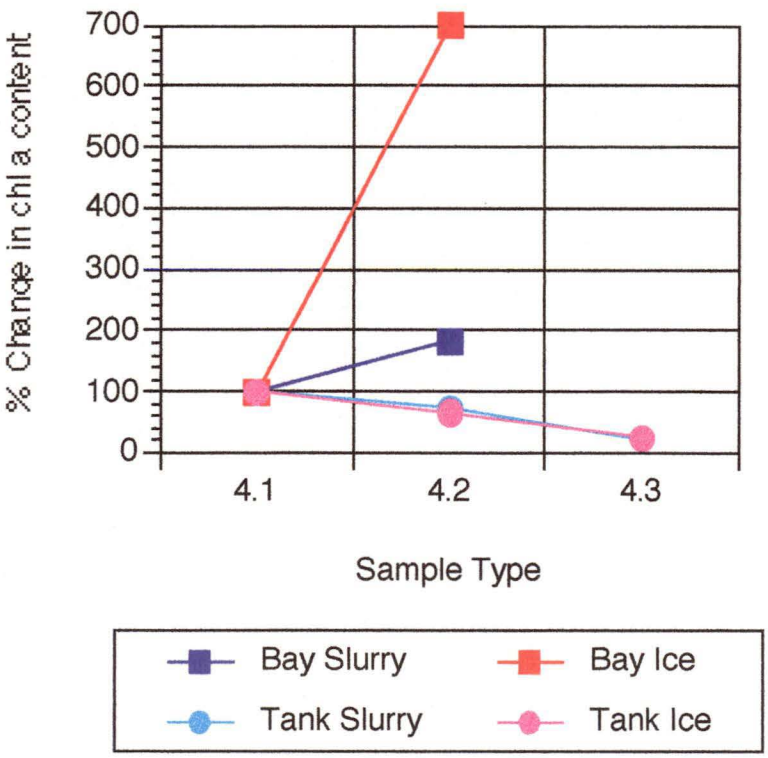


Figure 4.21 Recirculating tank experiment without agitation (Expt 5).

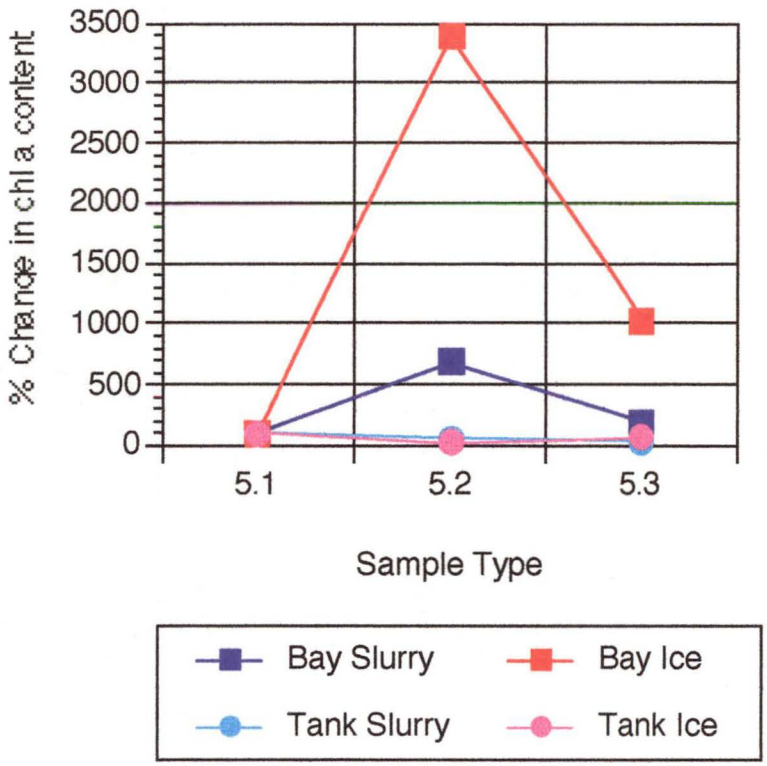
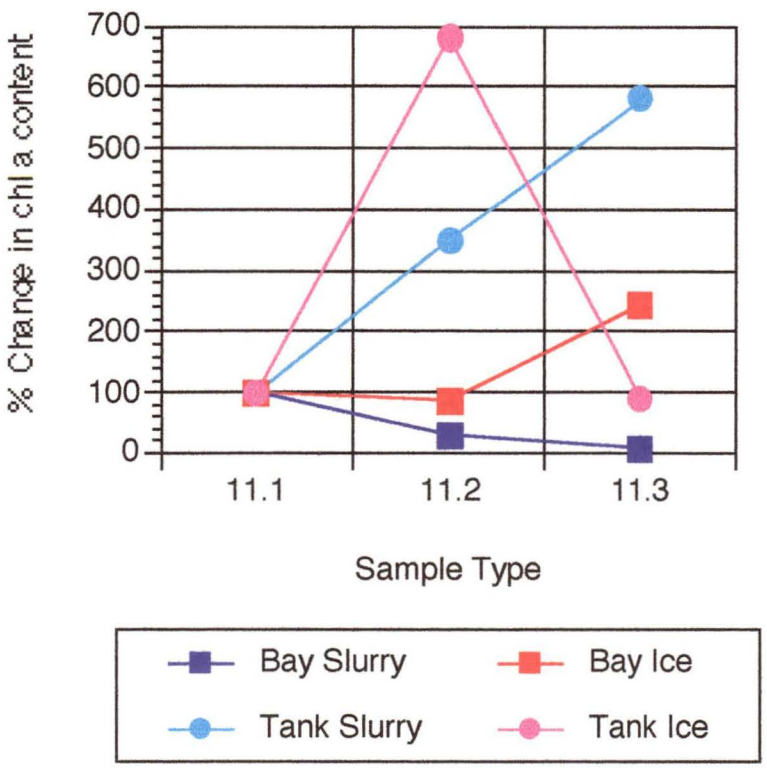


Figure 4.22 Recirculating tank experiment without agitation (Expt 11).



Flow-through with Agitation

The biomass in the tank samples from Experiment # 7 was at a maximum in the first tank slurry sample collected (Figure 4.23). This was 4 x higher than the biomass initially present in the water. Subsequent ice and slurry samples were comparable to, or slightly higher than the initial water sample. The biomass maximum from the bay samples was also found in the first slurry samples collected. Biomass from sample 7.5 I was also high (6 times the initial bay water biomass). Subsequent slurry and ice biomass samples (as with the tank) were very much reduced. There was only one flow-through experiment with agitation, as a result Student's t-Test analysis was not conducted.

Figure 4.23 Flow-through tank experiment with agitation (Expt 7). Note labelling on the x-axis of Figure 4.23, extends from the fourth eight hour period to the sixth. This is a result of high air temperatures which prevented ice formation until 24 hours after commencement of the experiment.

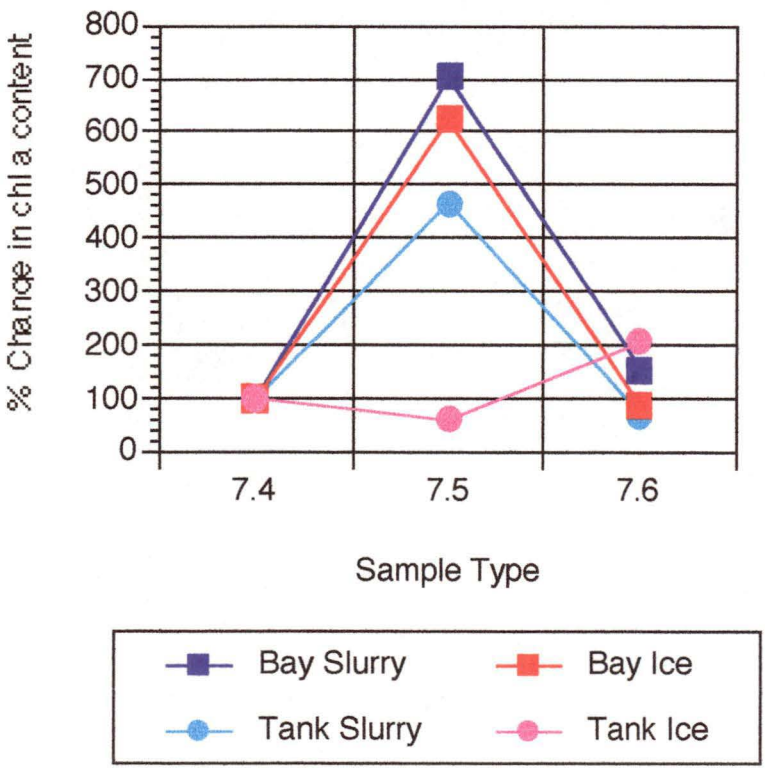


Figure 4.24 Flow-through tank experiment with agitation (Expt 9).

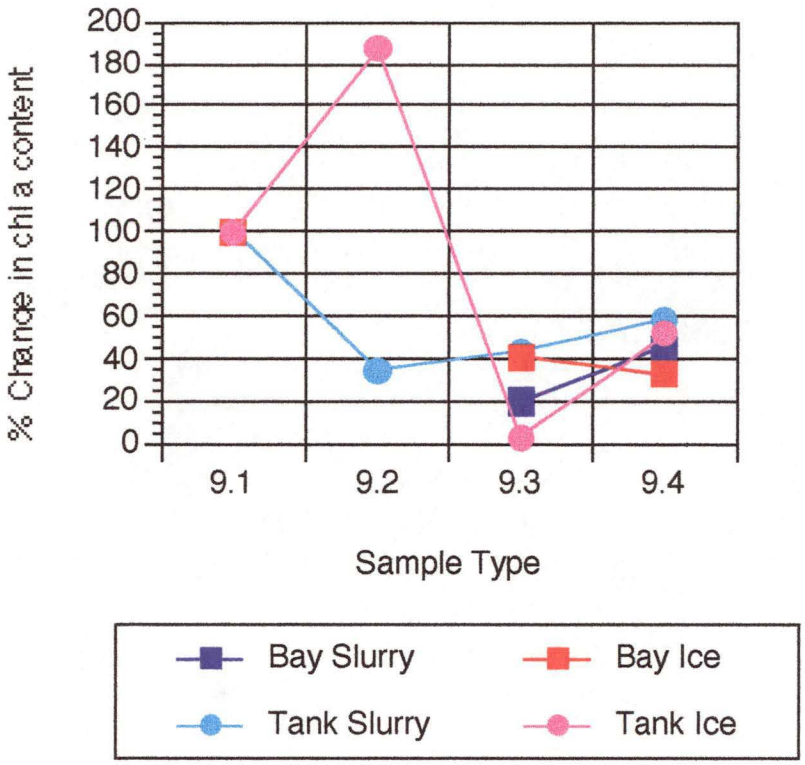
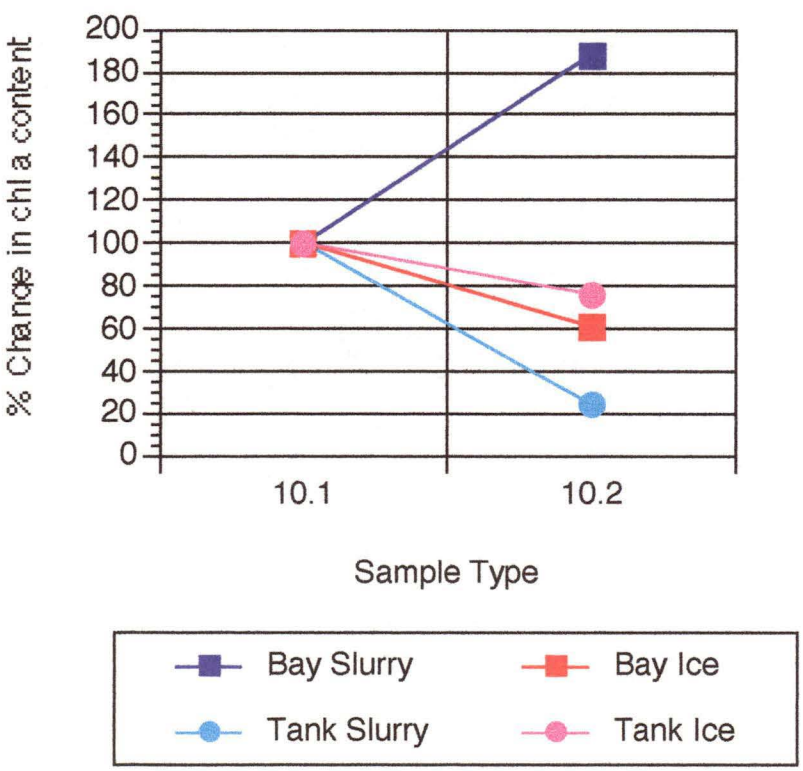


Figure 4.25 Flow-through tank experiment with agitation (Expt 11).



Flow-through without Agitation

In Experiment # 9 (Figure 4.24) biomass in slurry and ice samples from the bay is roughly half the initial level in the water column. Biomass in the slurry sample from Experiment # 11 is twice the original water value. Similarly the maximum biomass is found in the first ice sample in Experiment # 9 (Figure 4.24), (at twice the level of the initial water). However, the biomass in the ice and slurry samples from the tank for Experiment # 11 is roughly half of the biomass from the original water sample.

4.3.5 CLUSTER ANALYSIS...

Data presented in this section complements species data presented in Section 4.3.3. Cluster analysis was used to determine the similarity of samples. The analysis compared individual species found in a sample to the species composition from other samples. These comparisons were conducted for each of the tank experiments (Figures 4.26 to 4.37) and for the entire data set (Figure 4.37). A dendrogram was generated with accompanying similarity scales (as described in Section 4.2.3). Pairing sequences for the various dendrograms are shown in Appendix 8.8.

Cluster diagrams are analysed in terms of: sample type (BW and TW, BS and TS, BI and TI), obviously bay and tank comparisons, and water, slurry and ice comparisons can also be made. Sampling time (x.1 = T_0 hrs, x.2 = T_8 hrs, x.3 = T_{16} hrs etc) is also compared. To facilitate interpretation of the dendrograms the following theories were proposed:

Recirculating with agitation:

- Bay and Tank samples should be well-mixed throughout the dendrogram
- Water, slurry and ice samples may be clearly separated
- Samples should be separated on basis of the sampling time (T_0, T_8, T_{16} etc)
- BW = TW, BS = TS and BI = TI are more alike in this type of experiment

Recirculating without agitation:

- Bay and Tank samples should form separate groups
- Water, slurry and ice samples may be clearly separated
- Samples should be separated on basis of the sampling time (T_0, T_8, T_{16} etc)
- BW ≠ TW, BS ≠ TS and BI ≠ TI are more alike in this type of experiment

Flow through with agitation:

- Bay and Tank samples should be well mixed throughout the dendrogram
- Water, slurry and ice samples may be clearly separated
- Samples should be separated on basis of the sampling time (T_0, T_8, T_{16} etc)
- BW = TW, BS = TS and BI = TI are more alike in this type of experiment

Flow Through without agitation:

- Bay and Tank samples should form separate groups
- Water, slurry and ice samples may be clearly separated
- Samples should be separated on basis of the sampling time (T_0, T_8, T_{16} etc)
- BW ≠ TW, BS ≠ TS and BI ≠ TI are more alike in this type of experiment

Recirculating with Agitation

The following four figures represent results of Cluster analysis of Recirculating tank experiment with agitation (Experiments # 1, 2, 6 and 8).

Parameters compared are:

1. B = Bay, T = Tank,
2. W = Water, S = Slurry, I = Ice,
3. T_0 = Beginning of Exp, T_8 = Second sampling, T_{16} = 3 rd etc,
4. Sample number (for reference to pairing sequence in Appendix 8.8)

Figure 4.26 Results of cluster analysis of Recirculating tank experiment with agitation (Expt # 1).

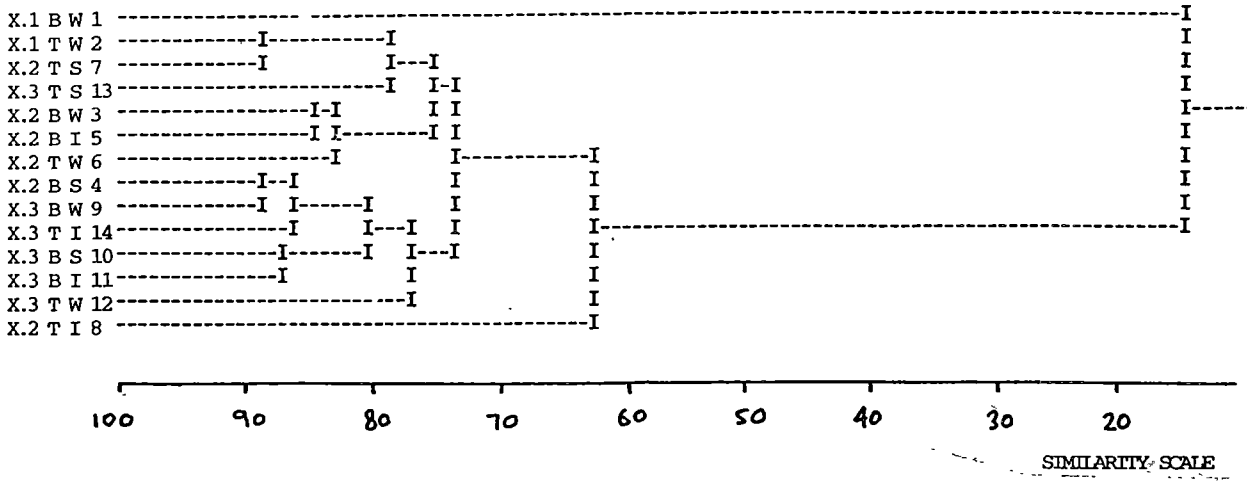


Figure 4.27 Results of cluster analysis of Recirculating tank experiment with agitation (Expt # 2).

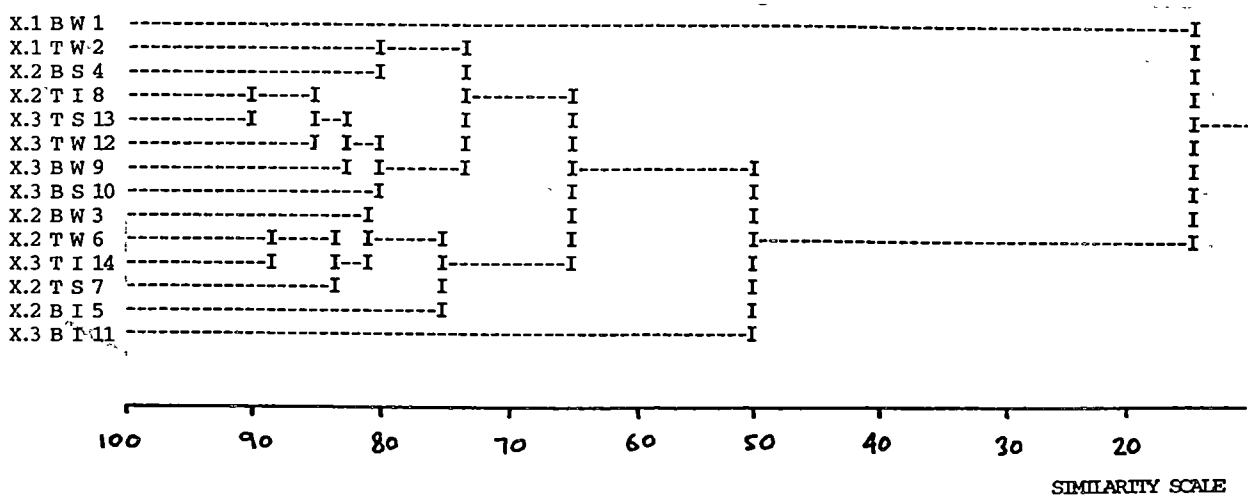


Figure 4.28 Results of cluster analysis of Recirculating tank experiment with agitation (Expt # 6).

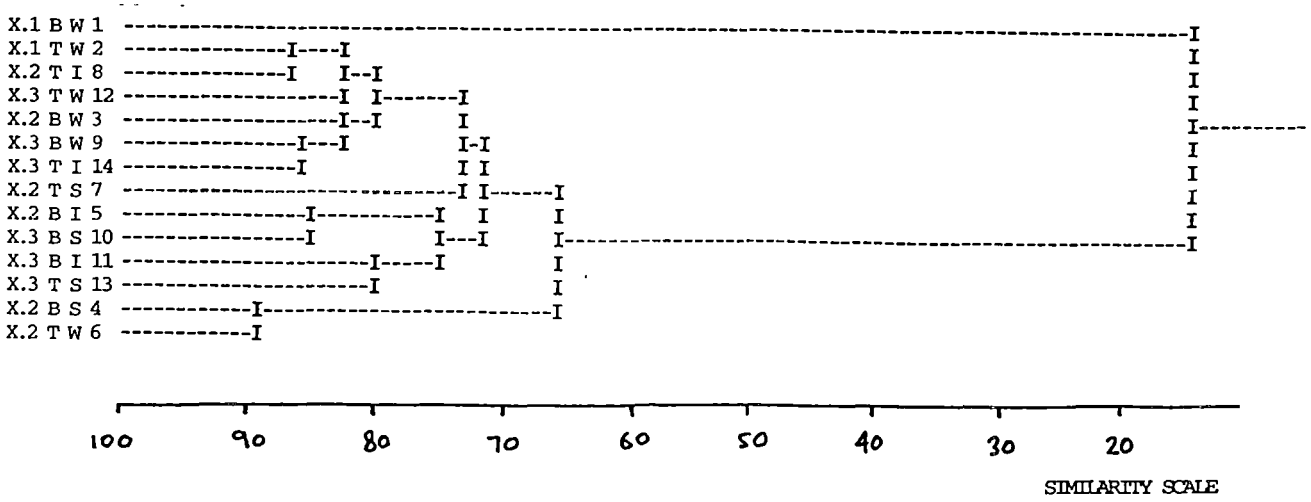
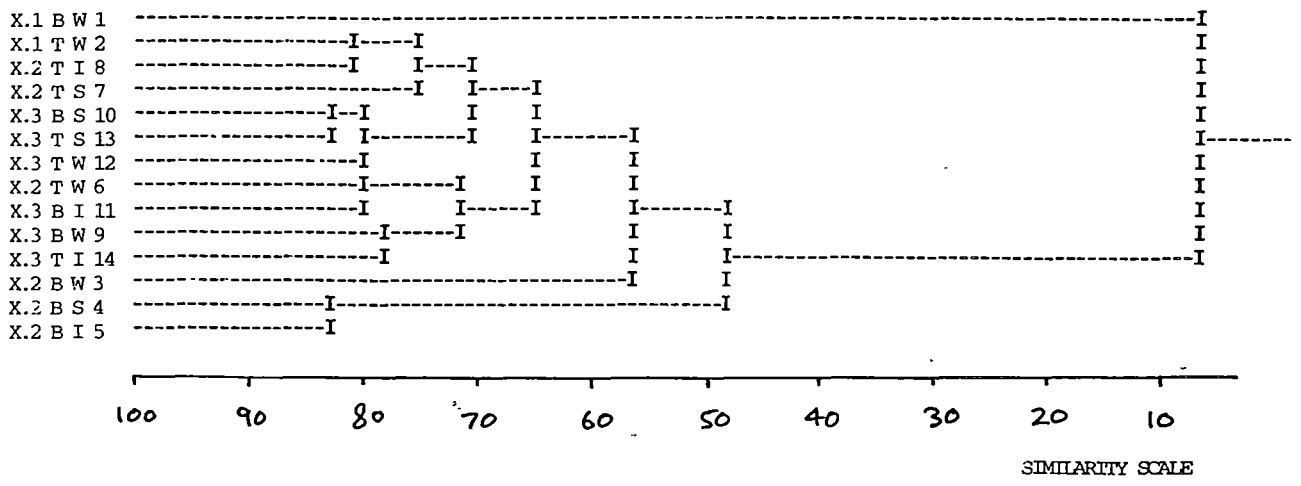


Figure 4.29 Results of cluster analysis of Recirculating tank experiment with agitation (Expt # 8).



No clear differentiation between bay and tank samples was evident. Water, slurry and ice samples were scattered throughout the dendrogram, with no evident separation. Separation on basis of sampling time was evident in Experiments # 1, # 2 and # 8. Grouping of BW and TW samples together was not evident nor was that of BS, TS and BI, TI. Thus Recirculation experiments with agitation only addressed half of the theories postulated.

Recirculating without Agitation

The following four figures represent results of cluster analysis of Recirculating tank experiment without agitation (Experiments # 3, # 4, # 5 and # 11). As with Recirculating experiments with agitation, four separate parameters are compared:

Figure 4.30 Results of Recirculating tank experiment without agitation (Expt # 3).

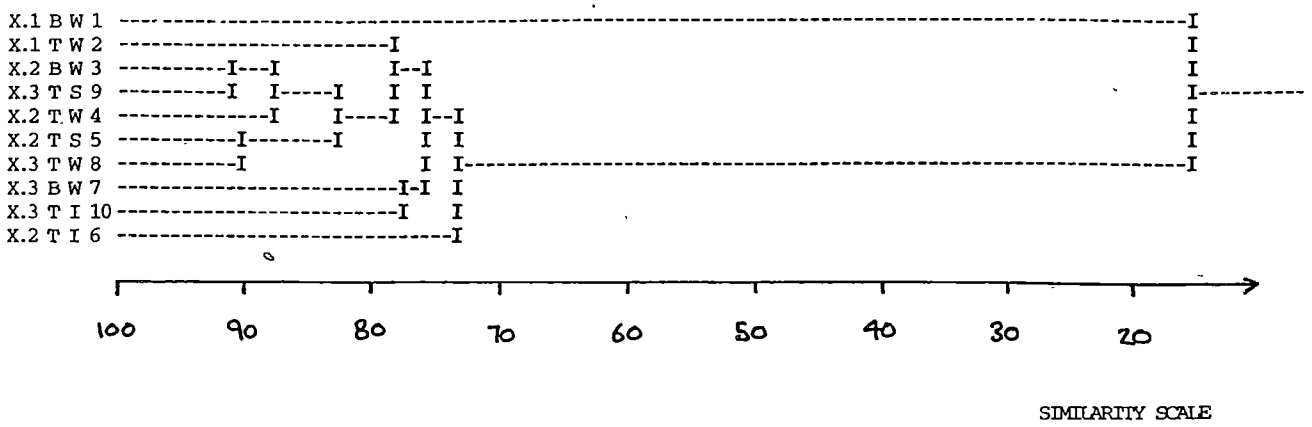


Figure 4.31 Results of Recirculating tank experiment without agitation (Expt # 4).

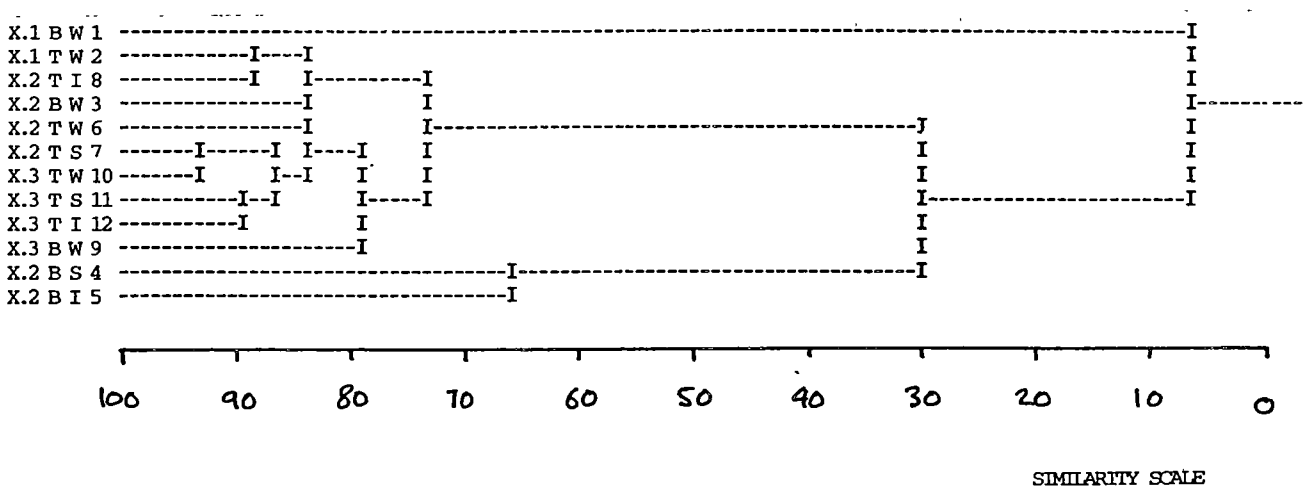


Figure 4.32 Results of Recirculating tank experiment without agitation (Expt # 5).

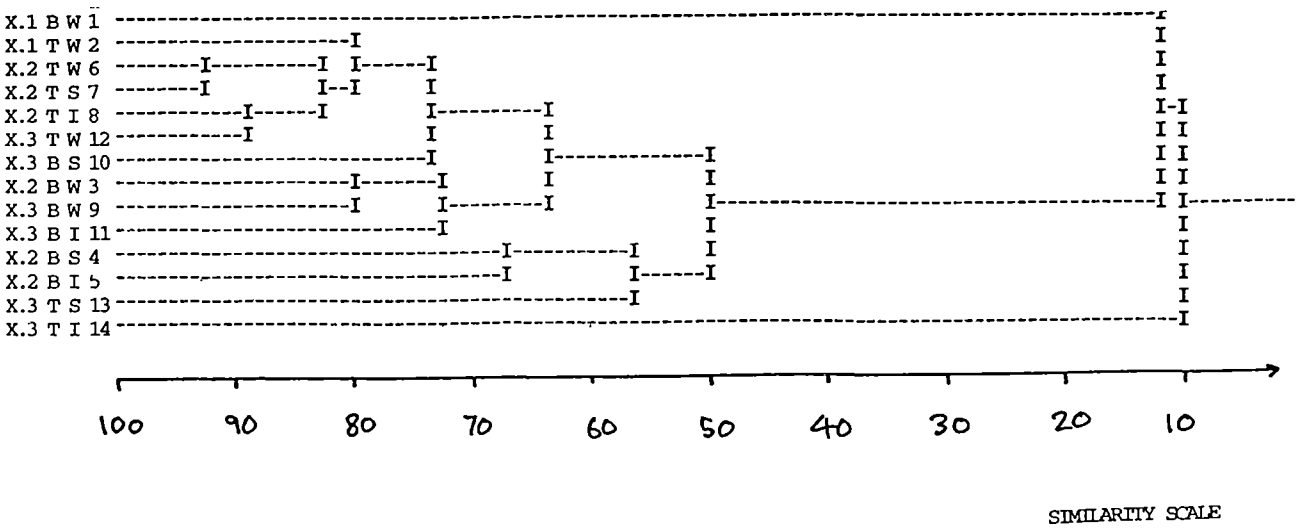
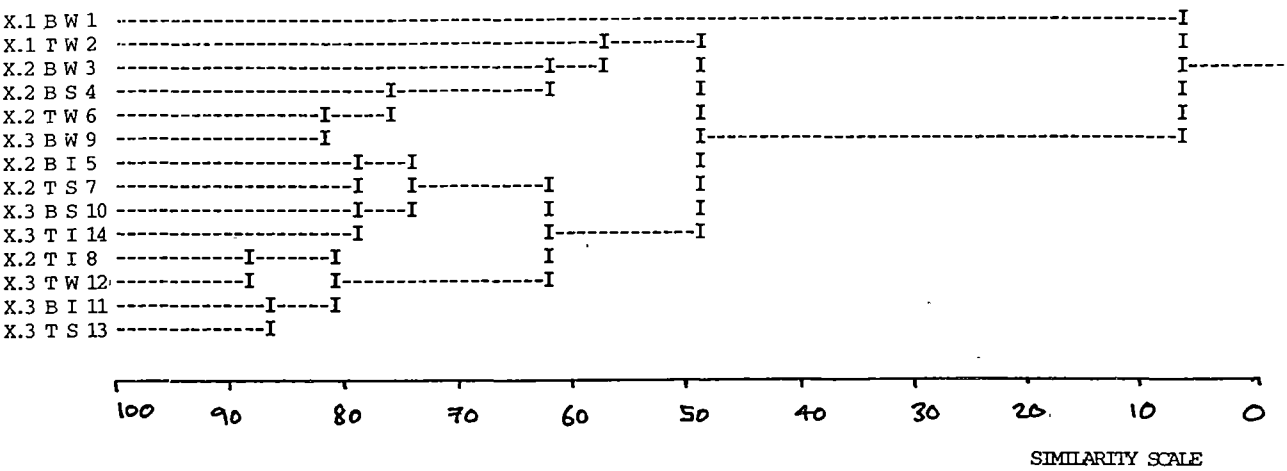


Figure 4.33 Results of Recirculating tank experiment without agitation (Expt # 11).

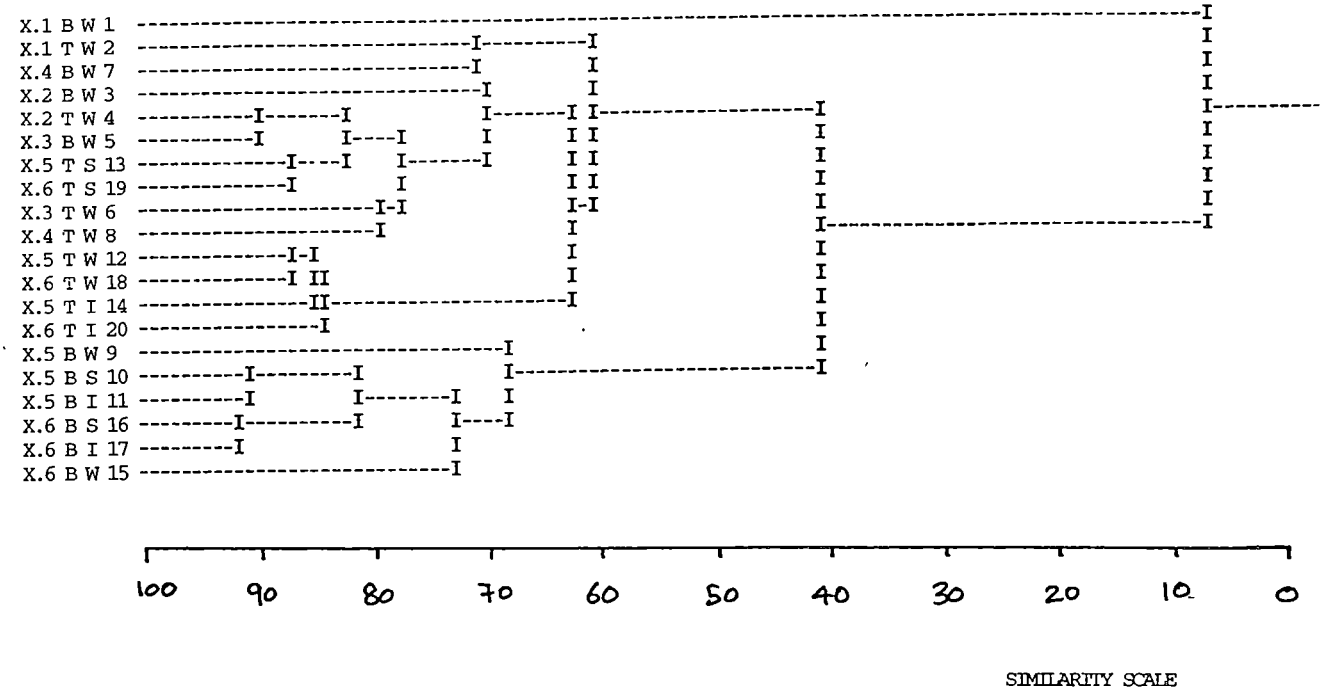


Bay and tank samples showed evidence of separate grouping in three of the recirculating experiments without agitation. Water, slurry and ice samples were well mixed. Separation on the basis of sampling time was not evident. Grouping of BW and TW samples together was not evident nor was BS, TS and BI, TI.

Flow-through with Agitation

The following figure represent results of cluster analysis of a flow-through experiment with agitation (Experiment # 7). As with previous experiments four parametres are compared. Bay and tank samples were clearly separated in this experiment, with a 40 % similarity value. Samples were not grouped according to sampling time or sample type (water, slurry or ice).

Figure 4.34 Results of Flow-through experiment with agitation (Expt # 7).



Flow-through without Agitation

The following four figures represent results of Cluster analysis of flow-through tank experiments without agitation (Experiments # 9 and 10). Again four separate parameters are compared.

Figure 4.35 Results of Flow-through tank experiment without agitation (Expt # 9).

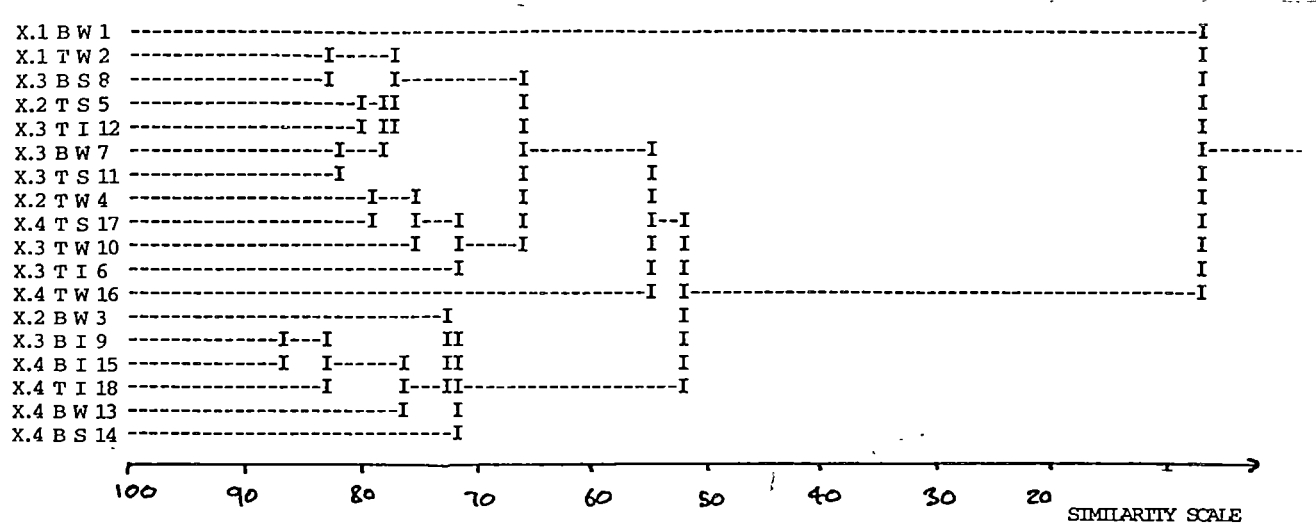
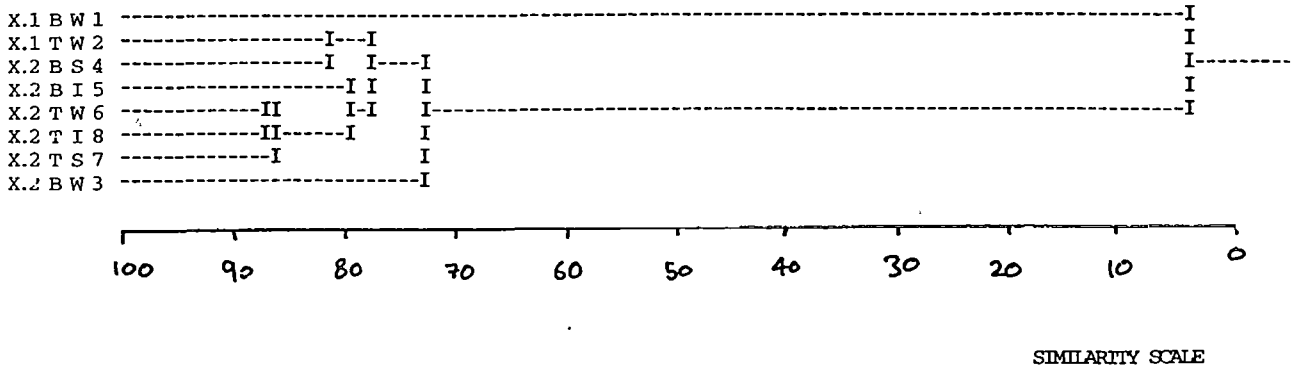


Figure 4.36 Results of Flow-through tank experiment without agitation (Expt # 10).



Bay and tank were grouped separately, as were water, slurry and ice samples. Separation of samples on a basis of sampling time was evident. No grouping of BW and TW, BS and TS, or BI and TI was evident.

Analysis of Entire Data Set

A dendrogram for the entire data set was generated and the following parameters were investigated:

Run type (Recirculating and Flow-through with or without agitation)
Sample type (water, slurry, ice)
Sample type (BW and TW, BS and TS, BI and TI)
Sample type (bay or tank)
Air temperature at time of sampling

The dendrogram portraying the entire data set is shown in figure 4.37. Only the corresponding sample number is shown on the dendrogram. Refer to Table 4.6 to relate this sample number to the above parameters.

Analysis of the complex and varied data set in this manner provided minimal concise information. The most striking result of the cluster analysis is related to succession and is discussed in the next section. Succession is indicated by later experiments being grouped towards the bottom of the dendrogram (refer Table 4.6).

Cluster analysis of individual tank runs provided more information than analysis of the entire data set.

Figure 4.37 Cluster Analysis of entire data set on basis of species type. Numbers on left-hand side of plot correspond to sample information provided in Table 4.6.

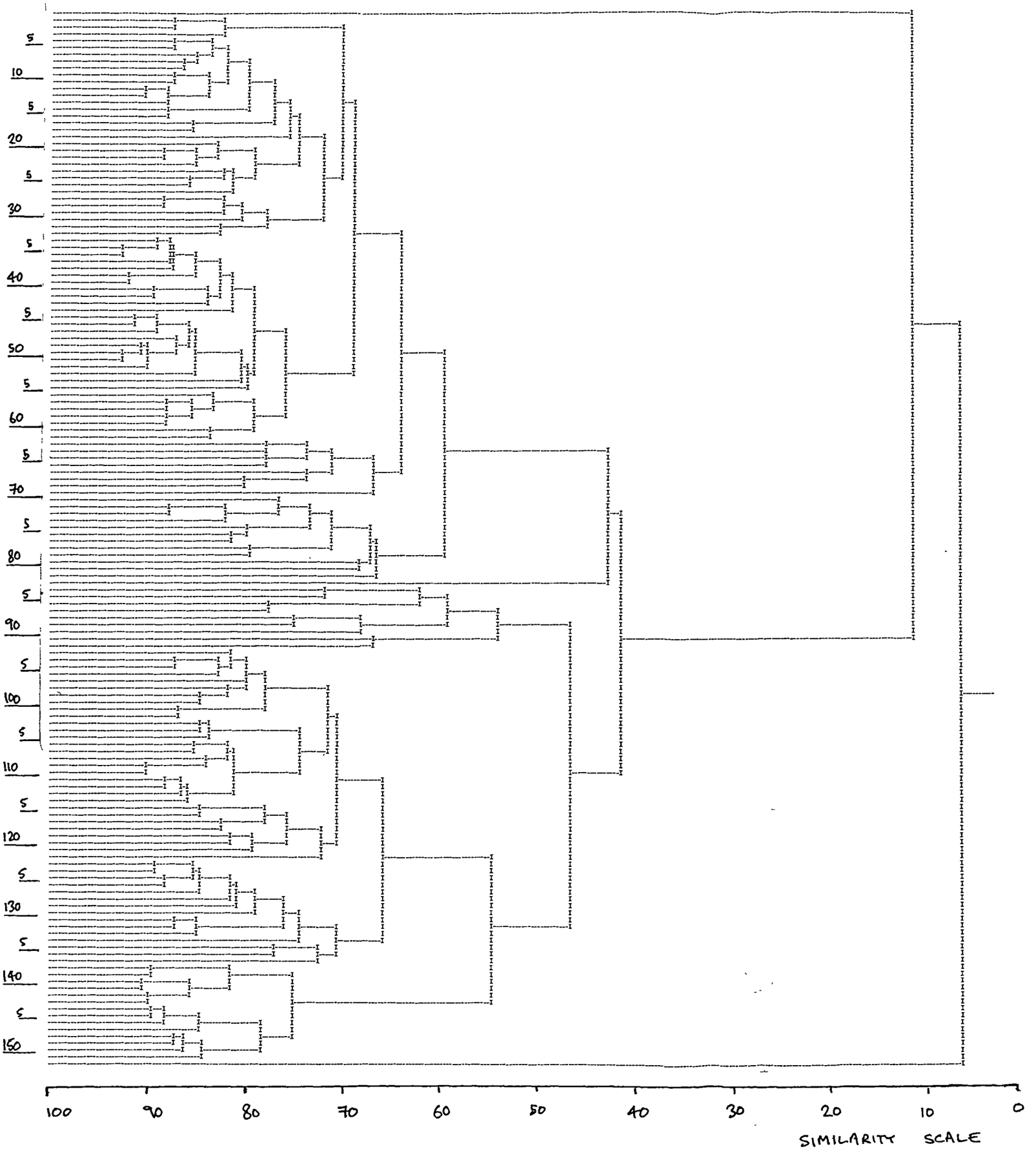


Table 4.6 Information relating to cluster analysis Figure 4 37. RC Ag = Recirculating with agitation, RC x Ag = recirculating without agitation, FT Ag = flow-through with agitation, FT x Ag = flow-through without agitation. W = water, S = slurry, I = Ice, B = Bay, T = Tank, Air Temp is in ° C.

Sample #	Expt Type	W, S, I	BW, TW, BS, TS, BI, TI	Bay, Tank	Air Temp	Expt #
1	RC Ag	W	BW	B	-10	1
2	RC Ag	W	TW	T	-10	1
3	RC Ag	S	TS	T	-10	1
4	RC x Ag	S	BS	B	-10	5
5	RC Ag	S	BS	B	-10	1
6	RC Ag	W	BW	B	-10	1
7	RC x Ag	W	TW	T	-5	4
8	RC x Ag	W	TW	T	-10	5
9	RC Ag	W	TW	T	-15	6
10	RC Ag	I	TI	T	-10	1
11	RC x Ag	I	TI	T	-5	4
12	RC Ag	S	BS	B	-10	2
13	RC x Ag	S	TS	T	0	3
14	RC x Ag	W	TW	T	0	3
15	FT Ag	W	TW	T	-10	7
16	FT Ag	W	BW	B	-10	7
17	RC Ag	S	BS	B	-10	1
18	RC Ag	I	BI	B	-10	1
19	RC Ag	W	BW	B	-15	6
20	RC x Ag	I	TI	T	0	3
21	RC Ag	I	TI	T	-15	6
22	RC Ag	S	TS	T	-10	8
23	RC Ag	W	BW	B	-15	6
24	RC Ag	W	TW	T	-15	6
25	FT Ag	S	TS	T	-10	7
26	FT Ag	S	TS	T	-10	7
27	FT x Ag	I	BI	B	-5	9
28	RC Ag	S	TS	T	-10	1
29	RC Ag	W	BW	B	-10	2
30	RC Ag	S	BS	B	-10	2
31	RC Ag	W	TW	T	-10	2
32	RC x Ag	W	BW	B	0	3
33	RC x Ag	W	TW	T	0	3
34	RC Ag	W	BW	B	-10	1
35	RC x Ag	I	TI	T	-5	4
36	RC x Ag	W	TW	T	-10	5
37	RC x Ag	S	TS	T	-10	5
38	RC x Ag	S	TS	T	-5	4
39	RC x Ag	S	TS	T	-5	4
40	RC x Ag	W	TW	T	-5	4
41	RC Ag	W	BW	B	-10	2
42	RC x Ag	W	BW	B	-10	5
43	RC x Ag	W	BW	B	0	3
44	RC x Ag	W	TW	T	-5	4
45	RC Ag	I	BI	B	-10	1
46	RC Ag	S	TS	T	-10	2
47	RC Ag	I	TI	T	-10	2
48	RC Ag	W	TW	T	-10	2
49	RC x Ag	W	BW	B	0	3
50	RC x Ag	S	TS	T	0	3
51	RC x Ag	W	BW	B	-5	4
52	RC x Ag	W	BW	B	-5	4
53	RC x Ag	W	TW	T	0	3
54	RC Ag	W	TW	T	-10	1
55	RC x Ag	W	BW	B	-5	4
56	RC x Ag	I	TI	T	0	3
57	RC x Ag	I	TI	T	-10	5
58	RC x Ag	W	TW	T	-10	5
59	RC Ag	S	BS	B	-15	6
60	RC Ag	W	TW	T	-15	6
61	FT Ag	W	BW	B	-10	7
62	FT x Ag	W	TW	T	-5	9
63	RC Ag	I	TI	T	-10	1
64	RC Ag	W	TW	T	-15	6
65	FT x Ag	W	TW	T	-5	9
66	FT x Ag	S	TS	T	-5	9
67	RC x Ag	S	BS	B	-15	11
68	RC x Ag	W	TW	T	-15	11
69	RC x Ag	W	BW	B	-15	11
70	RC x Ag	W	BW	B	-10	5
71	RC Ag	W	TW	T	-10	1
72	RC Ag	W	TW	T	-10	2
73	RC Ag	I	TI	T	-10	2
74	RC Ag	S	TS	T	-10	2
75	RC Ag	W	BW	B	-10	2
76	RC Ag	W	BW	B	-15	6
77	RC Ag	I	TI	T	-15	6
78	RC Ag	I	BI	B	-10	2
79	RC x Ag	I	BI	B	-10	5

Sample #	Expt Type	W, S, I	BW, TW, BS, TS, BI, TI	Bay, Tank	Air Temp	Expt #
80	FT Ag	W	TW	T	-10	7
81	RC x Ag	W	BW	B	-15	11
82	RC Ag	S	TS	T	-15	6
83	RC Ag	I	BI	B	-10	2
84	RC x Ag	S	BS	B	-5	4
85	FT Ag	W	BW	B	-10	7
86	RC x Ag	I	BI	B	-5	4
87	FT Ag	W	BW	B	-10	7
88	RC x Ag	S	BS	B	-10	5
89	RC Ag	W	BW	B	-10	8
90	RC x Ag	I	BI	B	-10	5
91	FT x Ag	W	TW	T	-5	9
92	RC x Ag	W	TW	T	-15	11
93	RC x Ag	W	BW	B	-10	5
94	FT x Ag	W	BW	B	-5	9
95	FT x Ag	W	BW	B	-15	10
96	FT x Ag	S	BS	B	-5	9
97	FT x Ag	W	TW	T	-5	9
98	FT Ag	W	TW	T	-10	7
99	RC Ag	S	TS	T	-10	8
100	FT x Ag	S	TS	T	-5	9
101	RC Ag	W	TW	T	-10	8
102	FT x Ag	I	TI	T	-5	9
103	RC x Ag	S	TS	T	-10	5
104	RC Ag	I	BI	B	-15	6
105	RC Ag	S	BS	B	-15	6
106	FT Ag	W	TW	T	-10	7
107	FT x Ag	S	BS	B	-5	9
108	FT Ag	I	TI	T	-10	7
109	FT Ag	W	TW	T	-10	7
110	RC Ag	W	TW	T	-10	8
111	FT Ag	I	TI	T	-10	7
112	FT x Ag	S	TS	T	-5	9
113	RC x Ag	I	BI	B	-15	11
114	FT x Ag	W	TW	T	-15	10
115	RC Ag	I	BI	B	-15	6
116	RC Ag	W	BW	B	-10	8
117	RC Ag	S	TS	T	-15	6
118	RC x Ag	S	TS	T	-15	11
119	FT Ag	W	BW	B	-10	7
120	RC Ag	W	TW	T	-10	8
121	RC Ag	I	TI	T	-10	8
122	FT x Ag	W	BW	B	-5	9
123	FT Ag	W	BW	B	-10	7
124	FT x Ag	I	BI	B	-5	9
125	FT x Ag	I	BI	B	-5	9
126	FT x Ag	I	TI	T	-15	10
127	RC x Ag	I	TI	T	-15	11
128	RC Ag	I	BI	B	-10	8
129	FT x Ag	S	BS	B	-15	10
130	FT x Ag	I	TI	T	-15	10
131	FT x Ag	W	BW	B	-5	9
132	FT x Ag	S	TS	T	-15	10
133	FT x Ag	W	TW	T	-15	10
134	FT x Ag	W	BW	B	-5	9
135	RC Ag	W	BW	B	-10	8
136	RC Ag	I	TI	T	-10	8
137	RC x Ag	S	BS	B	-15	11
138	FT Ag	S	BS	B	-10	7
139	FT Ag	I	BI	B	-10	7
140	FT Ag	S	BS	B	-10	7
141	FT Ag	I	BI	B	-10	7
142	RC Ag	S	BS	B	-10	8
143	FT x Ag	S	BS	B	-5	9
144	RC Ag	I	BI	B	-10	8
145	FT x Ag	W	BW	B	-15	10
146	RC x Ag	S	TS	T	-15	11
147	RC x Ag	I	BI	B	-15	11
148	FT x Ag	I	BI	B	-15	10
149	RC x Ag	W	TW	T	-15	11
150	RC x Ag	I	TI	T	-15	11
151	RC x Ag	W	BW	B	-15	11
152	RC x Ag	I	TI	T	-10	5

4.3.6 SPECIES SUCCESSION...

This section is included to indicate the change in the dominant diatom species in Newcomb Bay, over the duration of the ice tank experiments. There is a shift in species distribution from the beginning of March until mid April 95, (the 6 week period over which the ice tank experiments were conducted), Figures 4.38 to 4.43.

Table 4.7 Dates of ice tank experiments with corresponding experiment number.

Experiment Number	Date of Experiment
1	03/03/95
2	09/03/95
3	13/03/95
4	19/03/95
5	21/03/95
6	27/03/95
7	28/03/95
8	03/04/95
9	05/04/95
10	07/04/95
11	14/04/95

Reviewing diatom assemblages in bay water is the clearest way to investigate succession (Figure 4.38). The dominant diatom species during the first six experiments was *Chaetoceros dichaeta*, and to a much lesser extent *Eucampia antarctica* and *Fragiliaropsis curta*. *Nitzschia stellata* dominated the bay water during experiment seven. *Navicula* species and *Chaetoceros dichaeta* dominated later bay water samples.

Seasonal species composition in newcomb bay is presented in Figures 4.38 to 4.43. Of particular interest is the high abundance of *Navicula* species noted in many ice and slurry samples from both bay and tank samples. *Nitzschia stellata* is present in high numbers in bay and slurry samples from Experiments 4, 5 and 6.

Figure 4.38 Succession graph for Bay Water (BW) samples at T_0 (zero hours).

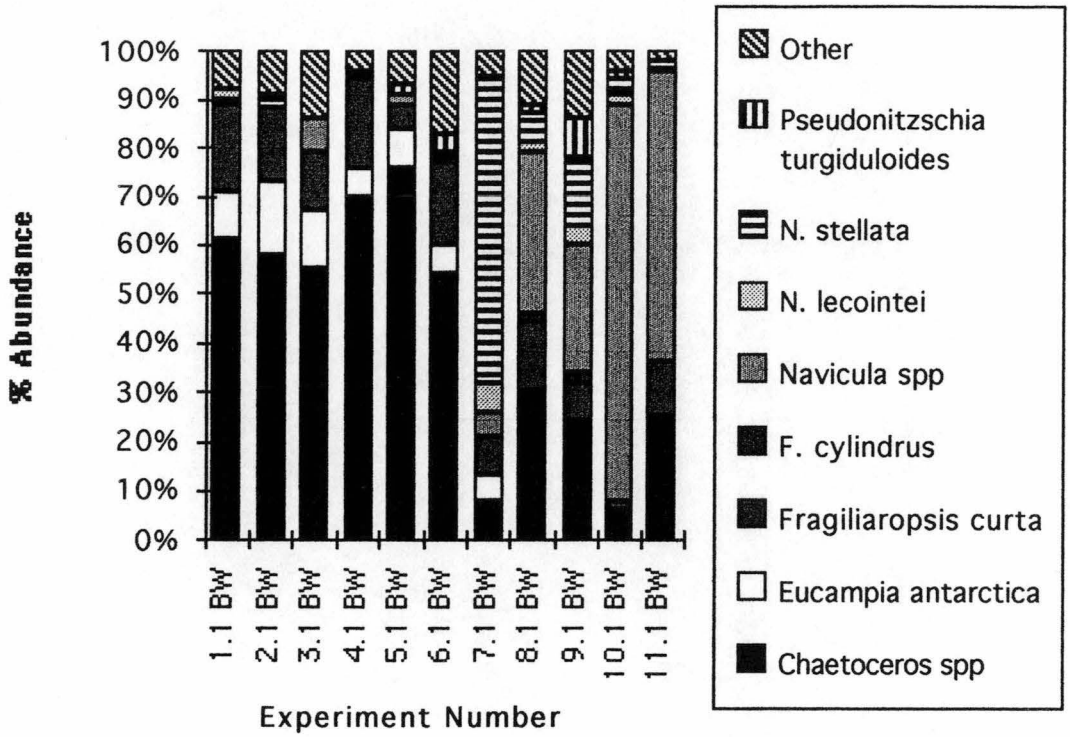


Figure 4.39 Succession graph for Tank Water (TW) samples at T_0 (zero hours).

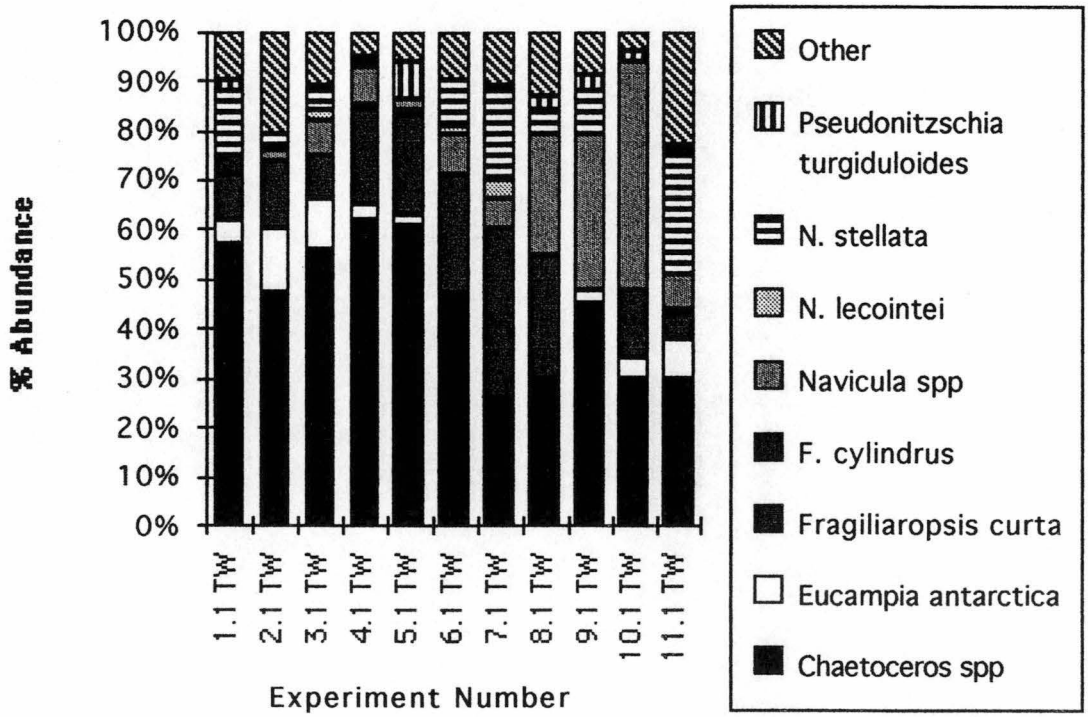


Figure 4.40 Succession graph for Bay Slurry (BS) samples at T_8 (eight hours).

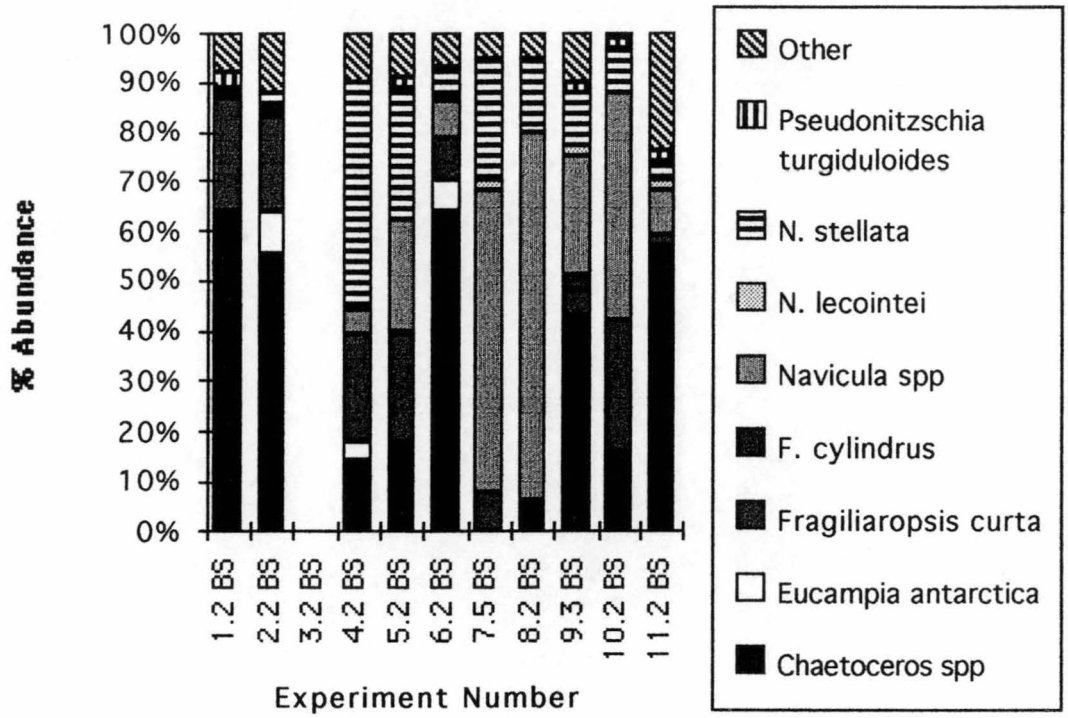


Figure 4.41 Succession graph for Tank Slurry (TS) samples at T_8 (eight hours).

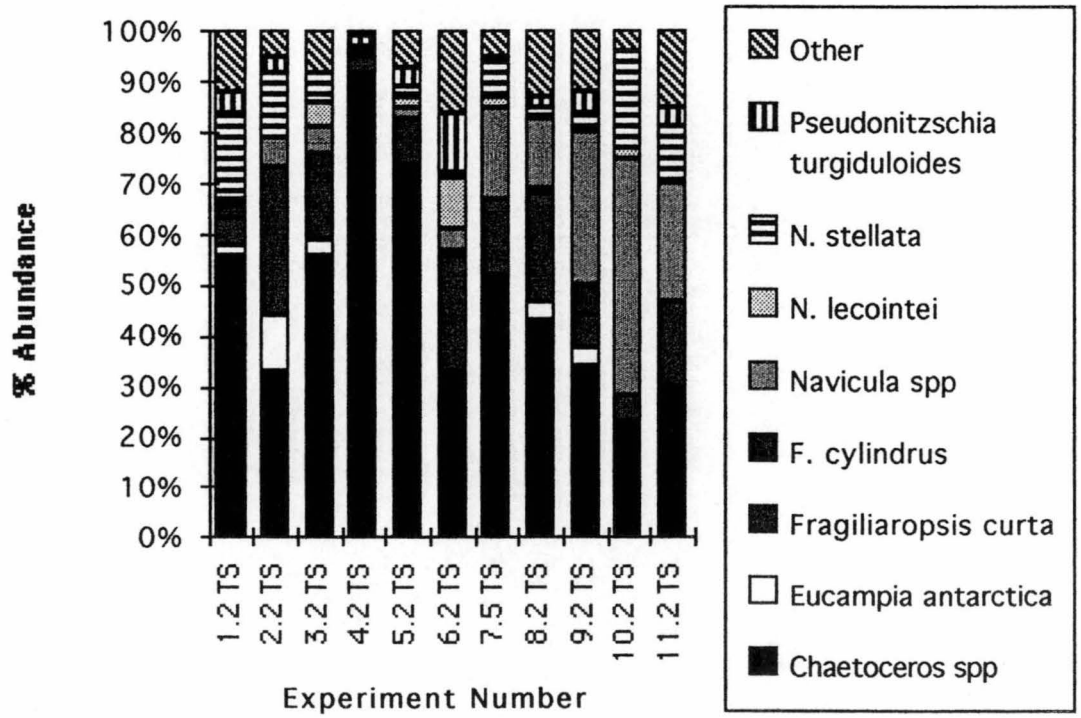


Figure 4.42 Succession graph for Bay Ice (BI) samples at T_8 (eight hours).

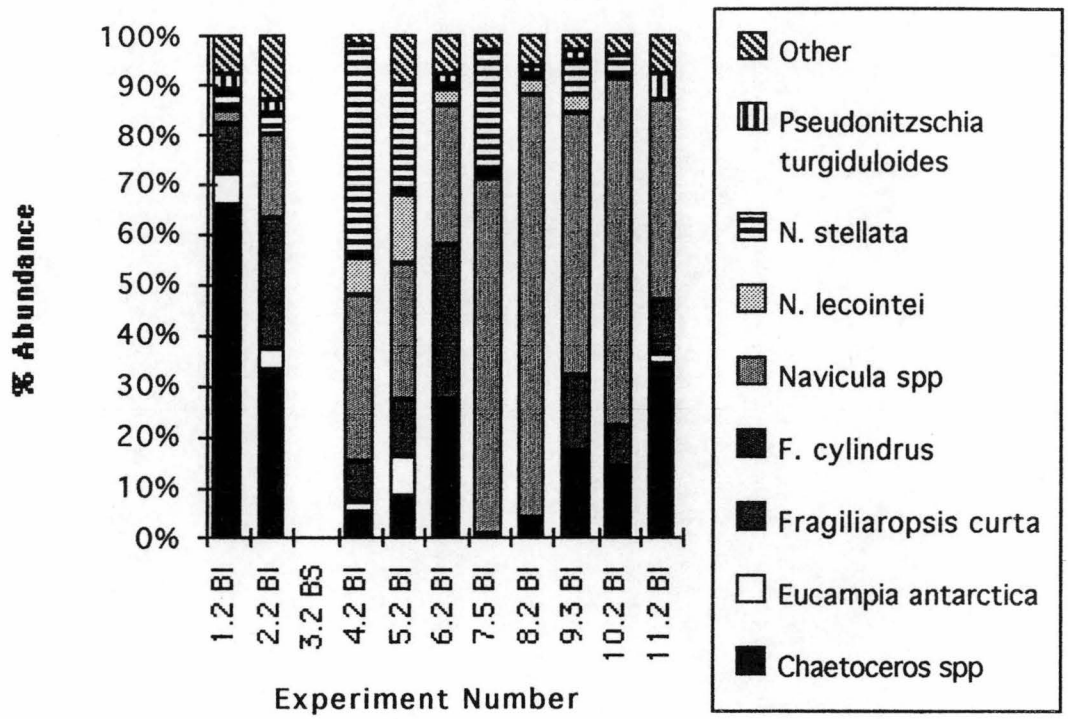
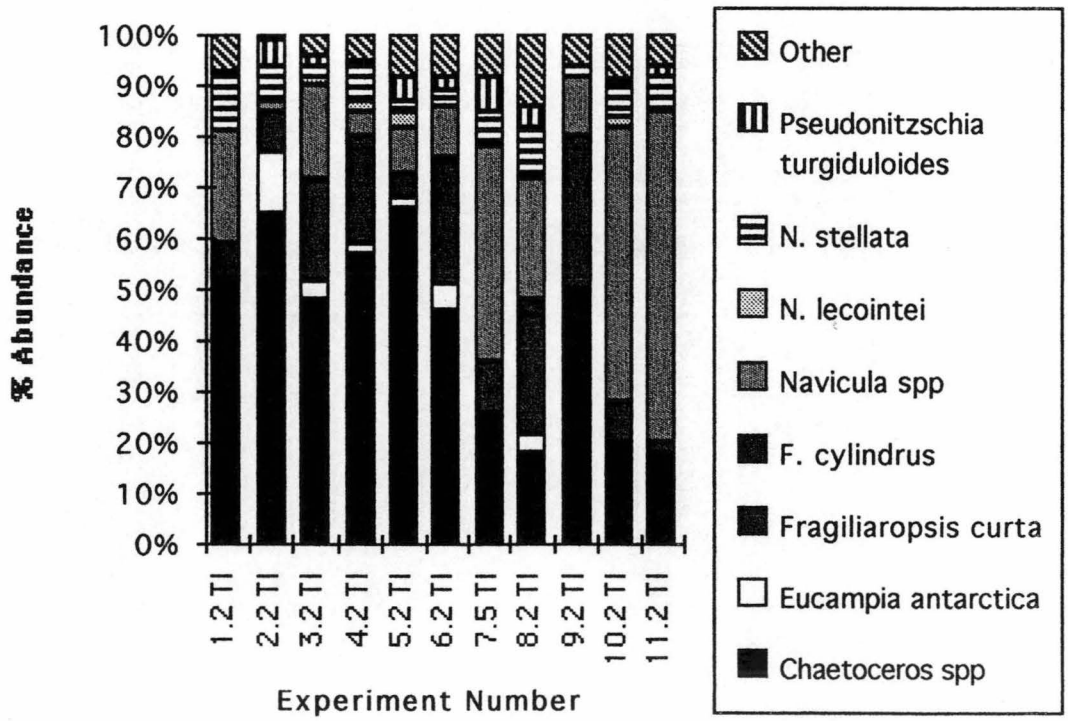


Figure 4.43 Succession graph for Tank Ice (TI) samples at T_8 (eight hours).



4.4 DISCUSSION

The field-based experimental ice tank enabled investigations into the processes which assist algal incorporation into sea ice. Four experimental designs were trailed in order to determine the importance of agitation in the natural system and to investigate the most effective and representative freezing regimes. An additional benefit the field-based experimental ice tank offered, was it provided useful design features which were incorporated into the laboratory-based tank described in Chapter 5.

4.4.1 FIELD TANK DESIGN...

The field-based ice tank was an effective system for generating sea ice. The ice tank and adjoining bay experienced very similar environmental conditions (wind and air temperatures). Sea ice formation in the tank occurred independently of the tank walls, all formation was on the water surface. Resultant sea ice was visually of indistinguishable structure compared to naturally-forming sea ice in the bay and in areas of newly forming ice elsewhere. Crystal size and orientation data was not investigated. Sea ice thickness in the tank was dependent on experiment type and air temperature. Recirculating experiments with agitation produced thick accumulations of frazil ice crystals (up to 10 cm in 16 hours). Experiments without agitation produced approximately 2 cm of frazil crystals underlain by up to 12 cm of congelation crystals.

4.4.2 TANK COMPARED TO BAY...

The bay and tank experienced the same surface energy balance as a result of input from wind, air temperatures and radiation from the sun. Bay water provided an ideal source for collection of tank water. However, some other parameters were not so closely linked. Water circulations patterns, tidal movement and wave-driven turbulence in the bay were all difficult to quantify and impossible to replicate in the tank. Various treatments in the tank were conducted in many ways independently

from bay processes. For example, Experiment # 3 was a Recirculating experiment without agitation. On this day wind speed was 20 knots, resultant bay water agitation by wave-driven turbulence and local currents would have resulted in a different agitation process to that tested in the tank.

The following discussion is based primarily on the slurry and ice formation processes in the tank, with few direct comparisons to processes occurring in the bay. Comparisons are made between initial bay water samples and initial tank water samples (these two types of samples should be relatively comparable). Differences in agitation between the bay and tank inevitably will cause variation between bay and tank, ice and slurry samples. Despite different formation mechanisms as discussed above, statistical analyses of biomass and species data (refer Section 4.3.5 and 4.4.5), suggest bay and tank samples were not significantly different.

4.4.3 SPECIES DISTRIBUTION...

The Casey tank investigated the incorporation of diatoms into artificially grown sea ice. Most authors have suggested that diatoms contribute to the majority of the phytoplankton in high latitude polar waters (Fryxell and Kendrick, 1988 and Xiuren *et al*, 1996). More fragile species were not investigated. The combination of the ice-melting process (refer, Garrison and Buck 1986) and the cleaning method described in Section 4.2.2, destroyed fragile species, biasing data towards more robust cells (diatoms). Diatoms remain easily speciated despite potential rupture of cell membrane.

Dominant diatom species found in bay and tank samples at Casey were: *Chaetoceros dichaeta, Eucampia antarctica, Fragiliaropsis curta, Nitzschia stellata* and *Navicula* species. *Chaetoceros dichaeta* was regularly the most abundant algal cell in the bay and tank samples.

Several authors have investigated diatom communities in Eastern Antarctic near-shore waters (Everitt and Thomas, 1986; McConville and Wetherbee, 1983; McMinn and Hodgson, 1993; Watanabe *et al* 1990). McConville and Wetherbee, (1983) provide one of the few investigations of algae in the near-shore environment at Casey Station. Results from their study indicate, *Navicula* species to be dominant in bottom ice and surface melt pool communities from Casey. Watanabe (1982), also found *Navicula* species to be amongst the dominant species from the Cape Bird, Ross Sea area. *Navicula glaciei* formed up to 10 % of diatom abundance in bottom ice communities in a subsequent study at Syowa Station

(Watanabe *et al*, 1990). The strong affiliation of this species with ice and slurry samples (up to 90 % abundance) found in this study, appears not to have been reported in preceding literature.

Watanabe *et al* (1990) found *Chaetoceros* species, *Nitzschia* and *Fragiliaropsis* species to dominate the near shore waters and sea ice around Syowa Station. Results from this chapter support the role of *C. dichaeta* as a dominant water and ice diatom in near-shore environments. *Fragiliaropsis curta*, while regularly present at 10 - 20% abundance levels in the Casey samples, was never the most dominant slurry or ice diatom.

4.4.4 BIOMASS...

Experiment Type

The following section compares ice thickness (Table 4.2) with biomass data (Appendix 8.7). Ice growth in Flow-through experiments without agitation (Experiments # 9 and # 10) and one recirculating experiment without agitation (# 11) resulted in the formation of congelation crystals (as a result of no paddle wheel agitation). Biomass data indicates that biomass decreased with the formation of congelation crystals. Tank ice biomass during frazil ice formation processes (early in Experiments # 9, # 10 and # 11), was one order of magnitude higher than later in the experiments (when congelation crystals were forming and growing). By comparison, experiments which used agitation, resulted in higher tank ice biomass later in the experiment.

Thus, while Flow-through experiments (# 9 and # 10) were continually exposed to new algal stock (as a result of the current of water), the absence of agitation in the tank resulted in a decrease in biomass in tank ice over time. Biomass data for the Flow-through with agitation (Experiment # 7), indicates biomass in ice was 2.5 times higher later in the experiment than earlier (Appendix 8.7). This is despite maximum ice thickness only reaching 1 cm. The importance of agitation in recirculating experiments is shown in Table 4.3. The mean biomass in tank ice in non-agitated recirculating experiments was lower than the mean value for the source water. The mean value for agitated experiments was higher in the tank ice than in the source water.

Student's t-Test analysis indicated that bay and tank samples from recirculating experiments were not significantly different. Such a result indicates that the

biomass in the tank water was similar to the biomass in the bay water at the commencement of the experiments. In addition, the enrichment in both bay and tank slurry/ice samples occurred to similar levels.

Comparing tank ice to bay ice in recirculating experiments without agitation generated the lowest P value of all comparisons ($P = 0.099$). This value is not significant ($P \leq 0.05$), however it probably indicates a trend. This trend may suggest that the biomass in tank ice and bay ice samples is different, and agitation of the water mass is a very important process for algal enrichment in sea ice.

Clearly, enrichment of algae into sea ice, hinges on more than simple availability of algae in the underlying water (as with Experiments # 9 and # 10). Agitation is a vitally important process.

Review of Experiments

The percentage change in chlorophyll a content over duration of tank experiments for both bay and tank samples is shown in Figures 4.15 to 4.25. It is possible bay samples experienced different water and wave agitation compared to the tank. Thus different biomass values may occur in bay and tank samples of similar type. Biomass in bay and tank water at T_0 was standardised to 100 % and subsequent bay and tank samples were expressed relatively to initial water values. This may also lead to bay and tank profiles differing.

Recirculation experiments with agitation show increased biomass in slurry and ice samples over initial levels in tank water (Figures 4.15 to 4.18); with a maximum 7 x increase in biomass being reached in Experiment # 6 (Figure 4.17).

Recirculation experiments without agitation (Figures 4.19 to 4.22) have a reduction in biomass in slurry and ice samples (as a result of increased settling of algae out of the tank water and formation of congelation ice crystals in calm water [refer Section 1.3.3]). Three of the four recirculating experiments (without agitation) show this. Tank slurry and tank ice biomass (Figures 4.20 and 4.21) are lower than the biomass in tank water at T_0. Wind speed during one recirculating experiment without agitation (Experiment 4.19) was 20 knots. This may have contributed to decreased sedimentation of algae in the agitation free environment and hence a greater inclusion of diatoms in the slurry and ice sample. However, biomass in tank slurry and ice samples is still lower than initial tank water values.

The Flow-through experiment with agitation has a result similar to recirculating experiments with agitation. However, even greater biomass increases may be possible as a result of the continually replenished algal stock. The tank slurry sample during the fifth sampling period contained up to 4 times the amount of chlorophyll a compared to initial tank water (Figure 4.23). This increase in biomass was noted early in the experiment. However, there was an agitation system failure somewhere between the fifth and sixth sampling periods. This may have contributed to the lower biomass noted in the later slurry and ice samples. In addition, only 1 cm of frazil crystals had formed in the tank by the end of the sixth sampling period (40 hours), thus frazil ice production was at a reduced rate compared to other experiments, (Table 4.3).

Flow-through experiments without agitation are shown in Figures 4.24 and 4.25. Depending on the effectiveness of a replenishing algal stock (in the absence of agitation) tank slurry and ice samples could show an increase or decrease in biomass over initial levels in the tank water. Up to twice the initial biomass in the water was found in the first tank slurry sample (Figure 4.24). All other samples had at least half the initial biomass. The second flow-through experiment without agitation (Figure 4.25) supports these results. The first tank slurry sample was drained from frazil crystals; later tank slurry and ice samples were comprised of columnar crystals (which tend not to incorporate biogical material).

Agitation is an effective way of increasing biomass in tank slurry and ice samples over initial water values. Replenishing algal stocks in water by flow-through current appears not to be an effective enrichment mechanism in the absence of agitation. Biomass in the water slurry and ice samples range between 0.10 and 3 ug chl a per litre (refer Appendix 8.7). These values are in a similar range to those reported elsewhere in the literature (Garrison and Close, 1993).

4.4.5 STATISTICAL ANALYSIS...

Cluster analysis was generated using species distribution data.

Individual Experiments

Grouping of samples appears dependent on the presence or absence of agitation. Recirculating and flow-through experiments with agitation have well mixed dendrograms with no clear separation of bay and tank samples, (refer Figures 4.26 - 4.29). Recirculating and Flow-through experiments without agitation present

dendrograms which show evidence of grouping bay and tank samples separately. This is particularly evident in Experiments 4, 5 and 9 (Figures 4.31, 4.32 and 4.35 respectively). Agitation within the tank apparently replicates more closely the processes occurring in the bay.

The Flow-through experiment with agitation shows a clear separation of bay and tank samples (Figure 4.34). This could be linked to the long duration of the experiment (40 hours). Bay algal assemblages may have changed over this period, due to water currents and tidal influences.

Using a similarity scale it is possible to estimate how similar the samples are to one another. Experiments with agitation result in more samples with similar algal assemblages, with grouping of samples split at around 65 - 75 % similarity in all experiments. Experiments without agitation cause a split of the majority of samples at between 30 and 70 % similarity. For example, bay ice and slurry samples from Experiment # 4 which was a recirculating without agitation experiment (Figure 4.31) were only 30 % similar to tank samples on a basis of species distribution. Thus the ice formation occurring in the tank was clearly quite different to the process in the bay.

Cluster Analysis of Entire Data Set

A series of investigations was conducted on the cluster analysis of the entire data set. The most striking result related to the grouping of later experiments in the lower half of the dendrogram (refer Table 4.6). This result can be attributed to species successional factors and is discussed in greater detail in the next section.

4.4.6 SUCCESSION...

Successional changes in diatom assemblages occur over a 6 week period from March until mid April, 1995, (Section 4.3.6). Different types of experiments probably resulted in different incorporation mechanisms of algae into ice and slurry samples. Thus Experiments # 1 and # 2 may, by nature, have a different population to # 3, # 4, # 5 etc; as a result different assemblages in slurry and ice samples may not be contributed exclusively to succession influences. The samples least affected by different ice formation processes are bay water samples and tank water samples, Figures (4.38 and 4.39).

Seasonal studies of algal assemblages have revealed a clear succession in dominant species within both sea ice and the water column (Bunt and Lee, 1970, Watanabe *et al* 1990, Dieckmann *et al* 1991b). Watanabe *et al* (1990) conducted a year-round survey of the bottom ice community in fast ice around Syowa Station. The most dominant diatom species in this study were found to be a variety of *Nitzschia* species (particularly *N. lecointei, N. stellata*) and *Pseudonitzschia turgiduloides*), although *Navicula glaciei* was found up to 10 % abundance in March, October and November. In addition, the authors found *Eucampia antarctica* to be regularly present at around 10 % abundance level in the internal ice environment. *Chaetoceros* species were also found in this environment but to a lesser extent. These results are from fast ice and not newly forming frazil. However, they indicate some of the important species in near-shore areas.

Many of the species deemed important in the study of Watanabe *et al* (1990) during March, April and May, were also found in high abundance in this successional study. A distinct succession of dominant species in bay water samples occurred over the six week period, Figure 4.38. *Chaetoceros dichaeta* dominated bay water samples for the majority of March. *Nitzschia stellata* then became the dominant diatom species in the water column, followed by *Navicula glaciei. Fragiliaropsis curta* was found in the all bay water samples at roughly 10 - 15 % abundance levels.

A representation of the succession of diatom species within slurry and ice samples is shown in Figures 4.40 to 4.43. Dominant species found in ice samples included: *Chaetoceros dichaeta, Navicula glaciei* and *Nitzschia stellata. Fragiliaropsis curta* and *Pseudonitzschia turgiduloides* were also present at up to 20 % abundance levels.

4.5 SUMMARY

The use of artificial ice tanks is constrained by many factors. Utilising such a tank in the Antarctic environment was invaluable in determining a suitable laboratory based system (adopted in Chapter Five). While the Antarctic environment assisted sea ice formation in the tank in many ways (air temperatures, exposure to wind and radiation, and as a useful source of water), it also limited some investigations. Such investigations included direct comparisons of tank and bay ice formation mechanisms. It was impractical to attempt to duplicate in the tank all the parameters acting on the bay. As a result, different processes occurred between the bay and the tank. Nevertheless, valuable results were generated from investigations of an artificial sea ice generation process in the natural environment.

Sea ice, which formed in the experimental tank was visually indistinguishable from naturally-formed sea ice from the bay and from areas of new ice formation elsewhere. Ice formed in the tank at similar rates to the bay. Detailed analysis of ice crystal morphology was not carried out.

Species data indicates *Chaetoceros dichaeta*, *Nitzschia stellata*, *Eucampia antarctica* *Navicula glaciei* and *Fragiliaropsis curta* to be dominant species. This result is supported by other studies of sea ice algal assemblages (Garrison and Buck, 1985; Garrison and Close, 1993 and Watanabe *et al* 1990). There was a strong affiliation of *Navicula glaciei* with ice and slurry samples (up to 90 % abundance). This appears to be the first time such a result has been reported in near-shore waters.

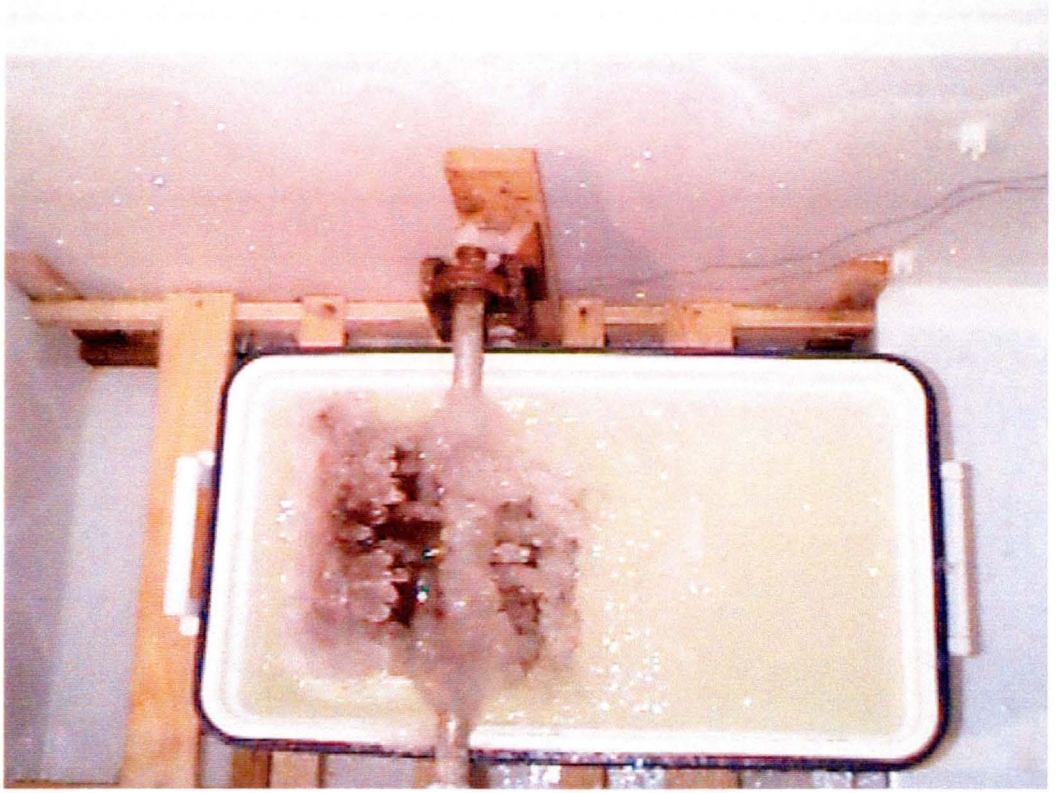
Biomass data (refer Section 4.4.4) suggests enrichment of algae into sea ice hinges on more than simple availability of algae in the underlying water (as with Flow-through experiments without agitation). Agitation is a vitally important process for algal enrichment in sea ice. Reference to biomass data strongly indicates biomass values decreased with the formation of congelation crystals. Biomass in bay and tank, slurry and ice samples, was regularly found to be up to 7 times the biomass in the initial corresponding water samples. Biomass in ice and water samples from this study were in the same range as reported elsewhere in coastal Antarctic environments (McConville and Wetherbee, 1983).

Student's t-Test analysis comparing sample biomass indicated that no tank samples from recirculating experiments with agitation, were significantly different to bay samples. Such a result indicates that the biomass in the tank water was similar to the biomass in the bay water, at the commencement of the experiments. In addition, enrichment in both bay and tank slurry/ice samples occurred at similar levels. This data is supported by cluster analysis of diatom assemblages which indicates recirculation experiments with agitation, most closely resemble the natural ice formation processes. Further, cluster analysis highlights the importance of agitation, as distinct groups formed when recirculating experiments without agitation were analysed.

Successional changes were evident amongst bay water samples. Initially *Chaetoceros dichaeta* dominated the bay water, followed by Nitzschia *stellata* and *Navicula glaciei.* This transfer in dominant species was reflected in bay and tank, slurry and ice samples.

5.0

Laboratory-based experimental sea ice tank...



5.1 EXPERIMENTAL DESIGN

Naturally occurring sea ice production and the resulting algal composition of various types of newly formed ice was investigated in Chapters 2 and 3. Results indicate a few key algal species dominate both the water column and the sea ice samples. These chapters suggested the importance of wind and wave-driven turbulence with regards to the types of new ice formed and the algal community they contain. The importance of these physical mechanisms is reviewed with a conceptual model (Figure 3.10). Buoyancy of phytoplankton species is suggested to be an influential biological factor in assisting algal inclusion in sea ice.

Chapter 4 describes the use of a sea ice tank based at Casey Station, Antarctica. The chapter investigates the appropriateness of using artificial tanks by comparing diatom inclusion in the tank to diatom inclusion newly forming sea ice in the adjoining bay. Results from this chapter indicate 'realistic' sea ice can by produced in experimental tanks. Agitation of the tank water was found to be of critical importance if the biomass values in the ice tank were to be comparable to the bay.

Questions which arose from the above investigation include:

1) Is algal inclusion in sea ice based on physical entrapment as a result of turbulent processes.
2) Does polar phytoplankton have special adaptations which facilitate its inclusion in sea ice.

Thus the aim of this part of the study was to investigate these questions with the aid of a laboratory-based ice tank. In addition, these experiments allowed a more detailed investigation of the use of artificial ice generation processes. Polar algal cultures, temperate phytoplankton stocks and microscopic glass beads were used in the investigation.

5.1.1 DESIGN OF ICE TANK...

Many factors had to be considered in the construction of a laboratory-based ice tank. These tanks need to replicate as closely as possible, natural conditions such as ice formation mechanisms and water agitation. The ice tank had to be enclosed in an environment of similar temperatures to those found naturally. Cooling of the water mass had to occur through surface contact with cool air. Ice production had to occur in a similar time frame to that of natural sea ice production. The water mass had to be agitated in a manner which produced similar ice to newly forming ice around Antarctica. Phytoplankton and other material introduced into the tank had to settle out of the water at a similar rate to natural settling rates.

Ice Tank

An environment similar to the Antarctic could only be reproduced in the laboratory by using a large freezer. The ice tank described in this chapter was comprised of an insulated water container inside a large chest freezer. Weissenberger and Grossmann (1998) describe a similar (although larger) design; they used a large capacity tank (3000 l) enclosed in a walk-in freezer. Earlier tank designs of Garrison *et al* (1989), Reimnitz *et al* (1993) were invaluable in addressing specific questions relating to ice harvesting mechanisms. However, the design of the tank described in this chapter (IASOS tank) and the Weissenberger and Grossmann (1998) design allow investigation of different aspects of algal incorporation in newly-forming sea ice. The design of the IASOS tank is discussed in greater detail in Section 5.2.

Temperature Control

Experiments were conducted to determine the best temperature régime for ice production. Temperature controls needed to be able to be adjusted to allow slow cooling, to equilibrate water temperatures in the tank to approximately the point of freezing, without inducing ice formation. In addition, the tank surrounds needed to rapidly attain and maintain temperatures of cf -15° C to replicate temperatures common during ice formation in the field.

Slow cooling capabilities were a particularly important consideration as many ice algal species may need to be exposed to cold water temperatures (cf -1.8° C) for days before producing chemicals which might assist their incorporation into newly forming ice (Raymond, personal communication, 1997). The method used to 'condition' phytoplankton is discussed in Section 5.2.2.

Agitation System

A similar paddle wheel agitation system was used in the laboratory and field based ice tanks. This agitation system proved to be a suitable method of producing frazil crystals from the agitated water mass. Shen (personal communication, 1996) also found that this means of agitation was appropriate for the formation of pancake ice in their ice mechanics tank. Due to the size of their tank (converted 25 m swimming pool) production of pancakes was possible. Production of pancakes was not possible in the tank described in this chapter due to its limited size, ie not enough surface area was available for the repeated collisions and separations required to produce pancake ice (Weeks and Ackley, 1982).

Visual inspection of the ice crystal type produced in laboratory-based ice tank revealed ice crystals indistinguishable in structure from those found in early sea ice production areas. Initial growth in the ice tank resulted in frazil ice crystals. As growth continued in the absence of agitation the crystals consolidated producing transition type ice. If growth was allowed to continue, congelation crystals formed (see Section 5.1.3 Design of Ice Tank Experiments). Examination of ice crystals under polarised light to accurately determine crystal orientation was not conducted.

Settling of Material out of Suspension

It was difficult to prevent settling of microscopic particles and some algal cells. Garrison *et al* (1989) found this to be a problem also. In an attempt to avoid this problem, phytoplankton cultures were released into the tank water approximately 30 minutes before the initial water samples were taken and the freezing initiated (phytoplankton had been equilibrated for two days in containers in the near freezing water). This allowed sufficient time for mixing of cultures throughout the water but not too much time for phytoplankton to settle out of suspension. Later experiments included a sediment trap on the bottom of the tank. Microscopic beads were found to have a much higher sedimentation rate than the Antarctic phytoplankton cultures and temperate phytoplankton cultures.

5.1.2 DESIGN OF ICE TANK EXPERIMENTS...

Ice tank experiments were designed to investigate the mechanisms assisting incorporation of polar phytoplankton species into sea ice. To facilitate this, polar phytoplankton cultures, temperate phytoplankton cultures, and minute glass

microspheres were used to investigate algal and particle enrichment in experimentally grown sea ice.

A variety of polar phytoplankton species was studied. All of these species were from cultured stocks at the Australian Antarctic Division (Kingston, Tasmania). The cultured species included (for example): known sea ice diatoms such as *Fragiliaropsis curta* and *F. cylindrus*, and planktonic species such as *Odontella weisflogii*. Cultured species ranged in size from the very small diatom *F. cylindrus* (2 μm x 5 μm) to large diatoms such as *Proboscia alata* (100 μm x 10 μm).

The incorporation of temperate phytoplankton into experimentally-grown sea ice was also studied. These experiments were included to investigate if polar phytoplankton had mechanisms which facilitated their uptake which similarly-sized and shaped temperate phytoplankton species did not.

Experiments using glass microspheres were also carried out. The aim of these experiments was to investigate whether the mechanism or mechanisms of incorporation into sea ice were purely physical processes that concentrated all particles in the water, or if the polar species had an adaptation which assisted their inclusion.

Several types of ice formation processes were designed, to determine the most representative freezing regime; these were: slow-forming frazil, fast-forming frazil and fast-freezing congelation.

- Slow-forming frazil ice was produced over 3 days. During this time air temperature in the tank surrounds was slowly decreased to approximately - 15° C. Agitation was constant.
- Fast-freeze congelation ice was produced by rapidly reducing the temperature in the tank in the absence of agitation.
- Fast-freeze frazil ice was produced by rapidly decreasing the air temperature in the chest freezer. Agitation ensured frazil ice production. Frazil ice production approached 10 mm/hr.

The majority of experiments involved the formation of fast-freeze frazil. During the above trial experiments, water containing temperate phytoplankton species was used to determine the most appropriate temperature settings for ice formation, the efficiency of the agitation system and the degree of settling. Water, slurry and ice samples were taken every two hours during the duration of the experiment. A sample was also collected from the bottom of the tank for the later experiments. This sample was used to determine the degree of settling. An outline of the sampling strategy is shown in Section 5.2.4.

5.1.3 COMPARISON of FIELD and LABORATORY ICE TANKS...

The design of the laboratory and field-based tanks was very similar. Both tanks were insulated from the sides and the bottom. Cooling could only occur from the top of the tanks. Both tanks were exposed to wind-driven heat-transfer and turbulence. The field-based tank used wind; whereas fans were used in the laboratory-based tank. Agitation of the water masses in the tank was provided in both cases by a paddle wheel which resulted in water being pushed through the ice unconsolidated matrix. The paddle wheel rotated at a rate of one complete turn in 2 seconds. Paddle width was altered to accommodate the smaller laboratory-based tank. The field-based tank had a greater capacity (250 l versus 45 l). The dimensions of the field tank were 500 mm x 500 mm x 1000 mm. The dimensions of the laboratory-based tank were 300 mm x 300 mm x 500 mm. Responsive temperature controls on the laboratory-based tank allowed close replication of temperatures common in the Antarctic.

5.1.4 BENEFITS THIS TANK OFFERS OVER OTHER SYSTEMS...

Early tank designs [Garrison *et al* (1989) and Reimnitz *et al* (1993)] gave valuable insights into ice harvesting mechanisms. However, the ice tanks had limitations with respect to duplicating the natural sea ice formation process; freezing did not occur from the water/air interface but from the sides, agitation was not representative and phytoplankton settling out of suspension was a significant problem.

Later experimental tanks addressed these concerns (eg Weissenberger and Grossmann 1998). The laboratory based tank described in this Chapter also addresses the above concerns. In addition, a similar system to this tank was tested in the Antarctic environment (Chapter 4). Several polar and temperate phytoplankton cultures were used to investigate inclusion of phytoplankton into forming sea ice. Inclusion of minute glass microspheres was also investigated. Algal cultures were equilibrated for 2 days near their freezing point to facilitate production of potential ice-active substances.

5.2 METHODS

5.2.1 CONSTRUCTION OF THE ICE TANK...

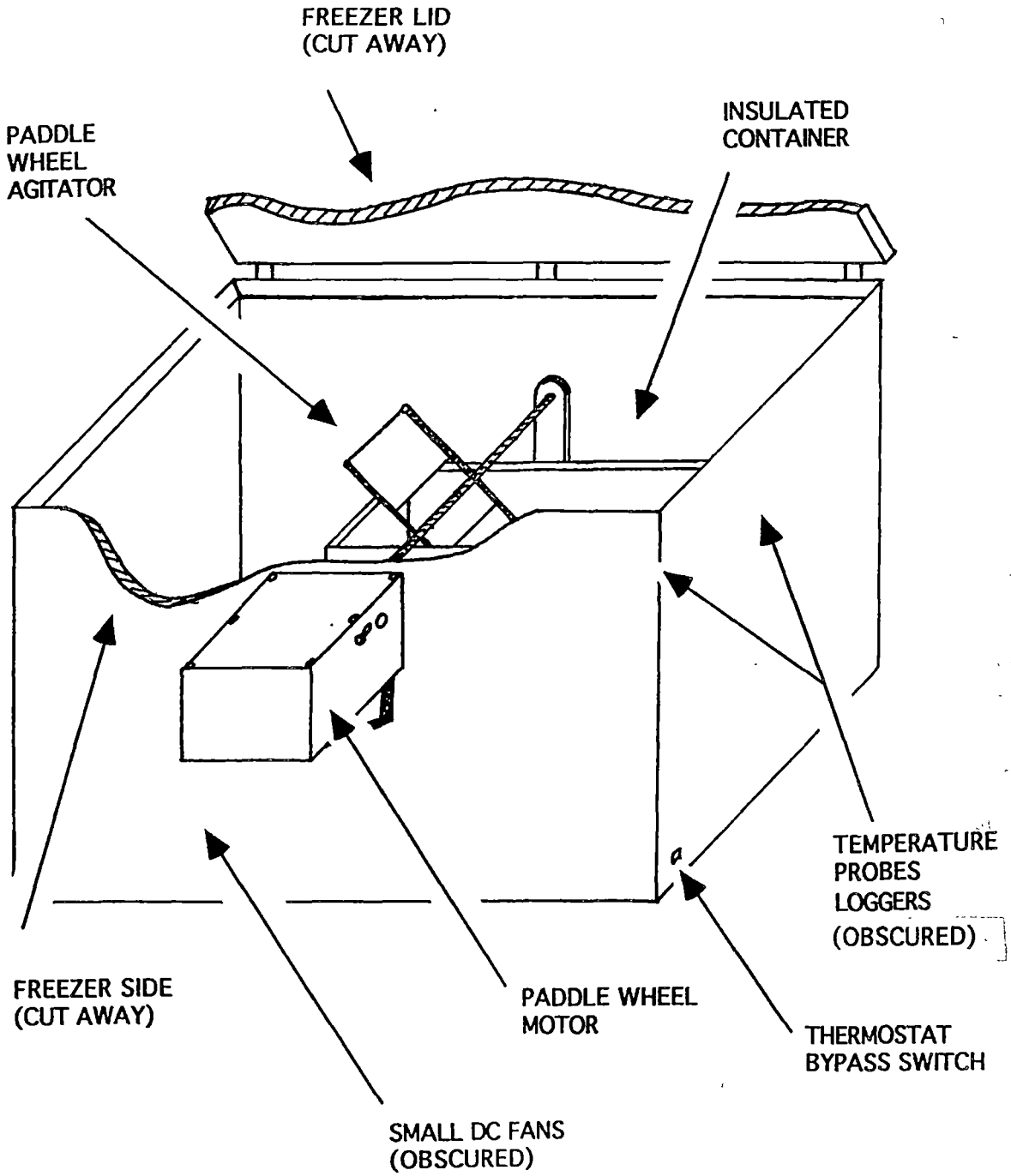
A 500 litre (1200 x 650 x 650 mm) chest freezer was modified to duplicate similar conditions to those found in the Antarctic pack ice (Figure 5.1). A thermostat bypass switch was fitted to the freezer so that it could always remain on. A temperature range which averaged around - 1.8° C could be attained using the thermostat switch. When this was bypassed, temperatures of around - 15° C were rapidly reached and maintained. A 45 l container (300 x 300 x 500 mm) which was insulated on all sides and the bottom, was mounted inside the freezer (polar water masses are only directly exposed to cold air at the water air interface; they do not cool from the sides or bottom), (Figure 5.2).

A motor was used to drive a paddle wheel which agitated the water body (Figure 5.3). Two fans were installed into the freezer to create a pattern of air circulation. The fans blew cold air over the surface of the water in the tank and recirculated back past the coldest part of the freeze. Agitation of the water surface by the fans was negligible compared to agitation provided by the paddle wheel. The fans were included to ensure circulation of cold air throughout the chest freezer.

During initial experiments, four temperature probes were inserted into the tank. Two of the probes (Tinytalk temperature recorders) recorded the air temperature and the water temperature, and stored the information for the duration of the experiment. The devices were programmed to record temperatures every 1-2 minutes. This information was invaluable in determining the appropriateness of the freezer settings. Information from these probes was up-loaded onto a computer and presented in a graphic form (Figure 5.4). Two other probes (Checktemp temperature probes) were used to give instantaneous air and water temperatures. Temperature profiles for a typical tank run are shown in Figure 5.4.

Once the tank was constructed, the three different experimental designs were trailed (refer Section 5.1.3). Fast-freeze frazil experiments were adopted for post trial experiments.

Figure 5.1 Diagram of ice mechanics tank



TANK DIMENSIONS (300 x 300 x 500 mm)

FREEZER DIMENSIONS (650 X 600 X 1200 mm)

Figure 5.1 Diagram of ice tank

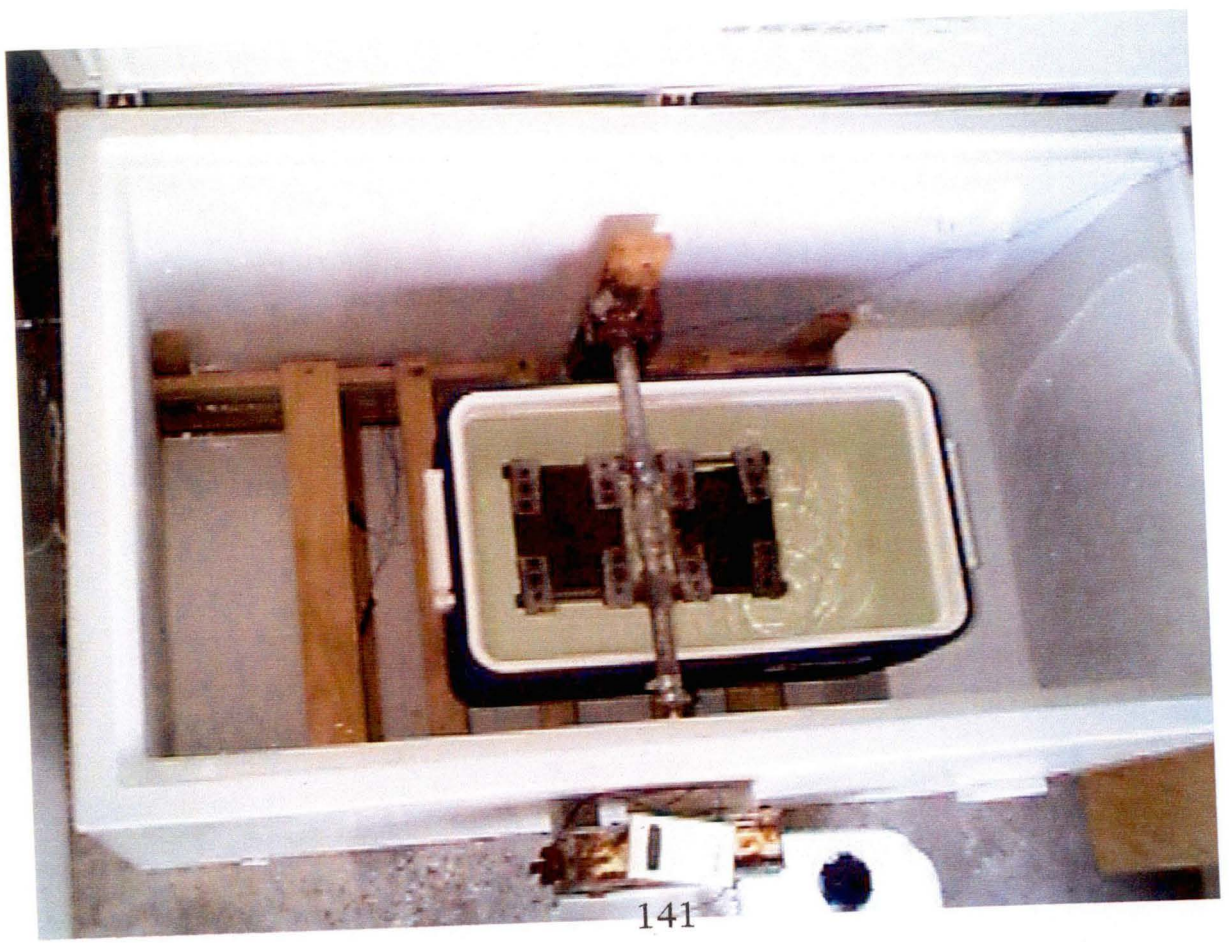
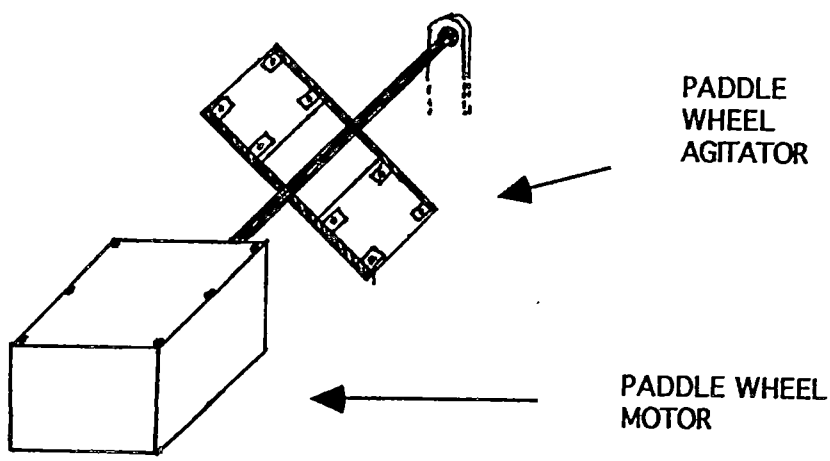


Figure 5.3 Diagram of agitation system. Paddle wheel motor consists of a small DC motor connected to a gearing system. Paddle wheel revolution was 30 cycles per minute. The paddle wheel motor had enough torque to maintain an ice-free area and continue agitation.



5.2.2 ALGAL CULTURING...

A number of polar phytoplankton species were cultured using stocks from the Australian Antarctic Division (AAD). The species were grown in F2 media (after, Guillard and Rhyther, 1962). The composition of F2 media is shown in Appendix 8.6.

A 20 l volume of media was produced. Each of the species was taken from AAD sub-cultures and placed into 3 litres of media. The phytoplankton species were kept in suspension on a shaker table at 2° C under optimal lighting conditions for approximately five weeks. Algal cultures were then stored in near freezing water (-1.0° C to - 2.0° C) for two days. The necessity for this step was explained in Section 5.1.3.

The species cultured were:

Fragiliaropsis curta
Nitzschia lecointei
Chaetoceros dichaeta
Fragiliaropsis cylindrus
Proboscia alata

Mixed cultures were also used; they contained:

Chaetoceros spp
Rhizosolenia spp
N. lecointei
Corethron spp
Odentella weisflogii
F. curta
Thalasiothrix spp
F. cylindrus
Navicula glaciei

Single experiments were carried out using a culture of small unidentified flagellates and a culture of bacteria. Volumes of the various algal cultures were added to 45 litres of 4.5 μm filtered sea water.

5.2.3 SEPARATION OF MICROSPHERES...

Two size ranges of microspheres were obtained from HST Technology, Technopark, Hobart, Tasmania (the size ranges were 1 - 53 μm and 53 - 106 μm). Using several stainless steel test sieves and a sieve shaker, it was possible to separate the beads into various size classes. The size classes were, 1 - 32 μm, 32 - 38 μm, 38 - 53 μm, 53 - 63 μm and 63 - 90μm.

Microspheres in the 1 - 32 μm size range, were inadequately separated by sieves. Attempts were made to separate microspheres into 1 - 10 μm, 10 - 20 μm and 20 -32 μm size ranges. Despite the use of a sieve shaker this separation proved to be unrealistically laborious. Consequently, microspheres in a broader size range of 1 - 32 μm were used.

5.2.4 SAMPLING FROM ICE TANK...

Sampling a typical tank experiment involved the following steps.

2 days prior	Place culture in container within tank (temp - 1° C to - 2° C)
- 30 min	Release culture to allow 30 min for mixing; position sediment trap
T_0	Turn on fast freeze switch; take water sample
T_4 (4 hr)	Take water, ice and slurry samples
T_6 (6 hr)	Take water, ice and slurry samples
T_8 (8 hr)	Take water, ice and slurry samples; collect sediment trap

Samples were collected in 100 ml bottles. To collect water samples these bottles were placed into the middle of the tank and allowed to fill. Ice samples were sectioned using a knife blade and scooped into a sieve. Immediate drainage from the sieve was collected; this formed the slurry sample (referred to as such as a result of very small ice crystals which were not separated by the sieve).

A small sediment trap was constructed. This trap consisted of a cylindrical container (diameter 100 mm x depth 20 mm) with a sealable lid. Upon completion of tank experiments, the trap could be sealed and removed. Algal material or microspheres could be gently resuspended and processed in the same manner as other samples.

Sample volume was limited by the size of the tank. Initial experiments involved collection of samples for biomass analysis. However, the sample volumes required to obtain useful data were too large to not affect the thermodynamics of the experiment, ie a large volume and hence surface area of very new ice would be required to obtain an accurate biomass measurement. The collection of ice for biomass measurements was not continued. This is discussed further in Section 5.4.

5.2.5 ANALYSIS of SAMPLES...

Microscopy of Polar Phytoplankton and Temperate Phytoplankton...

A Zeiss Telaval 31, inverted microscope was used to analyse the samples. Water, slurry and ice samples were collected and three replicate counts of algal cell numbers were made. Depending on cell concentration, usually a minimum of three rows of the settling chambers were counted per replicate count. A minimum of 400 cells per replicate was counted.

Microscopy of Microspheres...

Samples containing microspheres were concentrated five-fold through settling and 10ml volumes of concentrate were then analysed using the inverted microscope. Three separate counts of 15 fields of view were analysed per sample, to determine the concentration of microspheres in the samples. A minimum of 400 microspheres per replicate count were sought.

Paired, Two Tailed t-Test Analysis...

Paired, two-tailed t-Tests assuming unequal variances, were conducted on microsphere or algal cell numbers, in all of the water, slurry and ice samples. A confidence level of $P \leq 0.05$ was used to test for significance. Comparisons resulting in a P value ≤ 0.05 are considered to be significantly different comparisons. Results are presented in Section 5.3.4 and in Appendix 8.5.

5.3.1 SUMMARY OF TANK EXPERIMENTS...

Table 5.1 Summary of ice tank experiments. TP - Temperate Phytoplankton, M - Microspheres, PC - Polar cultures, FFF - fast-freeze frazil, FFC - fast-freeze congelation, SFF - slow-freeze frazil.

Expt Type	Expt Description		Times which samples were collected after experiment commenced	Section Discussed
TP		FFC	4, 6, 8	4.3.3
TP		SFF	24, 45, 65	4.3.3
TP		FFF	4, 6, 8	4.3.3
M	FFF	20 - 32 μm	4, 6, 8	4.3.4
M	FFF	32 - 38 μm	4, 6, 8	4.3.4
M	FFF	38 - 53 μm	4, 6, 8	4.3.4
M	FFF	53 - 63 μm	4, 6, 8	4.3.4
M	FFF	63 - 90 μm	4, 6, 8	4.3.4
PC	FFF	*N. lecointei* #1	4, 6, 8	4.3.5
PC	FFF	*N. lecointei* #2	2, 4, 6	4.3.5
PC	FFF	*F. cylindrus* #1	4, 6, 8	4.3.5
PC	FFF	*F. cylindrus* #2	2, 4, 6	4.3.5
PC	FFF	*F. curta*	2, 4, 6	4.3.5
PC	FFF	*C. dichaeta*	2, 4, 6	4.3.5
PC	FFF	*P. alata*	2, 4, 6	4.3.5
PC	FFF	Flagellate	2, 4, 6	4.3.5
PC	FFF	Bacteria	4, 6, 8	4.3.5
PC	FFF	Multi-species #1	4, 6, 8	4.3.6
PC	FFF	Multi-species #2	2, 4, 6	4.3.6

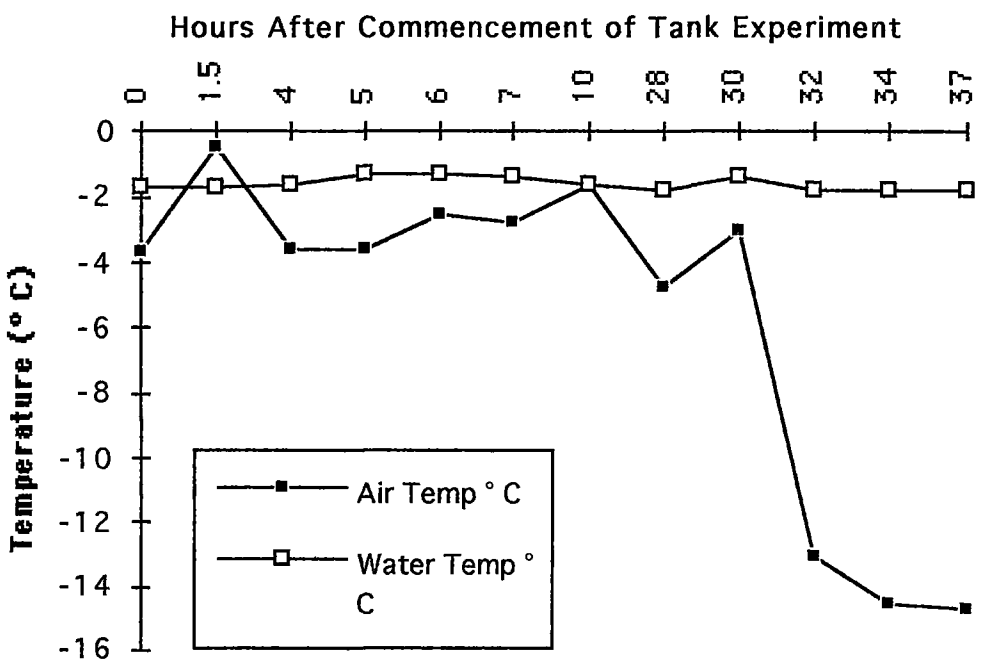
5.3.2 TANK EXPERIMENT INFORMATION...

The first three experiments show examples of:

1) rapidly freezing the water with no agitation; this experiments was labelled 'fast-freeze congelation' (FFC).
2) slowly freezing the water in the tank whilst agitating; this experiment was labelled 'slow-freeze frazil' (SFF).
3) rapidly freezing the water in the tank whilst agitating; these experiments were labelled 'fast-freeze frazil' (FFF).

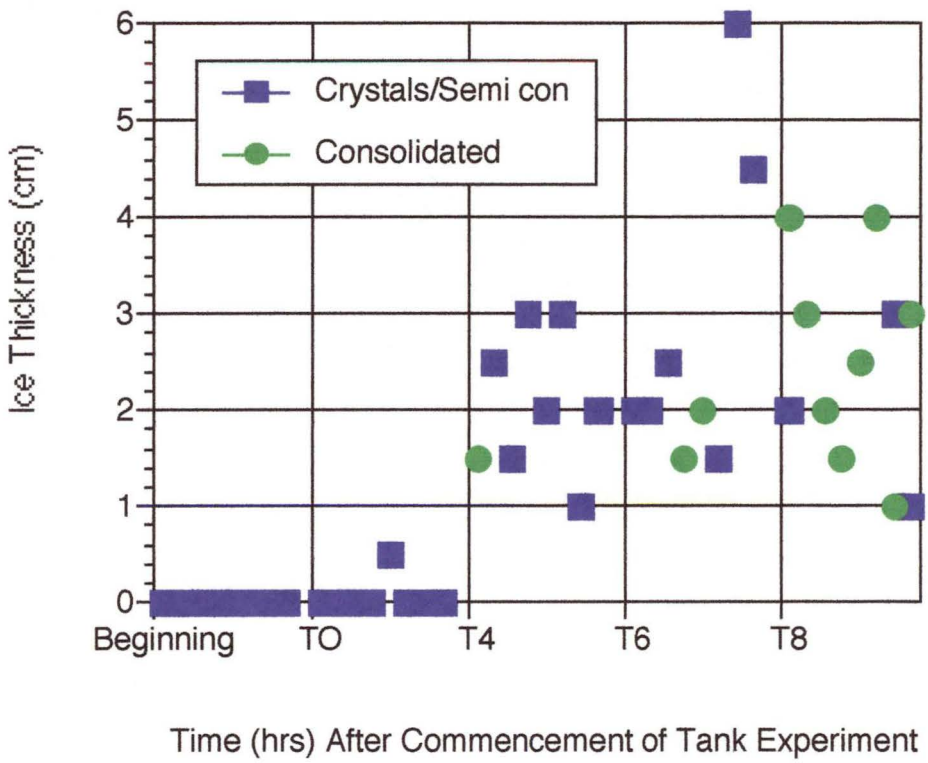
Fast-freeze frazil experiments most closely resembled the naturally occurring newly forming ice. Consequently fast-freeze frazil experiments were used for subsequent ice tank experiments. A typical temperature time series for a fast-freeze frazil experiment is shown below (Figure 5.4). Tinytalk temperature probes were used to measure the air and water temperatures in the ice tank. These probes recorded temperatures every two minutes for the duration of the experiment.

Figure 5.4 Shows a typical temperature series for a fast-freeze frazil experiment. The x-axis is not linear. There is a rapid decrease in air temperature after 30 hours, this is a result of the fast-freeze switch being activated. The water temperature remains relatively constant at - 1.8° C throughout the experiment.



Ice growth plots are shown in Figure 5.5. Agitation of the water mass and the short duration of the fast-freeze frazil experiments resulted almost entirely in frazil crystal formation.

Figure 5.5 Representation of the average ice growth during a fast-freeze frazil experiment. The y-axis represents ice thickness in cm. The x-axis represents time in hours after commencement of tank experiment. The graph shows the results of 8 different experiments (ie for each time there are 8 measurements). Blue squares represent semi-consolidated ice or crystals. Circles represent consolidated ice.



5.3.3 INCORPORATION OF TEMPERATE PHYTOPLANKTON

Initial ice tank experiments were conducted using temperate phytoplankton stocks. Phytoplankton was collected from the Derwent River estuary and refrigerated in the dark at 2° C for a brief period until required (usually < 24 hrs). Phytoplankton composition in the water was typically 55 % pennate diatoms, 15 % centric diatoms and 30 % flagellates. Results of FFC and SFF experiments are shown, as are FFF experiments.

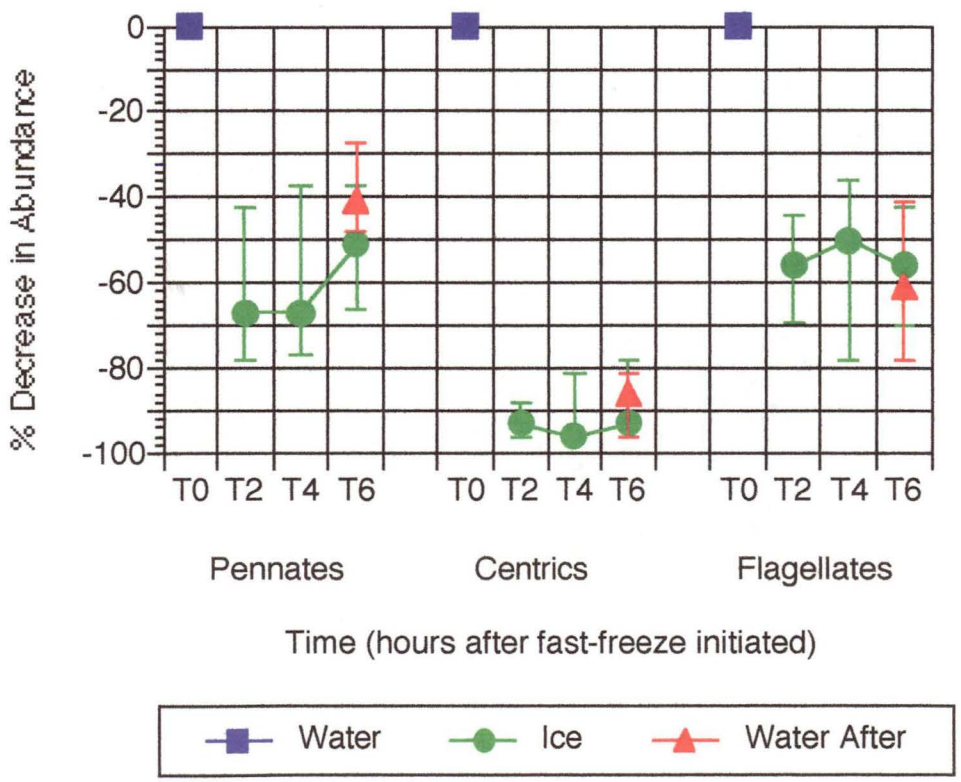
Paired, two tailed t-Tests were conducted using temperate phytoplankton numbers to compare samples. Summary information of statistical data is represented in Appendix 8.5. The following results use a P value ≤ 0.05 to test for significance. Any comparisons resulting in a $P \leq 0.05$ are considered to have a significantly different number of algal cells or microspheres.

Interpretation of Figures 5.6 - 5.8.

Three replicate counts of phytoplankton in the water at T_0 were recorded. These values were averaged and standardised to 100%, this value was then shown as zero on the figures. The numbers of algal cells in the subsequent ice and water samples were expressed as a decrease or increase relative to this value. Three replicate counts were recorded for ice and water $_{after}$ samples during the FFC and SFF experiments. Twelve replicate counts for the number of algal cells in the ice samples were recorded for the FFF experiment. Error bars represent the total range of algal cell numbers.

Fast-freeze congelation experiments

Figure 5.6 Fast-freeze congelation experiment using abundance of temperate phytoplankton species. The y-axis represents percentage decrease over initial cell concentrations in the water at T_0 (Time = 0 hrs). The x-axis represents time in hours after commencement of tank experiment. Four separate samples were collected, at 0 hrs, 2 hrs, 4 hrs and 6 hrs. A separate plot is shown for pennate diatoms, centric diatoms and flagellates.



Comparing initial phytoplankton levels in the water at T_0 with the levels in the water at the end of the experiment ($Water_{After}$) indicates that phytoplankton quickly settled out of suspension. Between 30 and 90 % less diatoms and flagellates were found in the ice samples compared to initial water samples.

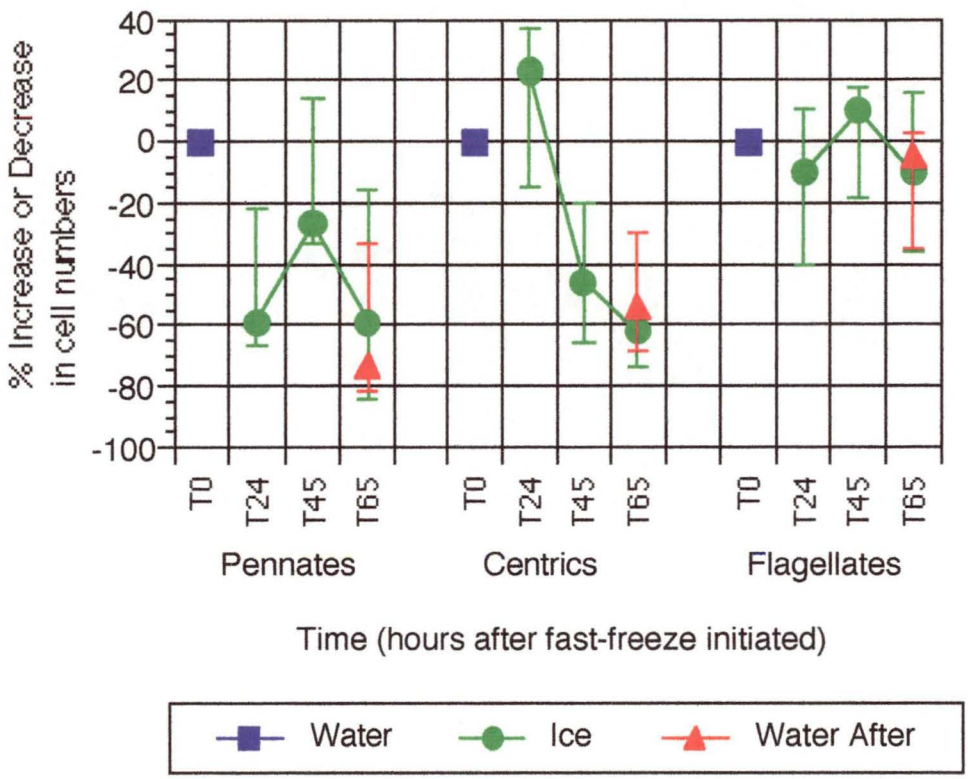
Table 5.2 Paired, two tailed t-Test analysis of samples in fast-freeze congelation experiment using temperate water algae. 'W' refers to water samples. 'I' refers to ice samples, 0, 2, 4 and 6 are all times in hours after commencement of the tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_{After}	I_2	I_4	I_6
Pennate Diatoms				
W_0	**0.02**	**0.01**	**0.01**	**0.02**
Centric Diatoms				
W_0	**0.01**	**0.01**	**0.01**	**0.01**
Flagellates				
W_0	**0.02**	**0.02**	**0.02**	**0.02**

The results of t-Test analysis of fast-freeze congelation samples indicate that all ice samples have significantly fewer algal cells than the initial water column sample (T_0). The number of flagellates, pennates and centric diatoms in the water at the end of the experiment was significantly lower than the number in the initial water column sample.

Slow-freeze frazil experiments

Figure 5.7 Slow-freeze frazil experiment using temperate phytoplankton species. The y-axis represents percentage increase or decrease over initial cell concentrations in the water pat T_0. The x-axis represents time in hours after commencement of tank experiment. Four separate samples were collected, at 0 hrs, 24 hrs, 45 hrs and 65 hrs. A separate plot is shown for pennate diatoms, centric diatoms and flagellates.



Eighty percent of pennate diatoms settled out of suspension (or were incorporated into the ice) during the experiment (T_0W versus W_{After}). Pennate diatoms were found in the ice samples at decreased levels compared to the initial water sample. However, there were slightly more pennate diatoms in the ice samples compared to the final water sample ($Water_{After}$). Sixty percent of centric diatoms settled out of suspension (or were incorporated into the ice) during the tank experiment. The number of centric diatoms in the ice at 24 hours was up to 40 % higher than the initially water sample. Centric diatom numbers decreased to 30 to 60% of the numbers in initial water sample. The number of flagellates in ice samples was similar to the initial water column number. Similarly only 5 % of flagellates settled out of suspension over the 65 hours of the experiment.

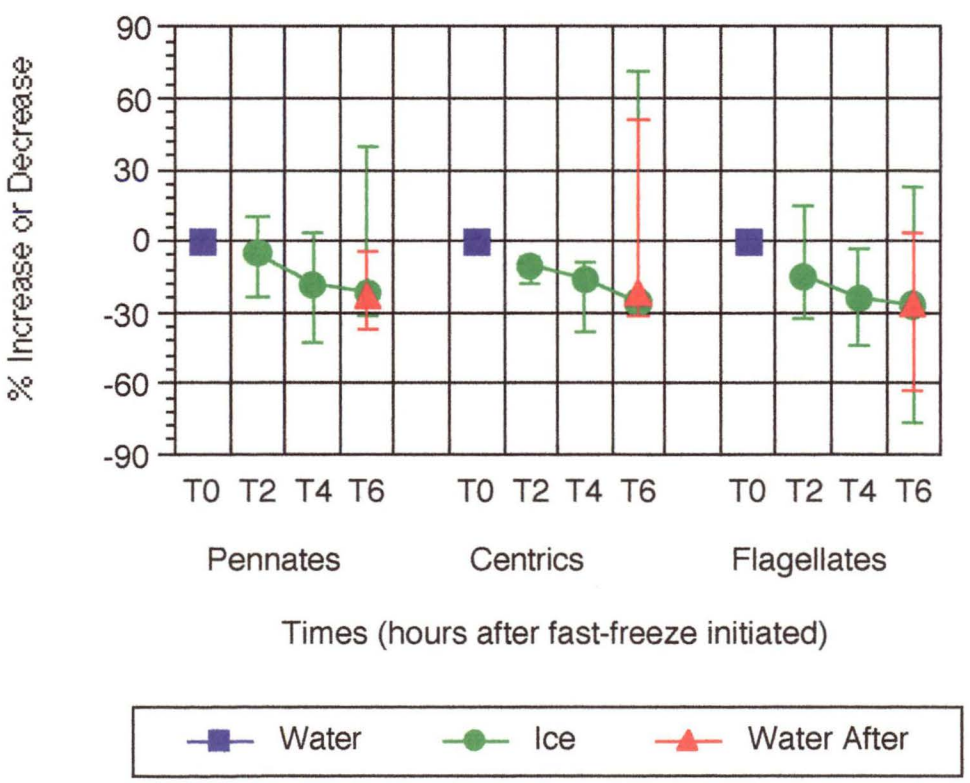
Table 5.3 Paired, two tailed t-Test analysis of samples in slow-freeze frazil experiment using temperate water algae. 'W' refers to water samples. 'I' refers to ice samples, 0, 24, 45 and 65 are all times in hours after commencement of the tank experiment. $P \leq 0.05$ confidence level is used.

	W_{After}	I_{24}	I_{45}	I_{65}
Pennate Diatoms				
W_0	0.13	0.15	0.59	0.24
Centric Diatoms				
W_0	0.23	0.41	0.24	0.22
Flagellates				
W_0	0.86	0.44	0.70	0.36

The results of t-Test analysis of slow-freeze frazil samples indicate no sample comparisons were significantly different.

Fast-freeze frazil experiments

Figure 5.8 Shows a Fast-Freeze Frazil experiment, using temperate phytoplankton species. The y-axis represents percentage increase or decrease over initial (cont...)



cell concentrations at T_0. The x-axis represents time in hours after commencement of tank experiment. Four separate samples were collected, at 0 hrs, 2 hrs, 4 hrs and 6 hrs. A separate plot is shown for pennate diatoms, centric diatoms and flagellates.

Combined results from three Fast-Freeze Frazil experiments are shown in Figure 5.8. The error bars encompass a large variation in algal cells numbers in the ice samples. However, general trends are evident. Pennate and centric diatoms, and flagellates in the water at the end of the experiment show a reduction over the initial water values. The number of cells in the water after samples is similar to the number in the ice samples from the 6 hour sampling period.

The importance of slurry samples was not fully appreciated at this stage. Thus, slurry samples were not collected.

Table 5.4 Paired, two tailed t-Test analysis of samples in fast-freeze frazil experiment using temperate water algae. 'W' refers to water samples. 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of the tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_{After}	I_4	I_6	I_8
Pennate Diatoms				
W_0	**0.03**	0.06	0.06	0.10
Centric Diatoms				
W_0	**0.01**	**0.05**	**0.03**	0.20
Flagellates				
W_0	0.40	0.53	0.13	0.51

The results of t-Test analysis of fast-freeze frazil experiment indicate that the number of pennate and centric diatoms in the water at the end of the experiment is significantly less than the number originally in the water column (T_0). The number of centric diatoms in the ice samples form T_4 and T_6 is significantly less than the number in the initial water sample.

5.3.4 INCORPORATION OF MICROSPHERES...

Glass microspheres were selected to cover a typical diatom size range. The size ranges used in the experiments were: 1 - 32 μm, 32 - 38 μm, 38 - 53 μm, 53 - 63 μm and 63 - 90 μm. Water and ice samples were collected from the ice tank and microsphere numbers were recorded. Three replicate counts were made of each sample. Replicate count information was used for Paired two-tailed t-Test analysis. Summary information of statistical data is represented in Appendix 8.5. The following results use a P value ≤ 0.05 to test for significance. Any comparisons resulting in a $P \leq 0.05$ are considered to have a significantly different in number of microspheres. The importance of slurry samples was not appreciated at this stage, consequently slurry was not collected.

Interpretation of Figures 5.9 - 5.13.

The number of microspheres in the water at the commencement of the experiments was up to 8000 % higher than the number in the water 4 hours into the experiment. Microspheres rapidly settled out of suspension. Consequently, the number of microspheres in the water 4 hours into the experiment was standardised to 100 % and shown as zero. The numbers of microspheres in the ice and water samples were expressed as a decrease or increase relative to this value. Water samples had a narrow range of error bars and consequently the error bars may not be seen on the figures. Three replicate counts were recorded for ice and water samples. Error bars represent the total range of microsphere numbers.

Microsphere Experiment # 1 (1 - 32 μm)

There were 3000 % more microspheres in the water sample at T_0 compared to the water sample from T_4. The number of microspheres in the ice samples varies between 50 % and 700 % of the number in the water sample from T_4 (Figure 5.9, overleaf). The general trend indicates ice samples contain roughly 2-3 times the number of microspheres than the corresponding water samples. The error bars on these measurements are large.

Figure 5.9 Microsphere Experiment #1 (1 - 32 μm). The x-axis represents sample time in hours after the fast-freeze switch was activated (ie T_4 = 4 hours after). The y-axis represents percentage increase or decrease over the initial number of microspheres in the water at T_4. The microspheres quickly settled leaving a relatively constant number in the water from T_4 to T_8.

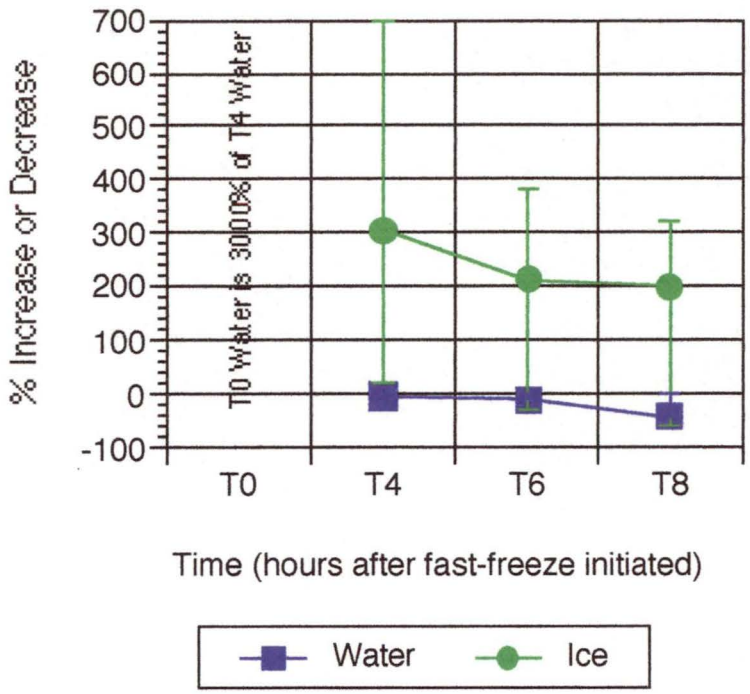


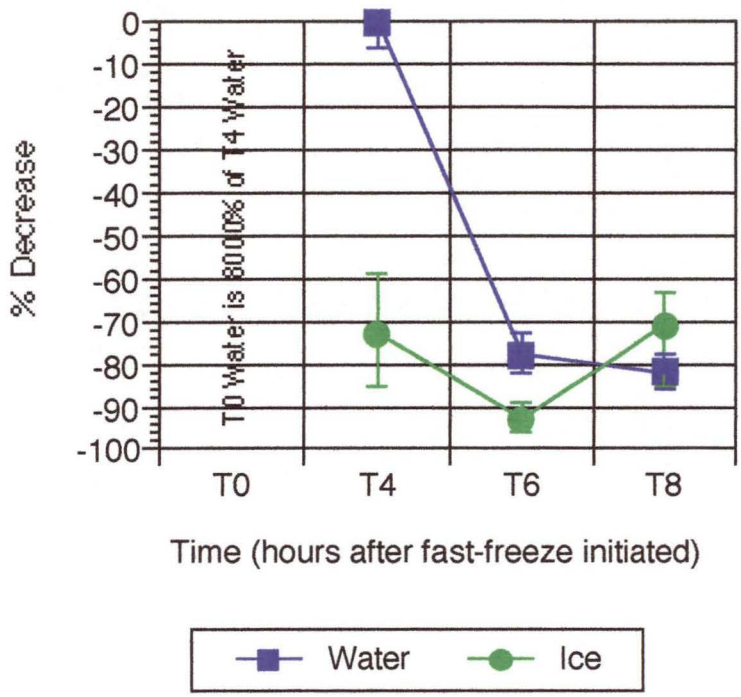
Table 5.5 Paired, two tailed t-Test analysis of Microsphere Experiment # 1. 'W' refers to water samples, 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_0	W_4	W_6	W_8	I_4	I_6	I_8
W_0		0.013	0.013	0.013	0.005	0.015	0.015
W_4			0.612	0.448	0.250		
W_6				0.737		0.264	
W_8							0.231

Paired two-tailed t-Test analysis compared the number of microspheres in the ice and water samples. Results indicated all water samples and ice samples have significantly less microspheres than the original water sample (Table 5.5). No other water samples or ice samples are statistically different.

Microsphere Experiment # 2 (32 - 38 μm)

Figure 5.10 Microsphere Experiment # 2 (32 - 38 μm). The x-axis represents sample time in hours after the fast-freeze switch was activated (ie $T_4 = 4$ hours after). The y-axis represents the percentage decrease over the initial number of microspheres in the water at T_4. The initial microsphere numbers in T_0 were 8000 % higher than the number in the water at T_4.



Microsphere numbers in the water at T_0 were 8000 % higher than at T_4. Microsphere numbers continued to decrease until T_6. The number of microspheres in the ice samples was generally lower than the corresponding water samples and much lower than the initial water sample from T_0.

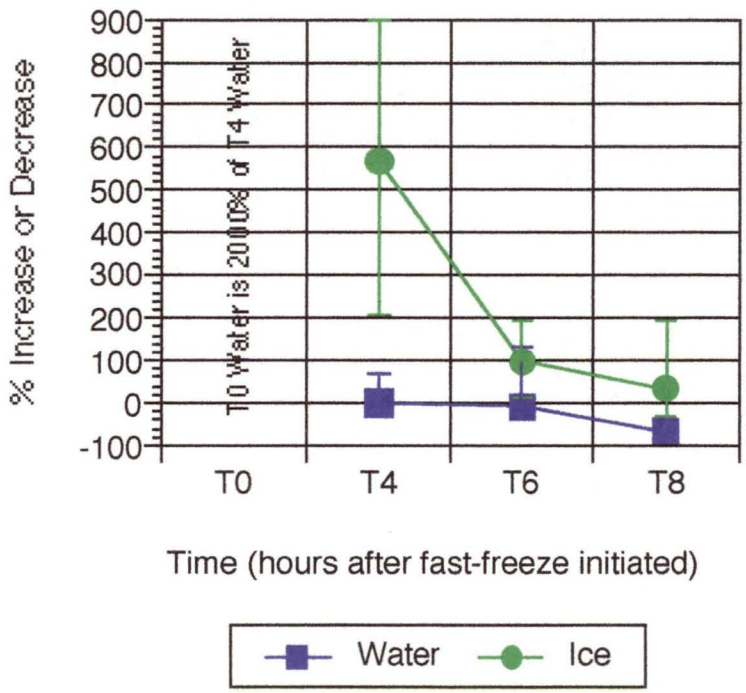
Table 5.6 Paired, two tailed t-Test analysis of Microsphere Experiment # 2. 'W' refers to water samples, 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_0	W_4	W_6	W_8	I_4	I_6	I_8
W_0		0.004	0.004	0.004	0.004	0.004	0.004
W_4			0.004	0.001	0.001		
W_6				0.234		0.028	
W_8							0.574

All water samples and ice samples had significantly less microspheres than the original water sample (Table 5.6). The number of microspheres in water samples at T_6 and T_8 is significantly lower than the number of microspheres in the water sample at T_4. The number of microspheres in ice samples at T_4 and T_6 is significantly lower than the number of microspheres in the corresponding water samples (T_4 and T_6).

Microsphere Experiment # 3 (38 - 53 μm)

Figure 5.11 Microsphere Experiment # 3 (38 - 53 μm). The x-axis represents sample time in hours after the fast-freeze switch was activated (ie $T_4 = 4$ hours after). The y-axis represents the percentage increase or decrease over the initial number of microspheres in the water at T_4.



Microsphere numbers in the water at T_0 were approximately 2000 % higher than those in the water at T_4. Microsphere numbers in the ice were initially high but quickly reduced to levels comparable with the corresponding water samples.

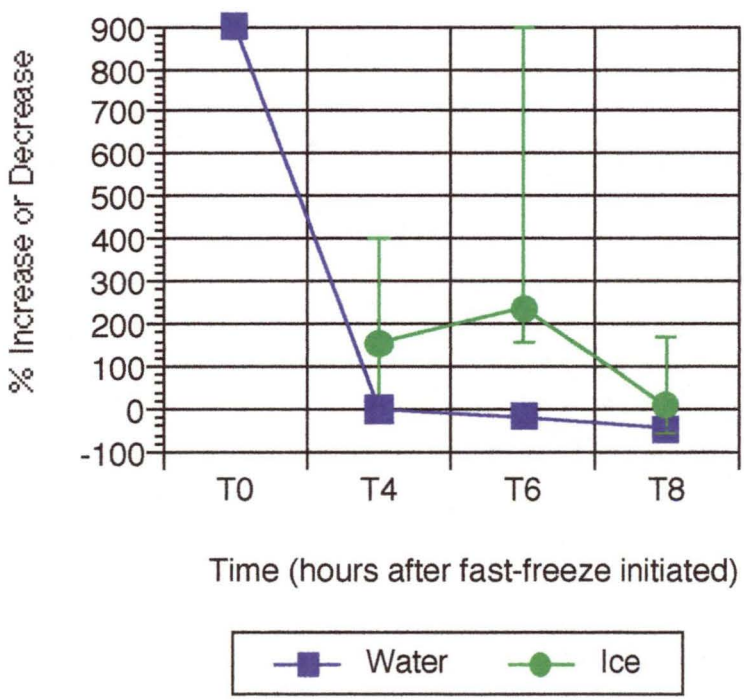
Table 5.7 Paired, two tailed t-Test analysis of Microsphere Experiment # 3. 'W' refers to water samples, 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_0	W_4	W_6	W_8	I_4	I_6	I_8
W_0		0.005	0.001	0.006	0.251	0.020	0.001
W_4			0.180	0.013	0.082		
W_6				0.533		0.455	
W_8							0.504

The numbers of microspheres in water samples from $T_{4,6,8}$ are significantly lower than the number of microspheres in the water samples from T_0. The numbers of microspheres in the ice samples from T_6 and T_8 are also significantly lower than the number found in the initial water sample. (Table 5.7). The water samples at T_8 has a significantly lower number of microspheres compared to the water sample collected at T_4. Ice samples did not contain a significantly different number of microspheres than the corresponding water samples.

Microsphere Experiment # 4 (53 - 63 μm)

Figure 5.12 Microsphere Experiment # 4 (53 - 63 μm). The x-axis represents sample time in hours after the fast-freeze switch was activated (ie $T_4 = 4$ hours after). The y-axis represents percentage increase or decrease over initial number of microspheres in the water at T_4.



Microsphere numbers at T_0 were 1000 % higher than the number in the water at T_4. Rapid initial settling of microspheres occurred. Microsphere numbers in the ice samples number up to 10 times the number in the underlying water samples.

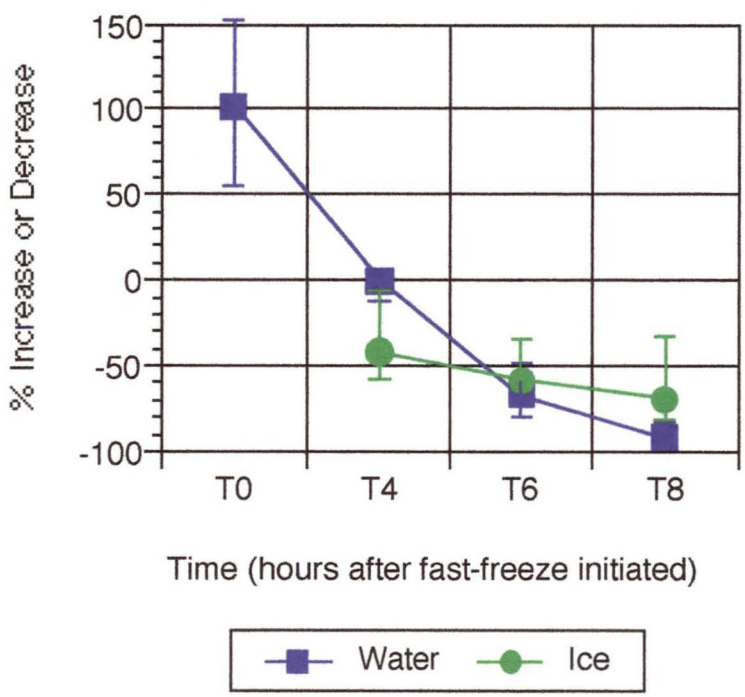
Table 5.8 Paired, two tailed t-Test analysis of Microsphere Experiment # 4. 'W' refers to water samples, 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_0	W_4	W_6	W_8	I_4	I_6	I_8
W_0		0.065	0.009	0.017	0.012	0.014	0.014
W_4			0.012	0.001	0.147		
W_6				0.157		0.538	
W_8							0.265

All ice samples and water from T_6 and T_8 are significantly different to initial water samples (Table 5.8). Water from T_6 and T_8 is also different to the water sample collected at T_4.

Microsphere Experiment # 5 (63 - 90 μm)

Figure 5.13 Microsphere Experiment # 5 (63 - 90 μm). The x-axis represents sample time in hours after the fast-freeze switch was activated (ie $T_4 = 4$ hours after). The y-axis represents percentage increase or decrease over initial number of microspheres in the water at T_4.



Settling of large microspheres is shown in Figure 5.13. The number of microspheres present in the ice and water samples are very low. Only 5 % of the microspheres

found in the initial water sample (T_0) are still in suspension in the water samples from T_8.

Table 5.9 Paired, two tailed t-Test analysis of Microsphere Experiment # 5. 'W' refers to water samples, 'I' refers to ice samples, 0, 4, 6 and 8 are all times in hours after commencement of tank experiment. $P \leq 0.05$ confidence level is used. Significant comparisons are shown in red.

	W_0	W_4	W_6	W_8	I_4	I_6	I_8
W_0		0.000	0.000	0.000	0.141	1.000	0.302
W_4			0.322	0.080	0.279		
W_6				0.176		0.210	
W_8							0.385

All water samples had a significantly lower number of microspheres compared to the initial number in the water at T_0. No comparisons between ice and water samples were found to be significant.

5.3.5 INCORPORATION OF ANTARCTIC PHYTOPLANKTON

The first four plots presented in this section highlight the importance of collecting slurry samples. Other earlier experiments, during which slurry samples were not collected, have not been included in this presentation of results. Figures 5.14 and 5.15 and Figures 5.16 and 5.17 present similar profiles for the number of *Nitzschia lecointei* and *Fragiliaropsis cylindrus* cells (respectively) in the water and ice samples. Only Figures 5.15 and 5.17 show slurry sample information. Up to eight times more algal cells are found in the slurry samples compared to the initial water samples (T_0) subsequent water samples and ice samples.

Interpretation of Figures 5.14 - 5.22.

The number of algal cells in the initial water sample was standardised to 100 % and shown as zero. The number of algal cells in the ice and water samples was expressed as a decrease or increase relative to this value. Some samples had a narrow range of error bars and consequently the error bars may not be seen on the figures. Three replicate counts were recorded for water, slurry, ice and settled water samples. Error bars represent the total range of algal cell numbers. Replicate counts were not carried out for the *Proboscia alata* (Figure 5.20) and flagellate (Figure 5.21) experiments, thus error bars and t-Test results are not shown. The relative percentage of algal cells in the water at the end of the experiment is shown by the blue squares in the T_8 category. The water samples shown in the 'end' category represents the relative percentage of algal cells which had settled out of suspension.

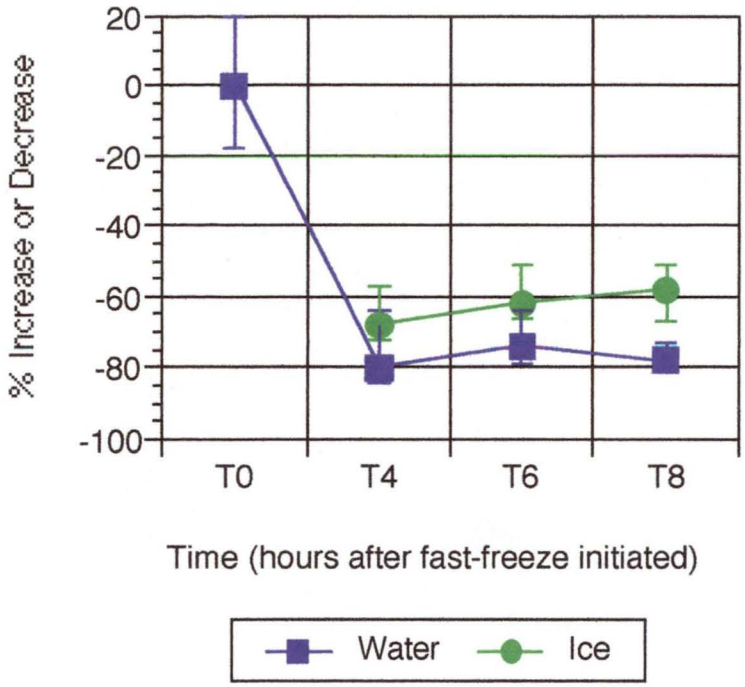
Nitzschia lecointei

There is an initial decrease in the concentration of algal cells in the water (T_0 - T_4), (Figure 5.14, overleaf). This is probably due to *N. lecointei* cells settling out of suspension. Concentrations of *N. lecointei* in the ice and underlying water are very similar. Slightly more cells are included in the ice. The number of *N. lecointei* cells in all the water and ice samples is very much lower than the number in the initial water sample at T_0.

Table 5.10 Results of Paired two-tailed t-Test analysis of *N. lecointei* Experiment # 1. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

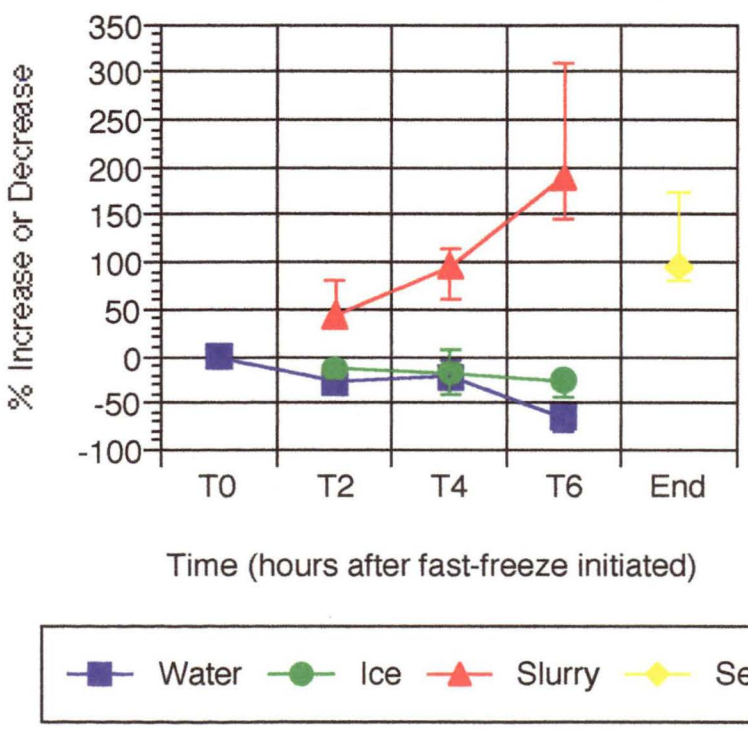
	W4	W6	W8	I4	I6	I8
W0	**0.03**	**0.04**	**0.04**	0.06	**0.04**	0.07
W4		0.78	0.62	0.62		
W6			0.64		0.17	
W8						**0.04**

Figure 5.14 *N. lecointei* Experiment #1. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 4, 6 and 8 hours).



Paired two-tailed t-Test analysis of *N. lecointei* numbers in ice and water samples indicates water samples from T_4 onwards, had significantly fewer *N. lecointei* cells. The ice sample from T_8 has significantly more *N. lecointei* than from the corresponding water sample.

Figure 5.15 *Nitzschia lecointei* Experiment # 2. The y-axis (cont...)



represents percentage increase or decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to the settled-water sample.

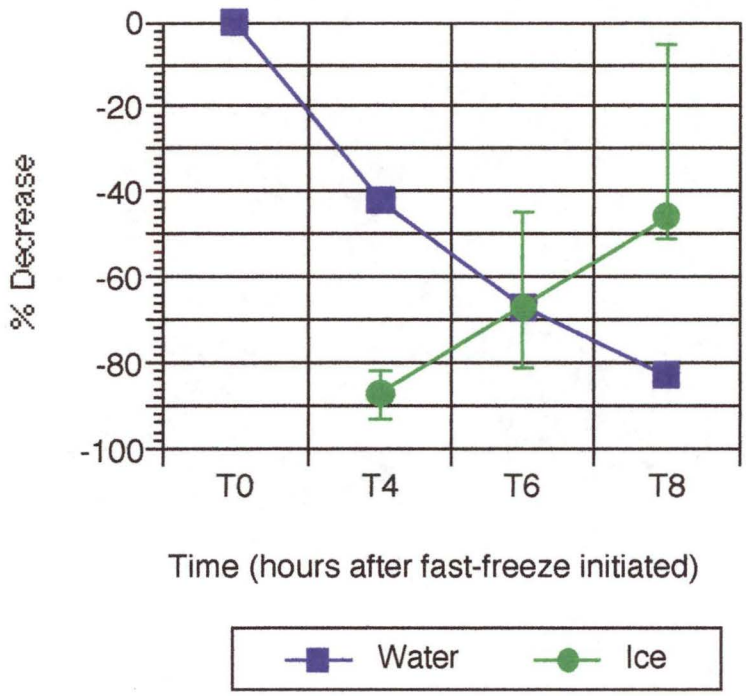
There is a increase in the number of algal cells in the slurry samples over the initial number in the water column. At T_2 there are roughly twice the number of cells in the slurry samples compared to water and ice samples of the corresponding time. At T_8 this has increased to roughly 8 times the number of cells in the slurry compared to the underlying water from T_8. The time series of ice and water samples concur with the previous experiment interms of reduction in cell numbers post the initial water sample. The amount of settling occurring over the duration of the experiment is indicated by the yellow marker on Figure 5.15. The importance of settling is discussed in Section 5.4.

Table 5.11 Results of Paired two-tailed t-Test analysis of *N. lecointei* Experiment # 2. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

	W2	W4	W6	Wset	S2	S4	S6	I2	I4	I6
W0	**0.01**	**0.04**	**0.00**	**0.04**	0.08	**0.03**	**0.02**	**0.03**	0.34	**0.02**
W2		0.54	**0.02**		**0.04**			0.16		
W4			**0.02**			**0.01**			0.52	
W6										**0.02**
S2								**0.05**		
S4									**0.01**	
S6										**0.01**

Statistical analysis of the second *N. lecointei* experiment reveals the water samples at T_8 have a significantly lower number of cells to the other water samples. The majority of slurry samples have a significantly higher number of cells compared to the underlying water and ice samples.

Figure 5.16 *Fragiliaropsis cylindrus* Experiment # 1. The y-axis represents percentage decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 4, 6 and 8 hours).



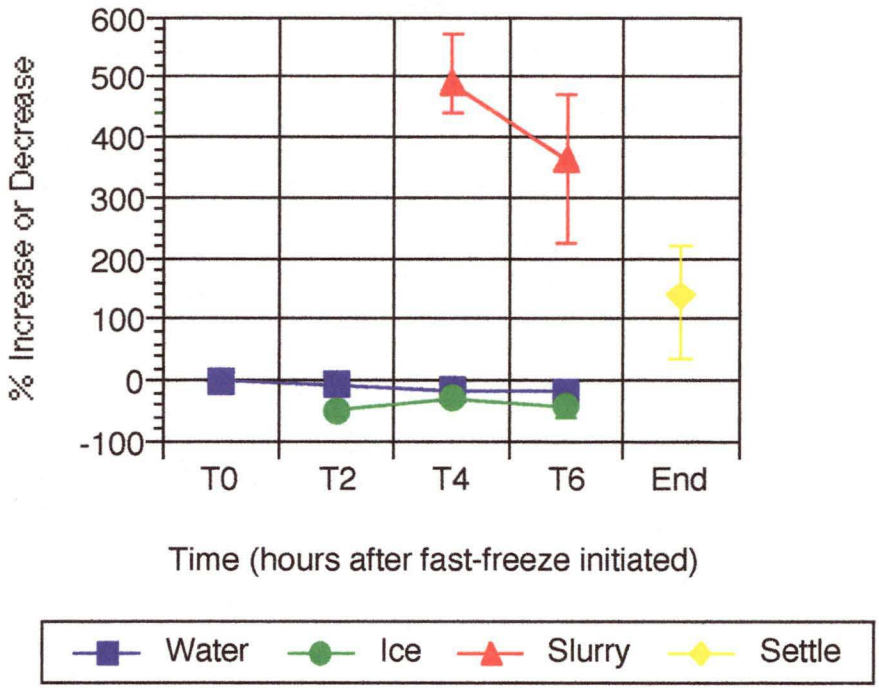
There is a decrease in the number of *F. cylindrus* in the water column after the initial water sample, Figure 5.16. This is probably due to settling of *F. cylindrus* out of suspension. The concentration of cells in the ice slightly increases above the numbers in the initial ice sample (T_2). However, the number of *F. cylindrus* cells in the ice remains lower than the initial number in the water column at T_0.

Table 5.12 Results of Paired two-tailed t-Test analysis of *F. cylindrus* Experiment #1. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

	W4	W6	W8	I4	I6	I8
W0	0.07	**0.00**	**0.00**	**0.00**	**0.01**	0.09
W4		**0.05**		0.06		
W6			0.18		0.86	
W8						**0.01**

The water and ice samples at T_4 and T_6 were significantly lower than the initial water samples at T_0. The ice sample at T8 had significantly more *F. cylindrus* cells compared to the water sample from the corresponding time.

Figure 5.17 *Fragiliaropsis cylindrus* Experiment # 2. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to settled water sample.



The second experiment using *F. cylindrus* (Figure 5.17) revealed high concentrations of algal cells in the slurry samples. Up to five times the number of cells in the water samples were found in the slurry samples. The number of *F. cylindrus* cells in ice samples was roughly half that in the corresponding water samples. Settling of *F. cylindrus* cells out of the water column is evident (refer to the 'settle' sample in Figure 5.17).

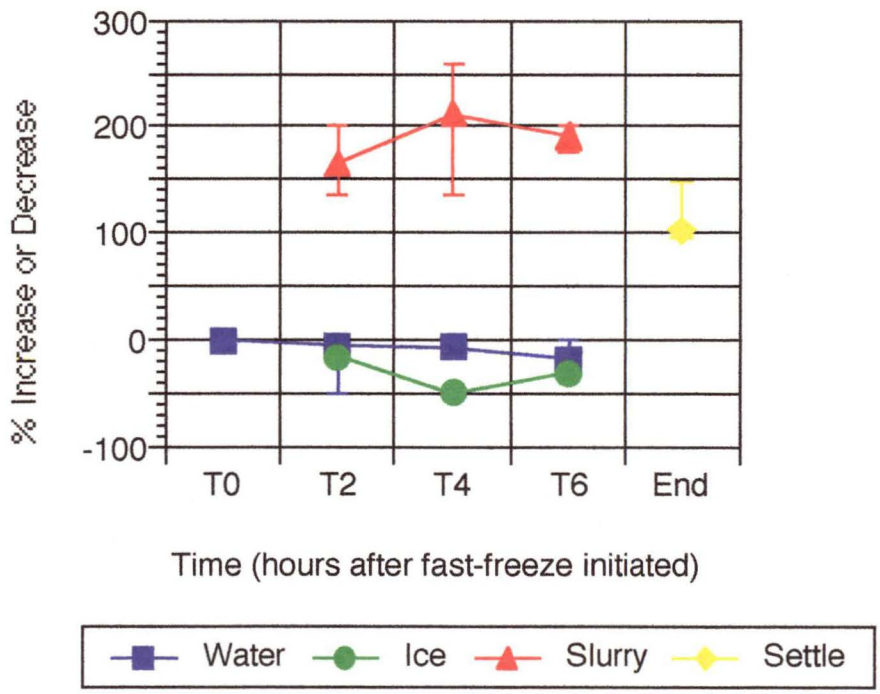
The results of t-Test analysis of *F. cylindrus* Experiment #2 are shown in Table 5.13 (overleaf). Slurry samples and the settled water sample have a significantly higher number of algal cells than the water and ice samples.

Table 5.13 Results of Paired two-tailed t-Test analysis of *F. cylindrus* Experiment #2. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

	W4	W6	W8	Wset	S4	S6	S8	I4	I6	I8
W0	0.93	0.11	0.13	**0.03**	No Data	**0.00**	**0.02**	0.10	0.25	0.25
W4		0.74	0.31		No Data			0.06		
W6			0.19			**0.00**			0.48	
W8							**0.03**			0.68
S4								No Data		
S6									**0.00**	
S8										**0.02**

Fragiliaropsis curta

Figure 5.18 *Fragiliaropsis curta* tank experiment. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to settled water sample.



Slurry samples were found to contain four times the number of *F. curta* cells present in underlying water, Figure 5.18. Water and ice samples were found to contain approximately the same number of algal cells. Twice the number of algal cells settled out of suspension.

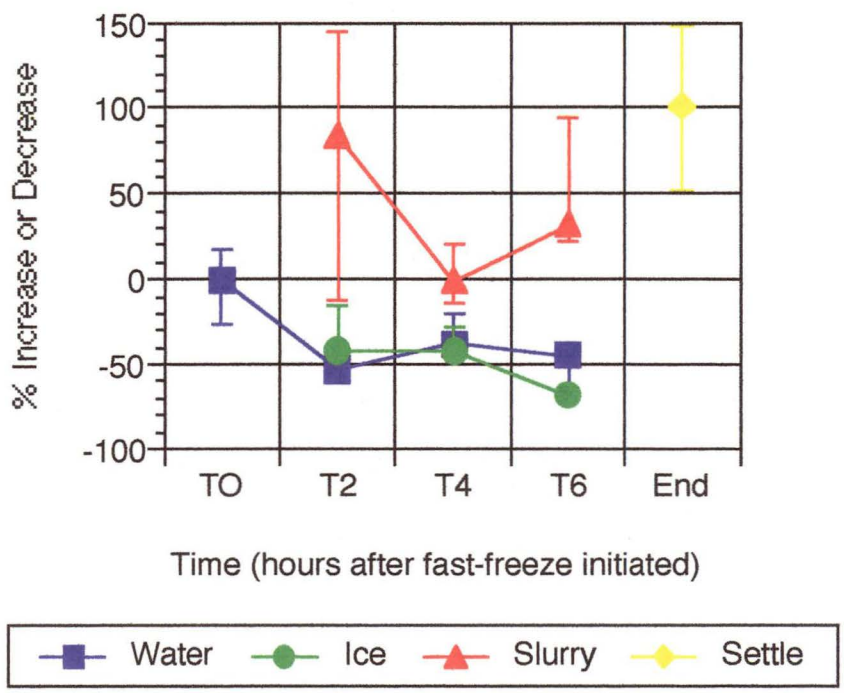
Table 5.14 Results of Paired two-tailed t-Test analysis of *F. curta* Experiment. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

	W_2	W_4	W_6	W_{set}	S_2	S_4	S_6	I_2	I_4	I_6
W_0	0.16	0.31	0.13	**0.01**	**0.01**	**0.01**	**0.00**	0.19	**0.04**	0.07
W_2		0.41	0.92		**0.01**			0.64		
W_4			0.35			**0.02**			**0.03**	
W_6							**0.00**			0.28
S_2								**0.02**		
S_4									**0.01**	
S_6										**0.00**

There were significantly more *F. curta* cells in the settled water sample than the initial water sample (T_0). Slurry samples had significantly more *F. curta* cells than both the ice and water samples (Table 5.15). Ice samples at T_6 are significantly different to water samples at T_0 and the water at T_6.

Chaetoceros dichaeta

Figure 5.19 *Chaetoceros dichaeta* tank experiment. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis represents time in hours after fast-freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to settled water sample.



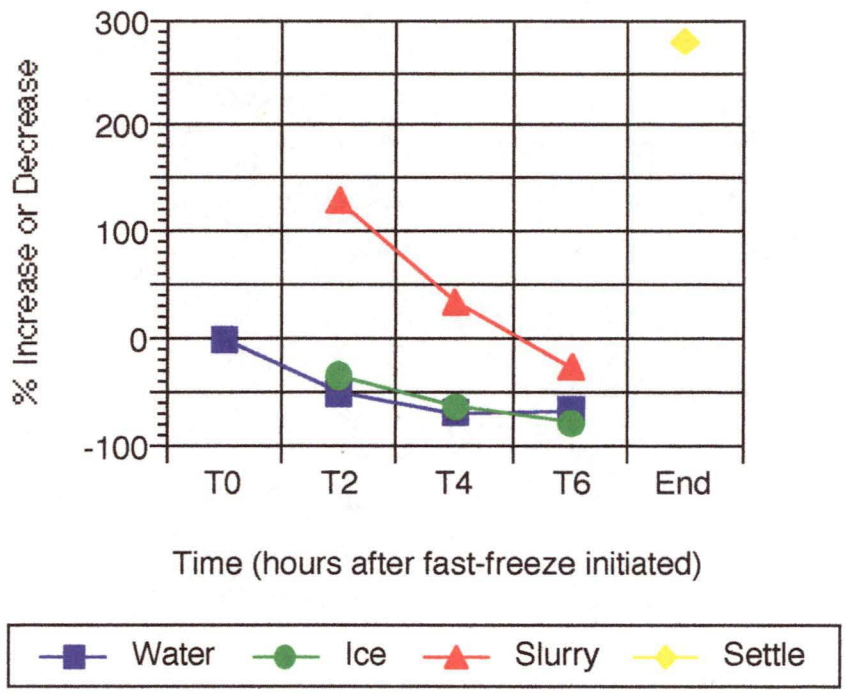
Slurry samples were found to contain up to four times the number of algal cells compared to the corresponding water samples. The large number of settled algal cells may account for the lower concentration of algal cells found in the T_4 and T_6 slurry samples. There was an initial decrease in the number of algal cells in the water, once again a reflection of the settling of cells and enrichment of cells into the ice.

Table 5.15 Results of Paired two-tailed t-Test analysis of *C. dichaeta* Experiment. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red. Overleaf...

	W_2	W_4	W_6	W_{set}	S_2	S_4	S_6	I_2	I_4	I_6
W_0	0.06	0.11	0.07	**0.02**	0.08	0.77	0.10	0.15	0.07	**0.02**
W_2		0.15	0.15		**0.05**			0.16		
W_4			0.24			**0.02**			0.70	
W_6							**0.04**			**0.05**
S_2								0.07		
S_4									**0.02**	
S_6										**0.03**

Paired two-tailed t-Test analysis reveals that the number of *Chaetoceros dichaeta* cells in the settled water sample ($Water_{set}$) is significantly higher than the number of cells in initial the water sample(W_0), (Table 5.16). Slurry samples from all times have a significantly higher number of *Chaetoceros dichaeta* cells compared to the water samples and ice at T_6 and T_8. Results of paired two-tailed t-Test analysis indicate a significant number of *C. dichaeta* cells settled out of suspension compared to their initial numbers in the water sample at T_0.

Figure 5.20 *Proboscia alata* experiment. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis is time in hours after fast freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to settled water sample.



The slurry sample at T_2 contained four times the number of *P. alata* cells compared to the corresponding water sample. There was a decrease in the number of cells in the slurry after this initial measurement. The high settled water value and the size of the diatom indicate that settling may responsible for the decrease in the number of cells in the subsequent slurry samples (ie there was a significant decrease in the number of *P. alata* in the water column).

Table 5.16 Results of Paired two-tailed t-Test analysis of *P. alata* Experiment. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

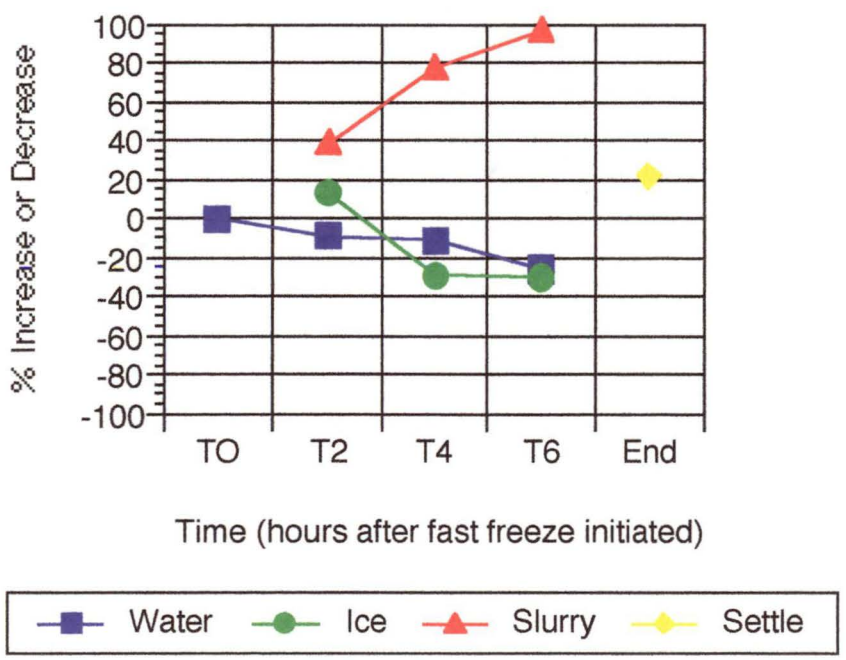
	W2	W4	W6	Wset	S2	S4	S6	I2	I4	I6
W0	**0.01**	**0.04**	**0.00**	**0.00**	**0.03**	**0.05**	0.10	**0.02**	**0.00**	**0.00**
W2		0.54	0.18		**0.02**			0.11		
W4			0.72			**0.00**			**0.03**	
W6							**0.01**			0.28
S2								**0.02**		
S4									**0.01**	
S6										**0.01**

Results of paired two-tailed t-Test analysis indicate all water and ice samples had a significantly lower number of *P. alata* cells compared to the initial water samples at T_0 (Table 5.17). Slurry samples at T_2 and T_4 are also significantly different to the initial water samples. The number of cells in the slurry samples was significantly higher than the number in the corresponding water and ice samples. A significant number of *P. alata* cells settled out of suspension compared to the initial water sample at T_0.

Flagellates

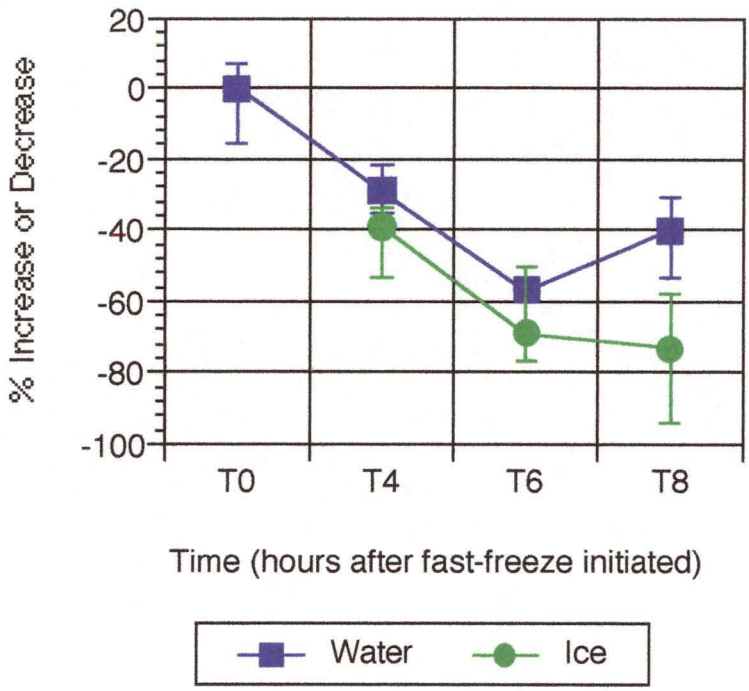
The flagellate experiment used small autotrophic flagellates (3 μm diameter). The inclusion of the flagellates into the interstitial slurry imitates the inclusion seen with diatoms, but to a lesser extent. Up to 2.5 times more flagellates were found in the slurry than in the corresponding water sample. Ice and water concentrations were similar. Only 20 % of flagellates settled out of suspension (or were incorporated into the ice) at the end of the experiment.

Figure 5.21 Flagellate experiment. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis is time in hours after fast freeze switch was activated (ie 2, 4 and 6 hours). 'End' refers to settled water sample.



Bacteria

Figure 5.22 Bacterial experiment. The y-axis represents percentage increase or decrease over cell numbers in water at T_0. The x-axis shows time in hours after fast-freeze switch was activated (ie 4, 6 and 8 hours).



A single experiment using bacteria was carried out. No slurry samples were collected. No increase in bacterial cells over initial numbers in the water (at T_0) was evident in the ice samples. A decline in bacterial numbers occurs with time in both the water and ice samples.

Table 5.17 Results of Paired two-tailed t-Test analysis of Bacteria Experiment. A confidence level of $P \leq 0.05$ is used to test for significance. Significant comparisons are shown in red.

	W4	W6	W8	I4	I6	I8
W0	**0.00**	**0.00**	**0.04**	**0.01**	**0.00**	**0.00**
W4		**0.04**	0.06	0.91		
W6			**0.01**		1.00	
W8						**0.03**

Water samples from T_4, T_6 and T_8 had significantly fewer bacterial cells compared to the initial water sample (T_0), (Table 5.17). The water samples from T_6 had a significantly lower number of bacterial cells compared to water samples from T_4. All ice samples had a significantly lower number of bacterial cells compared to the initial

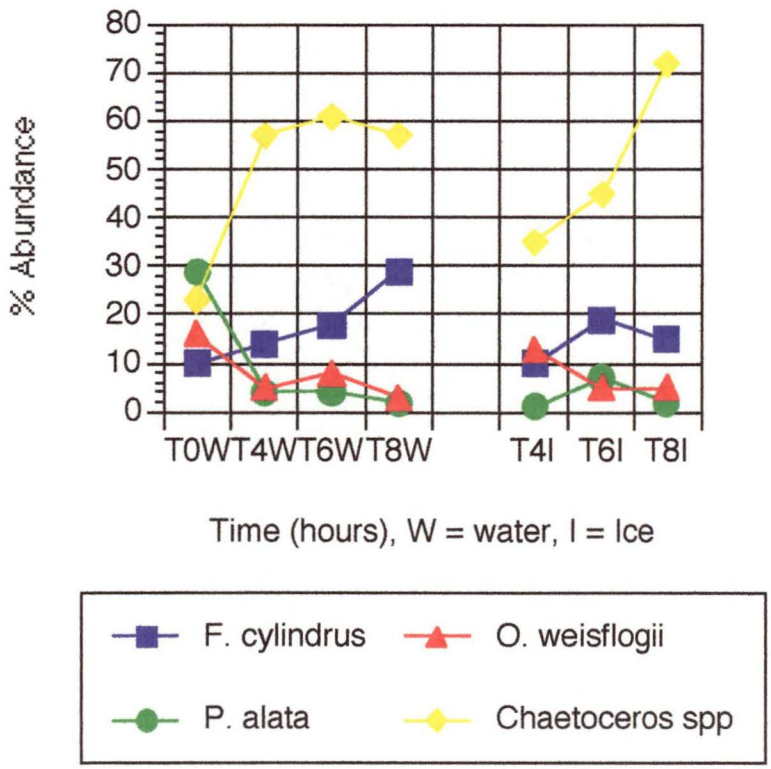
water samples. The ice samples at T_8 has significantly lower number of bacterial cells compared to the water sample for T_8.

5.3.6 MULTI-SPECIES EXPERIMENTS...

Cultures containing up to twelve polar diatom species were released into the ice tank. The Figures below show the percentage abundance for the four most abundant species in each of the two experiments. Results of the first experiment are shown in Figure 5.23 and Table 5.18. Results of the second experiment are shown in Figure 5.24 and Table 5.19. Replicate counts were not carried out, consequently error bars are not shown. Slurry samples were collected during the second experiment but not the first.

Multi-species Experiment # 1

Figure 5.23 Multi-species Experiment # 1. The y-axis represents percentage abundance. The x-axis shows time in hours after fast-freeze switch was activated (ie 4, 6 and 8 hours). 'W' refers to water samples. 'I' refers to ice samples.



The first multi-species figure shows *Chaetoceros* to be the dominant species in the water column and ice samples. *Proboscia alata* is more dominant in the initial water sample at T_0, but this species quickly settles out of suspension. It comprises only approximately 5 % of algal abundance in later water and ice samples. Similarly *O.*

weisflogii is not as abundant in subsequent water and ice samples compared to the initial water sample. The abundance of other species is shown in Table 5.18.

Table 5.18 Species abundance expressed as a percentage of 100, in Multi-species Experiment # 1.

	T_0W	T_4W	T_4I	T_6W	T_6I	T_8W	T_8I
Chaetoceros simplex	3	2	17	3	6	1	4
Chaetoceros species	23	57	35	61	45	57	72
Fragiliaropsis curta	6	11	4	3	7	3	2
F. cylindrus	10	14	10	18	19	29	15
Navicula glaciei	5	5	5	2	5	4	4
Nitzschia lecointei	8	2	15	1	6	0	0
Odontella weisflogii	16	5	13	8	5	3	5
Proboscia alata	29	4	1	4	7	2	2
Dinoflagellate species	2	0	1	0	0	1	0

Multi-species Experiment # 2

Figure 5.24 Multi-species Experiment #2. The y-axis represents percentage abundance. The x-axis shows time in hours after the fast-freeze switch was activated (ie 4, 6 and 8 hours). 'W' refers to water samples. 'S' refers to slurry samples. 'I' refers to ice samples.

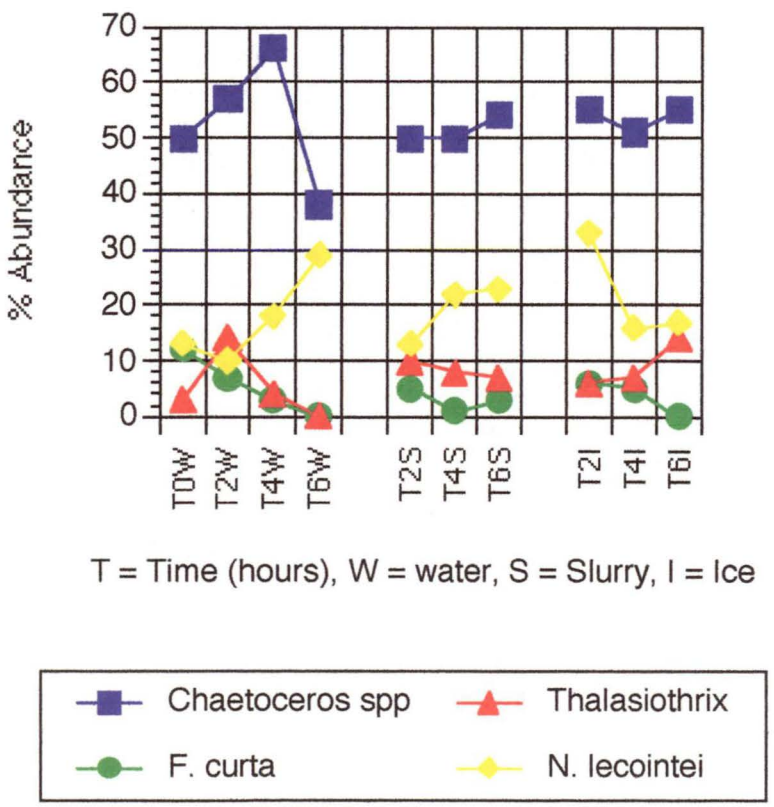


Table 5.19 Species abundance in Multi-species Experiment # 2.

	T_0W	T_2W	T_4W	T_6W	T_2S	T_4S	T_6S	T_2I	T_4I	T_6I
Chaetoceros spp	50	57	66	38	50	50	54	55	51	55
C. criophillum	4	0	0	0	5	1	1	0	5	0
Corethron spp	2	0	1	0	0	1	3	0	0	0
F. curta	12	7	3	0	5	1	3	6	5	0
F. cylindrus	6	6	7	14	6	7	4	0	5	7
N. lecointei	13	10	18	29	13	22	23	33	16	17
N. subcurvata	2	3		0	5	2	2	0	9	0
Rhizosolenia spp	6	3	0	19	6	6	0	0	2	5
Thalasiothrix	3	14	4	0	10	8	7	6	7	14
Other	2	0	1	0	0	2	3	0	0	2

The second multi-species figure shows *Chaetoceros* to be the dominant species. *Fragiliaropsis cylindrus*, *P. alata* and *O. weisflogii* combine to account for most of the rest of the algal community. The relative abundance of *Chaetoceros* species increases in the water samples as other species settle out of the water. *Chaetoceros* species are also dominant in the ice comprising up to 70 % of the algal entrained. *Proboscia alata* and *O. weisflogii* quickly settle out of the water column. The abundance of other species is shown in Table 5.19.

5.4 DISCUSSION

The aim of this chapter was to investigate whether phytoplankton inclusion in sea ice results from physical or biological mechanisms. It sought to clarify results of Chapter 3 which suggested phytoplankton buoyancy (as a result of wind or wave agitation or natural buoyancy) is linked to the extent of its incorporation in ice. It also compared the extent of polar phytoplankton inclusion in sea ice versus temperate species inclusion. This chapter further investigated the use of artificial sea ice tanks as a tool for investigating algal inclusion in sea ice.

5.4.1 CONSTRUCTION OF THE ICE TANK...

The design of the ice tank described in this chapter was structured in order to replicate the natural environment as closely as possible. Ice growth rates in the ice tank were consistent with growth rates in areas of newly forming sea ice in Antarctica (Weeks and Ackley, 1982). Visual examination of tank ice crystal size and shape, indicated crystals were similar to naturally forming frazil crystal. Optical examinations of ice crystals to determine crystal orientation was not carried out. Weissenberger and Grossmann (1998) also found it was possible to reproduce ice formation processes in the laboratory. In addition, they believed size, shape and orientation of the ice crystals, were not influenced by tank effects.

The temperature regime used in the tank effectively produced ice at growth rates of around 5 -10 mm/hr (Figure 5.4 shows water and air temperature time series). Slow-cooling of the water in the tank was possible by adjusting the thermostat control switch. Fast-freezing of water in the tank was induced by by-passing the thermostat.

Paddle wheel agitation allowed formation of frazil crystals for the duration of the eight hour experiments. Without agitation, transition and congelation crystals rapidly formed and enrichment of phytoplankton in the ice was greatly reduced. The ice tank and agitation system design was structured to enable an investigation into algal incorporation as a result of water moving (or being pushed) through the newly-forming ice. While the tank was not designed to produce waves, a similar physical

process was occurring (albeit on a smaller scale). Results of experiments revealed that the action of water moving through the ice, enriched algal or microsphere numbers in slurry samples up to 10 times over the corresponding numbers of algal cells and microspheres in the water column (Sections 5.4.4 and 5.4.5). These results concur with Weissenberger and Grossmann (1998) who found wave action resulted in algal enrichment of up to 9 times. The processes they describe, were the mixing of floating ice crystals with underlying water and pumping of water into the underlying ice matrix by periodical expansion and compression of the slush ice layer (both being responsible for this wave induced enrichment of algal cells). In the same way that aggregated frazil crystals filter algal cells in Langmuir Cells (Martin, 1981), ice crystals act as a filter to phytoplankton in a propagating wave field. Or on a smaller scale the filtering process also occurs when water is pushed through the ice matrix by a paddle wheel. Wave field enrichment is not a new concept (refer Ackley *et al* 1987, Shen and Ackermann 1988), but until this study and the work of Weissenberger and Grossmann (1998) its effects had not been duplicated in the laboratory using algal cultures.

While Reimnitz *et al* (1993) suggest that ice crystals do not have adhesive properties, it appears to be undisputed that, frazil ice crystals can at least, act as a filter for algal cells (Martin 1981). Garrison *et al* (1989), on the other hand indicated that the number of algal cells incorporated into newly-forming ice is a function of the number of encounters between ice crystals and cells.

5.4.2 SAMPLING STRATEGY...

Weissenberger and Grossmann (1998) suggest that the efficiency of the incorporation of ice algae changes over the duration of ice tank experiments. They suggest that ability of water to penetrate the ice decreases as the ice becomes more consolidated. This is reflected strongly in the results of the ice tank described in this chapter, and is discussed in greater detail in the 'efficiency versus time' part of Section 5.4.5. Sampling times were altered (from T_4, T_6 and T_8 to T_2, T_4 and T_6) to encompass more of the inclusion process.

Slurry Samples

During the early tank experiments slurry samples were not collected. Ice samples were collected in such a way which allowed some interstitial fluid drainage. Thus combining the later slurry and ice samples is not an appropriate way to relate later experiments to the earlier ones. The importance of sample the slurry is well

demonstrated in Figures 5.14 to 5.17 (refer Section 5.3.5). As described previously, slurry samples refer to the water which drained immediately from ice samples collected. As the majority of ice samples collected were, at most, loosely consolidated, there was always a fair amount of drainage.

Settling of Phytoplankton out of Suspension

Settling of phytoplankton out of suspension was evident in several experiments (eg *Proboscia alata* experiment [Figure 5.20]). Reimnitz *et al* (1993), also describe settling of phytoplankton species in their experimental ice tank. Settling occurs in artificial sea ice tanks because it is difficult to replicate the fine scale water circulation patterns of the natural environment. In the natural environment water circulation patterns such as Langmuir Circulation (Bainbridge, 1957; Stavn, 1971; Garrison *et al* 1989) operate to considerable depths (20-30 m). Thus algae, which may initially sink out of suspension, are re-suspended when caught in the up-welling leg of the Langmuir Cell.

It is difficult to relate the extent of settling seen in this tank to the extent to which this occurs in the natural environment. Later experiments included a sediment trap which provided an approximate estimate of the extent of settling in the tank. (see Section 5.2.4 for more details). Earlier experiments involved the release of algal cultures into the tank up to two days before the experiment was initiated. This technique was adopted to promote potential production of ice active substances, in near freezing water (Raymond *et al*, 1997). However, a large percentage of algal cells settled out of suspension over the two day period. Later experiments involved the release of algae into the ice tank 30 minutes prior to T_0. Algae was equilibrated for 2 days in a sealed container in near freezing water, rather than in tank.

5.4.3 INCORPORATION OF TEMPERATE PHYTOPLANKTON

No enrichment of temperate water phytoplankton was evident in the fast-freeze congelation experiment (Figure 5.6). The numbers of pennate diatoms, centric diatoms and flagellates in the ice samples were between 10 % and 50 % of the number of cells in the initial water sample (at T_0). The number of cells in the water samples also reduced with time. Weissenberger and Grossmann (1998) also found that in the absence of waves or current, the chlorophyll concentration in the ice was no different to water. Hence no enrichment occurred in non-agitated water.

Algal abundance in slow-freeze frazil experiments was presented in Figure 5.7. Pennate and centric diatoms settled out of suspension over time, flagellates became

relatively more abundant. Numbers of pennate diatoms and flagellates were maximised at T_{45}. Centric diatom numbers were highest at T_{24}. All of these numbers were much lower than those of the initial cell numbers in the water (T_0), however no samples were considered significantly different (Table 5.3). The increased abundance of the flagellates late in the experiment can be explained by the motile nature of the flagellates which allows them to avoid settling out of suspension.

The results of fast-freeze frazil experiments are presented in Figure 5.8. General trends indicate that enrichment of temperate phytoplankton species into ice does not occur even in a well agitated environment. Slurry samples were not collected. Enrichment of polar phytoplankton species in the ice samples from Section 5.3.5 was also not evident. In the absence of slurry samples it is difficult to ascertain whether temperate phytoplankton species are enriched in sea ice.

5.4.4 INCORPORATION OF MICROSPHERES...

The incorporation of sediment in sea ice is well documented (Babic *et al* 1992, Barnes *et al* 1991, Shen and Ackermann 1988, Wollenburg *et al* 1991 and Ackermann *et al* 1990). Their studies are directly relevant to the study of algal enrichment in sea ice. Clay, silt and sand particles encompass the size range as bacteria and algae (Reimnitz *et al* 1990).

Microspheres used in this experiment covered the range of 1 μm to 90 μm, with a specific gravity of 2.6. This compares with the specific gravity of the opaline cell walls of diatoms, with a specific gravity of about 2.0, (comprising up to 50 % of their dry weight), Strickland, (1965) from Smayda (1970). The specific gravity of cytoplasm in marine organisms varying from 1.03 to 1.10 (Jacobs 1935, from Smayda 1970). The specific gravity of sea water varies from about 1.021 to 1.028 (Smayda, 1970). The net result being both microspheres and diatoms are denser than sea water. Microspheres were approximately 50 % denser than equivalent sized algae.

Settling out of Suspension

Rapid settling out of suspension is evident for microspheres (Figures 5.9 - 5.13). Some reduction in microsphere numbers in the water samples can be linked to their inclusion in the ice, however, most of the decrease is related to a high rate of settling out of suspension. In a four-hour period between introduction of microspheres into the tank and the second set of samples large decreases in microspheres numbers were found (Table 5.20)

Table 5.20 Decrease in microsphere numbers between T_0 and T_4.

Size Range	Extent of Settling	Figure #
1 - 32 μm	96.6 %	5.9
32 - 38 μm	98.8 %	5.10
38 - 53 μm	96.6 %	5.11
53 - 63 μm	90.0 %	5.12
63 - 90 μm	50.0 %	5.13

Settling of large-sized microspheres (> 53 μm) was so rapid that the extent of decrease shown above is most probably inaccurate, as most of the settling would have occurred before the T_0 water sample was collected (this sample was collected 30 minutes after the microspheres were released into the tank [this allowed sufficient time for mixing]). These results concur with the findings of Reimnitz *et al* (1993), who found sand-sized particles > 53 μm suffered a high settling rate which precluded interaction with rising frazil in their experimental tank. To counter this they introduced larger particles immediately prior to 'slush' ice accumulation. The different dimensions of the tank described in this chapter prevented thorough mixing of larger particles or cultures if they were introduced immediately prior to the T_0 water sample was collected.

Inclusion of Microspheres

Microsphere numbers in ice were up to 20 times the number of microspheres in the water samples from corresponding water samples (Figures 5.9 - 5.13). Microspheres in the size range of 1 - 32 μm and 53 - 63 μm were most consistently enriched in the ice (generally 10 times the level over the water column samples). This result was not reflected in the 32 - 38 μm category or with microspheres greater than 63 μm in size. These results are consistent with Garrison *et al* (1983) whose population comparisons from field samples have indicated that no size selection occurs when organisms are incorporated into frazil ice from the water column. However, Wollenburg *et al* (1991) indicate that the bulk of sediment entrained in sea ice was finer that 16 μm. This may be a result of the bulk of material in the water column at the time being in that size category.

5.4.5 INCORPORATION OF ANTARCTIC CULTURES...

Section 5.3.5 provides results of polar phytoplankton, flagellate and bacterial experiments. Cell numbers in water, slurry, ice and sediment trap samples were compared using Paired two-tailed t-Test analysis. In the majority of samples results of

this analysis indicated slurry samples contained significantly higher number of algal cells compared to both the water and ice samples. In addition the settled water sample regularly contained a significantly higher number of algal cells compared to the initial water sample.

Slurry Samples

The percentage increase or decrease of *Nitzschia lecointei* is shown in Figures 5.14 and 5.15. Water and ice samples show a similar time series shape. Slurry samples in Figure 5.15 show a steady increase with time, reaching up to 6 times the number in the underlying water of the corresponding time and 3 times the number which was originally found in the water (T_0).

Similarly Figures 5.16 and 5.17 show slurry samples containing between 4 and 6 times higher numbers of *Fragiliaropsis cylindrus* compared with the water and ice samples. Experiments during which slurry samples were not collected clearly mask some details of algal inclusion.

Slurry samples in subsequent experiments follow a similar pattern to discussed already. Increases in cell numbers in slurry samples of between 2 and 3 times over the level in corresponding and initial water samples are seen. Almost without exception slurry samples contained a significantly higher number of algal cell compared with the corresponding water samples.

The slurry sample from T_6 in the *P. alata* experiment (Figure 5.20) was not significantly different to the water sample from this time or the initial water sample. This diatom is relatively large ($\approx$100 μm x $\approx$10 μm) when compared with the other cells reviewed. Numbers of *P. alata* in the slurry samples (and in the water and ice samples) steadily decrease over the duration of the experiment. This raises two important issues: settling and maximum incorporation efficiency (ie efficiency versus time). These are discussed in turn below.

Settling Out of Suspension

The number of cells which settle onto the bottom of the tank during the duration of the experiments is shown in Figures 5.15 and 5.17 to 5.21. This is a rather simple technique but well describes the approximate extent of settling out of suspension. Settling, as measured by this method (per volume), is uniformly twice that of the level of cells in the T_0 water sample (for *N. lecointei, F. cylindrus, F. curta* and *C. dichaeta*). However the amount of sedimentation in the *P. alata* experiment is higher (4

times). Unlike the other algae investigated in this section, *P. alata* is not normally a sea ice algal species (Medlin and Priddle 1990). Settling occurs to a lesser extent in the flagellates experiment (Figure 5.21). The is only a 20 % increase in number of flagellates found in the settled water samples compared to the initial water sample. The motile nature of flagellates appears to enable them to resist settling out of suspension more effectively than the diatom species. Reductions in the number of algal cells later in the experiment results from both settling and incorporation of algal cells into the ice.

Efficiency Versus Time

The highest number of cells are found in the slurry samples either two or fours hours after commencement of the tank experiment (Figures 5.17 to 5.20). As the ice in the tank consolidates the ability of water to penetrate the ice decreases. Thus less phytoplankton is pushed into the ice matrix. Per volume of ice the number of algal cells will be less in later samples. This is shown in Figures 5.17, 5.18 and 5.20. Weissenberger and Grossmann (1998) also found the enrichment mechanism was most effective early in their experiments. This is not the case with the *N. lecointei* experiment # 2 (Figure 5.15). This experiment shows a peak in cell numbers at T_6, but the ice at this time was still unconsolidated (unlike in other experiments). As a result of the early maximisation of inclusion efficiency, the sampling routine was changed from T_4, T_6 and T_8 in early experiments to T_2, T_4 and T_6 in later experiments.

Bacteria

Bacteria are not enriched in newly forming sea ice (Figure 5.22). The number of bacteria cells in the ice is the same or less than in the water. The maximum number of bacteria cells in the ice are found at T_4. The number of cells at T_6 and T_8 is roughly half that of T_4. This finding is consistent with the work of Weissenberger and Grossmann (1998). They found physical enrichment of bacteria within the ice was negligible. They propose bacteria cells were not physically incorporated by rising ice crystals, but co-incorporated along with algal cells or aggregates (Weissenberger and Grossmann 1998, Grossmann and Gleitz 1993, and Grossmann 1994).

Bacterial maxima in the ice (at T_4) may result from the early peak in efficiency of enrichment processes described above and by Weissenberger and Grossmann (1998).

5.4.6 MULTI-SPECIES EXPERIMENTS...

The percentage abundance data for the first multi-species experiment is shown in Figure 5.23. Despite *Chaetoceros* species being less abundant than *P. alata* at T_0, it was the dominant species in the water for the remainder of the experiment. This is due to rapid sedimentation of *P. alata* and *O. weisflogii*. The presence of spines on the *Chaetoceros* species would contribute to the buoyancy of the cells (refer Smayda, 1970, for a review of suspension and sedimentation). *Chaetoceros dichaeta* was incorporated into the ice samples preferentially over other species. The relative abundance of *F. cylindrus* in the water column increased with time, as *C. dichaeta* was incorporated into the ice and *P. alata* and *O. weisflogii* were settled out of the water.

The second multi-species experiment (Figure 5.24) again plots the percentage abundance of the four most abundant species against time. *Chaetoceros dichaeta* again dominates the water and ice samples. Aside from a relative decrease in *C. dichaeta* cells at T_6, the percentage abundance of the cells is constant throughout. Despite its reputation of being a sea ice diatom, *Fragiliaropsis curta* rapidly settled out of suspension and was found in negligible levels in slurry and ice samples. *Nitzschia lecointei* was found in increased numbers in the slurry and ice samples over the initial levels in the water column.

5.5 SUMMARY

The mechanisms by which particles become entrained in sea ice have been the topic of ever-increasing interest. Initially, the focus of such studies was directed towards sediment investigations [eg Benson and Osterkamp (1974), Larson (1980), Naidu (1980), Osterkamp and Gosink (1982), Osterkamp and Gosink (1984)]. Despite twenty-five years of such studies, little information is available about the physical or biological enrichment processes for algae. A variety of mechanisms has been discussed since the pioneering work of Garrison *et al* (1983, 1989), these include: nucleation, scavenging, wave field pumping, water circulation patterns and ice active substances.

Results of the study herein indicate that water moving through an ice matrix is an effective enrichment mechanism. This process is capable of enriching algal numbers in slurry samples up to 10 times over the level in underlying water. Similarly, the same mechanism resulted in up to 20 times enrichment of microspheres. Both these results occurred over short duration (8 hours), and a small spatial scale (a small experimental tank). Increases in both temporal and spatial parameters could well attribute to greater enrichment factors as often seen in the Antarctic sea ice. Results from this experimental tank were consistent with those of Weissenberger and Grossmann (1998) who attributed a potential 9 - 53 times enrichment to wave field pumping and water circulation experiments respectively.

Microspheres were enriched in ice samples at levels far exceeding those of temperate phytoplankton species and even exceeding some of the Antarctic species. This indicates that enrichment processes in ice were not likely to be driven entirely by biological mechanisms but can be a product of physical processes. This finding is supported by many authors who have reported sediment enrichment in sea ice (Osterkamp and Gosink 1982, 1984). Polar phytoplankton cultures are enriched in sea ice slurry at levels exceeding water column concentrations. Due to the absence of slurry samples from the temperate phytoplankton experiments it is difficult to ascertain whether polar species are more efficiently incorporated into sea ice than temperate phytoplankton cultures. Biological mechanisms cannot be excluded as playing an

important role in the enrichment process. Polar phytoplankton species such as *P. alata* are probably less buoyant than some other species. Consequently this species settled out of suspension and was less effectively incorporated into the ice and slurry.

A diverse size range of microspheres was enriched in the ice samples. However larger microspheres (> 53 μm) rapidly settled out of suspension. This indicates that size selectivity may be an important factor in potential for incorporation of microspheres. Buoyancy and shape may be important in determining potential for algal incorporation and avoidance of settling. Phytoplankton settled out of suspension in all of the tank experiments. Results of paired two-tailed t-Test analysis indicate almost without exception, significantly more phytoplankton cells per volume were found in the settled water sample compared to the initial tank water sample.

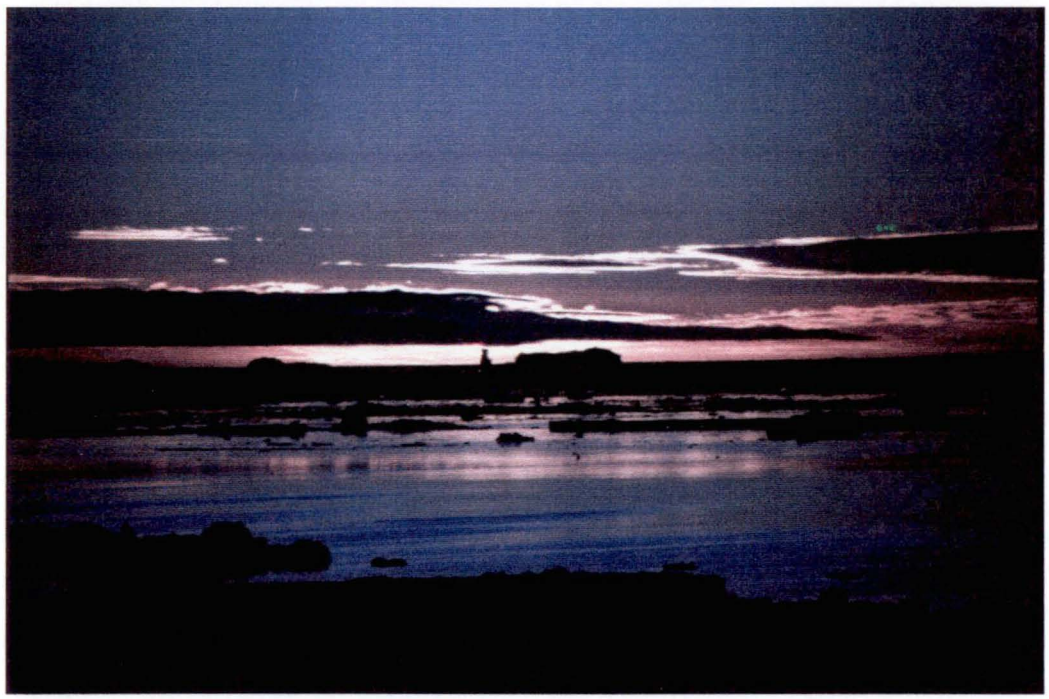
Common sea ice diatoms such as: *Fragiliaropsis curta, F. cylindrus* and *Chaetoceros dichaeta* were found to be enriched in slurry samples. Enrichment of bacteria into ice samples was not evident. Weissenberger and Grossmann (1998) also found bacteria were not effectively enriched in laboratory produced sea ice (unless they were co-entrapped using any algal culture).

The importance of sampling slurry as distinct from ice or a combination of ice and slurry was demonstrated. Slurry samples were consistently found to contain significantly higher numbers of algal cells compared with initial water samples and ice, and water samples from corresponding times (using, paired, two tailed t-Tests, assuming unequal variances and a $P \leq 0.05$ confidence level).

The use of laboratory-based experimental ice tanks is an effective method for investigating processes occurring in the natural environment.

6.0

Conclusions...



CONCLUSIONS

This study investigated the processes that facilitate the incorporation of phytoplankton into newly forming sea ice. In addition, it examined why different algal species are incorporated into different ice types. The use of field and laboratory-based experimental ice tanks facilitated investigation into specific aspects of phytoplankton inclusion in newly forming ice, without the logistic difficulties of operating in the Antarctic pack ice. While there is still scope for further studies, several important results have emerged from these experiments.

Results from laboratory and field experiments confirmed that phytoplankton were actively incorporated into newly forming sea ice. The extent of incorporation (enrichment) of algae from the underlying water was 10 - 1000 times in the natural environment (Chapters 2 and 3) and up to 10 times for laboratory-based studies (Chapters 4 and 5).

Within the pack ice, the concentration of phytoplankton in the water column at 5 and 10 m beneath both nilas and pancake ice was found to be very similar. However, the concentration of ice algae varied significantly, depending on whether it had been incorporated into nilas or pancake ice. Wave and wind-driven turbulence during pancake ice formation contributed to an increase in algal cells near the surface of the water column and consequently in the ice and slurry samples. Thus the degree of agitation in the water column at the time of new ice formation effects the number of algal cells incorporated.

Ice tank experiments supported these results. Results from non-agitated experiments indicated the number of Antarctic and temperate phytoplankton cells were significantly lower in the ice and slurry samples compared to the initial number of algal cells in the water. Results from agitated experiments indicate that Antarctic phytoplankton species and microspheres were enriched in ice and slurry samples up to 10 times the number present in the underlying water samples. No enrichment was evident in combined ice and slurry samples from experiments which used temperate phytoplankton species.

The importance of sampling slurry as distinct from ice, or a combination of ice and slurry, was demonstrated. Slurry samples were consistently found to contain significantly higher numbers of Antarctic algal cells compared with the initial water and ice samples, and water samples from corresponding times. Combined ice and slurry samples from agitated recirculating experiments from Casey had up to 7 times the biomass as compared to the initial water samples. Incorporation of bacterial cells into the ice and slurry samples was not evident. Twice the number of flagellates were found in slurry samples compared to underlying water samples in laboratory-based experiments.

While physical agitation of the water column was found to be important for algal inclusion into sea ice, the experiments discussed in this thesis show that biological factors also influence the extent of algal inclusion. Cluster and MDS analysis of natural diatom species distributions separated grease and some nilas samples from the remaining samples (Chapter 2). There is evidence of preferential incorporation of small sized cells over medium sized cells in grease ice (Chapter 2). Results from the field-based study described in Chapter 3 indicate *Chaetoceros dichaeta* was found in higher abundances in nilas compared to the underlying water column. This species has a lightly silicified frustule with four long setae (spines) and consequently it is likely to have a higher buoyancy than the more compact and more heavily silicified *Fragiliaropsis curta*. In a calm or lightly agitated system, therefor, *C. dichaeta* is more likely to be high in the water column and more available for 'scavenging' by ice crystals. In contrast, in agitated systems *Fragiliaropsis curta* dominated ice samples and upper water column samples. The zonation in species distribution in the water column at the grease ice site described in Chapter 3 (Figure 3.10), suggests the penetration of water currents to 35 m. This is indicative of a Langmuir type water circulation pattern. This process combined with wave-driven turbulence, may have been responsible for the incorporation and enrichment of algae at the grease ice site as a result of phytoplankton-rich water movement through the new ice matrix.

Results from field sampling of new ice indicate 1 to 4 species typically combined to account for > 80 % of algal abundance in newly forming ice samples (Chapters 2 and 3). Results from tank experiments support the low diversity of dominant algal species within ice and slurry samples with 1 to 4 species forming up to 90 % of species abundance in samples collected from tank experiments at Newcomb Bay, Casey, Antarctica (Chapter 4). Laboratory-based tank experiments indicated size-selective incorporation of microspheres into the ice occured. The largest (and heaviest) microspheres (> 63 μm) rapidly settled out of suspension. Smaller diameter microspheres were enriched in the ice samples up to 10 times the number in water samples from corresponding times (Section 5.3.4). Phytoplankton settled out of

suspension in all of the tank experiments. Antarctic phytoplankton species such as *Proboscia alata* are probably less buoyant than some other species. Consequently this species settled out of suspension and was less effectively incorporated into the ice and slurry (Section 5.3.5).

In summary, the incorporation and enrichment of algae into newly forming Antarctic sea ice is not purely a physical or biological process but a combination of both. The enrichment of algae into sea ice hinges on more than just the availability of algae in the underlying water; agitation is a vitally important process. Physical processes which act to enrich the algae include scavenging, filtering by the ice matrix and size selectivity. In calm conditions, initial algal incorporation probably results from ice crystals scavenging the more buoyant algal cells. In an agitated system wind and wave-driven turbulence leads to enrichment as a result of water being 'pumped' through the ice matrix, by either wave field activity and/or small scale water circulation patterns (eg Langmuir cells). Scavenging may occur once the ice consolidates and lessens the efficiency of wind and wave-driven turbulent processes. Some size selectivity occurs discriminating against incorporation into the ice of large microspheres (> 63 μm) and large diatoms (*Proboscia alata*); in addition there is evidence of preferential incorporation of small diatoms in grease ice samples. Biological processes which assist the incorporation of phytoplankton into newly forming ice include, the size of the phytoplankton cells and their ability to remain suspended the upper water column in the absence of agitation.

7.0

References...

7.0 REFERENCES

Ackermann NL, Shen HT and Sanders B, 1990 Experimental studies of sediment enrichment of artic ice covers due to wave action and frazil entrainment. *IAHR Ice Symposium Espoo* 86-96

Ackley SF, 1979 Mass balance aspects of the Weddell Sea pack ice. *J Glaciol* **24** 391 - 406

Ackley SF, 1982 Ice scavenging and nucleation: two mechanisms for incorporation of algae into newly-formed sea ice. *EOS* **63** 54

Ackley SF, Buck KR and Taguchi S, 1979 Standing crop of algae in the sea ice of the Weddell Sea region. *Deep Sea Res* **26** 269 - 281

Ackley SF, Dieckmann GS & Shen H, 1987 Algal and Foram incorporation into new sea ice. *EOS Trans Amer Geophys Union* **63** 1736

Ackley SF and Sullivan CW, 1994 Physical controls on the development and characteristics of Antarctic sea ice biological communities - a review and synthesis. *Deep Sea Res* **41** 1583 - 1604

Allison I and Worby AP, 1994 Seasonal changes of sea ice characteristics off East Antarctica. *Ann Glaciol* **20** 195 - 202

Ahn I, Chung H, Kang S and Kang S, 1997 Diatom composition and biomass variability in nearshore waters of Maxwell Bay, Antarctica, during the 1992/1993 austral summer. *Polar Biol* **17** 123 - 130

Babic M, Bjedov G, Shen H, 1992 Sediment laden oscillatory flow in an idealised porous media. *Transport in Porous Media* **7** 187 - 204

Bainbridge R, 1957 The size, shape and density of marine phytoplankton concentrations. *Biol Reviews* **32** 91 - 115

Barnes PW, Kempema EM and Reimnitz E, Source, characteristics, and significance of sediment pellets formed on the sea ice of the artic basin. CRREL Monograph 90-1. pp 106 - 108

Barry JP, 1988 Hydrographic patterns in McMurdo Sound Antarctica and their relationship to local benthic communities. *Polar Biol* **8** 377 - 91

Benson CS and Osterkamp TE, 1974 Underwater ice formation in rivers as a vehicle for sediment transport. In: Hood DW and Kelley EJ (eds) *Oceanography of the Bering Sea*, Inst of Marine Sci, Univ of Alaska, Fairbanks

Bray JR, Curtis JT 1957 An ordination of the upland forest communities of Southern Wisconsin. *Ecol Monogr* **2** 325 - 349

Bunt JS and Lee CC, 1970 Seasonal primary productivity in Antarctic sea ice at McMurdo Sound in 1967. *J Mar Res* **28** 304 - 320

Clarke DB and Ackley SF, 1984 Sea ice structure and biological activity in the Antarctic marginal ice edge zone. *J Geophys Res* **89** 2087 - 2095

Clarke DB, Ackley SF and Kumai M, Morphology and ecology of diatoms in sea ice from the Weddell Sea CRREL Monograph 84-5. pp 1 - 41

Cota GF, Legendre L, Gosselin M and Ingram RG, 1991 Ecology of bottom ice algae: I Environmental controls and variability. *J Mar Syst* **2** 257 - 277

Daly SF and Stolzenbach KD, 1984 The dynamics of frazil ice formation. In: Ice Symposium 1984, *Proceedings International Association of Hydraulic Research*, Hamburg **1** 161 - 172

Davidson AT and Marchant HJ, 1992 Protist abundance and carbon concentration during a *Phaeocystis*-dominated bloom at an Antarctic coastal site. *Polar Biol* **12** 387 - 395

Decho AW, 1990 Microbial ex-polymer secretions in ocean environments: Their role(s) in food web and marine processes. *Oceanogr Mar Biol Ann Rev* **28** 73 - 153

Dieckmann GS, Rohardt G, Hellmer H and Kipfstuhl J, 1986 The occurrence of ice platelets at 250 m depth near the Filchner Ice Shelf and its significance for sea ice biology. *Deep-Sea Research* **33** 141 - 148

Dieckmann GS, Lange MA, Spindler M and Ackley SF, 1988 The foraminifera *Neogloboquadrina pachyderma* in sea ice of the Weddell Sea. *EOS* **69** 1262

Dieckmann GS, Spindler M, Lange MA, Ackley SF and Eicken H, 1991 Antarctic sea ice: a habitat for the foraminifer *Neogloboquadrina pachyderma. J of Foram Res* **21** 182 - 189

Dieckmann GS, Lange MA, Ackley SF and Jennings JC, 1991b The nutrient status in sea ice of the Weddell Sea during winter: Effects of sea ice texture and biological activity *Polar Biol* **11** 449 - 456

Dieckmann GS, Wang Z, Stürcken-Rodewald M, Klauke U, Koch V and Schmidt W, 1993 Water column biology (phytoplankton and nutrients). In: Spindler M, Dieckmann G, Thomas D (eds) The expedition ANTARKTIS X/3 of RV "Polarstern" in 1992 *Rep Polar Res* **121** 51 - 58

Eicken H, 1992 The role of sea ice in structuring Antarctic ecosystems *Polar Biol* **12** 3 - 13

El-Sayed SZ, 1988 Seasonal and inter-annual variability's in Antarctic phytoplankton with reference to krill distribution. In: Sahrhage D(ed) *Symposium on Antarctic ice and water masses* Scientific Committee on Antarctic Research, Cambridge pp 35 - 54

Everitt DA and Thomas DP, 1986 Observations of seasonal changes in diatoms at inshore localities near Davis Station, East Antarctica. *Hydrobiol* **139** 3 - 12

Everson IR, 1988 The Antarctic food web. In: Walton DWH (ed) *Antarctic Science* Cambridge University Press, Cambridge pp 113 - 124

Fogg GE, 1983 The ecological significance of extracellular products of phytoplankton photosynthesis. *Botanica Mar* **26** 3 - 14

Fryxell GA and Kendrick GA, 1988 Austral spring microalgae across the Weddell Sea ice edge: spatial relationships found along a northward transect during AMERIEZ 83. *Deep Sea Research* **35** 1 - 20

Garrison DL, 1991 Antarctic sea ice biota. *American Zoology* **31** 17 - 33

Garrison DL and Buck KR, 1985 Sea ice algal communities in the Weddell Sea: species composition in ice and plankton assemblages. In: Gray JS and Christiansen ME (eds): *Marine Biology of Polar Regions and Effects of Stress on Marine Organisms* John Wiley and Sons pp 103 - 122

Garrison DL and Buck KR, 1986 Organism losses during melting: a serious bias in sea ice community studies. *Polar Biol* **6** 237 - 239

Garrison DL and Buck KR, 1989 Protozooplankton in the Weddell Sea Antarctica: Abundance and distribution in ice-edge zone. *Polar Biol* **9**

Garrison DL and Buck KR, 1991 Surface-layer sea ice assemblages in Antarctic pack ice during the austral spring: environmental conditions primary production and community structure. *Mar Ecol Prog Ser* **75** 161 - 172

Garrison DL and Close AR, 1993 Winter ecology of the sea ice biota in Weddell Sea pack ice. *Mar Ecol Prog Ser* **96** 17 - 31

Garrison DL, Ackley SF and Buck KR, 1983 A physical mechanism for establishing algal populations in frazil ice. *Nature* **306** 363 - 365

Garrison DL, Sullivan CW and Ackley SF, 1986 Sea ice microbial communities in Antarctica. *Bioscience* **36** 243 - 250

Garrison DL, Buck KR and Fryxell GA, 1987 Algal assemblages in Antarctic pack ice and in ice edge plankton. *J Phycol* **23** 564 - 572

Garrison DL, Close AR and Reimnitz E, 1989 Algae concentrated by frazil ice: evidence from lab experiments and field measurements. *Ant Sci* **1** 313 - 316

Gleitz M, Bathmann UV and Lochte K, 1994 Build up and decline of summer phytoplankton biomass in the Eastern Weddell Sea, Antarctica. *Polar Biol* **14** 413 - 422

Gløersen P, Campbell WJ, Cavalieri DJ, Comiso JC, Parkinson CL and Zwally HJ (eds), 1992 *Arctic and Antarctic sea ice, 1978 - 1987: satellite passive-microwave observations and analysis.* NASA, Washington DC, 290 pp

Gordon AL and Cosimo, 1988 Polynyas in teh Southern Ocean. *Sci Am* June 70 - 76

Gow AJ, Ackley SF, Weeks WF and Govoni JW, 1982 Physical and structural characteristics of Antarctic sea ice. *Ann Glaciol* **3** 113 - 117

Grant WS and Horner RA, 1976 Growth responses to salinity differences in four Arctic diatoms. *J Phycol* **12** 180 - 185

Gradinger R and Ikavalko J, 1998 Organism incorporation into newly forming Arctic sea ice in the Greenland Sea. *J Plank Res* **20** 871 - 886

Grossi SM, Kottmeier ST and Sullivan CW, 1984 Sea ice microbial communities III, Seasonal abundance of microalgae and associated bacteria McMurdo Sound Antarctica. *Microbial Ecology* **10** 231 - 242

Grossmann S (1994) Bacterial activity in sea ice and open water of the Weddell Sea, Antarctica: a microautoradiographic study. *Microb Ecol* **28** 1 - 18

Grossmann S and Gleitz M (1993) Microbial responses to experimental sea ice formation: implications for the establishment of Antarctic sea ice communities. *J Exp Mar Biol Ecol* **173** 273 - 289

Guillard RR and Ryther JH (1962) Studies of marine planktonic diatoms. In: Werner D (ed) *The biology of diatoms*, Blackwell Sci Publ, Oxford 372 - 469

Hanley TOD and Tsang G, 1984 Formation and properties of frazil in saline water. *Cold Reg Sci Technol* **8** 209 - 221

Hasle G, 1969 *Hvalrad Skr* **52** 1 - 168

Hasle G and Fryxell G, 1970 Diatoms: cleaning and mounting for light and electron microscopy. *Trans Am Microsc Soc* **89** 469 - 474

Helmke E and Weyland H, 1995 Bacteria in sea ice and underlying water of the Eastern Weddell Sea in Midwinter. *Mar Ecol Prog Ser* **117** 269 - 287

Hobbs P, 1974 *Ice Physics* Oxford University Press 837 p

Horner RA, 1976 Sea ice organisms. *Oceanogr Mar Biol Ann Rev* **14** 167 - 182

Horner RA, 1977 History and recent advances in the study of sea ice biota. In: Dunbar MJ (ed) *Polar Oceans* Arct Inst N Am Calgary/CDN pp 269 - 283

Horner RA, 1985 *Sea Ice Biota.* CRC Press, Boca Raton, pp 220

Horner RA, 1991 Ice associated ecosystems. In Medlin LK and Priddle J (eds): *Polar marine diatoms* BAS Cambridge pp 9 - 14

Horner RA, Ackley SF, Dieckmann GS, Gulliksen B, Hoshiai T, Legendre L, Melnikov IA, Reeburgh WS, Spindler M and Sullivan CW, 1992 Ecology of sea ice biota: habitat terminology and methodology. Polar *Biol* **12** 417 - 427

Hoshiai T, 1977 Seasonal change in sea ice near Syowa station, Antarctica In. Dunbar MJ (ed) *Polar Oceans* Arct Inst N Am, Calgary/CDN, 307 - 317

Jeffries MO, Worby AP, Morris K and Weeks WF, 1997 Seasonal variations in the properties and structural composition of sea ice and snow cover in the Bellingshausen and Amundsen Seas, Antarctica. *J Glaciol* **43** 138 - 151

Kang SH and Fryxell GA, 1991 Most abundant diatom species in water column assemblages from five leg 119 drill sites in Prydz bay Antarctica: distribution patterns. *Proceedings of the Ocean Drilling Program Sciresults* **119** 645 - 666

Kawamura A and Ichikawa T 1984 Distribution of diatoms in a small area in the Indian Sector of the Antarctic. In: *Proceedings of the 6 th Symposium on Polar Biology.* National Institute of Polar Research, Japan, pp 25 - 37

Kempema EW, Reimnitz E and Hunter RE, 1986 Flume studies and field observations in the interaction of frazil and anchor ice with sediments. *US Geological Survey Open-File Report* 86 515 - 548

Knox GA, 1994 *The biology of the Southern Ocean.* Cambridge University Press, Cambridge, pp 444

Kottemeir ST and Sullivan CW, 1987 Sea ice microbial communities, Bacterial production in annual sea ice of McMurdo Sound Antarctica. *Mar Ecol Prog Ser.* **35** 175 - 186

Larson L, 1980 Sediment laden sea ice: Concepts problems and approaches. In: Schell DM (ed) *Beaufort Sea Winter Watch: Ecological Processes in the Nearshore Environment,* Special Bulletin 29, Univ of Alaska, Fairbanks

Laubscher R, Perissinotto R and McQuaid C, 1993 Phytoplankton production and biomass at frontal zones in the Atlantic sector of the Southern Ocean. *Polar Biol* **13** 471 - 481

Ligowski R, 1987 Sea ice microalgae community of the floating ice in the Admiralty Bay (South Shetland Islands). *Pol Polar Res* **8** 367 - 380

Lizotte MP and Sullivan CW, 1991 Photosynthesis-irradiance relationships in microalgae associated with Antarctic pack ice: evidence for in situ activity. *Mar Ecol Prog Ser* **71** 175 - 184

McConville MJ and Wetherbee R, 1983 The bottom-ice microalgal community from annual ice in the inshore waters of East Antarctic. *J Phycol* **19** 431 - 439

McConville MJ, 1985 Chemical composition and biochemistry of sea ice microalgae. In: Horner RA (ed) *Sea Ice Biota.* CRC Press, Boca Raton, pp 21 - 82

McMinn A and Hodgson D, 1993 Summer phytoplankton succession in Ellis Fjord, Eastern Antarctica. *J Plankt Res* **15** 925 - 938

Martin SA and Kauffman P, 1981 A field study and laboratory study of wave dampening by grease ice. *J Glaciol* **27** 283 - 314

Medlin LK and Priddle J, 1990 *Polar marine diatoms* BAS, Cambridge, pp 214

Meguro H, Ito K and Fukushima H, 1967 Ice flora (bottom type): A mechanism of primary production in polar seas and the growth of diatoms in sea ice. *Arctic* **20** 114 - 133

Melinkov I, 1995 An in situ experimental study of young sea ice formation on an Antarctic lead. *J Geophys Res Ocean* **100** 4673 - 4680

Melinkov I, 1998 Winter production of sea ice algae in the Western Weddell Sea. *J Mar Sys* **17** 195 - 205

Naidu S, 1980 An alternative conceptual model for sediment concentration in frazil ice of North Arctic Alaska. In: Schell DM (ed)*Beaufort Sea Winter Watch: Ecological Processes in the Nearshore Environment,* Special Bulletin 29, Univ of Alaska, Fairbanks

Osterkamp TE and Gosink JP, 1982 Selected aspects of frazil ice formation and ice cover development in turbulent streams, Paper presented at the *Workshop on the hydraulics of ice covered rivers*, Edmonton, Alberta, Canada

Osterkamp TE and Gosink JP, 1984 Observations and analysis of sediment laden sea ice. In: Barnes PW, Scholl DM and Reimnitz E (Eds) The Alaska Beaufort Sea: Ecosystems and Environments. Academic Press, San Francisco, CA pp 73 - 94

Painter TJ, 1983 Algal polysaccharides. In: Aspinall GO (ed) *The Polysaccharides 2*, Academic Press, New York, 195 - 285

Palmisano AC and Sullivan CW, 1982 Physiology of sea ice diatoms I, Response of three polar diatoms to a simulated summer - winter transition. *J Phycol* **18** 489 - 498

Parsons TR, Maita Y *et al*, 1984 Determination of chlorophylls and total carotenoids: spectrophotometric method. *A Manual of Chemical and Biological Methods for Seawater Analysis*. Pergamon Press, New York, pp 270

Perry FGJ and Pounder ER, 1958 Crystal orientation in ice sheets. Can *J Phys* **36** 494 - 502

Phytoplankton Manual, Monographs on Oceanographic methods. In : Sournia A (ed) UNESCO, Paris, 1978, pp 337

Poulin M, Cardinal A and Legendre L, 1983 Response of a community of ice diatoms to a salinity gradient (Hudson Bay). *Mar Biol* **76** 191 - 202

Price IR, 1990 Marine Plant Life In: Clayton MN and King RJ (eds) *Biology of Marine Plants* Longman Chesire, Sydney, pp 501

Priddle J, Croxall JP, Everson I, Heywood RB, Murphy EJ, Prince PA and Sear CB, Large-scale fluctuations in distribution and abundance of krill - a discussion of possible causes. In: Sahrhage D (ed) *Antarctic Ocean and resources variability.* Springer-Verlag Berlin pp 169 - 182

Raymond JA, Sullivan CW and DeVries AL, 1997 Ice-active substances from Antarctic sea ice diatoms. *Polar Biol* **14** 71 - 75

Reimnitz E, Kempema E, Weber W, Clayton J and Payne J, 1991 Suspended-matter scavenging by rising frazil ice. In: Ackley S, Weeks S (eds) Sea ice properties and processes. CRREL Monograph 90-1. pp 97 - 100

Reimnitz E, Clayton JR, Kempema EW, Payne JR and Weber WS, 1993 Interaction of rising frazil with suspended particles: tank experiments with applications to nature. *Cold Reg Sci Technol* **21** 117 - 135

Round FE, Crawford RM and Mann DG, 1990 *The diatoms: Biology and Morphology of the genera.* Cambridge University Press, Cambridge

Scott P, 1992 Diatom composition in Antarctic sea ice. B.S., Univ of Tasmania, Hobart, Tasmania, Australia, 121 pp

Scott P, McMinn A and Hosie G, 1994 Physical parameters influencing diatom community structure in eastern Antarctic sea ice. *Polar Biol* **14** 507 - 517

Shen H and Ackermann NI, 1988 Wave induced sediment enrichment in ice covers. *EOS* **69** 1262

Smayda TJ, 1970, The suspension and sinking of phytoplankton in the sea. *Oceanogr Mar Biol Ann Rev* **8** 353 - 414

Smith WO and Nelson DM, 1985 Phytoplankton bloom produced by a receding ice edge in the Ross Sea: spatial coherence with the density field. *Science* **227** 63 - 166

Spindler M and Dieckmann G, (1990) Distribution and abundance of the planktic foraminifer *Neogloboquadrina pachyderma* in sea ice of the Weddell Sea *Polar biol* **5** 185 - 191

Stavn HR, 1971 The horizontal-vertical distribution hypothesis: Langmuir circulations and *Daphnia* distributions. *Liminol Oceanogr* **16** 453 - 466

Steemann - Nielsen E, 1977 *Marine photosynthesis with special emphasis on ecological aspects* Elsevier Oceanography Series 13 Elsevier Amsterdam 141 p

Stringer WJ, Barnett DG and Godin RH, 1984 Handbook for sea ice analysis and forecasting. *Naval Env Pred Res Fac,* CA

Sullivan CW, 1985a Sea ice bacteria: Reciprocal interaction of the organisms and their environments In: RA Horner (ed) *Sea ice biota* CRC Press Boca Raton Florida pp 159 - 171

Sullivan CW, 1985b Sea ice microbial communities: distribution abundance and diversity of ice bacteria in McMurdo Sound Ant 1980. *Appl Environ Micro* **49** 788 - 795

Sullivan CW and Palmisano AC, 1984 Sea ice microbial communities: distribution, abundance and diversity of ice bacteria in McMurdo Sound, Antarctica, in 1980. *Appl Env Microbiol* **47** 788 - 795

Syvertsen E and Kristan S, 1993 Ice algae during EPOS, leg 1: assemblages, biomass, origin and nutrients. *Polar Biol* **13** 61 - 65

Untersteiner (1986) *The Geophysics of Sea Ice* Plenum Press New York pp 523

Vincent WF, 1988 *Microbial ecosystems of Antarctica* Cambridge University Press Cambridge 304 p

Waite A, Ficher A, Thompson P and Harrison P, 1997 Sinking rate versus cell volume relationships illuminate sinking rate control mechanisms in marine diatoms. *Mar Ecol Prog Ser* **157** 97 - 108

Watanabe K, 1982 Centric diatoms communities found in the Antarctic sea ice. *Antarct Rec* **74** 119 - 126

Watanabe K and Satoh H, 1987 Seasonal variations of ice algal standing crop near Syowa Station East Antarctica in 1983/84. *Bulletin of the Plankton Society of Japan* **34** 143 - 164

Watanabe K, Satoh H and Hoshiai T, 1990, Seasonal variation in ice algal assemblages in the fast ice near Syowa Station in 1983/84. In Kerry KR, Hempel G (eds) *Antarctic ecosystems: ecological change and conservation.* Springer-Verlag, Berlin 136 - 142

Weeks WF and Ackley SF, 1982 The growth structure and properties of sea ice. CRREL Monograph 82-1. pp 130

Weissenberger J and Grossmann S, 1998 Experimental formation of sea ice: importance of water circulation and wave action for incorporation of phytoplankton and bacteria. *Polar Biol* **20** 178 - 188

White WB and Peterson RG, 1996 An Antarctic circumpolar wave in surface pressure, wind, temperature and sea ice extent. *Nature* **380** 699 - 702

Wilson DL, Smith WO and Nelson DM, 1986 Phytoplankton bloom dynamics of the Wetsren Ross Sea ice edge -I. Primary productivity and species specific productivity. *Deep Sea Res* **33** 1375 - 1387.

Wollenburg I, Pfirman S and Lange MA, 1991, Sediment in Eurasian Arctic sea ice CRREL Monograph 90-1. pp 102 - 106

Worby AP and Massom RA, 1995 The structure and properties of sea ice and snow cover in East Antarctic pack ice. Antarctic CRC Research Report No.7. Hobart TAS. pp 190

Worby AP, Jeffries MO, Weeks WF, Morris K and Jana R, 1996 The thickness distribution of sea ice and snow cover during late winter in the Bellinghausen and Amundsen Seas, Antarctica. *J Geophy Res* **101** 441-455

Worby AP, Massom RA, Allison I, Lytle VI and Heil P, 1998 East Antarctic sea ice: a review of its structure properties and drift. *Ant Res Ser* **74** 41-67

World Meteorological Organisation, 1970 *WMO sea ice nomenclature, terminology, codes and illustrated glossary.* WMO/OMM/BMO 259, TP 145. Geneva, World Meteorological Organisation

Wright SW, Jeffrey SW, Mantoura RFC, Llewellyn CA, Bjørnland, Repeta D, Welschmeyer N, 1991 Improved HPLC method for the analysis of chlorophylls and carotenoids from the marine phytoplankton. *Mar Ecol Prog Ser* **77** 183 - 196

Wright SW, Thomas DP, Marchant HJ, Higgins HW, Mackey MD and Mackey DJ, 1996 Analysis of phytoplankton of the Australian sector of the Southern Ocean: comparisons of microscopy and size frequency data with interpretations of pigment HPLC data using the 'CHEMTAX' matrix factorisation program. *Mar Ecol Prog Series* **144** 285 - 298

Xiuren N, Zilin L, Genhai Z and Junxian S, 1996, Size fractionated biomass and productivity of phytoplankton and particulate organic carbon in the Southern Ocean. *Polar Biol* **16** 1 - 11

Zwally HJ, Cosimo JC, Parkinson CL, Campbell WJ, Carsey FD and Gloersen P, 1983 Antarctic sea ice 1973 - 1976: Satellite Passive - Microwave Observations NASA SP-459 Washington pp 224

8.0

Appendices...

8.0 APPENDICES

8.1 Species cited in thesis

8.2 Chapter 2 Species abundance information

8.3 Chapter 3 Species abundance information

8.4 F2 Media

8.5 Students t-Test Analysis data for Chapter 4

8.6 Chapter 4 Species abundance information

8.7 Chapter 4 Biomass raw data

8.8 Chapter 4 Cluster Analysis Pairing Sequences

8.9 Chapter 5 Students t-Test Analysis data

8.1 Species Cited

Species	Citation
Actinocyclus actinochilus	(Ehrenberg) Simonsen
Asteromphalus hecpactis	(Brebisson) Ralfs
A. hookerii	Ehrenberg
A. hyalinus	Karsten
Atlanticum bulbossum	
Berkeleya rutilans	
Chaetoceros dichaeta	Ehrenberg
C. spp	-
Cocconeis spp	-
Corethron criophilum	Castracane
Corethron spp	-
Dactyliosolen antarcticus	Castracane
Dinoflagellate spp	-
Dinoflagellate cyst	-
Entomeneis kjellmanii	
Eucampia antarctica	(Castracane) Manguin
Fragilariopsis curta	(VH) Hudtedt
F. cylindrus	(Grunow) Krieger
F. kerguelensis	(O'Meara) Hasle
F. ritscheri	(Hustedt) Hasle
Leptocylindrus mediterraneus	H. Peragallo
Navicula directa	Cleve
N. glaciei	Van Heurck
N. spp	-
Nitzschia angulata	Hasle
N. closterium	(Ehrenberg) W. Smith
N. lecointei	Van Heurck
N. lineata	(Castracane) Hasle
N. lineola	Cleve
N. obliquecostata	(Van Heurck) Hasle
N. peragallii	Hasle
N. pseudodonana	Hasle
N. separanda	(Hustedt) Hasle
N. stellata	Manguin
N. sublineata	Hasle
N. vanheurckii	(M. Per) Hasle
Pinnularia spp	-
Pleurosigma spp	-
Porosira spp	-
Proboscia alata	Brightwell
Pseudonitzschia turgiduloides	(Hasle) Hasle
Rhizosolinea spp	-
Silicoflagellate spp	-
Stelarima microtrias	Hasle and Sims
Synedra spp	-
Thalassiothrix spp	-
Thalasiosira gracilis	(Kars.) Hustedt
T. gravida	Cleve
T. lehgtinosa	(Jan.) Fryxell
T. oestrupii	(Ost.) Hasle
T. radicans	
T. tumida	(Jan.) Hasle
Tintinid spp	-
Tricotoxin spp	-

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	C N	C SU	C 5	C 10	E N	E SU	E 5	E 10
Actinocyclus actinochilus			1					
Asteromphalus hecpactis					0			
A. hookerii								
A. hyalinus								
Atlanticum bulbossum								
Berkeleya rutilans			1					1
Chaetoceros dichaeta	17		12	16	35		47	67
C. sp	1		3	1				1
Cocconeis spp								
Corethron spp			2		0			1
Dactyliosolen antarcticus					1			
Dinoflagellate spp								
Dinoflagellate cyst								
Entomeneis kjellmanii								
Eucampia antarctica								2
Fragilariopsis curta	53		55	41	33		35	11
F. cylindrus	12		7	9	9		7	1
F. kerguelensis					3			
F. ritscheri	1		1		1			
Navicula directa			1					
N. glaciei								
N. spp								
Nitzschia angulata	1		3	5	5			
N. closterium	1						1	
N. lecointei	5		4	4	0		3	3
N. lineata								
N. lineola								
N. obliquecostata					1			
N. peragallii								
N. pseudodonana					1			
N. separanda				1	1			2
N. stellata	1			3	4			2
N. sublineata	3		2	2	0		1	
N. vanheurckii								
Pinnularia spp								
Pleurosigma spp								
Porosira spp	1						1	
Proboscia alata								
Pseudonitzschia turgiduloides	1		7	16	2		4	4
Rhizosolinea spp					1			
Silicoflagellate spp			1					1
Stelarima microtrius								
Thalassiothrix spp								
Thalasiosira gracilis					1			
T. gravida	2		1	2			1	1
T. oestrupii								
T. radicans								
T. tumida	1							
Tintinid spp				1			1	
Tricotoxin spp			1					
Other	1		1				1	3

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	I N	I SU	I 5	I 10	J N	J SU	J 5	J 10
Actinocyclus actinochilus	1							
Asteromphalus hecpactis							1	
A. hookerii	3							
A. hyalinus								
Atlanticum bulbossum							1	
Berkeleya rutilans	2							
Chaetoceros dichaeta	14			54	20		17	30
C. sp				4			2	6
Cocconeis spp								
Corethron spp					1			
Dactyliosolen antarcticus					2		9	19
Dinoflagellate spp								
Dinoflagellate cyst								
Entomeneis kjellmanii					1			
Eucampia antarctica	1			1				
Fragilariopsis curta	38			16	30		29	10
F. cylindrus	9			1	11		7	9
F. kerguelensis	3				2		9	
F. ritscheri	1				1		2	1
Navicula directa								
N. glaciei								
N. spp	1							
Nitzschia angulata	3				5		9	2
N. closterium								
N. lecointei				2	0		3	1
N. lineata								
N. lineola								
N. obliquecostata					0			
N. peragallii								
N. pseudodonana					2			
N. separanda				2	3		1	
N. stellata	1			2	1		2	1
N. sublineata	2			1	1			
N. vanheurckii	1							
Pinnularia spp								
Pleurosigma spp								2
Porosira spp	1			1				1
Proboscia alata				1				
Pseudonitzschia turgiduloides	15			7	17		6	13
Rhizosolinea spp	1				0			
Silicoflagellate spp				1				1
Stelarima microtrius								
Thalassiothrix spp					1		1	
Thalasiosira gracilis					1			
T. gravida	1							4
T. oestrupii								
T. radicans								
T. tumida								
Tintinid spp								
Tricotoxin spp								
Other	2			7			1	

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	O N	O SU	O 5	O 10	S1 P	S1 SU	S1 5	S1 10
Actinocyclus actinochilus								
Asteromphalus hecpactis							2	2
A. hookerii		3						
A. hyalinus								
Atlanticum bulbossum								
Berkeleya rutilans					2			
Chaetoceros dichaeta	21	65	25	20	51	21	32	11
C. sp	5	3	1		7	2		
Cocconeis spp		3						
Corethron spp					1			
Dactyliosolen antarcticus		.8	10	6		8	3	18
Dinoflagellate spp								
Dinoflagellate cyst				1				1
Entomeneis kjellmanii				1				
Eucampia antarctica								
Fragilariopsis curta	53	13	30	19	19	26	22	25
F. cylindrus	8		4	14	1	7	1	3
F. kerguelensis	1		5	1	5	4	10	15
F. ritscheri	2	3	1	4	1		1	
Navicula directa								
N. glaciei								
N. spp								
Nitzschia angulata	7	3	6	7	1	7	1	2
N. closterium								
N. lecointei					1			
N. lineata								
N. lineola							1	
N. obliquecostata			1			1		1
N. peragallii								
N. pseudodonana			1	3			1	3
N. separanda	1		3	2	1	4	2	
N. stellata	1	3	3	1				
N. sublineata			1	2	3		5	
N. vanheurckii							1	
Pinnularia spp								
Pleurosigma spp								
Porosira spp								1
Proboscia alata								
Pseudonitzschia turgiduloides			9	16	7	17	17	14
Rhizosolinea spp	1							
Silicoflagellate spp								1
Stelarima microtrius								
Thalassiothrix spp				1				
Thalasiosira gracilis				1		2	1	1
T. gravida	1							
T. oestrupii						1		
T. radicans								
T. tumida								
Tintinid spp								
Tricotoxin spp				1				
Other								2

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	S2 P	S2 SU	S2 5	S2 10	R1 P	R1 SU	R1 5	R1 10
Actinocyclus actinochilus				1	1			
Asteromphalus hecpactis								1
A. hookerii				1		1		
A. hyalinus		1						
Atlanticum bulbossum								
Berkeleya rutilans								
Chaetoceros dichaeta	17	33	45	27	26	29	36	49
C. sp	2						6	3
Cocconeis spp								
Corethron spp	1	1		1		1	1	
Dactyliosolen antarcticus	2	8	2		2	5	7	8
Dinoflagellate spp								1
Dinoflagellate cyst						1		1
Entomeneis kjellmanii				1				
Eucampia antarctica								
Fragilariopsis curta	39	20	18	16	30	25	11	14
F. cylindrus	6	1	2	9	6	7	13	2
F. kerguelensis	18	9	8	14	5	5	2	3
F. ritscheri		1	2	2	5	1	3	
Navicula directa								
N. glaciei								
N. spp								
Nitzschia angulata	7	7	4	2	6	7	2	1
N. closterium				2				
N. lecointei			2			1	2	1
N. lineata								
N. lineola								
N. obliquecostata								
N. peragallii			1					
N. pseudodonana		2	1	1	1	3	1	
N. separanda		3			1	3		1
N. stellata	2	2			1			
N. sublineata	1		2	1	1		1	1
N. vanheurckii		1						
Pinnularia spp								
Pleurosigma spp	1							
Porosira spp				1				
Proboscia alata	1	1						1
Pseudonitzschia turgiduloides	3	8	11	20	13	9	12	8
Rhizosolinea spp		1			1	1		
Silicoflagellate spp				1				3
Stelarima microtrius								
Thalassiothrix spp					1		1	
Thalasiosira gracilis	3	1						
T. gravida			1		1	1		1
T. oestrupii			1				1	
T. radicans								
T. tumida							1	
Tintinid spp								
Tricotoxin spp								
Other	1	1						1

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	R2 P	R2 SU	R2 5	R2 10	R3 P	R3 SU	R3 5	R3 10
Actinocyclus actinochilus							1	1
Asteromphalus hecpactis								
A. hookerii				—			1	
A. hyalinus								
Atlanticum bulbossum						1		
Berkeleya rutilans								
Chaetoceros dichaeta	19	30	5	16	62	1	21	27
C. sp	1				2			2
Cocconeis spp					1		1	1
Corethron spp	1							1
Dactyliosolen antarcticus	11	16	1	12		4	19	16
Dinoflagellate spp					1			
Dinoflagellate cyst							1	
Entomeneis kjellmanii	1					1	1	
Eucampia antarctica								
Fragilariopsis curta	31	23	30	22	2	36	9	10
F. cylindrus	7	7	4	4	7	4	4	1
F. kerguelensis	5	4	3	2		7	3	
F. ritscheri	5	1	2	6		1	3	
Navicula directa	1		1		2	1		
N. glaciei							2	3
N. spp								
Nitzschia angulata	5	1	3		1	5	3	4
N. closterium								
N. lecointei		1			2		2	6
N. lineata								
N. lineola					1		2	
N. obliquecostata	1		1					
N. peragallii								
N. pseudodonana	1				2			
N. separanda			2	4		1		1
N. stellata					1			1
N. sublineata								
N. vanheurckii								
Pinnularia spp								
Pleurosigma spp					1			
Porosira spp							1	
Proboscia alata	1					1		
Pseudonitzschia turgiduloides	7	13	39	30	7	31	19	23
Rhizosolinea spp			2			0		
Silicoflagellate spp	1	1				1	1	1
Stelarima microtrius								
Thalassiothrix spp	1		2					
Thalasiosira gracilis								
T. gravida	1	3	1	4				
T. oestrupii						2	1	
T. radicans								
T. tumida						1		
Tintinid spp								
Tricotoxin spp	1		4					
Other	1				8	2	5	2

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	U1 DP	U1 SU	U1 5	U1 10	U2 DP	U2 SU	U2 5	U2 10
Actinocyclus actinochilus								1
Asteromphalus hecpactis								
A. hookerii								
A. hyalinus	4	1		1	1	1	1	
Atlanticum bulbossum						2	1	
Berkeleya rutilans								
Chaetoceros dichaeta	50	19	18	28	27	23	29	27
C. sp	2	1	1	4	2	3		2
Cocconeis spp				1		1		
Corethron spp		1						
Dactyliosolen antarcticus		13	9	9	9	2	4	6
Dinoflagellate spp								
Dinoflagellate cyst			1		1		1	
Entomeneis kjellmanii		1						
Eucampia antarctica								
Fragilariopsis curta	5	13	16	17	18	8	16	3
F. cylindrus	5	5	3	2	6	1	2	3
F. kerguelensis	11	7	11	1	7	4	7	2
F. ritscheri		1		3		2	1	2
Navicula directa				2	1	3	2	
N. glaciei			1			7		3
N. spp	1							
Nitzschia angulata	3	5	13	6	3	4	12	5
N. closterium				1				
N. lecointei	3	6	4	2	2	4	5	5
N. lineata								
N. lineola				1				
N. obliquecostata						1		
N. peragallii								
N. pseudodonana			1	2	1	1		
N. separanda				1	3			
N. stellata			1			5		
N. sublineata		1				1		1
N. vanheurckii								
Pinnularia spp								
Pleurosigma spp			1					
Porosira spp								
Proboscia alata		1				1		2
Pseudonitzschia turgiduloides		23	17	12	15	19	7	19
Rhizosolinea spp								
Silicoflagellate spp	1						1	1
Stelarima microtrius			1					
Thalassiothrix spp								
Thalasiosira gracilis								
T. gravida				3		1		1
T. oestrupii		3		3	1		3	
T. radicans								
T. tumida								
Tintinid spp	4							5
Tricotoxin spp	1	1				1	2	
Other	10	1	2	1	2	5	6	12

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	U3 DP	U3 SU	U3 5	U3 10	V1 DP	V1 SU	V1 5	V1 10
Actinocyclus actinochilus		1		1				
Asteromphalus hecpactis								
A. hookerii								
A. hyalinus	6	1			1	1		
Atlanticum bulbossum								1
Berkeleya rutilans								
Chaetoceros dichaeta	2	21	28	34	16	20	12	36
C. sp				4			1	
Cocconeis spp								
Corethron spp					1			
Dactyliosolen antarcticus	2	11	6	12	2	4	15	7
Dinoflagellate spp				1				
Dinoflagellate cyst			1					
Entomeneis kjellmanii				1				
Eucampia antarctica								
Fragilariopsis curta	14	21	11	11	26	11	25	9
F. cylindrus		5		2	6	3		4
F. kerguelensis	32	11	22	5	10	7	8	14
F. ritscheri	8			1		1	2	
Navicula directa		1		1			2	
N. glaciei		1						
N. spp	2							
Nitzschia angulata	6	3	11	2		5	5	1
N. closterium					5			1
N. lecointei	6	1	1	3	3	1	11	3
N. lineata		1						
N. lineola								
N. obliquecostata								
N. peragallii								
N. pseudodonana		1			4	5		
N. separanda	2	1				1		3
N. stellata		1		2	2		2	1
N. sublineata		1						1
N. vanheurckii								
Pinnularia spp	2							
Pleurosigma spp								
Porosira spp								
Proboscia alata								1
Pseudonitzschia turgiduloides	6	18	12	4	21	9	10	13
Rhizosolinea spp								
Silicoflagellate spp				1				
Stelarima microtrius								2
Thalassiothrix spp								1
Thalasiosira gracilis								
T. gravida	2					3		1
T. oestrupii		1			3	27	5	1
T. radicans								
T. tumida								
Tintinid spp						2		
Tricotoxin spp							1	
Other	10	1	8	15			1	

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	V2 SU	V2 5	V2 10	V3 S	V3 SU	V3 5	V3 10	X1 G
Actinocyclus actinochilus								
Asteromphalus hecpactis								
A. hookerii								
A. hyalinus	2		3				1	
Atlanticum bulbossum			4		1			
Berkeleya rutilans								
Chaetoceros dichaeta	50	27	39	49	27	24	37	
C. sp	2	1	1	1	3		3	
Cocconeis spp		6			1	2	1	
Corethron spp								
Dactyliosolen antarcticus		1	2	3	6	11	7	
Dinoflagellate spp	1							
Dinoflagellate cyst								
Entomeneis kjellmanii	2			1				1
Eucampia antarctica								1
Fragilariopsis curta	6	27	12	19	35	26	7	70
F. cylindrus	2	8		3	4	7	2	5
F. kerguelensis	6	1	13	3	5	7	7	
F. ritscheri	1	2	1	4	1	2	1	3
Navicula directa							2	
N. glaciei		1						
N. spp	1	3						
Nitzschia angulata	2	4		2	3	5	2	
N. closterium			2					
N. lecointei	5	5	3		4	2		
N. lineata								4
N. lineola								
N. obliquecostata								1
N. peragallii								
N. pseudodonana	1	2	2	1		1		
N. separanda		2			3	1		
N. stellata				1				7
N. sublineata	2	1					3	2
N. vanheurckii								
Pinnularia spp								1
Pleurosigma spp								1
Porosira spp								
Proboscia alata					1	1	1	
Pseudonitzschia turgiduloides	7	5	8	11	6	9	17	2
Rhizosolinea spp							1	
Silicoflagellate spp				1		2		
Stelarima microtrius	1							
Thalassiothrix spp								
Thalasiosira gracilis								
T. gravida							1	
T. oestrupii				1			1	
T. radicans								
T. tumida								1
Tintinid spp	1		2					
Tricotoxin spp				1				
Other	8	4	8	1			6	3

8.2 Section 2 species abundance information (expressed as a percentage of 100).

Species	X1 SU	X2 G	X2 SU	X3 G	X3 G
Actinocyclus actinochilus		1		2	
Asteromphalus hecpactis					
A. hookerii					
A. hyalinus					
Atlanticum bulbossum					
Berkeleya rutilans					
Chaetoceros dichaeta					
C. sp					
Cocconeis spp				1	
Corethron spp					
Dactyliosolen antarcticus	2				
Dinoflagellate spp					
Dinoflagellate cyst					
Entomeneis kjellmanii		1	4		
Eucampia antarctica				1	1
Fragilariopsis curta	40	81	64	70	34
F. cylindrus		3	6	13	5
F. kerguelensis					
F. ritscheri					
Navicula directa					
N. glaciei	18				
N. spp	0				
Nitzschia angulata	3				3
N. closterium					
N. lecointei	3	1		1	
N. lineata		8		7	2
N. lineola					
N. obliquecostata	2				
N. peragallii					
N. pseudodonana					
N. separanda					
N. stellata	17				
N. sublineata			2	2	1
N. vanheurckii		1			
Pinnularia spp					
Pleurosigma spp					
Porosira spp		1	8	2	3
Proboscia alata					
Pseudonitzschia turgiduloides	3				
Rhizosolinea spp					
Silicoflagellate spp					
Stelarima microtrius		3			
Thalassiothrix spp					
Thalasiosira gracilis					
T. gravida					
T. oestrupii	3		4		49
T. radicans					
T. tumida	8	2	10	1	2
Tintinid spp					
Tricotoxin spp					
Other		2	2		

8.3 Chapter 3 species abundance information (expressed as a percentage of 100)

Nilas Ice Sample	Ice	Slurry	4m	10m	20m	35m
Chaetoceros dichaeta	72	54	48	31	50	23
Chaetoceros spp		1	1	2	2	1
Corethron criophilum		2		1	1	
Distephanos speculus						
Fragiliaropsis curta	11	20	10	24	19	16
Fragiliaropsis cylindrus	1	8	8	4	6	23
Gymnodinium spp			1			3
Picoplanktonic Flagellate spp	9	2	1	5	2	11
Loricate choanoflagellate			1	2		1
Leptocylindrus mediteraneus	4	3	9	5	9	5
Nitzschia closterium		1	1	2		
Nitzschia lecointei	1	7	12	2	6	6
Nitzschia pseudodonana					1	
Proboscia alata						1
Pseudonitzschia turgiduloides		2	4	3	1	5
Rhizosolenia spp			2	3	1	2
Thalassiosira gravida				3		1
Thalassiosira lengtinosa						
Tricotoxin spp						
Dinoflagellate spp	2			2	2	
Other			2	11		2
Grease Ice Sample	**Ice**	**Slurry**	**4m**	**10m**	**20m**	**35m**
Chaetoceros dichaeta	9	22	27	28	20	27
Chaetoceros spp				8		1
Corethron criophilum			1			
Distephanos speculus					1	
Fragiliaropsis curta	73	54	31	34	21	6
Fragiliaropsis cylindrus	7	7	1	3	13	19
Gymnodinium spp		1	3	4	4	2
Picoplanktonic Flagellate spp		5	15	13	19	37
Loricate choanoflagellate			4	2	3	
Leptocylindrus mediteraneus	7	7	4	4	3	1
Nitzschia closterium			1			
Nitzschia lecointei		2	2		3	5
Nitzschia pseudodonana						
Proboscia alata						
Pseudonitzschia turgiduloides			1			
Rhizosolenia spp		1				
Thalassiosira gravida			6	1	3	
Thalassiosira lengtinosa						
Tricotoxin spp					4	
Dinoflagellate spp			3	1	4	2
Other	4	1	1	2	2	

8.3 Chapter 3 species abundance information (expressed as a percentage of 100)

Nilas Ice Sample	50m	65m	80m	95m	110m	125m
Chaetoceros dichaeta	21	39	27	22	28	29
Chaetoceros spp		3				
Corethron criophilum	1					
Distephanos speculus				2		
Fragiliaropsis curta	28	15	25	8	6	33
Fragiliaropsis cylindrus	17	21	19	22	40	22
Gymnodinium spp			1	1		4
Picoplanktonic Flagellate spp	9	5	9	18	8	1
Loricate choanoflagellate		1	3	3		
Leptocylindrus mediteraneus	10	2	4	6	6	
Nitzschia closterium				1		
Nitzschia lecointei	5	5		7		7
Nitzschia pseudodonana						
Proboscia alata			1			
Pseudonitzschia turgiduloides	2	2		3		
Rhizosolenia spp	2	1		4		
Thalassiosira gravida	1	1	1			
Thalassiosira lengtinosa						
Tricotoxin spp			3		2	
Dinoflagellate spp	1		2	2	1	2
Other	3	5	5	1	9	2
Grease Ice Sample	**50m**	**65m**	**80m**	**95m**	**110m**	**125m**
Chaetoceros dichaeta	23	49	60	46	44	40
Chaetoceros spp	1		3	8		
Corethron criophilum						
Distephanos speculus						
Fragiliaropsis curta	9	5	7	3	23	19
Fragiliaropsis cylindrus	18	11	4	12	4	6
Gymnodinium spp	1	1	1	1		1
Picoplanktonic Flagellate spp	25	11	4	3	9	19
Loricate choanoflagellate	3		1	3	4	
Leptocylindrus mediteraneus	8	7	9	10	8	5
Nitzschia closterium		1	1			
Nitzschia lecointei	4	5		3		
Nitzschia pseudodonana	1					
Proboscia alata						
Pseudonitzschia turgiduloides	1		4	1	2	
Rhizosolenia spp		3				
Thalassiosira gravida	1	2			2	2
Thalassiosira lengtinosa						
Tricotoxin spp	1					2
Dinoflagellate spp	2	3	4	2	1	2
Other	2	2	2	8	3	4

8.4 F2 Media

F2 Media (Guillard and Rhyther, 1962)		
Trace Metals		
	$NaNO_3$	150 g/l
	Na_2SiO_3	13.04 g/l
	$CuSO_4$	0.0196 g/l
	$Na_2MoO_4.2H_2O$	0.0126 g/l
	$MnCl_2.4H_2O$	0.36 g/l
	KH_2OPO_4	5.675 g/l
	$CoCl_2.6H_2O$	0.022 g/l
	$ZnSO_4.7H_2O$	0.044 g/l
	Fe EDTA	15.8 g/l
	Citric Acid	9.0 g/l
Vitamins		
	Thiamine	0.2 g/l
	Biotin	0.001 g/l
	B12	0.001 g/l

8.5 Students t-Test Analysis Data for Chapter 4

Slow Freeze Frazil t-Test: Two-Sample Assuming Unequal Variances

Pennates

	W0	W4	W6	W8	I4	I6	I8
1	40	13	3	4	6	6	9
2	22	14	6	7	9	16	10
3	15	6	9	10	11	35	21
Mean	26	11	6	7	9	19	13
Variance	166	19	9	9	6	217	44
n	3	3	3	3	3	3	3

Centrics

	T0	T4	T6	T8	I4	I6	I8
1	38	1	0	3	2	2	4
2	15	2	1	7	16	9	7
3	12	2	9	11	19	11	10
Mean	22	2	3	7	12	7	7
Variance	202	0	24	16	82	22	9
n	3	3	3	3	3	3	3

Flagellates

	T0	T4	T6	T8	I4	I6	I8
1	19	11	9	11	6	9	7
2	11	19	11	12	10	11	9
3	8	20	16	13	12	13	10
Mean	13	17	12	12	9	11	9
Variance	32	24	13	1	9	4	2
n	3	3	3	3	3	3	3

Fast Freeze Congelation t-Test: Two-Sample Assuming Unequal Variances

Pennates

	W0	W6	I2	I4	I6
1	100	59	33	33	49
2	120	14	25	30	14
3	80	7	11	10	15
Mean	100	27	23	24	26
Variance	400		124	156	
n	3	3	3	3	3

Centrics

	W0	W6	I2	I4	I6
1	100	14	7	4	7
2	90	5	5	15	15
3	110	10	3	0	3
Mean	100	10	5	6	8
Variance	100		4	60	37
n	3	3	3	3	3

Flagellates

	W0	W6	I2	I4	I6
1	100	39	44	50	44
2	100	20	12	14	14
3	100	17	13	28	14
Mean	100	25	23	31	24
Variance			331	321	300
n	3	3	3	3	3

8.5 Students t-Test Analysis Data for Chapter 4

Fast Freeze Frazil t-Test: Two-Sample Assuming Unequal Variances

Pennates

	W0	W6	I2	I4	I6
1	100	27	41	73	41
2		67	78	114	84
3		18	33	79	66
Mean	100	37	50	89	64
Variance			576	490	466
n	3	3	3	3	3

Centrics

	W0	W6	I2	I4	I6
1	100	46	123	54	38
2	100	70	137	80	70
3	100	60	161	74	50
Mean	100	59	140	69	53
Variance			369	185	261
n	3	3	3	3	3

Flagellates

	W0	W6	I2	I4	I6
1	100	95	90	110	90
2	100	103	111	118	116
3	100	65	120	138	116
Mean	100	88	107	122	107
Variance			237	208	225
n	3	3	3	3	3

8.5 Students t-Test Analysis Data for Chapter 4

1 - 32 um Microspheres t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	741	13	12	9	132	82	86
2	695	19	8	6	65	50	48
3	505	12	18	18	13	7	11
Mean	647	15	13	11	70	46	48
Variance	15652	14	25	39	3559	1416	1406
n	3	3	3	3	3	3	3

32 - 38 um Microspheres t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	7960	125	23	32	46	12	47
2	7004	110	20	24	30	8	32
3	8712	105	18	21	16	2	16
Mean	7892	113	20	26	31	7	32
Variance	732784	108	6	32	225	25	240
n	3	3	3	3	3	3	3

38 - 53 um Microspheres t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	66	2	15	8	59	33	19
2	57	2	7	6	40	12	8
3	53	4	5	6	20	5	4
T0 - T4							
Mean	59	3	9	7	40	17	10
Variance	44	1	28	1	380	212	60
n	3	3	3	3	3	3	3

53 - 63 um Microspheres t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	104	48	24	4	44	44	44
2	84	48	16	4	28	20	15
3	68	40	8	8	20	8	8
Mean	85	45	16	5	31	24	22
Variance	325	21	64	5	149	336	364
n	3	3	3	3	3	3	3

63 - 90 um Microspheres t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	57	15	11	9	47	103	66
2	55	11	9	6	28	37	12
3	51	9	8	4	9	23	5
Mean	54	12	9	6	28	54	28
Variance	9	9	2	6	361	1825	1114
n	3	3	3	3	3	3	3

Bacteria t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	255	162	122	189	170	122	94
2	245	147	105	175	150	105	65
3	225	128	92	171	123	92	21
Mean	242	146	106	178	148	106	60
Variance	233	290	226	89	556	226	1351
n	3	3	3	3	3	3	3

N. lecontiei Expt #1 t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	85	30	19	10	25	33	33
2	65	13	17	14	21	25	27
3	48	11	12	19	19	17	21
Mean	66	18	16	14	22	25	27
Variance	343	109	13	20	9	64	36
Observations	3	3	3	3	3	3	3

N. lecointie Expt #2 t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	Wsettle	S4	S6	S8	I4	I6	I8
1	142	112	115	30	261	176	210	440	114	110	98
2	133	94	82	64	264	242	276	326	105	144	101
3	130	97	104	45	360	192	261	386	122	88	76
W0 - W4											
Mean	135	101	100	46	295	203	249	384	114	114	92
Variance	39	93	282	290	3171	1185	1197	3252	72	796	186
n	3	3	3	3	3	3	3	3	3	3	3

P. alata t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	Wsettle	S4	S6	S8	I4	I6	I8
1	45	24	30	22	186	148	60	36	28	18	11
2	49	29	13	13	188	106	66	46	39	19	11
3	49	18	15	16	182	112	74	36	32	18	9
Mean	48	24	19	17	185	122	67	39	33	18	10
Variance	5	30	86	21	9	516	49	33	31	0	1
n	3	3	3	3	3	3	3	3	3	3	3

F. curta t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	Wsettle	S4	S6	S8	I4	I6	I8
1	232	161	180	168	346	536	602	498	144	87	123
2	171	159	163	113	336	454	534	466	136	94	118
3	168	93	141	141	420	404	432	506	171	87	110
Mean	190	138	161	141	367	465	523	490	150	89	117
Variance	1304	1497	382	756	2105	4441	7321	448	336	16	43
n	3	3	3	3	3	3	3	3	3	3	3

F. cylindrus Expt #1 t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	I4	I6	I8
1	100	58	33	17	13	33	54
2	115	72	45	27	18	55	95
3	97	49	22	17	7	19	49
Mean	104	60	33	20	59	36	66
Variance	93	134	132	33	134		
n	3	3	3	3	3	3	3

F. cylindrus Expt #2 t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	Wsettle	S4	S6	S8	I4	I6	I8
1	411	436	411	385	397	No data	385	477	161	323	399
2	428	507	398	363	491	No data	406	560	216	291	357
3	409	322	395	297	292	No data	300	367	209	284	200
Mean	416	422	401	348	393	No data	364	468	195	299	319
Variance	109	8710	72	2097	9910	No data	3150	9373	896	432	11002
n	3	3	3	3	3	No data	3	3	3	3	3

Chaetoceros dichaeta t-Test: Two-Sample Assuming Unequal Variances

	W0	W4	W6	W8	Wsettle	S4	S6	S8	I4	I6	I8
1	381	173	261	164	666	720	369	616	290	253	155
2	246	151	207	182	788	611	330	438	191	162	106
3	332	157	182	183	544	402	302	428	208	191	118
Mean	320	160	217	176	666	578	334	494	230	202	126
Variance	4670	129	1630	114	14884	26114	1132	11188	2802	2161	652
n	3	3	3	3	3	3	3	3	3	3	3

	1.1 BW	1.1 TW	1.2 BW	1.2 BS	1.2 BI	1.2 TW	1.2 TS	1.2 TI	1.3 BW	1.3 BS	1.3 BI	1.3 TW	1.3 TS	1.3 TI	2.1 BW	2.1 TW	2.2 BW
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	61	57	80	63	66	72	56	52	56	48	50	45	56	54	58	47	41
C. dichaeta	0	4	0	0	2	0	4	7	0	0	0	0	0	0	0	9	0
Cocconeis spp	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Corethron spp	0	0	0	0	2	1	0	0	0	0	1	0	0	0	0	0	1
Dactyliosolen spp	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Eucampia antarctica	10	5	2	1	6	4	2	0	2	3	3	11	17	1	15	13	2
Fragiliaropsis curta	18	9	10	23	10	6	5	1	22	21	17	18	10	23	15	14	30
F. cylindrus	1	4	0	0	0	1	3	6	1	2	0	0	0	3	0	0	1
F. ritscheri	1	0	0	2	0	0	0	0	2	0	0	0	0	2	0	2	0
Navicula spp	0	0	3	1	3	0	0	22	1	6	3	0	1	4	0	2	3
Nitzschia angulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
N. lecointei	2	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	3
N. lineata	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
N. stellata	0	13	2	1	4	0	17	12	2	2	8	14	5	10	3	3	5
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	0	2	0	3	3	6	4	0	6	3	4	6	4	1	0	0	6
Synedra spp	5	1	0	3	2	4	3	0	2	9	8	3	1	2	1	1	1
Silicoflagellate spp	1	2	0	1	1	2	1	0	0	6	2	0	3	0	2	5	1
Thalassiosira gravida	0	2	2	1	1	0	1	0	0	0	0	0	0	0	2	0	0
Thallasiothrix spp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Unidentified Pennates	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0
Unidentified Centrics	0	0	1	0	0	1	3	0	6	0	0	0	2	0	3	3	2
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	2.2 BS	2.2 BI	2.2 TW	2.2 TS	2.2 TI	2.3 BW	2.3 BS	2.3 BI	2.3 TW	2.3 TS	2.3 TI	3.1 BW	3.1 TW	3.2 BW	3.2 TW	3.2 TS	3.2 TI
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
Chaetoceros spp	55	33	38	33	65	75	58	16	68	67	43	55	56	71	69	56	48
C. dichaeta	0	0	4	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Cocconeis spp	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0
Corethron spp	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactyliosolen spp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	9	4	10	11	12	9	3	2	4	6	7	12	10	9	0	3	4
Fragilliaropsis curta	19	26	33	29	8	4	15	47	13	11	30	12	9	13	12	17	19
F. cylindrus	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	1
F. ritscheri	3	2	1	0	0	0	0	7	0	0	2	0	0	0	0	2	0
Navicula spp	1	17	1	6	2	0	5	7	2	2	2	7	7	0	3	5	18
Nitzschia angulata	0	0	0	1	0	0	0	0	1	0	0	7	1	0	0	0	0
N. lecointei	1	0	0	0	0	0	2	1	1	1	2	0	2	0	0	5	2
N. lineata	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	3	4	9	13	7	1	9	11	0	6	9	0	5	4	4	6	2
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	0	3	0	3	5	2	3	2	0	2	0	0	0	0	4	0	2
Synedra spp	1	5	1	0	0	0	0	2	6	0	1	0	5	2	0	0	1
Silicoflagellate spp	0	2	2	2	1	1	3	0	1	1	0	2	0	1	0	3	0
Thalassiosira gravida	0	0	1	0	0	7	1	1	3	3	1	1	1	0	6	3	0
Thallasiothrix spp	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Unidentified Pennates	0	3	0	0	0	0	0	1	0	0	0	0	3	0	0	0	0
Unidentified Centrics	2	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0
Dinoflagellate spp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

	3.3 BW	3.3 TW	3.3 TS	3.3 TI	4.1 BW	4.1 TW	4.2 BW	4.2 BS	4.2 BI	4.2 TW	4.2 TS	4.2 TI	4.2 BW	4.3 TW	4.3 TS	4.3 TI	5.1 BW
Berkeleya rutilans	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	76	63	70	70	70	62	67	14	5	76	92	57	67	85	77	81	76
C. dichaeta	1	0	0	2	0	0	0	2	0	0	1	0	4	3	0	0	0
Cocconeis spp	7	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0
Corethron spp	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
Dactyliosolen spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	6	1	5	0	6	3	6	4	2	1	0	2	4	0	2	4	8
Fragiliaropsis curta	0	18	16	5	18	19	15	21	8	1	3	21	5	7	3	5	5
F. cylindrus	3	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
F. ritscheri	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Navicula spp	1	4	2	14	1	8	1	5	33	0	0	5	0	0	4	3	2
Nitzschia angulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. lecointei	1	3	0	2	0	1	0	0	7	1	0	2	1	0	2	1	0
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	2	2	2	0	1	1	10	46	43	11	2	8	3	2	5	0	0
N. sublineata	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	1	2	2	2	0	0	0	0	0	1	2	0	2	1	2	3	2
Synedra spp	0	0	0	1	3	2	0	2	0	1	0	1	0	2	2	0	0
Silicoflagellate spp	1	3	0	1	0	0	1	1	0	1	0	0	0	0	0	0	1
Thalassiosira gravida	0	3	2	0	0	0	0	0	0	0	0	0	3	0	2	0	0
Thallasiothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	1	2
Unidentified Pennates	0	0	1	0	0	2	0	1	0	2	0	3	0	0	0	2	2
Unidentified Centrics	0	0	0	0	0	0	0	2	1	4	0	0	0	0	0	0	2
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	5.1 TW	5.2 BW	5.2 BS	5.2 BI	5.2 TW	5.2 TS	5.2 TI	5.3 BW	5.3 BS	5.3 BI	5.3 TW	5.3 TS	5.3 TI	6.1 BW	6.1 TW	6.2 BW	6.2 BS
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Chaetoceros spp	61	42	17	8	82	74	66	41	51	33	63	35	0	54	47	37	64
C. dichaeta	0	1	0	4	0	0	3	2	3	5	2	0	0	0	0	6	2
Cocconeis spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corethron spp	1	0	0	0	0	0	0	0	0	0	0	0	0	4	0	1	0
Dactyliosolen spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Eucampia antarctica	2	0	1	8	1	0	2	1	4	5	0	0	0	6	0	2	6
Fragiliaropsis curta	20	0	21	11	6	9	5	4	9	15	9	30	0	17	24	27	9
F. cylindrus	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0
F. ritscheri	1	1	0	6	0	1	1	2	1	2	0	0	0	8	1	5	1
Navicula spp	2	17	22	27	2	2	9	27	5	13	8	35	0	1	8	6	7
Nitzschia angulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. lecointei	0	8	0	14	0	2	3	8	0	5	5	0	0	1	2	1	1
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	0	18	27	22	1	2	2	6	17	8	6	0	0	0	9	3	6
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	8	2	2	0	4	4	5	4	3	4	3	0	0	4	0	4	0
Synedra spp	0	0	2	0	0	0	0	0	0	2	0	0	0	0	3	0	1
Silicoflagellate spp	1	1	2	0	1	3	1	0	2	2	0	0	0	0	1	1	2
Thalassiosira gravida	2	5	2	0	2	2	1	0	0	1	1	0	0	0	1	0	0
Thallasiothrix spp	0	0	0	0	0	0	1	0	0	1	1	0	0	5	1	3	0
Unidentified Pennates	0	4	3	0	1	1	1	5	2	4	0	0	0	0	0	1	0
Unidentified Centrics	1	0	0	0	0	0	0	0	3	0	0	0	0	0	1	2	1
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	6.2 BI	6.2 TW	6.2 TS	6.2 TI	6.3 BW	6.3 BS	6.3 BI	6.3 TW	6.3 TS	6.3 TI	7.1 BW	7.1 TW	7.2 BW	7.2 TW	7.3 BW	7.3 TW	7.4 BW
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	27	66	33	46	42	24	32	55	37	35	8	26	62	52	58	48	20
C. dichaeta	0	0	0	3	1	0	3	0	2	1	0	0	4	0	0	4	0
Cocconeis spp	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1
Corethron spp	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
Dactyliosolen spp	0	0	0	0	0	0	0	0	2	0	0	0	0	2	2	4	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	0	3	0	5	1	1	2	2	0	3	5	0	12	2	1	0	0
Fragiliaropsis curta	31	9	23	24	21	25	18	20	19	32	8	34	0	14	15	4	26
F. cylindrus	0	0	1	1	4	2	2	0	2	4	0	0	0	0	0	4	4
F. ritscheri	1	4	3	2	0	3	0	0	0	0	2	0	0	0	0	0	1
Navicula spp	28	12	4	10	15	36	24	7	17	11	5	6	8	8	7	16	23
Nitzschia angulata	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. lecointei	3	2	10	0	2	4	7	1	1	2	6	4	2	6	4	4	3
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	1	2	1	3	2	3	0	3	13	1	63	18	2	4	4	8	7
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	2	0	12	3	5	1	2	10	3	6	0	1	2	7	2	8	6
Synedra spp	1	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Silicoflagellate spp	1	0	5	1	2	0	1	0	0	1	0	1	0	0	1	0	0
Thalassiosira gravida	0	0	2	0	0	0	6	0	3	0	0	5	8	3	2	0	9
Thallasiothrix spp	0	0	0	1	2	1	1	1	0	0	0	1	0	0	3	0	0
Unidentified Pennates	0	1	0	0	1	0	0	1	0	2	2	0	0	0	0	0	0
Unidentified Centrics	1	0	5	1	2	0	1	0	0	1	0	4	0	0	0	0	0
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	7.4 TW	7.5 BW	7.5 BS	7.5 BI	7.5 TW	7.5 TS	7.5 TI	7.6 BW	7.6 BS	7.6 BI	7.6 TW	7.6 TS	7.6 TI	8.1 BW	8.1 TW	8.2 BW	8.2 BS
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	43	0	0	0	34	52	26	11	0	0	28	49	30	30	30	15	5
C. dichaeta	0	0	0	0	7	0	3	0	0	0	2	0	0	3	1	2	3
Cocconeis spp	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0	0
Corethron spp	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Dactyliosolen spp	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	0	3	0	0	0	0	0	0	0	1	3	1	2	0	0	0	1
Fragiliaropsis curta	11	0	8	0	6	15	10	12	0	0	10	17	18	14	25	5	0
F. cylindrus	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	2	0
F. ritscheri	0	2	3	1	0	2	1	2	0	1	0	0	0	0	1	3	0
Navicula spp	25	45	60	70	42	18	42	56	90	80	45	12	39	33	24	22	74
Nitzschia angulata	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0
N. lecointei	3	0	2	1	2	2	0	3	0	0	7	0	2	2	0	10	0
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	4	46	25	25	3	8	7	10	10	17	2	16	0	6	5	29	14
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	7	0	0	0	0	0	7	3	0	0	0	0	4	2	3	4	1
Synedra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Silicoflagellate spp	1	1	0	0	0	0	0	0	0	0	0	2	0	1	0	0	1
Thalassiosira gravida	6	2	1	1	2	1	3	0	0	0	2	2	4	5	3	5	1
Thallasiothrix spp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Unidentified Pennates	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Unidentified Centrics	0	0	0	0	0	0	1	0	0	0	0	0	0	1	4	0	0
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0

	8.2 BI	8.2 TW	8.2 TS	8.2 TI	8.3 BW	8.3 BS	8.3 BI	8.3 TW	8.3 TS	8.3 TI	9.1 BW	9.1 TW	9.2 BW	9.2 TW	9.2 TS	9.2 TI	9.3 BW
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	4	24	43	18	19	40	8	43	38	13	24	45	25	51	34	50	38
C. dichaeta	0	3	5	3	7	0	5	0	1	0	0	0	0	1	0	0	0
Cocconeis spp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corethron spp	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Dactyliosolen spp	0	0	0	0	3	0	0	0	0	2	6	0	0	0	2	0	0
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Eucampia antarctica	0	1	4	4	0	0	2	5	4	0	0	3	0	4	4	0	0
Fragiliaropsis curta	0	9	21	23	17	8	13	5	14	10	8	0	1	5	6	30	10
F. cylindrus	0	2	1	3	3	1	0	9	1	16	2	0	2	0	6	0	0
F. ritscheri	4	2	1	0	0	0	0	0	0	0	4	0	0	9	0	0	2
Navicula spp	84	48	14	24	38	43	51	27	29	37	26	31	45	13	30	12	30
Nitzschia angulata	0	0	0	0	0	0	0	2	0	0	0	2	0	0	0	0	0
N. lecointei	3	6	0	0	0	2	5	4	0	1	4	0	0	4	0	0	7
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	1	3	2	10	5	1	10	1	4	13	14	9	18	5	4	2	6
N. sublineata	0	1	0	0	0	1	1	0	0	0	0	0	0	3	0	2	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	2	0	2	4	5	0	0	0	4	6	8	3	4	0	4	0	2
Synedra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silicoflagellate spp	0	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0
Thalassiosira gravida	1	1	3	0	1	0	0	2	0	2	4	4	0	0	6	2	0
Thallasiothrix spp	0	0	0	1	0	1	0	1	1	0	0	0	2	1	0	0	1
Unidentified Pennates	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Unidentified Centrics	0	0	3	4	0	3	5	0	2	0	0	1	3	1	0	2	3
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	2	0	0	0	1	2	0	0	0	0	2	4	0	0

	9.3 BS	9.3 BI	9.3 TW	9.3 TS	9.3 TI	9.4 BW	9.4 BS	9.4 BI	9.4 TW	9.4 TS	9.4 TI	10.1 BW	10.1 TW	10.2 BW	10.2 BS	10.2 BI	10.2 TW
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	43	17	65	34	41	5	3	14	34	52	11	6	30	37	15	14	25
C. dichaeta	0	0	1	0	0	1	0	0	2	3	0	1	2	2	0	1	0
Cocconeis spp	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0
Corethron spp	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0
Dactyliosolen spp	0	0	1	1	3	0	0	0	0	0	0	0	0	0	0	0	2
Entomeneis kjellmanni	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	0	0	2	0	1	0	0	0	0	10	0	0	4	0	1	0	0
Fragiliaropsis curta	5	15	1	17	8	18	1	8	4	3	20	2	14	7	26	8	1
F. cylindrus	3	0	1	0	11	0	0	2	0	0	2	0	0	0	0	0	0
F. ritscheri	6	0	0	5	0	0	0	0	9	0	0	0	1	2	0	0	2
Navicula spp	24	52	12	38	27	44	71	56	4	11	50	81	46	31	46	69	54
Nitzschia angulata	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. lecointei	2	4	3	2	2	2	3	3	3	0	3	2	0	2	0	1	0
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	11	7	0	1	0	24	17	12	42	11	2	3	0	10	9	4	11
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	2	2	1	1	0	2	2	0	0	4	0	2	2	2	2	0	3
Synedra spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Silicoflagellate spp	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Thalassiosira gravida	1	2	8	0	0	1	3	0	0	3	11	0	0	4	1	3	2
Thallasiothrix spp	1	1	1	0	1	1	0	1	1	3	1	1	0	1	0	0	0
Unidentified Pennates	0	0	1	0	0	2	0	2	0	0	0	0	0	0	0	0	0
Unidentified Centrics	0	0	2	0	5	0	0	0	0	0	0	1	1	0	0	0	0
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

	10.2 TS	10.2 TI	11.1 BW	11.1 TW	11.2 BW	11.2 BS	11.2 BI	11.2 TW	11.2 TS	11.2 TI	11.3 BW	11.3 BS	11.3 BI	11.3 TW	11.3 TS	11.3 TI
Berkeleya rutilans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chaetoceros spp	23	20	25	30	43	57	34	62	30	17	55	18	7	19	6	18
C. dichaeta	1	4	0	10	0	3	0	8	4	0	0	3	0	0	2	0
Cocconeis spp	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0
Corethron spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactyliosolen spp	3	3	0	10	14	11	2	0	5	0	5	15	0	0	1	0
Entomeneis kjellmanni	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Eucampia antarctica	0	0	0	8	0	0	2	0	0	1	2	0	0	0	0	1
Fragiliaropsis curta	5	8	11	5	32	2	11	5	17	2	0	11	12	1	0	12
F. cylindrus	0	0	0	1	0	0	0	0	0	0	0	3	0	0	0	3
F. ritscheri	0	0	0	0	0	0	1	0	2	3	0	0	0	0	0	2
Navicula spp	47	54	60	7	2	9	40	21	23	65	29	38	78	76	84	47
Nitzschia angulata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
N. lecointei	2	2	0	0	2	2	0	0	0	0	1	3	0	0	0	2
N. lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. obliquecostata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N. stellata	19	6	2	25	2	4	0	0	11	7	0	0	0	2	0	11
N. sublineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0
Pinularia spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pleurosigma spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pseudonitzschia turgiduloides	0	1	0	1	0	2	5	0	4	2	0	0	0	1	0	0
Synedra spp	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Silicoflagellate spp	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Thalassiosira gravida	0	1	2	1	1	0	2	2	2	0	6	6	0	1	3	1
Thallasiothrix spp	0	1	0	0	1	1	3	1	2	0	2	3	2	0	0	2
Unidentified Pennates	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Unidentified Centrics	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	1
Dinoflagellate spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0

8.7 Biomass data for Chapter 4 (chl a ug/l)

Tank Run	Sample Type	chl a ug/l
1.1	BW	0.66
	TW	0.10
1.2	BW	0.18
	BS	0.11
	BI	0.25
	TW	1.09
	TS	0.06
	TI	0.23
1.3	BW	0.81
	BS	1.05
	BI	0.28
	TW	0.55
	TS	0.27
	TI	0.18
2.1	BW	1.44
	TW	1.43
2.2	BW	1.41
	BS	0.55
	BI	0.64
	TW	0.62
	TS	0.58
	TI	5.67
2.3	BW	4.29
	BS	0.56
	BI	0.88
	TW	0.70
	TS	1.68
	TI	0.79
3.1	BW	7.25
	TW	1.18
3.2	BW	1.26
	TW	0.44
	TS	0.40
	TI	0.14
3.3	BW	7.03
	TW	0.22
	TS	1.14
	TI	0.74
4.1	BW	2.11
	TW	3.50
4.2	BW	1.63
	BS	3.87
	BI	14.76
	TW	5.03
	TS	2.59
	TI	2.30
4.3	BW	1.97
	TW	0.41
	TS	0.80
	TI	0.89

Tank Run	Sample Type	chl a ug/l
5.1	BW	0.61
	TW	3.61
5.2	BW	2.13
	BS	4.24
	BI	20.85
	TW	2.74
	TS	2.14
	TI	0.99
5.3	BW	3.01
	BS	1.15
	BI	6.29
	TW	1.64
	TS	1.22
	TI	2.68
6.1	BW	1.21
	TW	0.45
6.2	BW	1.45
	BS	0.12
	BI	0.11
	TW	0.26
	TS	1.21
	TI	1.49
6.3	BW	1.48
	BS	1.07
	BI	0.92
	TW	1.70
	TS	0.33
	TI	3.00
7.1	BW	4.34
	TW	0.70
7.2	BW	0.97
	TW	0.42
7.3	BW	0.36
	TW	1.01
7.4	BW	1.05
	TW	0.50
7.5	BW	2.69
	BS	7.40
	BI	6.55
	TW	2.25
	TS	2.32
	TI	0.42
7.6	BW	5.35
	BS	1.62
	BI	0.64
	TW	0.48
	TS	0.34
	TI	1.03

8.7 Biomass data for Chapter 4 (chl a ug/l)

Tank Run	Sample Type	chl a ug/l
8.1	BW	0.36
	TW	0.38
8.2	BW	1.47
	BS	2.50
	BI	1.53
	TW	0.41
	TS	0.53
	TI	0.70
8.3	BW	0.57
	BS	0.75
	BI	1.59
	TW	0.13
	TS	1.23
	TI	0.78
9.1	BW	2.85
	TW	1.24
9.2	BW	0.57
	TW	0.22
	TS	0.43
	TI	2.31
9.3	BW	2.08
	BS	0.57
	BI	1.25
	TW	1.09
	TS	0.50
	TI	0.04
9.4	BW	0.95
	BS	1.32
	BI	1.67
	TW	0.77
	TS	0.40
	TI	0.64
10.1	BW	0.59
	TW	0.87
10.2	BW	2.37
	BS	1.10
	BI	0.36
	TW	0.35
	TS	0.22
	TI	0.66
11.1	BW	1.21
	TW	0.19
11.2	BW	0.58
	BS	0.34
	BI	1.04
	TW	0.39
	TS	0.66
	TI	1.29
11.3	BW	0.27
	BS	0.09
	BI	2.96
	TW	0.31
	TS	1.10
	TI	0.17

Recirculating Experiments with Agitation

Pairing Sequence for Experiment # 1

Item	Joins Item	At Distance
4	9	0.119
2	7	0.119
10	11	0.138
4	14	0.143
3	5	0.158
3	6	0.178
4	10	0.201
2	13	0.218
4	12	0.243
2	3	0.252
2	4	0.273
2	8	0.380
1	2	0.853

Pairing Sequence for Experiment # 6

Item	Joins Item	At Distance
4	6	0.113
2	8	0.142
9	14	0.151
5	10	0.160
3	9	0.184
2	12	0.184
11	13	0.207
2	3	0.212
5	11	0.266
2	7	0.281
2	5	0.299
2	4	0.357
1	2	0.878

Pairing Sequence for Experiment # 2

Item	Joins Item	At Distance
8	13	0.098
6	14	0.118
8	12	0.152
6	7	0.171
8	9	0.173
3	6	0.193
8	10	0.205
2	4	0.206
3	5	0.254
2	8	0.270
2	3	0.354
2	11	0.494
1	2	0.842

Pairing Sequence for Experiment # 8

Item	Joins Item	At Distance
4	5	0.176
10	13	0.177
2	8	0.196
6	11	0.204
10	12	0.205
9	14	0.222
2	7	0.246
6	9	0.287
2	10	0.296
2	6	0.352
2	3	0.427
2	4	0.513
1	2	0.916

Recicrulating Experiments without Agitation

Pairing Sequence for Experiment # 3

Item	Joins Item	At Distance
3	9	0.097
5	8	0.107
3	4	0.126
3	5	0.178
2	3	0.226
7	10	0.229
2	7	0.250
2	6	0.274
1	2	0.861

Pairing Sequence for Experiment # 4

Item	Joins Item	At Distance
7	10	0.077
11	12	0.115
2	8	0.125
7	11	0.139
2	3	0.168
6	7	0.171
6	9	0.217
2	6	0.266
4	5	0.345
2	4	0.702
1	2	0.931

Pairing Sequence for Experiment # 5

Item	Joins Item	At Distance
6	7	0.086
8	12	0.114
6	8	0.178
3	9	0.210
2	6	0.212
2	10	0.272
3	11	0.280
4	5	0.342
2	3	0.374
4	13	0.447
2	4	0.508
1	2	0.895
1	14	0.913

Pairing Sequence for Experiment # 11

Item	Joins Item	At Distance
8	12	0.126
11	13	0.144
6	9	0.189
8	11	0.198
10	14	0.225
5	7	0.225
4	6	0.253
5	10	0.268
5	8	0.392
3	4	0.395
2	3	0.438
2	5	0.525
1	2	0.962

Flow-through Experiment with Agitation

Pairing Sequence for Experiment # 7

Item	Joins Item	At Distance
16	17	0.093
10	11	0.102
4	5	0.112
13	19	0.136
12	18	0.140
12	14	0.158
12	20	0.170
4	13	0.183
10	16	0.191
6	8	0.215
4	6	0.229
10	15	0.278
2	7	0.300
3	4	0.309
9	10	0.327
3	12	0.378
2	3	0.396
2	9	0.600
1	2	0.932

Flow-through Experiment without Agitation

Pairing Sequence for Experiment # 9

Item	Joins Item	At Distance
9	5	0.138
2	8	0.175
9	18	0.179
7	11	0.183
5	12	0.202
4	17	0.213
5	7	0.220
2	5	0.233
9	13	0.238
4	10	0.253
3	9	0.273
4	6	0.282
3	14	0.284
2	4	0.340
2	16	0.448
2	3	0.480
1	2	0.922

Pairing Sequence for Experiment # 10

Item	Joins Item	At Distance
6	8	0.136
6	7	0.145
2	4	0.200
5	6	0.212
2	5	0.233
2	3	0.280
1	2	0.979

Recirculating Experiments T-test Two-Sample Assuming Unequal Variances

	BW	BS	BI	TW	TS	TI
1	0.66	0.11	0.25	0.10	0.06	0.23
2	1.44	1.05	0.28	1.43	0.27	0.18
3	1.21	0.55	0.64	0.45	0.58	5.67
4	0.36	0.56	0.88	0.38	1.68	0.79
5		0.12	0.11		1.21	1.49
6		1.07	0.92		0.33	3.00
7		2.50	1.53		0.53	0.70
8		0.75	1.59		1.23	0.78
Mean	0.92	0.84	0.77	0.59	0.74	1.61
Variance	2.48E-05	5.81E-05	3.22E-05	3.35E-05	3.24E-05	3.50E-04
n	4	8	8	4	8	8

Recirculating without agitation T-test Two-Sample Assuming Unequal Variances

	BW	BS	BI	TW	TS	TI
1	7.25	3.87	14.76	1.18	0.40	0.14
2	2.11	4.24	20.85	3.50	1.14	0.74
3	0.61	1.15	6.29	3.61	2.59	2.30
4	1.21	0.34	1.04	0.19	0.80	0.89
5		0.09	2.96		2.14	0.99
6					1.22	2.68
7					0.66	1.29
8					1.10	0.17
Mean	2.80	1.94	9.18	2.12	1.26	1.15
Variance	9.21E-04	3.91E-04	7.01E-03	2.91E-04	5.57E-05	8.47E-05
n	4	5	5	4	8	8